Local and regional processes in macroinvertebrate communities in shallow lakes

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Lokale en regionale processen in macroinvertebratengemeenschappen in ondiepe vijvers

Frank Van de Meutter

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General introduction

Shallow lakes – alternative stable states

Shallow lakes are usually defined as lakes that can be largely colonized by macrophytes (average lake depth < 3 m), and that do not stratify for long periods during summer (polymictic lakes, Scheffer 1998). Especially the intense sediment-water interaction and the potentially large impact of aquatic vegetation make the functioning of shallow lakes different from that of their deep counterparts. Because these lakes are shallow and polymictic, they are particularly prone to anthropogenic disturbance such as eutrophication, which is why they are considered to be under global threat (Lodge et al. 1998).

Not all shallow lakes are characterized by an abundant vegetation of macrophytes. On the contrary, in many regions the majority of the lakes now typically are devoid of any vegetation, with the water being murky brown or green. The observation that shallow lakes can occur in two distinct states, which may suddenly shift from one to another, has given rise to the theory of alternative stable states in shallow lakes (Scheffer et al. 1993). In this theory, two distinct lake types are distinguished, depending on the nutrient load of the lakes: a clear-water state with abundant submersed vegetation at low nutrient loads, and a turbid water state with high algal biomass at high nutrient loads (Scheffer et al. 1993, Scheffer 1998). At intermediate nutrient loads, both alternative lake states are possible, their stability and resilience depending on their nutrient load. This theory has stimulated much research, which has shown that these alternative states are distinct attractors, each stabilized by multiple feedback mechanisms (summarized in Scheffer 1998, see Fig. 1). The clear-water, highly vegetated lake state is stabilized by high zooplankton grazing rates, high piscivorous fish abundances, and low planktivorous and benthivorous fish abundances. The turbid, vegetationless lake state tends to be stabilized by light limitation due to algal blooms, low zooplankton grazing rates, and high planktivorous and benthivorous fish densities. The conservative strength of these feedback loops is illustrated by the study of Cottenie et al. (2001), which showed that, in a network of 34 shallow lakes in De Maten, lakes apparently in a random sequence belonged to different lake states, reflecting strong differences in macrophyte cover, water transparency and zooplankton community structure, despite the fact that they are strongly sequentially interconnected.

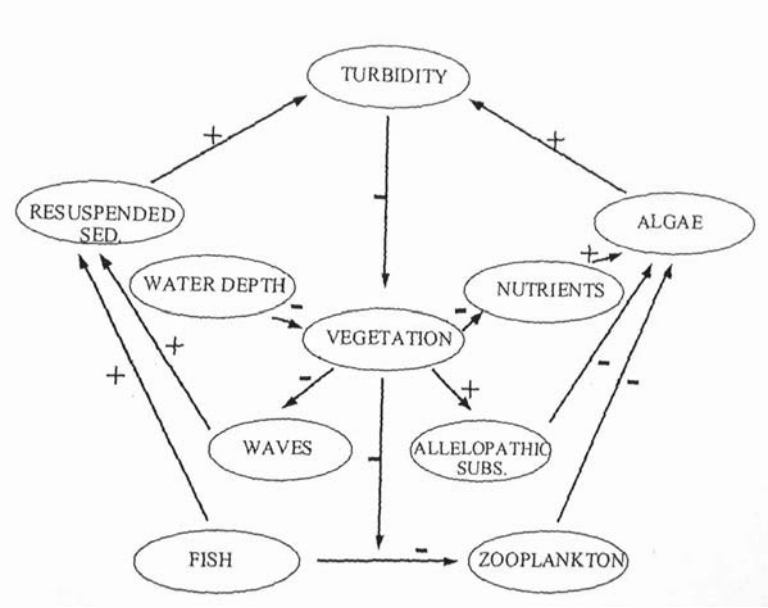

Fig. 1. Positive and negative feedback mechanisms in a shallow lake (from Scheffer et al. 1993).

Macroinvertebrates and turbidity

As appears from the schematic representation of all actors involved (Fig. 1), macroinvertebrates do not actively participate in the basic model of alternative stable states in shallow lakes. Still, macroinvertebrate community composition and structure may be strongly affected by lake state. It is widely recognized that macrophytes increase the diversity of habitats and resources and reduce the susceptibility of macroinvertebrates to fish predators (Crowder and Cooper 1982, Diehl and Kornijów 1998). Hence, a strong positive relationship between taxonomic diversity and density of macroinvertebrates with abundance of macrophytes and thus lake state is commonly observed (Cyr and Downing 1988, Blindow et al. 1993, Cheruvelil et al. 2002).

As the effect of macrophytes on macroinvertebrate community structure is so obvious, ecologists have only rarely made any further efforts investigating what other factors may contribute to the observed differences in macroinvertebrate community structure among clear-water and turbid lakes. However, given that many fish species rely on vision to detect their prey (Guthrie and Muntz 1993), it is plausible that water turbidity itself may drastically affect predator-prey interactions, and hence also the community structure of macroinvertebrates. The majority of studies that have investigated predator-prey interactions in relation to water turbidity in lakes and ponds have focused on the fish's perspective. For example, Reid et al. (1999) showed that turbidity may reduce prey detection distance and hence predation rates by fish, although

compensatory mechanisms such as altered foraging behaviour may exist that partially counter these effects (Vøllestad et al. 1986). This impaired vision of predatory fish by water turbidity may cascade changes into lower trophic invertebrate communities, as shown by Wissel et al. (2003), who found elevated numbers of *Chaoborus* and strongly different zooplankton community compositions among lakes with a different water colour. Water turbidity is, however, likely to not only influence the predator but also the prey. Prey that detects its food and predators visually may not only suffer from a lower foraging efficiency in turbid water, but in addition may also encounter difficulties in judging the risk of predation. The net result of impaired vision in both predator and prey is therefore not so easy to predict. Abrahams and Kattenfeld (1997) hypothesized that in turbid systems, antipredator behaviour of the prey will become ineffective and will occur less frequently. Several studies found support for this hypothesis in fish-fish predator-prey systems (Abrahams and Kattenfeld 1997, Jacobsen and Perrow 1998, Snickars et al. 2004), but whether these results can be generalized to other combinations of predators and prey is yet unknown. From this first hypothesis, Abrahams and Kattenfeld (1997) derived that predator-prey interactions in turbid water should be primarily characterized by the direct effects of predator consumption of prey, while in clear-water systems, direct as well as indirect effects of the predator on the prey's growth rate through behavioural modification may be important. Therefore, the turbidity state of a lake might not only influence the prey's survival, but also its key life history characteristics and behaviour. This idea, however, has not yet been tested.

The key players in creating and maintaining alternative lake states are macrophytes, phytoplankton, fish and zooplankton. There is, however, some evidence that macroinvertbrates may also play a (minor) role. Jones and Sayer (2003), for example, showed that macroinvertebrate periphyton grazers contribute in maintaining the high macrophyte biomass of clear-water lakes. Alternatively, macroinvertebrates might also indirectly affect lake state by interfering with the local fish or zooplankton community. Benthivorous fish, for instance, can strongly increase a lake's turbidity by their specific foraging habits for benthic macroinvertebrates (mainly Chironomidae). As macroinvertebrates only rarely occur in the lake pelagic (with the exception of *Chaoborus* midge larvae), interactions of macroinvertebrates with the pelagic zooplankton (*Daphnia*,…) may seem less likely at first sight. However, even in clear-water lakes with relatively low densities of zooplanktivorous fish, *Daphnia* is probably only able to maintain its high population densities because they evolved a wide variety of behavioural anti-predator mechanisms against fish such as horizontal migration from

open water at night to macrophyte beds in the day, and vertical migration from near-surface water layers at night to lower water layers during the day (De Meester et al. 1999, Burks et al. 2002). Especially in shallow lakes with abundant macrophytes (clear-water lakes), horizontal migration may be the prevalent strategy (Burks et al. 2002). For the *Daphnia*, however, macrophyte beds are not the preferred habitat, as suggested by the strong avoidance of macrophytes by *Daphnia* in the absence of fish, and the observation that *Daphnia* often congregate at the edges of macrophyte beds, rather than actually entering them. Besides detrimental effects of chemical substances released by plants (Sütfeld et al. 1996, Burks et al. 2000) or the low availability of food (algae) in macrophyte beds (Burks et al. 2002), it has been suggested that predation by littoral macroinvertebrate predators may account for this avoidance behaviour (Burks et al. 2002). In a field experiment using large enclosures, Burks et al. (2001b) were able to show that the presence of predatory dragonfly larvae in vegetation can reduce overall *Daphnia* abundance. These findings strongly suggest a role for littoral macroinvertebrates in regulating local *Daphnia* population dynamics. As the *Daphnia* are thus under predation risk from both pelagial fish and littoral macroinvertebrates, it is likely that they have developed strategies to cope with both risks at the same time.

Local and regional determinants of community composition

When discussing the possible mechanisms regulating macroinvertebrate community composition and structure, we so far assumed lakes and lake communities to be closed and isolated, and regulated by local lake-specific factors (macrophytes, fish, turbidity, …). It is widely recognized, however, that other ecological processes occurring at a higher scale may be important in structuring communities (Wiens 1989, Levin 1992). Species interactions can occur in a network of local communities, so-called metacommunities, where they affect colonization probabilities and extinction patterns at the local scale (Levins and Culver 1971, Mouquet and Loreau 2002, Leibold et al. 2004). Different metacommunity models exist, which differ in their assumptions concerning the importance of local biotic and abiotic interactions, environmental heterogeneity, and regional dispersal constraints in regulating local species persistence (see Fig. 2). For example, neutral models of metacommunity assembly assume that extirpation arises from stochastic processes that operate on functionally equivalent species (Hubbell 2001). Patch dynamic models often ignore environmental heterogeneity and instead emphasize regional colonization-extinction dynamics among homogeneous patches (Wilson 1992, Shurin and Allen 2001). Species-sorting models (Leibold 1998, Leibold et

al. 2004) assume that the partitioning of species among variable environments operates according to individual adaptations and that interspecific fitness varies among patches. Finally, the mass-effect framework (Mouqet and Loreau 2002) differs from species sorting models by assuming that dispersal rates exceed the rate at which environmental conditions exclude taxa such that migrants influence assemblage composition in recipient habitats.

Modeling metacommunity dynamics of whole macroinvertebrate communities is unlikely to be very successful, as different groups of macroinvertebrates have very different means and intensities of dispersal. The adults of many aquatic insects may fly from one to another habitat or perform multiple flights, whereas other macroinvertebrate taxa rely on external means of transport such as wind (aquatic spiders), or animal vectors (Acari, Gastropoda, Hirudinea, Mollusca, …). The general view is that metacommunity dynamics will be more intense and far-reaching in active than in passive dispersers. Therefore, this subdivision according to dispersal mode seems important in studying macroinvertebarte metacommunity structure. The idea that metacommunity dynamics are more intense in active than in passive dispersers has been tested in a comparison of distribution patterns of passively dispersing Microcrustacea and actively dispersing aquatic beetles in small ponds (Rundle et al. 2002). No difference in distribution patterns could be shown, possibly because all ponds were relatively old, implying a high accumulative chance for colonization, and because dispersal rates in Microcrustacea may be higher than previously thought (Louette and De Meester 2005). More pronounced differences in distribution and community structure between active and passive dispersers may be expected in young habitats or when distance-similarity relationships are studied instead of simple species occurrence and distribution data. The latter type of studies is scarce, but seems more promising, as shown by the nice distance-similarity relationship observed in passively dispersing aquatic snail communities in Swedish lakes (Bronmark 1985).

The type of metacommunity model or the spatial pattern of community structure that can be expected in a metacommunity of ponds or lakes may change dramatically when ponds or lakes are connected by water flow. Cottenie et al. (2003) showed that community composition of passively dispersing Microcrustacea in a large set of interconnected ponds depended significantly upon the network of lake connections and the dispersal rates observed in these connections. This was not totally unexpected, since passive transport of individuals through the lake connections probably amply outnumbered passive dispersal of resting stages over land (Michels et al. 2001).

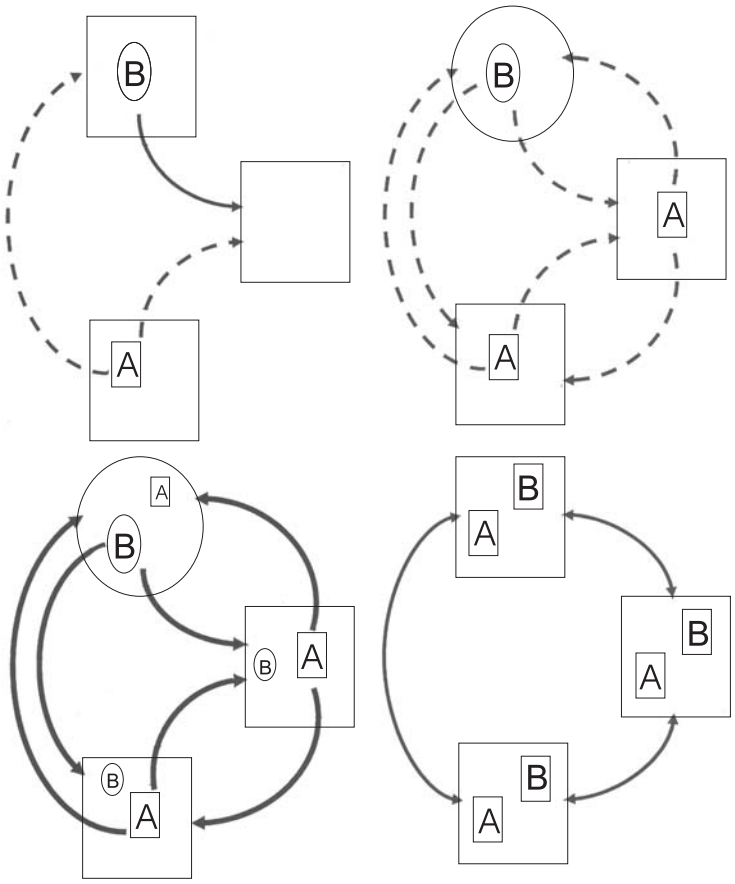

Fig. 2. Schematic representation of the four paradigms for metacommunity theory for two competing species with populations A and B. Arrows connect donor populations with potential colonization sites, shown as large boxes or ovals. Solid arrows indicate higher dispersal than dashed arrows and either unidirectional movement (single-headed arrow) or bi-directional movement (double-headed arrows). The degree to which a species is the competitive dominant in a site is shown by the matching of the smaller box or oval (denoting its habitat type niche) with the site symbol. The four paradigms illustrated are (a) patch-dynamics, (b) species-sorting, (c) mass-effects and (d) neutral. In (a) the patch-dynamics paradigm is shown with conditions that permit coexistence: a competition-colonization trade-off is illustrated with species A being a superior competitor but species B being a superior colonist. In (b) species are separated into spatial niches and dispersal is not sufficient to alter their distribution. In (c) mass effects cause species to be present in both source and sink habitats; the smaller letters and symbols indicate smaller sized populations. In (d) all species are currently present in all patches; species would gradually be lost from the region and would be replaced by speciation (from Leibold et al. 2004).

Whether and to what extent macroinvertebrates are transported among different lakes through lakes connections is not known. However, dispersal through lake connections probably also outnumbers dispersal over land in many passively dispersing macroinvertebrates, and metacommunity dynamics and spatial community similarity patterns may therefore also reflect the lake connections network for this category of

macroinvertebrates. In actively dispersing macroinvertebrates, however, dispersal over land and through lake connections may be more balanced or in favour of the former and, therefore, the outcome for metacommunity dynamics might be totally different from that observed in zooplankton (see Fig. 3 for a schematic representation).

Finally, while the relative importance of local and regional factors is still a hot topic in community ecology, there is growing evidence that yet another alternative mechanism may be important: habitat selection. Habitat selection is the ability of an individual to judge the quality of a particular habitat, and to adapt its colonization behaviour accordingly. Habitat selection in ponds and lakes has been demonstrated in an increasing number of actively dispersing taxa, including frogs, mosquitoes and water beetles (o.a. Resetarits and Wilbur 1989, Blaustein 1999, Rieger et al. 2004). Habitat selection behaviour in these groups reflects a high aversion of habitats with fish, which is in line with the observation that all species considered suffer highly from fish predation. So far, distribution patterns in the field have been explained by the latter process of species sorting by the predators, but if habitat selection is truly widespread, the same patterns may be generated by habitat selection itself.

Lentic macroinvertebrates

Lentic macroinvertebrates, i.e. macroinvertebrates inhabiting standing freshwater habitats, are a notoriously diverse group of organisms, both functionally and taxonomically. A multitude of classes of several different phyla are commonly presented at one site: Arachnida, Bivalvia, Gastropoda, Hirudinea, Insecta, Oligochaeta, Turbellaria,… making that macroinvertebrates often constitute a substantial part of the local biodiversity in ponds and lakes. Within these taxonomic units, species may be classified into very different functional groups according to their food, feeding habits, locomotion or which life cycle stages occur in the water (Usseglio-Polatera et al. 2000). This high overall diversity may make it difficult to distinguish general patterns from noise, and probably is one of the reasons why lentic macroinvertebrate communities have received comparatively little interest from researchers. Other difficulties associated with research on macroinvertebrates are their strongly patchy local distributions and the high seasonal species turnover, which makes accurate sampling difficult and time-consuming. Finally, sorting and identifying macroinvertebrates is tedious and time-consuming and requires quite some expertise. Altogether this probably explains why still little is known on the functioning and the driving mechanisms behind community structure and composition in lentic macroinvertebrates (Diehl and Kornijów 1998).

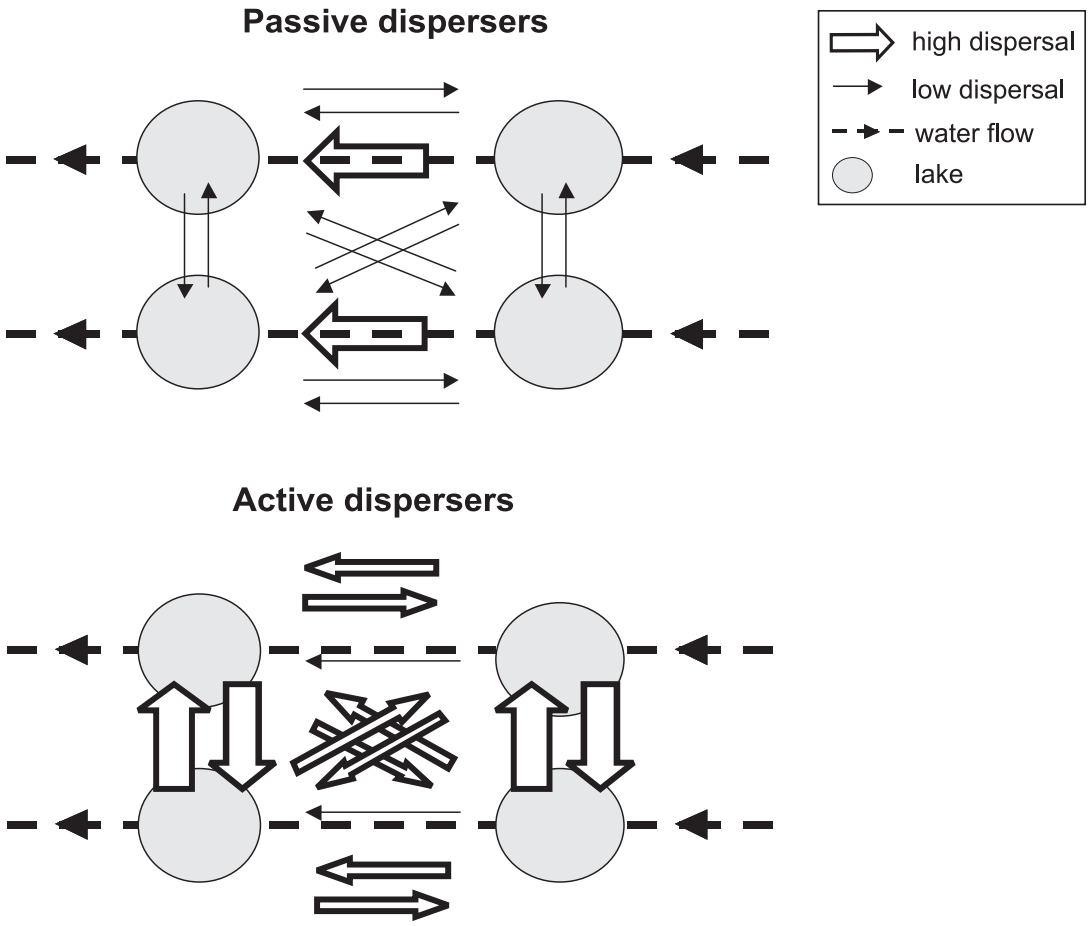

Fig. 3. A priori expected importance of over land dispersal and dispersal by passive transport through lake connections in passive and active dispersers.

Sampling lentic macroinvertebrates

As appears from above, adequate sampling of lentic macroinvertebrates can be problematic. A multitude of samplers has been developed, especially for sampling sediment-inhabiting macroinvertebrates (see Sutherland 1996, Schloesser and Nalepa 2002 for overviews), but general standardized methods for sampling macroinvertebrates that live in the water column are lacking. So far, many different methods are applied, which may differ depending on the focal macroinvertebrate group. As for sediment-inhabiting macroinvertebrates, specific samplers (grabbers) have been proposed (Turner and Trexler 1997, Marklund 2000) but their use is limited. Most studies applied sampling methods using a dip net. Dip nets can be used to sample the contents of a sampling tube along a transect (Urban 2004), or the sampling effort can be standardized in both space (number of net sweeps, length of the net sweeps, length of a transect; e.g. Johnson and Goedkoop 2002, Rundle et al. 2002) and time (e.g. Reid et al. 1995). In a

paper where they compared most of the above-mentioned methods, Reid et al. 1995 concluded that, in terms of repeatability and representability of the fauna, dip net sampling standardized in space or time performed among the best.

The focus of this thesis is on littoral non-benthic macroinvertebrates. This includes all epibenthic, epidetritic, epiphytic and free-swimming macroinvertebrates. Following the suggestion of Reid et al. 1995, we chose for a sampling procedure using a D-shaped net (23 cm x 23 cm, 500 µm mesh) that was standardized in both space and time. To obtain representative samples of the lakes, we first monitored microhabitats in all lakes in 2001 to characterize the composition of their littoral zone. For all major microhabitats represented (riparian vegetation, emergent vegetation, submerged vegetation, open water), a score on 10 reflecting its presence in the littoral zone was given. Next, we selected in each lake a 75 meter transect parallel to the shoreline that proportionally included all major microhabitats present in the lake. In each lake, the same transects have been sampled on all occasions. Sampling occurred in a zigzag line along this transect and was limited in time to 10 minutes. The time (minutes) spend sampling in one microhabitat type accords to its lake-specific proportion in the littoral zone and in the transect (double-check). To satisfy to the proportional sampling of microhabitats as set out at the start of the sampling, transects often were discontinuous and interrupted by walks between patches of microhabitats. Both the duration and the length of the sampling trajectory in our studies are (much) longer than in most other studies on pond and lake macroinvertebrates. This was done to increase repeatability and robustness of the samples, to overcome patchiness of macroinvertebrates both within and among microhabitats and to obtain more reliable estimates of taxon richness and diversity. On the other hand, this extensive sampling also comes at a cost: much time was spend in sorting an identifying the large samples. All samples were fully sorted (without taking subsamples). On average 2,243 ± 348 macroinvertebrates were present in one sample, resulting in a total of 410,000 macroinvertebrates that were sorted and identified during this PhD.

Study system

The pond complex studied in this thesis is part of the nature reserve De Maten, situated in the Northeastern part of Belgium near Genk. The reserve nowadays covers an elongated area of approximately 300 ha which stretches for some 3.5 km along a Northeast-Southwest axis. De Maten is an open, diverse landscape consisting of sand dunes, dry *Calluna* heath land and small patches of woodland intermixed with meadows,

marshes and ponds. As this reserve is situated on the southern edge of the Campine tableland, many small streams that fountain on top of the tableland cross the reserve. The pond complex is a typical example of an ancient chain of fishing ponds, which were created by the construction of dykes on the streams and peat-digging. The upstream pond is situated at 55 m above sea level, whereas the downstream ponds in the Southwestern end of the system are situated at approximately 40 m above sea level. This altitudinal difference of 15 m creates a unidirectional flux of water through the pond complex. The main sources of water are two rivulets, which provide almost half of the water to the system, the rest being derived from ground water. Historically, all lakes in the reserve were used for extensive fish farming. Consequently, every 4-5 years, the lakes were alternatingly drained to harvest the fish. Fish farming in De Maten was gradually pared down as the protected area of the reserve increased, and finally stopped in 1990 (Daniëls 1998). In order to preserve the diverse landscape of De Maten, the area is now continuously managed (grazing by Galloway cattle, cutting of sods, cutting of woodlots). To counter the excessive fish densities and the continuous enrichment of the lakes causing eutrophication, and to create suitable foraging habitat for marsh birds and waders, an alternating cyclic drainage regime in all lakes with a frequency of 3-4 years was installed in 1993.

Lake drainages of shallow lakes are widely applied, both in fish farming, lake management and lake restoration projects. The prevailing idea is that lake drainages of deteriorated lakes (turbid lakes, excessive fish densities, …) will revalue local biodiversity and nature values. Lake drainages seem particularly successful in this respect for aquatic macrophytes and zooplankton, both of which may readily reestablish from dormant resting stages in the lake sediment (Woltemade 1997, Hosper 1998). How lake drainages affect local macroinvertebrate communities is, however, not known.

Outline of the thesis

This thesis consists of three major parts. A first part deals with the interaction of the local factors turbidity and predation with lake macroinvertebrates and macroinvertebrate community structure in lakes. In the second part we investigate to what extent the presence of littoral invertebrate predators may interfere with horizontal migration behaviour of *Daphnia*. Finally, in the third part we focus on the effects of the regional process dispersal and the interaction between dispersal and the drainages of the lakes, on structuring lake macroinvertebrate (meta)communities.

Macroinvertebrates and turbidity

Shallow lakes can occur in two alternative stable states, a clear-water state and a turbid state. This is associated with separate assemblages of fish, zooplankton and plants. Little is known about whether macroinvertebrate assemblages differ across both stable states and, if so, what mechanisms may take part in this. We performed a descriptive study to evaluate within and among lake variation in macroinvertebrate community structure in a subset of 3 clear-water and 3 turbid lakes in De Maten (**chapter one**). The specific aim of this study is to look for effects of turbidity on macroinvertebrate community structure independent of the apparent differences in macrophyte cover, which is known to greatly affect the presence of lentic macroinvertebrates, by comparing different microhabitats (open water, pondweed and reed) that occur in both the clear-water and turbid lakes.

In **chapter two** we use a combination of a behavioural laboratory experiment and a large-scale field enclosure experiment to evaluate how water turbidity affects behaviour, growth and survival of damselfly larvae (*Ischnura elegans*) in the presence of fish in the predictive framework of two hypotheses postulated by Abrahams and Kattenfeld (1997). According to the first hypothesis, prey organisms should relax antipredation behaviour towards visual predators, as visual prey detection is constrained by turbidity. The second hypothesis is directly derived from the first hypothesis, and states that prey organisms will be decreasingly indirectly affected by the presence of a visual predator (e.g. growth), but increasingly by direct effects of the predator (survival) at increasing levels of turbidity.

Macroinvertebrates and horizontal migration behaviour of Daphnia

To seek refuge against predation by fish, *Daphnia* can undertake diel horizontal migration from open water to macrophytes. Despite the fact that macrophytes provide relatively good protection against fish predation (Burks et al. 2001a), often *Daphnia* are observed to not enter the macrophytes, but instead to stay at the edges of the macrophyte stands. Littoral predatory dragonfly larvae were shown to potentially incur a high predation cost on *Daphnia*, and possibly *Daphnia* learned to recognize and avoid these predators.

In **chapter three** we first establish the spatial pattern of predation risk of *Daphnia magna* in open water versus vegetated environments to predation by the opportunistic littoral macroinvertebrate predator *I. elegans*. Next, we test whether *Daphnia* shows a spatial response in its horizontal distribution towards the presence of *I. elegans* larvae in

the macrophytes, and evaluate whether this response is adaptive with respect to the spatial pattern of predation risk by *I. elegans*. In **chapter four** we extend the study of the previous chapter to the situation where also pelagic *Chaoborus* predators are present. More detailed, we first study spatial patterns of predation risk of *Daphia* to both *Chaoborus and I. elegans* predators. After that, we investigate the spatial distribution (open water/ macrophytes) of *Daphnia* in response to the presence of *Chaoborus, I. elegans* or both, and evaluate whether the observed responses are adaptive with respect to the respective spatial patterns of predation risk in each situation.

Macroinvertebrate dispersal: combined effects of dispersal mode and lake drainage on (re)colonization and community structure in shallow lakes

In the third part of the thesis we used a combination of direct (colonization experiment, direct assessment of dispersal rates) and indirect (community similarity patterns) methods to study the intensity and importance of dispersal of lentic macroinvertebrates in De Maten. Special attention is devoted to the different potential pathways that are available in De Maten (dispersal over land by flight and dispersal through lake connections) and the way actively and passively dispersing macroinvertebrates, respectively, make use of this. Additionally, we took opportunity of the fact that the lakes in De Maten were drained in the framework of the management of the reserve, to evaluate how this affected macroinvertebrate community structure in these lakes, and to what extent these drainages affected the spatial metacommunity pattern of macroinvertebrates.

In **chapter five** we report on an intensive day and night sampling of the connecting overflows and rivulets among the lakes in De Maten. We further try to relate the rate of dispersal and the composition of the assemblage of dispersing macroinvertebartes to the physical properties of the connecting elements, the time the organisms were caught (day/ night), and to the composition of the macroinvertebrate assemblage in the upstream source lakes. In **chapter six** we evaluate what the consequences are of the lake drainages for the local macroinvertebrate communities in a subset of six lakes in De Maten. Lake drainages are common use in fish farming practices, and also are increasingly used as a way to restore eutrophicated lakes. The results of this study are discussed against the background of the high among lake connectivity in De Maten and a general appreciation of this restoration practice with respect to conservation of macroinvertebrate diversity is given. In **chapter seven** we investigate the relative contribution of environmental factors and connectivity among the

lakes on macroinvertebrate community structure in the lakes of De Maten. Hence, this chapter integrates all earlier discussed local and regional determinants of macroinvertebrate community structure. We specifically test for differences in the spatial pattern of community similarity, and in the contribution of local versus regional factors in community structure between actively and passively dispersing macroinveretbrates.

In **chapter eight** we report on a field experiment using fishless containers that were placed throughout the reserve of De Maten to investigate dispersal and colonization properties of lentic macroinvertebrates. The composition of the colonizing assemblage of macroinvertebrates in the containers is compared to that of the lakes in De Maten, and tested for distance-similarity relationships among containers and between containers and lakes. The results are discussed in the framework of the growing idea that macroinvertebrates do not randomly colonize, but actively select habitats from the landscape.

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Chapter I

The effect of turbidity state and microhabitat on macroinvertebrate assemblages: a pilot study of six shallow lakes

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[adjusted version of Hydriobiologia, in press]

Abstract

*Shallow lakes can occur in two alternative stable states, a clear-water state and a turbid state. This is associated with separate assemblages of fish, zooplankton and plants. Little is known about whether macroinvertebrate assemblages differ across both stable states. This study investigated this in a connected set of three turbid and three clear-water shallow lakes. To overcome confounding effects of differences in spatial structure of macrophytes in turbid and clear-water lakes, we sampled three microhabitats that occurred in both alternative stable states: open water, sago pondweed (*Potamogeton pectinatus*) and reed (*Phragmites australis*). Univariate analyses indicated no differences in the number of organisms, taxon richness or diversity between turbid and clear-water lakes. Multivariate analysis, however, indicated a trend towards differences in the macroinvertebrate community structure of both stable states. Two taxa explained a significant amount of the variation between both lake types, both of which preferred the clear-water lakes. The number of organisms and the taxon richness were higher in reed than in the other microhabitats, but diversity and evenness did not differ among the microhabitats. Multivariate analyses separated all three microhabitats. Four taxa, mainly detritus feeders and collector-gatherers, explained most of the variation in the data and preferred the reed microhabitat. The effects of turbidity state (11.8 % explained variance) and microhabitat (24 % explained variance) on the macroinvertebrate assemblages were largely independent from each other (2 % shared variance). Although macroinvertebrates are not implemented in the initial theory of stable states, our results indicate different assemblages across both stable states.*

Introduction

The structure and functioning of ecosystems can vary considerably across environmental gradients (e.g. hydroperiod: Wellborn et al. 1996, Stoks and McPeek 2003; oxygen: Diaz and Rosenberg 1995; nutrients: Jeppesen et al. 2003, Smith 2003; ammonia: Krupa 2003). The change in the structure and functioning of ecosystems, however, often does not parallel the change over the environmental gradient. Nonlinear behaviour of ecosystems to a linear gradient in environmental factors, resulting in multiple ecosystem states, may be common in nature (Scheffer et al. 2001, Dent et al. 2002, McClanahan et al. 2002, Foley et al. 2003) and applies particularly well to shallow freshwater ecosystems (Scheffer et al. 1993, Hosper 1998).

Shallow lakes can occur in two alternative stable states, depending on the nutrient load of the lakes: a clear-water state with abundant submersed vegetation at low nutrient loads and a turbid water state with high algal biomass at a high nutrient load (Scheffer et al. 1993, Scheffer 1998). At intermediate nutrient loads, both alternative states are possible. These alternative states have been found to be distinct attractors, each stabilized by multiple feedback mechanisms (Scheffer et al. 1993), and their stability depends on the nutrient load. The plankton and fish community of shallow lakes play a major role in the creation and stability of these alternative equilibria, and both exhibit a typical and well-studied community structure associated with each of the stable ecosystem states (Jeppesen et al. 2000, Cottenie et al. 2001). The epiphytic macroinvertebrate community was largely ignored in the initial model of Scheffer (1993), but recent evidence suggests an important role for epiphytic macroinvertebrate periphyton grazers in maintaining the high macrophyte biomass of clear-water lakes (Jones and Sayer 2003). However, little is known on how the structure and dynamics of the epiphytic macroinvertebrate community varies with the alternative equilibria in shallow lakes.

Differences in food sources, predators and seasonal dynamics support a division of lentic macroinvertebrates in benthic and epiphytic macroinvertebrates (Diehl and Kornijów 1998). The taxonomic diversity and density of epiphytic macroinvertebrates are related to (1) the density and growth form of the macrophytes they live on (Cyr and Downing 1988, Lillie and Budd 1992, Cattaneo et al. 1998, Taniguchi et al. 2003), (2) the abundance and functional diversity of the fish community (Osenberg and Mittelbach 1989, Diehl 1995) and their interaction with macrophytes (Crowder and Cooper 1982, Diehl and Kornijów 1998) and to a lesser extent (3) water turbidity (Timms and Boulton 2001, Chessman 2003). All these factors are directly linked with the theory of alternative

stable states (Scheffer et al. 1993) and suggest that the epiphytic macroinvertebrate community may differ considerably between the alternative equilibria in shallow lakes.

This study investigates the epiphytic macroinvertebrate communities of three shallow lakes in the turbid state and three shallow lakes in the clear state. To minimize confounding variation from spatially related variables such as water quality, chemistry and dispersal, six neighbouring and highly connected lakes were selected (Cottenie et al. 2001). This study focused on differences caused by turbidity state, without taking into account the effects of differences in macrophyte density and growth form between the alternative states that have been studied extensively before. Therefore, three microhabitat types were sampled that occurred in all lakes irrespective of its turbidity state: the open water, sago pondweed (*Potamogeton pectinatus*), and reed (*Phragmites australis*).

Material and methods

Study area

The study area is the nature reserve “De Maten”, a protected heath land of about 300 ha situated in the northeastern part of Belgium. The reserve comprises 34 shallow lakes, connected to each other by a complex network of rivulets and overflows (Michels et al. 2001, Cottenie and De Meester 2001). The shallow lakes were created in the 15th century by peat-digging and the construction of dikes in the valleys of two parallel small streams. For centuries, the lakes were used for extensive fish farming, but this stopped in 1991.

Despite the high degree of connectivity, the shallow lakes display striking differences in turbidity, macrophyte cover and zooplankton community structure (Cottenie et al. 2001, Cottenie and De Meester 2003). Based on a hierarchical clustering analysis of zooplankton data from all lakes in “De Maten”, Cottenie and De Meester (2001) classified the lakes into three different groups. This subdivision in lake groups based on zooplankton data was associated with striking differences in other biotic and abiotic factors such as turbidity, percentage submerged vegetation and fish density. According to the theory of alternative equilibria in shallow lakes (Scheffer et al. 1993), lakes with turbid water, a high fish density and sparse vegetation were classified as turbid water lakes; lakes with clear water, low fish densities and lush vegetation were classified as clear water lakes. A third group of lakes with intermediate characteristics exists in this system, but this will not be further discussed (Cottenie et al. 2001).

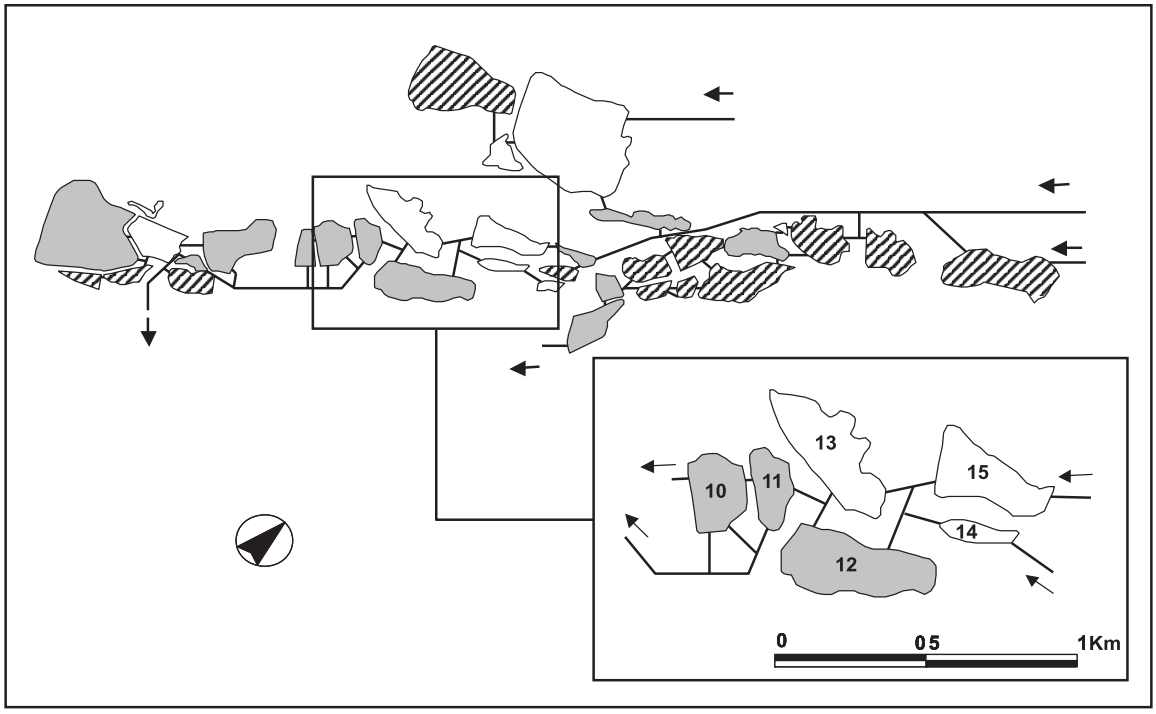

Fig. 1. Map of the lake complex in the study area 'De Maten', with an enlarged detail of the six selected shallow lakes. Lakes are classified based on zooplankton community structure (Cottenie et al. 2001) as turbid water lakes (gray), intermediate lakes (shaded) or clear-water lakes (white). Solid lines connecting lakes represent rivulets and overflows. Small arrows indicate the directions of water flow. The large arrow indicates to the North.

For this study, a cluster of six neighbouring lakes was selected, three lakes (lakes 10, 11 and 12) of the turbid water group, and three lakes (lakes 13, 14 and 15) of the clear-water group (Fig. 1). New environmental data collected during the sampling year 2001 confirmed the turbidity state of each lake (see Table 1).

Sampling

Samples were taken from 23 to 25 July 2001 in three different microhabitat types that occurred in both lake types: the open water zone, sago pondweed and reed. Each habitat type was sampled at three different localities within each lake. Reed was absent from lake 10 and sago pondweed could not be sampled in lakes 11 and 13. To take quantitative samples of macroinvertebrates, we used a large plastic sampling pipe (length 92 cm, diameter bottom 58 cm, diameter top 69.5 cm) that was open at both ends and that was placed vertically in the water column. To standardize the volume of water sampled, each sample was taken at a water depth of 80 cm. We selected sample

Table 1. Turbidity state-related environmental variables for the six lakes measured on 23 July 2001. Snell depth is the deepest point under water (in cm) at which a mini Secchi disk, lowered in a gray PVC tube (8 cm diameter) filled with lake water, can be seen. 'Submersed vegetation' is the percentage cover of submersed macrophytes along a transect of 75 m parallel to the lake shore. Chlorophyll a was measured spectrophotometrically using methanol extraction (Talling and Driver 1963).

Lake	Turbidity state	Snell depth (cm)	Chlorphyll a ($\mu g\ l^{-1}$)	Submersed vegetation
10	Turbid	11	339.16	10
11	Turbid	11	172.36	0
12	Turbid	13	230.74	5
13	Clear	47	18.07	20
14	Clear	38	12.51	30
15	Clear	56	15.29	20

localities with a standard vegetation density per pipe volume of approximately 7-10 grams per pipe volume dry weight of sago pondweed, and a density of 15-20 stems of reed. The content of the sampling pipe was sampled with a net (mesh size 500 µm) and fixed on 4 % formaldehyde.

Sample processing

In the laboratory, all invertebrates were sorted in white trays, identified and counted. Invertebrates were identified to the following taxonomic levels: Acari, Aranea, Asellidae, Chaoboridae, Chironomidae, Coleoptera, Coleoptera larvae, Culicidae, Diptera (comprising mainly Ceratopogonidae, Dixidae, Limoniidae and Tipulidae), Hirudinea, Lepidoptera, Oligochaeta, *Sialis* and Trichoptera. Ephemeroptera and Hemiptera were identified to species level and Mollusca and Odonata to genus level. Female *Sigara falleni* and *S. striata* water bugs were pooled into one group (*Sigara* females) since they are difficult to distinguish morphologically (Nieser 1982). When needed, a stereomicroscope (Olympus, SZX-ILLK200) at 20x magnification was used for identification. The identification level for the different taxa is essentially a balance between the extra information obtained and the time that is needed for identification. Identification followed the key of De Pauw and Vannevel (1993) for most of the taxa and Gysels (1991) for Ephemeroptera, Nieser (1982) for Hemiptera, and Heidemann and Seidenbush (1993) and Norling and Sahlén (1997) for Odonata. For each sample, the following four characteristics were selected: the number of organisms, the number of

taxa (taxon richness) and the diversity and evenness measures of Shannon-Wiener (Shannon and Weaver 1963).

Statistical analysis

We started with univariate analyses. Separate nested analyses of variance (Nested ANOVA) were used to identify differences between the different turbidity states and microhabitats for the four studied characteristics of macroinvertebrate assemblages. Turbidity state and microhabitat were included as fixed effects and lake nested in turbidity state was set as a random effect. The number of organisms and the diversity and evenness indices of Shannon-Wiener were log-transformed prior to analysis to meet the assumptions of ANOVA. Significant effects of the ANOVA were further explored with post-hoc Tukey HSD tests (Sokal and Rohlf 1995).

For multivariate analysis, the abundances (ind./m^2) of all taxa were square-root transformed to minimize the effect of high densities (ter Braak and Smilauer 2002). Because in this study, lakes were nested in de turbidity factor, we cannot correct properly for the interdependence of the replicate samples within each lake using al observations. Therefore, we averaged replicate samples of every combination of lake and microhabitat in all analyses. Because Detrended correspondence analyses (DCA) indicated relatively short gradients within the data (all gradients < 3 SD), linear ordination methods were applied. To partition the variance in the macroinvertebrate assemblages into a turbidity state component and a microhabitat component, we used the procedure of Borcard et al. (1992) using Redundancy analysis (RDA). First, the RDA was performed with both turbidity state and microhabitat as environmental predictors. This quantifies total variance explained by both variables. Second, separate partial RDA analyses were run with only turbidity state or microhabitat as the key variable, and the other variable as a covariable. This quantifies the conditional effects, i.e. the amount of variance explained by the variable that is not yet explained by the other variable, of both variables. As microhabitats within one lake still may be interdependent, we additionally performed separate RDAs with turbidity as the environmental predictor for each microhabitat.

To explicitly explore the variation in the macroinvertebrate assemblages associated with the a priori defined groups, discriminant analyses (DA) were applied. A stepwise forward selection procedure was then used to identify the taxa that best supported the subdivision by the environmental variables of the ordination model. Two analyses were run, one based on the different microhabitats, and one based on the

turbidity states. Species that explain a significant proportion of the variation in macroinvertebrate communities among the microhabitats and between the turbidity states, respectively, were selected. Because in (R)DA the number of environmental factors (taxa) cannot exceed the number of samples-1, we restricted the list of taxa in both analyses to the 13 most abundant taxa: Acari, *Caenis horaria*, *Caenis robusta*, *Chaoborus*, Chironomidae, *Cloeon dipterum*, *Cymatia coleoptrata*, Diptera, *Ischnura*, Physidae, *Sigara* females, *Sigara* juveniles and *Sigara striata*. We did not perform DA analyses separating the turbidity states per microhabitat since than only 3 taxa could be included in the analyses.

