Scale dependency of processes structuring metacommunities of cladocerans in temporary pools of High-Andes wetlands.

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Abstract

Metacommunity structure can be shaped by a variety of processes operating at different spatial scales. With increasing scale, the compositional variation among local communities (beta diversity) may reflect stronger environmental heterogeneity, but may also reflect reduced exchange of organisms between habitat patches. We analyzed the spatial architecture of a metacommunity of cladoceran zooplankton in temporary pools of High Andes wetlands, with the objective of explaining the spatial dependency of its structure. The spatial distribution of the pools is hierarchical and highly discontinuous: pools are clustered within small wetlands, which lay scattered over valleys that are separated from each other by mountain ridges. We studied a total of 59 pools, belonging to six different wetlands in four different valleys. We assessed pool environmental heterogeneity and sampled active communities and dormant propagule banks of cladoceran zooplankton. Environmental heterogeneity proved very high within wetlands and showed almost no increase with increasing spatial scale. Conversely, diversity partitioning analyses indicated an increase in beta diversity with spatial scale, especially among valleys. Variation partitioning on environmental data and spatial RDA models suggested environmental heterogeneity as the most important generator of beta diversity within wetlands. At the largest spatial scale, beta diversity manifested itself mainly as a differentiation of species occurrence patterns among valleys, which could not be entirely explained by environmental variables**.** Our study thus presents a case where environmental control seems to be the dominant metacommunity structuring process at the smallest spatial scale, whereas neutral processes and dispersal limitation are the most likely generators of beta diversity at the largest spatial scale.

Introduction

The importance of spatial scale has increasingly been recognized in community ecology, especially during the latest decades (Menge and Olson 1990). Compositional variation among communities (beta diversity) can be generated by different factors and the relative importance of these factors can vary among spatial scales. One important reason for this is that environmental variables that shape communities may differ in their range of variation among spatial scales. Some variables show large variation at small spatial scales and will therefore generate high community dissimilarity in relatively small areas. Other important variables only show substantial variation at large spatial scales, and will thus only give rise to community variation in large study areas (Borcard et al. 2004).

A second major driver of beta diversity is dispersal limitation. Since the breakthrough of the metacommunity concept (Leibold et al. 2004, Holyoak et al. 2005), community ecology has increasingly considered alternative models of spatial dynamics in explaining patterns of diversity within and among communities at the landscape scale. Dispersal plays a pivotal albeit different role in all these models. Neutral and patch dynamics models, which assume absence of species interactions with the environment (Hubbell 2001), strongly rely on dispersal limitation to explain patterns of (meta-) community structure. Conversely, the species sorting model (sensu Leibold et al. 2004, Cottenie 2005, also referred to here as 'environmental control') explains community composition by the interaction of species niches with the abiotic and biotic environment and assumes that dispersal limitation does not prevent species from tracking environmental gradients in space and time. In the case of mass effects, massive fluxes of

individuals from source to sink communities can overwhelm the effect of local conditions and species interactions (Vanschoenwinkel et al. 2007, Guelat et al. 2008). The potential of organisms to disperse among habitat patches within metacommunities depends on the distance and type of connections among patches (Shurin et al. 2009). As spatial scale is intrinsically related to the among-patch distances and the physical structure of the landscape, it is expected to be very important in determining the type and strength of alternative types of spatial dynamics that shape metacommunity structure (Dumbrell et al. 2008).

The revival of diversity partitioning techniques (Lande 1996, Veech et al. 2002, Jost 2007) has strongly enhanced research on the spatial organization of biodiversity in metacommunities. For spatially nested datasets, these techniques allow researchers to decompose overall gamma diversity (total regional diversity) into alpha diversity (mean local diversity) and components of beta diversity for each level of spatial scale. Although this approach has considerably increased our knowledge on the overall architecture of metacommunity biodiversity for several organism groups in a variety of landscape contexts (Stendera and Johnson 2005, Diekotter et al. 2008, Lindo and Winchester 2008), it often has limited power in explaining the observed patterns when standing on its own. Parallel to the development of diversity partitioning, an increasing number of studies have tried to link patterns of community variation with existing metacommunity paradigms (Holyoak et al. 2005) by partitioning community variation into spatial and environmental components (Borcard et al. 1992, Cottenie 2005), using direct gradient ordination techniques (Legendre et al. 2005). These studies have increased our understanding of processes underlying metacommunity patterns, but the large majority of

these studies are confined to a single spatial scale. In order to get a better understanding of the link between the structure and dynamics of metacommunities at different levels of spatial scale, there is a need for an integrated approach through the combined application of these two analytical frameworks.

Due to their discontinuous distribution at multiple spatial scales, temporary pools of small wetland systems in the high Andes provide an interesting model system for the study of scale dependency in aquatic invertebrate metacommunities. The spatial distribution of pools in this part of the Andes is hierarchical: pools are clustered within small wetlands and are to a variable degree connected with each other during periods of high rainfall. These wetlands lay scattered over valleys that are separated from each other by mountain ridges with height of approximately 450 m. For this study, we focused on cladoceran zooplankton, a group of passively dispersing aquatic invertebrates (Bohonak and Jenkins 2003) for which time-integrated species lists of individual pools can readily be obtained through the analysis of dormant propagule banks (Vandekerkhove et al. 2005a, 2005b). We sampled pools at three levels of spatial scale (i.e., within-wetlands, within valleys and among valleys) with the aim of dissecting the spatial architecture of the metacommunity and evaluating the relative importance of alternative metacommunity processes for each level of spatial scale. We expected beta diversity among pool communities to increase with increasing spatial scale and set out to explore if that increase corresponded to community patterns that should emerge from stronger environmental control along more pronounced environmental gradients, or whether these patterns were more consistent with the patterns that were expected to emerge as a result of stronger dispersal limitation. At the smallest spatial scale, the scale of individual

wetlands, we expect little dispersal limitation, given the small size of the wetlands and short distances among pools; conversely, flooding events may lead to homogenization of communities through mass effects. At the intermediate and largest spatial scales, we expect an increase in the importance of dispersal limitation, given the larger among-patch distances, lower hydrological connectivity and the presence of mountain ridges between valleys. In addition, broader environmental gradients may potentially enhance beta diversity at these larger scales, as long as environmental control is not impeded by dispersal limitation (Leibold and Norberg 2004).

