

Diversity increases biomass production for trematode parasites in snails

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Increasing species diversity typically increases biomass in experimental assemblages. But there is uncertainty concerning the mechanisms of diversity effects and whether experimental findings are relevant to ecological process in nature. Hosts for parasites provide natural, discrete replicates of parasite assemblages. We considered how diversity affects standing-stock biomass for a highly interactive parasite guild: trematode parasitic castrators in snails. In 185 naturally occurring habitat replicates (individual hosts), diverse parasite assemblages had greater biomass than single-species assemblages, including those of their most productive species. Additionally, positive diversity effects strengthened as species segregated along a secondary niche axis (space). The most subordinate species—also the most productive when alone—altered the general positive effect, and was associated with negative diversity effects on biomass. These findings, on a previously unstudied consumer class, extend previous research to illustrate that functional diversity and species identity may generally both explain how diversity influences biomass production in natural assemblages of competing species.

Keywords: diversity; productivity; biomass; parasitic castrators; parasite communities; *Cerithidea californica*

1. INTRODUCTION

At first glance, the equation, $1_A + 1_B > 1_A$, might appear to be self-evident. But this is the contested and basic premise behind a questioned value of biodiversity, namely whether multi-species assemblages outperform single-species assemblages for some aggregate ecosystem functions. One potential function of biodiversity is the production of biomass (Schlapfer & Schmid 1999; Loreau *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2006; Cardinale *et al.* 2006; Stachowicz *et al.* 2007). For several free-living trophic groups, diverse assemblages have higher standing-stock biomass than does the average single species when alone (Cardinale *et al.* 2006; Stachowicz *et al.* 2007). However, it is not completely clear what mechanisms underlie these positive effects of species diversity. Indeed, diverse assemblages frequently do not have greater total standing-stock biomass than does the single species with the greatest standing stock when it is alone (Cardinale *et al.* 2006). Thus, the diversity–production relationship might be explained by the simple effect of diverse assemblages tending to include ecologically important species (Aarssen 1997; Huston 1997; Tilman *et al.* 1997; Loreau 2000; Cardinale *et al.* 2006). Here, we examine diversity versus production for a natural assemblage of parasitic castrators. We study how castrator diversity within a host affects the production of castrator standing-stock biomass. We do this to test the universality of patterns uncovered in the studies of free-living communities (no parasitic groups have previously been adequately studied), as well as to examine whether the study of these

simple parasite assemblages can clarify the mechanisms by which species diversity influences ecological process in general.

Trematode parasitic castrators in individual snails provide unexplored assemblages very suitable for investigating the effect of diversity on biomass production. The natural replication provided by individual hosts (as discrete habitat patches) has the simplicity that is typically only available in laboratory settings. In their first intermediate host snail, trematodes are parasitic castrators, a distinctive consumer strategy (Lafferty & Kuris 2002). Following infection, a single trematode reproduces asexually and exploits the entire reproductive budget of the host, as is typical for castrators (Kuris 1974; Baudoin 1975). Therefore, when more than one trematode species infects the same host, resource limitation drives strong interspecific competition, usually leading to the exclusion of competitively subordinate species (Lim & Heyneman 1972; Lie 1973; Combes 1982).

In general, functional differences between species should drive the effects of species diversity on their aggregate function (Tilman *et al.* 1997; Hooper *et al.* 2002; Naeem & Wright 2003). Because a single trematode infection theoretically uses all the resources (host reproductive effort) available for a parasitic castrator, it is unclear whether complementary mechanisms of resource acquisition among multiple trematode species could cause diversity to increase parasite biomass. However, recent analyses have indicated that trematode species differ in how they allocate resources (McCarthy *et al.* 2002; Hechinger *et al.* 2008). Also, trematode species can vary in spatial distribution within their host, i.e. they vary

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in what host tissues they infect (e.g. Cable 1956; Holliman 1961; DeCoursey & Vernberg 1974; Yoshino 1975; Kuris 1990; Sousa 1993; Hechinger *et al.* 2008). Variation in host tissue use by trematodes might reflect variation in resource use or acquisition. If so, then any variation in the effects of species diversity on productivity might be best explained by functional diversity, reflecting the degree of resource use complementarity.