The significance of the explained variance by the environmental predictors in the RDA and of the variance explained by the selected taxa in the forward analysis, were determined by Monte Carlo randomization tests (9,999 permutations). DCA, RDA and DA were performed in CANOCO 4.5 (ter Braak and Smilauer 2002). Analysis of variance was performed using Statistica 6.0 (Statsoft 2001).

Results

Univariate analysis

On average 76 (SE: 11) organisms were collected in a sample. Samples of lakes with a different turbidity state did not support a different number of organisms ($F_{1, 29} = 1.13$, $P = 0.35$) or a different number of taxa ($F_{1, 29} = 0.61$, $P = 0.48$). The mean number of organisms differed among microhabitats ($F_{2, 29} = 13.15$, $P < 0.001$), and was significantly higher in reed than in the open water and sago pondweed (Fig. 2A). Taxon richness followed the same pattern and was also higher for reed samples than for open water and pondweed samples ($F_{2, 29} = 14.37$, $P < 0.001$; Fig. 2B; see also Appendix 1 for an exhaustive list of taxon occurrences in the samples).

Lake turbidity and microhabitat state did not affect Shannon-Wiener diversity or evenness (all $P > 0.18$, Fig. 2C-D). Shannon-Wiener diversity displayed variable patterns over microhabitats depending on lake origin (lake(turbidity state) x microhabitat: $F_{5, 29} = 5.20$ $P = 0.002$; Figs. 2C). Shannon evenness differed among lakes as a result of very low evenness values in lake 13 (lake(turbidity state): $F_{4, 29} = 3.78$, $P = 0.011$; Tukey HSD lake 13 vs. all other lakes all $P < 0.05$, other $P > 0.50$).

Multivariate analysis

RDA showed that turbidity state and microhabitat together explained 37.8 % ($P = 0.012$) of all variance in the data. Variation partitioning estimated the unique variance explained

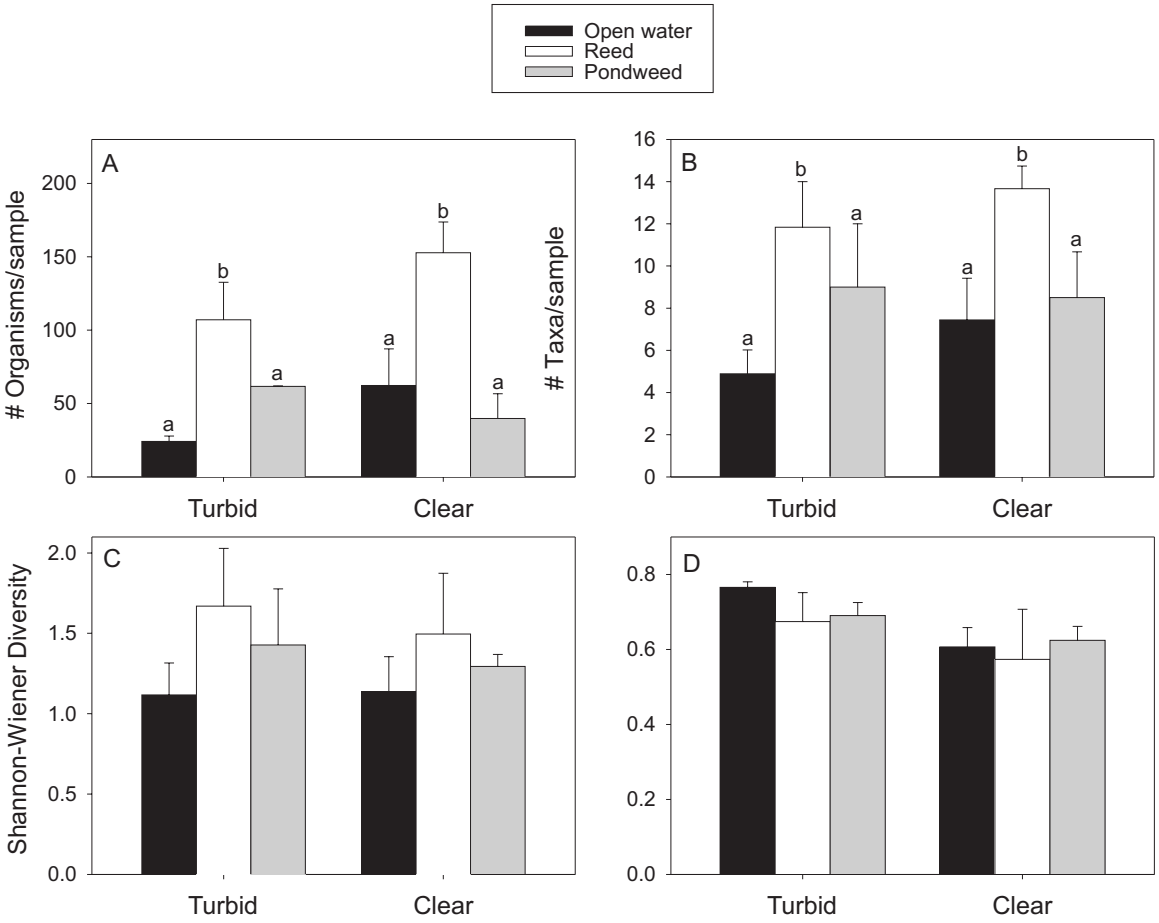

Fig. 2. Characteristics of the macroinvertebrate assemblages in function of turbidity state and microhabitat. The following parameters are shown: (A) the number of organisms per sample, (B) taxon richness, (C) Shannon-Wiener diversity and (D) Shannon-Wiener evenness. When ANOVA indicated significant differences between the groups, each group differing in the post-hoc tests was given a different letter (a or b). Bars represent means (based on lake means) + 1 standard error.

by turbidity state as 11.8 % (P = 0.068) and by microhabitat as 24.0 % of all variance (P = 0.018). Another 2 % of the variance was shared between both variables. Separate RDAs per microhabitat type indicated that turbidity explained 34.4% (P = 0.094), 20 % (P = 0.90) and 17.4 % (P = 0.99) of the variation in open water, reed and pondweed, respectively.

Forward selection in the DA with turbidity state as a priori grouping factor identified two taxa, *Chaoborus* and Diptera, that explained a significant portion of the variance along this axis (Table 2). Both of these taxa were more numerous in lakes of the clear-water state.

The DA-ordination between the different microhabitats resulted in a subdivision of the open water group from the reed and pondweed group along axis 1 and the

distinction of the reed (and open water) group from the pondweed group along axis 2 (Fig. 4). Four taxa explained a significant amount of variation between the three microhabitats (Table 3). *Caenis horaria*, *Caenis robusta* and Diptera all had reed as the preferred microhabitat; *Sigara* females were relatively abundant in both reed and pondweed but not in open water.

Discussion

This study demonstrates that turbidity state and microhabitat generated structure in the macroinvertebrate assemblages of the studied shallow lakes. The effects of turbidity state and microhabitat were largely independent, as shown by their additive effects in the univariate analysis, and their low percentage of shared variation in the RDA. Macroinvertebrate assemblages of clear and turbid lakes were numerically and structurally similar (univariate analyses), but tended to be characterized by other taxa. Macroinvertebrate assemblages of different microhabitats were also structurally similar, but differed in the densities of macroinvertebrates and in the presence of key taxa.

Variation partitioning suggested that microhabitat was a more important structuring force in macroinvertebrate assemblages than turbidity state, while the microhabitat-specific effect of turbidity only tended to be significant in open water and not in reed or pondweed. However, the effect of turbidity state may have been underestimated in these analyses. First, as turbidity state had a lower number of groups (2) than microhabitat (3) in this analysis, the amount of explained variation in macroinvertebrate community structure will more likely be lower for turbidity state than for microhabitat. Second, the vegetation-specific analyses were based on only 2 replicas per turbidity group in pondweed, on 2 and 3 groups in reed and on 3 groups per turbidity state in open water. This implies very low power and thus a low probability of detecting significant differences between turbidity groups. Third, the studied shallow lakes are highly connected by overflows and are situated closely to each other (Figure 1, Michels et al. 2001). Preliminary results indicated relatively frequent dispersal rates of macroinvertebrates between these lakes, both through the connecting overflows and through air (F. Van de Meutter unpublished results, Chapter V). High dispersal rates could lead to source-sink dynamics (MacArthur and Wilson 1967) or a ‘rescue effect’ (Brown and Kodric-Brown 1977) between lakes of a different turbidity state. This may prevent some taxa from going extinct in their suboptimal habitat, making the distinction among lakes of a different turbidity state in the study system less pronounced than in more isolated habitats. Finally, this study selected for similar microhabitats in different

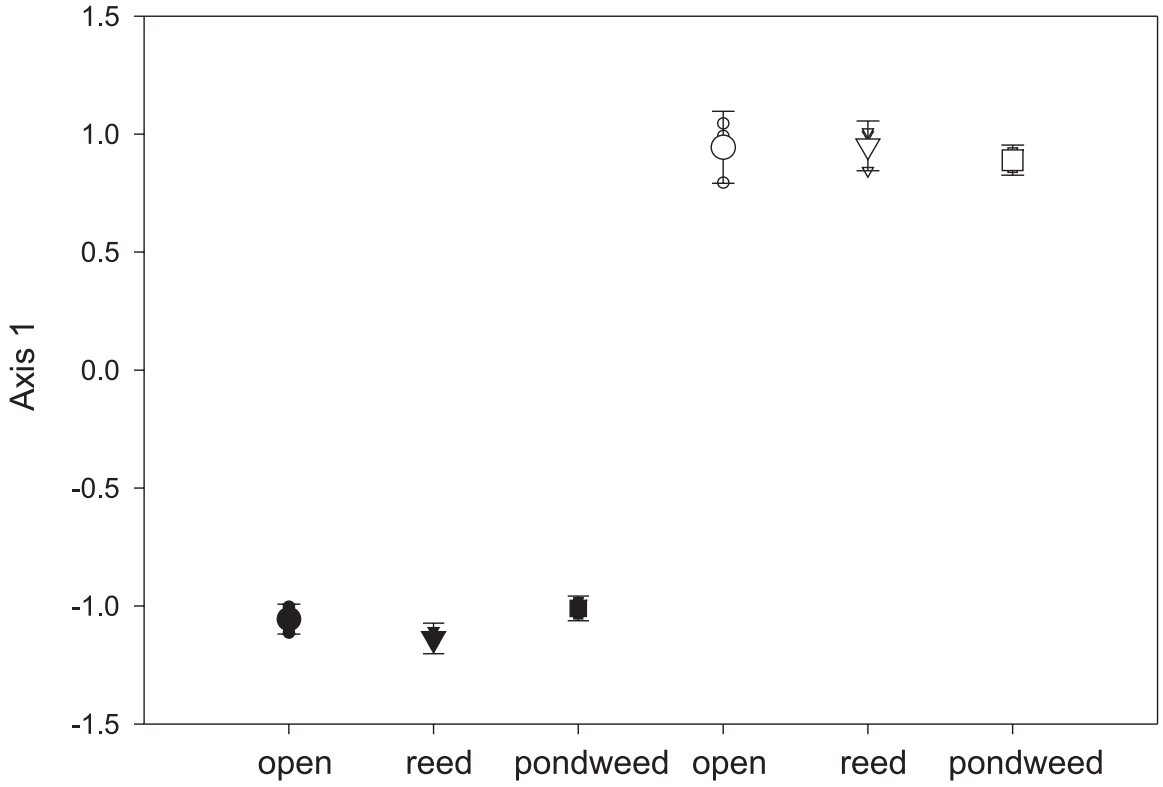

Fig. 3. Scatterplot of the scores on axis 1 of the DA based on turbidity state for all combinations of turbidity state and microhabitat. Small symbols represent lake averages for every combination of microhabitat and turbidity state. Large symbols represent the means per combination of microhabitat and turbidity state and are given ± 2 standard errors.

lake types, not taking into account the fact that they differ in relative abundance according to lake type. Clear-water lakes usually have a considerably higher submerged macrophyte cover than turbid water lakes (Table 1, Scheffer 1993). This should result in larger epiphytic macroinvertebrate communities with a higher number of taxa in clear-water than in turbid lakes (van den Berg et al. 1997, Piezcynska et al. 1998) as a result of species-area relationships (MacArthur and Wilson 1967). Given these strong limitations, we consider the low p-values of the turbidity effect in the general RDA ($P = 0.068$) and in the RDA for open water ($P = 0.094$) as strong indications that – even when correcting for differences in vegetation cover – clear-water and turbid lakes contain different macroinvertebrate communities.

Despite the fact that macroinvertebrates may interact with the establishment and maintenance of alternative equilibria in shallow lakes (Jones and Sayer 2003), studies that focus on macroinvertebrate community structure in lakes with a different turbidity state are still scarce. Several studies have shown an effect of lake trophic status, which

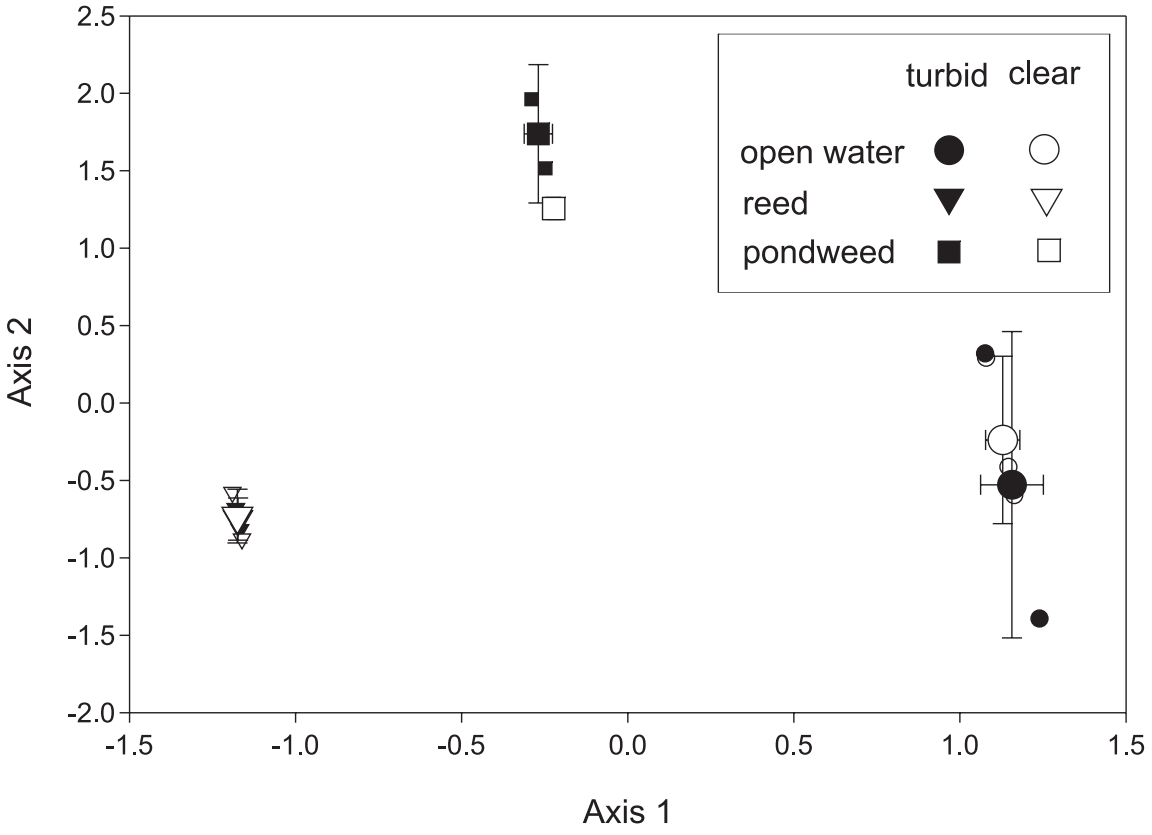

Fig. 4. Scatterplot of the lake means scores on axis 1 and axis 2 of the DA-ordination based on microhabitat for each combination of turbidity state and microhabitat. Small symbols represent lake means for every combination of microhabitat and turbidity state. Large symbols represent the means per combination of microhabitat and turbidity state and are given ± 2 standard errors.

can be considered a rough proxy for turbidity state (Scheffer et al. 1993), on benthic macroinvertebrate communities (Kornijów 1988, Brodersen et al. 1998, Brodersen et al. 2001, Tolonen et al. 2001). Similar studies on epiphytic macroinvertebrates are rare (but see van den Berg et al. 1997, Pieczynska et al. 1998). Van den Berg et al. (1997) reported a change in macroinvertebrate community composition and density along with a turbidity-induced change in the dominating macrophyte species. They, however, did not disentangle the effect of changes in plant density and growth form, from that of turbidity state. The study by Pieczynska et al. (1998) found that macroinvertebrate assemblages and the number of macroinvertebrates retrieved from four species of submerged macrophytes, associated more closely with lake trophic state than with plant species.

Despite the tendency for a subdivision by DA of the macroinvertebrate assemblages between turbid and clear-water lakes, we could not demonstrate differences in the number of organisms, taxon richness or diversity between turbid and

clear-water lakes. This suggests that the shift to the other turbidity state results in the substitution of individual taxa rather than in the net gain or loss of taxa per unit of volume. However, it should be noted that the use of a more detailed level of identification might reveal some community differentiation that remained hidden within the used higher-level taxonomic groups. In this study, *Chaoborus* midge larvae were more abundant in clear-water than in turbid lakes. This contrasts with previous studies that reported higher numbers of *Chaoborus* in turbid-water lakes than in clear-water lakes (Cuker 1993, Jeppesen et al. 2003). The latter observation was explained by the lower predation pressure of visually hunting fish on *Chaoborus* due to reduced visibility with increasing turbidity (Gregory and Levings 1998, Sweka and Hartman 2003). Because *Chaoborus* species differ strikingly in their habitat preference (Berendonk et al. 2003), species differences among studies may explain the different pattern observed.

The structuring role of microhabitats for the macroinvertebrate assemblages was mainly due to numerical and taxon differences between open water and the two other microhabitats. Reed housed the richest macroinvertebrate assemblage. Reed beds accumulate high amounts of detritus and macrophyte litter that may support a high number of detritus feeders and collector-gatherers (Varga 2001, Warren et al. 2001). This was also reflected in the taxa that were found to prefer the reed microhabitat: the ephemeropterans *Caenis horaria* and *C. robusta* apply a collector-gatherer feeding strategy (Elliott et al. 1988), and most of the Diptera families (Ceratopogonidae, Dixidae, Limoniidae, Tipulidae) are detritus feeders (Schmedtje and Colling 1996). Pondweed and open water had a comparable number of organisms, taxon richness and diversity, and differed mainly in the composition of their macroinvertebrate assemblages. This is surprising since the sampling method was biased towards epiphytic macroinvertebrates, which are lacking in the open water. This unexpected similarity between pondweed and open water samples is likely due to small stands of *Chara* sp. (clear-water lakes) and filamentous algae (turbid lakes) that were unintentionally included in the sampling. Another explanation could be that the sampling procedure may have stirred up the upper sediment layer in open water samples, which is unprotected by vegetation or plant roots, to a higher extent than in samples in the vegetated microhabitats. However, the number and abundance of benthic taxa (Chironomidae, Oligochaeta) in open water samples did not differ from those in other samples, which invalidates the latter hypothesis.

The finding that macroinvertebrate assemblages tend to differentiate between both stable turbidity states adds to the growing list of studies showing that assemblages

Table 2. Results of the forward selection procedure on the DA with turbidity state as the a priori grouping factor. Taxa that explain a significant portion of the variance between turbidity states are presented. P-values were obtained with Monte Carlo randomization tests using 9,999 permutations and test whether a taxon explains more than 0 % of the variation between turbidity states. Positive and negative scores of a taxon with DA axis 1 indicate a higher abundance in ponds of the clear and turbid water state, respectively (see Fig. 3).

Taxon	Marginal explained variance	P	Score on axis 1
Chaoborus	0.605	0.0016	0.894
Diptera	0.428	0.010	0.72

Table 3. Results of the forward selection procedure on the DA with microhabitat as the a priori grouping factor. Taxa that explain a significant portion of the variance between different microhabitats are presented. P-values were obtained with Monte Carlo randomization tests using 9,999 permutations. Positive scores on axis 1 indicate higher abundances in open water; negative scores on axis 2 indicate higher abundances in reed (see Fig. 4).

Taxon	Marginal explained variance	P	Score on axis 1	axis 2
Diptera	0.298	0.0073	-0.690	-0.300
Caenis horaria	0.268	0.0082	-0.668	-0.226
Sigara females	0.195	0.045	-0.537	0.305
Caenis robusta	0.191	0.052	-0.585	-0.038

of other organisms, like zooplankton and fish, clearly differentiate between both lake types (e.g. Cottenie et al. 2001, Jeppesen et al. 2000). The major difference being that, according to the widely accepted model of Scheffer et al. (1993), the latter organisms are widely recognized to play a key role in maintaining the two stable states, while macroinvertebrates are not assumed to play such a role. Interestingly, Jones and Sayer (2003) provided data that support a model where fish are the prime determinants of macroinvertebrate community structure in shallow lakes, through a cascading effect of predation on grazing invertebrates influencing the biomass of periphyton and hence, plants. Therefore, the reported differentiation in macroinvertebrate assemblages may not just be a by-product of biotic and abiotic variables associated with the two stable states as suggested by the model of Scheffer et al. (1993), but may actually be part of the

cascading mechanisms underlying the stable states. Another recent study, using mesocosms with three invertebrate species, indicated that predator-prey interactions among invertebrates, irrespective of fish, macrophytes and zooplankton, might also generate alternative stable community states in low and high productivity lakes (Chase 2003). Clearly, our understanding of the key mechanisms structuring macroinvertebrate assemblages in shallow lakes is rapidly evolving. In our opinion, two types of studies are needed to allow further advances. There is a need for more field studies to evaluate the generality of discrete macroinvertebrate assemblages in turbid and clear-water shallow lakes, as reported here. Ideally, such studies should span several years to assess the stability of these assemblages. Next, mechanistic studies should clarify whether such assemblages are by-products or key components of stable equilibrium states of shallow lakes and whether or not their stability is purely maintained by intrinsic interactions.

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Appendix 1. Frequency of occurrence of all taxonomic groups for every combination of microhabitat and pond number. Frequency ranges from 0 (present in none of the samples) to 3 (present in all of the samples). "*" indicates that only two samples were taken.

Lake Type	TURBID							CLEAR							
Microhabitat	OPEN			REED		PONDWEED		OPEN			REED			PONDWEED	
Lake Nr.	10	11	12	11*	12	10	12	13	14	15	13	14	15	14	15
OLIGOCHAETA	2	1	3	2	3	1	3	2	0	2	3	0	2	0	3
HIRUDINEA	2	0	1	2	0	0	1	0	0	0	0	1	1	0	0
MOLLUSCA															
Gyraulus sp.	0	0	0	0	0	0	0	2	0	0	0	1	3	0	1
Lymnea sp.	0	0	1	0	0	1	0	1	1	0	1	1	2	1	1
Physa sp.	0	1	0	2	0	3	0	1	0	0	0	0	3	0	2
Sphaerium sp.	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
ACARI	2	1	0	1	1	2	1	1	2	1	1	0	1	0	1
ARANEA	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
EPHEMEROPTERA															
Caenis horaria	1	0	0	2	3	3	2	3	3	0	3	3	3	3	3
Caenis robusta	0	0	0	0	2	1	1	1	2	0	3	3	3	1	2
Cloeon dipterum	0	0	0	2	1	0	0	3	0	0	3	3	2	1	1
ODONATA															
Erythromma sp.	0	0	0	0	1	2	2	2	1	0	1	0	0	1	0
Ischnura sp.	0	0	0	2	3	3	0	2	3	0	2	3	1	2	0
Libellula sp.	0	0	0	0	0	1	0	0	1	0	1	2	0	0	0
Sympetrum sp.	0	0	0	0	0	0	0	1	0	0	2	1	0	0	0
HEMIPTERA															
Nepa cinerea	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Micronecta meridionalis	1	0	0	2	0	0	1	0	0	0	0	0	0	0	0
Cymatia coleoptrata	2	1	0	2	1	1	1	0	0	0	0	1	1	0	1
Sigara falleni	1	1	0	1	0	0	0	1	0	0	1	0	1	0	1
Sigara striata	1	3	0	2	1	3	0	1	0	0	0	1	3	0	2
Sigara juveniles	2	2	0	0	3	3	0	3	2	3	3	1	3	1	3
Sigara females	1	3	0	2	1	3	1	0	0	0	1	1	3	1	2
Ilyocoris cimicoides	0	0	0	0	0	0	0	0	1	0	0	1	2	0	0
DIPTERA															
Chaoboridae	0	0	0	0	0	0	0	1	2	1	0	2	2	2	1
Chironomidae	3	3	3	2	3	2	3	3	3	3	3	3	3	3	3
Culicidae	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Other Diptera families	0	0	0	2	3	1	0	1	2	1	3	2	2	2	3
MEGALOPTERA															
Sialis sp.	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
TRICHOPTERA	0	0	0	0	1	0	1	1	1	0	1	2	2	1	1
COLEOPTERA															
adults	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0
juveniles	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Chapter II

Water turbidity affects predator-prey interactions in a fish-damselfly system

Frank Van de Meutter, Robby Stoks and Luc De Meester

[Oecologia, in press]

Abstract

Community structure may differ dramatically between clear-water and turbid lakes. These differences have been attributed to differences in the cascading effect of fish on prey populations, due to the reduced efficiency of fish predation in the presence of macrophytes. However, recent theoretical ideas suggest that water turbidity may shape predator-prey interactions, and it is predicted that prey will relax its antipredation behaviour in turbid water (H1). As a result, the nature of predator-prey interactions is expected to shift from both direct and indirect in clear water to dominantly direct in turbid water (H2). We tested these ideas in a fish-damselfly predator-prey system. In a first behavioural experiment, we looked at antipredation behaviour of damselfly larvae isolated from habitats that differ in turbidity, in the presence of fish in clear and turbid water. As predicted in H1, the larvae were more active in turbid than in clear water. In a complementary enclosure experiment, we reared larvae in a clear-water pond and a turbid pond, respectively, and manipulated the origin of the larvae (clear-water, turbid pond), fish presence (absent, present), and vegetation density (sparse, abundant). In both ponds, fish had a direct negative effect on survival of the larvae, which was mitigated in the presence of vegetation. In the fish treatment, the change in average body mass tended to be higher in the turbid than the clear pond, suggesting indirect effects of fish were mitigated in the turbid pond. This was supported by a negative effect of fish on effective growth rate of larvae in the clear pond, but not in the turbid pond. These results are compatible with the idea that predator-prey relationships are mainly governed by direct effects in turbid water, and by direct and indirect effects in clear water.

Introduction

Predation is a key factor shaping transitions in community structure along the freshwater habitat gradient going from small temporal ponds to large permanent lakes (Wellborn et al. 1996). The transition between temporary habitats that contain few predators and permanent fishless habitats that contain large invertebrate predators, and the transition between permanent fishless and fish-containing lakes, have been well-studied (Wellborn et al. 1996, Stoks and McPeek 2003a,b). Interestingly, there is also overwhelming evidence for a structuring role of fish predation within permanent fish-containing lakes, associated with water turbidity (Moss 1990, Scheffer et al. 1993, Scheffer 1998). Shallow fish-containing lakes can occur in two alternative stable states: a clear-water state with abundant submersed vegetation and a turbid water state with high algal biomass (Scheffer et al. 1993, Scheffer 1998). The former state is the only one possible at low nutrient loads, whereas the latter predominates at high nutrient loads. At an intermediate nutrient level, both states can occur, and are stabilized by a suite of feedback mechanisms (Scheffer et al. 1993, Scheffer 1998). There is much evidence that the community composition of fish, macroinvertebrates and zooplankton may change dramatically with the turbidity state of the lake (reviewed by Scheffer 1998, Van de Bund and Van Donk 2002, Cottenie and De Meester 2003, Van de Meutter et al. in press, Chapter I). Traditionally, these differences have been largely attributed to the sheltering property of aquatic vegetation in clear-water lakes (Timms and Moss 1984, Lammens 1989). However, given that many fish species rely on vision to detect their prey (Guthrie and Muntz 1993), it is plausible that water turbidity itself may drastically affect predator-prey interactions, and hence community structure.

The majority of studies that have dealt with predator-prey interactions in relation to water turbidity in lakes and ponds have focused on the top predator's perspective. For example, Reid et al. (1999) showed that turbidity may reduce prey detection distance and hence predation rates by fish, although compensatory mechanisms such as altered foraging behaviour may exist that partially counter these effects (Vøllestad et al. 1986). Water turbidity is, however, likely to not only influence the predator but also the prey. Prey that detects its food and predators visually may not only suffer from a lower foraging efficiency in turbid water, but in addition also may encounter difficulties in judging the risk of predation in a situation where both prey and predator detection is impaired. Abrahams and Kattenfeld (1997) hypothesized that in turbid systems, antipredator behaviour of the prey will become ineffective and will occur less frequently. Several studies found support for this hypothesis in fish-fish predator-prey systems

(Abrahams and Kattenfeld 1997, Jacobsen and Perrow 1998, Jacobsen et al. 2004). From this hypothesis, Abrahams and Kattenfeld (1997) derived a second hypothesis that predator-prey interactions in turbid water will be primarily characterized by the direct effects of predator consumption of prey, while in clear-water systems, direct as well as indirect effects of the predator on the prey's growth rate through behavioural modification will be important. Therefore, the turbidity state of a lake might not only influence the prey's behaviour, but also its key life history characteristics like growth and survival. To our knowledge, the second hypothesis of Abrahams and Kattenfeld (1997) has never been tested.

In this study, we looked at the effects of water turbidity on behaviour, growth and survival of *Ischnura elegans* (Van der Linden 1820), a damselfly that occurs over a wide range of permanent aquatic habitats in Belgium. *I. elegans* larvae are opportunistic intermediate predators in the littoral zone of ponds and lakes, and often suffer from predation by fish (Corbet 1999). In a first experiment, we evaluated the first prediction of Abrahams and Kattenfeld (1997) in an invertebrate prey species by testing whether *Ischnura* larvae relax their antipredator behaviour under risk of predation in turbid water compared to clear water. To get insight in how former exposure to turbid water may shape this behavioural response, we used both field-caught larvae that originated from a clear pond and from a turbid pond, and reared half of the larvae from each pond in the laboratory in clear or turbid water prior to the experiment. In a complementary life history experiment we investigated how these behavioural interactions translate into survival and growth rate of larvae in the field under clear-water and turbid conditions, thus explicitly evaluating the second hypothesis of Abrahams and Kattenfeld (1997). In each experiment, larvae of *I. elegans* from the clear-water and the turbid pond were reared in the presence or absence of fish in enclosures set up in both a clear-water and a turbid pond. Because, in addition to turbidity, the presence of aquatic vegetation may impair the detection of prey and predators in natural systems (Crowder and Cooper 1982, Burks et al. 2001), we crossed the fish treatment with high and low density of vegetation in each pond.

Material and methods

A. Behaviour experiment

Experimental design

The effect of water turbidity on behaviour of larvae of the damselfly *I. elegans* was studied in a common garden experiment in the laboratory. The experiment had a 2 x 2 x

2 design with all combinations of three different factors related to turbidity: the pond of origin (clear, turbid), rearing turbidity in the laboratory (clear, turbid) and actual turbidity during the behavioural trials. *I. elegans* larvae of instar F-1 and F-2 (with F-0 as final instar) were collected on 27 May 2003 in the nature reserve De Maten (Northeast Belgium). This reserve consists of 34 shallow ponds that exhibit a wide range in turbidity levels. Larvae were collected in two neighboring ponds: turbid pond 12, and clear-water pond 13 (Cottenie et al. 2001, Van de Meutter et al. in press, Chapter I). They were brought to a climate room (20°C, L:D 16:8) and were placed in aerated 10 L buckets (40 larvae/pond; 10 larvae/bucket). Half of the larvae from each pond (20 individuals) were reared in water of their native pond; the other half was transplanted to buckets with water of the pond with the other turbidity state. In each bucket, we placed 10 stems (length 15 cm) of plastic aquatic vegetation (mimics of *Ceratophyllum*, Plantastics©) to provide some structure to the habitat. We daily visually checked water turbidity in the turbid water buckets, and added small amounts of bentonite clay on two occasions (day 8 and day 18) to maintain turbidity at the original level. Larvae were kept in the laboratory for 21 days and were daily fed *Daphnia magna* ad libitum.

From 16 to 20 June, we scored the behaviour of all larvae in random order once in clear water and once in turbid water, but never on the same day. Observations were done in 10 L aquaria (24 cm x 34 cm x 13 cm high) in which two compartments were created: a small compartment (10 cm x 10.5 cm) attached to the wall of the aquarium, separated from a large compartment by transparent Plexiglas walls and a small piece of netting. This way, both visual and olfactorial cues could be transmitted from one compartment to the other. Ten minutes before the start of an observation trial, a pumpkinseed *Lepomis gibbosus* was added to the small compartment to assure the presence of fish cue. As we were interested in the behaviour of the larvae in the presence of fish, fish and fish cue were present in all observation trials. At the start of the experiment, the fish was transferred to the large compartment, and 25 adult *Daphnia* sp. were added to the small compartment. Next, an *I. elegans* larva was gently released into the small compartment and allowed to settle down for 1 minute. After this acclimatization period, its behaviour was recorded for 10 minutes. We recorded six distinct behaviours (cf. Johansson et al. 2001): walking (frequency and duration), swimming (frequency and duration), orienting the head towards a *Daphnia* (frequency), advancing towards a *Daphnia* (frequency and duration), striking towards a *Daphnia* with the labial mask to catch it (frequency) and catching a *Daphnia* (frequency). Following the advice of Davies-Colley and Smith (2001), and given the fact that we focused on the visual aspects of

turbidity, we did not measure turbidity in our experiments in NTU (nephelometric units) but with a Snell tube. A Snell tube consists of a mini Secchi disk (Ø 5 cm) that can be lowered in a long PVC tube filled with water. Snell depth is given as the mean of the measurements of the maximal depths (in cm) at which a black cross on the disk can be seen when lowering the disk in the tube and the depth at which the cross can be seen again when lifting the disk. Turbid water in our experiment had a Snell depth of 15 cm, corresponding to that of the turbid pond where the larvae originated from, and was created at the start of each day by adding bentonite clay to dechlorinated tap water. To maintain turbidity levels throughout the experiments, we stirred up the water between all behavioural trials.

Statistics

Prior to analysis, the original behavioural variables were log(Y+1)-transformed to eliminate positive relationships between means and variances of all variables. As the six behavioural variables are potentially highly correlated, we performed a Principal Component Analysis (PCA), which extracts new orthogonal variables as a linear combination of the original variables. We obtained three new variables (PC1-PC3) with eigenvalues >1 that together explained 87.5 % of the original variance in the behavioural dataset. PC1 (55.9 % explained variance) had high positive loadings (> 0.75) from duration and frequency of walking and is further referred to as +WALK. PC2 (22.4 % of the explained variance) had strong negative loadings (< -0.75) from all variables related to foraging (advancing towards *Daphnia*, striking towards *Daphnia*, catching *Daphnia*) and is hereafter named -FORAGE. PC3 (9.2 % explained variance) was highly positively correlated (> 0.75) with duration and frequency of swimming (hereafter +SWIM). To evaluate the effect of origin of the larvae, rearing turbidity and actual turbidity during the experiment on the newly extracted behavioural variables, we performed a 3-way Repeated Measures MANOVA. Origin (clear pond / turbid pond) and rearing turbidity (clear/ turbid) were used as categorical predictors. For each of the three behavioural PCs, we treated the score of a given larvae in clear and in turbid water as repeated measures, giving in total three sets of repeated measures as dependent values. When significant, the rmMANOVA is followed by rmANOVAs for each PC variable.

B. Enclosure experiment

Experimental setup

To study the effects of fish and vegetation density on larval survival and growth rate of the damselfly *I. elegans* in ponds with a contrasting turbidity state, we set up an enclosure experiment in the same turbid (pond 12) and clear-water pond (pond 13) as the ones we sampled for *I. elegans* larvae in the behavioural experiment. To take into account possible acclimatization of the larvae to their native pond, we did a reciprocal transplant by rearing half of the larvae in their own pond and the other half in the pond with contrasting turbidity state. This resulted for each pond in eight different treatment combinations of origin (pond 12, pond 13), fish presence (absent, present) and vegetation density (sparse, abundant). Each treatment combination was replicated in three enclosures, giving a total of 24 enclosures in each pond. Within each individual enclosure, all larvae originated from the same pond. Enclosures were placed in three different blocks (A, B, C) of eight. Within each block, the eight different treatment combinations were randomly assigned to the enclosures.

Enclosures had a cylindrical frame (diameter 50 cm, height 90 cm) of chicken wire (mesh size 2 cm). Within this frame, we placed a cylindrical nylon bag (mesh size 1 mm) with the same size and shape. To prevent the bags from collapsing, we fixed them tightly to the frame at 30 cm and at 60 cm. A plastic dish (diameter 50 cm) served as bottom. Each enclosure was attached to three 1.5 m PVC pipes that were drilled into the sediment. One block of eight enclosures was placed in both ponds on 31 July (block A), one on 1 August (block B) and one on 2 August (block C). One pumpkinseed *Lepomis gibbosus* (standard length 5.8-6.3 cm) was added to the fish treatments on 24 August. The diet of pumpkinseeds in “De Maten” of this size class consists largely of macroinvertebrates, containing mainly chironomids, ephemeropterans and zygopterans (Declerck et al. 2002). All pumpkinseeds originated from pond 27 of De Maten, a pond with a turbidity state intermediate to that of pond 12 and pond 13, and were inoculated to the enclosures directly after capture. The time lag of three weeks between installing the enclosures and applying the fish treatment served to permit colonization of prey organisms for the damselfly larvae and the fish.

The vegetation treatments were created by adding sago pondweed *Potamogeton pectinatus*. Sago pondweed is a common submersed macrophyte species in the shallow ponds of De Maten, and it occurs in both clear-water and turbid ponds. Sago pondweed was collected in pond 27, rinsed carefully on white plastic dishes to remove all large macroinvertebrates and divided into small (30 g wet weight) and large (300 g wet weight)

portions. On 24 August, we added a large portion to all enclosures of the high-density macrophyte treatment and a small portion to the low-density macrophyte treatment.

On 26 August, we added 20 damselfly larvae to each enclosure. The resulting density corresponds to natural damselfly densities in both ponds. Two species of damselfly, *I. elegans* and *E. najas*, make up more than 90% of the damselfly community in both ponds and occur at a ratio of 3/1 in pond 13 and of 2/1 in pond 12. Therefore, enclosures with larvae originating from pond 13 consisted of 15 *I. elegans* and 5 *E. najas*, whereas larvae originating from pond 12 consisted of 13 *I. elegans* and 7 *E. najas*. Larvae were collected by sampling the littoral of both ponds and placed in the enclosures on the same day. Only instars F-4 to F-1 larvae were used in the experiment. The experiment ran for 21 days. Afterwards, the entire content of each bag was preserved in 4% formaldehyde. Unfortunately, three enclosures in the turbid pond (treatment combinations: high vegetation - no fish - origin clear pond [2] and low vegetation - fish - origin turbid pond [1]) were damaged during the experiment. Data of these enclosures were excluded from all following analyses.

Growth rate and survival

For every enclosure, we quantified the growth rate of *I. elegans* as [ln(mean final dry mass) - ln(mean initial dry mass)]/21 d. For this metric is not an actual measure of individual growth rate of larvae, we will hereafter refer to it as the change in average body mass (CABM). Mean initial dry mass of the *I. elegans* that were inoculated in the enclosures was determined on an additional sample of 50 individuals from each pond. Mean final dry mass for an enclosure is the mean dry mass of *I. elegans* larvae that were retrieved from that enclosure after 21 days. Dry mass was determined with a Mettler-Toledo HL52 microbalans to the nearest 10 µg after drying larvae at 50°C for 48 hours. Prior to drying, head width was measured with an Olympus SZX-ILLK200 stereomicroscope to the nearest 10 µm. Head width is an accurate measure of total body length of damselflies (Corbet 1999) and was measured to detect possible size selective foraging of the fish. Survival was calculated as the number of *I. elegans* larvae retrieved at the end of the experiment divided by the initial number of *I. elegans* larvae added to the enclosure (which was different depending on pond origin).

Statistics

To test for possible effects of experimental pond, fish, vegetation and pond of origin on the survival of *I. elegans* larvae, we performed a 4-way ANOVA using the mixed

procedure in SAS 8.02 (SAS institute 2001). Experimental pond, fish, vegetation and pond of origin were used as categorical predictors; survival was the dependent variable. In three treatment combinations with fish, changes in average body mass could only be estimated from a single replicate (the remaining cages having zero survival). This may yield unreliable estimates of larval growth. To circumvent this problem, we sacrificed the origin treatment (data were pooled, not averaged). Effects of experimental pond, fish and vegetation on changes in average body mass of *Ischnura* larvae were analyzed using a 3-way ANOVA with fish, vegetation and experimental pond as categorical predictors and CABM as the dependent variable. Since our experiment has an unreplicated randomized block design, 'Block' (3 levels: A, B and C) was included in both models as a random main effect (Underwood 1997). In both analyses, however, no significant effect of Block was present (both $p > 0.12$), and we pooled the MS of the Block factor with the error MS. Specific effects of interest were further explored using contrast analysis comparing least square means.

Apparent differences in changes in average body mass between larvae from enclosures with fish and without fish can originate either from differences in effective growth rate in the presence of fish or from size selective foraging of the fish. To differentiate between both, we performed an Analysis of Covariance (ANCOVA) on the final mass of the larvae, with head width as the covariate and fish treatment as the categorical factor. Because larvae from the same enclosure are statistically dependent, we included the categorical factor "Enclosure" nested in the Fish treatment and its interaction with Head width (Enclosure[fish]*Head width) to correct for this. To obtain a linear relationship between the variables, final mass was log10-transformed. Head width is known to be constant per larval stage and independent of food regime (McPeek 1990). If final mass of *I. elegans* larvae still differs among fish treatments after correction for head width, then this mass difference is not caused by size selective foraging of the fish and truly reflects differences in effective growth rate.

Results

A. Behaviour experiment

In general, *I. elegans* larval behaviour was clearly affected by the turbidity during the experiment (rmMANOVA: $F_{3, 58} = 7.21$, $P < 0.001$), but not by rearing turbidity ($F_{3, 58} = 2.09$, $P = 0.11$), nor by the origin of the larvae ($F_{3, 58} = 0.15$, $P = 0.94$). Univariate rmANOVAs showed that larvae that originated from the turbid pond walked more when the water was turbid, but not those that originated from the clear-water pond (+WALK:

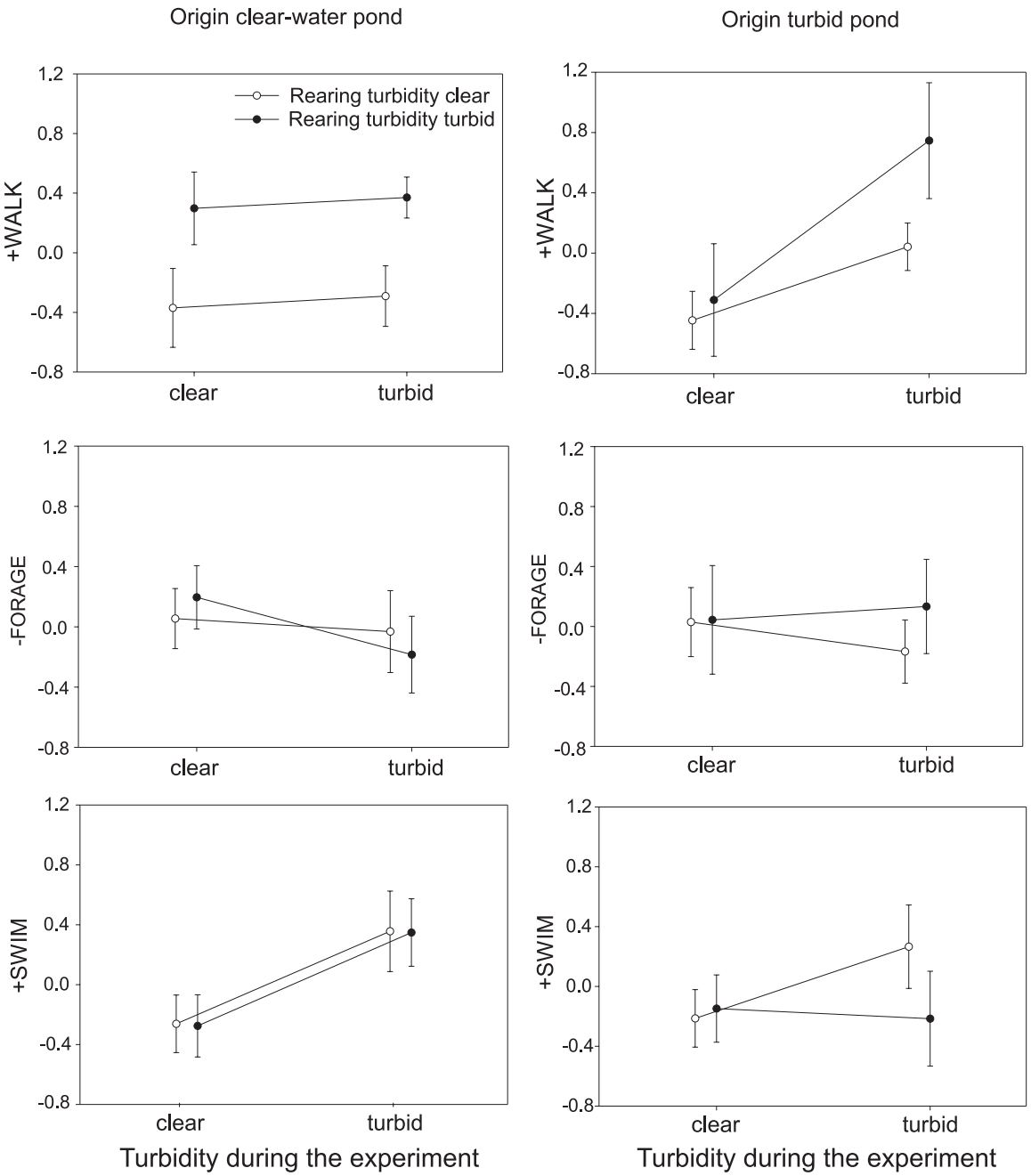

Fig. 1. The behavioural principal components +WALK, -FORAGE and +SWIM of *Ischnura* larvae in the presence of fish in function of turbidity during the experiment, rearing turbidity and pond of origin. Left panels give values for larvae from the clear-water pond, right panels for larvae of the turbid pond. Means are given ± 1 standard error.

