



Increasing intraspecific diversity increases predictability in population survival in the face of perturbations

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It has been proposed that biodiversity can be important for ecosystem functioning and act as an insurance against perturbations and environmental fluctuations. To date, theoretical work supports this idea but direct experimental evidence is still to some extent ambiguous and debated. The main reason for this debate – and the lack of strong empirical support – is due to unavoidable experimentally and statistically inherent variance reduction effects. Here we present the results of an experimental study that circumvents earlier hidden treatments. By random draws without replacement, we collected 180 full-sibling batches of an amphipod from a large pool of possible parents. Assembled amphipod populations with diversity levels ranging from one to ten were exposed to either a single perturbation (nutrient enrichment) or two combined perturbations (nutrient enrichment and desiccation). The results show that the variance in the number of surviving individuals decreased with increasing diversity in the combined perturbations treatment. Predictability in population survival thus seemed to be higher in more diverse assemblages. Our results, together with a simple model suggest that variance-decreasing effects can be due to actual real world statistical sampling effects of increasing diversity.

The accelerating loss of global biodiversity (Pimm et al. 1995) and the hypothesised connection between biodiversity and the functioning of ecosystems (Schulze and Mooney 1993) have triggered a rapid succession of studies focusing on the functional consequences of changing biodiversity (Loreau et al. 2002, Hooper et al. 2005). To date, the general validity of conclusions derived from many biodiversity–ecosystem function (BEF) experiments have been widely debated (Huston 1997, Wardle 1998, Huston et al. 2000, Kaiser 2000, Loreau et al. 2001, Cameron 2002, Huston and McBride 2002). The main reason for this debate has been the design of many BEF experiments: factors other than changes in diversity can explain effects of the various processes and functions studied. Such factors include abiotic factors and statistical artefacts (Huston 1997).

One of the most highly discussed features within the BEF framework concerns that of the sampling effect. The sampling effect refers to an increasing probability of including species with particular traits when species richness of experimental manipulations

increases. This sampling effect is restricted to positive effects of increasing biodiversity on ecosystem functions and is a special case of the more general selection effect (which can also be negative; Hector et al. 2002, Bruno et al. 2005). In terms of terrestrial plants, this means that productivity is higher in more diverse assemblages when there is a positive covariance between the productivity of a species and its competitive ability, and the chance to include highly productive species increases with species richness (Hector et al. 2002). The sampling effect has sometimes been considered a simple and valid mechanism linking species richness and ecosystem processes (Tilman et al. 1997), while others have viewed it as a statistical hidden treatment (Huston 1997). An alternative explanation of increasing functioning with increasing richness involves facilitation and niche differentiation, (which can be grouped together as complementarity effects, Loreau 2000). Complementarity can mean that diverse communities show increased performance compared to when species are studied in isolation. Further, as manipulated richness

increases so does the similarity among replicates within richness levels, the so called “variance reduction effect”. This arises because species pools are finite, and can cause confounding between the manipulated factor diversity and unavoidable statistical properties of the examined diversity treatments (Huston and McBride 2002). High similarity among replicates at high diversity levels can result in decreasing variance among those replicates (Wardle 1998). It is therefore hard to evaluate whether decreasing variance is the effect of stabilizing properties of increasing species richness or an effect of the “variance reduction effect”. It should be noted, however, that it can be ecologically relevant to compare diverse assemblages with their less-diverse counterparts (Naeem and Li 1998) since this reflects patterns of species loss observed in nature.

We argue that the true functional consequences of high biodiversity may be due to valid statistical properties of natural populations. According to the central limit theorem (CLT), the means of multiple samples from any original distribution will approximate a normal distribution, and the variance of this distribution is inversely related to the number of observations in each sample. In other words, the variance should decrease with increasing number of traits, individuals or groups sampled from the (original or “parent”) population. The mean, however, should be unaffected (in the absence of resource partitioning or positive interactions), and this should be independent of the distribution of traits within “parent” populations. All sampled groups of high diversity will always include some traits from the “parent” population that are favourable, while sampled groups of low diversity will have either favourable or non-favourable traits. The mean performance across sampled groups will therefore average out and will be unaffected by levels of diversity within the sampled groups.