Here, we examine the patterns and mechanisms underlying how naturally occurring trematode parasitic castrator diversity affects parasite standing-stock biomass. This allows a test of the generality of diversity effects on ecosystem function. This is because secondary consumers have scarcely been considered in diversity versus ecosystem function studies (Balvanera *et al.* 2006), and, to our knowledge, there have been no studies examining the effects of parasite diversity on parasite biomass among individual hosts. We found that diverse trematode castrator assemblages had greater levels of biomass and impacted resources more than did single-species assemblages, and that this was not simply a result of including the most productive species. Additionally, our findings indicate that functional traits of species—in this case, complementary use of space—can drive the effects of species diversity.

2. MATERIAL AND METHODS

As presented in detail in Hechinger *et al.* (2008), we studied the biomass of trematode parasite assemblages that castrate the California horn snail, *Cerithidea californica* (Haldeman 1840). We dissected 594 snails that were haphazardly collected from tidal channels and intertidal flats at Carpinteria Salt Marsh, CA, USA (34.40° N, 119.53° W), in winter and summer 2005. We quantified standing-stock biomass for each encountered trematode assemblage except for those single-species assemblages that we frequently encountered. For such assemblages, we stopped quantifying biomass after sampling at least 20 of that species assemblage. Additionally, we excluded those assemblages with immature infections (those that have only begun to grow). We identified the trematode species following Martin (1972) and R. F. Hechinger & T. Huspeni (unpublished manuscript).

We quantified parasite standing-stock biomass and snail tissue mass in 185 replicate habitats (i.e. individual host snails): 159 single-species and 26 mixed-species assemblages. Single-species assemblages included 15 species of larval trematode parasites. Mixed-species assemblages included 17 types of species-pair combinations involving 13 species (table 1). We did not encounter any triple- and quadruple-species assemblages, which are exceedingly rare (Martin 1955; Yoshino 1975; Sousa 1993).

We quantified trematode biomass (wet weight) in snails as detailed in Hechinger *et al.* (2008). Briefly, we did so by (i) separating and weighing the infected regions of each snail (figure 1a), (ii) using serial cross sections (figure 1b) to quantify trematode tissue proportional volume in the infected regions, (iii) multiplying the trematode proportion by the mass of the infected region, and (iv) ensuring that tissue densities for trematode and snail tissue were at least approximately equal. For mixed-species assemblages, we calculated aggregate mass. Because infections are inter-mixed, we were unable to separately calculate each species'

individual contribution to aggregate mass. We determined snail tissue mass by subtracting the trematode mass from the entire infected snail's directly weighed soft tissue mass.

To compare average biomass for mixed- and single-species assemblages, we used a general linear model (GLM; table 2) that statistically controlled for any effects of snail size, habitat and season (variables previously determined to affect trematode relative mass in snails; Hechinger *et al.* 2008). We incorporated assemblage (the particular species or species combination) as a random factor nested within the fixed-factor diversity level (mixed- or single-species assemblage). We used the 'expected mean square' approach (Quinn & Keough 2002) to incorporate random effects. This approach had the advantage of allowing us to test the diversity effect using the appropriate denominator mean squared error and degrees of freedom, while also providing unbiased least-squares means (LSMs) for the particular species and species combinations (which we were also particularly interested in, used below). All two-way interactions were non-significant (all $p > 0.17$) and not used in the final GLMs. To determine the effect of trematode diversity on resource levels (snail tissue), we performed the parallel analysis with snail tissue mass as the response variable (table 3). We used JMP v. 7 for these analyses.

To examine whether any positive diversity effects were solely due to mixed-species assemblages including more productive species, we expressed the effect of diversity as the proportional change in the standing stock of each of the 17 types of mixed-species assemblages compared with (i) the average of the standing stocks characterizing their constituent species when in single-species assemblages and (ii) the standing stock characterizing the constituent species that achieved the largest mass when it was alone. The second is a test for 'transgressive overyielding' (Trenbath 1974; Fridley 2001; Hector *et al.* 2002), and directly examines whether mixed-species assemblages produce more biomass than any of the constituent species when alone. We used the GLM LSMs for the standing stock characterizing each type of mixed- and single-species assemblage. We used the LSMs for each pair combination as replicates ($n = 17$) instead of each encountered mixed-species assemblage ($n = 26$). Although this decreased our sample size, it removed pseudo-replication issues.

