Connectivity and cladoceran species richness in a metacommunity of shallow lakes

KARL COTTENIE AND LUC DE MEESTER
Laboratory of Aquatic Ecology, Katholieke Universiteit Leuven, Ch. De Beriotstraat 32, B-3000 Leuven, Belgium

SUMMARY
1. The shallow ponds of the nature reserve ‘De Maten’ form a metacommunity, in which individual ponds are highly interconnected via a system of overflows and rivulets. This study reports on the relations between cladoceran species richness and (a) connectivity patterns and (b) local environmental variables.
2. No relation was found between local species richness and three connectivity variables or dispersal pathways.
3. Spatial configuration was related to richness, but was confounded by environmental variables for 2 of 3 years. In those 2 years, there was a significant linear relation between Secchi disc depth and species richness, suggesting an important impact of the clearwater/turbid state alternative equilibria in shallow lakes in determining cladoceran richness. Only in the year in which environmental variables were unimportant did connectivity between the ponds influence species richness.
4. These results suggest that local environmental variables related to the clearwater/turbid state alternative equilibria in shallow lakes are important in determining cladoceran species richness. Connectivity and dispersal of individuals between the different ponds only act secondarily by increasing the general species richness within a pond through dispersal from ponds with different environmental conditions.

Keywords: cladocera, connectivity, metacommunity, shallow lakes, species richness

Introduction
Understanding factors that control species richness in local habitats is a central question in both applied and fundamental research. Some important local factors influencing zooplankton species richness have been identified: lake area and primary productivity (Dodson, 1991, 1992; Dodson, Arnott & Cottingham, 2000), lake depth (Keller & Conlon, 1994), latitude (Hebert & Hann, 1986), acidity (Brezonik, Crisman & Schulze, 1984; Locke, 1992), nutrients (Leibold, 1999; Jeppesen et al., 2000), toxins (Yan et al., 1996), predation (Brooks & Dodson, 1965), competition (Shurin, 2000) and climate (Stemberger et al., 1996).

All the above-mentioned studies approach lakes as closed units. However, interactions between ponds through dispersal and colonisation can influence species richness as well. Individual species differ in their probability of colonising patchy habitats because of differential abilities to disperse and the effects of varying distances between habitats. As a result, sites exhibiting similar environmental conditions may have dissimilar species composition because of differential chances of colonisation and extinction for each species. Terrestrial ecologists have found these ideas useful for understanding systems containing highly fragmented habitats (Fahrig & Merriam, 1994). Several studies have considered how readily individuals may move between patches and the longevity of populations in patches with various degrees of connectivity (the effect of landscape structure on species use, ability to move and risk of mortality on the movement rate of individuals among habitat patches in the
It is believed that more isolated patches will have reduced rates of colonisation, equal or higher extinction rates, and as a consequence, a more depauperate community. Changes in the degree of isolation (through changes in patch connectivity) may lead to changes in population viability and community composition. Ecologists have applied these ideas of patch or metapopulation dynamics to issues of conservation (see Saunders, Hobbs & Margules, 1991 for a review).

Moreover, recent modelling by Loreau & Mouquet (1999) showed that immigration can increase local species diversity in competitive communities open to interactions with other communities. In similar but isolated communities without immigration, competition for space would lead to the exclusion of all but one species.

Studies of the effect of lake area on diversity implicitly suppose lakes to be habitats open to immigration and extinction (island biogeography’s equilibrium hypothesis; MacArthur & Wilson, 1967), but a higher diversity in larger lakes can also be explained by more subdivisions (niches and habitats) in larger lakes or to sampling phenomena. We know of only four studies that explicitly integrate the spatial configuration and connectivity between lakes (regional approach) in their study of diversity patterns. Dodson (1992) included in his regression model the number of lakes within 20 km as an important factor contributing to diversity, suggesting that immigration sources are important. van den Brink, Van Katwijk & Van der Velde (1994) studied species composition in 100 mature floodplain lakes in relation to flood frequency, but found no relation. Havel, Eisenbacher & Black (2000) found a positive relation between connectivity of scour basins with the Missouri River and species richness, and then illustrated the importance of connections with immigration sources for local diversity. Shurin et al. (2000) found that local species richness was positively related to the number of species in the surrounding area, a result that may suggest a large role for dispersal limitation.

