LETTERS

Effects of biodiversity on the functioning of trophic groups and ecosystems

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Over the past decade, accelerating rates of species extinction have prompted an increasing number of studies to reduce species diversity experimentally and examine how this alters the efficiency by which communities capture resources and convert those into biomass[1,2]. So far, the generality of patterns and processes observed in individual studies have been the subjects of considerable debate[3–7]. Here we present a formal meta-analysis of studies that have experimentally manipulated species diversity to examine how it affects the functioning of numerous trophic groups in multiple types of ecosystem. We show that the average effect of decreasing species richness is to decrease the abundance or biomass of the focal trophic group, leading to less complete depletion of resources used by that group. At the same time, analyses reveal that the standing stock of, and resource depletion by, the most species-rich polyculture tends to be no different from that of the single most productive species used in an experiment. Of the known mechanisms that might explain these trends, results are most consistent with what is called the 'sampling effect', which occurs when diverse communities are more likely to contain and become dominated by the most productive species. Whether this mechanism is widespread in natural communities is currently controversial. Patterns we report are remarkably consistent for four different trophic groups (producers, herbivores, detritivores and predators) and two major ecosystem types (aquatic and terrestrial). Collectively, our analyses suggest that the average species loss does indeed affect the functioning of a wide variety of organisms and ecosystems, but the magnitude of these effects is ultimately determined by the identity of species that are going extinct.

Whereas one of the most striking features of our planet is its great variety of life, one of the most pervasive environmental changes of our time is the global loss of this biological diversity[8,9]. Considerable uncertainty exists about current rates of extinction, but estimates place it somewhere between two and three orders of magnitude higher than rates found in the fossil record[10,11]. Biologists have long pondered the environmental effects of species extinction. Even so, it was not until the 1990s that research efforts began to formalize the hypothesis that species diversity might influence the fluxes of energy and matter that are fundamental to all ecological processes, including those that control the abundance, biomass and distribution of organisms. Seminal studies suggested that species loss does, in fact, decrease how productive communities are and how efficiently they capture and consume limited resources[12–14]. But the interpretation of these studies provoked considerable debate[3–7], and subsequent work produced several counterexamples that questioned the generality of these biodiversity effects[15–19]. As a result, it has been argued that the consequences of biodiversity loss are likely to be idiosyncratic, differing quantitatively and qualitatively between trophic groups and ecosystems[20–23].

After more than a decade of research, a sufficient number of studies have now emerged to permit rigorous testing of whether there are indeed general effects of biodiversity on ecosystem functioning. Here we present a formal meta-analysis of 111 field, greenhouse and laboratory experiments that have manipulated the diversity of species for a wide variety of organisms and ecosystems (see Supplementary Information). We focused on experiments that varied the richness of three or more species in a given trophic group t and measured either of two response variables: the aggregate abundance or biomass of all species in t (referred to as 'standing stock') and/or the total amount of resources depleted by t from a known resource pool (see Methods). Data were summarized for four trophic groups: first, microalgal, macroalgal or herbaceous plants assimilating nutrients or water; second, protozoan or metazoan herbivores consuming live algal or herbaceous plant tissue; third, protozoan or metazoan predators consuming live prey; and fourth, bacterial, fungal or metazoan detritivores consuming dead organic matter. Diversity effects were quantified with two complementary metrics. First, for each experiment i, we calculated the proportional difference in the response variable y between the mean value of the most species-rich polyculture $\bar{p}$ and the mean value of these same species grown in monoculture $\bar{m}$ as the log response ratio $LR_{\bar{m}} = \ln(y_{i\bar{p}}/y_{i\bar{m}})$. This unitless metric allows us to test whether there is a significant change in y with increasing richness when averaged across all species used in an experiment. We then calculated a complementary metric that quantifies the proportional difference between the mean value of the most species-rich polyculture and that of the taxon having the highest (lowest) mean value of y in monoculture $\hat{m}$, as $LR_{\hat{m}} = \ln(y_{i\bar{p}}/y_{i\hat{m}})$, where $y_{i\hat{m}}$ is the highest (lowest) value when $LR_{\hat{m}} > 0$ (< 0). Testing whether $LR_{\hat{m}} > 0$ is analogous to tests for 'transgressive' overyielding, which are widely used to assess whether diverse polycultures are any more productive than the single most productive species[24].

Our analyses reveal quite general and consistent mean effects of species diversity on the aggregate abundance or biomass of species in a trophic group, with cascading effects on the resources used by that group. For $LR_{\bar{m}}$, we found that species richness positively affected the standing stocks of all four trophic groups considered, increasing the abundance or biomass of plants, herbivores, predators and detritivores (Fig. 1a). Higher diversity within each group was also associated with more complete depletion of resources (Fig. 1b). Experimentally increasing plant, predator and detritivore diversity all led to greater decreases in nutrients/water, prey, and dead organic

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matter, respectively. There was a similar tendency for increasing herbivore diversity to decrease the amount of living plant matter ($P = 0.08$ for a mixed-model analysis of variance; see Methods). In total, 67 of 76 experiments recorded positive values of $LR_{\bar{m}}$ for standing stock (88%), and 54 of 70 recorded positive values for resource depletion (77%).

When $LR_{\bar{m}}$ was modelled as a function of trophic group, we found no significant difference in the average diversity effect size between the four trophic groups for either response variable (Table 1). Furthermore, we found no significant difference in the average diversity effect size between studies performed in aquatic and terrestrial ecosystems (Fig. 1c, d and Table 1). This degree of consistency is remarkable given that the experiments spanned a wide variety of life forms (bacteria, fungi, plants and animals) and many of Earth's major ecosystems (lakes, streams, oceanic coastal habitat, temperate grasslands and forests; see Supplementary Information). Although studies are certainly not invariable in their conclusions, our results suggest that variation among studies is not consistent with previously proposed differences between trophic levels or ecosystems[19–22].

One of the major controversies in biodiversity research concerns the fact that some species exert stronger control over ecological processes than others[3]. Thus, a primary question when interpreting the average effect of species diversity is whether a diverse polyculture performs any differently than the single 'best' species (that is, the species having the greatest influence over a process). Our analyses show that the standing stock of, and resource depletion by, the most diverse species polyculture is statistically indistinguishable from that of the single species that achieves the highest level of these response variables in monoculture. Specifically, $LR_{\hat{m}}$ did not differ from zero for any of the four trophic groups (Fig. 1a, b) or for either of the two ecosystems (Fig. 1c, d). These conclusions hold true even if we apply a liberal test, considering only studies in which $LR_{\bar{m}} > 0$ ($P = 0.13$ for standing stock, 0.27 for resource depletion). Of the known mechanisms by which species diversity can affect ecosystem functioning, these results are most consistent with what is called the 'sampling effect' of biodiversity, in which communities comprising more species have a greater chance of being dominated by the most productive taxa. Note, however, that confirmation of this mechanism requires data on the covariance between competitive dominance in polyculture and the performance of species in monoculture[24,25]—data that are not generally reported. There has been much controversy about whether the sampling effect is best interpreted as a 'real' biological mechanism that operates in nature or as an artefact of experiments that use random draws of species to assemble experimental communities[1,3,6]. Until this debate is resolved, the relevance of the sampling effect for predicting the functional consequences of extinction is open to debate.