To examine whether functional traits of the trematode species could explain the positive effects of species diversity, we calculated an index for 'functional diversity'. This index was based upon the degree of complementary use of space by trematodes in mixed-species assemblages (table 1). Most of the trematode species occupy the gonadal space of the snail host visceral mass (Yoshino 1975; Kuris 1990; Sousa 1993; Hechinger *et al.* 2008). However, they differ to the extent that they also use surrounding tissues in the snail visceral mass (Hechinger *et al.* 2008). Furthermore, some trematode species do not use the visceral mass at all and occupy a completely disjunct area—the mantle (Martin 1955; Yoshino 1975; Sousa 1993; Hechinger *et al.* 2008). Therefore, we calculated functional diversity as the number of tissues not shared by the constituent species divided by the total number of tissues used by the constituent species. We used ordinary least-squares regression to determine whether functional diversity explained species diversity effects on trematode standing-stock biomass.

Because one trematode species (*Probolocoryphe uca*) was particularly associated with negative effects of diversity

Table 1. Diversity effects of the particular mixed-species parasitic castrator assemblages on parasite standing stock and impact on resources (host snail tissue) for the various encountered trematode species-pair combinations and details of spatial resource use.

mixed-species assemblage ^a	<i>n</i>	proportional change of ^b				tissue sites ^d			spatial complementarity ^c
		standing-stock mass compared with		impact on resource compared with		sp. 1	sp. 2	mixed	
		average sp.	largest sp.	average sp.	largest sp.				
ACAN–AUST	1	−0.019	−0.077	−0.007	−0.017	g	g + dg + bvm	g + dg + bvm	0.67
ACAN–LGXI	1	0.378	0.314	0.067	0.065	g	g + dg	g + dg	0.50
AUST–EUHA	2	0.059	−0.040	−0.001	−0.009	g + dg + bvm	g	g + dg + bvm	0.67
AUST–HIMA	4	0.110	0.129	0.027	0.023	g + dg + bvm	g	g + dg + bvm	0.67
AUST–HIMB	3	−0.017	−0.011	−0.029	−0.055	g + dg + bvm	g + bvm	g + dg + bvm	0.33
AUST–PROB	1	0.282	−0.025	0.087	−0.074	g + dg + bvm	g + dg + bvm	g + dg + bvm	0.00
AUST–STIC	2	0.783	0.569	0.212	0.192	g + dg + bvm	g	g + dg + bvm	0.67
CATA–LGXI	1	0.635	0.453	0.164	0.147	m	g + dg	m + g + d	1.00
EUHA–SMCY	1	0.419	0.288	0.065	0.044	g	g + dg + bvm	g + dg + bvm	0.67
HIMA–LGXI	1	0.275	0.164	0.059	0.044	g	g + dg	g + dg	0.50
HIMA–PROB	1	−0.078	−0.307	−0.051	−0.230	g	g + dg + bvm	g + dg + bvm	0.67
HIMB–PROB	3	0.219	−0.077	0.049	−0.086	g + bvm	g + dg + bvm	g + dg + bvm	0.33
HIMB–RENB	1	0.950	0.566	0.140	0.093	g + bvm	m	m + g + bvm	1.00
PARO–PROB	1	−0.211	−0.377	−0.124	−0.281	g + bvm	g + dg + bvm	g + dg + bvm	0.33
PROB–SMCY	1	0.263	−0.150	0.122	−0.069	g + dg + bvm	g + dg + bvm	g + dg + bvm	0.00
RENB–SMCY	1	1.082	0.982	0.133	0.131	m	g + dg + bvm	m + g + dg + bvm	1.00
SMCY–SMMI	1	0.842	0.413	0.187	0.129	g + dg + bvm	g	g + dg + bvm	0.67
sum or mean	26	0.351	0.165	0.065	0.003				
mean without PROB ^e		0.458	0.312	0.085	0.065				

^aSpecies codes: ACAN, *Acanthoparyphium spinulosum*; AUST, *Austrobilharzia* sp.; CATA, *Catantropis johnstoni*; EUHA, *Euhaplorchis californiensis*; HIMA, *Himasthla rhigedana*; HIMB, *Himasthla* sp. B; LGXI, large xiphocercaria; PARO, *Parorchis acanthus*; PROB, *Probolocoryphe uca*; RENB, *Renicola buchmanii*; SMCY, small cyathocotylid; SMMI, small microphallid; STIC, *Stictodora hancocki*.