The nature reserve ‘De Maten’ in Belgium includes a set of 35 interconnected ponds, with very different ecological characteristics (Cottenie et al., 2001). Michels et al. (2001b) described the connectivity pattern within this system and measured dispersal rates of zooplankton in the overflows and rivulets. Relatively high dispersal rates of on average 70 (Daphnia sp.) to 900 (Chydoridae) individuals per hour were observed, which corresponds to an influx of on average 0.1% of the local population size per day (Michels et al., 2001b). In this system with ecologically different, yet highly interconnected ponds, we related species richness patterns in cladoceran communities to different local and regional variables, to determine the important structuring variables, and generate hypotheses regarding cladoceran richness in highly interconnected systems. We drew up different working hypotheses, based on possible interactions between the ponds.

Hypothesis 1: The connecting elements are the dispersal routes for cladoceran species, resulting in a pattern in which the species composition of downstream ponds is a subset of upstream pond species composition.

Hypothesis 2: Local richness is related to (a) connectivity variables: ponds at the end of the system are more species rich; (b) spatial configuration of the ponds; (c) local environmental factors.

We used species richness as a diversity estimate. Two types of richness were determined for each pond, in an effort to differentiate between two types of mechanisms. Local richness per pond is based on species richness estimated from a single sample. This represents the number of species coexisting in the water column at the same time (Shurin et al., 2000). This is the number of species that possibly interact with each other, and is thus the correct way to test whether local interactions limit diversity of coexisting species. Total richness per pond is based on estimates of multiple samples. It is a measure of the potential species pool for a specific pond, and may contain information about stability or resilience (Arnott, Magnuson & Yan, 1998). We also determined regional richness as the total number of species found during the study.

Methods
Study area: ‘De Maten’ (Belgium)

The ponds studied are part of the nature reserve ‘De Maten’ (Genk, Belgium) and cover an area of 200 ha [for additional information on the system and a map of the ponds see Cottenie et al. (2001) and Michels et al. (2001a,b)]. There is an altitude difference of 15 m between the highest and the lowest pond. The marshes were converted to fish ponds around 1400
by peat-digging and the building of dikes. Fish farming stopped in 1991, but all ponds except pond 1 still contain fish (Cottenie et al., 2001). The ponds are connected to each other through a system of overflows and rivulets. The main sources of water are two rivulets, one of which mainly feeds a subset of ponds located in the NW corner of the area. The ponds are also fed by groundwater. At the other end of the nature reserve, the outflow of water is again diverted to the Stiemerbeek, the main rivulet.

**Cladoceran sampling**

Quantitative zooplankton samples were collected with a 12-L Schindler–Patalas sampler from the pelagic zone in June–July for three consecutive years. Four random samples at different locations were taken in the pelagic zone of each pond. The samples from a given pond were combined, filtered through a 64-µm mesh net and preserved in 5% formaldehyde (final concentration). Total sample volume was adjusted to 60 mL and subsamples of 2 mL were taken using a 4-mm pipette. Cladoceran individuals were identified to the species level under a stereomicroscope at 90× magnification. A second subsample of 3 mL was taken to count additional individuals of species with less than 50 individuals counted in the first subsample. The following keys were used: Flößner (1972), Flößner & Kraus (1986), Smirnov (1996). All identified individuals were included in the analyses. Because of conservation management practices, some ponds were empty during some sampling periods and consequently no zooplankton data are available for pond 20 in 1996, 4 in 1997 and 11, 14 and 20 in 1998.