Material and methods

Study area

Our study area is located in the Tunari mountain range (Cordillera del Tunari, between 17° 19’ 19’’ and 17° 10’ 56’’ South latitude and 66° 08’ 53’’ and 66° 22’ 43’’ West longitude), at altitudes between 4000 and 4400 m, and is part of the eastern Andes mountain range (Figure 1). It is a mountainous area, with numerous small wetlands (locally called ‘bofedales’) that lay scattered over the valleys and mountain slopes (Coronel et al. 2004). Most of the wetlands in this area contain small temporary fishless pools, of which the total number typically varies between one and eight, although pools can be more numerous in some of the larger wetlands. The region is subject to a rainy season from October to March and a dry season from April to September. During the

latter period, the pools fall dry. We refer to Coronel et al. (2004) for a more detailed description of the limnological features of the pools.

Sampling design

We sampled a total of 59 pools in four different valleys of the Tunari mountain range, i.e., Taquiña, Toro, Saito and San Ignacio. The aim of our sampling design was to study cladoceran community variation at three levels of spatial scale, i.e. at the level of individual wetlands, individual valleys and multiple valleys. Full hierarchical sampling would have required us to sample all pools in the entire set of wetlands in each of the studied valleys, an effort that was unfeasible in the context of the present study. We therefore applied a different approach still allowing us to capture variation among communities at the three levels of spatial scale. For this, we collected two sets of samples (Figure 1). For a first dataset (further referred to as the WTL dataset), we sampled all pools in 6 different wetlands, i.e. two wetlands in Taquiña (Tq1, with 6 pools and Tq2, with 7 pools), two wetlands in Toro (Tr1, with 6 pools and Tr 2, with 5 pools), one wetland in Saito (S1, with 5 pools) and one wetland in San Ignacio (SI1, with 6 pools). The total number of pools sampled for the WTL dataset thus equals 35 from 6 wetlands (Figure 1). For the second dataset (the VALLEY dataset), we selected a number of wetlands in each of the four valleys (13 wetlands in Taquina, 7 in Toro, 5 in Saito and 4 in San Ignacio) and in each of these wetlands we sampled one haphazardly chosen pool (Figure 1), including one randomly selected pool of each wetland from the WTL dataset.

In total, the VALLEY dataset consists of 29 sampled pools from 29 wetlands. The WTL dataset thus represents cladoceran community variation within wetlands, whereas the VALLEY dataset represents community variation among wetlands at the valley scale. When combined, the WTL & VALLEY dataset also represent variation among valleys at the regional scale; there are 59 pools from 29 wetlands located in 4 valleys.

Sampling

We assessed a variety of pool characteristics during a sampling campaign in February-March 2004, in the middle of the wet season. We determined the concentration of nitrates (NO_3), total phosphate (TP), alkalinity, pH, conductivity, macrophyte coverage, phytoplankton chlorophyll a, thickness of the sludge layer on the sediments, pool depth and pool surface area. In addition, we assessed the density of potential predators (cyclopoid copepods, mites, and larvae of the coleopteran genera *Ranthus* (Colymbetinae) and *Hydroporus* (Hydroporinae). We refer to Coronel et al. (2004) for methodological details on the collection of the data for these variables.

We collected cladoceran community data in two different ways: (1) by taking snap shot samples of active communities at one sampling occasion, and (2) through the analysis of dormant egg banks. These two methods have been shown to be complementary in their ability to detect cladoceran species (Vandekerkhove et al. 2005a). We sampled active communities during February-March 2004 by collecting water with a tube sampler (75 mm diameter and 1.5 m length) from different places (vegetated and

non-vegetated areas) in the pool and by filtering this water through a 30-µm Nitex mesh. The total filtered volume ranged between 3 and 15 L. Samples were preserved in sucrose-formaldehyde solution (5% final concentration). We collected sediments with dormant eggs at the beginning of the dry season of 2006 (26-29th of June). In each pool, we collected sediment at ten haphazardly chosen locations using a KC-sediment core sampler (0.7 meter long plexi-glass tube of 5.2 cm diameter). Only the upper three centimeters of each core were retained (approximately 100 g wet weight per sample). A total amount of one kilogram sediment per pool was collected. Immediately after collection, samples were wrapped in aluminum foil and transported to the lab in a cooler box.

Sample analysis

We analyzed active community samples by counting a total of at least 300 individuals from sub-samples. For the analysis of the sediment samples, we first isolated dormant eggs by means of the Onbé-Marcus method as modified by Vandekerkhove et al. (2004). After storage in the dark at 4 °C, we first removed gross material (mostly vegetal debris) using sieves of 1 mm and 500µm. Dormant eggs retained by a sieve of 63-µm mesh were then isolated from fine debris by the sugar flotation method, following three steps: 1) filtration through a 48-µm mesh, 2) centrifugation of the residual in a sugar solution (1000 g table sugar in 1000 ml distilled water) at 3000 rpm for three minutes, and 3) washing of the supernatant over a 48-µm size mesh using tap water. The isolated dormant eggs (at least 300 eggs whenever possible) were next sorted according to morphology,

identified and counted under a stereo microscope (Olympus SZX12). To allow identification to species level, we incubated unknown dormant egg types individually in 30-ml multi-well plates containing the Aachener Daphnien Medium (ADAM medium) at a conductivity of 30 µs cm^{-1}. Multi-well plates were placed in an incubator at 20°C with a photoperiod of 14 hours light and 10 hours dark. Incubation medium was refreshed every five days. For a period of two months, we checked all multi-well plates every four days for emerging hatchlings. Hatchlings were transferred to 50-ml vessels and fed *Scenedesmus obliquus* (100.000 cells ml^{-1}) until a developmental stage at which they could be identified. We used the keys of Pagui (1995) and Smirnov (1996) for identification.

Data analysis

We compiled species lists for each of the pools, combining data from the active community snapshot samples and the dormant propagule banks, and derived a presence-absence dataset from these lists. We also calculated relative abundances of species using the data from the snapshot samples of active communities.