Our use of log response ratios to quantify the effects of species diversity could be criticized on grounds that these ratios compare only two ends of a continuum (highest versus lowest diversity). Because the highest levels of diversity differ between experiments (range 3–72 species) and tend to be higher in studies of terrestrial organisms than in those of aquatic organisms ($t = 4.64$, $P < 0.01$; 12.2 ± 9.6 species for terrestrial studies, 5.4 ± 8.1 for aquatic studies (means $\pm$ s.d.)), it is useful to ask how the general form of the diversity effect changes across levels of species richness. For 57 of 76 experiments that measured standing stock of a trophic group, and 51 of 70 experiments that measured resource depletion, species were manipulated at three or more levels of richness. This allowed us to fit data from each study to the Michaelis–Menten function $Y = Y_{\max}S/(K + S)$, where Y is the standing stock of, or resource depletion by, a trophic group standardized relative to the mean value of all monocultures $y_{\bar{m}}$ (that is, $Y = y_S/y_{\bar{m}}$ where y_S is the value of y at richness level S). $Y_{\max}$ is therefore the maximum proportion by which Y increases or decreases relative to the average one-species system, and K describes how quickly Y approaches $Y_{\max}$ with increasing diversity. This function was an excellent fit to the data (median $R^2 = 0.84$), and better than several other models (see Methods). Thus, we used maximum-likelihood estimates of $Y_{\max}$ and K to compare key features of the diversity–function curves across systems.

We found no significant differences in $Y_{\max}$ or K between aquatic and terrestrial ecosystems (Fig. 2 and Table 1), which indicates that

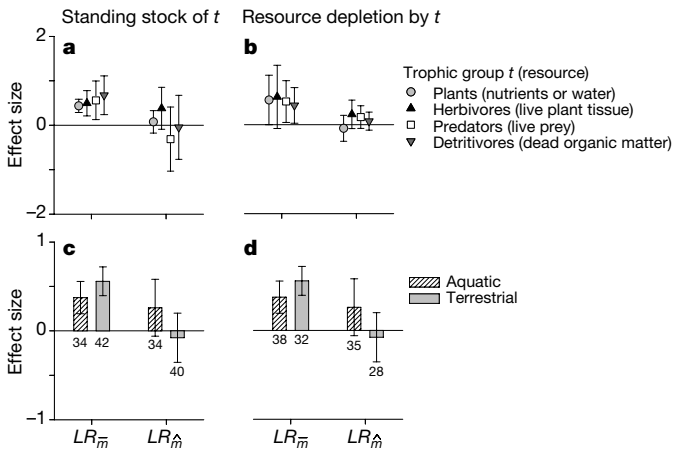

Figure 1 | Effects of species richness on the standing stock abundance or biomass of trophic group *t*, and the depletion of resources consumed by *t*. Data are means and 95% CI for two log response ratios that estimate the diversity 'effect size' from experiments. $LR_{\bar{m}}$ compares the mean value of the response variable y in a polyculture with the mean value of y averaged across the same species in monoculture. $LR_{\bar{m}} > 0$ indicates that more diverse polycultures achieve higher standing stock (**a**, **c**) and deplete resources more fully (**b**, **d**) than the average monoculture. $LR_{\hat{m}}$ compares the mean value of y in a polyculture with that of the species with the highest (for $LR_{\hat{m}} > 0$) or lowest (for $LR_{\hat{m}} < 0$) mean value of y in monoculture (see the text). $LR_{\hat{m}} = 0$ indicates that polycultures perform no differently than monocultures of the most productive species. Results are divided between four trophic groups (**a**, **b**) and two ecosystem types (**c**, **d**).

Table 1 | Statistical comparison of diversity effect sizes

Variable	Among trophic groups		
	d.f.	*F*	*Pr* > *F*
$LR_{\bar{m}}$			
Standing stock of *t*	76	0.43	0.73
Resource depletion by *t*	70	0.09	0.96
$LR_{\hat{m}}$			
Standing stock of *t*	74	0.98	0.41
Resource depletion by *t*	63	0.86	0.47
Variable	**Among ecosystems**		
	d.f.	*F*	*Pr* > *F*
$LR_{\bar{m}}$			
Standing stock of *t*	76	2.27	0.14
Resource depletion by *t*	70	0.13	0.72
$LR_{\hat{m}}$			
Standing stock of *t*	74	3.24	0.08
Resource depletion by *t*	63	1.62	0.21
Standing stock of *t*			
$Y_{\max}$	55	1.93	0.17
K	55	2.74	0.10
Resource depletion by *t*			
$Y_{\max}$	50	1.73	0.19
K	50	1.09	0.30

Results for $LR_{\bar{m}}$ and $LR_{\hat{m}}$ are from separate mixed-model analyses of variance that compare how richness in trophic group *t* influences the standing stock abundance or biomass of *t*, and the depletion of resources consumed by *t* among trophic groups (plants, herbivores, predators and detritivores) or ecosystems (aquatic and terrestrial). $LR_{\bar{m}}$ is the log ratio comparing the mean value of the response variable y in a polyculture with the mean value of y for the same species in monoculture. $LR_{\hat{m}}$ is the log ratio comparing the mean value of y in a polyculture with that of the species having the highest (for $LR_{\hat{m}} > 0$) or lowest (for $LR_{\hat{m}} < 0$) mean value of y in monoculture (see the text). Results for $Y_{\max}$ and K compare the maximum-likelihood parameter estimates for curves characterizing the diversity–function relationship (see Fig. 2) in aquatic and terrestrial ecosystems. All *F* values are non-significant, indicating that the effects of species richness on standing stocks and resource depletion do not differ between trophic groups or ecosystems.

the qualitative form of the diversity–function relationship is consistent across these habitat types (data were insufficient to make similar comparisons among trophic groups). With few exceptions, the curves were positive but decelerating, with values of Y_{max} being greater than the null expectation of unity (that is, $y_S > y_{\bar{m}}$, $t = 14.0$ and $t = 11.9$ for standing stock and resource depletion, respectively, both $P < 0.01$) and values of K being greater than 0 ($t = 6.7$ and $t = 5.5$ for standing stock and resource depletion, respectively, both $P < 0.01$). Asymptotic estimates of Y_{max} suggest that the most diverse species polyculture would achieve 1.9-fold the standing stock of the average monoculture (95% confidence interval (CI) 1.6–2.2) and 1.8-fold the resource depletion (95% CI 1.5–2.1). Estimates of K indicate that half of the maximum value for both standing stock and resource depletion is achieved by the average species monoculture (mean for standing stock, 0.98 (95% CI 0.68–1.28); mean for resource depletion, 0.89 (95% CI 0.56–1.22)). However, the decelerating nature of these curves suggest that although a small number of species can maintain more than half the function, a disproportionately high number of species is required to maintain functions near maximal values.