Just as species diversity has been shown to influence various aspects of ecosystem functioning, within-species diversity has also been shown to affect function in terms of seagrass resistance to disturbance and stress (Hughes and Stachowicz 2004, Reusch et al. 2005), the associating arthropod community of plants (Wimp et al. 2004, Johnson et al. 2006), and settling success in a marine invertebrate (Gamfeldt et al. 2005). Furthermore, Johnson and Agrawal (2003) argue that the diversity of prey populations can influence the variation in population sizes among replicate communities. We used a large, genetically diverse, population of the amphipod *Gammarus duebeni* to examine how changes in within-species richness affect survival of individuals following a pulse perturbation. *Gammarus duebeni* is a common inhabitant of northern Atlantic rock pools (Ganning 1971), and has a high tolerance to shifting salinities and temperatures (Gaston and Spicer

2001). We tested the hypothesis that increasing richness will enhance predictability in population survival and decrease variance in the number of surviving individuals among populations, while the mean survival among populations remains unaffected.

Methods

Gammarus duebeni individuals were sampled from multiple rock pools on four different islands of the Swedish west coast (58°54'N, 11°7'W) in October 2004. Approximately 250–300 individuals were sampled from each island. Pairs that were in precopula position at the time of sampling were removed. All the remaining non mating individuals were put together in a large tank to create a “random mating population” (RMP) to reduce inbreeding effects caused by mating of individuals from the same rock pool. The RMP consisted of approximately 1000 individuals. The RMP was monitored daily and pairs swimming in precopula mating position were removed and isolated in separate plastic containers (200 ml). The plastic containers were placed on a light table to make sure that the females in the pairs were not already pregnant, i.e. carrying eggs. Pairs with pregnant females were removed. We are not aware of any reports of multiple paternity in *G. duebeni*. Furthermore, in other studies we have checked for the occurrence of females that give birth to several subsequent broods without intermediate mating with males. Fifteen females were kept isolated for up to 12 months after hatching of the last brood without producing new eggs (unpubl.). The amphipods were fed with fish flakes (Sera Flora, www.sera.de) dissolved in water and administered with a pipette two times per week. Water was exchanged once a week. Salinity was approximately 15‰ (measured as total salt conductivity) and prepared by mixing filtered seawater (35‰) with freshwater. Both the RMP and the isolated mating pairs were kept in a 12:12 light:dark regime at a temperature of 20°C. Pairs were sampled from the RMP during 10 days to a total of 300 pairs. After mating, the pregnant females were isolated from the males in new plastic containers. From the time when the eggs turned orange (indicating they are about to hatch, pers. obs.), the plastic containers with pregnant females were monitored at least twice per day. Hatched juveniles were removed to a separate plastic container. Sibling-groups, hereafter referred to as “families”, from females that produced 10 or more juveniles were kept while families with fewer than 10 individuals were discarded. A total of 216 families were produced and kept for two weeks under the described conditions. *Gammarus duebeni* is a routinely outbreeding invertebrate and, therefore, all offspring from one parent pair were always full siblings. Consequently, we assumed

that genetic variability within broods was substantially less than that among broods (sensu Gamfeldt et al. 2005).

We created four different levels of intraspecific diversity by putting a total of 10 individuals into each of 40 new plastic containers (500 ml). The lowest diversity level consisted of 10 juveniles from the same family (i.e. all siblings, diversity level 1). The second diversity level contained five juveniles from two different families each (diversity level 2), the third level contained two juveniles from five different families (diversity level 5), and the highest diversity level contained one juvenile from each of 10 different families (diversity level 10). After sampling individuals from any one family (for diversity levels: 1, 2, 5 or 10), that family was discarded. This method ensured that there could be no “sampling diversity effect” due to juveniles from particular families. In our design, all diversity treatments and replicates were independent. By sampling offspring from parent pairs drawn from a large population, without replacement, we applied a design that avoided the nested factor of specific identity of families and could therefore directly evaluate the effects of changing richness on ecosystem function (in our case survival). Each diversity level had five replicate containers. Due to logistical constraints in obtaining *Gammarus* juveniles we were limited in the number of diversity levels and replicates we could use (with correspondingly reduced power for statistical tests as a result). The experiment was maintained in a 12:12 light:dark regime at 20°C.