For every pond-year combination, local richness was computed as the number of species present, including rare species. The mean local richness per pond was measured, averaging the local diversities across years. Total richness was computed as the total number of species found in a specific pond. As sampling intensity can have important influences on richness measures (Arnott et al., 1998), we included in our analyses on total richness only those ponds that were sampled all 3 years (30 ponds).

All statistical analyses mentioned below were carried out with S-Plus 2000 (professional version, Insight 2001). Normality of the data was tested with a Kolmogorov–Smirnov goodness-of-fit test (Sokal & Rohlf, 1995), and equality of variances in a two-sample test with an F-test (Sokal & Rohlf, 1995). As some data did not meet all the assumptions, we used only non-parametric tests for testing Hypothesis 1.

**Hypothesis 1 – connections are dispersal pathways**

If the connecting elements are the dispersal routes for the cladoceran species, then the likelihood of species dispersal between adjacent ponds will follow the current direction. The coefficient of species dispersal direction, DD (x1 → x2), equals a(b - c)/(a + b + c)^2, with x1 the upstream pond; x2 the downstream pond; a: number of species that pond x1 and x2 have in common; b: the number of species found in x1 but not in x2; c: the number of species found in x2 but not in x1 (Legendre & Legendre, 1998; pages 763–765). This coefficient of species dispersal will be positive when the number of species found in the upstream but not in the downstream pond is bigger than the number of species found in the downstream but not in the upstream pond. The coefficient of species dispersal direction was computed for all upstream–downstream pond couples, and we used a one-sample Wilcoxon signed ranks test (Sokal & Rohlf, 1995) to test if the median is positive (one-sided test).

**Hypothesis 2 – local versus regional processes**

If the connection through overflows and rivulets results in the transportation of species from upstream to downstream ponds, the species richness in downstream ponds is expected to be higher than that in upstream ponds and dependent on the number of ponds that flow into the pond. We thus hypothesise a positive relation between species richness in a particular pond and the number of possible source ponds, defined as the number of ponds which are a direct source of individuals and water to a pond (local source load). Moreover, in a linear system, this can lead to a steady downstream increase in species richness. The total source load is the cumulative number of ponds that provide possible cladoceran species for a particular pond, and very similar to the definition of lake order (Riera et al., 2000). The ponds that were not connected to any other pond (ponds 1 and 34) were given a total source load of 0. The ponds that are at the origin of the subgroups (ponds 32, 17, 20) were given an arbitrary load of 1 (the ponds...
receive water from a rivulet, but there are no other ponds within 1 km of these ponds. The ponds connected to these ponds get load 2, etc. A third variable (upstream richness) is the total richness of directly connected upstream ponds, as this might also influence the diversity in the downstream ponds. These three variables (local source load, total source load and upstream diversity) constitute the first set of explanatory variables (connectivity variables).

The second set of explanatory variables pertains to the spatial configuration of the ponds, taking into account the connecting elements (Michels et al., 2001a; flow rate model). The effective geographical distances between all the ponds in the system were computed, taking into account the connecting elements, the flow rates in those elements and the current direction. The matrix of spatial variables was then constructed in the following way (Pinel-Alloul, Niyonsenga & Legendre, 1995; Legendre & Legendre, 1998): (1) the effective geographical distances between all pairs of ponds were transformed into $xy$ coordinates with principal coordinate analysis, which Michels et al. (2001a) showed to have a clear hydrological interpretation by identifying three pond subgroups, (2) the obtained $x$ and $y$ values were centred by subtracting the respective mean of all values for both variables, and (3) a third degree polynomial was constructed to incorporate not only linear gradient patterns between the landscape and species richness, but also more complex features like patches or gaps (cubic trend regression, Legendre & Legendre, 1998). This second set of variables thus consists of nine variables ($x, y, x^2, y^2, xy, x^3, y^3, x^2y, xy^2$, spatial variables).