Geographic distance and environmental heterogeneity across spatial scales

We calculated the mean geographic distance among pairs of pools for each of the three levels of spatial scale, i.e. among pools within each individual wetland (WTL dataset), among pools belonging to different wetlands within each valley (VALLEY dataset) and

among pools belonging to different valleys (WTL & VALLEY datasets combined). In the same way, we used the environmental variables to estimate environmental heterogeneity among pairs of pools at the within-wetland, the within-valley and the among-valley scales as standardized Euclidean distances.

Beta diversity of cladoceran communities across spatial scales

We estimated beta diversity at each spatial scale following two different approaches: (1) as the mean Bray-Curtis dissimilarity among pairs of pools, similar as for geographic distance and environmental heterogeneity (i.e. among pools within wetlands using the WTL dataset; among pools from different wetlands within valleys using the VALLEY dataset, and among pools from different valleys). These calculations were done on the presence-absence data derived from the species lists as well as on the relative abundance data of the active communities; (2) through the application of diversity partitioning on species richness of the species lists and true Shannon diversity (Jost 2007) estimated from the abundance data of active communities. Species richness was partitioned in the additive way, with $\gamma = \alpha + ß1 + ß2 + ß3$ (Lande 1996), where α refers to the average richness in local communities (samples), gamma refers to the total richness observed in the entire set of samples, and ß1, ß2 and ß3 refer to the beta diversity at each of the studied spatial scales: ß1 = among pools within wetlands; ß2 = among wetlands within valleys; ß3 = among valleys. Additive diversity partitioning has the advantage that beta components can directly be quantitatively compared with each other and with alpha and gamma diversity. True Shannon diversity can only be partitioned in a multiplicative way, but we transformed its alpha, beta and gamma components logarithmically (i.e., into

Shannon entropies) so as to make them relate additively (Jost 2007). A full hierarchical partitioning analysis, including the three levels of spatial scale, could only be performed on a subset of the WTL dataset, including all data of Taquina and Toro, but excluding the data of Saito and San Ignacio. Incorporation of the latter valleys in the analysis would have resulted in a bias, underestimating average beta diversity among wetlands (ß2), because there were only data available for one wetland in each of these valleys. To assess the robustness of these results and fully exploit the size of our dataset, we performed additional partitioning analyses on our complete dataset, estimating beta diversity among pools within each of the wetlands in the WTL dataset (ß1) as well as among pools belonging to different wetlands in each of the valleys of the VALLEY dataset. Logically, the latter variable incorporates both within- and among-wetland beta diversity within valleys and should equal ß1 + ß2. Diversity partitioning calculations were performed with the software PARTITIONv3 (Veech and Crist 2009).

Explaining the beta components

A commonly applied method to quantify the relative importance of alternative metacommunity processes consists of the decomposition of community variation into an environmental (E) and a spatial (S) component. Using direct gradient ordination techniques (mostly redundancy analysis), statistical models are first constructed for both components and their relative contributions to community variation are subsequently assessed through variation partitioning (Borcard et al. 1992). Upon correction for the spatial component, a significant environmental component (E/S) is generally accepted to represent environmental control. A significant spatial component, after control for the

268 environmental component (S/E), can indicate neutral processes, historic events and
269 dispersal limitation (Legendre & Legendre 1998, Cottenie 2005), assuming that all
270 important environmental variables have been measured. The co-occurrence of significant
271 environmental and spatial components has been considered as being indicative of mass
272 effects (Cottenie 2005), although it can also represent species sorting that is partially
273 constrained by dispersal limitation (Ng et al. 2009).

274 To explain beta diversity in the cladoceran communities, we applied variation
275 partitioning on redundancy analysis models (RDA) for each of the three levels of spatial
276 scale, separately. For the largest spatial scale, i.e. the inter-valley scale, we constructed an
277 environmental and a spatial model using the entire dataset (VALLEY and WTL datasets
278 combined, $n = 59$). The environmental model (E) was constructed by applying the
279 forward selection procedure of Blanchet et al. (2008) to the environmental variables. For
280 the spatial model (V), we constructed a matrix representing valley identity using 3
281 dummy variables. With variation partitioning (Borcard et al. 1992, Legendre et al. 2005,
282 Peres-Neto et al. 2006), we then tested the marginal and unique contributions of the
283 environmental and spatial models in explaining community variation.

284 We applied the same procedures to the VALLEY dataset ($n = 29$) for the study of
285 beta diversity at the within-valley scale. However, the spatial model consisted here of two
286 components: (1) a V-component, representing valley identity using dummy variables (see
287 above), and (2) an S-component consisting of Moran's eigenvector maps (MEM) that
288 describe the spatial relationships among pools within individual valleys. MEM analysis
289 produces a set of orthogonal spatial variables, derived from the geographic coordinates of
290 the study sites (Dray et al. 2006). These variables represent spatial variation across a

291 range of spatial scales and can be used as explanatory variables in direct gradient analysis
292 to model spatial relationships in community data. In the S-matrix, the MEM variables
293 were arranged in blocks, each block corresponding to one valley. Within these blocks,
294 pools from the other valleys received the mean value 0. The structure of the blocks of
295 MEM variables in S were similar to that used to test the presence of different spatial
296 structures at different times, shown in Appendix C of Legendre et al. (2010). Appendix 1
297 provides an R function that can be used for the construction of a staggered matrix of
298 MEM spatial eigenvectors for sites that are spatially clustered. The type of MEM
299 variables computed in the present study were formerly called principal coordinates of
300 neighbour matrices (PCNM: Borcard and Legendre 2002, Borcard et al. 2004, Dray et al.
301 2006).
302 Using the VALLEY dataset, we then applied variation partitioning to assess the
303 unique contributions of the S- and E- model components to community variation, while
304 controlling for the V-component. An identical procedure was applied for studying
305 cladoceran variation at the intra-wetland scale using the WTL dataset (n = 36).
306 All analyses were carried out on the species lists (active and dormant community
307 data combined) as well as on the relative abundance data from the active communities.
308 We did the analyses on these two datasets because they should cast light on different
309 aspects of metacommunity dynamics. Indeed, we expect that analyses on abundance data
310 of active communities should mainly stress the impact of environmental gradients or
311 mass effects, because they emphasize shifts in the relative success of species and also
312 because these data were collected simultaneously with the environmental data. Analyses