Turbidity during Experiment x Origin: $F_{1, 126} = 4.29$, $P = 0.040$; Fig. 1). Walking depended also on the rearing turbidity of the larvae: larvae that were reared in turbid water walked more than larvae that were reared in clear water ($F_{1, 126} = 10.38$, $P = 0.002$). Larvae also swam more in turbid water than in clear water, regardless of their origin or their rearing

turbidity (+SWIM: $F_{1,\ 126}$ = 5.57, P = 0.02; other P > 0.25; Fig. 1). Finally, foraging behaviour of the larvae was not modified by the turbidity during the experiment or by the rearing turbidity state they experienced (-FORAGE: all P > 0.29, Fig. 1).

B. Enclosure experiment

Survival

The results of the ANOVA on survival data of *Ischnura* larvae are summarized in Table 1. Survival of *Ischnura* larvae was determined by an interactive effect of fish and vegetation, which on itself depended on experimental pond (Experimental Pond x Fish x Vegetation, p = 0.0005). In the clear-water pond, fish had an overall negative effect on survival of *Ischnura*, whereas in the turbid pond, fish had a negative effect on *Ischnura* survival at low vegetation density, but not at high vegetation density (Fig. 2). The lack of an effect of fish on *Ischnura* survival at high vegetation density in the turbid pond resulted from a combination of reduced survival in the absence of fish (contrast, turbid pond vs. clear pond for the no-fish & high vegetation density treatment; p = 0.0001) and a tendency for increased survival in the presence of fish (contrast, clear pond vs. turbid pond for the fish & high vegetation density treatment: p = 0.081). Ponds also differed in the way larvae of a different origin were affected by vegetation density (Experimental Pond x Origin x Vegetation, p = 0.0014). In the clear-water pond, larvae originating from the clear-water pond were better at surviving at high vegetation density than at low vegetation density, whereas larvae originating from the turbid pond showed little difference. In the turbid pond, larvae from both origins were similarly affected by vegetation density.

Change in average body mass

The results of the ANOVA on the data for CABM of *Ischnura* larvae are summarized in Table 1. In both ponds, fish had a negative effect on CABM of *Ischnura* larvae at low vegetation density, but not at high vegetation density (Fish x Vegetation, p = 0.037, Fig. 3). However, when corrected for head widths, only a weak negative trend of fish at low vegetation density on final mass of *Ischnura* was present (ANCOVA; Fish: $F_{1,110}$ = 2.85, p = 0.094; Fish x Head width: $F_{1,110}$ = 2.04, p = 0.16). This suggests that size selective foraging of the fish may have contributed to the apparent differences in growth rate of the *Ischnura*. CABMs in the fish treatments tended to be slightly higher in the turbid than in the clear-water pond (Experimental Pond x Fish, p = 0.10). This was supported by a negative fish effect on final size-corrected body mass of larvae in the clear pond

Table 1. Results of the ANOVAs on survival and change in average body mass (CABM) of *Ischnura* larvae in the enclosure experiment.

Survival			**CABM**		
Treatment	$F_{1,36}$	p	Treatment	$F_{1,31}$	p
Experimental Pond (P)	1.49	0.23	Experimental Pond	0.34	0.57
Origin (O)	0.51	0.48	Fish	1.61	0.21
Fish (F)	45.82	<0.0001	Vegetation	1.53	0.23
Vegetation (V)	6.06	0.021	P x F	2.81	0.10
P x O	2.44	0.13	P x V	0.11	0.74
P x F	5.90	0.022	F x V	4.74	0.037
P x V	3.65	0.067	P x F x V	0.41	0.53
O x F	3.45	0.074			
O x V	0.16	0.70			
F x V	34.20	<0.0001			
P x O x F	0.086	0.77			
P x O x V	12.69	0.0014			
P x F x V	15.92	0.0005			
O x V x F	2.35	0.14			
P x O x V x F	1.69	0.21			

(ANCOVA; Fish: $F_{1,174} = 4.21$, $p = 0.042$; Fish*Head width: $F_{1,174} = 6.14$, $p = 0.014$; Fig. 4A), but not in the turbid pond (ANCOVA; Fish: $F_{1,110} = 3.05$, $p = 0.083$; Fish*Head width: $F_{1,110} = 2.04$, $p = 0.16$; Fig. 4B). This indicates lower effective growth of larger larvae in the presence of fish in the clear-water pond, but not in the turbid pond.

Discussion

We showed that the invertebrate predator *Ischnura* changes its behaviour according to the turbidity of the water. Moreover, we found indications that growth and survival of *Ischnura* larvae were differently affected by the presence of fish and vegetation in a clear-water pond compared to a turbid water pond. Taken together, our results suggest that predator-prey interactions differ between clear-water ponds and turbid water ponds. We discuss these findings in more detail and confront them with the predictions of Abrahams and Kattenfeld (1997).

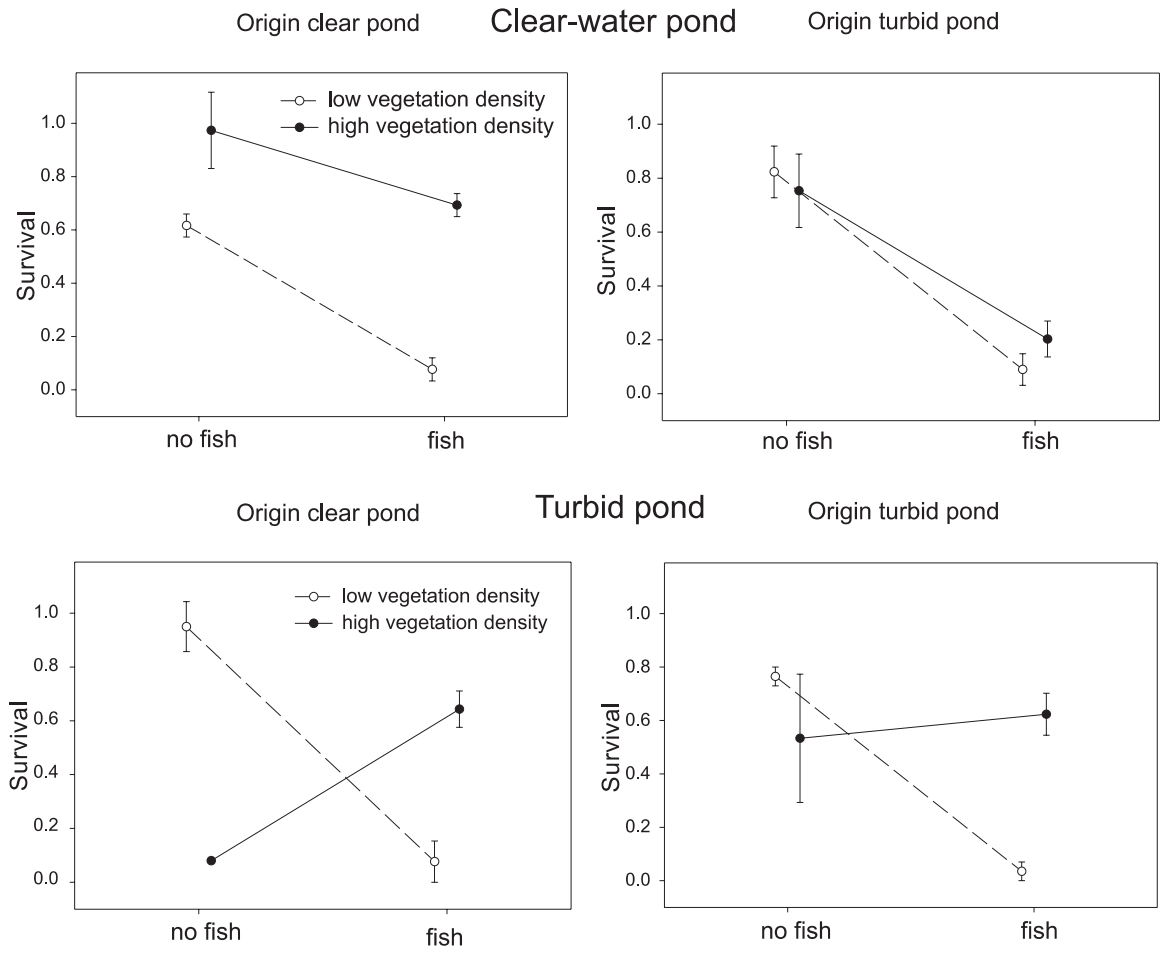

Fig. 2. Survival of *Ischnura* larvae of the enclosure experiment in the clear-water pond (upper panels) and in the turbid water pond (lower panels) in function of fish, vegetation density and pond of origin. Left panels show the results for the larvae that originated from the clear-water pond; right panels show the results for larvae originating from the turbid pond. Symbols represent the means of 3 replicate enclosures except for the 'high vegetation-no fish-origin clear pond' and the 'low vegetation-fish-origin turbid pond' treatment combinations in the turbid pond, the means of which were based on 1 and 2 enclosures, respectively. Means are given ± 1 standard error.

According to the first prediction by Abrahams and Kattenfeld (1997), antipredation behaviour of prey should be less pronounced in turbid water than in clear water. One of the most common antipredator behaviours in prey is the reduction of activity in the presence of predators (Lima 1998). In damselflies, foraging, walking and swimming behaviour have been shown to attract the attention of predators, leading to increased predation and mortality (Convey 1988, McPeek 1990, Baker and Smith 1997, Elkin and Baker 2000). By consequence, these behaviours are generally decreased in the presence of a predator (e.g. McPeek 1990, Stoks et al. 2003). Translated to our

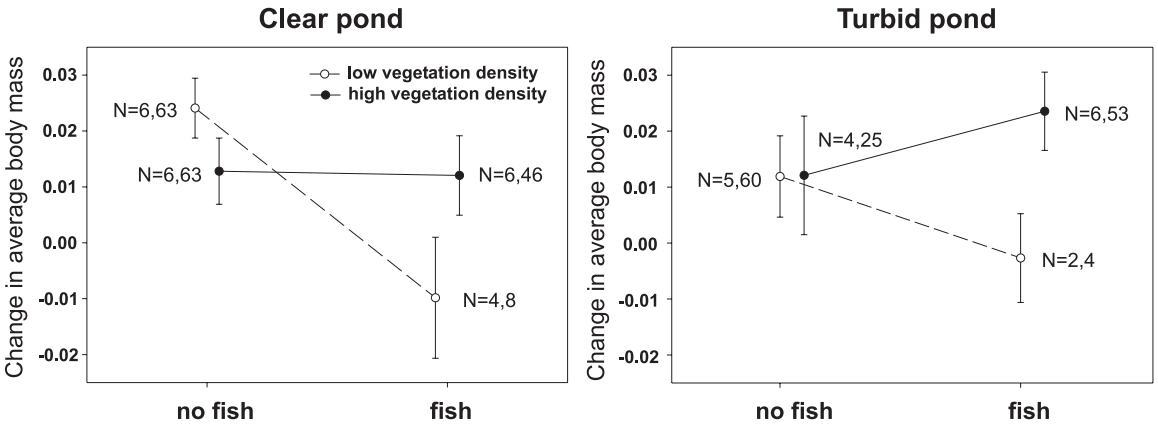

Fig. 3. Change in average body mass of *Ischnura* larvae of the enclosure experiment in the clear-water pond (left) and in the turbid pond (right) in function of fish and vegetation density. For each mean, the number of enclosures followed by the total number of individuals on which the means are based are given. Means are given ± 1 standard error.

model system, the hypothesis of Abrahams and Kattenfeld (1997) thus would be that in the presence of a fish, *Ischnura* should show more foraging, walking and swimming activity in turbid water than in clear water. Overall, our results are in accordance with this hypothesis for walking and swimming. Similarly, Heads (1985) observed that *Ischnura* larvae moved more in the dark than in the light, and suggested that this was because larvae felt safer under the cover of dark. In contrast with the first hypothesis, foraging activity did not differ between clear and turbid water. Although decreased foraging in the presence of fish has been observed in several species of damselflies (e.g. McPeek 1990, Stoks and Johansson 2000, Stoks and McPeek 2003b) this response is not always present in *I. elegans* (Slos and Stoks in review). This is consistent with the fact that *Ischnura*, a fast growing species, tries to avoid large reductions in growth rate in the presence of a predator as opposed to other damselfly genera like *Enallagma* (McPeek et al. 2001, McPeek 2004).

The behaviour of *Ischnura* in clear versus turbid water was to a large extent determined by its former experiences with turbidity. Larvae that were reared for three weeks in turbid water showed a higher walking activity than larvae that were reared in clear water. This probably indicates carry-over effects (Sih et al. 2003), where organisms that have adapted their behaviour to a particular risk environment may to some extent carry over this behaviour when confronted with a new environment, as was shown before in *Ischnura* (Slos and Stoks in review). Strikingly, pond origin still affected some responses to water turbidity, even though all larvae had been kept for three weeks in the

laboratory. Larvae from the turbid pond walked more in turbid water than in clear water, but larvae from the clear pond showed no difference. We acknowledge that both ponds where the larvae originated from may differ in other aspects than turbidity, so it cannot be claimed that the differences are related to the turbidity of the ponds. If turbidity is the factor involved, it is still unclear whether these origin-specific responses to turbidity stem from carry-over effects following acclimatization to the turbidity of the original pond, or have a genetic basis. Genetic differences, however, seem unlikely since only 10 meters separate both ponds. More detailed quantitative genetic research is needed to verify this.

Overall, our results of the enclosure experiment are compatible with the second hypothesis of Abrahams and Kattenfeld (1997): direct effects of fish on *Ischnura* (survival) were apparent in both the turbid and the clear-water pond, indirect effects of fish on *Ischnura* (CABM, effective growth rate) tended to be stronger in the clear-water pond than the turbid pond. Survival of *Ischnura* at low vegetation density was strongly negatively affected by the presence of fish in both ponds. This confirms our premise that pumpkinseed is an effective predator of *Ischnura* and can have severe negative effects on its survival, as shown in other studies (McPeek 1998). In the turbid but not in the clear-water pond, however, the fish effect tended to be relaxed when the vegetation density was high. Previous studies have shown a mitigating effect of vegetation on fish predation on macroinvertebrates and zooplankton (Crowder and Cooper 1982, Gilinsky 1984, Burks et al. 2001). Dense vegetation beds prevent visual detection of prey by fish and may also physically hinder the fish to catch their prey (Crowder and Cooper 1982, Burks et al. 2001). In our study, the mitigating effect of vegetation on fish predation tended to be strengthened when the protective effect of vegetation was combined with a reduced visibility due to water turbidity. In the turbid pond, high vegetation density had a negative effect on survival of *Ischnura* in the absence of a predator, possibly suggesting increased intraspecific interactions (Elkin and Baker 2000). Unfortunately, we do not have data on *Ischnura* behaviour under different combinations of macrophyte density and turbidity that could support this. Finally, larvae originating from the clear-water pond were better at surviving at high versus low vegetation density in the clear-water pond, whereas survival of *Ischnura* originating from the turbid pond benefited only weakly from high vegetation density. Possibly, larvae from the clear-water pond were better adapted at surviving in a clear-water & high vegetation density environment, since it is their natural environment.

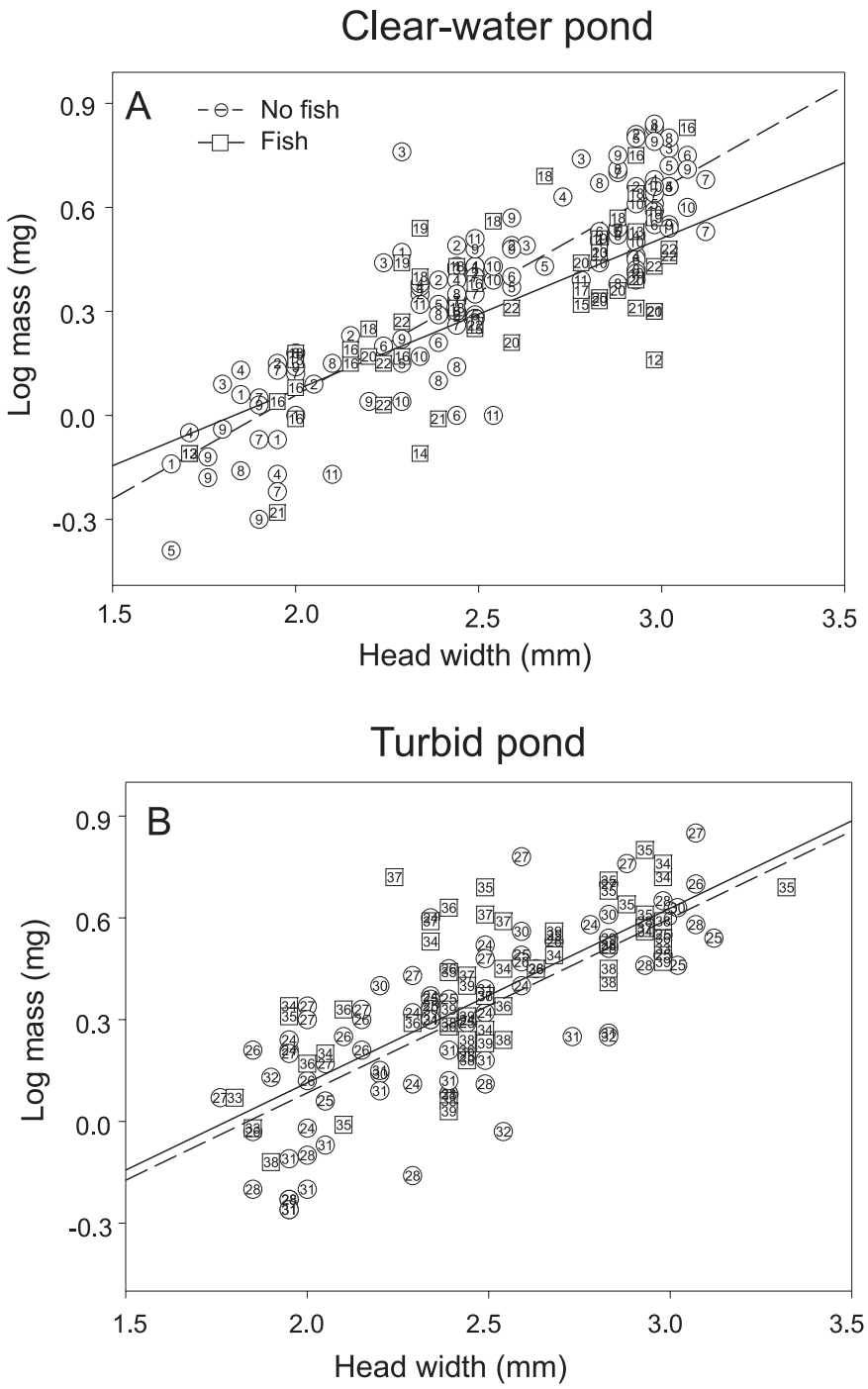

Fig. 4. Scatterplot of final head widths versus the logarithm of final mass for *Ischnura* larvae in the enclosure experiment in the clear-water lake (A) and the turbid lake (B). Squares represent larvae of the fish treatment, circles represent larvae of the non-fish treatment. For each larva, the enclosure to which it belonged is indicated with the enclosure number. As all different enclosures encompass a relatively large section of the range in head widths, as well as to indicate general trends in the data, regressions based on individual larvae are given (note that the ANCOVA corrects for dependent larvae per enclosure). Dashed lines represent the regressions for the non-fish treatments, solid lines represent the regression for the fish treatments.

CABMs in the fish treatments tended to be higher in the turbid than in the clear-water pond, suggesting indirect effects of fish were mitigated in the turbid pond (Fig. 3). Accordingly, fish negatively affected size-corrected body mass in the clear pond, indicating lower effective growth rate in the presence of fish. However, fish did not affect body mass in the turbid pond (Fig. 4). We suggest that the fish-induced lower effective growth rate in clear but not in turbid water could result from the combination of two factors. First, damselfly larvae and fish partially compete for the same food sources like chironomids and cladocerans (Thompson 1978, Declerck et al. 2002), and food availability to damselfly larvae probably was lower in the presence of fish. In the turbid pond, prey densities may have remained more elevated than in the clear-water pond because of constrained visual predation by the fish. Second, in nature prey is often aggregated in patches (Diehl and Kornijów 1998), and foraging success may be linked to walking behaviour to find patches with prey (Baker 1982). In the presence of fish, *Ischnura* walked less in clear water compared to turbid water, which may result in reduced feeding rates and growth rates. The same two factors may also explain why CABMs were reduced in the presence of fish at low vegetation density, but not at high density. The sheltering effect of high vegetation density may have increased prey availability and possibly *Ischnura* mobility towards optimal foraging patches. The fact that the effect of fish on final corrected mass of *Ischnura* larvae in the low vegetation treatment was only weak, suggests that other mechanisms such as size selective predation by fish could also have contributed to the observed pattern, however, the number of surviving individuals (9) in the low vegetation treatment may have been too low to detect the subtle differences for corrected final body mass.

In conclusion, our results confirm the importance of aquatic vegetation in shaping predator-prey interactions in clear-water versus turbid lakes, while also showing that turbidity itself may drastically affect predator-prey interactions by changing prey behaviour and possibly life history. More specifically, our results demonstrate that an invertebrate, *Ischnura*, shows a more risky higher activity in turbid water compared to clear water, and are compatible with the idea that fish-*Ischnura* interactions are primarily mediated by direct effects in turbid water, and increasingly by both direct and indirect effects in clear water. Further research should focus on the possible consequences of the observed patterns of altered life history for population dynamics and community structure in prey populations of clear-water and turbid lakes.

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Chapter III

Behavioural linkage of pelagic prey and littoral predators: microhabitat selection by *Daphnia* induced by damselfly larvae

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[Oikos (2004) 107: 265-272]

Abstract

Only recently ecologists started treating the previously separately considered benthic, littoral and pelagic zones of lake ecosystems as closely connected compartments. Here we study a link between organisms belonging to a different compartment - namely the pelagic and the littoral - through behaviour in a series of laboratory experiments. Waterfleas of the genus Daphnia *are inhabitants of the pelagic zone and suffer a high predation pressure from syntopic vertebrate predators (mainly fish). Presumably to escape this predation, they sometimes migrate in the day to the littoral to seek refuge within macrophytes and return to the pelagic at night. Zygopterans from the genus* Ischnura *do commonly co-occur in ponds with* Daphnia *and are known as opportunistic predators of* Daphnia*. In two initial experiments in microcosms in the lab we showed that* Ischnura *larvae are littoral predators strongly associated with macrophytes. Although we found that predation rates of individual* Ischnura *larvae on* Daphnia *are approximately 1.5 fold lower in macrophytes compared to open water, total predation from* Ischnura *on* Daphnia *per unit area is tenfold higher within macrophytes than in open water, making the open water a safer place for* Daphnia *with regard to* Ischnura *predation. In a third microcosm experiment we monitored horizontal distribution of* Daphnia *in the absence, presence and odour only of* Ischnura *larvae. After 2 hours, on average 10 % less* Daphnia *remained within the vegetation when* Ischnura *larvae or only their odour were present compared to when* Ischnura *or* Ischnura *odour were absent. We interpret this as a behavioural anti-predation response of* Daphnia *to the presence of* Ischnura *larvae that seems primarily chemically mediated. The observed horizontal migration of the pelagic prey driven by the littoral predator may couple both lake compartments and may interact with the predator-prey relationships within the pelagic.*

Introduction

Predation has the potential to intensely couple the dynamics of a predator and its prey (Kerfoot and Sih 1987, Begon et al. 1996). Mostly predator and prey inhabit the same well-defined habitat and contribute to a habitat specific discrete food web. However, food webs and their dynamics can be strongly influenced by the behaviour and movement of prey and predators across habitats or even ecosystems (e.g. benthic-pelagic coupling in marine systems, Boero et al. 1996; terrestrial-aquatic linkage, Nakano and Murakami 2001). Within lentic freshwater ecosystems, several studies have linked pelagic and littoral habitats by showing a coupling of benthic prey to pelagic fish through migration of the predator (Schindler and Scheuerell 2002 and references therein). Recently, there is also an increasing interest in the coupling between the pelagic and the littoral due to mobility of the prey from the pelagic to the littoral (e.g. MacIsaac et al. 1992).

In recent years it has become apparent that planktonic *Daphnia* - a typical and important member of the pelagic food web (Scheffer 1998) - exhibits diel horizontal migration behaviour (DHM) between the pelagic and the littoral (Kvam and Kleiven 1995, Lauridsen and Buenk 1996). DHM seems primarily an anti-predation strategy, driven by the presence of pelagic predators (Burks et al. 2002), and may be reversed when small zooplanktivorous fish hide in the littoral from pelagic piscivores (concept of behavioural cascade, Romare and Hansson 2003). Migrating to the littoral, however, may impose a new threat: mortality due to littoral predators. The possible influence of littoral predators on *Daphnia* and their DHM has repeatedly been stressed, but empirical data to the subject are scarce. We know of two studies that strongly suggested a negative effect of littoral odonates on *Daphnia* densities in the field (Blois 1985, Burks et al. 2001b), indicative of a direct pelagic-littoral link. Unfortunately, these studies did not make behavioural observations. As such, it is not known whether *Daphnia* recognizes the presence of littoral predators and responds with adaptive anti-predation strategies. Induced anti-predation behaviour may influence the impact of littoral predators and may impact the DHM itself. No studies have yet focused on this behavioural link between pelagic prey and littoral predators.

In this study we examine horizontal distribution patterns of *Daphnia magna* in the absence and presence of *Ischnura elegans*, a common littoral zygopteran predator. We first investigate microhabitat choice and predation effectiveness of *Ischnura* larvae in relation to the presence or absence of macrophytes. *Ischnura* larvae are hypothesized to prefer macrophytes above open water conditions. Under the hypothesis of adaptive anti-predation behaviour of the pelagic prey to the littoral predator, we expect *Daphnia* to

avoid macrophytes that are inhabited by *Ischnura* more than macrophytes without *Ischnura*. More specifically, as visual communication is often hampered in aquatic habitats and prey mostly relies on chemical cues (Brönmark and Hansson 2000), we expect *Daphnia* to avoid macrophytes in the presence of *Ischnura* odour. Because the combination of visual, mechanical and chemical stimuli may be stronger than the presence of chemical cues only, we hypothesize the response of *Daphnia* to the actual presence of *Ischnura* to be stronger or equal than to the presence of *Ischnura* odour only.

Material and methods

Experiment 1: Microhabitat choice of Ischnura elegans

This experiment was conducted to determine microhabitat use of *Ischnura elegans* van der Linden (Odonata: Zygoptera). Final instar *Ischnura* larvae were collected in a small fishless pond in Overijse, Belgium on 21 November 2002 and kept on dechlorinated tap water in an environmental chamber (T = 20°C, Dark/Light = 18/6h) until the day of the experiment. Larvae were fed *Artemia sp*. nauplii every other day until three days before the experiment. The experiment itself was conducted on 26-28 November 2002 in half-filled 36 l glass aquaria (L = 49cm W = 30cm H = 24cm). In each aquarium, we distinguished a peripheral and a central oval zone each equaling half of the surface of the tank. To test for microhabitat preference (open water vs. macrophytes) of *Ischnura*, we used three different macrophyte treatments: no macrophytes (NOMF), macrophytes in the peripheral zone (MFP) and macrophytes in the central zone (MFC). In the macrophyte-infested zone, we installed a total of 24 shoots of plastic *Ceratophyllum demersum* (Plantastics) and 8 shoots of natural *Ceratophyllum demersum*. Each shoot measured 12 cm in length, producing a PVI (Percent Volume Infested) of 17% (Canfield et al. 1984). Natural macrophytes proved very fragile and difficult to handle. Therefore, we used the combination of plastic and natural macrophytes to provide the larvae with both olfactorial and visual stimuli of macrophytes. In treatments with macrophytes, the zone containing the macrophytes was bordered with green netting (1 cm mesh). This netting was considered a part of the heterogenic structure of the macrophytes itself and therefore we did not use it in the controls.

At the start of the experiment, 16 larvae of *Ischnura* were placed in the central zone together with 20 *Daphnia magna* Straus in order to mimic a natural situation with prey present. Neither *Ischnura* larvae nor *Daphnia* were hampered in their locomotion by the netting that bordered the macrophytes (F. Van de Meutter personal observation).

Daphnia species are known to be natural prey items of *Ischnura* (Thompson 1978, Corbet 1999). The position of the *Ischnura* larvae was then monitored at 1 h intervals for a total of 6 h. The experiment was replicated 10 times, with replicate observations of all treatments spread out equally and carried out over three days in random order (N = 4 day 1, N = 3 day 2 and N = 3 day 3).

To test for time and macrophyte treatment effects on macrophyte preference of *Ischnura* larvae, we used repeated measures analysis of variance (rmANOVA) with macrophyte treatment as between group and the number of *Ischnura* in the central zone at subsequent hourly counts in the same aquarium as the repeated measures. Day was used as a random block factor (Newman et al. 1997). We also performed a two-way analysis of variance (2-way ANOVA) with macrophyte treatment as grouping factor and the final number of *Ischnura* larvae in the central zone after 6 h as dependent variable. Day was used as a random block factor. We tested our hypothesis of preference of macrophytes with two a priori contrasts (Sokal and Rolff 1995). A first contrast tested for more *Ischnura* in the central zone in NOMF treatments versus MFP treatments. A second contrast tested whether there were more *Ischnura* in the central zone in MFC treatments versus NOMF treatments. Given the clear directionality of these contrast tests, we report 1-sided p-values.

Experiment 2: Predation rate of Ischnura *on* Daphnia *in presence or absence of macrophytes*

In small 2 l plastic aquaria (L = 17,5 cm W = 11,5 cm H = 15 cm), filled with dechlorinated tap water to the same level as experiment 1 (12 cm), we tested predation efficiency of *Ischnura* in the presence and absence of macrophytes. We used *Ischnura* larvae that were collected on the same date and pond as in experiment 1. The experiment was conducted on 10-11 December 2002. In aquaria with macrophytes, we used identical macrophyte densities (PVI = 17%) as in the peripheral zone of the experimental tanks in experiment 1, but now macrophytes were present in both the central and peripheral zone. Ten minutes before the start of the experiment, we introduced 20 *Daphnia magna* in the central area of the aquaria. The experiment started when a total of 12 final instar *Ischnura* larvae were added to the aquaria. To obtain a uniform distribution of the larvae, we divided each aquarium in 12 equal-sized cells by placing a grid above it. One larva was lowered in the center of each cell. The number of living *Daphnia* in each tank was recorded at 20 minutes intervals for 2 h. At the end of the experiment, all remaining *Daphnia* were alive, indicating that no natural mortality

occurred during the experiment and that all missing *Daphnia* could be attributed to predation. We performed the experiment on two consecutive days, with four replicate observations on each date. Except for two individuals, all *Daphnia* in all treatments were eaten after 2 h. Therefore, we used the number of *Daphnia* eaten by *Ischnura* after 20 minutes as the response variable. Additionally, we assessed in which 20 minutes interval 50% of the *Daphnia* were eaten.

To test for the influence of macrophytes on *Ischnura* predation rate on *Daphnia*, we used 1-way ANOVAs that were blocked for time (Day). One analysis considered the number of *Daphnia* preyed upon after 20 minutes, and the other analysis the 20 minutes interval at which 50% of the *Daphnia* were eaten. Data for the latter analysis were skewed towards high values and were therefore log10-transformed to normalize them.

Experiment 3: Effect of Ischnura *on the horizontal distribution of* Daphnia

In this experiment, we tested the response in horizontal distribution of *Daphnia* to the absence of *Ischnura* larvae, the presence of *Ischnura* larvae themselves and of their odour only. *Daphnia* used in the experiment consisted of a mixture of eight different clones, randomly hatched from resting eggs from a fish lake with a moderately developed littoral zone (Oud-Heverlee, Belgium; Cousyn et al. 2001). The clones were reared separately on *Scenedesmus acutus* and intermixed on the day of the experiment. Only parthenogenetic females carrying their first brood were used to equalize *Daphnia* size across treatments and experiments. Final instar *Ischnura* larvae were collected at a small fishless pond in Overijse, Belgium on 9 December 2002 and treated further as in experiment 1. The experiment was conducted on 17, 18 and 20 December 2002. We used the same aquaria and applied the same compartments as in experiment 1. We used the same macrophyte density of 32 shoots (24 plastic, eight natural), all placed in the peripheral zone. Since *Daphnia* rely on both structural and olfactorial stimuli for detecting macrophytes (Pennak 1973, Lauridsen and Lodge 1996) and since real macrophytes were very fragile and difficult to handle, we used a combination of plastic and natural *Ceratophyllum demersum*.

Three different predator treatments were applied: presence of *Ischnura* larvae themselves, odour only of *Ischnura* larvae, and no larvae or odour of *Ischnura* larvae present. In treatments with *Ischnura* present, 16 larvae were placed in the peripheral zone, 30 minutes before the experiment started. To prevent the larvae from eating *Daphnia*, their labial mask used for capturing prey was tied to the head with a fine thread. Larvae treated as such still advanced towards and attacked prey but were

unable to catch *Daphnia* (F. Van de Meutter personal observation). In treatments with *Ischnura* odour, 3 l of *Ischnura* conditioned medium was added to the macrophyte zone, 5 minutes before the experiment started. *Ischnura* medium was created by keeping 30 *Ischnura* larvae without food for 24 h in a tank filled with 9 l of dechlorinated tap water. The number of animals in the tank equals maximal densities of *Ischnura* observed in the field (F. Van de Meutter unpublished data). The procedure to make the medium is similar to the commonly used protocol for creating fish medium in experiments concerning anti-predation behaviour with *Daphnia* (De Meester 1993). At the start of the experiment, we added 20 *Daphnia* to the central zone of each aquarium. The response variable was the visually determined number of *Daphnia* in the central zone recorded at 15 minutes time intervals for 2 h. We chose for the 2 h duration because a pilot experiment and literature data showed a stabilization of the response within 2 hours (Lauridsen and Lodge 1996, Burks et al. 2001a, F. Van de Meutter personal observation). All aquaria were placed in a row and the position of the different treatments was assigned randomly. Since *Daphnia* of both aquaria placed at the ends of the row aggregated in the outer corners - probably due to differing light conditions - these aquaria were left out from the analysis. The experiment was blocked for time because conducting all 10 replicates on the same day was logistically not possible (N = 4 on day 1, N = 3 on day 2 and N = 3 on day 3).

We used a repeated measures analysis of variance (rmANOVA) to test for time and predator effects on horizontal position of *Daphnia*. Predator treatment was treated as between group and the number of *Daphnia* in the central zone on different times as the repeated measure of the within group variable. Day was used as a random block factor. Final counts (after 2 h) of the number of *Daphnia* in the central zone were tested for predator treatment effects with a two-way analysis of variance (2-way ANOVA). Predator treatment was used as grouping variable and Day as random block factor. Specific hypotheses were tested using a priori contrasts. Because we expect *Daphnia* to avoid macrophytes with *Ischnura,* a first contrast tested for higher numbers of *Daphnia* in the central open zone in treatments with *Ischnura* or *Ischnura* odour present versus the absence of *Ischnura*. A second contrast tested whether the avoidance of macrophytes by *Daphnia* was stronger when *Ischnura* were present versus presence of *Ischnura* odour only. Given the clear directionality of these contrast tests, we report 1-sided p-values. All statistical analyses were performed in SAS 8.02 (SAS Institute inc.).

Results

Experiment 1: Horizontal distribution of Ischnura elegans

The number of larvae inhabiting the central zone of the tank differed significantly among treatments and stayed constant during the six hours of the experiment (rmANOVA, Macrophytes: $F_{2,25} = 664.38$, $P < 0.001$; Time: $F_{5,125} = 0.84$, P = NS; Time x Macrophytes: $F_{10,125} = 1.6$, P = NS; Day and all interactions with Day had $P > 0.243$; see Fig. 1). The treatment with macrophytes present in the central zone contained significantly more *Ischnura* in the central zone than the control without macrophytes (97% vs. 30%; Contrast [MFC] vs. [NOMF]: $F_{1,25} = 657.91$, $P_{1\text{-sided}} < 0.001$). The treatment with macrophytes in the peripheral zone harbored significantly less *Ischnura* in the central zone than the control (5% vs. 30%; Contrast [MFC] vs. [NOMF]: $F_{1,25} = 92.28$, $P_{1\text{-sided}} < 0.001$). Hence, we can conclude that more than 95% of the larvae occupied the macrophyte microhabitat whenever it was present, independent of whether it was located at the peripheral zone or in the middle of the aquaria. When macrophytes were absent, larvae occurred more in the peripheral zone of the aquaria (about 70%), probably because they seek refuge in the corners.

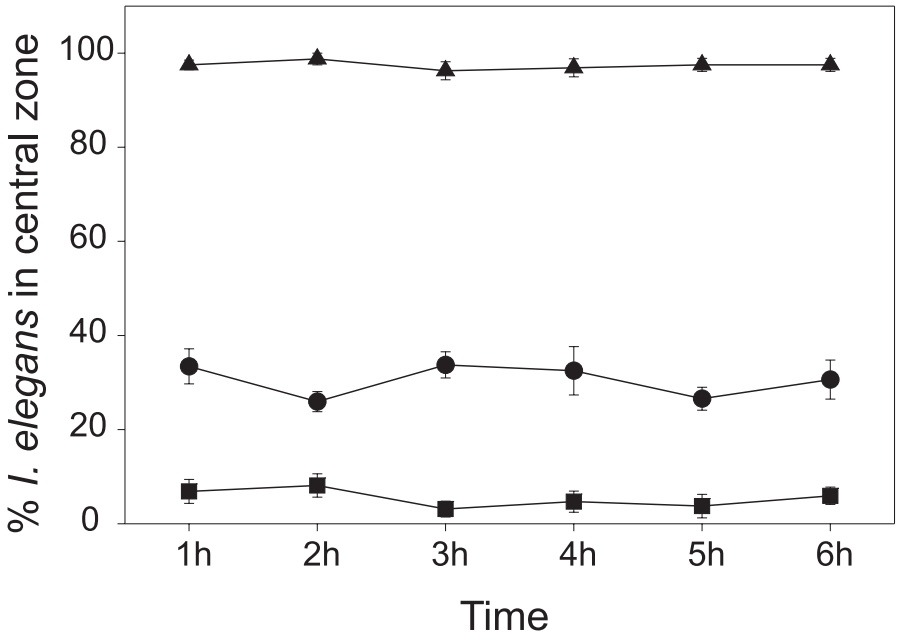

Fig. 1. Influence of microhabitat treatment on percentages (±1 SE) of *Ischnura* larvae occupying the central area of the aquaria for six consecutive hours. (● = no macrophytes, ▲ = macrophytes in the central zone; ■ = macrophytes in the peripheral zone).

Experiment 2: Predation rate of Ischnura *on* Daphnia *in the presence or absence of macrophytes*

The predation rate after 20 minutes of *Ischnura* on *Daphnia* differed significantly between macrophyte treatments. In the absence of macrophytes, *Ischnura* ate more *Daphnia* (11.5 ± 0.87) than in the presence of macrophytes (7.88 ± 0.88) (Macrophytes: $F_{1,12} = 10.65$, P = 0.0072; Day and Day x Macrophytes: $F_{1,12} < 4.57$, P = NS). This lower predation rate in the presence of macrophytes was also reflected in a shorter time needed to eat 50% of the *Daphnia* in the absence (25 ± 3.3 minutes) than in the presence (47.5 ± 8.4 minutes) of macrophytes (Macrophyte treatment: $F_{1,12} = 10.43$; P = 0.0076; Day and Day x Treatment: $F_{1,12} < 1.85$, P = NS).

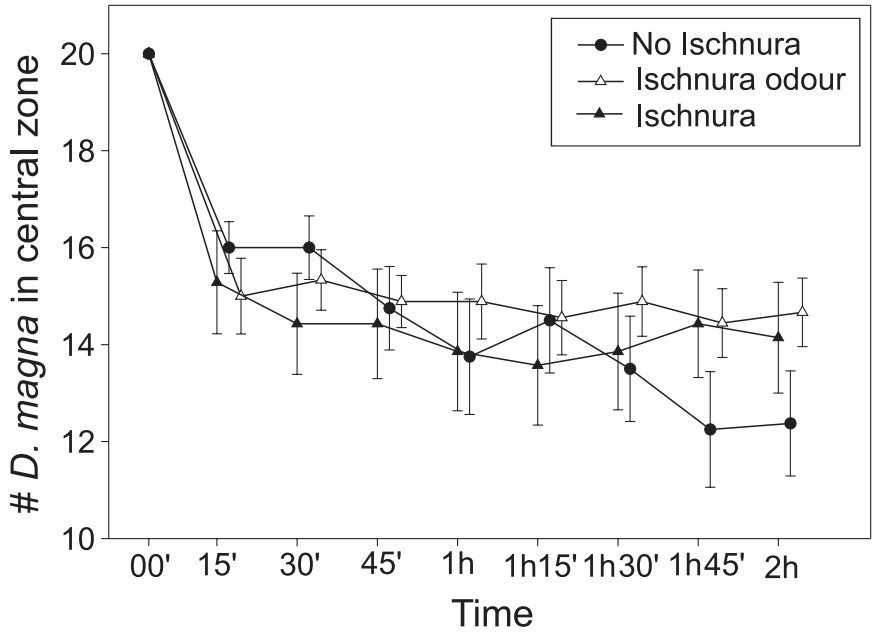

Fig. 2. Influence of predator treatment (No *Ischnura*, *Ischnura* odour, 16 *I. elegans* larvae present) on *Daphnia* horizontal distribution. Data represent the number of *Daphnia* (± 1 SE) in the central zone of the tanks. The peripheral zone had macrophytes, the central zone not. (△= *Ischnura* odour, ▲ = *Ischnura* larvae, ● = no *Ischnura*).

Experiment 3: Horizontal distribution of Daphnia magna *in the absence, presence or odour-only of* Ischnura

This last experiment tested for the response over time of *Daphnia* to the presence and odour-only of the predator *Ischnura*. The responses to each of the three predator

treatments showed a different pattern over time (rmANOVA, Treatment $F_{2,15} = 0.208$, $P = 0.82$; Time $F_{7,105} = 4.132$, $P < 0.001$; Time x Treatment $F_{14,105} = 2.012$, $P = 0.023$; Day and all interactions with Day had $P > 0.13$). Figure 2 shows a diverging pattern over time with treatments with *Ischnura* (IS) and *Ischnura* odour-only (ODIS) quickly stabilizing and the control without *Ischnura* (NOIS) still declining after 2 h. Overall, after 2 hours more *Daphnia* were present in the central open zone in treatments with *Ischnura* compared to controls (Contrast [ODIS and IS] vs. [NOIS]: $F_{1,15} = 3.46$, $P_{1\text{-sided}} = 0.041$). In the presence of *Ischnura* or *Ischnura* odour-only, *Daphnia* clearly avoided further entering the vegetation in the peripheral zone. After two hours, this resulted in 62 % of the *Daphnia* residing in the central open area for the control and 71 % and 73 % for predator and predator odour treatments, respectively. We detected no difference between the *Ischnura* and *Ischnura* odour only treatment (Contrast [ODIS] vs. [IS]: $F_{1,15} = 0.008$, $P_{1\text{-sided}} = 0.47$).

Discussion

Our results show a behavioural response of a pelagic prey (*D. magna*) towards a strictly littoral predator (*I. elegans*). Moreover, our two initial experiments suggest the adaptiveness of this antipredation behaviour. A behavioural response of *Daphnia sp.* to invertebrate predators has so far only been demonstrated to syntopic pelagic predators [e.g. *Anisops, Chaoborus, Notonecta* (De Meester et al. 1999, Kleiven et al. 1996, Nesbitt et al. 1996, Barry 2000)].

We showed *Ischnura* to be strongly associated with macrophytes. Several observational studies reveal the same pattern in the field (Olson et al. 1995, Weatherhead and James 2001). Macrophytes offer a relatively safe environment against fish predation (Crowder and Cooper 1982, Dionne et al. 1990, Diehl 1993) and often harbor large densities and diversities of macroinvertebrates (Diehl et al. 1992, Olson et al. 1995) that may serve as prey items for *Ischnura* (Thompson 1978, Corbet 1999) making it an ideal habitat for damselfly larvae. In the field, this pattern may be enhanced since many damselfly species exhibit endofytic egg-laying behaviour (Corbet 1999).