Thus, our meta-analysis of 111 experiments conducted over more than a decade reveals two consistent results. First, as researchers have experimentally reduced the richness of species of a variety of organisms inhabiting numerous types of ecosystems, the average effect of diversity loss is to decrease the abundance or biomass of the focal trophic group, leading to less complete depletion of resources used by that group. Second, it is equally general that these average effects of species diversity on ecosystem functioning are best explained by the loss of the most productive species from a diverse community. There are at least two implications of these findings. First, from the perspective of basic research, our results present a new challenge to biologists. A fundamental tenet of biodiversity theory is that species must use resources in different ways to coexist stably[26,27]. When species do coexist by such niche differentiation, theory predicts that diverse polycultures will produce more biomass and capture a greater fraction of limited resources than even the 'best' species monoculture[28,29]. The balance of evidence from experiments does not seem to support this, and understanding why there is a divergence between empirical and theoretical conclusions is one of the foremost challenges in this field. It may be that experiments have been performed at smaller spatial or shorter temporal scales than are the focus of theory, or that experiments do not meet equilibrium assumptions of theory. Second, our results re-emphasize a long-standing dilemma in the field of conservation biology—one that must soon be resolved. Biologists have long known that certain species exert much stronger control over ecological processes than others, but predicting which species these are in advance of extinction has proven difficult at best. A key challenge for future research is to detail more accurately how the traits that determine vulnerability to extinction are related to functional dominance in communities. Until that time, our finding that key aspects of ecosystem functioning decline consistently with the average species loss suggests that a precautionary approach to preserving as much biodiversity as possible is warranted.

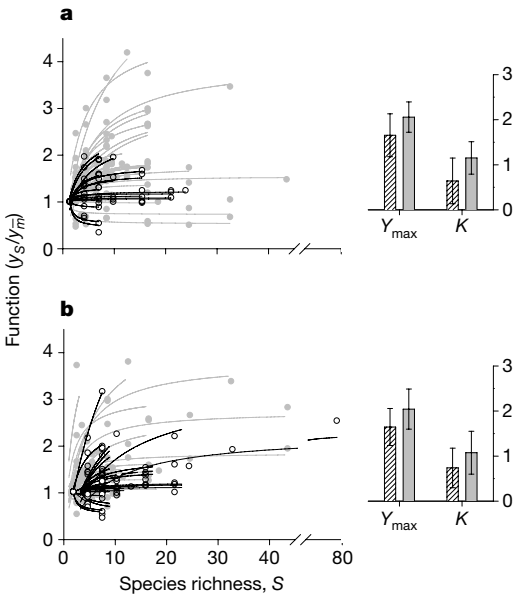

Figure 2 | The general form of the diversity–function relationship. Effects of species richness on the standing stock abundance or biomass of trophic group t (**a**), and the depletion of resources consumed by t (**b**). Each curve corresponds to data from a single study fitted to $Y = Y_{max}S/(K + S)$, where Y is the proportional change in the dependent variable with increasing richness S, Y_{max} is the asymptotic estimate of Y, and K is the value of S at which $Y = Y_{max}/2$. Sample sizes are 18 and 27 aquatic (black circles and lines), and 37 and 23 terrestrial studies (grey circles and lines) in **a** and **b**, respectively. Insets show the mean and 95% CI for the maximum-likelihood parameter estimates (hatched, aquatic; grey, terrestrial).

METHODS

Selection of studies. We searched the literature for studies that experimentally manipulated the richness of three or more species in a given trophic group t and then measured a direct effect of richness on the standing stock of all species in t and/or the total depletion of resources by t. Standing stocks were calculated as the aggregate abundance or biomass of all organisms in t per unit area or volume. Depletion of resources was calculated as an instantaneous rate of consumption (for example, metabolic estimates of consumption of organic matter by bacteria or fungi), the difference between a known initial and a measured final resource concentration (for example, the depletion of soil nitrogen by plants), or the difference between treatments and zero-species controls (for example, the capture of prey by predators). Because our focus was on how richness affects ecosystem functioning at a given moment, we did not include studies focusing on community stability (that is, how diversity affects temporal variation in a dependent variable or invasibility). In all, we reviewed 184 papers, amassing data from digitized figures or tables or by acquiring original data from the authors of 58 studies reporting results from 111 experiments that met our criteria (see Supplementary Information).

Analyses of the diversity 'effect size'. We used two log response ratios to quantify the diversity effect size in each experiment (see the text for the equations). $LR_{\bar{m}}$ was used to characterize the mean effect of diversity, testing whether the average of all replicates from the highest diversity treatment was different from the average response of these same species when grown in monocultures. In contrast, $LR_{\hat{m}}$ was used to test whether the average response of the highest diversity treatment was any different from that of the species having the highest (if $LR_{\hat{m}} > 0$) or lowest (if $LR_{\hat{m}} < 0$) value in monoculture. Two or more replicates of each monoculture were run for 59 of 63 experiments that measured resource depletion (94%), and 62 of 74 experiments measuring standing stock (84%). For these, we used the average value of replicates in our calculation of $LR_{\bar{m}}$. For the small remainder of studies that had only $N = 1$ replicate for each monoculture, we used the point estimate.

Log ratios are the most widely used metrics in meta-analyses for two reasons: first, they estimate a proportional difference between treatments that can be readily compared between studies, and second, they have sampling properties that are known to be normal and that are robust to bias from small sample sizes[30]. Mixed-model analyses of variance were used to test whether log response ratios differed from zero and to compare the mean values of these response ratios between trophic groups and ecosystem types. The general statistical model was $y_i = \mu + \tau_i + b_i + \varepsilon_i$, where y_i is $LR_{\bar{m}}$ or $LR_{\hat{m}}$ for each response variable, τ_i is a fixed categorical effect (trophic group or ecosystem type), b_i is the random effect associated with experiment i (with errors that are distributed normally and independently, $N[0, \sigma_b^2]$), and ε_i is the residual error. An important decision in meta-analyses is whether to standardize effect sizes by the variance of an experiment, giving greater weight to studies with higher 'certainty'. We performed analyses with and without weighting, and these led to identical conclusions. Here we present unweighted results because these allow the more realistic, but also more variable, field studies to have the same influence on our conclusions as greenhouse and laboratory studies that tend to have higher replication and smaller variance.