313 on species lists should do better at reflecting distribution patterns of species; we expect
314 them to be more powerful in revealing patterns related to dispersal limitation.
315 All species data (both abundance and presence-absence) were Hellinger
316 transformed prior to analysis (Legendre and Gallagher 2001). After this transformation,
317 RDA and variation partitioning are based on the Hellinger distance, which is appropriate
318 for community composition data (presence-absence or abundance), instead of being based
319 on the inappropriate Euclidean distance; the Hellinger distance computed on presence-
320 absence data is monotonically related to the Ochiai distance, which is also appropriate for
321 community composition data. Of the environmental variables, conductivity, surface area,
322 sediment depth and chlorophyll *a* were log-transformed. Densities of predatory
323 invertebrate taxa tended to be positively correlated with each other; we summarized
324 overall predator density with the first principal component (i.e., sample scores vector) of
325 a standardized principal component analysis (eigenvalue: 39%). All statistical analyses
326 were performed in R (v2.8.1; R Development Core Team 2008), using the rda and varpart
327 functions of the vegan library (Oksanen et al. 2005, Peres-Neto et al. 2006), the
328 forward.sel function of the packfor library (Dray et al. 2007); MEM spatial
329 eigenfunctions were computed using the PCNM function of the PCNM library (Legendre
330 et al. 2009).
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333 ## Results
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335 **Geographic distance and environmental heterogeneity across spatial scales**

The mean geographic distance among pools increased by more than one order of magnitude between successive levels of spatial scale (Figure 2A). The distance among pools within wetlands (cf. WTL dataset) averaged 0.063 km. The mean distance among pools of different wetlands within valleys (cf. VALLEY dataset) was 0.69 km, whereas pools from different valleys were located at an average distance of 19.7 km from each other (WTL and VALLEY datasets combined).

The mean Euclidean distances for environmental variables were highly variable, both among individual wetlands as well as among valleys (Figure 2B). We detected no tendency for higher heterogeneity at the valley scale compared to the wetland scale. Heterogeneity among pools from different valleys also tended to be only slightly higher than the within-wetland or within-valley means. See Appendix 2 for summary statistics of the environmental variables.

Patterns of beta diversity across spatial scales

Although highly variable among wetlands and among valleys, mean Bray-Curtis dissimilarities based on species lists tended to increase with spatial scale (Figure 2C). In contrast, no such tendency was observed for Bray-Curtis dissimilarity based on the relative abundance of species in active communities (Figure 2D).

A full hierarchical diversity partitioning analysis on the richness data of the Taquina and Torro subset showed a substantial contribution of both the smallest (β_1 =

359 7.2) and largest spatial scales (β_3 = 5.5 species) to gamma diversity (Figure 3A, left
360 pane). Beta diversity among wetlands within valleys (β_2 = 3.5 species) was relatively
361 small. The additional partitioning analyses on the WTL and VALLEY datasets (Figure
362 3A, right pane) also showed a relatively high average beta diversity among pools within
363 wetlands (β_1) compared to the average beta diversity among pools from different
364 wetlands at the valley level ($\beta_1+\beta_2$). According to a paired t-test, both estimates of beta
365 diversity could not be shown to differ significantly, indicating only a minor contribution
366 of β_2 to gamma diversity.
367 We observed similar patterns for Shannon diversity of active communities (Figure
368 3B). Full hierarchical diversity partitioning also indicated a small contribution of β_2 and a
369 relatively large contribution of β_1 and β_3. Average beta diversity at the valley level
370 ($\beta_1+\beta_2$; cf. VALLEY dataset) showed no significant difference with the average beta
371 diversity within wetlands (β_1; WTL dataset) according to a paired t-test ($p > 0.05$).
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374 **Community variation and environmental gradients**
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376 Appendix 3 gives a list of the species and their abundances in samples of active
377 communities and dormant propagule banks. Species lists differed significantly among
378 valleys (V- and V/E-components in Table 1A), with valley identity explaining up to 10.6
379 % of the community variation. pH proved to be the only significant environmental
380 variable (cf. E in Table 1A), although the effect of this variable was strongly reduced
381 upon correction for inter-valley differences (E/V in Table 1A left). Within valleys and

382 wetlands, MEM models showed no evidence for spatial patterns. Environmental models
383 explained significant fractions of the variation in the species lists of the WTL and
384 VALLEY datasets, but these effects became non-significant when the identity and MEM
385 models of valleys and wetlands were taken into account, respectively (Table 1B and C
386 left).

387 At the inter-valley scale, the composition (in terms of relative abundance) of the
388 active cladoceran communities was significantly explained by both the environmental
389 model (consisting of the variables alkalinity, sludge depth and surface area) and by valley
390 identity, although the environmental model explained approximately 3 times more
391 variation than valley identity (See E-, V-, E/V- and V/E-components in Table 1A right).
392 Analyses at the within-valley level using the VALLEY dataset (Table 1B right) revealed
393 no significant spatial patterns, whereas the explanatory power of the environmental
394 model was low (only marginal effects were significant). At the within-wetland level
395 (WTL dataset, Table 1C right), the marginal and conditional effects of the environmental
396 model (based on alkalinity, surface area, sludge depth and the predation gradient)
397 amounted to 21% and 17% of explained variation, respectively. At this level of spatial
398 scale, the MEM model explained no community variation.

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401 ## Discussion

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403 In our study, geographic distances among pools increased with more than one order of
404 magnitude across each level of spatial scale. This, however, translated only in a weak

405 increase in environmental heterogeneity and some individual wetlands encompassed a
406 degree of pool heterogeneity similar to what was present at the landscape scale. We found
407 this high within-wetland heterogeneity to concur with relatively high cladoceran beta
408 diversity, both in terms of species richness and Shannon diversity. With RDA analysis,
409 we were able to uniquely explain part of the variation among active communities by
410 environmental gradients, which suggest that this beta diversity at the wetland scale is
411 structured by environmental heterogeneity (Cottenie and De Meester 2004). Part of the
412 unexplained beta diversity may also have originated historically by chance (cf. priority
413 effects; Louette and De Meester 2007, Loeuille and Leibold 2008), although we have no
414 specific data to further support this idea. We found no evidence for spatial community
415 patterns within wetlands.