We also illustrated experimentally that *Ischnura* preyed upon *Daphnia* at a higher rate in open water than within macrophytes. Other studies either reported similar results (Convey 1988) or did not find a significant impact of structural heterogeneity on predation rate of odonates on *Daphnia* (Burks et al. 2001b). The latter result may be due to an insufficiently long exposure time to detect differences. Convey (1988) attributed the lower predation rate within macrophytes to lower encounter rates in the presence of

structural heterogeneity, as capture efficiency was significantly higher from perches (wooden sticks) compared to in the open water.

Combining information from our two initial experiments strongly suggests open water to be a safer place than macrophytes for *Daphnia* with regard to *Ischnura* predation. We can calculate the absolute impact (T) of *Ischnura* predation in both habitats as T = H x P, with H the percentage of the *Ischnura* in each habitat, and P a measure of predation impact per individual predator or per surface area. In our experiment, the latter can be expressed as the number of *Daphnia* (N_D) eaten per *Ischnura* per surface area (S = 2.0125 m^2) in 20 minutes [P = N_D / (S x *Ischnura* x 20 min)]. Based on our data, this results for the macrophyte habitat in 0.95 x 7.88/(2.0125 x 10^{-2} x 16 x 20) = 23.25 *Daphnia*/(m^2 x *Ischnura* x 20 min). For the open water we get 0.05 x 11.5 / (2.0125 x 10^{-2} x 16 x 20) = 1.79 *Daphnia*/(m^2 x *Ischnura* x 20 min). Hence, there is a tenfold higher absolute predation impact after 20 minutes of *Ischnura* on *Daphnia* in macrophyte habitat compared to open water. Therefore, the open water is the safer environment for *Daphnia* in respect to damselfly predation, and avoidance of macrophytes inhabited by damselflies is an appropriate and adaptive response.

An important question that arises is to what extent these results interact with earlier findings on horizontal migration behaviour and what importance they can have on fish-*Daphnia* and odonate-*Daphnia* interactions. Very little is known yet about what impact littoral invertebrate predators may have on pelagic daphnid populations. Blois (1985) analyzed the larval diet of three anisopteran larvae and showed a strong predatory link to pelagic *Daphnia*. Burks et al. (2001b) demonstrated in a field enclosure experiment that an assemblage of odonate larvae at a macrophyte density of 20 PVI can negatively influence densities of a natural pelagic daphnid community. Avoidance behaviour of *Daphnia* to macrophytes containing *Ischnura* larvae may reduce mortality rates from predation by odonates, but no data on this issue exist from the field. Several field studies report that *Daphnia* in fish lakes often aggregate at the edges of macrophyte beds instead of entering them, but a relationship with the presence of littoral predators has not been firmly established yet (Lauridsen and Buenk 1996, Lauridsen et al. 1998).

Our results suggest that the behavioural coupling between both compartments is chemically mediated. The horizontal distribution of *Daphnia* did not differ between macrophytes that contain *Ischnura* themselves and macrophytes that contain *Ischnura* odour only. Although some studies report visually and mechanically mediated predator detection in zooplankton (Bollens et al. 1994), kairomones released by predators seem

to be among the most important cues for *Daphnia* to detect their predators and to exhibit anti-predation behaviour (Tollrian and Harvell 1999). The chemical features of the kairomones of a set of different fish species (Cyprinidae, Esocidae, Gasterosteidae) were shown to be identical, indicating that their active compounds are very similar (von Elert and Pohnert 2000). *Daphnia* indeed shows identical anti-predator behaviour to kairomones of all above mentioned fish families (von Elert and Pohnert 2000). In contrast to the situation with fish, anti-predator behaviour induced by macroinvertebrate predators seems more predator-specific. Nesbitt et al. (1996) showed that *Daphnia* responds with different diel vertical migration behaviour to the presence of *Notonecta* or the presence of *Chaoborus*. This suggests that *Daphnia* can distinguish between different macroinvertebrate predators and exhibit differential and adaptive anti-predation behaviour. Studies towards the chemical nature and characteristics of these macroinvertebrate kairomones are scarce (but see Parejko and Dodson 1990).

From our results, it is impossible to discern whether *Daphnia* exhibits horizontal migration behaviour away from macrophytes in the presence of the *Ischnura* kairomone or whether *Daphnia* responds directly to a gradient of kairomones (higher concentrations of the kairomone in the macrophytes) with a directional escape behaviour. We did not prevent mixing with the rest of the water volume in the aquaria when adding the kairomone infested water to the macrophytes, and it is not certain whether some gradient was maintained through the experiment. However, such a gradient in predator cues may exist in the field, as shown by Turner and Montgomery (2003) in a field experiment with a snail and its fish predator. Since *Ischnura* larvae are tightly associated with macrophytes (this study), is it plausible to imagine that a permanent gradient of kairomones may stretch out from *Ischnura* inhabited macrophyte zones. Finally, it is unclear whether our results still hold in a natural environment with a cocktail of chemical predator cues present. Field experiments on DHM of *Daphnia* combining two or more predator (cues) are needed to verify this.

Compared to horizontal migration behaviour induced by fish, the response to damselfly larvae is smaller and in the opposite direction [Fish: 35-50% more animals in the vegetation (Lauridsen and Lodge 1996, Burks et al. 2001a); Damselfly: 10% less in the vegetation (present study)]. To the extent that we can compare the horizontal migration behaviour induced by fish with horizontal migration behaviour induced by macroinvertebrates, we put forward four non-exclusive explanations for the stronger response induced by fish compared to macroinvertebrates. First, zooplanktivorous fish are syntopic pelagic predators. Second, fish may constitute a more important predator in

regulating population dynamics of large-bodied *Daphnia* (Luecke et al. 1990, Boersma et al. 1996, Gliwicz et al. 2000). Third, macrophytes themselves have a strong repellent effect on *Daphnia* (Pennak 1973, Dorgelo and Heykoop 1985, this study). This repellent effect is based on both structural and chemical characteristics of macrophytes (Lauridsen and Lodge 1996, Burks et al. 2001a). The scope for an additional repellent effect from damselflies that inhabit these macrophytes is therefore likely to be smaller than the reverse effect of fish induced DHM into the macrophytes. And fourth, it is possible that the concentration of the fish kaïromone is higher than that of the macroinvertebrate kaïromone, hence producing a higher response.

An important consequence of horizontal migration behaviour is that the pelagic and the littoral of a lake become connected. In many experimental and field studies on horizontal migration behaviour, littoral zone inhabitants have been neglected even though their potential importance has repeatedly been stressed (e.g. Burks et al. 2001b, Burks et al. 2002). The repeated invasions of the littoral by *Daphnia* may have important direct and indirect effects on the littoral zone food web, as they represent extra prey, predators or competitors, depending on the focal littoral species. To what extent DHM of *Daphnia* promotes a strong coupling between the pelagic and the littoral lake habitats needs to be further quantified.

Further research should be oriented towards the joint effects of pelagic and littoral predators on horizontal migration behaviour in *Daphnia*. Nothing is known so far about *Daphnia* horizontal migration behaviour when confronted with both fish and littoral predators. It is expected that the benefit of escaping pelagic predators must be balanced with the costs of confronting predators in the littoral zone. Estimates of the relative costs related to predation, competition and abiotic stress of staying in the pelagic or moving to the littoral are needed to be able to model the relative fitness in both habitats in the field. Such studies may help us understand the timing and pattern of DHM in zooplankton.

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Chapter IV

Spatial avoidance of littoral and pelagic invertebrate predators by *Daphnia*

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Abstract

Studies on spatial avoidance behaviour of predators by prey often ignored the fact that prey typically face multiple predators which themselves interact and show a spatial pattern in abundance and predation rates. In a series of laboratory experiments we investigated predation risk and horizontal migration of the cladoceran Daphnia magna *between open water and vegetation in response to two important invertebrate predators with a contrasting spatial distribution: pelagic* Chaoborus *and vegetation-associated* Ischnura*. As expected, predation risk by* Chaoborus *was higher in open water due to higher numbers and higher predation rates of* Chaoborus*, while for* Ischnura*, predation risk was highest in the vegetation due to higher densities but despite lower predation rates of* Ischnura*. In accordance with this,* Daphnia *moved into the vegetation in the single presence of the pelagic* Chaoborus*. In the single presence of* Ischnura*, however,* Daphnia *showed no response. We hypothesize this may be the result of a constitutive behaviour of* Daphnia *to avoid pelagic fish, which impedes a response to the open water. In the combined predator treatment,* Daphnia *migrated to the open water zone. The increased risk of predation in the vegetation, due to a facilitating effect of* Chaoborus *on* Ischnura *predation rates is believed to have caused this migration of the* Daphnia*. This response of* Daphnia *declined through time and* Daphnia *moved toward the vegetation. A decline in the activity of the* Ischnura *larvae through time may have switched the risk balance in favor of the vegetation environment.*

Introduction

Predation is one of the key factors that control the structure and dynamics of natural ecosystems (Kerfoot and Sih 1987, Wellborn et al. 1996). Recently, two important insights have contributed to our understanding of the role of predators in communities. First, both lethal and non-lethal effects of predators on their prey may drastically change when other predators are present (Sih et al. 1998, Relyea 2003). The net effect of combined predators depends mainly on the relative risk of each predator to the prey, on the directionality of the prey response to each predator in isolation and on predator-predator interactions (Relyea 2003). An emerging pattern is that prey generally perceive the risk of combined predators as being similar to the risk of the most dangerous predator in the pair, and not as a summed or averaged predation risk (Relyea 2003). Second, predators are not passive sources of risk to which prey responds, but active participants in predator-prey interactions, which may respond strategically to prey behaviour (Lima 2002). For example, allowing predators to move in response to prey movements may not only radically alter standard expectations with respect to prey behaviour, but may also reveal new classes of behavioural phenomena that occur at larger spatial scales than traditionally considered in lab studies (Lima 2002). Especially, when looking at spatial interactions between predators and prey, taking into account those new insights may be rewarding. Although situations where prey face several predator species simultaneously, are expected to be quite common in nature (Schoener 1989, Polis and Strong 1996), empirical tests of these ideas are largely lacking (but see Kohler and McPeek 1989, Nesbitt et al. 1996, Eklov and Werner 2000).

Spatial avoidance behaviour has been particularly well documented in cladocerans of the genus *Daphnia* and can include both vertical migration (De Meester et al. 1995, Boersma et al. 1998, De Meester et al. 1999) and horizontal migration (Lauridsen and Buenk 1996, Lauridsen and Lodge 1996, Burks et al. 2002). Several invertebrate predators with contrasting habitat use have been shown to induce vertical migration in *Daphnia.* In the presence of only larval *Chaoborus* predators and only adult *Notonecta* predators and their kairomones, upward and downward movements of *Daphnia* have generally been reported, respectively (reviewed by De Meester et al. 1999). So far, only Nesbitt et al. (1996) confronted *Daphnia* with both types of predators. In the presence of both *Chaoborus* and *Notonecta*, the upward migration response to the presence of *Chaoborus* was modified in the daytime (but not at night) to simultaneously avoid areas occupied by each predator. Several invertebrate predators have striking horizontal patterns of distributions in ponds (Voss and Mumm 1999, Marklund et al.

2001, Hampton 2004) and may have a large predation impact on cladoceran communities, especially in fish-free habitats (Wissel et al. 2000, Burks et al. 2001b, Steiner and Roy 2003, Blumenshine and Hambright 2003). The resulting horizontal pattern of predation risk could lead to horizontal avoidance behaviour by *Daphnia*. This has been shown for *Daphnia* and its damselfly predator *Ischnura*, where *Daphnia* increasingly avoided vegetation inhabited by *Ischnura* (Van de Meutter et al. 2004, Chapter III).

In this study, we present results on horizontal migration behaviour of *Daphnia* in response to the presence of two invertebrate predators, the phantom midge *Chaoborus* and the damselfly larva *Ischnura*, which may also prey upon *Chaoborus*. In addition, we also considered the spatial distribution of the predators and the resulting predator-predator interactions as advocated by Lima (2002). Both *Chaoborus* and *Ischnura* predators are common inhabitants of ponds and lakes throughout Western Europe and are known to prey upon *Daphnia* (Blois 1985, Tollrian and Dodson 1999). *Chaoborus* larvae are considered pelagic, where they can survive even in the presence of fish because of vertical migration behaviour (Voss and Mumm 1999, Berendonk et al. 2003). We therefore predicted them to prefer the open water above the vegetation-dominated littoral zone. *Daphnia* has been shown to escape from *Chaoborus* cues by swimming away (Kvam and Kleiven 1995, but see Roozen and Lurling 2001). We therefore predicted that *Daphnia* would migrate to the vegetated littoral as a response to the presence of *Chaoborus* in the open water. In contrast to the pelagic *Chaoborus*, larvae of the damselfly *Ischnura* are strongly associated with aquatic vegetation, and *Daphnia* has been shown to avoid vegetation inhabited by *Ischnura* (Van de Meutter et al. 2004, Chapter III). Because *Chaoborus* and *Ischnura* probably induce responses in opposite spatial directions, we expect a spatial response away from the more risky predator (Relyea 2003). We evaluated this hypothesis by explicitly quantifying the relative risk imposed by each predator species in each environment, and by confronting this with the spatial response of *Daphnia* in the presence of both predators.

Material and methods

Final instar larvae of *Chaoborus obscuripes* van der Wulp (Diptera: Nematocera) were isolated from a small fishless pond in Neervelp, Belgium and were kept in a climate room (T = 20°C, L/D: 18/6) on the original pond water. Final instar larvae of *Ischnura elegans* van der Linden (Odonata: Zygoptera) were collected in a small fishless pond in Overijse, Belgium and kept on dechlorinated tap water in the same climate room as the

Chaoborus larvae. Both species were fed brine shrimp *Artemia* spec. nauplii ad libitum every other day until three days before the experiments took place. We choose not to feed the *Chaoborus* with *Daphnia* since *Daphnia* behaviour may be strongly affected by the chemicals released by crushed conspecifics (Pijanowska 1997), and could possibly lead to an overestimation of the effect of the predators on *Daphnia* behaviour. *Daphnia magna* Straus used in the experiments consisted of a mixture of seven clones, randomly hatched from resting eggs from a fish lake with a weakly developed littoral zone (Oud-Heverlee, Belgium; Cousyn et al. 2001). Both *Chaoborus* and *Ischnura* are present in the lake from which the *Daphnia* originated. The climate room in which the experiments were run has no windows and is lightened by 4 evenly distributed rows of fluorescent lamps attached to the ceiling. This way, a relatively uniform light regime of about 250 lux is obtained.

Experiment 1: Chaoborus *horizontal distribution*

This experiment was conducted to quantify microhabitat use of the *Chaoborus* larvae. *Chaoborus* larvae were collected on 25 November 2002. The experiment was conducted on 3-5 December 2002, in 36 l glass aquaria (49 cm long x 30 cm wide x 24 cm high), half-filled with aged tap water. We closely followed the methodology we previously used to determine microhabitat use in *Ischnura* damselfly larvae (Van de Meutter et al. 2004, Chapter III). In each aquarium, we distinguished a peripheral and a central oval zone each equaling half of the bottom surface. To test for microhabitat preference (open water vs. vegetation) of *Chaoborus*, we used three different vegetation treatments: no macrophytes (NOMF), macrophytes in the peripheral zone (MFP), and macrophytes in the central zone (MFC). By including the last two treatments, we can eliminate spurious associations with the vegetation, due to position preferences of the *Chaoborus* larvae within the aquaria. In the vegetation zone, we installed a total of 24 shoots of plastic *Ceratophyllum demersum* (Plantastics®) and eight shoots of natural *C. demersum*. Each shoot measured 12 cm in length, producing a PVI (Percent Volume Infested) of 17% (Canfield et al. 1984). Natural vegetation proved very fragile and difficult to handle. Therefore, we used a combination of plastic and natural vegetation to provide the larvae with both olfactorial and visual stimuli of vegetation. In treatments with vegetation, the zone containing the vegetation was bordered with green netting (1 cm mesh). This netting was considered part of the heterogenic structure of the vegetation itself and therefore we did not use it in the controls.

At the start of the experiment, 20 larvae of *Chaoborus* were placed in the central zone together with 20 *Daphnia magna* in order to mimic a natural situation with prey present. Both predator and prey densities used in this experiment occur under natural conditions in the field (Horppila et al. 2000, F. Van de Meutter unpublished results). Neither *Chaoborus* larvae nor *Daphnia* were hampered in their movements by the netting that bordered the vegetation (F. Van de Meutter personal observation). The position of the *Chaoborus* larvae was monitored at 1 h intervals for a total period of 6 h. An additional, final observation was made after 24 h. All three experimental treatments were replicated 10 times over a period of three days. The experiment was run in 10 aquaria at a time and one treatment was randomly assigned to one of the different aquaria. Before starting a new experiment, we thoroughly cleaned all aquaria and replaced the water (as is the case in all next experiments).

To test for effects of the vegetation treatment and time on the horizontal distribution of *Chaoborus* larvae, we used a repeated measures analysis of variance (rmANOVA) (Sokal and Rohlf 1995), with vegetation treatment as the between group variable and the subsequent counts of the *Chaoborus* larvae in the central zone as the repeated measures. Date and aquarium were used as random block factors. We additionally tested for an effect of vegetation treatment on final counts of *Chaoborus* after 24 h using a 3-way ANOVA that also included date and aquarium as random block factors. When significant results were obtained, Tukey-HSD tests were used for identifying significant differences among pairs of vegetation treatments.

Experiment 2: Chaoborus *predation on* Daphnia

In this experiment we addressed the question to what extent the presence of *Ischnura* and vegetation influences the predation activity of *Chaoborus* on *Daphnia*. *Chaoborus* and *Ischnura* larvae were collected on 31 October 2003. Since *Ischnura* can prey upon both *Daphnia* and *Chaoborus* (Corbet 1999), we tied their labial mask, which is used for capturing prey, to their heads with a fine thread. Larvae treated this way still attack prey items but are unable to catch them (F. Van de Meutter, personal observation).

The experiment was conducted in small plastic aquaria (32 cm long, 17 cm wide and 19 cm high) from 6 till 9 November 2003. We used smaller aquaria compared to the experiment testing horizontal migration of *Daphnia* experiments (see below) to facilitate the observation of predator-prey interactions between *Chaoborus* and *Daphnia*. We performed a randomized full-factorial experiment with vegetation (absent/present) and *Ischnura* treatments (0/6). We applied the same vegetation density as in the other

experiments by installing 24 stems of *C. demersum* (18 plastic, 6 natural) within the aquaria and added six *Ischnura* larvae to the respective treatment combinations. Ten *Daphnia* were added to each aquarium, ten minutes before the experiment started. *Daphnia*, *Chaoborus* and *Ischnura* densities (per unit water volume) were similar to those in the experiment testing for horizontal migration of *Daphnia*.

The predation experiment started by adding four *Chaoborus* larvae to each aquarium and lasted two hours. All *Chaoborus* and *Daphnia* were replaced before starting a new experiment. Predation activity was quantified in two different ways. First, by counting the number of *Daphnia* preyed upon by *Chaoborus* during four consecutive 30 minutes time intervals (at 30, 60, 90 and 120 minutes). Second, by counting the number of attacks of *Chaoborus* on *Daphnia* within a ten minutes time interval after 30 minutes and after 90 minutes. Only two out of four aquaria could be monitored for *Chaoborus* attacks at a time and we monitored each pair of randomly chosen aquaria for five minutes. At every 30 minutes time interval and prior to recording *Chaoborus* behaviour, we replaced all eaten *Daphnia* by living ones. A *Chaoborus* attack was defined as a definite striking movement with the post-antennal filaments at a *Daphnia* in close proximity to the larva (Swift and Fedorenko 1975). The experiment was replicated 10 times using 10 blocks of all of the four treatment combinations. Within each block, treatment combinations were assigned randomly to the different aquaria. Treatment effects on the number of eaten *Daphnia* at the subsequent time intervals were analyzed using repeated measures analysis of variance (rmANOVA). The *Ischnura* and vegetation treatments were used as between group variables, and the number of eaten *Daphnia* at the different time intervals as the repeated measures. Block was added as a random effect. The number of attacks by *Chaoborus* was analyzed analogously, with the number of attacks at 30 minutes and at 90 minutes as the repeated measures. To satisfy ANOVA assumptions, the number of attacks by *Chaoborus* on *Daphnia* was $\log_{10}$-transformed.

Experiment 3: Ischnura *predation on* Daphnia

In this experiment we examined to what extent predation activity of *Ischnura* on *Daphnia* depended on the presence of *Chaoborus* larvae and vegetation. *Chaoborus* and *Ischnura* were collected on 31 October 2003. We used the same small experimental aquaria as in the previous experiment and applied four different treatment combinations of vegetation (absent/present) and *Chaoborus* (0/4). In treatments with vegetation present, we applied identical vegetation densities as in all other experiments. In

treatments with *Chaoborus* larvae present, we added four *Chaoborus* larvae from which we cut off the postantennal filaments three days before the experiment started. *Chaoborus* larvae were anaesthetized in CO_2-saturated water prior to the operation. This operation is non-lethal and larvae treated this way continue attacking *Daphnia* but are unable to catch them (F. Van de Meutter, personal observation). Together with the *Chaoborus* larvae, we inoculated 10 *Daphnia* to each aquarium. Ten minutes after we added the *Daphnia* and *Chaoborus* larvae, we started the experiment by placing six *Ischnura* larvae in the aquaria. We ran four aquaria at a time and recorded the number of eaten *Daphnia* every 30 minutes for two hours. At every 30 minutes time interval, we replaced all eaten *Daphnia* by living ones. Therefore, all treatments started with the same number of *Daphnia* at the beginning of each 30 minutes time interval.

After 30 minutes, and again after 90 minutes, we recorded in each aquarium predation activity of all six *Ischnura* larvae on *Daphnia* for a period of five minutes. *Ischnura* larvae catch their prey from ambush or after a short approach to the prey (Corbet 1999). During each five minutes observation period, we recorded four behaviours that are directly related to predation activity: the number of head orientations towards prey, advances towards prey, strikes with the labial mask towards prey, and prey captures (Stoks and Johansson 2000). Since only two aquaria could be monitored at a time, one pair of randomly chosen aquaria was monitored five minutes later than the other pair. Each treatment was replicated ten times in ten blocks with all four treatments. The experiment ran from 12 until 15 November 2003.

Treatment effects on the number of eaten *Daphnia* at the subsequent time intervals were analyzed using repeated measures analysis of variance (rmANOVA). The *Chaoborus* and vegetation treatments were used as between group variables, and the number of eaten *Daphnia* at the different time intervals as the repeated measures. Block was added to the analysis as random effect. The set of four behavioural variables of predation activity was first collapsed into a single variable with a Principal Component Analysis (Sokal and Rohlf 1995). We ran the same rmANOVA model as before, but now with the principal component reflecting predation activity at 30 minutes and at 90 minutes as the repeated measures.

Experiment 4: Daphnia *horizontal migration experiment*

We conducted this experiment to determine the response in horizontal distribution of *Daphnia* to the presence of both *Chaoborus* and *Ischnura*. *Ischnura* larvae were collected on 3 March 2003 and *Chaoborus* larvae on 4 March 2003. We used the same

large aquaria as in experiment 1 and applied the same compartments, but all aquaria had only vegetation present in the peripheral area (MFP of the first experiment). This situation resembles most the natural situation, with vegetation present in the littoral zone. We used four different predator treatments in this experiment: no predators present, *Chaoborus* present, *Ischnura* present and both *Chaoborus* and *Ischnura* present. To prevent the *Chaoborus* larvae from eating the *Daphnia*, we cut off their postantennal filaments as in the previous experiment. *Ischnura* larvae were treated as in the second experiment to prevent them from eating *Daphnia* or *Chaoborus*.

The experiment was conducted on 11, 12, 13 and 17 March 2003. One hour before the experiment started, we introduced 10 *Chaoborus* into the central open water-zone and 16 *Ischnura* into the peripheral vegetation-zone of the aquaria. These densities (16 *Ischnura*/ 18 l and 10 *Chaoborus*/ 18 l) are equal to maximal densities of these predators we recorded in Belgian fishponds (F. Van de Meutter unpublished data). The experiment started when 20 *Daphnia* were added to the central zone of the aquaria. The response variable was the visually determined number of *Daphnia* in the central open-water zone recorded every 15 min for 2 h. We chose for an observation period of 2 h because previous experiments and literature data showed a stabilization of the response within two hours (Lauridsen and Lodge 1996, Burks et al. 2001a, F. Van de Meutter personal observation). In treatments with *Chaoborus* larvae present, we also recorded the number of *Chaoborus* in the central zone every 15 minutes for 2 h.

Ten aquaria were placed in a row and the different treatments were assigned randomly to aquaria. Because *Daphnia* in the aquaria placed at both ends of the row aggregated in the corners, probably due to different light conditions, we omitted these aquaria from analysis. The experiment was blocked for aquarium position and day because conducting all 32 replicates on the same day was logistically not possible. Eight replicates were run of each of the four treatments.

We tested for effects of the presence of the predator (*Chaoborus* and *Ischnura*) and day on the horizontal distribution of *Daphnia*, using a rmANOVA with *Chaoborus* and *Ischnura* treatments as between group variables and the subsequent number of *Daphnia* in the central zone of an aquarium as the repeated measures. Day and aquarium position were included as random block factors. We also performed a four-way analysis of variance (4-way ANOVA) with *Chaoborus* and *Ischnura* as independent variables (date and aquarium position were included as random block factors) and the final number of *Daphnia* in the central zone after 2 h as dependent variable. When the interaction between treatment effects was non-significant, we pooled the interaction term

MS with the error term MS (Crawley 2002). All statistical analyses were performed using STATISTICA 6.0 (Statsoft Inc. 2002). Means are given with 1 standard error.

Experiment 5: Foraging effort of restrained Ischnura

Daphnia's horizontal distribution in the presence of both restrained predators showed a strikingly changing pattern over time, suggesting a temporal pattern in predation risk by the predator *Ischnura* (Experiment 4, see further in the results section). Since we did not record predation attempts (foraging effort) of *Ischnura* during this experiment, we did an additional experiment testing whether foraging effort of *Ischnura* with restrained mouthparts on *Daphnia* in the presence of *Chaoborus* changes within a 2 hours time interval. *Ischnura* and *Chaoborus* larvae were obtained from the same ponds as in experiment 4 on 24 March 2004 and treated similarly. The experiment was run on 29 March in the same experimental containers as experiment 3 (predation experiment with *Ischnura*) and had the same vegetation cover and vegetation density as in experiment 4. First, we added 10 *Daphnia* and 4 *Chaoborus* to the aquaria. After ten minutes, we added six *Ischnura* larvae. Both *Chaoborus* and *Ischnura* larvae were treated to be unable to catch the *Daphnia* as in experiment 4. *Ischnura* foraging behaviour (walk, orient, strike) was recorded during 5 minutes intervals at 30, 60, 90 and 120 minutes. The experiment was replicated five times.

The set of three behavioural variables of predation activity was first collapsed into a single variable with a Principal Component Analysis (Sokal and Rohlf 1995). We ran an rmANOVA with the behavioural PCs at 30, 60 90 and 120 minutes as the repeated measures to test for differences in foraging effort over time.

Predation Risk

To validate whether the observed behaviour of *Daphnia* (Experiment 4) was adaptive with respect to predation risk by its predators, we calculated the actual predation risk to *Daphnia* for all 6 combinations of microhabitat (open water/ vegetation) and predator species (*Chaoborus*/ *Ischnura*/ *Chaoborus* + *Ischnura*). Predation risk is a function of both predator density and predation rate of the predators (Hampton 2004). In this study, we defined predation risk (PRI) as the number of *Daphnia* that is preyed upon (considering the *Daphnia* density used in our experiments) in the first hour of the predation experiments by *Chaoborus* and/ or *Ischnura*. We calculated PRI according to the following formula:

$PRI_{i,j} = N(CH)_{i,j} . PR(CH)_{i,j} + N(IS)_{i,j} . PR(IS)_{i,j}$

Indices i and j represent all combinations of microhabitat (i) and predator treatment (j). PR(CH) and PR(IS) are the per capita predation rates per unit surface area for *Chaoborus* and *Ischnura* respectively, and are based on predation rates obtained in the predation experiments. Note that predation rate not only varies with microhabitat but also may depend on the presence of the other predator. N(CH) and N(IS) are the mean numbers per unit aquarium bottom surface area of *Chaoborus* and *Ischnura* that were actually present in the specific microhabitat during the *Daphnia* horizontal migration experiment. In combinations with only one predator species present, the number of the other predator is zero, and its term in the equation will become 0.

Results

Experiment 1: Horizontal distribution of Chaoborus

The number of *Chaoborus* present in the central zone of the aquaria differed significantly among vegetation treatments and showed a different pattern over time across the vegetation treatments (rmANOVA, Vegetation: $F_{2, 16} = 9.503$, $P = 0.002$; Time: $F_{6, 96} = 7.309$, $P < 0.001$; Time x Vegetation: $F_{12, 96} = 3.42$, $P < 0.001$). The treatment with vegetation present in the peripheral zone (MFP) contained significantly more *Chaoborus* in the central open water-zone than the control without vegetation (NOMF) (69 ± 4 % vs. 41 ± 4 %: Tukey HSD test: $P < 0.001$). Both in the MFP treatment and the control, numbers of *Chaoborus* in the central zone stayed constant over time. In the treatment with vegetation present in the central zone (MFC), *Chaoborus* larvae gradually evaded this zone to the open water. After 24 h, 36 ± 5 % of the *Chaoborus* larvae occurred in the central zone containing vegetation (MFC), 38 ± 3% were in the central zone in the control, but much more (69 ± 5 %) of the *Chaoborus* larvae occurred in the central zone when the vegetation was in the peripheral zone (3-way ANOVA: Vegetation: $F_{2, 12} = 30$, $P = 0.002$, blocks and other treatments $P > 0.30$; Post-hoc Tukey HSD: MFP vs. MFC: $P < 0.001$; MFP vs. NOMF: $P = 0.99$). In the control without vegetation, *Chaoborus* showed a bias toward the peripheral zone of the aquaria (63 %, one-sample t-test with reference value 50 %: $t_9 = -4.628$, $P = 0.001$).

We can conclude that eventually ca. 65% of the *Chaoborus* larvae occupied the open water-zone independent of whether the vegetation were located in the peripheral zone or in the central zone of the aquaria.

Experiment 2: Chaoborus *predation on* Daphnia

Chaoborus ate less *Daphnia* in the presence of vegetation ($F_{1, 24}$ = 17.10, P < 0.001, Figs. 1A-B). The presence of *Ischnura* larvae did not influence the number of *Daphnia* eaten by *Chaoborus* ($F_{1, 24}$ = 0.178, P = 0.68). Predation intensity remained relatively constant over all treatments during the two hours of the experiment (Time: $F_{3, 72}$ = 1.677, P = 0.18). Attack frequency of *Chaoborus* on *Daphnia* yielded similar results: attack frequency was in general lower within vegetation and was unaffected by the presence of *Ischnura* (Vegetation: $F_{1, 24}$ = 14.783, P < 0.001, *Ischnura*: $F_{1, 24}$ = 0.730, P = 0.40). Attack frequency declined over time in open water, but remained at constant low level in the vegetation treatment (Time x Vegetation: $F_{1, 24}$ = 4.883, P = 0.037, Figs. 1C-D). Successful predation events were very infrequent during the five minutes monitoring bouts of predation activity and were not further analyzed. Out of 32 attacks, only four were successful.

Experiment 3: Ischnura *predation on* Daphnia

Predation by *Ischnura* on *Daphnia* was lower in the presence of vegetation ($F_{1, 27}$ = 23.542, P < 0.001, Figs. 2A-B). The number of *Daphnia* eaten by *Ischnura* per 30 minutes time interval declined over time averaging 6.3 ± 0.3 during the first hour of the experiment and 3.6 ± 0.3 over the second half (Time: $F_{3, 81}$ = 38.99, P < 0.001, Figs. 2A-B). Predation tended to be higher in the presence of *Chaoborus*, but this trend was not significant ($F_{1, 27}$ = 2.620, P = 0.12). During the first hour, we notice a distinct facilitating effect of *Chaoborus* on *Ischnura* predation ($F_{1, 27}$ = 7.741, P = 0.010), which was manifest in the vegetation treatment (Tukey HSD P < 0.001) but not in the open water treatment (Tukey HSD P = 0.92). During the second hour, *Chaoborus* did no longer affect *Ischnura* predation ($F_{1, 27}$ = 0.31, P = 0.58).

The principal component analysis on the four original predation behaviours extracted one new variable that explained 68 % of the original variance. This new variable was highly positively correlated with all four original variables: advances (0.77), head orientations (0.85), strikes (0.87), and captures (0.81) and therefore will be further referred to as predation activity.

Predation activity was negatively affected by the presence of vegetation ($F_{1, 27}$ = 5.817, P = 0.023, Figs. 2C-D). Overall, predation activity after 90 minutes was lower than predation activity after 30 minutes ($F_{1, 27}$ = 7.042, P = 0.013, Figs. 2C-D). The presence of *Chaoborus* larvae had no effect on *Ischnura* predation activity ($F_{1, 27}$ = 0.172, P = 0.68).

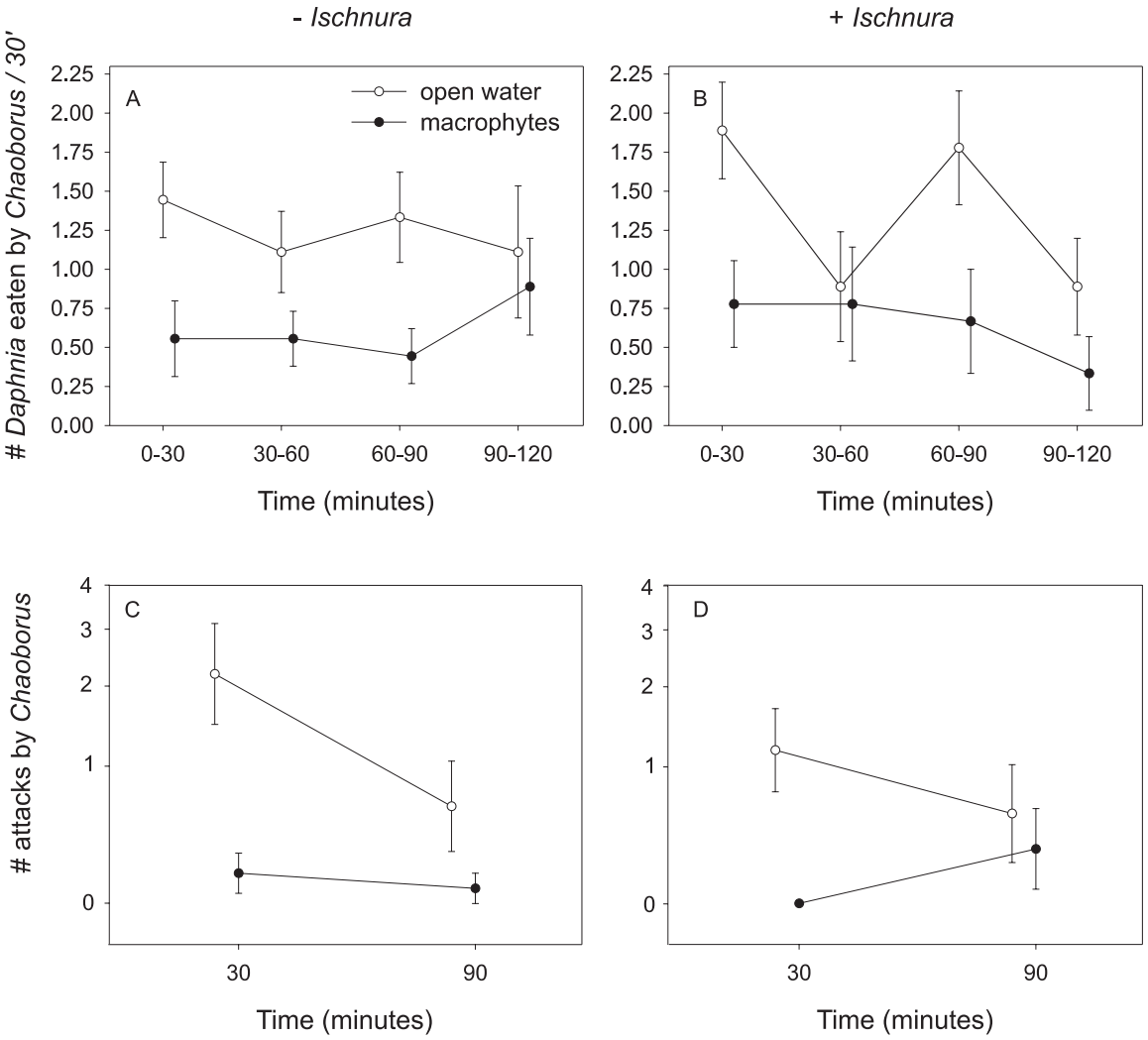

Fig. 1. Top panels show the number of *Daphnia* eaten by four *Chaoborus* larvae at four consecutive 30 minutes time intervals in the absence (A) and presence (B) of *Ischnura*. Bottom panels show the log-transformed number of attacks of four *Chaoborus* larvae within a five minutes time interval after 30 minutes and 90 minutes in the absence (C) and presence (D) of *Ischnura*. Filled symbols represent the vegetation treatment, open symbols represent the open water treatment. Means are given ± 1 standard error.

Experiment 4: Horizontal distribution of Daphnia

The number of *Daphnia* in the central open water-zone was determined by a complex interaction between the presence of *Ischnura*, *Chaoborus*, and time (rmANOVA, Time x *Ischnura* x *Chaoborus*: $F_{7, 175} = 2.952$, $P = 0.006$, Fig. 3). This interaction reflected a temporal pattern in the magnitude of the interaction between both predator treatments. After 15 minutes, we noticed a significant interaction between the *Ischnura* and the *Chaoborus* treatment (*Ischnura* x *Chaoborus*: $F_{1, 22} = 7.396$, $P = 0.013$): the number of *Daphnia* in the central open water-zone was higher than the control without predators

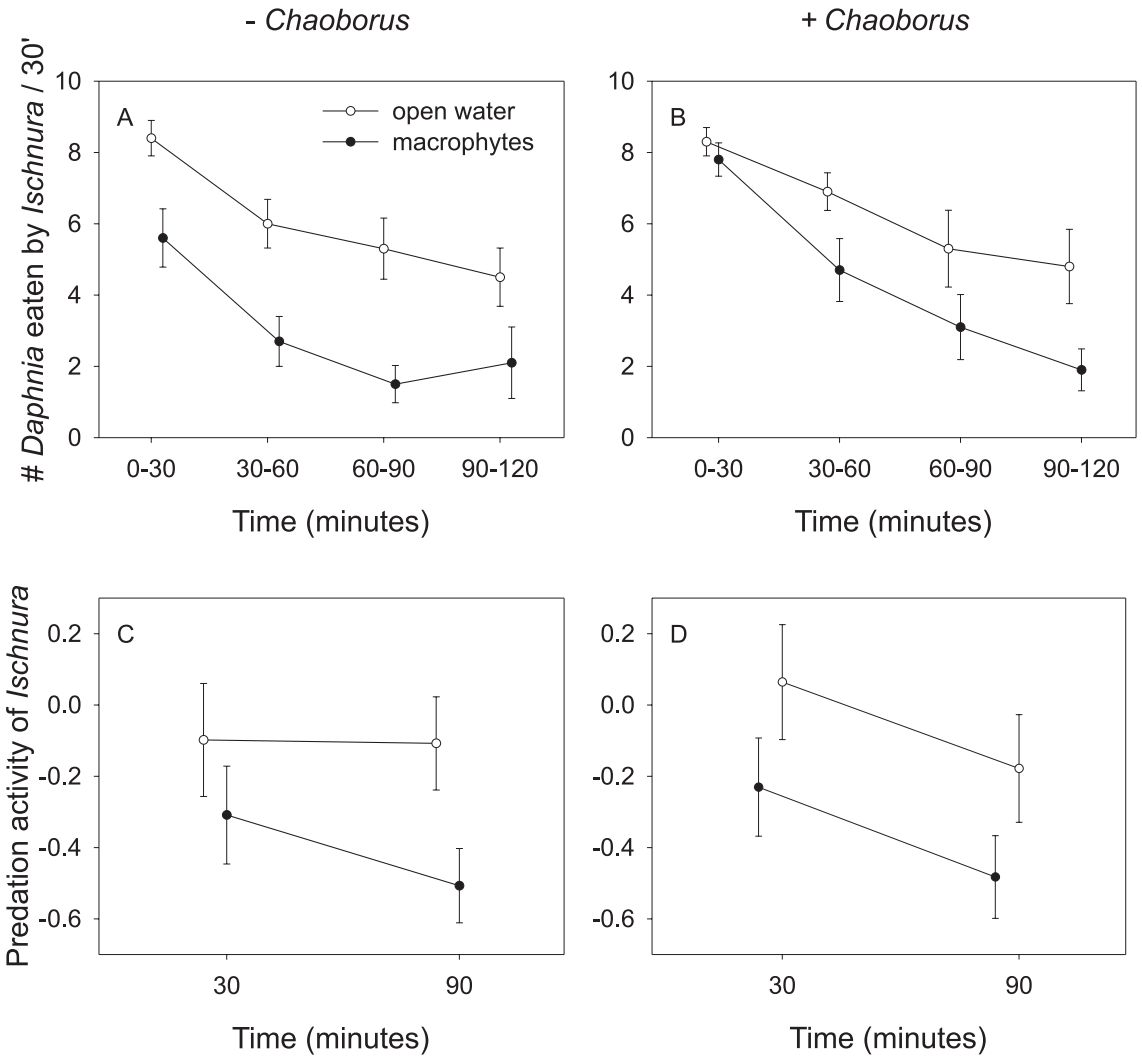

Fig. 2. Top panels show the number of *Daphnia* eaten by six *Ischnura* at four consecutive 30 minutes time intervals in the absence (A) and presence (B) of *Chaoborus*. Bottom panels show predation activity of the six *Ischnura* at 30 minutes and 90 minutes in the absence (C) and presence of *Chaoborus*. Predation activity is a principal component based on four behaviors monitored during five minutes after 30 minutes and after 90 minutes. Filled symbols represent the vegetation treatment, open symbols represent the open water treatment. Means are given ± 1 standard error.

when both *Ischnura* and *Chaoborus* were present, but tended to be lower than the control in the presence of only *Chaoborus*, and did not differ from the control in the presence of *Ischnura* only (Fisher LSD post-hoc, comparing control against *Chaoborus* + *Ischnura*: P = 0.032, against *Chaoborus*: P = 0.085, against *Ischnura*: P = 0.44). Eventually, after 2 h, both predator treatments no longer interacted ($F_{1, 22}$ = 1.048, P =

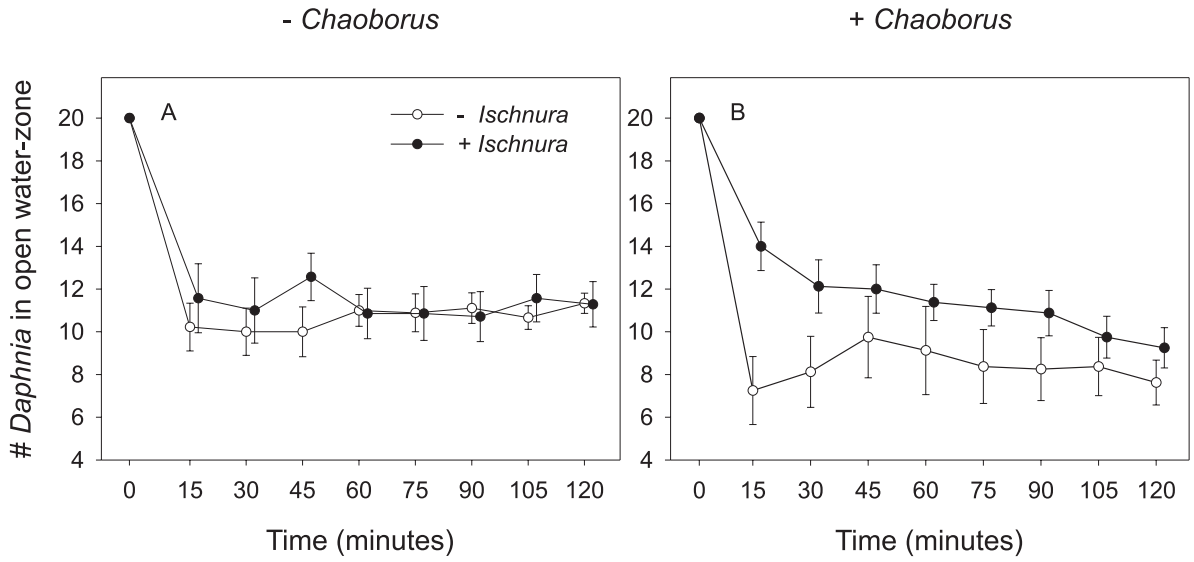

Fig. 3. The number of *Daphnia* in the central open water-zone through time is presented in the absence (A) and in the presence (B) of *Chaoborus*. Filled symbols represent the treatment with *Ischnura* larvae present in the macrophytes, open symbols represent the treatment with *Ischnura* absent. Note that on Time 0, 20 *Daphnia* were introduced in the open water-zone. Means are given ± 1 standard error.

0.32), but a negative main-effect of *Chaoborus* on the number of *Daphnia* in the central open water-zone remained, irrespective of the presence of *Ischnura* (ANOVA, *Chaoborus*: $F_{1,\ 22}$ = 9.083, P = 0.006). However, only in the single presence of *Chaoborus*, significantly less *Daphnia* stayed in the open water zone compared to the control (Tukey HSD, P = 0.031) whereas in the combined predator treatment this trend was not significant (Tukey HSD, P = 0.361).