Curve fitting. To characterize the general form of diversity–function relationships, we fit data from each study to three nonlinear functions that have

previously been used in the literature (log, power and hyperbolic). The Michaelis–Menten version of the hyperbolic function was the best-fitting model for the majority of studies (44% compared with 35% for power and 21% for log functions), and had the highest explanatory power (mean $R^2 = 0.71$, median 0.84). However, all three functions led to identical conclusions. For data fitted to the power function $\log(y) = m\log(S) + b$, m was positive (function increases with diversity, 95% CI 0.15–0.32 for standing stock and 0.11–0.29 for resource depletion) and did not differ between aquatic and terrestrial studies ($P > 0.26$ for both). For the log function $Y = b + m\log(S)$, m was positive (95% CI 0.25–0.66 for standing stock and 0.20–0.49 for resource depletion) and did not differ between aquatic and terrestrial studies ($P > 0.25$ for both).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Global Genetic Change Tracks Global Climate Warming in Drosophila subobscura

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Global Genetic Change Tracks Global Climate Warming in *Drosophila subobscura*

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Comparisons of recent with historical samples of chromosome inversion frequencies provide opportunities to determine whether genetic change is tracking climate change in natural populations. We determined the magnitude and direction of shifts over time (24 years between samples on average) in chromosome inversion frequencies and in ambient temperature for populations of the fly *Drosophila subobscura* on three continents. In 22 of 26 populations, climates warmed over the intervals, and genotypes characteristic of low latitudes (warm climates) increased in frequency in 21 of those 22 populations. Thus, genetic change in this fly is tracking climate warming and is doing so globally.

Climate change is altering the geographic ranges, abundances, phenologies, and biotic interactions of organisms (*1*, *2*). Climate change may also alter the genetic composition of species, but assessment of such shifts requires genetic data sampled over time (*2*–*5*). For most species, time series of genetic data are nonexistent or rare, especially on continental or global scales (*5*). For a few *Drosophila* species, however, time-series comparisons of chromosomal inversions are feasible (*4*, *6*–*8*) because these adaptive polymorphisms were among the first genetic markers quantified in natural populations (*9*). Consequently, historical records of inversion frequencies in *Drosophila* spp. provide opportunities for evaluating genetic sensitivity to changes in climate and other environmental factors (*4*, *8*, *10*, *11*). Time-series data (13 to 46 years, mean = 24.1 years) of chromosomal-arrangement frequencies and of climate are now available for 26 populations of the cosmopolitan species *D. subobscura* on three continents. Here we examine whether ambient temperatures have warmed at these sites and also whether genotypes characteristic of low latitudes have increased in frequency.

Drosophila subobscura is native to the Old World, where it is geographically widespread from North Africa to Scandinavia (*12*). It has a rich complement of chromosomal arrangements (inversions) on its five acrocentric chromosomes (*12*). Over the past half-century, inversion frequencies have been scored at many sites in the Old World. The frequencies of most inversions change clinally with latitude and thus with climate (*13*, *14*). These climatic clines must be maintained dynamically by natural selection because the gene flow within continents is very high (*15*). Therefore, temporal shifts in inversion frequencies should be sensitive indicators of adaptive responses to climate change (*4*, *10*, *11*).

In the late 1970s, *D. subobscura* was accidentally introduced (*16*) into South America and soon thereafter (*17*) into North America. It spread explosively on both continents (*18*). Geneticists soon (1981 in South America, 1985 to 1986 in North America) began surveying inversion frequencies of these introduced populations at different latitudes (*19*, *20*). On both continents they detected incipient latitudinal clines in chromosome inversion frequencies that almost always had the same sign with latitude as in the Old World, supporting the inference that these clines are adaptive (*18*, *21*). Some other traits of these introduced flies show rapid clinal evolution as well (*22*, *23*).

To obtain comparative data on contemporary chromosome-arrangement frequencies, we and colleagues have revisited many of the historical sampling sites in both the Old and New World. Initial studies with *D. subobscura* reported that "warm-climate" inversions have increased in frequency at several European sites and proposed that these shifts reflect climate warming, but these studies did not investigate continent-scale correlations with climate (*10*, *11*, *24*, *25*). Our analyses here investigate whether the magnitude and direction of genetic shifts actually parallel those in climate, and whether they do so on all three continents.

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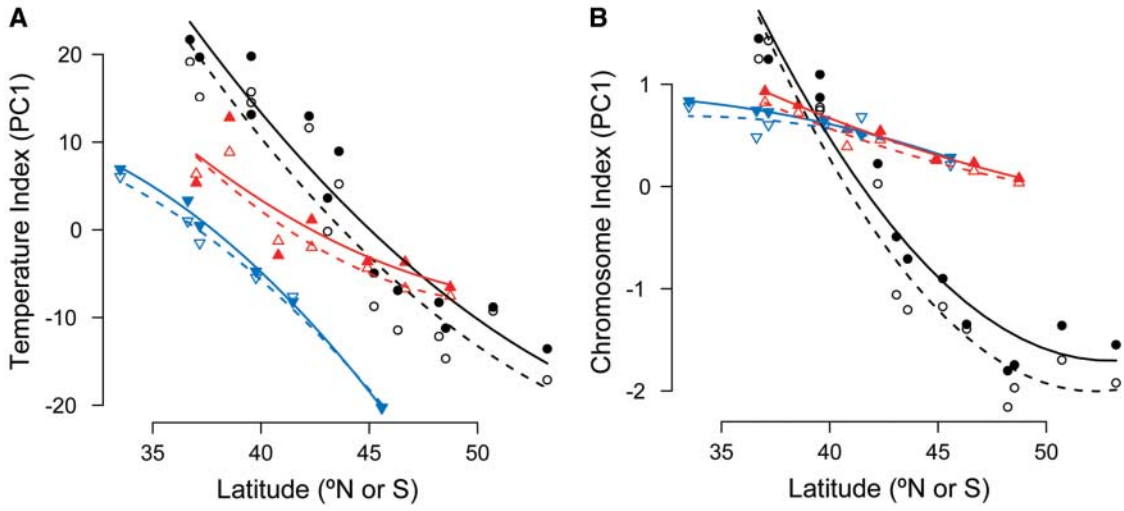

Fig. 1. Temporal shifts in temperature and in chromosome inversion frequencies at different latitudes on three continents. (**A**) A climate temperature index (T_{PC1}) is inversely correlated with latitude for 26 sites on three continents and has increased from the historical (open symbols, dashed regression lines) to contemporary samples (filled symbols, solid regression lines). Black, European sites; red, North American sites; and blue, South American sites. Regression lines are for second-degree orthogonal polynomials. (**B**) A chromosome index (Ch_{PC1}) is inversely related to latitude and has increased from the historical to contemporary samples (see text).