^bEffects of mixed-species assemblages are expressed relative to the effects characterizing their particular constituent species when in single-species assemblages (the average of their constituents and to the single constituent with the largest effect). The absolute values for mixed- and single-species assemblages were least-squares means from general linear models (tables 2 and 3).

^cSpatial niche complementarity = (no. of tissues not shared by the constituent species)/(total no. of tissues used by the constituent species).

^dg, gonad; dg, digestive gland; bvm, basal visceral mass; m, mantle; tissue use from (Hechinger *et al.* 2008).

^ePROB is the outlier species (by far the largest when alone, yet the most competitively inferior) excluded for these calculations.

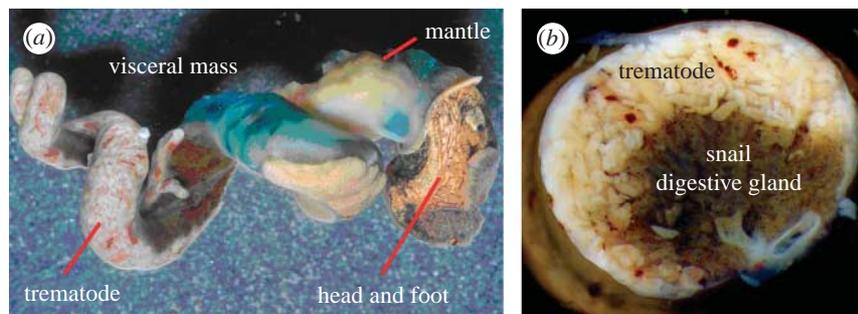


Figure 1. Trematode parasitic castrator infections in the snail, *Cerithidea californica*. (a) Snail (shell removed) infected with the trematode, *Himasthla rhigedana* (photo credit: Todd Huspeni). The trematode has completely castrated the snail, replacing the snail gonadal tissue. (b) Cross section through the visceral mass of a snail infected by the trematode, *Cloacitrema michiganensis*.

Table 2. Results of GLM assessing how trematode standing stock^a is affected by trematode assemblage diversity and other predictor variables.

predictor	d.f.	SS	F-ratio	r ²	p
diversity (mixed or single species)	1	0.032	12.42 ^b	0.15	0.0004 ^c
assemblage (diversity) ^d	30	0.151	4.21	0.46	<0.0001
season (summer or winter)	1	0.028	23.08	0.13	<0.0001
habitat (channel or flat)	1	0.012	10.31	0.06	0.0016
total tissue weight	1	0.170	141.95	0.49	<0.0001
full model	34	0.838	20.61	0.82	<0.0001
residual	150	0.179			

^aTrematode mass was fourth-root transformed in analysis to meet assumptions of normality and homogeneity of variance.

^bThe synthesized denominator mean squared error for this test was 0.0026, with 59.1 d.f.

^cOne-tailed *p*-value.

^dThe particular species (15 spp.) or species-pair combination (17 types) as a random factor nested within if an assemblage was mixed or single species.

Table 3. Results of GLM assessing how trematode resource level (snail tissue) is affected by trematode assemblage diversity and other predictor variables.

predictor	d.f.	SS	F-ratio	r ²	p
diversity (mixed or single species)	1	0.009	7.55 ^a	0.09	0.0039 ^b
assemblage (diversity) ^c	30	0.070	3.86	0.44	<0.0001
season (summer or winter)	1	0.008	13.24	0.08	0.0004
habitat (channel or flat)	1	0.003	5.42	0.03	0.0212
total tissue weight	1	0.564	936.67	0.86	<0.0001
full model	34	0.932	45.51	0.91	<0.0001
residual	150	0.090			

^aThe synthesized denominator mean squared error for this test was 0.0012, with 62 d.f.

^bOne-tailed *p*-value.

^cThe particular species (15 spp.) or species-pair combination (17 types) as a random factor nested within if an assemblage was mixed or single species.

on standing-stock biomass, we examined diversity effects of mixed-species assemblages both with and without this species.