Environmental variables included lake depth, lake area, phytoplankton abundance, total fish density, macroinvertebrate diversity, submerged macrophytes, turbidity, pH, conductivity, oxygen, phosphate and nitrate concentration. For details on sampling procedure, we refer to Cottenie et al. (2001; in press). Area, chlorophyll $a$, phosphate and nitrate concentration were log$_10$-transformed for normality. All independent variables were standardised to the same scale after transformation by subtracting the mean for that variable and dividing by the standard deviation, as this allows direct comparisons of regression coefficients.

As some fish density values are missing because of management constraints, and fish densities are shown to be an important structuring factor of cladoceran species, a number of ponds were left out of the analysis (1996: ponds 1, 4, 5, 20; 1997: ponds 1, 2, 3, 4, 5, 21; 1998: ponds 1, 5, 11, 14, 20, 29). We also removed ponds with extreme outliers, i.e. absolute values larger than three times the standard deviation from the mean (outside a 0.997 confidence interval around the mean for normally distributed variables). The removed ponds were pond 21 in 1996 for pH, pond 18 in 1997 for total fish density, and pond 18 and 32 for pH and conductivity, respectively.

For every set of variables, we looked at the linear relation of the variables with local cladoceran richness using multiple linear regression. We selected a parsimonious set of variables with a forward–backward stepwise procedure, using Akaike’s information criterion (AIC; Venables & Ripley, 1999). The selected variables were then fitted and checked for multicollinearity with the variance inflation factor, which should be less than 10 (Neter et al., 1996; p. 387). We also selected the most parsimonious set of variables for the three variable sets together (three connectivity variables, nine spatial variables, and 12 environmental variables), which we also checked for multicollinearity.

We also tested the linear relation between total cladoceran richness and both the connectivity and spatial set of variables, following the procedure outlined above. We did not test for linear relations with the environmental variables, as they give momentaneous information, while total species richness is integrated over several sampling dates.

**Results**

**Hypothesis 1 – connections are dispersal pathways**

No direct evidence for downstream transportation of species was found. All dispersal direction coefficients were symmetrical around 0, both for the total richness values ($n = 44, z = 0.79, P = 0.21$) as for local richness levels of the three different years (1996: $n = 42, z = 0.71, P = 0.24$; 1997: $n = 43, z = 0.09, P = 0.47$; 1998: $n = 33, z = -0.13, P = 0.55$).

**Hypothesis 2 – local versus regional processes**

No significant linear relation between the set of connectivity variables and local cladoceran richness could be found for any year (Tables 1–3). For spatial and environmental variables, we found a difference between 1996 and 1997 versus 1998. The selected
spatial variables explained approximately 25% of the variance in all 3 years, but when all sets of variables were selected, only in 1998 was a spatial variable selected (Table 3). For the environmental variables, the selected variables explained approximately 45% of the variance in 1996 and 1997, and twice Secchi disc depth was selected, together with depth in 1996 and oxygen concentration in 1997 (Fig. 1). In 1998, only 24% of the variation was explained by the selected environmental variables, of which only pH was significant in the final model, and selected again when all variable sets were used together.

No significant linear relation was found between total cladoceran richness and either connectivity or spatial variables.

**Discussion**

A study of diversity patterns stands or falls with the estimator used to determine diversity. We used as estimator the richness of a standard subvolume of a quantitative sample, and defined rarity in relation to the subvolume sampled, because we were interested in the number of species that possibly interacted with...
each other, with densities greater than approximately 0.08 individuals per litre. This underestimates the total number of species present in the habitat (Arnott et al., 1998, 1999). It is very much possible that for Hypothesis 1 (connections are dispersal pathways) particularly the very rare species are important in estimating species richness in downstream ponds with species extinctions buffered by continuous dispersal, but this will not interfere with the claim that local processes are more important than regional processes.