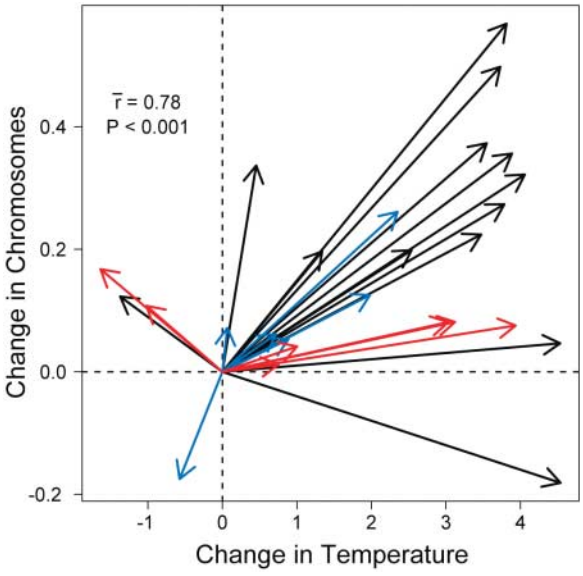

Fig. 2. Change in the direction of the chromosome index over time parallel those in the temperature index at 22 of 26 sites (upper right and lower left quadrants). Black, European sites; red, North American sites; and blue, South American sites.

Table 1. Spearman's rho correlation coefficients (95% confidence limits) for the relation between indices for chromosomes (Ch_{PC1}) and for climate (T_{PC1}) for old and for new samples on three continents. $^{**}P < 0.01$, *** $P < 0.001$.

Sample	Europe	South America	North America
Old	0.94***	0.49	0.93**
	(0.806, 0.982)	(−0.53, 0.930)	(0.584, 0.990)
New	0.95***	1.00***	0.93**
	(0.838, 0.985)	(1, 1)	(0.584, 0.990)

Table 2. Estimated equatorial shift (in degrees of latitude) between old and new samples from 10,000 bootstrapped replications of chromosome clines and of temperature clines. Values show means ± SE, with the 95% confidence limits indicated in parentheses.

Sample	Europe	South America	North America
Chromosomes	−0.884 ± 0.1721	−1.089 ± 1.4785	−0.757 ± 0.2612
	(−1.221, −0.547)	(−3.987, 1.809)	(−1.268, −0.245)
Temperatures	−1.106 ± 0.2095	−0.545 ± 0.1872	−0.735 ± 0.4275
	(−1.516, −0.696)	(−0.912, −0.178)	(−1.573, 0.103)

Historical data on inversion frequencies of *D. subobscura* in the Old and New Worlds were drawn from the literature (*11*). Between 1997 and 2004, contemporary estimates of inversion frequencies were scored from flies at the same (or very nearby) populations (*26*), during the same seasons as the original samples (*11*, *27*). Contemporary samples were also obtained in 2004 for seven populations in North America (*26*) (table S1). In all samples, each of the five acrocentric chromosomes was examined and scored for chromosomal arrangements, according to standard procedures (*26*). We analyzed 50 arrangements, including 21 that show significant latitudinal clines in the Old World and all 18 arrangements present in the New World (*27*).

Rather than analyzing frequency shifts of individual inversions, we developed a genome-wide index based on frequencies (p_i) of all inversions on the five acrocentric chromosomes. Specifically, we applied a principal component analysis to the centered and unscaled frequencies (after transformation by $2\sqrt{p_{ij}}$) of the scored arrangements on all chromosomes for the 52 (population × time) samples (*26*). Here we analyze the first principal component, which accounts for 45.8% of the variance.

To determine whether climates had shifted between samples at the study sites, we developed an index of ambient temperature. We compiled monthly mean temperatures from the nearest recorded weather station for the 4-year period before each sample and then computed a principal component index of the centered, unscaled monthly means for each site and period (*26*). The temperature index (T_{PC1}) reflects overall temperature and accounts for 79.8% of the variation.

T_{PC1} is inversely correlated with latitude on the three continents (Fig. 1A, table S2). Within continents, we found no significant heterogeneity among slopes between temporal samples ($F_{[4,17]} = 0.313$, $P = 0.865$), and so we used analysis of covariance to fit a common slope to

compute the between-sample effect (*28*). T_{PC1} increased significantly between samples ($F_{[1, 25]} = 28.8$, $P = 1.22 \times 10^{-6}$), consistent with global climate warming. Indeed, T_{PC1} increased at 22 of 26 sites. Shifts were larger in Europe (Fig. 1A), probably reflecting the longer sample intervals there and the broader range of climates (Fig. 1A).

A genomewide, principal component index of chromosome arrangement frequencies (Ch_{PC1}) was computed for all sites (*26*). Ch_{PC1} is inversely related not only to latitude (Fig. 1B, table S2), but also to T_{PC1} on all three continents (Table 1). Thus, Ch_{PC1} serves as a genetic indicator of the local climate. Because we found no significant differences in slope between temporal samples within continents ($F_{[4,17]} = 1.03$, $P = 0.419$), we fit a common slope within each continent and carried out an analysis of covariance (*29*). If the observed climate warming (Fig. 1A) is having a genetic impact, then genotypes associated with low latitudes (i.e., high Ch_{PC1} scores, Fig. 1B) should have increased in frequency between samples. In 24 of the 26 populations, this was indeed the case ($F_{[1,25]} = 22.7$, $P = 1.99 \times 10^{-6}$) (Fig. 1B). Within-site shifts in the direction of the chromosome index paralleled those of the temperature index in 22 of 26 sites (Fig. 2, sign-test, $P = 5.3 \times 10^{-5}$; Rayleigh test of uniformity, $\bar{r} = 0.78$, $P = 6.8 \times 10^{-8}$). Moreover, chromosome frequencies shifted toward a more low-latitude pattern in 21 of the 22 sites that warmed over the sample interval (upper right quadrant, Fig. 2). Thus, inversion frequencies have changed in step with climate on three continents.

In effect, genotype frequencies and climate at a given site have become more equatorial over the sample intervals (Figs. 1 and 2). Consequently, we rescaled the magnitude of these shifts (*26*) in terms of equivalent degrees of latitude (*4*). For temperature and for genotypes on all three continents, the observed shifts are equivalent to moving the historical sample site ~1° of latitude closer to the equator (Table 2).

Drosophila subobscura is experiencing detectable climate warming on three continents (Fig. 1A). Environmental warming appears to have had a genetic impact on these flies, because frequencies of chromosome inversions associated with warm latitudes have increased in parallel with climate on these continents (Fig. 2). This genetic shift is exceptionally rapid (*25*) and is detectable even for samples separated by fewer than two decades. Genetic shifts paralleling climate warming have been reported recently for a few other insects (*3*, *4*, *8*, *30*), although on more limited geographic scales. In no example to date, however, is it clear whether the observed shifts at given sites reflect local selection, a progressive invasion of genotypes from low latitudes, or both (*11*). Similarly, it is unclear whether the observed genetic changes reflect thermal (*8*, *31*) or seasonal selection (*5*), or correlates thereof.

The increasing numbers of examples documenting genetic (*2*–*5*, *8*, *10*, *11*), as well as phenotypic (*1*, *2*) responses, to recent climate change are not surprising from an evolutionary perspective, but nonetheless are disturbing from ecological or economic ones, because such changes signal inevitable disruptions in the distributions, population dynamics, and community interactions of organisms (*1*, *2*). Nevertheless, the ability of *D. subobscura* (*10*, *24*, *25*)—and probably other species with short generation times (*3*, *4*, *8*, *32*)—to respond genetically and rapidly to imposed environmental shifts may partially buffer their persistence in a globally warming world (*5*).