We ensured that we met assumptions regarding normality and variance homogeneity by inspecting plots of residuals versus predicted values and normal quantile plots with Lilliefors' curves (Kutner *et al.* 2005). All tests concerning diversity effects are one tailed, commensurate with the directional hypothesis of positive diversity effects. This is further justified by two recent, thorough and quantitative meta-analyses (Balvanera *et al.* 2006; Cardinale *et al.* 2006), which found that, across the board, diversity effects on the standing stock range from positive to zero. Because our tests were based on *a priori* defined hypotheses, we present nominal *p* values. But we note that all tests deemed significant at the 0.05 level remain so when controlled for

multiple comparisons by holding the false discovery rate (Benjamini & Hochberg 1995) to 0.05 for our family of 10 diversity tests.

3. RESULTS

Trematode standing stock varied among single-species assemblages and also among the particular combinations of mixed-species assemblages (GLM, $F_{30,150} = 4.2$, $p < 0.0001$; tables 1 and 2). The average mixed-species assemblage in our sample had 41.3 per cent greater trematode standing stock than did the average single-species assemblage in our sample (*t*-test on GLM LSMs, $t_{59} = 3.5$, $p_{\text{one-tailed}} = 0.0004$; figure 2; table 2).

The combined trematode and host mass did not differ significantly between mixed- and single-species assemblages (GLM, $F_{1,150} = 1.8$, $p = 0.18$). But hosts containing mixed-

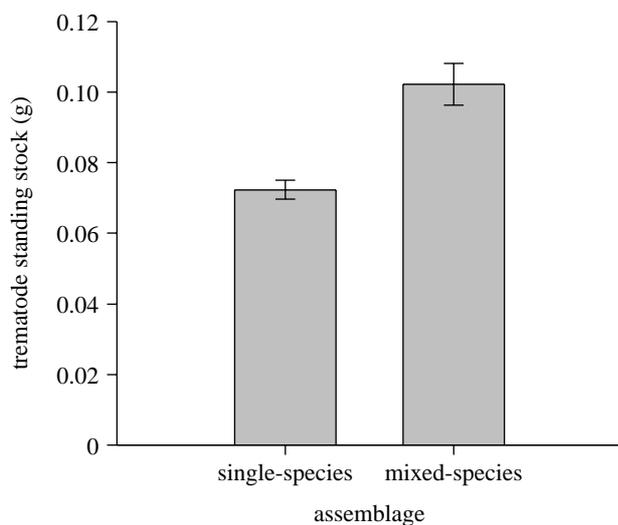


Figure 2. Average trematode standing-stock biomass of 159 naturally occurring single- and 26 mixed-species parasite assemblages. Data are LSMs (± 1 s.e.m.) from a GLM controlling for the effects of season, habitat and snail mass.

species assemblages had 8.3 per cent less snail tissue than did hosts with single-species assemblages (t -test on GLM LSMs, $t_{62}=2.7$, $p_{\text{one-tailed}}=0.0039$; table 3).

We then compared the standing stock of mixed-species assemblages directly to the standing stock achieved by their constituent species when in single-species assemblages. On average, mixed-species assemblages had 35.1 per cent greater standing stock than the average of their constituent species when alone (one-sample t -test, $t_{16}=3.8$, $p_{\text{one-tailed}}=0.0008$; figure 3*a*). Furthermore, mixed-species assemblages ‘transgressively overyielded’ (Trenbath 1974; Fridley 2001; Hector *et al.* 2002), having on average 16.5 per cent greater standing stock than that characterizing their single largest constituent species when it was alone ($t_{16}=1.9$, $p_{\text{one-tailed}}=0.036$; figure 3*b*).

Sometimes, mixed-species assemblages had a lower standing stock than did their most productive species when alone (figure 3*d*). This effect was most strong and common for mixed-species assemblages including the trematode, *P. uca* (figure 3*d*; table 1). *Probolocoryphe uca* is an outlier species in terms of productivity, taking a remarkable 39 per cent of the infected snail tissue mass when it is alone (Hechinger *et al.* 2008). Excluding assemblages with *P. uca* from analysis, diversity more strongly affected parasite standing stock (figure 3*a,b*). In this case, mixed-species assemblages had 45.8 per cent greater standing stock than the average of their constituent species when alone (one-sample t -test, $t_{11}=4.0$, $p_{\text{one-tailed}}=0.0010$; figure 3*a*). Additionally, when compared with the standing stock characterizing their single largest constituent when alone, mixed-species assemblages not including *P. uca* had 31.3 per cent greater standing stock ($t_{11}=3.5$, $p_{\text{one-tailed}}=0.0024$; figure 2*b*).