416 Beta diversity at the valley scale was not higher than at the scale of individual
417 wetlands, despite the larger average distance among pools. Within valleys, environmental
418 heterogeneity could not be shown to be consistently higher than within wetlands and
419 RDA-models were not able to significantly explain beta diversity. This suggests that
420 environmental control was probably not more important as a generator of beta diversity at
421 this spatial scale than at the within–wetland scale. Absence of spatial patterns also
422 suggests no important dispersal limitation within valleys.

423 In contrast, beta diversity among valleys tended to be relatively high. RDA-
424 analyses demonstrated strong differences among valleys, especially for species lists. Such
425 pattern may be generated by neutral dynamics and dispersal limitation but could also
426 indicate control of community composition by large-scale environmental gradients.
427 RDA-analysis on species lists indeed suggested an association between species

distribution patterns with a pH-gradient, but variation partitioning revealed that the importance of large scale pH variation could not be unequivocally evaluated, whereas among-valley community differentiation proved robust. Reduced exchange of propagules among pools at this scale is indeed very plausible given the morphology of the landscape (mountain ridges, lack of hydrological connections) and the distance among valleys (ranging between 4 and 28 km). Large mammals can be important vectors for the dispersal of cladoceran dormant eggs (Vanschoenwinkel et al. 2008) and in our study area llamas (*Lama glama*) are good candidates for such zoochorous dispersal. Llamas are mainly herded in the vicinity of farms, and these animals may therefore be responsible for much of the exchange of dormant eggs between pools within wetlands and among wetlands within valleys but they may be less important as dispersal vectors among valleys.

The RDA-analyses on the relative abundance data of active communities also indicated a tendency towards differentiation among valleys, but this pattern was considerably weaker than with species lists. One can indeed expect that analyses on abundance date will be less efficient in revealing patterns of species distributions because they tend to be confounded mainly by abundance patterns of dominant and subdominant species. However, analyses on abundance data may be more sensitive to responses of communities to environmental gradients because they emphasize shifts in the relative success of species that perform differently along these gradients. In our study, the abundance data were explained by similar sets of environmental variables at the largest and smallest of spatial scales (e.g., alkalinity, surface area and sludge depth). The effect of these variables at the largest spatial scale seemed, however, mainly a reflection of their

451 effect at the smallest scale. Indeed, the gradient lengths of these variables were only
452 slightly larger at the inter-valley scale than at the wetland scale, and variation partitioning
453 based on the entire dataset showed that the explanatory power of the environmental
454 model (E) was only slightly reduced when among-valley differences were controlled for
455 (E/V; see Table 1). This indicates that large scale environmental heterogeneity was of
456 minor importance in explaining cladoceran community variation.
457 Several field studies have attempted to assess the relative importance of
458 environmental control, mass effects, neutral processes and dispersal limitation for
459 zooplankton metacommunities. These studies were performed on a variety of water body
460 types with different connectivity levels at a wide range of spatial scales. From these
461 studies, environmental control seems to come out most frequently as the dominant
462 metacommunity organizing principle. In a system of neighboring, strongly interconnected
463 ponds, Cottenie et al. (2003) found that environmental control generated an important
464 degree of differentiation among communities despite the homogenizing effect of water
465 exchange via rivulets. In a set of temporary rock pools on a small scale rock shelf,
466 Vanschoenwinkel et al. (2007) also concluded environmental control to be the most
467 important metacommunity structuring process, together with some indications for mass
468 effects among interconnected pools. Pandit et al. (2009) showed the degree of
469 environmental control to depend on the degree of habitat specialization. At a much larger
470 spatial scale, Beisner et al. (2006) mainly found evidence for environmental control in a
471 set of connected lakes. Although abundance data showed spatial patterns, these patterns
472 were not reflected in the presence- absence dataset, as abundant zooplankton species were
473 not restricted in their spatial distribution. Based on a meta-analysis of a large number of

474 datasets of a variety of organism groups along a broad gradient of spatial scales, Cottenie
475 (2005) concluded that passive dispersers overall tend to track well environmental
476 heterogeneity, although the importance of dispersal limitation appears to increase at the
477 expense of environmental control at the larger spatial scales, probably because species
478 fail to reach habitat patches that match with their niche requirements.
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481 **Conclusions**
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483 To our knowledge, our study is the first to simultaneously study metacommunity
484 structure of a passively dispersing animal group at three levels of spatial scale along a
485 range of inter-patch distances of at least two orders of magnitude. The detailed analysis of
486 resting egg banks also allowed us to complement data obtained from active community
487 samples to establish a high quality presence-absence dataset, yielding reliable species
488 distribution patterns that are otherwise difficult to obtain from active community
489 sampling alone (Vandekerkhove et al. 2005a). Our study presents a case where
490 environmental control seems to be the dominant metacommunity structuring process at
491 the smallest spatial scale. However, an increase in spatial scale coincided with a marked
492 increase in beta diversity that appears to be mainly generated by dispersal limitation. Our
493 study is well in line with the results of Cottenie (2005) but illustrates that larger spatial
494 scales need not necessarily be associated with stronger environmental gradients, which in
495 some cases may also explain the lack of evidence for stronger environmental control at
496 these scales. Extrapolation of spatial diversity patterns in cladoceran metacommunities to

497 other organism groups should be done with caution. However, we believe that our results
498 may represent general patterns that also hold for other aquatic organisms with similar life
499 history and dispersal strategies (e.g., short generation times, production of dormant
500 stages, passive dispersal). Candidate organism groups are large branchiopods and small
501 invertebrate taxa like rotifers, ostracods, turbellarians, and nematodes.
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613 an old idea. - Oikos 99: 3-9.