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28. The change in temperature across latitudes and continents over time was examined with a stratified linear model [a second-degree polynomial slope (Fig. 1A) provided a marginally better fit than a simple linear model]. We could not reject homogeneity of slopes within continents over time, so we fitted a common slope for the two time periods within each continent and carried out analysis of covariance, testing for a significant change among time samples over the within-site mean squares.
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Supporting Online Material

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Waking Experience Affects Sleep Need in *Drosophila*

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Sleep is a vital, evolutionarily conserved phenomenon, whose function is unclear. Although mounting evidence supports a role for sleep in the consolidation of memories, until now, a molecular connection between sleep, plasticity, and memory formation has been difficult to demonstrate. We establish *Drosophila* as a model to investigate this relation and demonstrate that the intensity and/or complexity of prior social experience stably modifies sleep need and architecture. Furthermore, this experience-dependent plasticity in sleep need is subserved by the dopaminergic and adenosine 3′,5′-monophosphate signaling pathways and a particular subset of 17 long-term memory genes.

Sleep is critical for survival, as observed in the human, mouse, and fruit fly (*1*–*3*), and yet, its function remains unclear. Although studies suggest that sleep may play a role in the processing of information acquired while awake (*4*, *5*), a direct molecular link between waking experience, plasticity, and sleep has not been demonstrated. We have taken advantage of *Drosophila* genetics and the behavioral and physiological similarities between

Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance

Hans O. Pörtner, *et al.*

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Climate Change Affects Marine Fishes Through the Oxygen Limitation of Thermal Tolerance

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A cause-and-effect understanding of climate influences on ecosystems requires evaluation of thermal limits of member species and of their ability to cope with changing temperatures. Laboratory data available for marine fish and invertebrates from various climatic regions led to the hypothesis that, as a unifying principle, a mismatch between the demand for oxygen and the capacity of oxygen supply to tissues is the first mechanism to restrict whole-animal tolerance to thermal extremes. We show in the eelpout, *Zoarces viviparus*, a bioindicator fish species for environmental monitoring from North and Baltic Seas (Helcom), that thermally limited oxygen delivery closely matches environmental temperatures beyond which growth performance and abundance decrease. Decrements in aerobic performance in warming seas will thus be the first process to cause extinction or relocation to cooler waters.

Climate change is projected to affect individual organisms, the size and structure of their populations, the species composition of communities, and the structure and functioning of ecosystems. Effects include poleward or high-altitude shifts in the distribution of ectothermic animals (*1*). A comprehensive mechanistic understanding has so far been lacking (*2*) but is needed for prediction of climate change effects. Physiological studies can address the mechanisms and reasons for the thermal sensitivity of organisms and their life stages.

In aquatic animals, a decrease in the capacity to perform aerobically (a drop in aerobic scope) characterizes the onset of thermal limitation at both ends of the thermal envelope [pejus thresholds T_p, fig. S1 (*3*–*6*)]. The reduction in aerobic scope is caused by limited capacity of circulatory and ventilatory systems to match oxygen demand. Such a constraint affects all higher functions (muscular activity, behavior, growth, and reproduction) and might thereby shape the long-term fate of species. Aerobic scope becomes minimal beyond low or high critical temperatures (T_c). Survival is then passive and time-limited, supported by anaerobic metabolism and protection of proteins and membranes by heat shock proteins and antioxidative defense. Thermal tolerance is hierarchical, with narrowing windows from molecular to cellular to systemic levels (*6*). Temperate species are able to acclimatize and shift the thermal window through changes in mitochondrial densities as well as other molecular to systemic adjustments of functional capacities (*3*, *6*–*10*) (fig. S1). Limits to acclimatization are set by trade-offs at various structural and functional levels that constrain the width of the thermal window, for example, through the trend to minimize energy turnover in relation to climate variability (*9*, *10*).

We investigated thermal limitation of the common eelpout, *Zoarces viviparus*, in its southernmost distribution area, the German Wadden Sea (part of the southern North Sea) during summer and thereby tested the ecological relevance of the concept of oxygen- and capacity-limited thermal tolerance (fig. S1). During the past 40 years, water temperatures in the German Bight increased by 1.13°C (at Helgoland Roads). Cold winters with sea surface temperatures (SSTs) around –1°C had occurred about once every 10 years up to 1944 but were experienced only once since 1960 (*11*). Models predict further SST increments for the next 90 to 100 years, by about 1.6° to 3.0°C in the northern and even by 3.0° to 3.9°C in the shallower southern North Sea (*12*), accompanied by rising sea levels (13 to 68 cm by 2050) and an increasing frequency of storm events (*13*).

Comparison of existing data sets indicates that field observations can be explained by the eelpout's physiological responses to warming (Fig. 1). The relative abundance of the nonmigratory eelpout decreases upon warming (5-year running means, data from 1954 to 1989, Fig. 1A) (*14*, *15*), reflecting a higher mortality in hot summers. Reduced field abundance coincides with reduced growth of laboratory-maintained, temperature-acclimated individuals (Fig. 1B). Individual growth is a key parameter shaping population growth and depends on aerobic scope. Lopsided growth curves result from the exponential rise in net aerobic scope upon warming, which is counterbalanced by the concomitant exponential rise in baseline metabolic costs (Fig. 1). Both abundance and growth begin to fall beyond upper pejus temperatures (T_p) (Fig. 1, C to E), reflecting the species-specific limits of acclimation capacity.

Pejus temperatures were derived from limitations in circulatory capacity (Fig. 1C), which occur before ventilatory limitations in eelpout (*Z. viviparus* and *Pachycara brachycephalum*) and Atlantic cod (*Gadus morhua*) (*4*, *16*–*18*). The loss of aerobic scope can also be derived from the shift of critical oxygen tensions, P_c, or concentrations, $[O_2]_c$. P_c or $[O_2]_c$ indicate oxygen limitation to the passive organism in hypoxia and the onset of anaerobic metabolism. Upon warming, $[O_2]_c$ reaches air saturation at T_c, where anaerobic metabolism begins in animals exposed to fully aerated waters (Fig. 1D). Aerobic scope thus begins to fall when $[O_2]_c$ starts to rise beyond T_p (Fig. 1E). Warming exacerbates oxygen limitations not only by the forced rise in oxygen demand, but also by reducing oxygen solubility (Fig. 1E).

The analysis of ecological responses in relation to 5-year running means of summer maxima, albeit improving the signal-to-noise ratio, may not precisely quantify temperatures and mechanisms effective in the field. Analysis of individual summers in long-term data series (*19*) should provide more detailed insight into cause-and-effect relationships (Figs. 2 and 3). The limited data set indicates that extreme temperatures of previous summers cause reduced abundance. Sampling took place in July, so the effects of the hottest season only become visible in the next year. Thermal limitation of aerobic scope may also translate into the next year by reducing the degree of successful fertilization and reproduction.