We tested for the effects of intraspecific diversity on variance in survival using two perturbation scenarios, one with nutrient overloading (lids on the containers) and one with nutrient overloading in combination with desiccation (no lids). Nutrient overload was accomplished using fish food tablets (as checked in a pilot experiment). The mechanism for this lethal effect is not known and will be examined in future experiments. Both desiccation (which increases salinity) and excess nutrients are common and natural stress factors in rock pools. Pools can become eutrophic due to input from the surrounding rocks during rains and through faeces from birds (Loder et al. 1996). After three weeks, when the water level in the desiccation treatment containers had reached around 33% of the original level, the numbers of surviving juveniles in all containers were counted. We analysed the results using linear regression. Since the hypothesized outcome of the experiment was a negative power function ($y = a \times x^{-b}$) (see our simple model in the Discussion) we log-transformed both variables prior to analysis. We also analysed our data using the coefficient of variation (CV).

Results

In concordance with our hypothesis, the mean number of surviving *Gammarus* among populations in the different treatments did not change with diversity (linear regression, $r^2 = 0.010$, $p = 0.68$ for the non-desiccation treatment; linear regression, $r^2 = 0.00016$, $p = 0.96$ for the desiccation treatment, Fig. 1). Further, there was no difference in total survival between the two treatments (two-sample t-test, $t_{38} = 1.31$, $p = 0.20$). In agreement with the model, we found that the variance decreased significantly with increasing diversity in the desiccation treatment (linear regression, $r^2 = 0.95$, $p = 0.024$, Fig. 2), supporting our hypothesis. There was not, however, any significant diversity effect on the variance in the non-desiccation treatment (linear regression, $r^2 = 0.40$, $p = 0.37$, Fig. 2). The inset in Fig. 2 shows the double-logged data on which all regression analyses were performed. The CV showed non significant changes with changing diversity for both the desiccation (linear regression, $r^2 = 0.39$, $p = 0.38$) and the non-desiccation treatment (linear regression, $r^2 = 0.75$, $p = 0.25$).

Discussion

Although the survival of individuals and populations is fundamental for all species and an important aspect of every ecosystem, the effects of changing intraspecific diversity on predictability and stability of survival has been overlooked to date. Our results showed that increased diversity increased the predictability of a key structuring process – survival of juveniles following a disturbance. In accordance with earlier theoretical and empirical work (McGrady-Steed et al. 1997, Naeem and Li 1997, Tilman et al. 1997, Yachi and Loreau

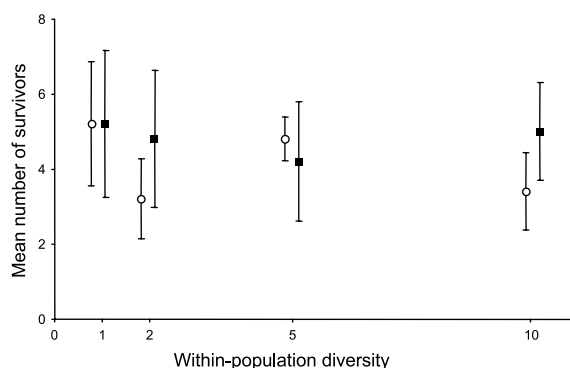


Fig. 1. The mean number of surviving *Gammarus* individuals per treatment and diversity level (\pm SE). There was no change in survival with changing diversity. Open circles represent the non-desiccation treatment and solid squares the desiccation treatment.