During the two hours of the experiment, the horizontal distribution of the *Chaoborus* larvae was not influenced by the presence of the *Ischnura* larvae, nor by time (rmANOVA, all P > 0.11). On average more C*haoborus* were present in the central open water-zone than among the vegetation (58 ± 4.64 %, N = 12, one sample t-test: t_{11} = 4.92, P < 0.001).

Experiment 5: Foraging effort of restrained Ischnura

The principal component analysis on the four original predation behaviours extracted one new variable that explained 70.3 % of the original variance. This new variable was highly positively correlated with the variables head orientations (0.93) and strikes (0.95), and to a lesser extent with approaches (0.58). *Ischnura* foraging effort on *Daphnia* declined significantly after the first 15' interval, and remained at a low level in the

subsequent time intervals (rmANOVA: $F_{3,12}$ = 7.251, p = 0.005; Tukey HSD: all combinations of foraging effort at the first time interval with foraging effort at later time intervals had p < 0.05).

Predation Risk

The calculated actual predation risks for each combination of predator species and microhabitat are presented in Fig. 4. Predation risk in the presence of *Ischnura* larvae was much higher within the vegetation compared to the open water (t_{14} = -213.77, P < 0.0001). A similar pattern was revealed for the combined predator treatment: predation risk within the vegetation was several times higher than in the open water (t_{14} = -73.40, P < 0.0001). In both cases, the clear pattern can be mainly accounted for by the strong preference of the *Ischnura* for the vegetated areas. In the single presence of *Chaoborus*,

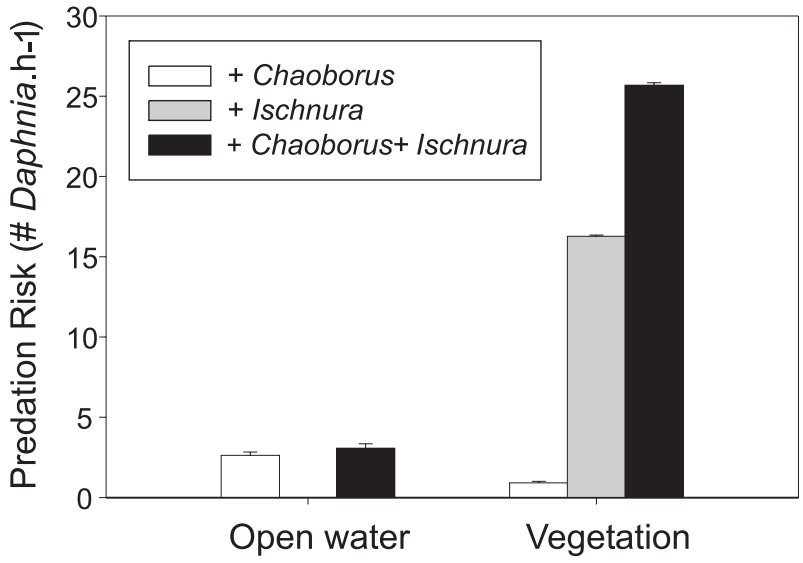

Fig. 4. Bars represent means (+ 1 standard error) of actual predation risk of *Daphnia* in the *Daphnia* horizontal migration experiment in the open water (left) and the vegetated (right) zone for each predator treatment. Predation risk is expressed as the number of *Daphnia* that is preyed upon within one hour.

the pattern was reversed and predation risk was higher in the open water environment than within the vegetation (t_{14} = 7.493, P < 0.0001).

Discussion

In shallow lakes, *Daphnia* has been observed to either avoid vegetated areas or to migrate horizontally toward the vegetation in the presence of fish during the day, but

often without entering it (Lauridsen et al. 1998, Burks et al. 2002). Different hypotheses have been put forward to explain the deterring effect of the vegetation, such as detrimental chemical substances released by the vegetation, scarcity of phytoplankton within vegetated zones, higher metabolic costs of *Daphnia* within the vegetated zones due to differing abiotic factors, or high levels of predation risk by littoral invertebrate predators (reviewed in Burks et al. 2002). In an experiment using large field enclosures, Burks et al. (2001b) could show a significant negative impact of a mixture of odonate larvae on total *Daphnia* abundance. Our findings build further on this by showing that the horizontal distribution of *Daphnia* in an environment consisting of an open water-zone and a vegetated zone is affected by the presence of damselfly larvae and *Chaoborus*, and by quantifying the predation impact of these invertebrate predators on *Daphnia* in an open water and a vegetated environment. It is expected that these results apply mainly to small and shallow lakes, where open water and vegetated areas are closely connected, and where *Daphnia*, *Chaoborus* and *Ischnura* are especially likely to interact with each other.

Chaoborus larvae are generally assumed to be typical pelagic predators (Tollrian and Dodson 1999, Voss and Mumm 1999), although no study has tested this yet experimentally. Indeed, our results indicate a preference of the *Chaoborus* for the open water zone, but this preference was, unexpectedly, rather weak (58-65 %). In shallow lakes with abundant vegetation, *Chaoborus* has been found to be as abundant in vegetation beds as in open-water areas (Schramm and Jirka 1989, Olson et al. 1995). Thus, the weak pattern in horizontal distribution of *Chaoborus* we observed probably is not an artifact, but may reflect a true spatial pattern in shallow lakes. We hypothesize that the premise that *Chaoborus* is strongly pelagic applies largely to deepwater lakes that have little vegetation and where *Chaoborus* exerts diel vertical migration to hypolimnetic waters to avoid fish predation (Voss and Mumm 1999, Liljendahl-Nurminen et al. 2002).

The weak preference of *Chaoborus* for the open water and especially its 2-fold higher predation rate in the open water resulted in a 3-fold higher predation risk for *Daphnia* in the open water (Fig. 4). In accordance, *Daphnia* increased its use of the vegetation zone in the presence of *Chaoborus*, at least in the absence of damselfly larvae (Experiment 4).

Although we expected staying in the vegetation to be costly due to predation from *Ischnura*, *Daphnia* exhibited no horizontal migration response toward the open water zone in the single presence of *Ischnura* (Experiment 4, Fig. 3A). In the *Ischnura*

predation experiment, *Daphnia* predation rates by *Ischnura* in the open water-zone exceeded those in the vegetation (Figs. 2A-B). On the other hand, *Ischnura* showed a strict preference for vegetation (Experiment 4), resulting in a much higher level of predation risk by *Ischnura* within the vegetation than in the open water (see also Van de Meutter et al. 2004, Chapter III). The lack of response of the *Daphnia* therefore is unexpected, and contrasts with earlier findings of a similar experiment by Van de Meutter et al. (2004, Chapter III) that indicated horizontal avoidance behaviour by *Daphnia* of vegetation that was inhabited by *Ischnura*. The set up in both experiments was very similar, with free-ranging prey and predators, and differed only in the set of *Daphnia* clones that was used. There is a large variability among *Daphnia* clones with respect to the traits for which they show a response to predators (Boersma et al. 1998). By isolating a random set of only seven (this study) or eight (earlier study) clones, by chance, clones responsive to *Ischnura* with horizontal migration behaviour may be differentially represented, which in turn may determine the outcome of the experiment.

Daphnia showed a tendency to stay longer in the open water-zone when both *Ischnura* and *Chaoborus* were present (Experiment 4, Fig. 3B). This is in line with the observation that in the predation experiment *Daphnia* suffered from higher predation by *Ischnura* when *Chaoborus* was present, primarily in the vegetation treatment (Experiment 3, Figs. 2A-B). Facilitation between predators is expected when prey experience conflicting anti-predator behaviours to each predator (Sih et al. 1998). *Daphnia* can avoid encounters with *Chaoborus* by decreasing its swimming speed (Weber and Van Noordwijk 2002), by migrating vertically to the water surface (Nesbitt et al. 1996, Weber and Van Noordwijk 2002), or by swimming away from the *Chaoborus* upon encounter (Kvam and Kleiven 1995). Decreased swimming speed cannot explain the increased predation by *Ischnura* in the combined predator treatment, since it would have reduced encounters with the primarily sit-and-wait predator *Ischnura*. Vertical migration of *Daphnia* to the water surface also could not have increased *Ischnura* predation on *Daphnia*, since *Ischnura* preferred the lowest plant parts rather than the water surface (F. Van de Meutter personal observations). Escape behaviour of *Daphnia* from *Chaoborus* at a micro scale level may have driven the *Daphnia* towards the *Ischnura* larvae. At a micro scale within the vegetation, *Chaoborus* and *Ischnura* did not occur in the same microhabitats. *Ischnura* larvae were mostly clinged to the vegetation or to the bottom of the aquaria, while *Chaoborus* larvae were planktonic and inhabited the open water space between the vegetation. This small-scale difference in microhabitat use between both predators in combination with avoidance behaviour by

Daphnia of the *Chaoborus* by swimming away upon encounter, may have increased encounter probability and frequency with the predators and hence increased the predation from *Ischnura* in the combined predator treatment. Unfortunately, we did not record *Daphnia* escape and swim behaviour during the predation experiment.

Relyea (2003) reviewed literature on prey responses to combinations of predators and concluded that, in general, prey respond to combined predators the same way as they respond to the more risky predator (based on prey responses) in the pair. His two main conclusions were that (1) when prey responses to individual predators alone are in the same direction, the prey response to the combination of both predators is in the same direction and varies in magnitude from the response to the less risky predator alone (21 % of studies) to a response that is equal to or even exceeds the response to the more risky predator alone (75 % of studies) and (2) when predators induce prey responses in opposite directions, prey responses were in 90 % of the cases directed away from the more risky predator and varied in magnitude from a response that was intermediate to the response to the more and the less risky predator alone to a response that was equal to the more risky predator alone. We hypothesized the spatial response of *Daphnia* to *Ischnura* and to *Chaoborus* to be oppositely directed, and therefore we a priori hypothesized the magnitude of the response to the combined predator treatment to be intermediate with regard to the responses to each predator separately or equal to the more risky predator. From our actual observations that *Daphnia* did not respond to the presence of *Ischnura* alone but migrated to the vegetation in the presence of *Chaoborus* (Experiment 4), the actualized expectation would be a similarly directed response to the combination of predators as to *Chaoborus* alone. Combining the two predators, however, resulted in a response to the open water-zone (away from the *Ischnura*) that was not observed before in one of the separate predator treatments (Fig. 3). This neither supports our a priori and actualized hypothesis based on the two invertebrate predators, nor matches the conclusions of Relyea (2003). As suggested above, we think that this unexpected spatial avoidance behaviour can be explained by a facilitating effect of *Chaoborus* predation by *Ischnura* in the vegetation (Fig. 2B). In the presence of both *Chaoborus* and *Ischnura*, the facilitating effect suddenly increases encounters with the predators and predation risk imposed by *Ischnura,* possibly reaching a threshold, resulting in a stronger avoidance of the vegetated zone by the *Daphnia*.

Altogether, our results may be best explained by invoking both the facilitating effect of *Chaoborus* on *Ischnura* predation in the vegetation, and a constitutive response

of the *Daphnia* toward the vegetation to avoid fish predation. At risk of predation, *Daphnia* could be more willing to enter the vegetation than to leave it because of a high selective pressure by fish in the open water in their source habitat. Although fish were not present in our experiments, they are far more important predators than *Ischnura,* and the *Daphnia* clones may have been selected toward a constitutive adaptive antipredator behaviour against fish. Therefore, in the presence of only *Ischnura*, *Daphnia* may have been reluctant to swim to the open water. In the presence of only *Chaoborus*, the avoidance behaviour is in the same direction as the constitutive behaviour to fish, and is strengthened. In the presence of both predators, the oppositely directed facilitating effect between the predators may override the fish effect, and the *Daphnia* will hesitate to enter the vegetation.

The response of *Daphnia* toward the open water in the combined predator treatment decreased over time and even disappeared near the end of the experiment. Previous studies on horizontal migration of *Daphnia* in response to fish did not detect such time effects (Lauridsen and Lodge 1996, Burks et al. 2001a). However, these studies used caged predators or kairomones, which does not allow for direct predator-prey interactions. In our experiment, *Chaoborus* and *Ischnura* could freely interact and respond to *Daphnia*, and changes in the nature of these interactions could change the behavioural response of *Daphnia*. In the predation experiments, predation by *Ischnura* as well as predation activity of *Ischnura* and *Chaoborus* declined over time. This decline cannot be explained by changing *Daphnia* densities, since *Daphnia* numbers were replenished at the start of each time interval. Possibly, the *Ischnura* and *Chaoborus* predators became satiated over time in our experiments. However, if satiation of the predators had caused the decline over time in the response of *Daphnia*, than this should also have lead to a declining response with time in the treatments with only one predator species present, which we did not observe. Moreover, satiation was excluded in the horizontal migration experiment with *Daphnia* since both predators were prevented from eating. An alternative explanation for the time-dependency of the response of *Daphnia* is that the *Ischnura*, even when they are prevented from eating the *Daphnia*, show declining predation activity within the 2 h duration of the experiment. In experiment 5 we could show that *Ischnura* foraging effort on *Daphnia* declined significantly after the first 15’ interval, and remained at a low level in the subsequent time intervals. Possibly, the *Ischnura* lower their effort to attack the *Daphnia* when experiencing their incapacity to catch their prey. Attacking prey with restrained mouthparts also might be energetically costly, and therefore may not be maintained at constant high level for 2 h. In summary,

we interpret the results of the combined predator treatment as follows; the horizontal migration response of the *Daphnia* toward the open water in the presence of *Chaoborus* and *Ischnura* was probably mediated by direct predator-prey interactions with the *Ischnura* (see above). We suggest that when these interactions became less frequent due to lower activity of the *Ischnura*, the predation risk in the open water by *Chaoborus* became more important. As a result, *Daphnia* gradually migrated to the vegetation. Note that at this point in time, our results are in accordance with the second conclusion of Relyea (2003).

Studies on prey responses to combinations of predators are still scarce, although this represents the more natural situation (Relyea 2003). Our results indicate that predator effects on prey spatial behaviour can vary considerably in strength and direction, depending on the presence of more than one predator. We suggest that interactions and facilitation effects among the predators can be important, and also may vary with the environment and with time. Clearly, more studies are needed that integrate data on spatial behaviour and spatial differences in predation efficiency of syntopic predators to fully understand predator-prey interactions. Such research should explicitly test spatial preferences of predator and prey organisms. Only by such studies a more general, widely applicable predictive framework of predator-prey interactions in a multipredator context may be generated.

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Chapter V

Lake-to-lake dispersal of lentic macroinvertebrates through lake-outlets

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[Unpublished manuscript]

Abstract

Little is known on dispersal of lentic macroinvertebrates. We quantified dispersal of lentic macroinvertebrates through lake connections in a highly connected lake system, and investigated the role of connection properties and timing (day, night) on dispersal rate. Furthermore, by comparing dispersal assemblages with the macroinvertebrate assemblages of source lakes, we tested whether dispersal was neutral or a taxon-specific process. Many taxa dispersed through the lake connections, probably mainly by passive transport. Taxon richness of the dispersing macroinvertebrate assemblage was proportional to taxon richness in the source lakes. The number of individuals that dispersed, however, was not related to source lake densities, possibly because of the highly patchy distribution of lentic macroinvertebrates within lakes and the relatively short sampling time. Baetidae, Chironomidae and Physidae exhibited higher dispersal rates, corrected for source pond densities, than a selection of seven other macroinvertebrate families, indicating that the extent of dispersal may be taxon-specific. None of the physical properties of the connections affected dispersal. The number of dispersing macroinvertebrates was higher during the night than during daytime. Of seven frequently dispersing families, Chaoboridae and Chironomidae showed higher dispersal during the night, probably resulting from diel vertical migration behaviour. Dispersal rates of actively and passively over-land dispersers through lake connections did not differ. Since over-land dispersal probably is more frequent in active dispersers compared to passive dispersers, dispersal through lake-outlets in connected lake systems may generate different spatial patterns compared to over-land dispersal with regard to the composition of communities and the genetic structure of populations.

Introduction

Understanding dispersal patterns has become an important issue in conservation biology and landscape management. Dispersal of individuals among habitats may not only affect the dynamics, persistence and genetic structure of local populations, but also may contribute to changes in community structure in both source and sink habitats (Slatkin 1985, Peckarsky et al. 2000, Bilton et al. 2001, Malmqvist 2002, Bohonak and Jenkins 2003). Despite its importance, the nature and extent of dispersal remain poorly known, mainly due to methodological problems such as the vague delineation of habitats and populations (e.g. many marine and terrestrial systems) or the insuperable difficulties involved with detecting or quantifying dispersing organisms (many insects and freshwater organisms) (Bilton et al. 2001, De Meester et al. 2002).

Freshwater invertebrates occur in habitats that represent discrete sites surrounded by an inhospitable terrestrial landscape. Dispersal between habitats is achieved using a variety of mechanisms such as active flight by adult insects, passive dispersal by animal vectors (phoretic dispersal), or passive dispersal by wind (Brown et al. 1997, reviewed in Bilton et al. 2001). Dispersal in freshwater invertebrates is notoriously difficult to obtain directly, and the extent and modes of dispersal remain poorly understood (Bilton et al. 2001, Hoffsten 2004). As a result, a large number of studies relies on indirect estimation of dispersal from colonization events (Brown et al. 1997, Rundle et al. 2002, Anderson and Smith 2004), or the use of markers like stable isotopes (Caudill 2003) and genetic markers (Meyran et al. 1997, Hughes et al. 1999, Kelly et al. 2001). Colonization events provide a relatively good view on the rate and extent of dispersal, but, generally, information about the origin of the organisms, the mechanism and the success of dispersal is lacking. Stable isotope labeling may enable one to track the origin of dispersing animals, but its applicability is largely restricted to systems that are clearly defined (unconnected) and have only a low number of source habitats. Apart from its undisputable merits in revealing unnoticed dispersal, genetic markers are not fully satisfactory for explaining, amongst others, metapopulation dynamics, density-dependent population dynamics or energy-flows between systems, since they do not provide estimates of actual dispersal rates, but rather indicate establishment success after dispersal. Relatively infrequent successful dispersal events may nullify differences in genetic structure of populations, but may be of little importance for acting upon (meta)community structure or population densities. Conversely, genetic differences among populations may remain, even though potential dispersal rates are high, as a consequence of low establishment success due to local interactions (De

Meester et al. 2002). Clearly, indirect approaches are not sufficient to fully understand dispersal patterns, making direct estimates essential (Bossart and Prowell 1998).

In connected lake systems, aquatic connecting elements (overflows, rivulets) constitute a potential pathway for invertebrate dispersal, additional to dispersal over land. Compared to the latter, dispersal through overflows and rivulets is well delineated and therefore relatively easy to quantify. Moreover, lake connections can be very important dispersal pathways, as has been shown for zooplankton, both in terms of numbers (high dispersal rates reported by Michels et al. 2001a, Walks and Cyr 2004), as well as in terms of its prominent effects on the structure and genetic composition of local populations (Michels et al. 2001b). For lentic macroinvertebrates, however, no quantitative studies exist that investigated the potential of lake connections for lake-to-lake dispersal.

This study aims at directly quantifying dispersal patterns of lentic macroinvertebrates through lake connections in a highly connected lake system. More specifically, we will consider three aspects of dispersal that have important ecological ramifications and are largely unknown for aquatic insects. First, we will evaluate whether dispersal through lake connections is taxon-dependent. Since dispersal over land generally occurs with a higher speed in active dispersers (flying insects) compared to passive dispersers (dispersal by wind or phoretic dispersal) (Brown et al. 1997, Hoffsten 2004), we will specifically evaluate whether dispersal through lake connections is neutral with respect to both groups of over-land dispersal modes. This is of crucial importance, for example in modeling dispersal in a metacommunity context. We will be able to evaluate the neutrality of dispersal by comparing the relationship between source lake densities and the number of dispersing individuals among different taxa. Second, the physical properties of connecting structures is a critical point that has received much attention for dispersal over land (Goodwin and Fahrig 2003, Wratten et al. 2003), but has largely been neglected in dispersal through water. The lake connections in our study system vary considerably in connection type (tube, rivulet), discharge rate, flow rate and length, which allows us to investigate the effect of these physical connection properties on macroinvertebrate dispersal rates. Third, dispersal patterns may show strong differences between day and night. In lotic systems, for example, it is well known that dispersal by drift is more frequent at night when animals suffer less predation risk by fish (Cowell and Carew 1976, McIntosh et al. 2002). In general, the documented differences between day and night in activity and vertical distribution of macroinvertebrates (Marklund et al. 2001, Berendonk et al. 2003) may change the actual subset of the

macroinvertebrate community that is prone to dispersal. To test for a diurnal pattern in dispersal, we sampled a subset of the lake connections during both day- and nighttime.

Material and methods

Study area

The study area is the Nature Reserve “De Maten”, a protected heath land of about 300 ha situated near Genk in the northeastern part of Belgium. The reserve comprises 34 small, shallow and eutrophic lakes, connected to each other by a complex network of rivulets and overflows (Michels et al. 2001b, Cottenie et al. 2003, Fig. 1). The lakes in the complex are in an approximately linear setting, with a 15 m drop between the most upstream and the most downstream lakes, causing a unidirectional water flow through the system. In spite of the high level of connectivity, the composition of the macroinvertebrate communities differs considerably among the lakes (Van de Meutter et al. in press, Chapter I).

Sampling dispersal through lake connections

All lake connections in “De Maten” start and end with an identical cylindrical tube (20 cm diameter) that canalizes the water at both the inlet and outlet. This allows for easy quantitative sampling of dispersal from lake to lake through these connections. Here fore, we used a 20 cm long PVC tube (diameter 20 cm) with a conical net (45 cm long, mesh size 500 µm) attached to its end, which was mounted on the end of the inlet of the receiving lake. Outlets were all situated near the water surface of the source lake; inlets were usually situated halfway the water column of the receiving lake.

In total, 28 lake connections were sampled once during daytime for a period of 6 h on 23, 24, 25 and 26 September 2003. To test for day/night differences in dispersal rates of macroinvertebrates through the lake connections, we sampled 20 out of these 28 lake connections in an identical way during nighttime (starting 2 h after sunset) on 28 and 29 September 2003. Climatic conditions during the entire sampling period were relatively constant with frequent showers and rain causing high flow rates. Dispersal samples were fixed on 4 % formaldehyde in the field and all macroinvertebrates were sorted in the laboratory. Identification of macroinvertebrates was achieved to family level, except for Actinedida, Hirudinea and Oligochaeta, which were not further identified. For convenience we will use the term “families” when referring to any of these taxa.

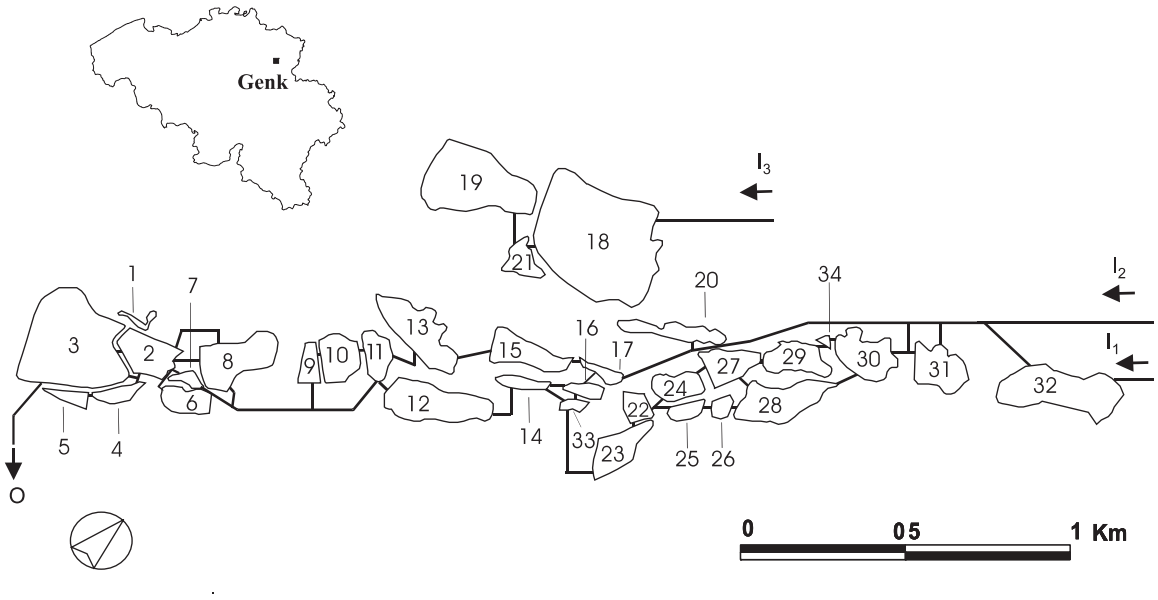

Fig. 1. Map of the lake complex at De Maten (Genk, Belgium) indicating all lake connections (solid lines) and the numbering of the lakes. I_1-I_3 represent inflows of water from outside the reserve, O represents the final outflow of the reserve. Arrows indicate the direction of the water current. Except for the connections 30-34 and 23-33, the water current is directed towards lakes with a lower numbering.

Physical properties of lake connections

We determined four physical properties of the lake connections that could possibly influence macroinvertebrate dispersal rates: length of the connection, flow rate, discharge volume and type of connection. Length refers to the total distance traveled by the water through the connection from one lake to the other, and was measured in the field (short connections) with a flexible ruler or calculated from topographical maps to the nearest 5 meters (connections > 30 m long). Flow rate was defined as the difference in time between adding salt to the water at the inflow of a connection and the resulting peak in connectivity measured at the outflow or at 30 meters from the inflow (long connections), divided by the distance between both. We calculated discharge volumes (l/s) as the product of the surface area of a cross-section of the water flow and the flow rate. Finally, we categorized lake connections in two groups: closed overflows through PVC tubes and small open rivulets that only lead water through a closed tube at their inlet and outlet. The physical properties of all sampled connections are summarized in Table 1.

Table 1. Physical properties of the 28 lake connections that were sampled. Source and sink lakes are indicated with their respective lake number (see Fig. 1).

Nr	Source lake	Sink lake	Length (m)	Flow rate (m/s)	Discharge volume (l/s)	Type
1	2	3	35	0.067	12.800	rivulet
2	2	4	50	0.020	3.360	rivulet
3	6	2	95	0.031	6.462	rivulet
4	7	2	45	0.158	4.958	rivulet
5	8	2	80	0.162	13.003	rivulet
6	8	2	190	0.125	12.250	rivulet
7	10	8	375	0.417	6.542	rivulet
8	11	10	25	0.556	5.667	tube
9	11	8	620	0.078	4.735	rivulet
10	12	11	80	0.189	15.167	rivulet
11	14	12	125	0.135	8.792	rivulet
12	15	13	80	0.202	3.110	rivulet
13	16	14	40	0.190	13.368	tube
14	17	15	40	0.104	6.147	tube
15	17	16	25	0.216	6.773	tube
16	23	33	225	0.357	3.821	rivulet
17	24	22	35	0.151	4.740	rivulet
18	27	24	10.5	0.162	5.072	tube
19	28	26	10	0.286	6.171	tube
20	29	27	10.5	0.175	5.495	tube
21	30	28	60	0.200	2.540	rivulet
22	30	34	4.5	0.450	6.165	tube
23	31	30	115	0.243	6.105	tube
24	32	17	1400	0.393	15.790	rivulet
25	32	20	1110	0.040	1.256	rivulet
26	32	30	455	0.200	3.533	rivulet
27	32	31	225	0.239	7.474	rivulet
28	34	29	5	0.556	6.500	tube

Lake Sampling

To be able to relate the composition of samples of dispersing macroinvertebrates through connections to the macroinvertebrate composition of the source lake, we sampled the macroinvertebrate community of 15 source lakes (see Table 1) once during

daytime between 18 and 22 September 2003. Samples were taken with a net (mesh size 500µm) and were standardized in both time (10 min) and length (sampling along a littoral trajectory of 75 m). Sampling trajectories were chosen to represent all major littoral microhabitats present in the lake. This sampling procedure includes epiphytic and free-swimming littoral macroinvertebrates, but excludes typical benthic species. Lake samples were treated identically as the samples of dispersing macroinvertebrates.

Statistics

To investigate the relationship between the number of dispersing macroinvertebrates and the number of macroinvertebrates in source lake samples, and between the number of dispersing families and the number of families in source lake samples, we applied regression analysis. Since only daytime samples of the lakes were available, nighttime data of dispersal were not used in these analyses. To test whether the extent of dispersal through the lake connections was neutral with respect to macroinvertebrate family (i.e. whether some families were overrepresented in the dispersal samples based on their relative abundance in the source lake), we performed an ANCOVA. We included Family as the categorical variable, with the number of dispersing individuals per family and per sample as the dependent variable and with the respective number of individuals of each family in the source lakes as the covariate. Significant effects of Family indicate that families have different corrected dispersal rates, and interactions between Family and the covariate indicate that the relation between the source density and dispersal rate differs between families. Significant effects of Family were further explored with post-hoc Fisher LSD tests. Because prevalence and density of animals in the source lakes were too low to do reliable statistical testing in many families, we selected the ten macroinvertebrate families with the highest median numbers in the source lakes for this analysis: Acroloxidae, Baetidae, Chironomidae, Coenagrionidae, Corixidae, Culicidae, Dixidae, Limoniidae, Physidae and Pyralidae. To test whether active over-land dispersers (Baetidae, Chironomidae, Coenagrionidae, Corixidae, Culicidae, Dixidae, Limoniidae and Pyralidae) exhibited different dispersal through lake outlets compared to passive over-land dispersers (Acroloxidae, Physidae), we repeated the ANCOVA, but with Dispersal mode and Family nested in Dispersal mode as the categorical predictors. Possible effects of connection type, connection length, discharge volume and flow rate on the number of dispersing macroinvertebrates and macroinvertebrate families were evaluated using an ANCOVA with the number of dispersing macroinvertebrates or macroinvertebrate families as the dependent variable, connection type as the categorical

predictor, and connection length, discharge volume, flow rate and number of individuals or families in the source lakes as the covariates. To test whether dispersal rates of macroinvertebrate families were differentially affected by the connection characteristics, we performed an ANCOVA with the numbers of dispersing individuals for seven macroinvertebrate families as the dependent, with Family and connection type as categorical predictors and with connection length, discharge volume, flow rate and the respective number of individuals of each family in the source lakes as the covariates. Since the effect of the connection characteristics on dispersal can only be evaluated for macroinvertebrates that frequently disperse, only seven families that occurred in five or more daytime dispersal samples were used in this analysis: Baetidae, Chironomidae, Corixidae, Lymnaeidae, Oligochaeta, Physidae and Pyralidae.

Differences between daytime and nighttime dispersal rates for the number of macroinvertebrates and the number of macroinvertebrate families were analyzed using repeated measures ANOVA (rmANOVA), with the respective rates of daytime and nighttime dispersal as the repeats. To test whether families showed a different pattern in daytime versus nighttime dispersal, we performed a rmANOVA with Family as the categorical variable and the number of dispersing individuals of the families during daytime and during nighttime as the repeats. The following five families that were present in at least five samples (day- or nighttime) were included in the latter analysis: Baetidae, Chaoboridae, Chironomidae, Oligochaeta and Physidae with 11, 5, 19, 8 and 10 samples included, respectively. Samples of connections where no individuals of a particular family were obtained during both daytime and nighttime sampling were excluded from the analysis for that family.

The numbers of macroinvertebrates in lakes and dispersal samples were Log(X+1)-transformed for all analyses to meet ANOVA and regression assumptions. Connection lengths were Log(X)-transformed. All analyses were performed using STATISTICA 6.0 (Statsoft 2001). Means are given ± 1 standard error.

Results

On average, 29.2 ± 10.9 macroinvertebrates and 3.3 ± 0.4 families of macroinvertebrates were found dispersing through the lake connections within a 6 h time interval. In total, 29 families of macroinvertebrates were observed to disperse through the lake connections, while 45 families were present in the lake samples (Table 2). Six families were found dispersing through the connections, while they were not observed in the lakes: Argyronetidae, Chaoboridae, Gammaridae, Gyrinidae, Hydropsychidae and

Table 2. Total number of individuals in the source lake samples (lakes), in daytime dispersal samples (day) and in nighttime dispersal samples (night) for all observed families of macroinvertebrates. The numbers of macroinvertebrates are based on 19 lake samples for the source lakes, and on 28 and 21 dispersal samples of the lake connections for daytime and nighttime dispersal sampling, respectively. Taxa indicated with † are not families, but represent higher order taxa.

Taxon	Source	Dispersal		Taxon	Source	Dispersal	
	Lakes	Day	Night		Lakes	Day	Night
Oligochaeta †	310	9	10	Noteridae	27	2	1
Hirudinea †	40	2	0	Haliplidae	48	12	0
Physidae	1321	18	7	Hydrophillidae	9	0	0
Lymnaeidae	227	9	4	Scirtidae	7	0	0
Planorbidae	287	9	18	Ecnomidae	1	0	0
Acroloxidae	799	4	1	Hydropsychidae	0	25	153
Sphaeriidae	4	1	0	Hydroptillidae	162	0	0
Argyronetidae	0	1	0	Leptoceridae	4	0	0
Actinedida †	51	1	9	Pyralidae	126	7	0
Argulidae	1	0	0	Chironomidae	2938	354	719
Asellidae	3	0	4	Simuliidae	0	66	312
Gammaridae	0	0	3	Ceratopogonidae	113	0	0
Baetidae	4832	38	63	Chaoboridae	0	0	64
Coenagrionidae	999	5	2	Culicidae	316	3	2
Aeshnidae	21	0	0	Dixidae	147	1	5
Libellulidae	8	0	0	Ephydridae	111	0	0
Nepidae	2	3	3	Limoniidae	345	13	1
Corixidae	821	6	0	Muscidae	5	9	1
Notonectidae	2	1	0	Sciomyzidae	12	0	0
Pleidae	12	0	0	Stratyomidae	1	0	0
Naucoridae	5	0	0	Syrphidae	2	0	0
Dytiscidae	3	2	3	Tipulidae	3	0	0
Gyrinidae	0	0	1				

Simuliidae. Of these, Argyronetidae, Gammaridae and Gyrinidae were only found once in the samples. Finally, Hydropsychidae and Simuliidae are typical rheophylic families (Schmedje and Colling 1996) and have never been found before in the lakes of De Maten, despite several large-scale sampling campaigns of all lakes (Van de Meutter F. unpublished results, Chapter VII). They likely originate from the lake connections

themselves instead of from the source lakes, and they were excluded from all further analyses.

Is dispersal neutral or selective with regard to taxon?

The number of dispersing macroinvertebrate families was positively related to the number of macroinvertebrate families in the source lakes ($F_{1, 13} = 8.59$, $p = 0.012$, adjusted $R^2 = 0.35$, Fig. 2a), but the total number of dispersing individuals did not depend on macroinvertebrate abundance in the source lake ($F_{1, 13} = 2.23$, $p = 0.16$, adjusted $R^2 = 0.08$, Fig. 2b). After correction for differences in family abundance in the source lakes, significant differences were detected in dispersal rates among the ten selected macroinvertebrate families (ANCOVA, Family: $F_{9, 200} = 3.02$, $p = 0.002$, Family x Nr. of individuals in source lake: $F_{9, 200} = 0.87$, $p = 0.55$, Fig. 3). Baetidae, Chironomidae and Physidae were more likely to disperse than Acroloxidae, Coenagrionidae, Corixidae, Culicidae, Dixidae, Limoniidae and Pyralidae (Fisher LSD: all $p < 0.05$), and Chironomidae were more likely to disperse than Baetidae and Physidae (Fisher LSD: both $p < 0.001$).

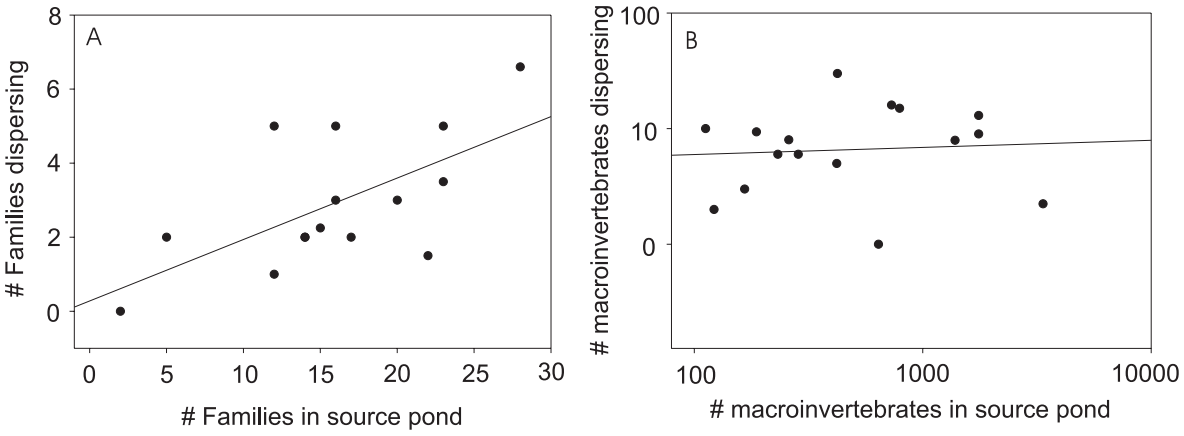

Fig. 2. The number of dispersing macroinvertebrate families (A) and the number of dispersing macroinvertebrate individuals (B) through the outgoing lake connections in relation to their numbers in the respective source lakes. Note that data of (B) are presented on a log-scale.

The comparison of a restricted set of active and passive over-land dispersers yielded no significant difference in dispersal rate through lake outlets between both (Dispersal mode: $F_{1, 7.79} = 0.44$, $p = 0.53$; Dispersal mode x Abundance source lake: $F_{1, 3.38} = 0.034$, $p = 0.86$).

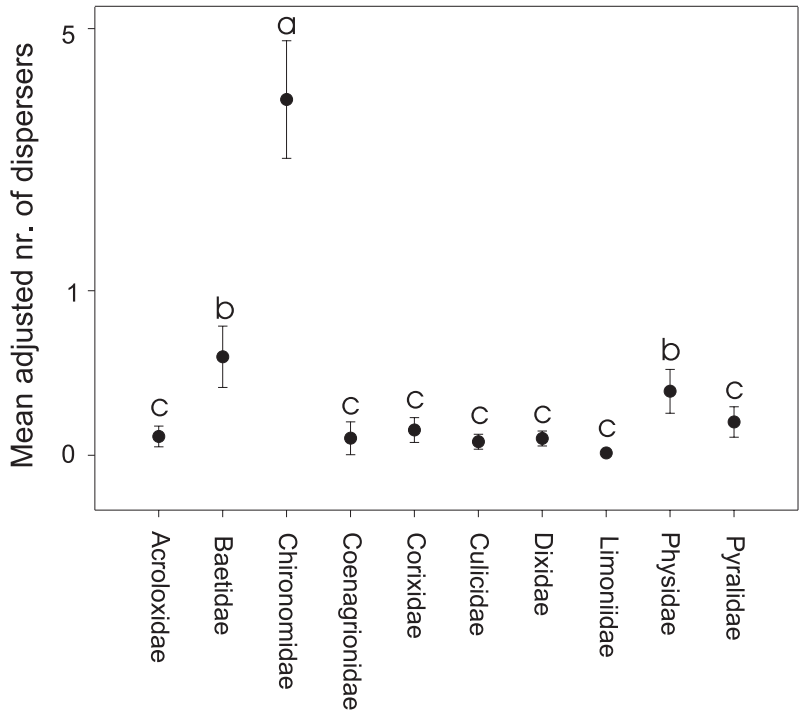

Fig. 3. Adjusted mean (± 1 standard error) dispersal rate (6 h) for the ten macroinvertebrate families with the highest median occurrences in the lakes. Adjusting was done taking the number of individuals in the source population into account using an ANCOVA. Family means that differ based on a LSD test are indicated with a different letter. Scaling of the y-axis is on a log-scale.

Do the physical properties of the connections affect dispersal?

Neither the number of dispersing individuals nor the number of dispersing families was influenced by connection length, connection type, discharge volume or flow rate (individuals: all $p > 0.16$, families: all $p > 0.36$) or any of their interactions (individuals: all $p > 0.53$, families: all $p > 0.37$). The comparison of seven frequently dispersing families revealed no family-specific differences in their relation with the physical properties of the connections (all interactions with family: $p > 0.35$).

Does dispersal differ between day and night?

The number of dispersing families did not differ between day and night (families: $F_{1, 13} = 1.87$, $p = 0.19$), but the number of dispersing individuals was higher during the night than during the day (individuals: $F_{1, 13} = 6.10$, $p = 0.028$). A restricted analysis only including the five most abundant families (Baetidae, Chaoboridae, Chironomidae, Oligochaeta and Physidae) showed a higher number of dispersing individuals during the night (Day/night: $F_{1, 36} = 4.91$, $p = 0.049$; Fig. 4), a trend that did not differ significantly among the five

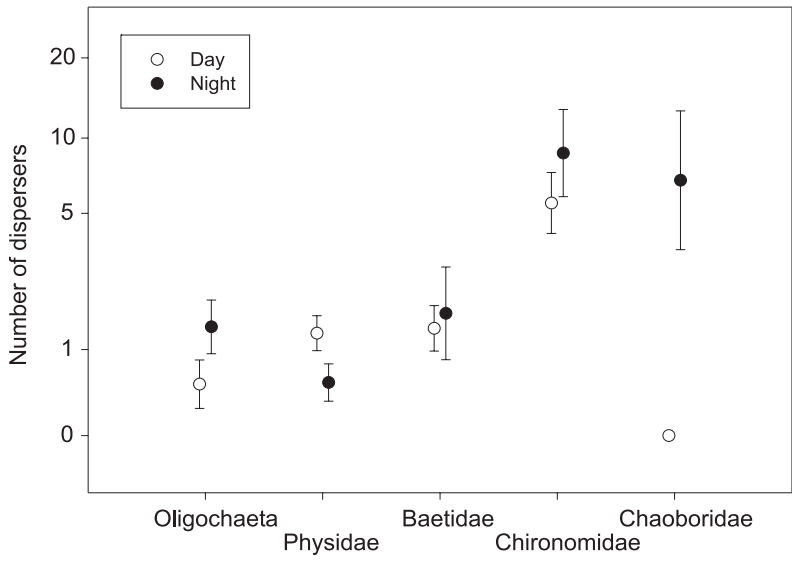

Fig. 4. Mean (± 1 standard error) daytime (open circles) and nighttime (filled circles) dispersal rate (6 h) for five selected macroinvertebrate families.

studied families (Family x Day/Night: $F_{4;\ 36} = 1.30$, $p = 0.29$). Dispersal, however, was only significantly higher during nighttime than during daytime for Chaoboridae and Chironomidae (Fisher LSD, Chaoboridae: $p = 0.040$; Chironomidae: $p = 0.048$). No significant differences between daytime and nighttime dispersal samples were observed for the other families (all $p > 0.64$).

Discussion

Lentic macroinvertebrates occur in isolated, island-like habitats, separated by inhospitable terrestrial landscape. Dispersal between habitats may be achieved by active or passive dispersal over land or, in connected systems, via aquatic connections between lakes (Bilton et al. 2001). Despite its importance for both local and regional dynamics of species, knowledge on the extent and the exact mechanisms of dispersal in the field is still poor (Bohonak and Jenkins 2003). Our results show the potential of lake connections for lentic macroinvertebrate dispersal between lakes and show characteristics of the dispersal patterns. Dispersal through water connections among our lakes was selective with regard to taxon, not influenced by the physical properties of the connections and, at least for some families, showed a diurnal pattern.

Is dispersal neutral or selective with regard to taxon?

Dispersal seemed to some extent neutral with regard to taxon. Taxon richness (family

level) of the source lake was a significant determinant of taxon richness of dispersing macroinvertebrates through the lake connections. Together with the fact that most of the abundant taxa were found dispersing, these results indicate that dispersal through lake connections probably is a general phenomenon in which all macroinvertebrate families to some degree participate.

Despite these general indications of dispersal to be neutral with respect to taxon, mean dispersal rates differed significantly among families, and were higher for Baetidae, Chironomidae and Physidae than for the other families. Dispersal through lake connections probably occurs as the transport of free-floating or swimming organisms in the upper water layers, and may therefore be more likely in organisms that frequently perform swimming or floating behaviour. This may explain the elevated dispersal rates of both Baetidae (frequent swims) and Chironomidae (several swimming species), but does not fit the non-swimming Physidae. Taxa that do not float nor swim probably disperse through the connections traveling on floating fragments of macrophytes. A relatively higher tendency of Physidae to occur on these floating fragments of macrophytes may have lead to higher dispersal rates, but we do not have data to confirm this. Alternatively, differences in dispersal rates of macroinvertebrate families may also occur because rheophylic species that only occur in the lake connections are transported to the lakes and join the dispersing lentic species from the source lakes. As a result, some families which have both lentic and rheophylic species in De Maten (e.g. Chironomidae, Oligochaeta,…) could show inflated numbers of dispersers. Most families, however, only consist of lentic species in De Maten (e.g. not in Baetidae, Corixidae, Physidae). Moreover, if dispersal were strongly influenced by the addition of rheophylic macroinvertebrates, we would expect an accumulating number of macroinvertebrates with increasing connection length, which we did not observe.

Contrary to our expectations, the number of dispersing macroinvertebrates was not related to their abundances in the source lakes. We suggest that the low predictive power of source lake abundances for explaining dispersal rates of macroinvertebrates may result from the highly patchy distribution of both freshwater microhabitats (e.g. macrophyte beds, patches of debris,…) and of macroinvertebrates within these microhabitats (Benoît et al. 1998, Diehl and Kornijów 1998). Our lake sampling technique integrates macroinvertebrate abundances of all littoral habitats over a large volume of water. However, the macroinvertebrate community that may actually be prone to transport through the lake connections is probably spatially confined to the area in the

direct proximity of the lake outlet, and may, by chance, deviate in terms of relative abundances of families from the integrated lake samples.