Thermal sensitivity is likely to be enhanced at large body sizes. In contrast to eelpout from the Baltic or from colder regions like the Russian White Sea, eelpout of the Wadden Sea only reach a maximum body length of about 23 cm at a maximum age of 3 to 4 years (*20*). A preliminary analysis of seasonal changes in size frequency distribution (fig. S2) shows that older specimens (larger than 20 cm) have low overall abundance and thus high mortality rates. High

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mortality of large fish is probably not associated with predation, which usually occurs during early life stages (*21*). Rather, the oxygen-limitation model predicts that temperature-dependent aerobic limits are experienced earlier by larger than by smaller individuals (*22*). In fact, thermal sensitivity of growth or exercise was found to be enhanced in large compared with small individuals of various fish species (*23*, *24*). Thermal limits depicted in Figs. 1 to 3 are valid for specimens of about 23 cm body length (compare with fig. S2). Mild summers, with temperatures regularly beyond T_p of large fish, can therefore be interpreted to cause mortality of this size class (Fig. 3 and fig. S2). Because of wider thermal windows in smaller specimens, these temperatures still allow for population growth, seen especially during the mild summer of 1998 (Fig. 2). The earlier loss in aerobic scopes of large individuals indicates that specimens do not grow beyond oxygen-dependent size limits set by temperature such that this size group displays low abundance all year round (fig. S2). The species finally experiences a net reduction in abundance (Fig. 1A) when smaller individuals are also affected and population loss during hot summers exceeds yearly population growth. In conclusion, harmful effects of warming set in beyond pejus temperatures. Only summers hotter than critical temperatures of the larger specimens (Figs. 1 to 3) entail the full range of thermal stress phenomena depicted in fig. S1. The mismatch in oxygen supply versus demand thus becomes effective at the ecosystem level before the onset of anaerobic metabolism or of thermal damage (Td in fig. S1) and also before critical thermal maxima (CT_{max}) traditionally determined in thermal biology (*25*).

Overall, the agreement of thermal limits operative in the field with the lab-determined pejus range supports previous studies, which interpreted thermal limitation in aquatic ectotherms to start with limited oxygen supply capacity (*3*–*6*, *16*–*18*) (Fig. 1, C to E). Accordingly, pejus limits are the earliest limits experienced by the whole organism in the field. Decrements in aerobic performance cause reduced growth and enhanced mortality first among larger specimens. A reduction in abundance results when all size groups of a population are affected. Residual variability in the data suggests that not only the temperature value itself but also the length of exposure is crucial in setting mortality. The data did not reveal an influence of the shift to milder winters. Further-

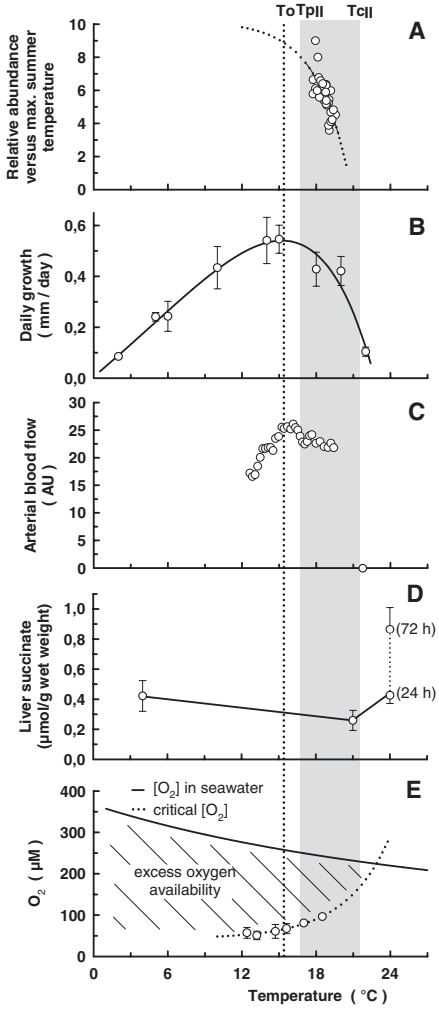

Fig. 1. Matching field and laboratory data reflect thermal limitation in eelpout in accordance with fig. S1. The shaded area characterizes the pejus range between upper T_p and T_c. (**A**) The negative correlation between summer water temperatures and relative abundance indicates heat-induced mortality of eelpout in the Wadden Sea [5-year running mean, recalculated from (*14*, *15*) and weather data licensed by DWD (Deutscher Wetterdienst)]. Data were fitted to $Ar(T) = Ar_{max}[1 - e^{k(T-T_o)}]$ where $Ar(T)$ is the relative abundance depending on temperature ($Ar_{max} = 10.173$; $k = 0.377$; $T_o = 20.853$, $r = 0.7130$, and $P < 0.01$). (**B**) Daily growth increments recalculated from (*31*) in relation to water temperature (mean ± SD). Data were fitted to the equation $dL(T) = F_1(T) + F_2(T) = (A_1 e^{B_1 T} + C_1) + (A_2 e^{B_2 T} + C_2)$ with $dL(T)$ = daily growth rates at maximum food supply. The first term, $F_1(T) = A_1 e^{B_1 T} + C_1$, represents the temperature dependence of aerobic processes supporting growth performance. The second term, $F_2(T) = A_2 e^{B_2 T} + C_2$, represents the parallel, exponential rise in processes limiting aerobic scope and thus growth capacity ($A_1 = 0.9901$, $B_1 = 0.0667$, $C_1 = -0.3953$, $A_2 = -0.1942$, $B_2 = 0.1299$, $C_2 = -0.3953$, $r = 0.9823$, and $P < 0.01$). (**C**) Arterial blood flow in relative units [measured by nuclear magnetic resonance (NMR) imaging techniques and recalculated as running means from (*4*)] reflects maximized circulatory oxygen supply at optimum temperature T_o and capacity limitation beyond T_{pll}. AU, arbitrary units. (**D**) Mismatch between oxygen supply and demand finally leads to the accumulation of succinate in the liver beyond T_{dl} [data are mean ± SD from (*32*)]. (**E**) At upper T_c, $[O_2]_c$ of the eelpout reaches air saturation oxygen concentrations. Water O_2 concentration (solid line) and $[O_2]_c$ (open circles, mean ± SE, fitted by dotted line) were recalculated from (*25*) for a salinity of 32 ‰. $[O_2]_c$ was fitted to $[O_2]_c(T) = C_1 T^{C2} + C_3$ ($C_1 = 4.33 \times 10^{-6}$, $C_2 = 5.56$, $C_3 = 48.19$, $r = 0.9764$, and $P < 0.01$). Reported data are valid for the largest specimens [between 23- and 25-cm body lengths (*25*)] found in the Wadden Sea.