As predicted, the effects of trematode diversity on production increased with the degree of spatial complementarity characterizing mixed-species assemblages (figure 3*c,d*). This occurred if the effects of diversity were expressed relative to the average of the constituent species ($r^2=0.30$, slope=0.70, $t_{15}=2.51$, $p_{\text{one-tailed}}=0.012$) or relative to the single largest constituent species ($r^2=0.44$, slope=0.78, $t_{15}=3.44$, $p_{\text{one-tailed}}=0.0018$), and also when *P. uca* was excluded and the diversity effects were compared

with the average constituent ($r^2=0.48$, slope=1.29, $t_{10}=3.1$, $p_{\text{one-tailed}}=0.0062$) or compared with the largest constituent ($r^2=0.46$, slope=0.99, $t_{10}=2.91$, $p_{\text{one-tailed}}=0.0078$; figure 3*c,d*). The effects of diversity approached complete additivity for those mixed-species assemblages with the maximal spatial complementarity (figure 3*c,d*; table 1). Specifically, on average, mixed-species assemblages involving both mantle and visceral mass dwellers had 1.9 times the average standing stock characterizing their constituent species when alone and 1.7 times the standing stock of their single largest constituent species when it was alone.

4. DISCUSSION

To our knowledge, this is the first examination of the effects of parasite diversity on parasite standing-stock biomass in individual hosts for any type of parasitic functional group (but see below). Thus, the positive diversity effects we uncovered support the generality of positive diversity effects on productivity.

Because trematode standing stock varied among single-species assemblages, the positive effects of species diversity could have arisen by highly productive parasite species being over-represented in mixed-species assemblages (akin to the issue of non-random selection of highly productive species in diversity experiments; Huston 1997). However, on average, a mixed-species assemblage had greater standing stock than either the average of its constituent species when alone or the largest constituent species when it was alone. This last result (transgressive overyielding) provides strong evidence that the positive effects of diversity on trematode standing stock are caused by something other than simply including highly productive species in mixed-species assemblages (Loreau 1998; Fridley 2001).

The greater biomass of most mixed-species assemblages compared with their most productive constituents indicates that these parasites have partially complementary mechanisms of resource acquisition or use. The increase in biomass with functional diversity (spatial complementarity) supports this hypothesis. This is surprising considering that each of these trematode species fully castrate their host when in single-species assemblages. Although single infections target and take the entirety of the same resource, diversity still had a positive effect on standing-stock biomass. Specific differences in the mechanism of resource extraction or strategy of resource use are probably directly related to the spatial partitioning that we quantified, and which explained the positive diversity effects on standing-stock biomass.

One trematode, *P. uca*, was strongly associated with negative diversity effects on standing-stock biomass. Although *P. uca* is by far the most productive species when alone (Hechinger *et al.* 2008), this parasite is also the most subordinate trematode in the competitive dominance hierarchy of this parasite guild (Kuris 1990). Thus, there is a simple potential explanation for the decrease in the standing stock for mixed-species assemblages involving *P. uca*: in such mixed-species infections, the dominant co-occurring species was in the process of competitively excluding *P. uca*, lowering the standing stock from the high level characterizing *P. uca* to the level characterizing the dominant species. This phenomenon—a competitive inferior being the most productive when alone—can also