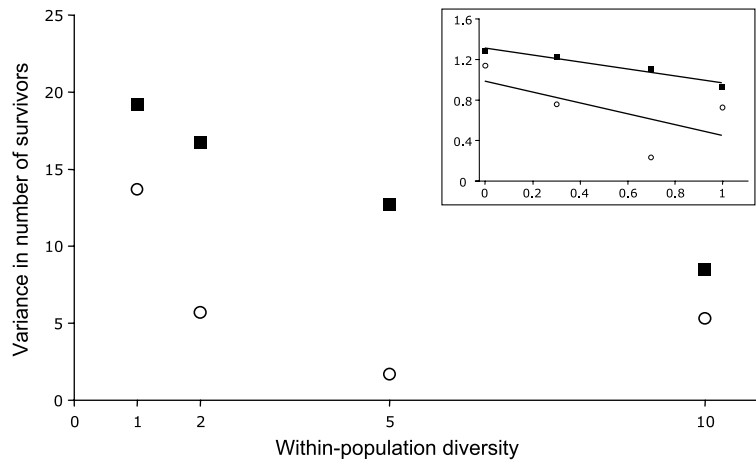


Fig. 2. Variance in the number of surviving individuals as a function of diversity. The variance in the desiccation treatment decreased significantly with increasing diversity in the desiccation treatment but not in the non-desiccation treatment. The inset in the figure shows the curves for the double-logged axes of the main figure, upon which regression analyses were performed. Symbols are as in Fig. 2.

1999), increasing diversity decreased the variance of the response variable and hence increased system predictability. This was a consequence of reduced spatial variation in genetic diversity among populations with increased diversity. We found that increasing diversity within populations of *Gammarus duebeni* decreased the variance in the number of surviving amphipods in the desiccation treatment (accepting our hypothesis), but not in the non-desiccation treatment (Fig. 2).

Our results were predicted by a simple model that showed the expected effects of increasing diversity on the variance in survival among populations (Fig. 3). In the model we created a population of 90 parent pairs (just as in our experiment) that show variation in a hypothetical trait that we were interested in, e.g. a trait that is important for survival in the face of a

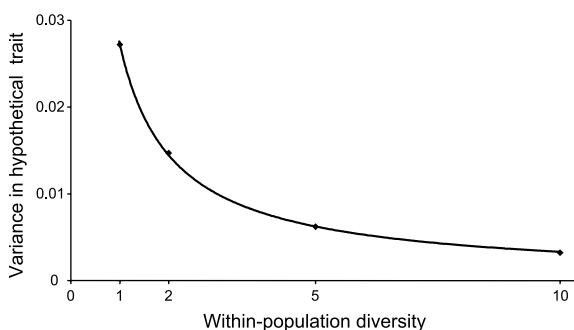


Fig. 3. Variance in a hypothetical trait among offspring populations (sampled from a created parent population consisting of 90 parent pairs with a Poisson distribution) as a function of diversity. The variance is negatively and inversely related to the number of sampled observations building up the means of the offspring populations.

perturbation. The distribution of this trait could come in any shape (normal, poisson, binomial etc). We then sampled parent pairs from this distribution to create offspring populations of diversity levels 1, 2, 5 and 10. Offspring populations in the lowest diversity level 1 contained offspring from only one parent pair (one sampling from the parent population) while offspring populations in the highest level 10 were made up of offspring from ten parent pairs (ten samplings from the parent population). Due to the effects of sampling many observations from a parent population (as described by the CLT), the variance decreased with increasing diversity in the form of a negative power function (except for the trivial case where there was no variation in the parent population). The variance was negatively and inversely related to the number of sampled observations building up the mean. Fig. 3 presents the variance among offspring populations originating from a Poisson distribution with $\lambda = 1$. It should be noted that identical results were obtained with other kinds of distributions (normal, left-skewed, right skewed) and wider ranges in the number of diversity levels (up to a diversity of 10000, figures not shown), as predicted by the CLT. With variation in the trait of interest, variance should always decrease with increasing diversity. The model thus suggested that a decreasing variance in survival among populations was due to real world statistical effects of increasing diversity.