It is surprising that we found for 2 of the 3 years no relation between local or total species richness per pond and any spatial or connectivity variables. In

<table>
<thead>
<tr>
<th>Selection of variables</th>
<th>Model</th>
<th>Variable</th>
<th>Value</th>
<th>SE</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connectivity</td>
<td>$R^2$ 0.10</td>
<td>Intercept</td>
<td>9.7</td>
<td>1.2</td>
<td>8.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$F_{(1, 24)}$ 2.6</td>
<td>Total load</td>
<td>-0.3</td>
<td>0.2</td>
<td>-1.6</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>P-value 0.123</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial</td>
<td>$R^2$ 0.29</td>
<td>Intercept</td>
<td>7.3</td>
<td>0.6</td>
<td>11.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$F_{(2, 23)}$ 4.6</td>
<td>$x^3$</td>
<td>-22.1</td>
<td>9.4</td>
<td>-2.3</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>P-value 0.021</td>
<td>$x^4$</td>
<td>-4.8</td>
<td>2.7</td>
<td>-1.8</td>
<td>0.090</td>
</tr>
<tr>
<td>Environmental</td>
<td>$R^2$ 0.24</td>
<td>Intercept</td>
<td>8.3</td>
<td>0.6</td>
<td>14.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$F_{(2, 23)}$ 3.6</td>
<td>Diversity</td>
<td>1.0</td>
<td>0.6</td>
<td>1.6</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>P-value 0.044</td>
<td>macroinvertebrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>-1.8</td>
<td>0.8</td>
<td>-2.1</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>All variables</td>
<td>$R^2$ 0.31</td>
<td>Intercept</td>
<td>8.1</td>
<td>0.5</td>
<td>15.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$F_{(2, 23)}$ 5.1</td>
<td>pH</td>
<td>-1.7</td>
<td>0.8</td>
<td>-2.1</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>P-value 0.015</td>
<td>$x$</td>
<td>6.1</td>
<td>2.7</td>
<td>2.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3 Results of forward–backward variable selection procedure using Akaike’s information criterion in multiple regression analyses of local cladoceran richness in 1998 versus four sets of explanatory variables. See Table 1 for further information.

Fig. 1 Local cladoceran richness versus the significant selected local environmental variables in 1996 (left column) 1997 (middle column), and 1998 (right column). Only the patterns for the variables selected in the multiple regression (see Tables 1–3) are shown.

1996 and 1997, spatial variables showed significant linear relations with species richness, but stepwise regression with all variable sets together showed this effect to be completely confounded by local environmental variables, as they were not selected in the final model. Upon closer examination, however, there are several plausible explanations. First, it is very likely that the differences in connectivity among ponds are too small to be detected in this system because connectivity is high throughout the pond system. The continuous dispersal with relatively high dispersal rates (Michels et al., 2001b) between the different components can be seen to result in a homogenisation of the species across all ponds. Moreover, the ponds of ‘De Maten’ have been present since the Middle Ages, so the existence of an extensive egg bank is very likely. The egg bank of each pond may harbour species available from the whole region. From the latter two observations, it follows that all ponds probably share the same species pool, with the number of species present dictated by chance events and local environmental factors. In 1996 and 1997, we could show the importance of turbidity through the inclusion of Secchi disc depth in the selected regression models for each year, which is an important variable in shallow lakes (see further). Thirdly, recent source-sink modelling work by Amarasekare & Nisbet (2001) showed that high rates of dispersal can undermine coexistence, and hence diversity, by reducing spatial variance in fitness. It is possible that when dispersal rates are very high, dispersing individuals (of an competitive inferior species) might drive some competitive superior species to extinction in the downstream population. However, we did not find that downstream species are a subgroup of upstream species (see Hypothesis 1). It is possible to check for the significance of the dispersal coefficients between two neighbouring ponds (Legendre & Legendre, 1998; p. 764), and we found not one upstream–downstream pond combination with an individual dispersal coefficient significantly different from zero.