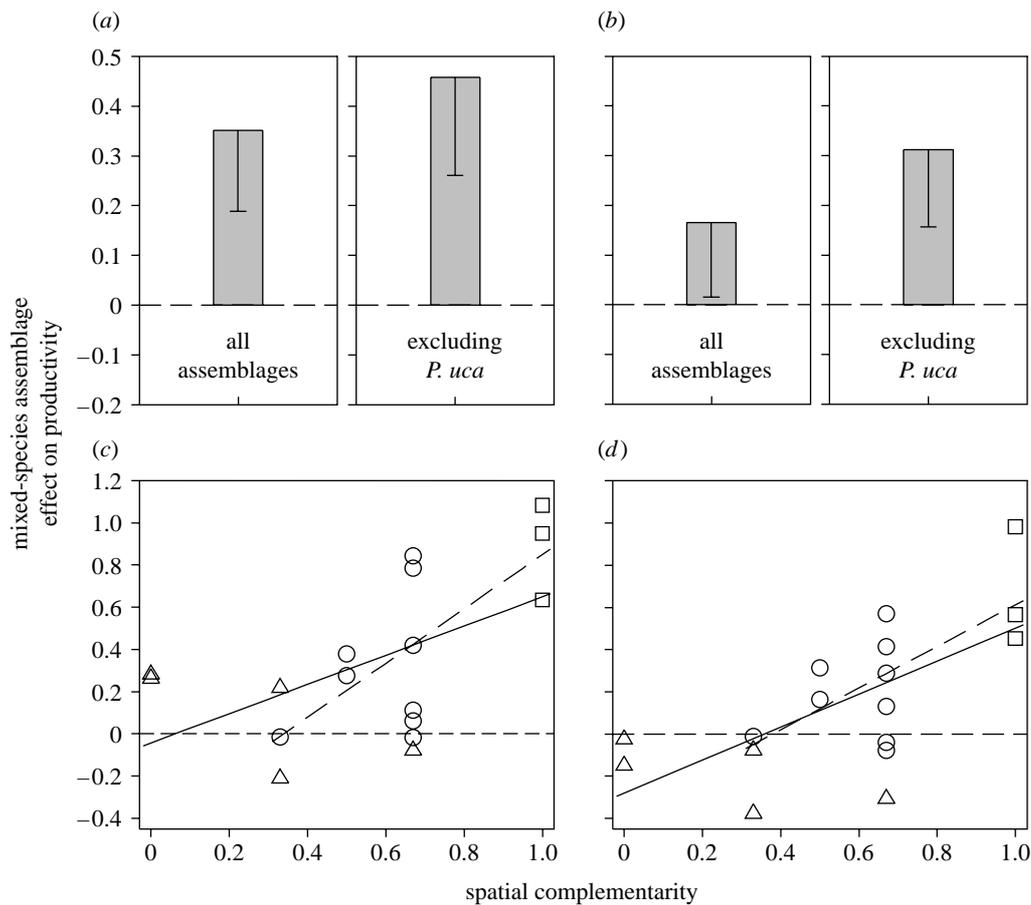


Figure 3. Effects of trematode parasite diversity on trematode standing-stock biomass. (a,b) Means and lower 95% confidence bounds for the proportional change in the standing stock of mixed-species assemblages compared with the standing stock characterizing their constituent species when alone. Data are shown for all types of encountered mixed-species assemblages ($n=17$) and for mixed-species assemblages not involving *Probolocoryphe uca* ($n=12$). (c,d) Stronger effects of species diversity on standing stock with increasing functional diversity. (a,c) display diversity effects on standing stock relative to the average standing stock characterizing the constituent species when alone, while (b,d) show diversity effects on standing stock expressed relative to the constituent species with the maximum standing stock when alone. Triangles in (c,d) assemblages involving *P. uca*, and squares indicate those involving one mantle-dwelling species and one visceral mass dweller (i.e. those with maximum spatial complementarity). The solid regression lines were fit using all mixed-species assemblages ((c) $y=0.69x-0.04$ and (d) $y=0.78x-0.28$) and the dashed regression lines are fit excluding assemblages with *P. uca* ((c) $y=1.29x-0.44$ and (d) $y=0.99x-0.37$). The horizontal dashed line in all plots represents the null hypothesis of no diversity effect.

occur in plant (Hector *et al.* 2002) and microalgal assemblages (Weis *et al.* 2007).

High diversity is usually a transient state within individual snails for these trematode assemblages. The strong interspecific competition characterizing these mixed-species assemblages typically leads to competitive exclusion of the subordinate species (Kuris 1990; Sousa 1993). Thus, in individual snails, mixed-species assemblages are relatively ephemeral. So, their higher standing stock may not reflect longer-term resource constraints. As competition is resolved, the higher biomass will decline to the level characterizing that of the dominant species when it is alone. In fact, some of the unexplained variations in trematode diversity effects may be due to quantifying standing stock for mixed-species assemblages that are at different points in the competitive exclusion process.