For any one snapshot at a small spatial scale, the sampling effect may not be interpreted as a mechanism explaining the effects of (e.g.) species richness on a given ecosystem function. Rather, at a particular time, it is more likely that a strong sampling effect would be interpreted as a certain species with particular traits

affecting ecosystem function, rather than richness per se. Having many species or genotypes in such a case does not, therefore, explain the level of the function. As is shown in experimental studies that manipulate the richness of primary producers and measure the response variable, productivity, at one point in time, the effect of species identity is often much stronger than the effects of species richness per se (Bruno et al. 2005, Hooper et al. 2005). On the other hand, a high richness of biological entities increases the statistical probability of containing those traits that significantly affect the function. In this sense, richness can be an important aspect of diversity to ensure ecosystem function, and would thus act as ecosystem insurance when the environment is changing and species are lost.

This view was supported by our results. In the face of both nutrient overloading and desiccation, *Gammarus* populations of higher diversity showed less variation in survivorship, pointing to higher predictability in population sustainability. Because we had unidirectional pulse perturbations in the form of nutrient overloading and desiccation, this lower variance in survival in high-diversity treatments was probably due to the higher probability that those treatments included individuals with favourable traits. In our view, this was a typical sampling mechanism. The CLT predicted that decreasing variance with increasing diversity should be a real-world inevitability. The reason for a lack of a significant effect in the non-desiccation treatment may be due to a lack of power to detect an effect in combination with a lower total stress level compared to the desiccation treatment. Variance in the traits that determined tolerance to an excess of nutrients may have been small in comparison for those for salinity tolerance. In a hypothetical case with multidirectional perturbations that vary over time, decreasing variance could result from complementarity in traits that are favourable under different conditions: a diverse set of genotypic individuals would provide a buffering effect (as discussed in Loreau 2000). We suggest that the increased predictability of increasing intraspecific diversity can often be due to real and valid statistical attributes of natural populations, just as statistical features for multispecies assemblages is suggested to be important over time for stability–diversity relationships (Doak et al. 1998). As shown by the central limit theorem, variance decreases as the number of observations drawn from a population increases.

The reasons underlying the non-significant effects of diversity on the CV for *Gammarus* survival are puzzling. This probably indicates that we have large variation in our data set, and that we cannot totally disentangle the effects on the variances from those on the means. Since the power of a test based on two estimates (the CV) is likely to be lower than that based on one estimate (the variance), the test on CV would

have needed larger effect sizes. The fact that the variance in survival decreased with increasing diversity does nonetheless support the hypothesis that diversity should increase the predictability in population persistence.

Our findings augment and extend recent studies that have also found effects of intraspecific variation. For example, high genetic diversity within component plant species reduced the rates of species diversity loss (Booth and Grime 2003), increased diversity within a plant affected the associating arthropod community (Johnson et al. 2006), Hughes and Stachowicz (2004) found that increasing genotypic diversity of seagrass meadows enhanced community resistance to disturbance by grazing geese, and Reusch et al. (2005) found increased resistance to thermal stress in seagrass meadows with higher intraspecific diversity. Further, it has been shown that within species diversity can affect settling success in the marine barnacle *Balanus improvisus* (Gamfeldt et al. 2005).

Our results suggest that variance-reduction effects in BEF studies may be real and general processes arising from the statistical properties of natural populations. Diverse assemblages of one or many species or groups have greater predictability of functions because they contain a wider range of attributes that can respond to environmental heterogeneity. This process occurs independently of any influence of diversity on the mean value of those functions. Manipulating intraspecific diversity provides opportunities to test many basic questions regarding the importance of biodiversity for the functioning of ecosystems. The benefits of preserving high within-species diversity could be substantial, as indicated by theory and our results.

Acknowledgements – Thanks to P. R. Jonsson, J. Havenhand and H. Hillebrand for helpful discussions which helped to improve the manuscript. This work was supported by the Swedish environmental protection agency through the Marbipp program, Formas through contract 210/2004-0285, the Colliander foundation, the Adlerbertska Science Foundation, and Helge Ax:son Johnsons Foundation.

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