614 Table 1. Partition of variation in cladoceran communities at three levels of spatial scale using partial RDA analysis on relative
615 abundance data of active communities and species lists (active communities and dormant propagule banks combined). E:
616 environmental model; S: spatial model component, constructed from MEM variables; V: model component representing the 4
617 individual valleys; WTL: model representing the 6 individual wetlands; E/(S+V): environmental model corrected for valley identity and
618 spatial patterns within valleys; E/(S+WTL): environmental model corrected for wetland identity and spatial patterns within wetlands.
619 R^2_{adj} (%): community variation explained by model, expressed in %; df_{model}: degrees of freedom of model; df_{res}: residual degrees of freedom.
620

	Species lists					Abundance data of active communities				
	R^2_{adj} (%)	df_{model}	df_{res}	F	P	R^2_{adj} (%)	df_{model}	df_{res}	F	P
A) Inter-valley scale										
WTL & VALLEY dataset, *n* = 59										
E	4.1[a]	1	57	3.49	0.001	9.9[d]	3	55	3.12	0.001
V	10.6	3	55	3.19	0.005	4.1	3	55	1.82	0.020
E/V	0.5	1	54	1.28	0.191	9	3	52	2.91	0.001
V/E	6.5	3	54	2.38	0.001	3.3	3	52	1.69	0.027
B) Within-valley scale										
VALLEY dataset, *n* = 29										

E	11.9 [b]	3	25	2.26	0.001	4.9 [e]	1	27	2.43	0.031
S	1.3	8	20	1.05	0.389	11.9	8	20	1.47	0.072
V	8.2	3	25	1.83	0.010	2.9	3	25	1.28	0.220
E/(S+V)	-0.5	3	14	0.97	0.526	-0.6	1	16	0.88	0.456
S/(E+V)	1.3	8	14	1.04	0.433	12.6	8	16	1.46	0.112
C) Within-wetland scale										
PLT dataset, *n* = 35										
E	8.5 [c]	1	33	4.15	0.001	20.8 [f]	4	30	3.23	0.001
S	-5.6	10	24	0.82	0.907	2.4	10	24	1.08	0.339
WTL	14.5	5	29	2.15	0.001	8.7	5	29	1.65	0.024
E/(S+WTL)	1.6	1	18	1.36	0.196	17.4	4	15	2.25	0.001
S/(E+WTL)	2.0	10	18	1.07	0.337	2.0	10	15	1.08	0.362

a: environmental model constructed from the environmental variable pH

b: environmental model constructed from the environmental variables chlorophyll a, depth and total phosphorus

c: environmental model constructed from the environmental variable pH

d: environmental model constructed from the environmental variables alkalinity, sludge depth and surface area

e: environmental model constructed from the environmental variable TN

f: environmental model constructed from the environmental variables alkalinity, sludge dept

627 **Legend to Figures**

628

629 Figure 1. Location of the four valleys sampled in the Tunari mountain range
630 (Cochabamba, Bolivia) and a schematic representation of the wetlands (represented by
631 ovals) and pools (represented by circles) sampled in each valley. Filled symbols represent
632 pools sampled for the VALLEY-dataset. Empty circles represent pools that were
633 additionally sampled for the WTL-dataset.

634

635 Figure 2. Average geographic distance (A), environmental heterogeneity (B) and
636 community dissimilarity (C,D) among pools across the three levels of spatial scale (i.e.,
637 within wetlands, among pools of different wetlands within valleys and among pools of
638 different valleys). Environmental heterogeneity was calculated as the standardized
639 Euclidean distance for the environmental variables. Community dissimilarities were
640 calculated as the Bray-Curtis distance among pools based on species lists (presence-
641 absence data derived from the combined data of active communities and dormant
642 propagule banks, C) as well as on the relative abundance data of the active communities
643 (D). Stars represent individual wetlands and valleys; circular symbols represent averages
644 and error bars denote the standard deviation.

645

646 Figure 3. Diversity partitioning results for species richness (A) and Shannon entropy (B).
647 The left panels in the graphs represent the results of a full hierarchical analysis on the
648 Taquina and Toro subset. The right panels represent average results for each wetland and
649 each valley in the WTL- and Valley-datasets, respectively. Alpha: local pool diversity;

650 Beta1: beta diversity among pools within wetlands; Beta2: beta diversity among wetlands
651 within valleys; Beta3: beta diversity among valleys. Beta diversity among pools in the
652 VALLEY-dataset comprises both Beta1 and Beta2 and is therefore denoted as
653 Beta1+Beta2. Error bars represent the standard deviation.
654