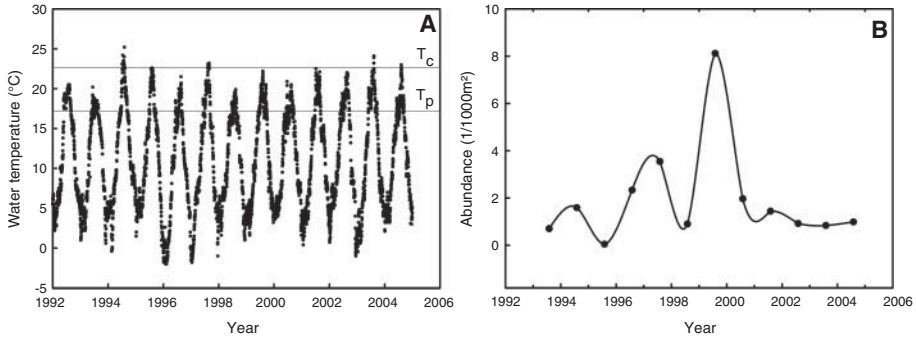

Fig. 2. (**A**) Daily water temperature (°C) in the Wadden Sea from 1992 to 2004 (DWD, Station Norderney). Putative upper critical (T_c) and pejus (T_p) temperatures of large eelpout (Fig. 1) are indicated by horizontal lines. (**B**) Total abundance (1/1000 m^2) of eelpout in the Wadden Sea sampling area during summers between 1993 and 2005. Data fitted by spline curve [Sigmaplot (*33*)].

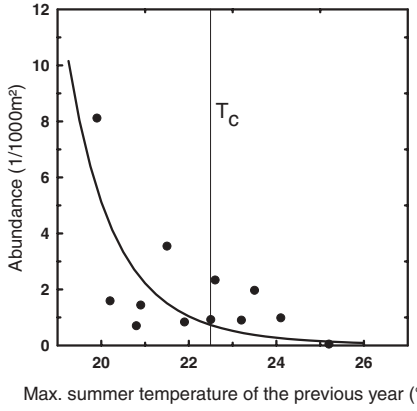

Fig. 3. Abundance of eelpout versus maximum summer temperature from the previous year (data from Fig. 2). Data were fitted to $A(T_{max-1}) = C_1 e^{C_2/C_3 T}$ where $A(T_{max-1})$ is the abundance depending on maximum temperature of the previous summer (fitting parameters $C_1 = 1.2 \times 10^{-7}$, $C_2 = 240.83$, $C_3 = 0.6686$, $r = 0.6599$, and $P < 0.01$). The putative upper T_c (Fig. 1) is indicated by a vertical line.

more, population growth depends on food supply, which in turn influences aerobic performance and thermal sensitivity. Potential additional components in field tolerance still need to be identified. However, we suggest that reduced aerobic performance beyond pejus limits enhances sensitivity to other, more obvious mechanisms eliciting mortality (predation, starvation, or disease). These influences would display their inherent variability and thereby enhance the variability in the temperature-dependence of abundance.

Matching thresholds in field and laboratory data highlight the ecological relevance of the concept of oxygen- and capacity-limited thermal tolerance. Adaptation to climate variability involves adjustments of functional capacity in general and, specifically, in the components of aerobic metabolism, of oxygen supply capacity, and of associated costs. Trade-offs in thermal adaptation processes and in organismic energy budget shape the width of thermal windows, with consequences for biogeography (*1*), growth performance, development, fecundity, recruitment, life-styles, and life-history evolution (*9*, *10*, *26*, *27*). Widths and locations of tolerance windows on the temperature scale may change or shift during ontogeny (*26*). At the ecosystem level, species-specific biogeographical ranges differ but overlap and imply that variable thermal windows and sensitivities cause variability in distribution shifts (*1*), species composition, seasonal timing, and associated mismatch phenomena in species interactions as in a food web. For example, the shift from larger (*Calanus finmarchicus*) to smaller (*C. helgolandicus*) copepod fauna in the southern North Sea caused reduced food availability for Atlantic cod (*G. morhua*) (*28*). This regime shift was largely determined by different thermal windows of the two copepod species (*29*). Warming-induced reductions of cod abundance are thus caused both directly [via thermal sensitivity of cod (*30*)] and indirectly [via the food web (*28*)] but based on the same physiological principles. Overall, the concept of oxygen- and capacity-limited thermal tolerance can provide an integrative framework for developing a cause-and-effect understanding of the influence of climate change and variability on marine ecosystems, including food web structure, recruitment success, and fish landings (*30*).

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34. Supported by the MarCoPoll program of the Alfred Wegener Institute.

Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5808/95/DC1
Materials and Methods
Figs. S1 and S2

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A Hexanucleotide Element Directs MicroRNA Nuclear Import

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MicroRNAs (miRNAs) negatively regulate partially complementary target messenger RNAs. Target selection in animals is dictated primarily by sequences at the miRNA 5′ end. We demonstrated that despite their small size, specific miRNAs contain additional sequence elements that control their posttranscriptional behavior, including their subcellular localization. We showed that human miR-29b, in contrast to other studied animal miRNAs, is predominantly localized to the nucleus. The distinctive hexanucleotide terminal motif of miR-29b acts as a transferable nuclear localization element that directs nuclear enrichment of miRNAs or small interfering RNAs to which it is attached. Our results indicate that miRNAs sharing common 5′ sequences, considered to be largely redundant, might have distinct functions because of the influence of cis-acting regulatory motifs.

Nucleotides 2 to 7 of microRNAs (miRNAs), known as "seed" sequences, are considered the most critical for selecting targets. Within a given species, highly related miRNAs sharing a common seed sequence are grouped into miRNA families, are predicted to have overlapping targets, and are considered to be largely redundant (*1*–*5*). Nevertheless, loss of function of miRNA family members with divergent 3′ end sequences results in overlapping but distinct phenotypes in *Caenorhabditis elegans* and in *Drosophila* (*6*, *7*). These distinct phenotypes often do not appear to be due to differences in miRNA expression patterns, which raises the possibility that distinct sequences within miRNA family members confer upon them characteristic functional properties. Here we describe a sequence motif that dramatically influences the posttranscriptional behavior of a human miRNA.

Examination of cell-cycle stage–specific miRNA expression patterns with a previously described oligonucleotide array (*8*) revealed substantial accumulation of miR-29 in mitotic HeLa cells (*9*). There are three human miR-29 paralogs: miR-29a, miR-29b, and miR-29c (fig. S1A). A highly specific Northern blot assay (fig. S1B) demonstrated that each exhibits a distinct expression pattern. miR-29a is constitutively expressed in all cell-cycle phases, miR-29b is present at low levels except in mitotic cells, and miR-29c is not detectable (Fig. 1A).

Human miR-29 family members are encoded by the miR-29b-1/miR-29a cluster and the miR-29b-2/miR-29c cluster (Fig. 1B). A fragment encompassing the miR-29b-1/miR-29a cluster was amplified by reverse transcription polymerase chain reaction (RT-PCR) after small interfering RNA (siRNA)–mediated inhibition of Drosha (which performs the first step in

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