The initial increase and decline in the standing stock characterizing these parasite assemblages warrants comparison with findings from previous work on microalgae and plants. Weis *et al.* (2007) found that niche partitioning can increase biomass production in diverse microalgal cultures, but only when comparisons were restricted to early successional time periods. Similar to

the trematode assemblages of our study, dominant species ultimately exclude subordinates, forming monocultures. Unlike the trematodes we studied, these equilibrium-state monocultures had more biomass than did early successional diverse assemblages, even though dominants were not the most productive species. To more thoroughly compare the processes operating in microalgal and trematode castrator assemblages, experiments could determine whether niche partitioning can increase biomass production by adding dominant microalgae to subordinate monocultures at carrying capacity. Transient positive effects of diversity do not appear to be the rule for plant assemblages, where the positive diversity effects tend to increase over time (e.g. Tilman *et al.* 2001; van Ruijven & Berendse 2005; and see meta-analysis by Cardinale *et al.* 2007). However, most plant biodiversity experiments are not conducted for more than a few years (van Ruijven & Berendse 2005; Cardinale *et al.* 2007). Perhaps the positive diversity effects would also be transient over the longer time scales potentially necessary for competitive exclusion to take place in these assemblages.

Although mixed-species assemblages may frequently be short lived within individual snails, such assemblages are

continually reformed in snail populations. Mixed-species assemblages appear to be always present in California horn snail populations, comprising from less than 2 per cent to over 20 per cent of encountered infections (Hunter 1942; Martin 1955; Sousa 1993; Lafferty *et al.* 1994). Thus, the ubiquitous presence of mixed-species assemblages, in conjunction with the large biomass that these trematodes contribute to estuaries (Kuris *et al.* 2008), indicates that the positive effect of diversity on trematode biomass within snails may scale up to have considerable influence at the ecosystem level.

Our findings bear on a general issue in parasitology and medicine concerning how variation in resource overlap can influence the production and pathology of a parasite species in mixed-species infections. In our study system, parasite assemblages are strongly interactive, with each species contending for the same ultimate resource (host reproductive effort). The production of one or both parasites was depressed by the presence of the other (apparent from the sub-additivity of mixed-species assemblages). Importantly, the severity of these negative effects was at least temporarily mediated by the degree of overlap in spatial resource use. This fits with the findings of a recent meta-analysis of mouse pathogen–parasitic worm co-infections. Although she did not measure total production, Graham (2008) found that the density of pathogens consuming red blood cells (RBCs) tends to decrease when in co-infections with parasitic worms expected to deplete the RBC resource (by blood feeding or causing haemorrhage). However, RBC-feeding pathogen density is not lower in co-infections with parasitic worm species that are thought not to decrease RBCs. Thus, ecological rules regarding how niche overlap affects competition may have broad implications for medicine and the dynamics of infectious disease.

In a previous comparative study of helminth parasites across vertebrate hosts, Poulin *et al.* (2003) considered whether productivity influences parasite species richness. They found that the mean diversity of parasites in a host species increased with the mean parasite biovolume (which was used to represent the productivity offered to parasites by a host species). This pattern is consistent with complementarity among parasite species (Poulin *et al.* 2003). However, complementarity may not be necessary because mean parasite biomass for the typical macroparasites used in that study does not represent the maximal parasite biomass supportable by a host (Poulin & George-Nascimento 2007). This is because such parasites do not grow to commandeer available resources (as opposed to parasitic castrators and pathogens; Lafferty & Kuris 2002) and mean numbers in a host are typically far lower than maximum numbers (Poulin & George-Nascimento 2007). Because mean measures for those parasites do not necessarily characterize assemblages experiencing competition, they are less subject to study focused on how niche complementarity influences diversity effects on productivity.

5. CONCLUSION

While much of the best work on the diversity–production relationship has been experimental, it is also important to examine the diversity patterns and processes in naturally occurring species assemblages (Chapin *et al.* 1997; Huston 1997; Loreau *et al.* 2001; Giller *et al.* 2004, and see Bracken *et al.* 2008). Unfortunately, the complexity of most natural systems makes them difficult to

replicate and analyse. The simple, replicated assemblages in individual hosts that we studied allowed consideration of naturally occurring patterns and the mechanisms by which species diversity influences ecological process. Including assemblages of differing species composition at our ‘high’ diversity level allowed us to examine functional diversity. This provided insight into the aspects of resource use that may drive the positive effects of diversity on standing-stock biomass. Future studies should manipulate trematode diversity to further clarify the mechanisms by which diversity influences standing stock and to confirm its causality. However, these findings provide further evidence that the effects of diversity on productivity are universal and rich—complementarity (functional diversity) and species identity probably both contribute to the influence of diversity on the aggregate function of assemblages.

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