655 Figure 1, Steven Declerck et al.

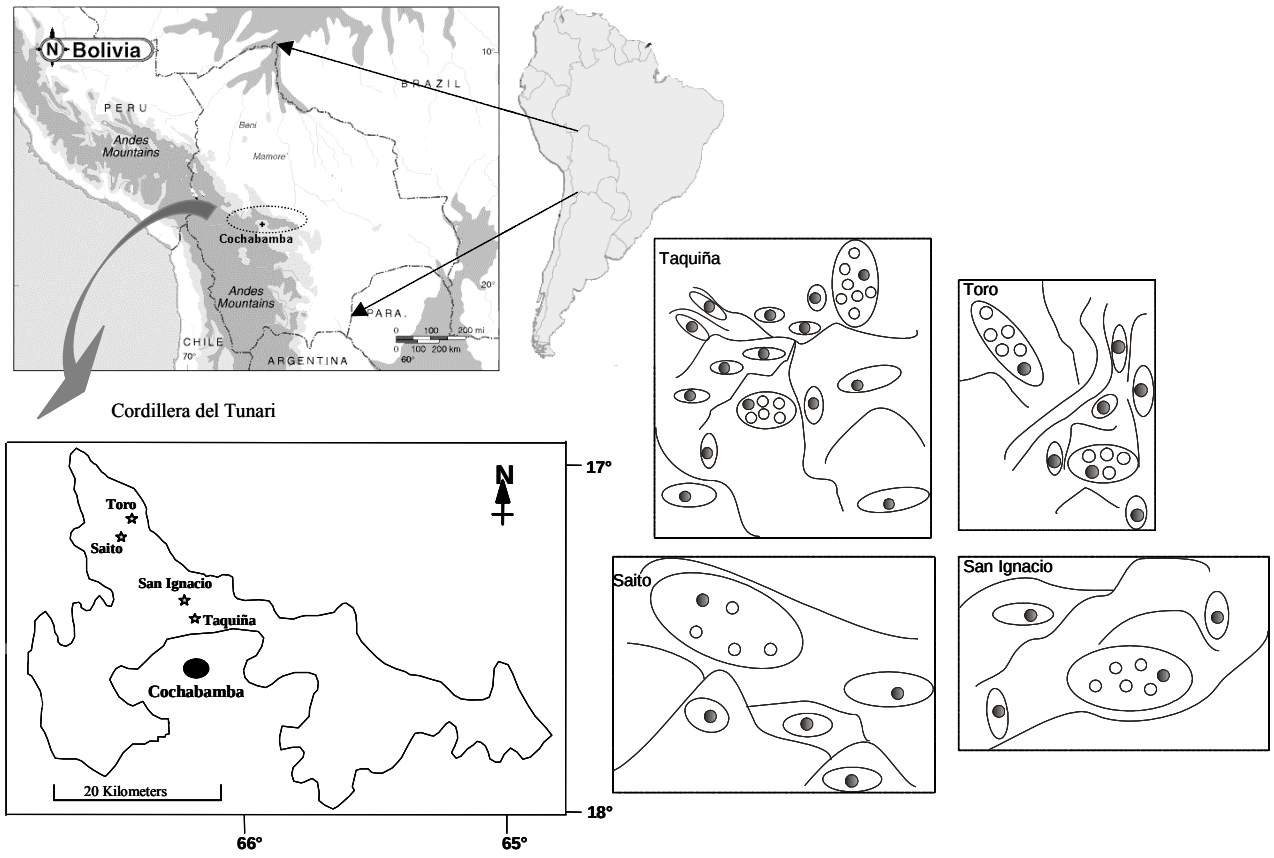

Figure 2, Steven Declerck et al.