Dispersal by lentic macroinvertebrates over land can be achieved actively or passively, with active dispersal (flight) assumed to be more rapid and efficient (Rundle et al. 2002). Based on colonization events in new habitats and the distribution of species across the landscape, several studies have concluded flightless organisms to exhibit lower colonization ability than actively dispersing organisms (Brown et al. 1997, Hoffsten 2004, F. Van de Meutter unpublished results, but see Rundle et al. 2002). In the present study, dispersal through the lake outlets did not differ significantly among active and passive over-land dispersers. Although the number of dispersing macroinvertebrates through the lake connections may seem low compared to dispersal of zooplankton in the same system (Michels et al. 2001a), dispersal through lake connections may constitute a potentially important dispersal pathway, especially in passive dispersers (e.g. Gastropoda, Mollusca, …) that do not disperse frequently over land. In these groups, the presence of direct connections may be a very important structuring factor at both the community and population level.

Do the physical properties of the connections affect dispersal?

Flow rate and discharge volume of lake outlets did not affect dispersal rate or taxon richness of the dispersing macroinvertebrate assemblage. This is somewhat counterintuitive, since one would expect the total number of macroinvertebrates transported to be related to the transported water volume. Again, we suggest this may result from the highly patchy distribution of both macrophytes and lentic macroinvertebrates within the lake, generating high variability in dispersal among lake connections. Also the range of discharge volumes of lake connections in De Maten is relatively small, and dispersal rates may be expected to differ among lake connections with more diverging discharge volumes. Open tubes and rivulets apparently showed a similar potency for transmitting dispersing invertebrates, and supported a similar dispersal rate and dispersing macroinvertebrate assemblage. We like to note, however, that rivulets in our system always started and ended with a tube, which implies that our statement merely applies to connection intersections, and not to rivulets as a whole.

Dispersing zooplankton in outlet streams suffers highly from predation by fish (Akopian et al. 1999) and macroinvertebrates (e.g. net-spinning Trichoptera: Eriksson 2001, 2002), leading to declining zooplankton densities with increasing distance to the source lake (Walks and Cyr 2004). For dispersing macroinvertebrates in our study, we

could not detect a relationship between density or community composition and the lengths of the connections. This is surprising, since both Hydropsychid caddisfly larvae (throughout the lake connections) and young-of-the-year pumpkinseeds *Lepomis gibbosus* (at the outlets of the source ponds) were abundant (F. Van de Meutter, personal observations, see also Table 2). Hydropsychid caddisflies, however, mainly feed on small seston (Harding 1997), and probably only to a lesser extent on macroinvertebrates. Pumpkinseeds are important macroinvertebrate predators (Declerck et al. 2002), but occurred mainly at the outlets, which makes their effect independent of connection length. In lake connections that support a dense fish population over their whole length, a negative impact on macroinvertebrate dispersal may be expected.

Does dispersal differ between day and night?

The number of dispersing macroinvertebrates was higher during the night than during the day. Higher dispersal rates probably mainly result from a higher number of individuals in the upper water layers, near the lake outlets. In line with this, higher dispersal rates during the night probably indicate diel vertical migrations of macroinvertebrates to the lake surface at night. Of seven frequently dispersing families, higher nighttime dispersal could be shown for Chaoboridae and Chironomidae. Both Chaoboridae and Chironomidae are known to perform diel vertical migrations from the lake sediment during daytime to the lake surface at night (Marklund et al. 2001, Berendonk et al. 2003), which is in agreement with the idea that increased dispersal results from vertical migrations towards the lake surface.

Knowledge on the dispersal of lentic macroinvertebrates, both in isolated and connected habitats, is still very scarce. This study expands the existing knowledge by showing that overflows and rivulets in a connected lake system constitute a potential important dispersal pathway for lentic macroinvertebrates, which selectively transports macroinvertebrates from lake to lake. For some families, day/night differences in dispersal may occur, probably related to diel migration patterns. Dispersal through lake outlets was equally frequent in macroinvertebrates that disperse passively and actively over land. Future research should focus on testing the relative importance of dispersal through lake connections on the one hand, and dispersal over land on the other hand, for generating genetic and community level patterns of diversity in connected versus unconnected lakes. Especially by comparing these patterns between actively and passively dispersing macroinvertebrates, a more comprehensive understanding of the extent of dispersal in lentic freshwater macroinvertebrates may be generated.

Acknowledgements

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Chapter VI

Rapid response of macroinvertebrates to drainage management of shallow lakes

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[Unpublished manuscript]

Abstract

Shallow lakes over the world are subject to drainages, either as part of fish farming practices to harvest the fish, or as part of lake restoration projects. Lake drainages of fish lakes are known to improve macrophyte and zooplankton diversity, but the effect on the macroinvertebrate community is poorly known. In this study, we investigated temporal trends in the macroinvertebrate community following lake drainage in six shallow connected lakes. We evaluated drainage effects for all macroinvertebrates at the family level and for Coleoptera, Hemiptera and Odonata at the genus or species level. After the drainages, diversity increased for all macroinvertebrates at the family level, and for *Hemiptera and Odonata. Taxon richness increased for Coleoptera, Hemiptera and Odonata. Recolonization of the lakes by the former inhabitants occurred within the first year after the drainages and was supplemented with a set of species that previously were rare or did not occur in the lakes. Changes in the environmental conditions of the lakes were small and transient, except for the decline in fish. The fact that species that occurred in the lakes before the drainages rapidly recolonized the lakes is attributed to the high connectivity in our study system. The occurrence of supplementary species probably was linked to the decline in fish predation, suggesting fish was a dominant factor in shaping the communities. Lake drainage has a positive effect on the diversity and richness of macroinvertebrates in shallow connected lakes. This positive effect seems largely due to a combination of a decline in fish predation following lake drainage and a high rate of recolonization*, *probably both by dispersal over land and via connections to non-drained lakes. Lake drainage therefore probably seems the most cost-effective lake restoration tool in shallow connected lakes. Other restoration tools may be more favourable in isolated lakes where recolonization may be constrained.*

Introduction

Shallow ponds and lakes are of paramount importance for biodiversity (Jeppesen et al. 1998, Williams et al. 2004, Declerck et al. in press). Human impact on these systems, such as artificial stockings with fish, eutrophication and pollution through wastewater and runoff, however, has increased rapidly during the last century, causing pervasive disturbances of the food web and ultimately the loss of species (Scheffer 1998, Gulati and Van Donk 2002). By necessity, actively restoring lakes has become an essential part of lake management and is increasingly supported by a rapidly growing scientific background (Hansson et al. 1998).

The theory behind lake restoration has emerged largely from the theory of alternative stable states in shallow lakes (Scheffer et al. 1993, Scheffer 1998). Shallow lakes can occur in two alternative stable states, depending on the nutrient load of the lakes: a clear water state with abundant submersed vegetation and low fish densities at low nutrient loads, and a turbid water state with high algal biomass and high fish densities at high nutrient loads (Scheffer et al. 1993, Scheffer 1998). These alternative states have been found to be distinct attractors, each stabilized by multiple feedback mechanisms (Scheffer et al. 1993). Restoration of turbid lakes essentially occurs by drastically forcing one or several of the above aspects of the turbid water state to that of the clear water state, e.g. decreasing nutrient loads, promoting the growth of submersed macrophytes, and fish removal. Either for its direct advantageous effects, reducing fish densities and promoting growth of macrophytes, or as a necessary step in other management practices like removal of nutrient-rich sediments, lake drainage is frequently applied as a tool for lake restoration. Its application, however, is far more general, since many shallow lakes worldwide that are used for fish farming also are frequently drained to harvest fish. Macrophytes and zooplankton, both of which may readily reestablish from resting propagules in the lake sediment, have been reported to respond positively in terms of biomass and species diversity to lake drainages (Scheffer 1998). In contrast, for macroinvertebrates, which contribute largely to the biodiversity of shallow lakes, little is known on how they are influences by lake drainages.

Lake drainages may affect macroinvertebrate communities in a direct and in an indirect way. Drainages will directly cause many macroinvertebrates to go locally extinct. Unlike macrophytes and zooplankton, macroinvertebrates rarely possess drought-resistant propagules accumulated in the lake sediment to recruit from after the drainages. The chances for recolonization of a particular taxon in the lake therefore will depend on the macroinvertebrate's presence in the regional source pool of nearby

ponds, and on taxon-specific dispersal abilities and frequencies (Bilton et al. 2001). Establishment and reproductive success of a species may be further affected by its relative order of arrival compared to other species (priority effects: Morin 1984, Belyea and Lancaster 1999). The lake drainages may also indirectly influence macroinvertebrates via changes in the local environmental conditions, such as the abundance of fish and macrophytes, nutrient dynamics and abiotic conditions. All of these factors have been reported to affect the presence of macroinvertebrates (Healy 1984, Ward 1992, Diehl and Kornijów 1998) and may jointly shape the identity of the newly established macroinvertebrate community in the lakes.

This study aims at investigating the effects of lake drainages on the macroinvertebrate community in a set of six shallow, closely connected lakes. Connected lakes differ from isolated ones amongst others in the fact that dispersal is enhanced in connected systems by passive transport of individuals through the lake connections (Michels et al. 2001, Van de Meutter et al. submitted, Chapter V). This may affect both the composition of the new community and the rate at which processes that determine community build-up and persistence take place. We studied the effects of the drainages on macroinvertebrates by analyzing the structure and composition of the macroinvertebrate community in the year before the lakes were drained as well as in the first and second year after the drainages. To evaluate whether the importance of direct versus indirect mechanisms, we also quantified changes in key environmental conditions for macroinvertebrates. The effects were studied at two levels and for different groups of the macroinvertebrate community. First, we focused on the macroinvertebrate community as a whole. Next, we studied three orders with a high number of species, the Coleoptera, Hemiptera and Odonata, in more detail.

Material and methods

Study Area

The study area was the nature reserve De Maten, an open, diverse landscape consisting of dry heath land, small patches of woodland, meadows and marshes situated in the Northeastern part of Belgium near Genk. The chief characteristic feature of the reserve is the 3.5 km long chain of 34 shallow, eutrophic lakes, which are fed by the water of three small rivers that enter the cluster of lakes from the northeast and leave as two new rivers again at the most southwesterly lakes (Cottenie et al. 2003). The lakes result from the conversion of marshes to lakes by peat-digging and the building of dikes in the Middle Ages. Since then, the lakes have been used for extensive fish farming, which

stopped at their gradual incorporation into the nature reserve from 1956 to 1991 (Daniëls 1998). Before, every 4-5 years, the lakes were drained to harvest the fish. This practice, however, was abandoned after the fish farming had stopped. In 1993, the management committee of the reserve decided to install an alternating cyclic drainage regime in all lakes with a frequency of 3-4 years, to create foraging opportunities for waders and ducks, and to reduce the density of the fish stock (mainly exotic species such as pumpkinseed *Lepomis gibbosus* and brown bullhead *Ameiurus nebulosus*) in the lakes. The present study focuses on six lakes in the reserve that were all drained from November 2001 until March 2002 in the framework of the current management policy.

Sampling and sample processing

We sampled six lakes of De Maten (lakes 3, 8, 12, 13, 14 and 15, for situation in the reserve see Cottenie et al. [2001]) for three consecutive years (2001, 2002, 2003) during the last ten days of May, July and September. This resulted in a series of nine samples for each lake. The samples taken in 2001 reflect the situation before the lakes were drained; samples of 2002 and 2003 represent the situation during the first and the second year after lake drainages. Inter-annual variation in the community structure of macroinvertebrates can either result from the lake drainages themselves or from temporal variation in other external factors such as climate or regional trends in species occurrences. To be able to separate both mechanisms that induce temporal variation in macroinvertebrate community structure, we included during each July sampling campaign six extra lakes that were drained one year earlier, during the winter of 2000-2001. For these lakes, 2001, 2002 and 2003 constitute the first, the second and the third year after their drainage.

We sampled macroinvertebrates with a D-shaped net (23 cm x 23 cm, 500 µm mesh). This sampling technique includes epiphytic, epidetritic and free-swimming macroinvertebrates, but excludes typical benthic species. All samples were standardized in both area covered (75 m shore line) and duration (10 minutes). Samples in a given pond were taken each time along an identical trajectory, which included all major lake microhabitats (macrophytes, open water, patches of detritus) in quantities reflecting their presence in the lake littoral. Samples were fixed in formaldehyde (4%). All specimens were counted and identified to family level, except for Actinedida, Hirudinea and Oligochaeta. Coleoptera (adults and larvae) were further identified to genus level, and Hemiptera (adults) and Odonata (larvae), were identified to species level. Because small specimens of some genera of Odonata cannot be distinguished morphologically, these

genera were pooled into two species groups (*Ischnura*/*Coenagrion* and *Libellula*/*Orthetrum*). Based on long-term field observations of adults, these groups presumably include four and two species, respectively, in De Maten

Environmental conditions in the lakes

The structure and composition of a macroinvertebrate community may be strongly dependent on the local environmental conditions in the lakes (Dunson and Travis 1991, Weatherhead and James 2001, Nicolet et al. 2004). The following abiotic and biotic variables that are known to affect macroinvertebrate presence were therefore monitored in all lakes together with the sampling of the macroinvertebrates: conductivity, pH, water transparency (as Snell depth), phytoplankton abundance (chlorophyll a), macrophyte coverage and fish abundance.

Conductivity (µSiemens) and pH were measured *in situ* using a WTW multiline F meter (Geotech ©). Water transparency was measured with a Snell tube. A Snell tube consists of a mini secchi disk (Ø 5 cm) that can be lowered in a long PVC tube filled with water. Snell depth is given as the mean of the maximal depth (in cm) at which a black cross on the disk can be seen when lowering the disk in the tube, and the depth at which the cross can be seen again when lifting the disk. A Snell tube is a convenient measurer of transparency when a conventional Secchi disk cannot measure water transparency because the Secchi depth is larger than the actual depth of the lake. Chlorophyll a concentrations were measured spectrophotometrically following the procedure of Talling and Driver (1963). Macrophyte coverage (%) of floating and submersed vegetation was monitored along the macroinvertebrate sampling trajectory in all six lakes and expressed as percentage coverage. Relative fish densities in each lake were estimated both by the number of juvenile fish collected during macroinvertebrate sampling with the D-net (mainly young-of-the-year [YOY] fish) and by sampling campaigns involving 22 lakes in the reserve with fykes in September 1998 (before the drainages) and September 2002 (after the drainages). For the latter, we placed two fykes in each lake, once for a period of two days and once for a period of three days. These data were recalculated to catch-per-unit-effort (CPUE), i.e. the number and biomass of fish per fyke per day.

Statistics

The same six lakes were monitored over nine occasions, therefore, all nine samples are repeated observations of the same set of subjects (lakes). Since the composition of a macroinvertebrate community inherently shows strong seasonal variation, which may

blur the general drainage effect, we separated the within-year effect from the among-year effect in all analyses.

Synoptic parameters of community structure (abundance, taxon richness and diversity) were analyzed for each macroinvertebrate group separately using univariate repeated measures ANOVAs (rmANOVAs), with two repeated factors; “Month” with May, July and September as the different repeats within years, and “Year” with the observations from 2001, 2002 and 2003 as the repeats. By including also the interaction term between the two repeated factors, we could not only test for differences within and among years, but also whether the among year effect (the drainage effect) was different for the different months. Tukey post-hoc tests were used to determine pair-wise differences among years. All univariate analyses were performed using mixed models in SAS version 8.2 (SAS Institute 2001).

To generate a more detailed view on taxon dynamics within each of the macroinvertebrate groups, we also performed multivariate analyses. We first tested for within year-effects that would blur the possible drainage effect in our data. Since within-year effects were apparent for all groups and for all years (all $p<0.03$), we tested for an among-year effect for each of the three months separately. To test for within-year and among-year effects, we applied linear constrained ordinations in combination with a permutation test in CANOCO 4.5 (ter Braak and Smilauer 2002) as advocated by Leps and Smilauer (2003). Prior to these tests, we checked the length of the dominant gradients in our data, using Detrended Canonical Correspondence Analysis (DCCA). Gradients <3 SD indicate a linear trend in the data, gradients >4 SD indicate a unimodal trend. All gradients in our data were smaller than 2.75 SD, hence we applied a linear ordination method, Redundancy Analysis (RDA), for all analyses. Significance of the inter-year effects was obtained by partialling out the variance among lakes and permuting the observations within lakes (999 permutations). When significant differences were obtained among years, we performed a Canonical Variates Analysis (CVA) with forward selection of variables to select the taxa that contributed most to the observed patterns. Taxa with the highest amount of explained variance were tested for significance using Monte-Carlo permutation tests (999 permutations).

Temporal changes in chlorophyll a concentrations, conductivity, macrophyte coverage, pH, Snell depth and the number of YOY fish were analyzed using separate rmANOVAs, with “Month” and “Year” as the repeated factors. The change in the number and biomass of fish between 1998 and 2002 was analyzed with a rmANOVA with the CPUE (number and biomass) of 22 lakes of De Maten in 1998 and 2002, respectively,

as the repeats, and with the timing of drainage as the categorical factor with two levels: the study lakes (6) that were drained during the winter of 2001-2002 and 16 extra lakes that were drained during the winter of 1999-2000.

Significant temporal patterns in macroinvertebrate community structure may originate either from the drainages of the lakes, or from regional inter-annual variation in community structure. If the latter were true, we would expect the observed patterns to be identical in the lakes that were drained in 2000 (extra lakes, only July data available) and the lakes that were drained in 2001 (study lakes). To test this, we compared temporal trends in the study lakes to the trends of the extra set of lakes using rmANOVAs, with timing of drainage (2000 or 2001) as the categorical predictor and "Year" (2001, 2002, 2003) as the repeated factor. If the observed year effects would truly reflect drainage effects, then we would expect a time lag of one year between both sets of lakes. This would be apparent as a significant interaction of Timing of drainage x Year. To reduce the amount of multiple testing, we only tested factors that showed a significant trend in our study lakes. Significant trends in our study lakes in the abundances, taxon richness and diversity for each of the four groups of macroinvertebrates were analyzed using rmANOVAs with "Year" (2001, 2002, 2003) as a single repeated factor.

Results

Environmental conditions

Means of the environmental variables of our study lakes for each sampling date are presented in Table 1. Chlorophyll a concentrations showed no significant changes among years (rmANOVA, Year: $F_{2,10}$=3.59, p=0.067), except for the month July, when a distinct peak of chlorophyll a was obtained in the second year after the drainages (Year*Month: $F_{4,20}$=5.58, p=0.005; Table 1). Both pH and Snell depth showed no general pattern through time (both p> 0.11). Conductivity declined after the drainages, but tended to reach the original level in the second year (Year: $F_{2,11}$=5.78, p=0.019; Tukey: 2001 vs. 2002: p=0.016, other p>0.13). Vegetation cover of submersed macrophytes increased the first year after the drainages, but decreased again the second year (Year: $F_{2,11}$=5.49, p=0.022; 2001 vs. 2002: p=0.024, 2002 vs. 2003: p=0.076, 2001 vs. 2003: p=0.99). The coverage of floating vegetation remained relatively constant ($F_{2,11}$=2.34, p=0.14).

Table 1. Means of the monitored environmental variables in our study lakes for May, July and September in 2001-2003. Presented are pH, Conductivity, Chlorophyll a, Snell depth, the number of YOY fish and percentage coverage of floating-leaved and submersed macrophytes. No macrophyte data are available for May 2001.

	2001			2002			2003		
	May	July	Sept	May	July	Sept	May	July	Sept
pH	8.07	8.42	6.90	7.44	7.10	7.76	7.36	8.32	7.52
Conductivity (µSiemens)	405.50	433.50	422.67	384.50	359.83	358.17	353.83	392.33	446.17
Snell depth (cm)	41.50	33.83	32.33	33.58	31.33	34.00	43.83	29.17	34.17
Chlorophyll a (µg/l)	19.81	77.38	32.90	10.08	17.14	42.86	10.19	189.04	27.80
YOY fish	36.83	29.50	18.50	17.67	13.17	4.50	95.50	19.50	29.17
Floating Macrophytes (%)	-	11.67	10.83	11.67	2.50	1.67	12.50	8.33	2.50
Submersed Macrophytes (%)	-	18.33	45.00	23.33	55.83	76.67	52.50	44.17	26.67

The number of YOY fish declined significantly the first year after the lake drainages (Year: $F_{2, 10}$=17.58, p=0.0005; Tukey: 2001 vs. 2002: p=0.0004), but increased again the next year (Tukey: 2001 vs. 2003: p=0.42, 2002 vs. 2003: p=0.096, Table 1). Both the number and the biomass of fish caught with fykes declined significantly from 1998 to 2002 (mean number CPUE ±1SE: 1998: 247±49, 2002: 25±9, $F_{1, 21}$=17.57, p=0.0004; mean biomass±1SE: 1998: 6.2±1.2 kg, 2002: 1.0±0.3 kg, $F_{1, 21}$=16.37, p=0.0006). The decrease in numbers and biomass of fish in 2002 did not differ betweenlakes that were drained in 1999-2000 and those that were drained in 2000-2001 (Timing of drainage* Year; numbers: $F_{1, 21}$=0.045, p=0.83; biomass: $F_{1, 21}$=0.38, p=0.54).

Macroinvertebrates

The results of the rmANOVAs on the macroinvertebrate data are summarized in Table 2 and Figure 1. Seasonal changes in taxon richness were observed in all four taxonomic groups. Additionally, there were consistent seasonal trends for abundance in Coleoptera and for diversity in Odonata.

The lake drainages had no effect on the abundances and the number of families of macroinvertebrates in the lakes. Family level diversity, however, increased significantly in the second year after the drainages. Multivariate analyses revealed a significant temporal gradient over years in the structure and composition of the macroinvertebrate community at the family level in May and July, but not in September (Table 3). In May, Anisoptera, Baetidae, Hirudinea, Naucoridae and Zygoptera showed higher abundances the second year after the drainages compared to the year before and the first year after the drainages (Table 4, Fig. 2A). Chironomidae were most abundant the year before the drainages and declined afterwards (Fig. 2A). In July, Culicidae were the only selected discriminating family; they increased the second year after the drainages (Table 4, Fig. 2B).

Coleoptera tended to increase in abundance and genera richness over the three years, but Coleoptera diversity remained unchanged. Multivariate analyses indicated among-year differences in the Coleoptera community in May and July, but not in September (Table 3). In May, the majority of the Coleoptera genera tended to be most abundant in the second year after the drainages (Fig. 2C). Discriminating genera were *Laccophilus* (abundant second year after the drainages) and *Rhantus* (abundant first year after the drainages) (Table 4). In July, each of the years was characterized by a different set of Coleoptera genera, but the Coleoptera community of the second year after the drainages (2003) differed most from the community of the other years (Table 3,

Fig. 2D). *Hyphydrus* and *Laccophilus* dytiscid beatles were most abundant the first year after the drainages, while *Rhantus* typically increased the second year after the drainages.

Table 2. Results of the rmANOVAs testing for effects of Year and Month on abundance, taxon richness and Simpson diversity for all macroinvertebrates, Coleoptera, Hemiptera and Odonata. F-values are given, with df = 2, 10 for main effects and df = 4, 20 for the interaction terms.

		Abundance	Taxon richness	Diversity
		F	F	F
All macroinvertebrates	Year	0.16	0.33	4.13*
	Month	3.31	13.99**	0.86
	Year*Month	0.90	0.76	1.82
Coleoptera	Year	2.95°	3.28°	0.57
	Month	33.55***	25.61***	2.33
	Year*Month	0.85	1.08	0.48
Hemiptera	Year	2.14	7.58**	7.46*
	Month	1.99	4.99*	3.09
	Year*Month	7.22***	2.85°	1.37
Odonata	Year	1.29	0.93	0.40
	Month	2.38	4.03*	8.88**
	Year*Month	1.41	3.64*	3.99*

° p<0.1, * p<0.05; ** p<0.01; *** p<0.001

The Hemiptera community showed an intriguing temporal pattern: the abundance of water bugs recovered rapidly from the drainages to the same level as before the drainages, but declined the second year after the drainages in July and September, but not in May (Year*Month, Table 2). The number of water bug species in the lakes, however, increased significantly the first year after the drainages, but decreased again to the original level the second year. Simpson diversity of Hemiptera increased gradually from the year before to the second year after the drainages. In contrast to these findings, multivariate analyses were unable to identify significant changes in the general structure and composition of the Hemiptera community with time over the three years (Table 3). The observed effects of the drainages on the odonate community were apparent in May, but were only subtle or absent in July and September. The number of odonate species increased the second year after the drainages in May, tended to increase the first year

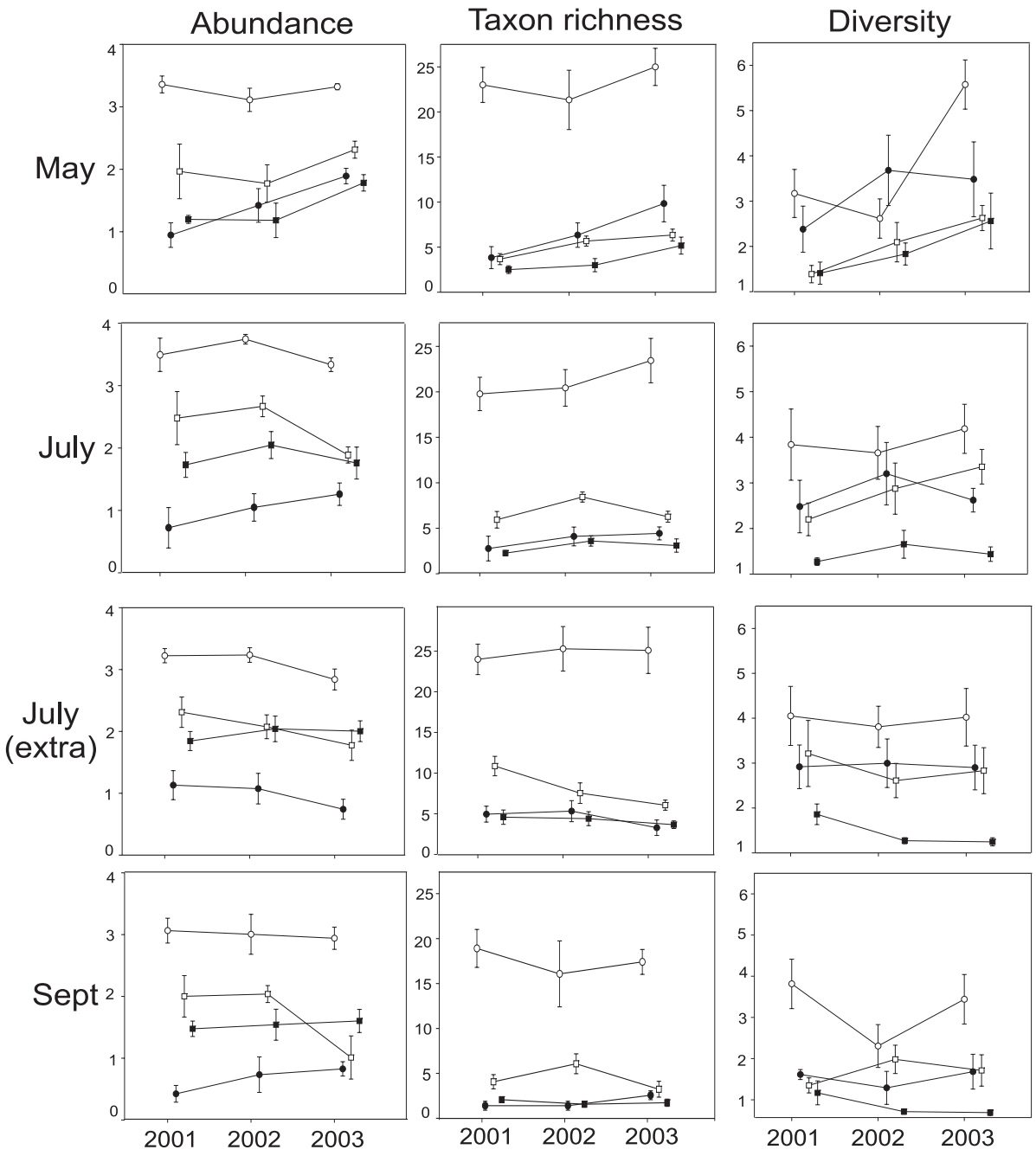

Fig. 1. Abundance (log-transformed), taxon richness and Simpson diversity of all macroinvertebrates (family-or-higher-level, ○), Coleoptera (genus-level, ●), Hemiptera (species level, □) and Odonata (species-level, ■) for three consecutive years (2001, 2002 and 2003) of six ponds that were drained during the winter of 2001-2002. Data are presented for May (first row), July (second row) and September (fourth row) separately. July-data of six extra lakes that were drained one year earlier (winter 2000-2001) are presented in row three.

after the drainages in July, but was unaffected by the drainages in September (Table 2, Fig. 1). Odonate diversity in May increased gradually over the three years, but odonate

diversity in July and September remained unchanged (Table 2, Fig. 1). Odonate abundances were not significantly affected by the drainages. Accordingly, multivariate analyses indicated significant changes in the odonate community in May, but not in July and September. In May, all odonate species performed at least temporarily better after the drainages than before it. Two groups of odonate species could be distinguished: a first group, characterized by *Lestes sponsa*, increased its abundance in the first year, and a second group, characterized by *Sympecma fusca* and *Ischnura/Coenagrion*, increased their abundances in the second year after the drainages (Fig. 2E).

When focusing on the July data of our study lakes, we could demonstrate only one significant inter-year pattern, namely for taxon richness of Hemiptera ($F_{2,10}$=4.14, p=0.049, other p>0.16): the number of species of Hemiptera tended to increase the first year after the drainages, but declined significantly the second year. This trend clearly differed from the trend we observed in the extra lakes (Timing of draining*Year: $F_{2,20}$=6.17, p=0.0082, Fig. 1), where we observed a steady decline throughout the years. This is in agreement with the expectation that these lakes would show a similar pattern as the study lakes, but shifted one year in time.

Discussion

Drainages of shallow lakes are applied worldwide, both in fish farming and in lake restoration projects. One of the main reasons for lake restoration is to increase local biodiversity, which could be demonstrated for bird, macrophyte and zooplankton communities (Scheffer 1998). Recent research, however, showed that biodiversity patterns in shallow lakes not necessarily are congruent among different taxonomic groups (Declerck et al. in press), which urges for more research on other, less-studied communities such as the rich macroinvertebrate community. This study shows that macroinvertebrate diversity increased as a result of lake drainage. For three species-rich insect orders, Coleoptera, Hemiptera and Odonata, we also observed a considerable increase in abundances and/or taxon richness. These changes were different among the specific groups and in some cases tended to be transient.

The distinct change over the three years in macroinvertebrate community structure and composition we observed in the study lakes in principle may be caused by the lake drainages themselves or by other sources of inter-year variation. We could demonstrate by a parallel analysis on a set of extra lakes, that the observed inter-year pattern in July was not general. Instead, the observed changes seemed shifted one year in time for the lakes that were drained one year earlier, which is consistent with the

Table 3. Results of the permutation test of the RDA for inter-year differences in community structure of all macroinvertebrates, Coleoptera, Hemiptera and Odonata among the years 2001, 2002 and 2003. Significance for all axes ($p<0.05$) indicates with 95% probability that the communities have changed over years. If a significant change among years was apparent, we also tested the significance of the first and the second axis (for interpretation, see Fig. 2).

	May				**July**				**September**			
	All axes		First axis	Second axis	All axes		First axis	Second axis	All axes		First axis	Second axis
	F-ratio	p	p	p	F-ratio	p	p	p	F-ratio	p	p	p
All macroinvertebrates	1.736	0.043	0.265	0.136	2.256	0.020	0.062	0.27	1.376	0.236	-	-
Coleoptera	2.586	0.026	0.052	0.245	2.72	0.006	0.02	0.632	1.716	0.09	-	-
Hemiptera	1.552	0.12	-	-	1.313	0.21	-	-	1.086	0.35	-	-
Odonata	3.723	0.001	0.001	0.094	1.070	0.388	-	-	0.295	0.892	-	-

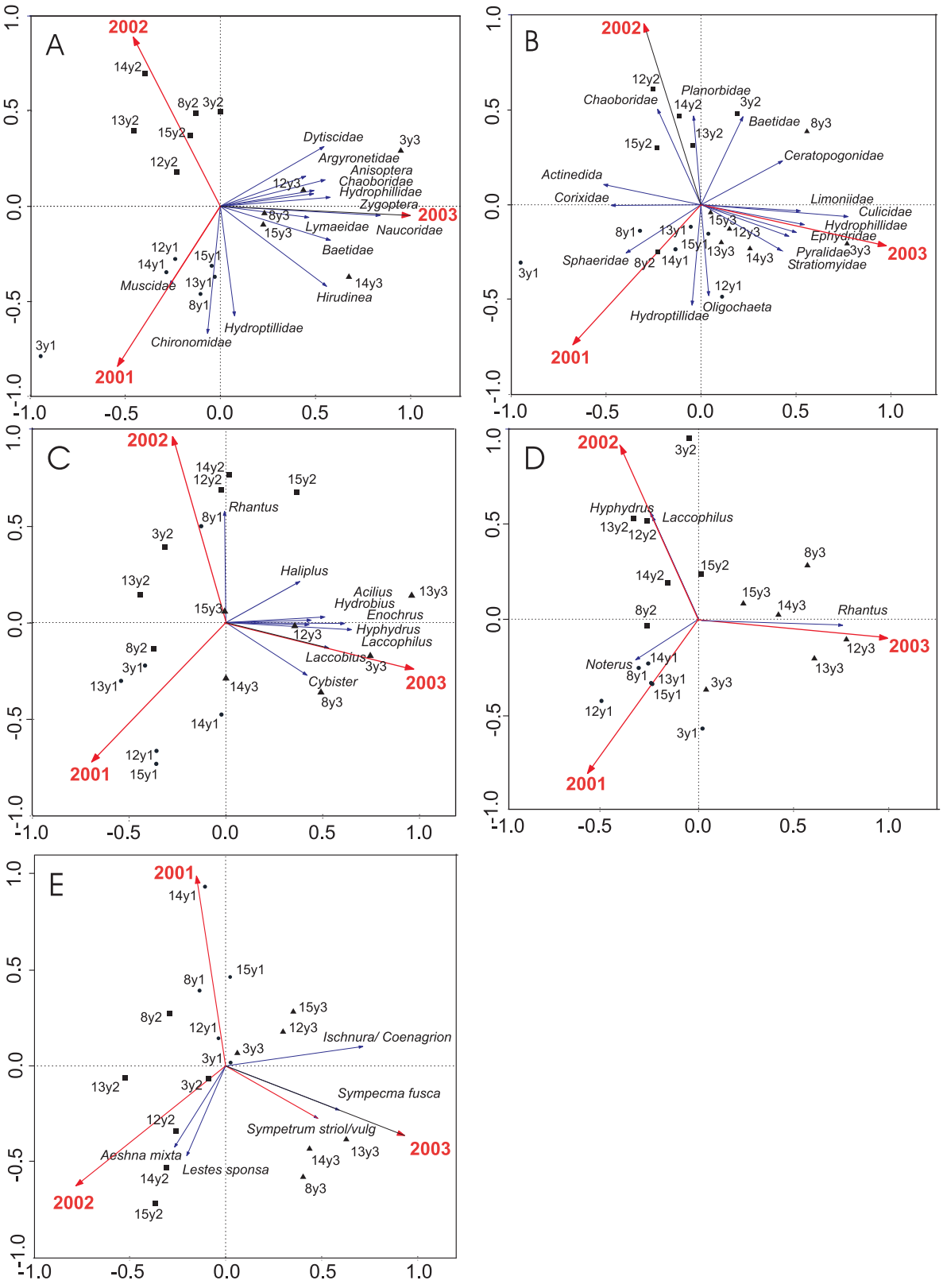

Fig. 2. (Legend on next page 140)

Fig. 2. Results of the Redundancy analysis on the macroinvertebrate data (family-or-higher-level) in May (A) and in July (B), on the Coleoptera data (genus-level) in May (C) and in July (D) and on the Odonata data in May (E). All six study lakes were drained from November 2001 to March 2002. Presented are the three sampling years (2001, 2002 and 2003), the individual samples, and the macroinvertebrate families. For clarity, only taxa with weights > 15 % are presented. Samples of 2001 are presented by ●, samples of 2002 by ■ and samples of 2003 by ▲. Sample names are a combination of lake number and year (for example 14y1 is the sample of pond 14 in 2001). Significance of the biplot axes is given in Table 3. Taxa that explain a significant amount of inter-year variation are given in Table 4.

notion that the changes are caused by the drainages. These results are based solely on the data of July, but we have no reason to assume that they do not apply to the other months as well. Together with the drastic nature of the drainages by virtually wiping out local macroinvertebrate communities, we therefore consider the lake drainages the strongest selective agent that was acting upon the lake communities during these years.

Lake drainage strongly affected the local environmental conditions in the lakes, but these changes were largely transient. Conductivity levels showed a transient decrease after the drainages, probably because refilling of the lakes through lake connections was supplemented with ion-poor rain water. Submersed macrophyte coverage increased transiently the first year after the drainages. Increased submersed macrophyte coverage following lake restoration is often related to increased water transparency (Hosper 1998, Scheffer 1998), but water transparency remained relatively unchanged in the present study. This may be due to relatively high levels of humic compounds, which limit the maximal transparency, and hence, growth of macrophytes in these lakes. The increase in submersed macrophyte coverage in the study lakes, probably resulted from the compaction and aeration of the lake sediments during the drainages, and the temporally lower water levels at the refilling of the lakes (Woltemade 1997). Fish densities were drastically reduced by the drainages. Fish are, however, known to migrate from lake to lake at a high rate through the lake connections in de Maten (S. Declerck unpublished results), and could rapidly recolonize the lakes after they were refilled. The number of juvenile fish already was back at its original level in the second year after the drainages, probably due to a combination of dispersal and increased survival of YOY fish. After two years, final densities of the fish community in the lakes were however still much below the densities prior to the lake drainages.

The years following the drainages, diversity of macroinvertebrate families increased, but the number of macroinvertebrates and the number of families remained relatively unchanged. From this, it appears that in every family, some species could rapidly (re)colonize the lakes. This contrasts with studies indicating differential

colonization rates among different macroinvertebrate families, with actively dispersing families colonizing sooner and more frequently than passively dispersing families (Brown et al. 1997, Bilton et al. 2001). The discrepancy between these studies and the current study probably originates from the fact that the lakes in our study are highly connected. The connections to the lakes that were not drained may have served as an important additional dispersal pathway, increasing the recolonization probability for actively and in particular passively dispersing families (Van de Meutter et al. submitted, Chapter V).

The families and higher-order taxa that benefited most from the drainages are Anisoptera, Baetidae, Culicidae, Hirudinea, Naucoridae and Zygoptera. Of these, Anisoptera, Hirudinea, Naucoridae and Zygoptera constitute large predatory species. Their increase probably can be attributed to decreased fish densities, since large invertebrates are particularly prone to fish predation (Wellborn et al. 1996). Chironomidae showed lower abundances after the drainages, although Chironomidae are amongst the most important prey of benthivorous fish. This is generally attributed to an increase in predation by invertebrate predators (Batzer et al. 2000).

In Coleoptera, abundances and the number of genera tended to increase after the lake drainages. Many aquatic beetles predominantly occur in fishless lakes (Fairchild et al. 2000), hence they were scarce in the fish lakes of De Maten before the drainages. Their high dispersal ability, however, allowed them to rapidly colonize the lakes from nearby ponds after the fish were decimated. Colonization by aquatic beetles may even be more enhanced by the fact that adult beetles can actively choose to colonize and reproduce in lakes where fish is scarce or lacking (Abjornsson et al. 1997).

The number of Hemipteran species increased significantly the first year after the drainages, but declined to initial levels in the second year. The decline occurred along with a drastic reduction in the abundances of Hemiptera. Multivariate analyses were unable to identify significant trends in the structure and composition of the Hemiptera community, probably due to high inter-lake variability in the composition of the newly colonized communities. Therefore, it is difficult to unequivocally identify the processes responsible for the observed patterns. Both environmental variables (Bendell and McNicol 1995, Downie et al. 1998), inter-specific interactions (Pajunen and Pajunen 1991) and the presence of fish (Bendell and McNicol 1995), are known to exert strong effects on the composition of a local Hemipteran community.

Odonates benefited both in terms of abundances and species richness from the lake drainages, although the extent of their increase and the temporal pattern over years differed among the three months. The most striking pattern, observed in May, was as

follows: before the drainages, spring species dominated the community. Spring species (e.g. members of genera *Anax*, *Coenagrion*, *Ischnura*) are species that develop to a full-grown larva during summer and autumn, and metamorphosize to adults the next spring,

Table 4. Results of the forward selection procedure in the Canonical Variates Analysis of taxa that explain a significant portion of the variability among years. Forward selection of taxa was only applied when RDA indicated significant inter-year effects (see Tabel 3). P-values were obtained by Monte-Carlo randomizations (999 permutations). Only taxa with p<0.05 are presented.

Group	Month	Taxon	Variance explained (%)	F	p
All macroinvertebrates	May	Naucoridae	38.1	6.763	0.005
		Hirudinea	29.4	4.586	0.019
		Chironomidae	29.3	4.556	0.012
		Baetidae	27.4	4.154	0.015
		Anisoptera	25.1	3.679	0.034
		Zygoptera	24.2	3.518	0.040
All macroinvertebrates	July	Culicidae	33.2	5.476	0.011
Coleoptera	May	*Rhantus*	25.5	3.763	0.032
		Laccophilus	24.5	3.579	0.038
Coleoptera	July	*Rhantus*	35.2	5.986	0.008
		Hyphydrus	23.9	3.450	0.046
		Laccophilus	22.4	3.170	0.049
Odonata	May	*Ischnura/Coenagrion*	49.7	7.672	0.001
		Sympecma fusca	30.4	3.690	0.029
		Lestes sponsa	25.5	2.936	0.049

or take several years to develop. The first year after the drainages, spring species were largely replaced by summer species. Summer species (e.g. members of *Aeshna*, *Lestes*, *Sympetrum)* are species that overwinter as an egg, complete their development in only a couple of months during spring and emerge to adults in summer. The second year after the drainages, both spring and summer species were abundant, with the exception of *Lestes sponsa* who decreased in numbers. The transient change in relative abundance of spring and summer species probably was due to a combination of drainage-related

changes in intra-guild predation and fish predation. Drainage directly eliminated spring species, but not summer species since these have overwintering eggs. As a result, the latter benefited from release of asymmetric intra-guild predation of summer species. Indeed, in spring, summer species will always be much smaller than spring species, often resulting in low survival rates and even exclusion of the former due to intra-guild predation (Morin 1984, Stoks and McPeek 2003). Also fish predation may have contributed to the transient relative increase in summer species. Due to their shorter larval life, summer species are more active and suffer more from fish predation than the less active spring species (Stoks and Johansson 2000).

Changes in the structure and the composition of the macroinvertebrate community after lake drainage were expected to result from a combination of both indirect and direct mechanisms: changes in local environmental conditions following lake drainage, and taxon-specific differences in extinction/colonization dynamics, respectively. Among the environmental conditions, the decline in fish predation probably was the most important factor, possibly strengthened by increased macrophyte coverage, which has a mitigating effect on fish predation (Crowder and Cooper 1982). The indirect effect of the decline in fish on the macroinvertebrate community was strong, and probably largely accounted for the observed changes in the macroinvertebrate community. Although direct mechanisms probably as well had strong effects on the macroinvertebrate community, these effects remained largely unnoticed due to the remarkably fast recolonization of most of the former lake inhabiting taxa. This high rate of recolonization probably can be attributed to the high level of connectivity in our study system, both over land (short distances among the lakes) as through aquatic connections among the lakes. In isolated lakes, recolonization of the former lake inhabitants may be (dispersal) limited, and direct and indirect effects of lake drainage may both contribute to changes in community structure and composition.

Although lake management in De Maten focuses mainly on the protection of birds and macrophytes, clearly macroinvertebrates benefited also from the drainage management in terms of species richness and diversity. This general positive effect was the result of the rapid recolonization of the former lake inhabitants due to the high connectivity to non-drained lakes, supplemented with a number of species that benefited from changes in he local lake conditions (mainly the decrease in fish) and managed to colonize the lakes. Apart from its positive effect on local biodiversity by accelerating recolonization of macroinvertebrates, the high level of connectivity among our lakes also causes a rapid and continuous inflow of nutrients and fish from outside which may

rapidly shift the lake back to its condition before the drainage. In a system of strongly connected lakes, therefore, lake restoration has to be repeated every couple of years. Since financial and human resources for conservation management often are limited, this can only be achieved if such restoration projects are cheap and do not require large efforts. Lake drainage meets these criteria. Additionally, the high extinction rates caused by drainage are counteracted by the rapid recolonization in connected systems, resulting in a high cost-efficiency.