Our observation that local species richness is not independently related to spatial factors in 1996 and 1997 contrasts with the results of Cottenie et al. (in press) on community composition of zooplankton. In this study, we found an important and significant spatial contribution to zooplankton community composition in the same pond system, independent of the environmental variables. In that study, we presented a model for the structuring of zooplankton communities in shallow, interconnected ponds, with a primacy of local environmental factors structuring the zooplankton community structure, and dispersal acting as a secondary force to make interconnected ponds more similar. Apparently, the impact on species composition does not translate to a similar pattern for species richness. However, we found for 1998 a significant relation between the spatial configuration

of the ponds and species richness, and a much lower impact of turbidity. This corresponds to the results found by Cottenie et al. (in press) for community composition. The results in this paper suggest a similar pattern: local environmental factors influencing species richness (1996 and 1997), and dispersal only apparent in the absence of important local factors associated with alternative stable states (1998).

We found for two of the three study years a relation between cladoceran richness and turbidity, measured as Secchi disc depth. In shallow lakes, turbidity is a very important structuring variable (Scheffer, 1998). Scheffer et al. (1993) developed a model of two alternative stable states in shallow lakes. One equilibrium state, which predominates at low nutrient concentrations, is characterised by abundant macrophytes and clear water (i.e. large Secchi disc depths). This state is stabilised by high zooplankton grazing rates, low planktivorous and benthivorous fish abundances and high piscivorous fish abundances. The other state is characterised by abundant phytoplankton and turbid water at relatively high nutrient concentrations. The turbid state is stabilised by light limitation because of algal blooms. At intermediate nutrient levels, both alternative stable states can occur. Cottenie et al. (2001) illustrated these two states to be present in 1996 in the studied pond system by relating differences in zooplankton community structure to local environmental variables. Jeppesen et al. (2000) also found a relation between species richness and turbidity, together with patterns in fish phytoplankton, and macrophyte richness. The relation between clear water and high cladoceran richness is probably indirectly caused by the presence of a more diverse and abundant submerged macrophyte vegetation associated with clear water (Van den Brink et al., 1994; Jeppesen et al., 2000). Submerged macrophytes offer refugia against fish predation, increase the habitat diversity and alter the phytoplankton composition. However, we observed no relation with macrophyte cover. This may be the result of the very coarse classes of macrophyte cover used by us, or to confounding the macrophyte effect in Secchi disc depth or phytoplankton densities. With respect to the latter possibility, however, we found no linear relation between macrophyte cover and either Secchi disc depth or phytoplankton abundances. In 1996, a relation with depth was found. This is surprising, considering the narrow depth range (0.3–1.50 m) of the ponds. Keller & Conlon (1994) also found a relation between zooplankton richness and depth, but their depth values ranged between 0.8 and 20 m.

The absence of a clear relation between differences in local species richness of Cladocera and connectivity in our pond system should not be interpreted to imply that connectivity does not have any influence on species richness. Connectivity may have an important effect on overall species richness of the system. Our data suggest that species richness is indeed relatively high. For instance, the values of species richness observed in the ponds of ‘De Maten’ are high when compared with the regression between cladoceran species richness and lake size compiled by Dodson (1991) and Dodson et al. (2000) (Fig. 2). Although our observational data do not allow us to exclude other causes of this effect, we here put forward the working hypothesis that this is the result of high connectivity providing local dispersal of species in our model system.

Acknowledgments

We thank Natuurpunt V.Z.W and Willy Peumans for giving us access to ‘De Maten’. We thank Nele Nuytten for help with species determinations, and M. Leibold and three anonymous reviewers for their helpful comments and valuable suggestions. K. Cottenie is supported by the Fund for Scientific Research – Flanders (Belgium). We acknowledge financial support by the National Fund of Scientific Research – Flanders (grant G.0358.01) and from EU project BIOMAN (EVK2-CT-1999–00046).

References


(Manuscript accepted 21 January 2003)