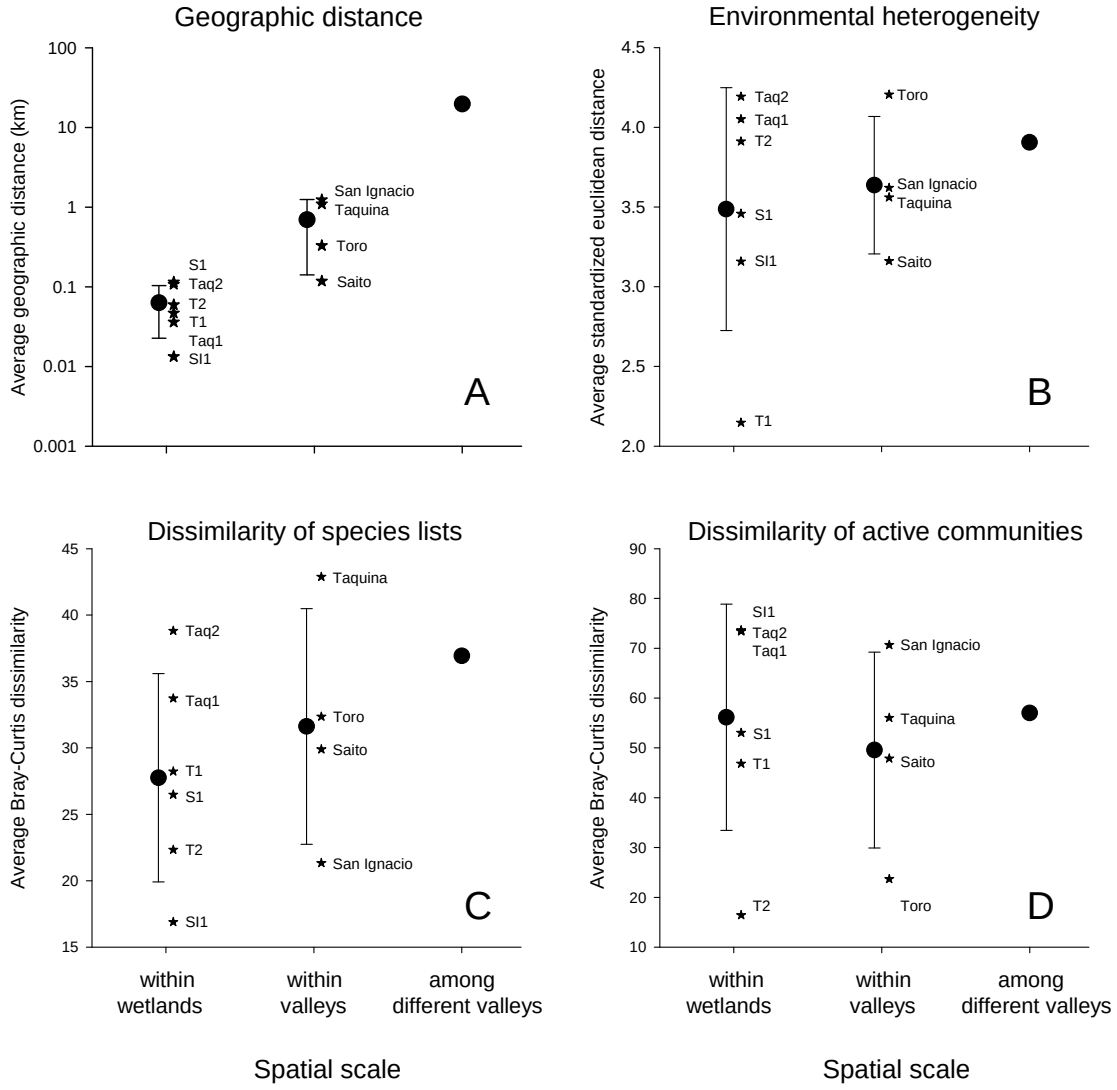

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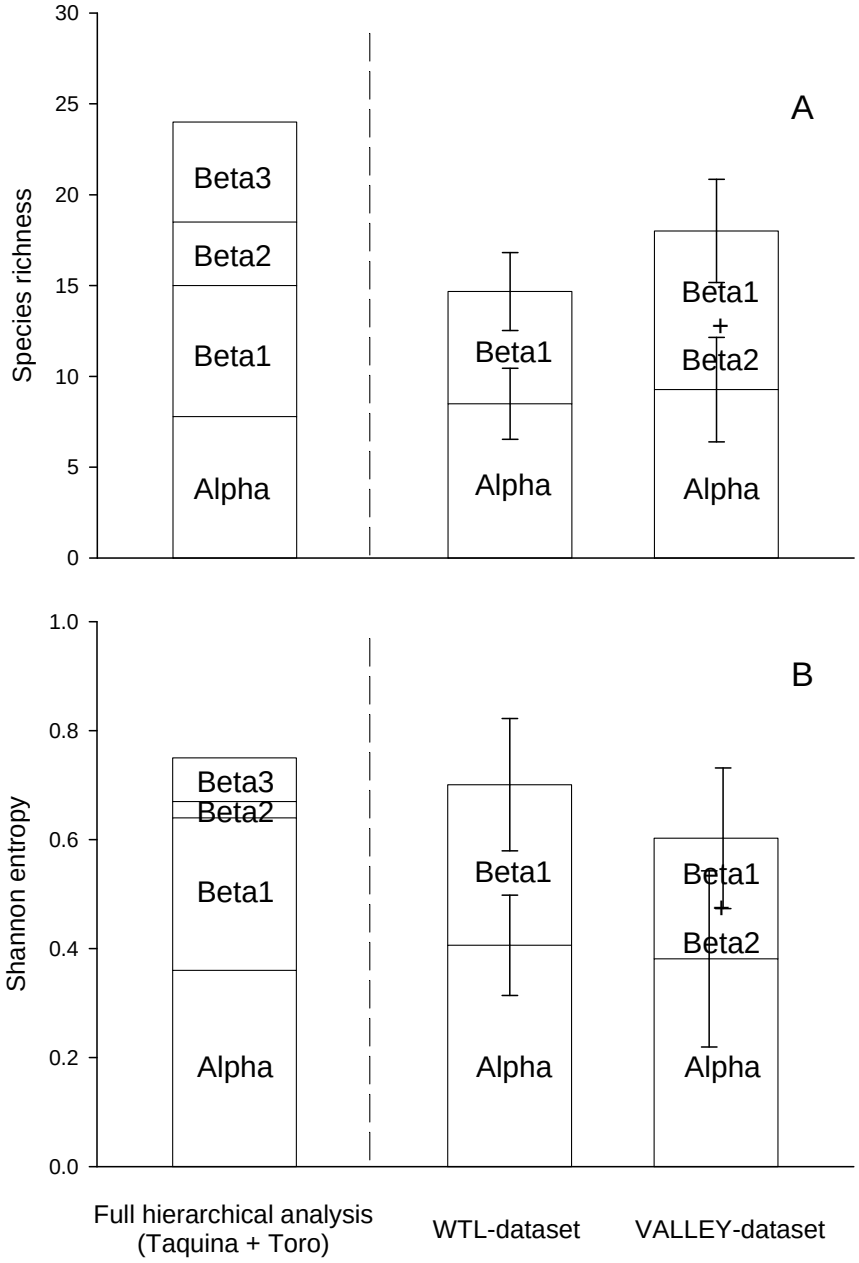
Figure 3, Steven Declerck et al.



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Appendix 2: Environmental variables with their medians, quartiles, minimum and maximum values. med: median; 25%: 25 percentile, 75%: 75 percentile; max: maximum recorded for the entire dataset.

	med	min	max	25%	75%
Chlorophyll a (µg/l)	11	2	154	5	18
Macropyte cover (%)	70	0	100	50	90
pH	7	5	10	7	8
Conductivity (µS/cm-1)	15	5	238	12	19
Alkalinity (mg/l CaCo3)	9	0	31	6	13
Oxygen (mg/l)	6	3	12	5	7
Total nitrates (mg/l)	0.08	0.01	0.36	0.05	0.13
Total phosphates (mg/l)	0.01	0.00	0.63	0.01	0.01
Transparency (m)	0.35	0.11	0.50	0.29	0.39
Depth (m)	0.19	0.03	0.46	0.13	0.27
Surface area (m2)	23	2	310	8	52
Sludge depth (m)	0.25	0.02	0.60	0.10	0.40
Altitude (m)	4327	4067	4429	4252	4383

Appendix 3: Occurrence and abundance of cladoceran species, as determined from the analysis of the dormant propagule banks and snap shot samples of the active communities. Occ: number of pools with the species; %occ: frequency of occurrence; med: median; 25%: 25 percentile, 75%: 75 percentile; max: maximum recorded for the entire dataset.

	Dormant propagule bank samples						Active community snap shot samples					
	occ	%occ	med[a]	25%[a]	75%[a]	max[a]	occ	% occ	med[b]	25%[b]	75%[b]	max[b]
Alona boliviana	3	5	0	0	0	35	1	2	0	0	0	62
Alona cambouei	42	69	13	0	36	159	39	64	1	0	3	28
Alona davidi	17	28	0	0	5	128	11	18	0	0	0	14
Alona glabra	4	7	0	0	0	22	0	0	0	0	0	0
Alona ossiani	55	90	34	12	72	247	47	77	2	0	9	59
Alonalla excisa	30	49	1	0	12	72	40	66	1	0	3	58
Bosmina huaronensis	0	0	0	0	0	0	1	2	0	0	0	0
Camptocercus aloniceps	23	38	0	0	12	60	18	30	0	0	0	44
Ceriodaphnia sp.	29	48	1	0	17	94	15	25	0	0	0	63
Chydorus brevilabris	52	85	22	11	41	119	56	92	18	4	88	500

Daphnia peruviana	5	8	0	0	0	4	4	7	0	0	0	2
Daphnia pulex	7	11	0	0	0	46	5	8	0	0	0	6
Ephemerophorus hibridus	7	11	0	0	0	162	2	3	0	0	0	18
Ephemeroporus cf. acanthodes	0	0	0	0	0	0	1	2	0	0	0	1
Graptoleberis testudinaria	11	18	0	0	0	41	8	13	0	0	0	10
Drepanothrix cf dentata	2	3	0	0	0	17	0	0	0	0	0	0
Macrothrix atahualpa	53	87	55	20	102	232	45	74	1	0	14	97
Paralona piagra	6	10	0	0	0	17	3	5	0	0	0	91
Pleuroxus caca	0	0	0	0	0	0	7	11	0	0	0	7
Pleuroxus cf aduncus	2	3	0	0	0	7	0	0	0	0	0	0
Pleuroxus sp1	6	10	0	0	0	32	0	0	0	0	0	0
Schapholeberis spinifera	1	2	0	0	0	4	1	2	0	0	0	1
Simocephalus mixtus	47	77	18	1	50	198	32	52	0	0	3	142
Streblocerus serricaudatus	5	8	0	0	0	55	0	0	0	0	0	0
Ilyocryptus cf spinifer	1	2	0	0	0	7	0	0	0	0	0	0

[a] number of dormant propagules per kilogram of sediment wet weight

[b] numbers of individuals per liter