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Chapter VII

Metacommunity structure of lentic macroinvertebrates: contrasting active and passive dispersers

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[Unpublished manuscript]

Abstract

Macroinvertebrates inhabiting ponds and lakes can be broadly categorized as active or passive dispersers. Because dispersal over land is assumed to be very limited in passive dispersers, connections between lakes may provide important additional dispersal corridors between lakes. Active dispersers can easily fly from lake to lake over land, and the additional contribution of dispersal via lake connections to dispersal may be negligible. As a result, metacommunities of active and passive macroinvertebrate dispersers may be differentially affected by lake connectivity and hence show different spatial structures. We investigated this hypothesis in a system of 34 connected lakes. In agreement with our expectation, we found that communities of passively dispersing macroinvertebrates were regionally structured according to a spatial model that incorporated the connections among the lakes, while active dispersers were not. Active dispersers, and surprisingly also passive dispersers, were also strongly structured conform the spatial model of Euclidian distances among the lakes, possibly suggesting that over-land dispersal as well was an important dispersal mechanism. These results confirm the importance of connectivity among habitats for metacommunity structure and dynamics, but also indicate that the net effect of connectivity on metacommunity structure depends on the prevailing mode of dispersal of the organisms.

Introduction

Massive evidence has accumulated that community structure and species occurrence may be strongly driven by local environmental factors (Morin 1999). However, it has been also recognized that sites exhibiting similar environmental conditions may have dissimilar species compositions because they are differentially accessible for dispersing organisms (e.g. Hanski and Gilpin 1996). Recently, much research has been addressed to the question which of these processes, local or regional, are more important in structuring communities, giving conflicting results (Karlson and Cornell 1998, Shurin 2000, Honnay et al. 2001, Cottenie et al. 2003). Obviously, the next step is to identify correlates associated with the relative importance of both processes. Intuitively, dispersal ability and connectivity among suitable habitats are strong candidates as they jointly determine the role of regional processes (Tscharntke and Brandl 2004).

The prevailing idea is that communities of organisms with low dispersal ability will be more strongly spatially structured than communities of frequently dispersing organisms (Johnson et al. 2001). Few studies, however, have empirically explored the link between dispersal ability (active vs. passive) and community structure. In communities of intertidal molluscs, Johnson et al. (2001) found that directly developing species (low dispersal ability) showed greater fine scale patchiness than the distributions of species with a larval dispersal stage. In freshwater systems, Rundle et al. (2002) failed to show differences in the distribution patterns of Microcrustacea (passive dispersers) and aquatic beetles (active dispersers) of small aged ponds. However, Microcrustacea may not be fully representative for passively dispersing invertebrates in general. First, colonization by Microcrustacea appears to be more rapid than previously thought (Caceres and Soluk 2002, Louette and De Meester 2005), and probably occurs more rapid than in any other group of passively dispersing macroinvertebrates. Second, once they are established, Microcrustacea may readily recolonize from the resting egg bank after long periods of absence, without the need of new colonizers from outside the habitat (Wolf and Carvalho 1989). These specific abilities are lacking in many other passively dispersing macroinvertebrate taxa (e.g. freshwater snails, Bronmark 1985), which therefore may be better suited to illustrate effects of dispersal mode on the spatial structuring of communities.

Community similarity among habitats may be strongly affected by connections, as this allows species to easily interchange among the habitats. In the specific situation of lakes connected by small streams or channels, dispersal may be strongly facilitated via these connecting elements. In passive dispersers, dispersal via these connecting

elements may outnumber the rare over-land transport (Michels et al. 2001, Van de Meutter et al. submitted, Chapter V). As a result, distributions and community similarity patterns of passive dispersers can be expected to correspond to the network of connections among lakes. Active dispersers, on the other hand, may frequently disperse from lake to lake both over land and through the lake connections, and their actual distribution and community similarity patterns depend on the relative contribution of each dispersal pathway.

To study the combined effects of dispersal mode (active, passive) and connectivity on community similarity patterns and on the relative contribution of local and regional processes in shaping metacommunity structure, we monitored during three years the macroinvertebrate communities in a cluster of connected lakes in De Maten (Belgium). Organisms inhabiting ponds and lakes are particularly suited for studying regional dynamics of community structure and composition. The fact that they inhabit discrete, island-like habitats in an inhospitable terrestrial matrix makes it easy to delineate different communities conform the theoretic ideas of metacommunities (Bilton et al. 2001). The study of lentic macroinvertebrates offers particular potential, as this is a very diverse group of organisms in which dispersal is achieved through active flight (many insects; active dispersers) or through passive transport (passive dispersers) by wind (water spiders) or animal vectors (aquatic mites, snails). The lakes of De Maten have been extensively studied in the past (e.g. Michels et al. 2001, Cottenie et al. 2001, Cottenie et al. 2003), with the focus on patterns and processes in the zooplankton (meta)community. Zooplankton dispersal occurs at a very high rate through the lake connections (Michels et al. 2001). Cottenie et al. (2003) showed that, besides local environmental factors, spatial factors (based on the configuration and intensity of dispersal through the lake connections) contribute significantly to variation in zooplankton community structure. In a previous study, we could show that also lentic macroinvertebrates disperse through the lake connections (Van de Meutter et al. submitted, Chapter V). Therefore, also the spatial structure of the macroinvertebrate metacommunity may be (partly) shaped according to the connectivity pattern among the lakes.

In the present study we want to investigate the following predictions with respect to the metacommunity structure of macroinvertebrates in De Maten: (1) As dispersal via the lake connections is probably relatively more important in passive dispersers compared to in active dispersers, we expect metacommunity structure of passive dispersers to be more affected by connectivity than metacommunity structure of active

dispersers (2) As active dispersers may easily fly from lake to lake, their distributions and spatial similarity patterns may be less spatially restricted than in passive dispersers in which dispersal may be mainly confined to the lake connections. Therefore, the relative contribution of spatial versus environmental factors in explaining metacommunity structure may be lower in active dispersers than in passive dispersers.

Material and methods

Study area

The study area is the nature reserve “De Maten”, a protected heath land of about 300 ha situated in the northeastern part of Belgium (Genk). The reserve comprises 34 shallow lakes, connected to each other by a complex network of rivulets and overflows (Fig. 1). The shallow lakes were created in the 15th century by peat-digging and the construction of dikes in the valleys of two parallel small streams. For centuries, the lakes were used for extensive fish farming, with an alternating draining regime with a rotation scheme of 4-5 years being installed. The fish farming practices stopped in 1991, but the alternating cyclic drainages continued as part of the management plan of the reserve to control local fish abundances and lake nutrient loads. Just before and during the present study, the lakes were all drained in two subsequent large draining campaigns: lakes 16-34 were

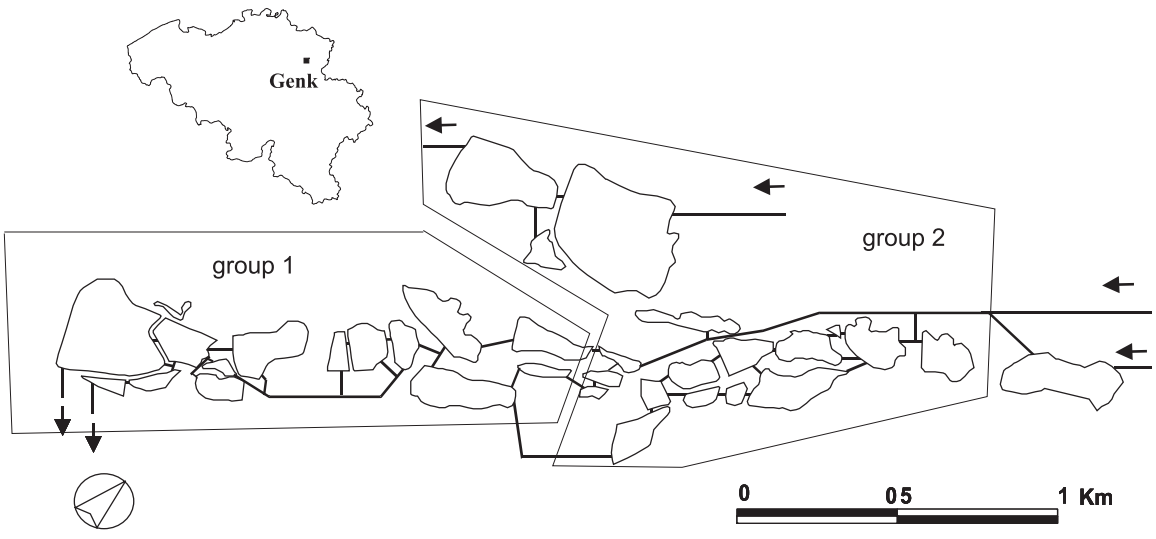

Figure 1. Map of the lake complex in our study area ‘De Maten’ with the lakes that were drained end 2000 (group 2) and the lakes that were drained end 2001 (group 1) indicated. Small arrows indicate the water current through the reserve; the large arrow directs to the North.

drained during winter 2000-2001 (group 2) and lakes 1-15 were drained during winter 2001-2002 (group 1). Drainings lasted for 2 up to 5 months (all ponds were refilled by the beginning of April).

Despite the high degree of connectivity, the shallow lakes display striking differences in environmental conditions turbidity, algal biomass, macrophyte cover and fish biomass. These ecological differences are largely in accordance with the theory of alternative states in shallow lakes with a turbid lake state characterized by high algal biomass, high zooplanktivorous fish biomass and low macrophyte cover, and a clear-water lake state characterized by opposite conditions (Scheffer et al. 1993).

Sampling and sample processing

We sampled the macroinvertebrate community in all 34 lakes of De Maten at the end of July for three consecutive years (2001, 2002 and 2003). Samples were taken with a D-shaped net (23 cm x 23 cm, mesh size 500 µm) and were standardized in both length (75 m shore line) and time (10 min). Samples in a given lake were taken each year along an identical trajectory, which included all major lake microhabitats (macrophytes, open water, patches of detritus) in quantities reflecting their presence in the lake littoral. This sampling procedure includes epibenthic, epiphytic, and free-swimming littoral macroinvertebrates, but excludes typical benthic species. All samples were preserved in the field in 4% formaldehyde. In the laboratory, all samples were fully sorted on white trays. Macroinvertebrates were counted and identified to family level, except for Coleoptera, Gastropoda, Hemiptera and Odonata, which were identified to species level.

The following environmental variables were measured for all lakes and all years: lake area, conductivity, chlorophyll a concentration, depth, densities of young of the year (YOY) littoral fish, oxygen concentration (O2), pH, Snell depth, water temperature, percentage open sediment (open), percentage cover by submersed vegetation, floating-leaved vegetation, *Lemna* sp., filamentous algae, emergent vegetation, tree roots and branches, and riparian vegetation and shading of the littoral by trees. Conductivity, oxygen concentration, pH and water temperature were measured with a WTW multiline F meter (Geotech ©). Water transparency was measured with a Snell tube (method as presented in Van de Meutter et al. in press, Chapter I). Chlorophyll a concentrations were measured spectrophotometrically following the procedure of Talling and Driver (1963). Macrophyte cover (%) of *Lemna* sp., filamentous algae, emergent, floating-leaved, riparian and submersed vegetation, the percentage cover by tree roots and branches, and the percentage open sediment, were monitored along the

macroinvertebrate sampling trajectory in all lakes. Shading by riparian trees was estimated as the percentage of the sampling trajectory that had tall trees (>10 m) within 10 m of the parallel shoreline. Relative YOY fish densities in each lake were estimated by the number of YOY fish collected during macroinvertebrate sampling with the D-net. The values obtained for each of these variables were summarized in an environmental variable matrix for each combination of year and lake group (6). Using Euclidian distance measures, dissimilarity matrices based on the standardized environmental variables were calculated for each environmental variable matrix.

Spatial parameters

Macroinvertebrates may disperse from lake to lake by active flight over land, or by swimming or passive transport through overflows and rivulets that connect the lakes. Both dispersal pathways result in a different spatial pattern of connectivity among the lakes. Using GIS software (Geomedia Professional Version 5.1, Intergraph 2003), we constructed three different spatial models to describe the connectivity patterns among the lakes in each lake group. Connectivity between lakes for the over land dispersal model ("Land model") was defined as the shortest Euclidian distance between the lakes. Connectivity between lakes for dispersal via the lake connections was defined as the shortest distance traveled through water from one lake to another ("Water model"). This model sums up the lengths of the connecting overflows or rivulets together with the shortest Euclidian lengths from lake-inlet to lake-outlet through the interspaced lakes. Non-connected lakes were given a connectivity value slightly higher than the maximum value obtained in the model. The third model also takes into account the connecting elements between the lakes, but assumes unlimited connectivity among neighbouring lakes and no interaction between non-connected or indirectly connected lakes ("Neighbouring model"). For both lake groups, all three models were constructed. The results of these models were summarized in six different square distance matrices.

Data management and analyses

To avoid an artificial community gradient, imposed by the asynchronous draining of two lake subclusters, to govern our data, we separately studied two sets of lakes in the study area. Due to lake management practices, six lakes were never sampled (Lakes 1, 9, 10, 11, 18, 33). Further, lake 4 was not sampled in 2003. This resulted in a total number of 9, 9 and 8 lakes for group 1 in 2001, 2002 and 2003, respectively, and 15 lakes for group 2 in all years. To avoid outliers (extremely high densities for some species) to

dominate our results, they were set to values just slightly higher than the highest value in the rest of the data set ("winsorizing", Sokal and Rohlf 1995, Cottenie et al. 2001). To further minimize the effect of high densities, all macroinvertebrate densities were square-root transformed. Before analysis, we classified all macroinvertebrates into active or passive dispersers. Aranea, Asellidae, Bivalvia, Gammaridae, Gastropoda, Hirudinea and Oligochaeta were classified as passive dispersers; Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Megaloptera, Odonata and Trichoptera were considered active dispersers.

To test for spatial patterns in the environmental conditions of the lakes, and for mutual relationships between the different spatial models, we performed MANTEL tests relating environmental dissimilarity matrices to different distance matrices and different distance matrices among each other. To evaluate which connectivity model best described macroinvertebrate metacommunity structure, hence, which dispersal pathway was most important in structuring the macroinvertebrate community in active versus passive dispersers and for the different years, we applied partial MANTEL tests between lake community dissimilarity and lake distance ('connectivity') controlled for environmental dissimilarity. We used Bray-Curtis dissimilarities to calculate macroinvertebrate community dissimilarity matrices. Euclidian distances and Bray-Curtis dissimilarities were calculated using Primer 5 for Windows Version 5.2.2. (Primer-E Ltd. 2001). All MANTEL and partial MANTEL tests were done using the freeware tool zt (Bonnet and Van de Peer 2002) and p-values were obtained by 9,999 permutations.

To be able to compare the relative contribution of spatial and environmental variables to macroinvertebrate community structure in active and passive dispersers, we first had to extract distance variables (coordinates) from the Land model and Water model distance matrices with Principle Coordinate Analyses using the DistPCoA freeware tool (Legendre and Anderson 1998). We chose not to include the Neighbouring model since this model is very difficult to summarize into a restricted number of coordinates. The Land model could be fully summarized in two axes (X, Y) for both lake groups, but the more complex Water model resulted in 7 (Ax1-Ax7) and 11 axes (Ax1-Ax11) in lake group 1 and 2, respectively. We combined the two axes of the Land model and the 7 axes of the Water model (group 1), and the two axes of the Land model and the first 10 axes of the Lake connection model (group 2) into one variable matrix, and added their quadratic terms to be able to test for non-linear dissimilarity-distance relationships. These data matrices were used in a forward selection Redundancy Analysis (RDA) procedure with as dependent matrix the macroinvertebrate abundances.

The number of selected axes ranged from 1-3 for group 1, and from 1-4 for group 2. In RDA, the sum of explanatory variables and covariates (environmental + spatial) cannot exceed the number of samples (lakes) -1. Therefore only the first two axes were used in the spatial explanatory matrix [S] for group 1. For group 2, the first three axes were included (for an overview of the selected axes per combination of year, lake group and dispersal group, see addendum 1).

Matrices of the full set of environmental variables were used as input in 12 forward selection RDA's (lake group x dispersal mode x year). The number of selected environmental variables ranged from 1 to 5 for group 1, and from 2 to 5 for group 2. For group 2, the first five environmental variables were included in the environmental matrix [E]. To keep the sum of variables in [E+S] lower than the number of lakes -1, the first four environmental variables were included for group 1 (for an overview, see Addendum 2).

To divide the variance in macroinvertebrate community structure into spatial and environmental components, the procedure of Borcard et al. (1992) was used. The total variance in the dependent matrix (macroinvertebrate abundances) was decomposed in different components: total variance explained [E+S], environmental variation [E], spatial variation [S], the fraction of variation that can be explained by the environmental variables independently of any spatial structure [E|S], and the fraction of the variation that can be explained by the spatial variables independently of any environmental variable [S|E]. Monte Carlo permutation tests (9,999 permutations) computed the significance for all the different components. We used the linear direct gradient analysis method RDA since preliminary Detrended Canonical Correspondence Analyses showed that the species respond linearly to gradients (maximal gradient lengths all smaller than 3 SD, Leps and Smilauer 2003). All ordination analyses were done with CANOCO for Windows Version 4.5 (ter Braak and Smilauer 2002).

Results

In lake group 2, all three spatial models were positively intercorrelated, indicating they all relfected a (partly) similar pattern of connectivity among the lakes (Table 1). In lake group 1, the Neighbouring model correlated highly positively with the Land and the Water model. The Land and Water model, however, were not correlated (Table 1).

Both lake groups differed in the presence of a spatial gradient in environmental conditions among the lakes. In lake group 1, no environmental gradient was present among the lakes (Table 2). Also the draining of the lakes end 2001 did not cause any

Table 1. MANTEL correlations (r) among the three connectivity distance models for lake group 1 (left, below diagonal) and 2 (right, above diagonal). P-values were obtained by 9,999 permutations. Significant correlations are in bold.

	Land		Water		Neighbouring	
	r	p	r	p	r	p
Land			**0.257**	**0.045**	**0.394**	**0.0001**
Water	-0.024	0.52			**0.573**	**0.0001**
Neighbouring	**0.454**	**0.0001**	**0.332**	**0.0001**		

change to this. In lake group 2, lakes that are directly connected (Neighbouring model) and lakes that are situated closely to each other (Land model) exhibited more similar environmental conditions. To a lesser extent this was also true for lakes that are indirectly connected (Water model, Table 2).

Table 2. MANTEL correlations (r) between the environmental dissimilarity matrices of 2001, 2002 and 2003 and each of the three connectivity distance models for lake group 1 and 2. P-values were obtained by 9,999 permutations. Significant correlations are in bold.

Group 1

	2001		2002		2003	
	r	p	r	p	r	p
Land model	0.040	0.36	-0.292	0.077	-0.067	0.41
Water model	-0.050	0.44	0.066	0.30	-0.035	0.48
Neighbouring model	0.060	0.45	0.179	0.17	-0.113	0.29

Group 2

	2001		2002		2003	
	r	p	r	p	r	p
Land model	**0.281**	**0.044**	**0.376**	**0.031**	**0.334**	**0.030**
Water model	0.193	0.067	**0.310**	**0.026**	0.175	0.14
Neighbouring model	**0.255**	**0.0029**	**0.204**	**0.010**	**0.209**	**0.010**

The spatial community similarity pattern in lake group 1 was highly variable among years, both in active and passive dispersers (Table 3). The community of active dispersers showed no pattern in 2001, was negatively correlated to the Water model in

2002, and was positively correlated with both the Land and Neighbouring model in 2003. Surprisingly, community similarity patterns in passive dispersers only were correlated with the Land model in 2001 and 2003. No general effect of the lake drainings could be shown from these data. More consistent patterns were observed in the larger lake group 2 (Table 4). Community similarity patterns in active dispersers were positively correlated with the Land model in all three years, and did not correlate with the Water or Neighbouring model. Community similarity patterns in passive dispersers correlated positively with all three models in all three years.

Table 3. Partial MANTEL correlations between macroinvertebrate dissimilarity matrices and three different connectivity matrices of lake group 1 are presented for active and passive dispersers. P-values were obtained by 9,999 permutations. Significant correlations are in bold.

Group 1						
Active dispersers						
	2001		2002		2003	
	r	p	r	p	r	p
Land model	-0.098	0.35	0.269	0.12	**0.313**	**0.037**
Water model	0.060	0.36	**-0.350**	**0.026**	0.284	0.056
Neighbouring model	-0.011	0.49	-0.048	0.41	**0.347**	**0.042**
Passive dispersers						
	2001		2002		2003	
	r	p	r	p	r	p
Land model	**0.368**	**0.032**	0.291	0.13	**0.428**	**0.050**
Water model	0.180	0.16	-0.047	0.41	0.017	0.42
Neighbouring model	0.200	0.13	0.193	0.17	0.033	0.47

Environmental and spatial factors could explain a significant proportion of the variation in community structure in both active and passive macroinvertebrates of both lake groups and for all years (Table 5, 6). Single effects of E and S, however, were seldom significant, mainly because a high proportion of the variance explained by E and S was shared between both. Significant contributions of the pure environmental component (E|S) were present in all combinations of lake group and dispersal mode: active dispersers of lake group 1 (2002) and group 2 (2003) and passive dispersers of lake group 1 (2001) and group 2 (2002). The contribution of the pure spatial component

Table 4. Partial MANTEL correlations (r) between macroinvertebrate dissimilarity matrices and three different connectivity matrices of lake group 2 are presented for active and passive dispersers. P-values were obtained by 9,999 permutations. Significant correlations are in bold.

Group 2						
Active dispersers						
	2001		2002		2003	
	r	p	r	p	r	p
Land model	**0.531**	**0.0085**	**0.501**	**0.011**	**0.359**	**0.039**
Water model	0.103	0.29	0.103	0.27	0.157	0.15
Neighbouring model	0.044	0.30	0.035	0.32	-0.009	0.46
Passive dispersers						
	2001		2002		2003	
	r	p	r	p	r	p
Land model	**0.474**	**0.0057**	**0.387**	**0.013**	**0.367**	**0.018**
Water model	**0.306**	**0.031**	**0.262**	**0.038**	**0.403**	**0.0019**
Neighbouring model	**0.260**	**0.0015**	**0.240**	**0.0028**	**0.161**	**0.036**

was significant only in active dispersers of lake group 2 in 2003. In both lake groups, the ratio of E|S on S|E (E/S) was consistently higher in passive dispersers than in active dispersers, except in group 1 in 2003. Although lakes of group 1 were drained end 2001, this did not notably alter the ratio of E/S.

Discussion

Based on their prevailing mode of dispersal, lentic macroinvertebrates can be categorized as either actively flying dispersers or passively transported dispersers. This study shows that this difference in dispersal mode may have drastic implications for the regional structuring of their respective communities. In agreement with our expectations, we found that, in a system of interconnected lakes, communities of passively dispersing macroinvertebrates were regionally structured according to a spatial model that incorporates the connections among the lakes, while active dispersers were not. Active dispersers, and surprisingly also passive dispersers, were strongly structured conform the spatial model of Euclidian distances among the lakes, possibly suggesting that over-land dispersal was an important dispersal mechanism in both groups.

Table 5. Variation partitioning and associated p-values of the macroinvertebrate data matrix of lake group 1 for the three different years and for active and passive dispersers into different components: E+S = total variation explained by all environmental and spatial variables together, E = variation explained by environmental variables, S = variation explained by spatial variables, E|S = pure environmental variation, S|E = pure spatial variation and E∩S = the spatial structuring in the species data that is shared by the environmental data. The ratio of E|S on S|E (E/S) is a measure to compare the relative importance of environmental variables versus spatial variables between active and passive dispersers and among years. Significant proportions are in bold.

Group 1

Active dispersers

	2001		2002		2003	
	Variance explained (%)	p	Variance explained (%)	p	Variance explained (%)	p
E+S	88.7	0.11	**92**	**0.0099**	91.7	0.18
E	**74.1**	**0.033**	**79**	**0.0019**	**72.5**	**0.042**
E|S	33.2	0.27	**44.6**	**0.048**	43	0.36
S	**55.5**	**0.016**	**47.4**	**0.019**	**48.8**	**0.019**
S|E	14.6	0.36	13	0.27	19.2	0.49
E∩S	40.9		34.4		29.6	
ratio E/S	2.273		3.431		2.240	

Passive dispersers

	2001		2002		2003	
	Variance explained (%)	p	Variance explained (%)	p	Variance explained (%)	p
E+S	**94.7**	**0.0046**	**94**	**0.040**	**96.1**	**0.033**
E	**85.8**	**0.0006**	**88.9**	**0.0055**	70.4	0.091
E|S	**39.3**	**0.018**	26.6	0.24	45.8	0.13
S	**55.4**	**0.0064**	**67.3**	**0.017**	**50.3**	**0.032**
S|E	9	0.21	5.1	0.63	25.6	0.22
E∩S	46.4		62.2		24.7	
ratio E/S	4.367		5.216		1.790	

Table 6. Variation partitioning and associated p-values of the macroinvertebrate data matrix of lake group 2 for the three different years and for active and passive dispersers into different components: E+S = total variation explained by all environmental and spatial variables together, E = variation explained by environmental variables, S = variation explained by spatial variables, E|S = pure environmental variation, S|E = pure spatial variation and E∩S = the spatial structuring in the species data that is shared by the environmental data. The ratio of E|S on S|E (E/S) is a measure to compare the relative importance of environmental variables versus spatial variables between active and passive dispersers and among years. Significant proportions are in bold.

Group 2

Active dispersers

	2001		2002		2003	
	Variance explained (%)	p	Variance explained (%)	p	Variance explained (%)	p
E+S	**72**	**0.028**	**73.3**	**0.048**	**82.9**	**0.0002**
E	**53.4**	**0.013**	**62.5**	**0.0032**	**61.2**	**0.0009**
E|S	29.9	0.22	30.3	0.22	**42.3**	**0.0008**
S	**42.1**	**0.010**	**43.1**	**0.015**	**40.6**	**0.0074**
S|E	18.6	0.22	10.8	0.61	**21.7**	**0.0084**
E∩S	23.5		32.3		18.9	
ratio E/S	1.608		2.806		1.949	

Passive dispersers

	2001		2002		2003	
	Variance explained (%)	p	Variance explained (%)	p	Variance explained (%)	p
E+S	**80.9**	**0.011**	**85.6**	**0.0049**	74.2	0.051
E	**75**	**0.0002**	**75.6**	**0.0004**	**61.1**	**0.013**
E|S	19.2	0.34	**40.7**	**0.019**	35.1	0.14
S	**61.7**	**0.0007**	**44.9**	**0.037**	**39.2**	**0.045**
S|E	5.9	0.84	10	0.25	13.2	0.45
E∩S	55.8		34.9		26	
ratio E/S	3.254		4.070		2.659	

The two subclusters of lakes gave different results on the relation between distance and community similarity in active and passive dispersers. In the smallest lake group 1 (8-9 lakes), correlations varied notably among years, and general trends were lacking. If anything, communities tended to be structured according to the over land dispersal model (Land model), especially the passively dispersing macroinvertebrates. In the larger lake group 2 (15 lakes), both actively and passively dispersing macroinvertebrates showed strong, clear-cut patterns, which were stable over all three years. According to our expectations, communities of active dispersers were strongly structured according to the Land model, suggesting dispersal over land as the main structuring dispersal mechanism. Communities of passively dispersing macroinvertebrates were significantly structured conform the Water and Neignbouring models. In other words, communities of passively dispersing macroinvertebrates were more similar when they were connected by lake connections, suggesting that dispersal via these connections was an important structuring dispersal mechanism.

Surprisingly, communities of passive dispersers were also strongly structured according to the Land model, both in lake group 1 (not in 2002) and 2. We see three possible interpretations for this. First, correlations with the Land model may be due to an artifact, as the Land and Water model were positively intercorrelated (lake group 2). In lake group 1, however, both models were not correlated. Second, the communities may be structured by a strong environmental gradient that is not corrected for in the environmental matrix. This may explain why both active and passive dispersers in both lake groups showed positive correlations with the Land model. If we assume that all the observed correlations with the Land model are false (autocorrelations), than communities of active dispersers would show no spatial structure (suggesting dispersal was not limited within the study area), while passive dispersers would be structured according to the Water and Neighbouring model only, which is what we expected. Although this scenario nicely fits our expectations, and though it is possible that some ecological differences among the lakes exist that were not corrected for, we cannot think of a candidate environmental variable that was not yet incorporated in the environmental matrix that could have imposed such a strong community gradient. Finally, the correlations of the community similarities with the Land distance model may truly be the result of over land dispersal in passive dispersers. Passively dispersing macroinvertebrates possibly were transported from lake to lake by cattle or amphibians. Whether this type of dispersal is sufficient to generate the observed community patterns, however, remains an open question.

The regional sets of communities of actively and passively dispersing macroinvertebrates appeared differentially affected by spatial and environmental factors according to the variation partitioning analyses. In active dispersers, the relative contribution of the spatial factors in shaping community structure - relative to the environmental factors - was higher than in passive dispersers. These findings contradict with our expectations and with the findings of Johnson et al. (2001), who demonstrated that spatial factors were more important in shaping community structure of species with a low dispersal ability compared to species with a high dispersal ability. However, the results of the variation partitioning should be taken with caution. In passive dispersers, the environmental and the spatial variance component on average shared a higher proportion of variation among each other compared to in active dispersers. This indicates that the spatial coordinates that best explained macroinvertebrate metacommunity structure to a large extent coincided with the gradients in the most important environmental variables. Unfortunately, it cannot be told which of these factors (spatial, environmental or both) accounted for the observed community gradient, which makes the E/S ratios difficult to interpret and compare. We can only safely say that communities of active and passive dispersers were differently affected by spatial and environmental factors, as previously emerged from the MANTEL tests.

Drainings probably completely wipe out local macroinvertebrate communities in lakes. In a previous study in De Maten, we found that the newly refilled lakes (after draining) were very rapidly recolonized by both actively and passively dispersing macroinvertebrates (Van de Meutter et al. unpublished manuscript, Chapter VI). It was hypothesized that the refilling of the lakes with water from non-drained neighbouring lakes, probably transporting macroinvertebrates from one lake to the other, had accelerated the speed of recolonization, especially in passive dispersers. Because lakes are refilled with the water of neighbouring lakes, this may also affect the way metacommunities are structured i.e. after the refilling communities may be to a higher extent structured according to the Water model or the Neighbouring model, especially communities of passive dispersers. Unfortunately, we only have reference data (prior to the drainings) of the small lake group 1. In this lake group, we found no indications that metacommunity structure was more structured conform the Water or Neighbouring models after the drainings.

Connectivity corridors among suitable habitat patches can strongly promote individual movement of organisms and hence intensify metacommunity dynamics (Michels et al. 2001, Forbes and Chase 2002, Cottenie and De Meester 2003). This is

supported by our findings that communities of passively dispersing macroinvertebrates were spatially structured according to the network of lake connections. The net effect of habitat connectivity on metacommunity structure, however, depended on the prevailing dispersal mechanism that is used by the organism group: metacommunity structure of macroinvertebrates that can fly from lake to lake was not affected by the network of lake connections. The degree of connectedness of lakes and ponds may therefore affect regional metacommunity dynamics of passive dispersers more than those of active dispersers.

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Addendum 1. Overview of the axes that were included in the spatial explanatory matrices [S] for each combination of lake group (1, 2), dispersal mode (active, passive) and year (2001, 2002 and 2003). Axes X and Y were derived from the Euclidian distance model ('Land model'); Axes 1-10 were derived from the lake connections model ('Water model').

group 1

Active			Passive		
2001	2002	2003	2001	2002	2003
Y^2	$Ax7^2$	Ax1	Ax4	X^2	Ax4
Ax4	Ax4	$Ax7^2$	Y^2	Ax6	Ax5

group 2

Active			Passive		
2001	2002	2003	2001	2002	2003
$Ax9^2$	Y^2	$Ax10^2$	Ax4	Ax3	Ax4
$Ax4^2$	Ax9	Ax3	Ax6	Ax2	$Ax9^2$
Y^2	$Ax7^2$	Ax10	Ax3	$Ax2^2$	Ax6

Addendum 2. Overview of the environmental variables that were included in the environmental variable matrices [E] for each combination of lake group (1, 2), dispersal mode (active, passive) and year (2001, 2002 and 2003). The variables presented are lake area (area), % cover by emergent vegetation (emergent), floating-leaved vegetation (floating), riparian vegetation (riparian), submersed vegetation (submers), filamentous algae (filam. algae), *Lemna* (*Lemna*), tree roots and branches (branches), % open sediment (open sediment), % shading by riparian trees (shade), chlorophyll a concentration (chlorophyll a), conductivity, lake depth (depth), pH, oxygen concentration (O2), Snell depth, densities of YOY fish and water temperature (temperature).

group 1

Active			Passive		
2001	2002	2003	2001	2002	2003
area	chlorophyll a	pH	shade	conductivity	O2
floating	branches	floating	open sediment	filam. algae	YOY fish
depth	depth	O2	floating	area	submers
shade	area	open sediment	depth	riparian	Snell depth

group 2

Active			Passive		
2001	2002	2003	2001	2002	2003
conductivity	riparian	riparian	conductivity	depth	riparian
Snell depth	open sediment	floating	*Lemna*	pH	shade
floating	Snell depth	shade	open sediment	*Lemna*	YOY fish
YOY fish	emergent	Snell depth	temperature	emergent	O2
riparian	pH	YOY fish	O2	O2	depth

Chapter VIII

Habitat selection drives macroinvertebrate community turnover along the hydroperiod gradient

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[Unpublished manuscript]

Abstract

Community turnover along the hydroperiod gradient is generally assumed to result from local selective mechanisms, abiotic limitation and species sorting, while the possibility of habitat selection behaviour is largely ignored. In this study, the colonizing assembly of macroinvertebrates in small, fishless habitats deviated strongly from that observed in a surrounding large set of fish lakes. By studying distance-similarity relationships, we could show that fish lakes did not act as sources for the colonization of the fishless habitats. We argue that neither abiotic limitation, nor species sorting due to invertebrate predators in the fishless habitats or due to fish in the fish lakes were able to explain the observed patterns. Our results indicate that strong habitat selection may substantially contribute to community turnover along environmental gradients observed in nature, thereby offsetting a direct role for abiotic limitation and species sorting.

Introduction

Much of community theory pertains to the question of how patterns of species presence and absence are generated along environmental gradients. The underlying processes thought to sequentially play a role in the composition of a community can be divided into three broad categories: processes linked with initial community assembly, abiotic limitation, and species sorting by biotic interactions (Belyea and Lancaster 1999). Traditionally, most empirical work has focused on the latter two processes (e.g. Connel 1961, McPeek 1990, Wissinger et al. 1996, 1999, Stoks and McPeek 2003a,b). The emerging picture from this research is that the specialization of species to deal with selection pressures associated with a particular part of an environmental gradient often precludes species to deal with the opposing selection pressures at other parts of the gradient. Although successful in explaining species turnover along gradients, this trade-off oriented view may be too simplistic, by ignoring preceding processes linked with initial community assembly. More specifically, given the expected trade-offs, it may pay for the organism to choose their habitat already in the phase of colonization.

Community assembly invariably starts with the colonization of the habitat by new species. Patterns and dynamics of colonization therefore are critical to the understanding of how natural species distributions are generated (e.g. priority effects: Shulman et al. 1983, Wilbur and Alford 1985; dispersal limitation: Caceres and Soluk 2002, Yu et al. 2004). In addition to the processes that apply to all biota, community assembly in active dispersers may also be directed by their capacity to actively select favourable habitats from the landscape to colonize. Although most of us are familiar with the idea that large-scale habitat selection is widespread in nature (e.g. aquatic versus terrestrial habitats, lotic versus lentic freshwater habitats), it is compelling to see how species are capable to select habitats with respect to fine-scale habitat variation along specific environmental gradients (e.g. habitat selection with respect to the presence of predators in frogs: Resetarits and Wilbur 1989; mosquitoes: Walton et al. 1990, Blaustein and Kotler 1993; aquatic beetles: Resetarits 2001). Given the rapidly growing number of studies that report fine-scaled habitat selection (e.g. Blaustein 1999), the role of direct interactions with the abiotic and biotic environment in structuring field patterns of species occurrence may be largely overestimated. Indeed, if habitat selection is general, many cases of species turnover are not directly driven by direct interactions, but rather by the avoidance of negative interactions through habitat selection. Yet, studies that include habitat selection as an alternative mechanism to explain species turnover

along gradients are scarce (but see Rieger et al. 2004, Almany 2004) or even nonexistent at the community level.

In freshwater habitats, the well-known hydroperiod gradient stretches from small, ephemeral ponds over temporary ponds with invertebrate top predators to large, permanent lakes with fish (Wellborn et al. 1996). Actively dispersing amphibians and macroinvertebrates show strong patterns of species and community turnover along this gradient (Wellborn et al. 1996, Snodgrass et al. 2000, Urban 2004, but see Baber et al. 2004). Several studies were successful in explaining these natural patterns of occurrence by reconstructing the species-specific selective environments along the hydroperiod gradient in a closely related species group (McPeek 1990, Wissinger et al. 1996, 1999, Stoks and McPeek 2003a,b). However, studies on an alternative explanation of patterns of species turnover along the hydroperiod gradient based on the mechanism of habitat selection are lacking.

In this study, we report on results that show that habitat selection may be an important determinant of macroinvertebrate community composition differences between small fishless ponds and large fish lakes. We monitored community assembly in small, fishless, artificial habitats that were placed in an area with abundant, shallow, well-studied fish lakes that were sampled intensively. The assembly of colonizing macroinvertebrates in the artificial fishless habitats consisted mainly of species that were never found in the fish lakes. By studying the area-similarity relationships between lakes and artificial habitats, and distance-similarity relationships among artificial habitats and between artificial habitats and fish lakes, we could demonstrate that colonization of the artificial habitats occurred independently of the spatial configuration of fish lakes, but was spatially structured according to some unknown - probably fishless - source habitats. Given that no specimens of the species that were lacking in the lakes were retrieved from a local large-scale fish gut analysis (Declerck et al. 2002), that random dispersal would incur enormous numeric losses in these species, and that the temporal pattern in biotic interactions strength and the temporal pattern in the occurrence of fish lake invertebrates did not match, predator-mediated species sorting was not a sufficient explanation for the disparate community compositions of small fishless habitats and fish lakes, suggesting this pattern was due to habitat selection.

Material and methods

Study area

Our study area is the nature reserve De Maten, an open, diverse landscape consisting of dry heath land, small patches of woodland and meadows situated in the Northeastern part of Belgium near Genk. The chief characteristic feature of the reserve is the 3.5 km long chain of 34 shallow fish lakes (surface area 0.12-12.2 ha), which are fed by the water of three small rivers (Cottenie et al. 2001, Fig. 1). Despite the fact that the lakes are all connected, they display striking differences in macrophyte, macroinvertebrate and zooplankton community structure (Cottenie et al. 2001, Cottenie and De Meester 2003, Van de Meutter et al. in press). Besides the lakes, some small (<10 m²) fishless ponds are present within and outside the study area.

Experimental containers

To study the community assembly of lentic macroinvertebrates against a different background of regional source communities, we placed a pair of containers at six different sites in the reserve at 10 m from the nearest lake. These sites were chosen so as to cover the full latitudinal and longitudinal range within the reserve, hence making distances between containers and source lakes largely independent of regional gradients in local factors (Fig. 1). We used black polyethylene containers with a volume of 600 l, measuring 75 cm in height and with an upper circular surface area of 0.95 m².

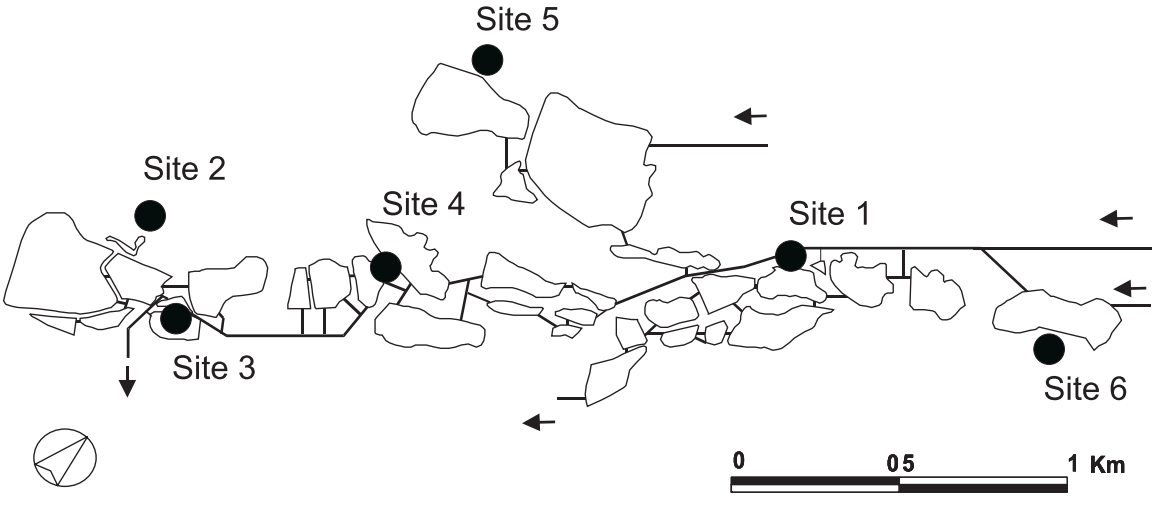

Fig. 1. Map of the fish lake complex in our study area 'De Maten'. Black dots represent the sites were pairs of containers were placed (sites 1-6). Small arrows indicate the water current through the reserve; the large arrow directs to the North.

All containers were filled with tap water on 24 July 2002 with the help of the local fire brigade. Water levels in all containers remained fairly constant during the whole experiment at 68 cm (i.e. 600 l volume). We provided no structure within the containers.

Data collection and processing

Containers were monitored for one year and were sampled seven times: after 2, 4, 6, 10, 14, 34 and 52 weeks. Containers of site 1 and 6 could not be sampled at week 2 and week 52, respectively. Sampling occurred using two complementary approaches. Firstly, we took 10 subsamples of the containers using a tube sampler (8 cm diameter, 65 cm height) to get reliable estimates of small and abundant invertebrates. To make sure that invertebrates at the bottom and on the sides of the containers were included, we stirred up the whole content of the containers before sampling using a large stick. The total sampled water volume in each container approximated 1/23 of the total water volume in the containers on each date. Secondly, we sampled all large or less numerous invertebrates (mainly Coleoptera, Heteroptera and Trichoptera) using a D-shaped net (24 cm x 24 cm, 500 µm mesh). Containers were sampled until three consecutive net sweeps yielded no new individuals. All macroinvertebrates collected this way were identified in the field and afterwards released again in their container of origin. Occasionally, one specimen was collected for checking field identifications in the laboratory. Tube sampler samples were preserved on 70 % ethanol in the field and counted and identified in the laboratory. Identifications were to the level of the species, except for Chironomidae, Culicinae, Hydrachnidia, Leptoceridae, Oligochaeta and Phrygaenidae that were not further identified. Before analysis, we recalculated the tube sampler data to whole containers and pooled these data together with the data of the net catches to obtain a single species frequency matrix for each container on each date.

To be able to assess differences in environmental conditions among the containers that might interfere with the colonization by macroinvertebrates, we measured chlorophyll a concentration, conductivity, pH and water transparency for each container on each sampling date. Possible shading effects on a container were noted as the absence (0) or presence (1) of trees (>4 m) within 5 m of the container for every of the four cardinal points. These values were summed up to a shading score of that container.

All 34 fish lakes in the reserve were sampled three times: in 16-18 July 2002, 17-18 September 2002 and 22-24 July 2003, corresponding approximately with week 0, 10 and 52 of the colonization experiment. Samples were taken with the same net as used for sampling the containers and were standardized in both time (10 min) and space

(sampling along a littoral trajectory of 75 m). Sampling trajectories were chosen to represent all major littoral microhabitats present in the lake. This sampling procedure includes epiphytic and free-swimming littoral macroinvertebrates, but excludes typical benthic species. Lake samples were preserved in 4 % formaldehyde in the field, and sorted and identified in the laboratory to the same level as the container samples.

To standardize the similarity measures among communities of lakes and containers for the different sampling dates, they were based on a restricted number of species that occurred in both lakes and containers on the given sampling date. Community similarity among containers was based on complete communities for all possible combinations of the different containers. Similarities in environmental conditions among different containers were calculated for all possible combinations of the different containers. Sampling in the containers (7 occasions) did not always coincide with sampling lake communities (3 occasions). Moreover, the lake samplings only give a snapshot of macroinvertebrate composition in lakes, whereas the container communities integrate colonization events over a continuous period of time. Therefore, we pooled the data mactrices for the three samplings per lake and calculated container-lake similarity measures by comparing the container communities for each date with the single pooled matrix of lake communities. We calculated Bray-Curtis similarities on square-root transformed species counts for comparing containers, on raw data for environmental conditions, and on square root-transformed and standardized species counts for comparing containers and lakes, using PRIMER 5 (2001). Geographical distances among containers, distances among containers and lakes and surface areas for all lakes were calculated using the GIS package Geomedia Professional 5.1 (Intergraph 2003).

Statistics

To investigate the relationship between lake area, distance between lakes and containers, and similarities between lake and container communities, we applied a nested repeated measures ANCOVA. Because 4 containers (sites 1 and 6) were not sampled on all dates, we restricted this analysis to the remaining 8 containers (4 sites). We used site and container nested in site as categorical variables, area and distance as covariables and the similarity values at each sampling date as the repeats. An analysis including all sites for only 5 sampling dates gave qualitatively similar results and only the former analysis will be given. To meet ANOVA assumptions, lake area was log(10)-transformed. To investigate the relationship between macroinvertebrate community dissimilarity among containers and distances among containers, we applied partial

MANTEL tests (Legendre and Legendre 1998) using the freeware tool zt (Bonnet and Van de Peer 2002). This analysis was corrected for environmental conditions in the containers. Associated p-values were obtained through 10,000 permutations.

Results

Macroinvertebrates rapidly colonized the containers: after two weeks on average 8.2 ± 1.2 (SE) taxa were present in a single container, increasing to 23.1 ± 1.5 (SE) after one year (Fig. 2). Species richness decreased in October (week 14), but continued increasing thereafter. After one year of colonization, the total number of macroinvertebrate taxa observed in the containers had accumulated to 78. Only a minority of these (33 taxa, 41 %) was also recorded in the fish lakes. When the taxa that were extremely rare in the lakes (<3 individuals recorded) are excluded, only 22 taxa (28 %) were in common. The fish lakes, however, also were taxon-rich, with a total of 125 taxa recorded. The newly assembled macroinvertebrate communities consisted mainly of actively dispersing taxa, with the exception of Acari (phoretic dispersal) and one *Argyroneta aquatica* (water spider, dispersal by wind). The environmental conditions recorded in the containers (chlorophyll a concentration, conductivity, pH and Snell depth) were all within the range of values recorded in the fish lakes.

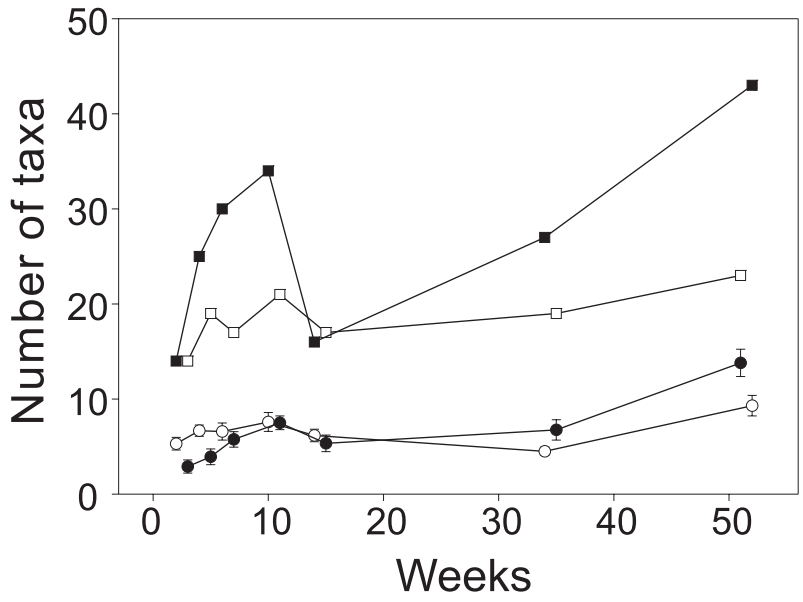

Fig. 2. Mean numbers of taxa per container (circles) and the number of taxa summed over all containers (squares) on each sampling date are presented for the species found in both containers and lakes (white) and for the species found in the containers only (black). Error bars represent ± 1 standard error.

Table 1. Results of the Repeated Measures nested ANCOVA on the similarity values between containers and lakes. Distance to the lakes and area of the lakes were used as covariates. Only containers that were sampled on all 7 sampling dates are included (N=8).

Effect	df	F	p
Site	3, 216	0.014	0.99
Container(Site)	4, 216	0.15	0.96
Area	1, 216	2.33	0.19
Distance	1, 216	1.15	0.29
Site x Area	3, 216	0.0058	0.99
Site x Distance	3, 216	2.89	0.036
Container(Site) x Area	4, 216	0.14	0.97
Container(Site) x Distance	4, 216	0.053	0.99
Time	6, 1296	2.77	0.011
Site x Time	18, 1296	0.93	0.54
Container(Site) x Time	24, 1296	0.40	0.99
Area x Time	6, 1296	0.96	0.45
Distance x Time	6, 1296	0.76	0.60
Site x Area x Time	18, 1296	0.33	0.99
Site x Distance x Time	18, 1296	1.92	0.012
Container(Site) x Area x Time	24, 1296	0.19	0.99
Container(Site) x Distance x Time	24, 1296	0.20	0.99

We could not detect a general relationship between similarity and distance between containers and lakes (Table 1). This relationship changed through time in a site-specific way (Site x Distance x Time: p=0.012). From Fig. 3A it appears that containers that were situated in the western part (containers 2-5) and containers situated in the eastern part of the reserve (containers 1 and 6) show opposite temporal trends between similarity and distance to the lakes. This suggests that a strong east-west gradient existed in the macroinvertebrate community composition of the lakes, which was passively reflected in the relationships between similarity and distance to the lakes. As these trends were oppositely directed for different sites, clearly lake macroinvertebrate assemblages did not actively affect the assemblage of macroinvertebrates that colonized the containers. Our results thus show that colonization of the fishless containers is independent of the nearby fish lakes, but depends on an additional source creating a strong East-West gradient. In agreement

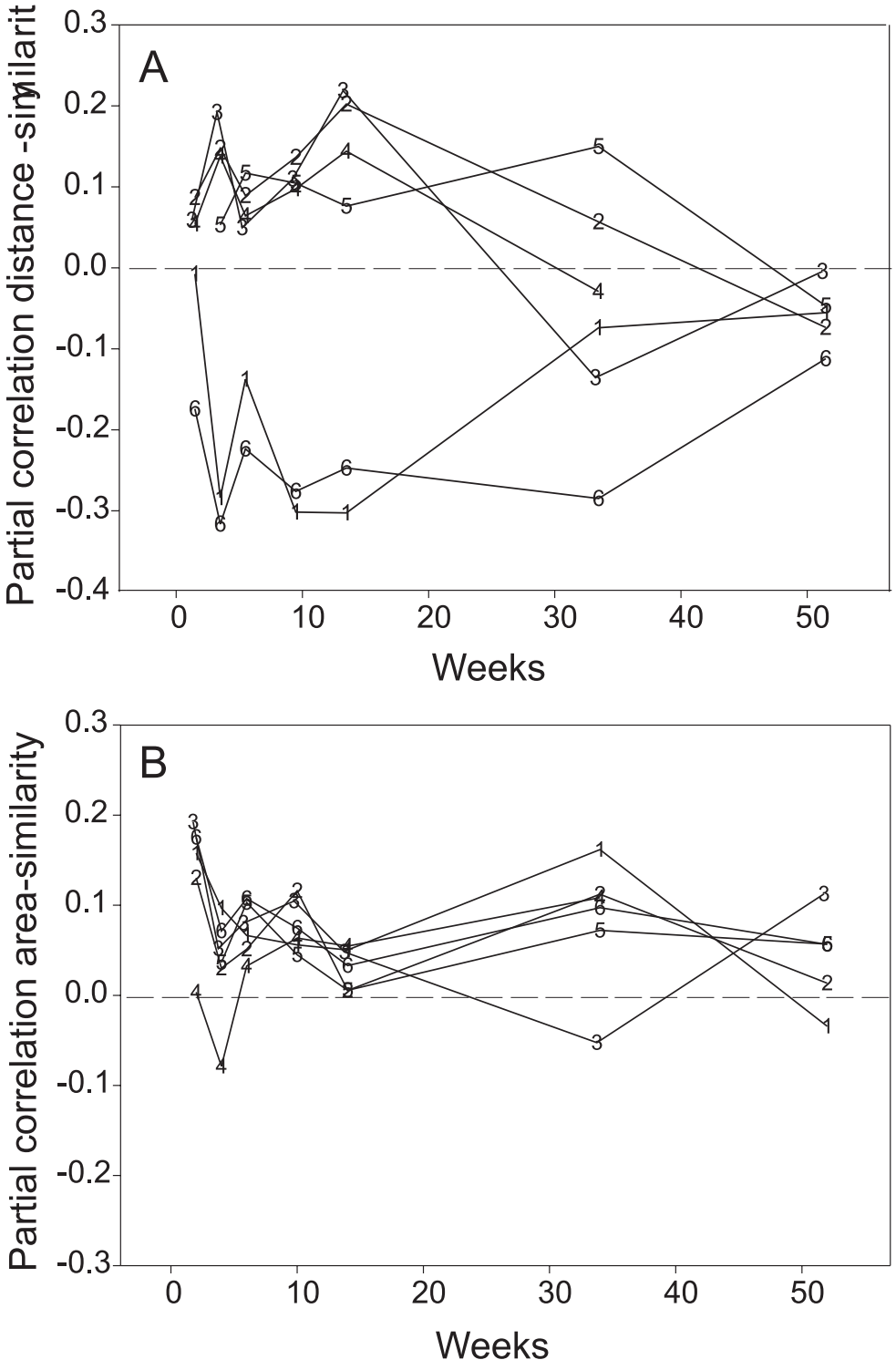

Fig. 3. Partial correlation coefficients between similarities of macroinvertebrate communities of containers and lakes calculated on a subset of taxa that occurred in both containers and lakes and (A) distances between container and lake communities (corrected for lake area), and (B) lake area (corrected for distances between container and lake communities), for each sampling date and each site. The dashed line represents the zero correlation line. Numbers refer to sites.

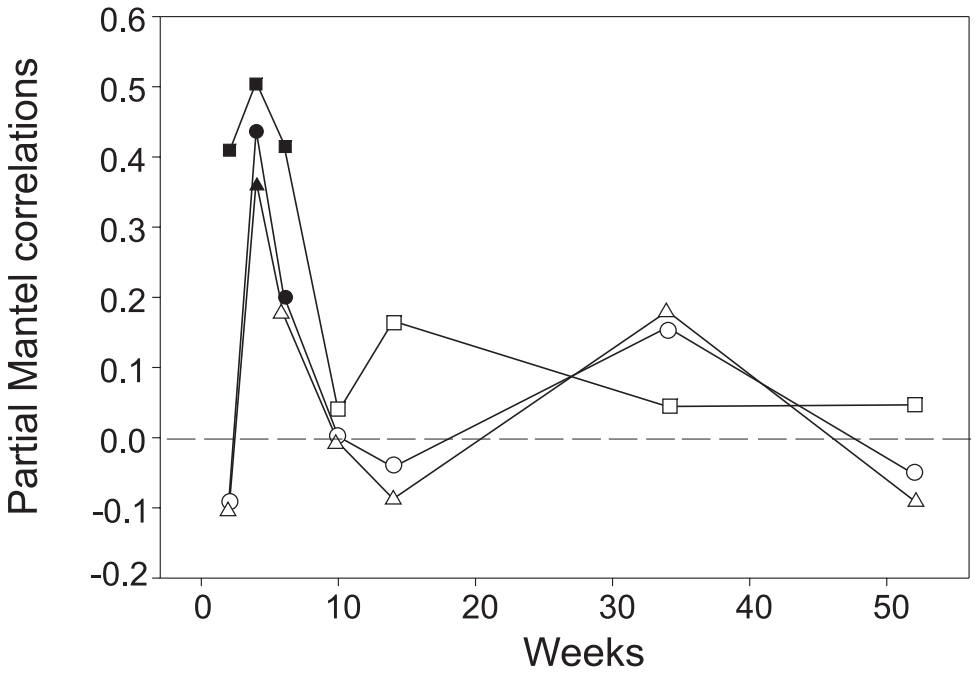

Fig. 4. Presented are partial MANTEL correlations between macroinvertebrate community dissimilarities and distances among containers for all taxa (circles), for taxa shared by containers and lakes (triangles) and for taxa that were only present in the containers (squares) for each sampling date. Filled symbols indicate significant correlations ($p<0.05$, 10,000 permutations). The dashed line represents the zero correlation line.

with our observation that the communities in the containers are not dependent on the neighbouring lakes, similarities among containers and lake communities were not affected by lake area (area and its interactions, all $p>0.19$).

Partial MANTEL correlations between dissimilarities and distances among container communities showed a striking pattern over time (Fig. 4). At first, community dissimilarity and distance were not correlated, except the subset of taxa that were only present in the containers, which showed a positive correlation. With colonization progressing for four to six weeks, all correlations were significantly positive, indicating that similarity among container communities declined with increasing distance between both. Thereafter, correlations decreased again and were non-significant. As the macroinvertebrates that colonized the containers possibly originated from two disparate types of source communities (fish lakes and fishless ponds), we additionally performed a similar analysis for two subsets of the community: macroinvertebrates occurring in both containers and lakes, and macroinvertebrates that only occurred in the containers. Both subgroups showed a pattern that was similar to that of the macroinvertebrate community as a whole: a transient positive relation between dissimilarity and distance at the early

start of colonization, but no significant correlation at the later stages (Fig. 4). In macroinvertebrates that only occurred in the containers, however, the significant correlation between dissimilarity and distance already showed up at week two, whereas in the other groups this only happened at week 4.

Discussion

Our data strongly suggests that the macroinvertebrates that colonized the fishless containers did not originate from the nearby fish lakes, even not the taxa that were recorded in both containers and lakes studied here. Three findings support this discrepancy between source pool and colonization assemblages in actively dispersing macroinvertebrates. First, the composition of the assemblage of species that colonized the fishless containers deviated strongly from the composition of the general source pool in the fish lakes. Of the taxa that colonized the containers, 59 % were not recorded from any of the fish lakes. Omitting rare species in fish lakes, this percentage increases to 72 %. All of these “new” species belong to aquatic Coleoptera and Heteroptera that are typical for temporary and/or fishless ponds (Savage 1989, Drost et al. 1992). These results are striking, given the fact that fish lakes were abundant in the immediate neighbourhood of the containers, species-rich and no doubt by far exceeded the potential source pool of macroinvertebrates of any other habitat type in the area. Second, community similarity between containers and lakes was not related to distance or lake area of the fish lakes, even when the analysis was restricted to a subset of species co-occurring in containers and lakes. Third, similarities among container communities in both the species subset that was not recorded from the lakes as in the species subset that was recorded from both containers and lakes, showed a consistent pattern with distance. From the latter two findings, it appears that the macroinvertebrates that colonized the containers originated from one or a set of other habitats than the fish lakes.

A possible mechanism that may explain the paradox between the presence of abundant nearby source pools and colonization from other sources, is that macroinvertebrates of fishless ponds (fish intolerant species) may disperse far more frequently than species of fish lakes. This idea has been proposed by several authors (Brown 1951, Wellborn et al. 1996), and is considered to be an adaptation to the generally more unstable character (pond drying) of fishless habitats. Strong empirical evidence, however, is largely lacking. It is clear that the taxa observed in the containers are good dispersers, in order to explain why such a high number of fish intolerant taxa

was retrieved from the containers, despite the low number of fishless pond source habitats in the neighbourhood. Given that these fish intolerant species abundantly disperse, how can we explain that more than half of the species that colonized the containers were not found in the lakes? Assuming that colonization occurs randomly, fish intolerant invertebrates should colonize lakes too. Given that the lakes make up a total water surface that is 69,427 times larger than that of all 12 containers together, and that per day each container was colonized by on average 1.61 adult Coleoptera or Heteroptera individuals that were never found in the lakes (based on the number of colonists at week 2), the enormous amount of 112,472 individuals of these macroinvertebrates is expected to colonize the lakes each day. However, 45 species that were retrieved from the containers could not be found in the lakes. Although this may suggest a strong role for species sorting by the fish predators (Wellborn et al. 1996), an extensive study during September 1999 on the diet composition of 826 fish of six species that make up more than 90% of the fish biomass in the lakes of De Maten (brown bullhead *Ameiurus nebulosus*, pumpkinseed *Lepomis gibbosus*, roach *Rutilus rutilus*, eastern mudminnow *Umbra pygmea*, topmouth gudgeon *Pseudorasbora parva* and gudgeon *Gobio gobio*) showed that none of the fish intolerant container taxa was presented (Declerck et al. 2002). Thus, it seems unlikely that each day the fish eats all fish intolerant species that colonize the fish lakes. Viewed from a different angle, if species sorting would be the only mechanism, it would entail the loss of 112,472 fish intolerant Coleoptera and Hemiptera from small fishless habitats per day in the nature reserve of De Maten. The nature reserve therefore would be a major death trap for animals that typically occur in fishless habitats. It is difficult to imagine that local populations of fish intolerant species can endure such high continuous costs and persist in the landscape of De Maten. Therefore, it seems likely that active habitat selection of fish intolerant species contributed to the strikingly disparate community composition of fish lakes and fishless containers.

From the classical view that species sorting is the driving force of community turnover along the hydroperiod gradient, the striking observation that container macroinvertebrate assemblages only consist for a small fraction of invertebrates that occur in the abundant nearby fish lakes, may reflect that the colonization of the containers by fish lake macroinvertebrates was constrained due to abiotic limitation and/or species sorting by invertebrate predators. Abiotic limitation, however, is unlikely since the values for environmental conditions recorded in the containers were within the range of values obtained in the fish lakes. If species sorting by large invertebrate

predators had caused the container communities to diverge from fish lake communities, we would expect this diverging pattern to coincide with the gradually increase of intensity of species sorting that is expected with increasing community development time (Mouquet et al. 2003). More specifically, we would than expect the number of fish lake species in the containers to decrease over time. However, from Fig. 2 it appears that the number of fish lakes species in the containers is constantly maintained at the same level. Possibly, fish lake invertebrate species were underrepresented simply because they infrequently disperse, as stated earlier. Habitat selection of the fish lake invertebrates, however, avoiding the small fishless containers, is an imaginable alternative mechanism that may account for this pattern.

In summary, our observations are best explained by strong habitat selection contributing to the disparate community types: fishless pond macroinvertebrates selectively colonize containers but not lakes, and possibly lake macroinvertebrates colonize lakes, but not ponds. An increasing number of freshwater macroinvertebrates has been shown to actively select habitats based on the absence or presence of the predator fish (Abjornsson et al. 2002, Blaustein 1999, Resetarits 2001). Abjornsson et al. (2002) showed that identical artificial pools only differing in the non-lethal presence of fish developed a different macroinvertebrate community. Large predatory insects that suffer much from fish predation strongly avoided the pools with fish, which cascaded into a different assemblage of non-predatory prey invertebrates. Since the lakes in De Maten all contain moderate to high densities of fish (Declerck et al. 2002), these lakes possibly were avoided by fish intolerant species, whereas the fish free containers were actively selected for. Yet, in our opinion, fish is only one criterion, and ecologists have only just started to explore the extensive list of possible habitat features that freshwater organisms may use to judge habitat suitability. For dispersing organisms that live in a restricted section of the hydroperiod gradient, watercolour, habitat size or vegetation cover may all be indicative of to which section of the gradient a particular habitat belongs. Which of these features was most important in shaping the observed differences in community structure and composition among lakes and containers in our study, however, cannot be derived from our data.

During the early stages of colonization, the similarity between container communities was a negative function of distance between containers, indicating dispersal limitation. After six weeks, the correlations converged towards zero. This pattern is concordant with the ideas of Mouquet et al. (2003) who state that the relative influence of local versus regional processes increases with community development,

largely because of increased biotic interactions. In this study, the different stages of community development succeeded each other at a surprisingly high rate. Mouquet et al. (2003) could demonstrate that this covaries negatively with the number of niches in the habitat. In our containers, both habitat heterogeneity and habitat size were minor, resulting in very low number of possible niches. Therefore, given the high rate of dispersal, species interactions may have been limiting colonization success already after four weeks of community development. This idea seems to contradict with the fact that species richness continued to increase after six weeks, suggesting communities were not yet saturated. However, community similarity in this study was both based on species presence and abundance, which may promptly reflect even slight interactive processes, while species richness data merely indicate the final state of species interactions (i.e. exclusion of species). Furthermore, during late fall, some tree leafs accumulated in all containers, providing extra niches and resources which could be colonized by new species, hence prolonging the time window for successful colonization.

Traditionally, ecologists have focused on present-day abiotic limitation and species sorting to explain patterns of community turnover along environmental gradients (Wellborn et al. 1996). This study adds to the growing number of studies reporting habitat selection in aquatic macroinvertebrates (Blaustein 1999, Abjornsson 2002), and indicates that this process may actually dominate in generating the observed patterns of community turnover along the hydroperiod gradient in nature. This urges for a reevaluation of the studies that have taken into account only direct local interactions as the explanatory mechanism for habitat endemism or species turnover along environmental gradients, by evaluating the possibilities for habitat selection processes. These findings do not imply that direct abiotic and biotic interactions no longer play a significant role in present-day community assembly. Rather, this shifts the emphasis of the role of direct interactions to the evolutionary past, and to the present-day stabilizing of habitat selection behaviour, for this behavioural trait would be leaky elseways. Apart from their fundamental scientific value, our findings may also have practical ramifications e.g. in conservation biology. This study shows that the colonization of small, fishless habitats occurs independent of the fact that fish lakes are close by, abundant and species-rich, and that colonization probably depends completely on the presence of other, fishless habitats. For example, this may profoundly alter the way we think about metacommunity dynamics of macroinvertebrate communities of ponds and lakes, as strict habitat selection may strongly decrease the number of available habitat patches or the connection strength of stepping stone networks for freshwater organisms, for only a

subset of the aquatic habitats may be truly used. The extent to which these ideas can be generalized to other systems remains unclear. Habitat selection may be particularly widespread in freshwater organisms, owing to the island like nature of ponds and lakes, and to the strong selective gradient along the hydroperiod gradient. More knowledge on what environmental and phylogenetic conditions may provoke the evolution of habitat selection are needed before we can actually judge whether our results can be considered to be rule in nature.

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General discussion

Macroinvertebrates and water turbidity

Shallow lakes can occur in two alternative stable states, a clear-water state and a turbid state, each characterized by specific communities of macrophytes, zooplankton and fish (Scheffer 1998). Macroinvertebrates also have been observed to exhibit two distinct communities associated with lake states, which is generally assumed to be mediated by changes in the abundance and composition of the macrophyte community (Diehl and Kornijów 1998). By controlling for differences in microhabitat (macrophyte species), we found indications that macroinvertebrate communities of clear-water and turbid lakes also may differ according to some other aspects related to lake state. A possible candidate for this is water transparency itself. Surprisingly, the fact that a lake's water transparency differs dramatically according to lake state has only scarcely inspired researchers to investigate the possible consequences of this for predator-prey interactions. This is even more surprising in light of the fact that the top predators in ponds and lakes are visual predators such as fish. A number of studies have investigated the effect of turbidity on prey detection by fish, and suggested that water turbidity may act as a protection against fish predation, although the effect may greatly depend on light availability (*Chaoborus*: Cuker 1993, Wissel et al. 2003, Horppila et al. 2004; *Daphnia*: Richmond et al. 2004; fish: Vogel and Beauchamp 1999, Sweka and Hartman 2003). However, turbidity may not only affect predation efficiency of predators, it is likely to also affect how prey respond to and cope with predators. Abrahams and Kattenfeld (1997) hypothesized that, with increasing turbidity, antipredator behaviour will become ineffective and will no longer be used. This hypothesis has been validated mostly in fish-fish predator-prey systems (Abrahams and Kattenfeld 1997, Snickars et al. 2004). We could show that the invertebrate littoral predator *Ischnura elegans* increased its use of conspicuous behaviours at high turbidity, suggesting it relaxed its antipredator behaviour. From the first hypothesis, Abrahams and Kattenfeld (1997) also derived that predator-prey interactions will be governed by both direct and indirect interactions in clear water, and mainly by direct interactions in turbid water. Translated to the prey's perspective, negative effects on a prey's life history (growth, mass) are expected to be more important in clear water compared to turbid water systems. The results of our enclosure experiment tended to be in accordance with this second hypothesis: fish tended to negatively affect *Ischnura* growth more in the clear-water lake than in the

turbid lake. Similarly, size-specific mass was negatively affected by fish in the clear-water lake, but not in the turbid lake. Overall, these results indicate that the effect of water turbidity alone may contribute to the observed differences in macroinvertebrate community structure between clear-water and turbid lakes.

Macroinvertebrates and horizontal migration behaviour of Daphnia

To avoid being eaten by fish, *Daphnia* evolved a broad scala of antipredator behaviours (Boersma et al. 1998, De Meester et al. 1999). In shallow lakes with abundant macrophytes, *Daphnia* have been observed to seek shelter in macrophyte beds during daytime, and to migrate to open water during the night. Despite the fact that macrophytes provide relatively good protection against fish predation (Burks et al. 2001a), often *Daphnia* are observed to not enter the macrophytes, but instead to stay at the edges of the macrophyte stands. That *Daphnia* does not accomplish maximal protection against predation by pelagial fish suggests that strong costs are associated with living in macrophyte stands. Several types of costs have been suggested, such as low food availability, detrimental chemicals released by the plants, and predation risk by invertebrate predators (Burks et al. 2002). Burks et al. (2001b) found that predatory larval dragonflies were able to reduce *Daphnia* densities in large field enclosures, implying predation by odonate larvae may be a severe cost of staying in macrophyte stands. We could show that *Daphnia* indeed avoid macrophytes more when they are inhabited by damselfly larvae, which may be the mechanism why *Daphnia* sometimes stays at the edges of the vegetation instead of entering it. However, we tested this in the absence of the pelagial predator fish(cue), which may either increase the scope for a behavioural effect of odonate larvae as the daphnids will be forced towards the macrophytes, or may override the observed response. Furthermore, the effect of the combined presence of odonate larvae and fish may be specific to a particular system as it probably depends on the effective cost that *Daphnia* experience when staying in either open water or macrophytes. That the presence of a pelagial predator may interfere with the effect of the littoral predator on *Daphnia* behaviour was shown in an experiment using pelagial *Chaoborus* and littoral Odonate larvae. Here, *Chaoborus* larvae were shown to induce horizontal migration behaviour in *Daphnia*, however, this response depended on whether damselfly larvae were present in the vegetation. These findings may be of particular interest in small fishless ponds where invertebrates are the main predators of *Daphnia*. Relatively few studies have been carried out on the functioning of small pond ecosystems, but our results indicate that, as in large shallow fish lakes,

intense predator-prey interactions and adaptive antipredator responses of *Daphnia* may be expected. As a matter of fact, the antipredator responses revealed by our study on fish lake *Daphnia* clones probably underestimate the fine-tuned responses that can be expected in *Daphnia* clones originating from fishless ponds. If *Daphnia* also fulfill the role of ecosystem engineers in small fishless ponds as in shallow lakes, invertebrate predators may be important secondary regulators of ecosystem state in ponds.

Dispersal of lentic macroinvertbrates

Little is known on dispersal and how it affects (meta)community dynamics in lake-inhabiting macroinvertebrates (Bilton et al. 2001). The possible modes of dispersal differ greatly among macroinvertebrate groups and between different systems. In isolated systems, the adults of many aquatic insects may fly from one to another habitat or perform multiple flights (‘active dispersers’), whereas other taxa rely on external means of transport such as wind or animal vectors (‘passive dispersers’). It is generally assumed that dispersal among isolated systems is far more frequent in active than in passive dispersers, possibly resulting in different patterns and intensities of metacommunity dynamics. In lakes and ponds that are connected by floodings, overflows or rivulets, transport by water may provide an additional means of dispersal to that in isolated systems, as shown for passively dispersing zooplankton (Michels et al. 2001). In an intensively connected set of lakes, we found littoral macroinvertebrates to disperse among lakes through lake connections. Moreover, actively and passively dispersing macroinvertebrates showed equal intensities of dispersal through the lake connections. The extra pathway for dispersal in these connected systems probably makes them to a higher extent resilient and resistant against local disturbances, as shown by the extremely rapid recolonization by macroinvertebrates of drained lakes. The typical set of both actively as well as passively dispersing macroinvertebrates occurring in the lakes of De Maten managed to recolonize the lakes within the first year after their refilling, probably helped by the high degree of connectivity to non-drained lakes. This original set of macroinvertebrates was in the first years supplemented by a new group of highly mobile, fish-intolerant species, which probably benefited from the decline in fish densities.

As expected, the assemblage of macroinvertebrates dispersing through the lake connections was found to reflect the composition and taxon richness of the macroinvertebrate community in the upstream source lakes. Consequently, dispersal through lake connections may make communities of connected lakes more similar

compared to those of unconnected lakes, as previously shown by Cottenie et al. (2003) for zooplankton. Interestingly, we found indications that the spatial community similarity patterns of actively and passively dispersing invertebrates were different, resembling the spatial network of lake connections in passive dispersers, whereas in active dispersers similarities reflected only the Euclidian distances among the lakes. This accords with the idea that in passive dispersers dispersal via the lake connections in De Maten is an important dispersal mechanism, whereas dispersal of active dispersers occurs mainly over land.

Differences in the metacommunity dynamics of active and passive dispersers may not only reflect differences in their dispersal ability, but may also stem from the ability of active dispersers to actively select habitats from the landscape to colonize. An increasing body of evidence suggests that habitat selection may be widespread in lentic macroinvertebrates (Blaustein 1999, Abjornsson et al. 2002). Yet, the currently prevailing view is that the local processes of abiotic limitation and species-sorting shape community differences of macroinvertebrates in ponds and lakes (Wellborn et al. 1996). We found that the colonization of small fishless tanks occurred independent of a large set of nearby fish lakes. Several findings strongly suggested that neither abiotic limitation nor predator-mediated species sorting can fully explain the observed pattern, leading to the conclusion that strong habitat selection may account for the observed pattern. Habitat selection as a mechanism creating differences in macroinvertebrate assemblages of different fish lakes is less likely, as the ecological differences among these lakes are notably smaller than between small temporary fishless ponds and large fish lakes. More research is needed, however, to understand under what circumstances habitat selection may be expected before we can actually predict its generality in nature.

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Summary

Chapter I: The effect of turbidity state and microhabitat on macroinvertebrate assemblages: a pilot study of six shallow lakes

Shallow lakes can occur in two alternative stable states, a clear-water state and a turbid state. This is associated with separate assemblages of fish, zooplankton and plants. Little is known about whether macroinvertebrate assemblages differ across both stable states. This study investigated this in a connected set of three turbid and three clear-water shallow lakes. To overcome confounding effects of differences in spatial structure of macrophytes in turbid and clear-water lakes, we sampled three microhabitats that occurred in both alternative stable states: open water, sago pondweed (*Potamogeton pectinatus*) and reed (*Phragmites australis*). Univariate analyses indicated no differences in the number of organisms, taxon richness or diversity between turbid and clear-water lakes. Multivariate analysis, however, indicated a trend towards differences in the macroinvertebrate community structure of both stable states. Two taxa explained a significant amount of the variation between both lake types, both of which preferred the clear-water lakes. The number of organisms and the taxon richness were higher in reed than in the other microhabitats, but diversity and evenness did not differ among the microhabitats. Multivariate analyses separated all three microhabitats. Four taxa, mainly detritus feeders and collector-gatherers, explained most of the variation in the data and preferred the reed microhabitat. The effects of turbidity state (11.8 % explained variance) and microhabitat (24 % explained variance) on the macroinvertebrate assemblages were largely independent from each other (2 % shared variance). Although macroinvertebrates are not implemented in the initial theory of stable states, our results indicate different assemblages across both stable states.

Chapter II: Water turbidity affects predator-prey interactions in a fish-damselfly system

Community structure may differ dramatically between clear-water and turbid lakes. These differences have been attributed to differences in the cascading effect of fish on prey populations, due to the reduced efficiency of fish predation in the presence of macrophytes. However, recent theoretical ideas suggest that water turbidity may shape predator-prey interactions, and it is predicted that prey will relax its antipredation behaviour in turbid water (H1). As a result, the nature of predator-prey interactions is

expected to shift from both direct and indirect in clear water to dominantly direct in turbid water (H2). We tested these ideas in a fish-damselfly predator-prey system. In a first behavioural experiment, we looked at antipredation behaviour of damselfly larvae isolated from habitats that differ in turbidity, in the presence of fish in clear and turbid water. As predicted in H1, the larvae were more active in turbid than in clear water. In a complementary enclosure experiment, we reared larvae in a clear-water pond and a turbid pond, respectively, and manipulated the origin of the larvae (clear-water, turbid pond), fish presence (absent, present), and vegetation density (sparse, abundant). In both ponds, fish had a direct negative effect on survival of the larvae, which was mitigated in the presence of vegetation. In the fish treatment, the change in average body mass tended to be higher in the turbid than the clear pond, suggesting indirect effects of fish were mitigated in the turbid pond. This was supported by a negative effect of fish on effective growth rate of larvae in the clear pond, but not in the turbid pond. These results are compatible with the idea that predator-prey relationships are mainly governed by direct effects in turbid water, and by direct and indirect effects in clear water.

Chapter III: Behavioural linkage of pelagic prey and littoral predators: microhabitat selection by *Daphnia* induced by damselfly larvae

Only recently ecologists started treating the previously separately considered benthic, littoral and pelagic zones of lake ecosystems as closely connected compartments. Here we study a link between organisms belonging to a different compartment - namely the pelagic and the littoral - through behaviour in a series of laboratory experiments. Waterfleas of the genus *Daphnia* are inhabitants of the pelagic zone and suffer a high predation pressure from syntopic vertebrate predators (mainly fish). Presumably to escape this predation, they sometimes migrate in the day to the littoral to seek refuge within macrophytes and return to the pelagic at night. Zygopterans from the genus *Ischnura* do commonly co-occur in ponds with *Daphnia* and are known as opportunistic predators of *Daphnia*. In two initial experiments in microcosms in the lab we showed that *Ischnura* larvae are littoral predators strongly associated with macrophytes. Although we found that predation rates of individual *Ischnura* larvae on *Daphnia* are approximately 1.5 fold lower in macrophytes compared to open water, total predation from *Ischnura* on *Daphnia* per unit area is tenfold higher within macrophytes than in open water, making the open water a safer place for *Daphnia* with regard to *Ischnura* predation. In a third microcosm experiment we monitored horizontal distribution of *Daphnia* in the absence,

presence and odour only of *Ischnura* larvae. After 2 hours, on average 10 % less *Daphnia* remained within the vegetation when *Ischnura* larvae or only their odour were present compared to when *Ischnura* or *Ischnura* odour were absent. We interpret this as a behavioural anti-predation response of *Daphnia* to the presence of *Ischnura* larvae that seems primarily chemically mediated. The observed horizontal migration of the pelagic prey driven by the littoral predator may couple both lake compartments and may interact with the predator-prey relationships within the pelagic.

Chapter IV: Spatial avoidance of littoral and pelagic invertebrate predators by *Daphnia*

Studies on spatial avoidance behaviour of predators by prey often ignored the fact that prey typically face multiple predators which themselves interact and show a spatial pattern in abundance and predation rates. In a series of laboratory experiments we investigated predation risk and horizontal migration of the cladoceran *Daphnia magna* between open water and vegetation in response to two important invertebrate predators with a contrasting spatial distribution: pelagic *Chaoborus* and vegetation-associated *Ischnura*. As expected, predation risk by *Chaoborus* was higher in open water due to higher numbers and higher predation rates of *Chaoborus*, while for *Ischnura*, predation risk was highest in the vegetation due to higher densities but despite lower predation rates of *Ischnura*. In accordance with this, *Daphnia* moved into the vegetation in the single presence of the pelagic *Chaoborus*. In the single presence of *Ischnura*, however, *Daphnia* showed no response. We hypothesize this may be the result of a constitutive behaviour of *Daphnia* to avoid pelagic fish, which impedes a response to the open water. In the combined predator treatment, *Daphnia* migrated to the open water zone. The increased risk of predation in the vegetation, due to a facilitating effect of *Chaoborus* on *Ischnura* predation rates is believed to have caused this migration of the *Daphnia*. This response of *Daphnia* declined through time and *Daphnia* moved toward the vegetation. A decline in the activity of the *Ischnura* larvae through time may have switched the risk balance in favour of the vegetation environment.

Chapter V: Lake-to-lake dispersal of lentic macroinvertebrates through lake-outlets

Little is known on dispersal of lentic macroinvertebrates. We quantified dispersal of lentic macroinvertebrates through lake connections in a highly connected lake system, and investigated the role of connection properties and timing (day, night) on dispersal rate.

Furthermore, by comparing dispersing macroinvertebrate assemblages with the macroinvertebrate assemblages of source lakes, we tested whether dispersal was neutral or a taxon-specific process. Many taxa dispersed through the lake connections, probably mainly by passive transport. Taxon richness of the dispersing macroinvertebrate assemblage was proportional to taxon richness in the source lakes. The number of individuals that dispersed, however, was not related to source lake densities, possibly because of the highly patchy distribution of lentic macroinvertebrates within lakes and the relatively short sampling time. Baetidae, Chironomidae and Physidae exhibited higher dispersal rates, corrected for source pond densities, than a selection of seven other macroinvertebrate families, indicating that the extent of dispersal may be taxon-specific. None of the physical properties of the connections affected dispersal. The number of dispersing macroinvertebrates was higher during the night than during daytime. Of seven frequently dispersing families, Chaoboridae and Chironomidae showed higher dispersal during the night, probably resulting from diel vertical migration behaviour. Dispersal rates of actively and passively over-land dispersers through lake connections did not differ. Since over-land dispersal probably is more frequent in active dispersers compared to passive dispersers, dispersal through lake-outlets in connected lake systems may generate different spatial patterns compared to over-land dispersal with regard to the composition of communities and the genetic structure of populations.

Chapter VI: Rapid response of macroinvertebrates to drainage management of shallow lakes

Shallow lakes throughout the world are subject to drainages, either as part of fish farming practices to harvest the fish, or as part of lake restoration projects. Lake drainages of fish lakes are known to improve macrophyte and zooplankton diversity, but the effect on the macroinvertebrate community is poorly known. In this study, we investigated temporal trends in the macroinvertebrate community following lake drainage in six shallow connected lakes. We evaluated drainage effects for all macroinvertebrates at the family level and for Coleoptera, Hemiptera and Odonata at the genus or species level. After the drainages, diversity increased for all macroinvertebrtaes at the family level, Hemiptera and Odonata. Taxon richness increased for Coleoptera, Hemiptera and Odonata. Recolonization of the lakes by the former inhabitants occurred within the first year after the drainages and was supplemented with a set of species that previously were rare or did not occur in the lakes. Changes in the environmental conditions of the

lakes were small and transient, except for the decline in fish. The fact that species that occurred in the lakes before the drainages rapidly recolonized the lakes is attributed to the high connectivity in our study system. The occurrence of supplementary species probably was linked to the decline in fish predation, suggesting fish was a dominant factor in shaping the communities. In summary, lake drainage has a positive effect on the diversity and richness of macroinvertebrates in shallow connected lakes. This positive effect seems largely due to a combination of a decline in fish predation following lake drainage and the high rate of recolonization amongst others via connections to non-drained lakes. Lake drainage therefore probably is the most cost-effective lake restoration tool in shallow connected lakes. Other restoration tools may be more favourable in isolated lakes where recolonization may be constrained.

Chapter VII: Metacommunity structure of lentic macroinvertebrates: contrasting active and passive dispersers

Macroinvertebrates inhabiting ponds and lakes can be categorized as active or passive dispersers. Because dispersal over land is assumed to be very limited in passive dispersers, connections between lakes may provide important additional dispersal corridors between lakes. Active dispersers can easily fly from lake to lake over land, and the additional contribution of dispersal via lake connections to dispersal may be negligible. As a result, metacommunities of active and passive macroinvertebrate dispersers may be differentially affected by lake connectivity and hence show different spatial structures. We investigated this hypothesis in a system of 34 connected lakes. In agreement with our expectations, we found that communities of passively dispersing macroinvertebrates were regionally structured according to a spatial model that incorporated the connections among the lakes, while active dispersers were not. Active dispersers, and surprisingly also passive dispersers, were also strongly structured conform the spatial model of Euclidian distances among the lakes, possibly suggesting that over-land dispersal as well was an important dispersal mechanism. These results confirm the importance of connectivity among habitats for metacommunity structure and dynamics, but also indicate that the net effect of connectivity on metacommunity structure depends on the prevailing mode of dispersal of the organisms.

Chapter VIII: Habitat selection drives macroinvertebrate community turnover along the hydroperiod gradient

Community turnover along the hydroperiod gradient is generally assumed to result from local selective mechanisms, abiotic limitation and species sorting, while the possibility of habitat selection behaviour is largely ignored. In this study, the colonizing assembly of macroinvertebrates in small, fishless habitats deviated strongly from that observed in a surrounding large set of fish lakes. By studying distance-similarity relationships, we could show that fish lakes did not act as sources for the colonization of the fishless habitats. We argue that neither abiotic limitation, nor species sorting due to invertebrate predators in the fishless habitats or due to fish in the fish lakes were able to explain the observed patterns. Our results indicate that strong habitat selection may substantially contribute to community turnover along environmental gradients observed in nature, thereby offsetting a direct role for abiotic limitation and species sorting.

Samenvatting

Hoofdstuk I: Effecten van waterhelderheid en microhabitat op de structuur van macroinvertebratengemeenschappen: een pilootstudie in zes ondiepe vijvers

Ondiepe vijvers kunnen voorkomen in twee sterk verschillende alternatieve stabiele toestanden: een helderwater toestand en een troebele toestand. Deze verschillende toestanden vertonen elk een typische gemeenschap van vis, zooplankton en waterplanten. Tot hiertoe is echter nauwelijks geweten of ook macroinvertebraten een verschillende gemeenschapsstructuur vertonen naargelang de helderheidstoestand van de vijver. We onderzochten dit in een onderling verbonden set van drie heldere en drie troebele vijvers. Doordat ook waterplantengemeenschappen, die sterk bepalend zijn voor de structuur van een macroinvertebratengemeenschap, doorgaans sterk verschillen tussen heldere en troebele vijvers, kan vaak niet eenduidig het effect van waterhelderheid onderscheiden worden. Om dit probleem te omzeilen bemonsterden we drie microhabitatten die we in beide vijvertoestanden aantroffen: open water, schedefonteinkruid (*Potamogeton pectinatus*) en riet (*Phragmites australis*). Univariate analyses toonden aan dat het aantal aangetroffen organismen, het aantal soorten, en de diversiteit van macroinvertebraten niet verschilden tussen heldere en troebele vijvers. Multivariate analyses daarentegen toonden aan dat de structuur van de macroinvertebratengemeenschap verschillend was in heldere en troebele vijvers. Negen taxa vertoonden significante verschillen tussen beide vijvertoestanden, waarvan er zeven talrijker waren in de helderwater toestand. De macroinvertebraten-gemeenschappen van microhabitatten vertoonden duidelijke onderlinge verschillen: het aantal aangetroffen organismen en het aantal soorten waren hoger in riet dan in de andere microhabitatten maar de diversiteit verschilde niet. Multivariate methodes toonden een duidelijk onderscheid tussen de microhabitatten. Acht taxa, voornamelijk detrivoren, waren talrijker in riet. De effecten van waterhelderheid (6.8 % verklaarde variantie) en microhabitat (13.1 %) op de macroinvertebratengemeenschappen waren nauwelijks gecorreleerd (1.5 % gemeenschappelijk). Deze studie toont aan dat ook macroinvertebratengemeenschappen een verschillende gemeenschapsstructuur vertonen naargelang de vijvertoestand, zelfs wanneer gecorrigeerd wordt voor verschillen in de waterplantengemeenschap.

Hoofdstuk II: Waterhelderheid beïnvloedt de predator-prooi interacties tussen vis en waterjufferlarven

De gemeenschapsstructuur van vijverorganismen vertoont vaak sterke verschillen tussen helderwater en troebele vijvers. Deze verschillen worden vaak geweten aan een verschillende impact van de predator vis op prooipopulaties in helderwater en troebele vijvers, doordat de talrijke waterplanten in helderwater vijvers de predatie-efficiëntie van vis verlagen. Recent werd gesuggereerd dat ook waterhelderheid op zichzelf predator-prooi interacties kan beïnvloeden. Zo verwacht men dat prooiorganismen hun antipredatiegedrag activiteit zullen verlagen bij toenemende watertroebelheid, doordat het zijn functie verliest bij verminderde zichtbaarheid voor de visuele predator vis (H1). Als gevolg hiervan verwacht men dat in helderwater vijvers zowel directe als indirecte predator-prooi interacties van belang zijn, terwijl in troebele vijvers bijna uitsluitend directe interacties een rol spelen. We testten de validiteit van beide ideeën in een vis-waterjufferlarve predator-prooi systeem. In een eerste gedragsexperiment bestudeerden we antipredatiegedrag van waterjufferlarven in aanwezigheid van vis voor larven uit een heldere en een troebele vijver in helder water en in troebel water. Zoals verwacht in H1, waren de larven actiever in troebel water dan in helder water. In een complementair veldexperiment werden larven opgekweekt in een heldere en een troebele vijver in enclosures met de volgende experimentele condities: oorsprong van de larven (helderwater vijver, troebele vijver), vis (aanwezig, afwezig) en densiteit aan waterplanten (weinig, veel). Vis had een sterk negatief effect op de overleving van waterjufferlarven in beide vijvers, al werd dit effect getemperd bij de hoge waterplantendensiteit. De massatoename van larven in enclosures met vis leek groter in de troebele vijver dan in de heldere vijver, in overeenstemming met de idee dat indirecte effecten minder belangrijk waren in de troebele vijver. De groottespecifieke massa van larven bleek ook negatief beïnvloed door vis in de heldere vijver, maar niet in de troebele vijver. Deze bevindingen zijn in overeenstemming met de idee dat predator-prooi interacties voornamelijk uit directe interacties bestaan in troebel water, en zowel directe als indirecte effecten behelzen in helder water.

Hoofdstuk III: Gedragsinteracties tussen pelagiale prooien en littorale predators: microhabitat selectie door *Daphnia* in aanwezigheid van waterjufferlarven

Lange tijd beschouwden ecologen de benthische, littorale en pelagiale zone van vijverecosystemen als strikt gescheiden compartimenten. In deze studie gaan we na of er een gedragslink bestaat tussen twee organismen die tot een verschillend

compartiment behoren: pelagiale *Daphnia* en littorale waterjufferlarven. Watervlooien van het genus *Daphnia* zijn typische bewoners van het pelagiaal en hebben sterk te lijden onder predatie door vis. *Daphnia* vertoont verschillende gedragingen om vispredatie te vermijden, waaronder dagelijkse horizontale migratie van het pelagiaal (’s nachts) naar littorale waterplanten (overdag). Waterjuffers van het genus *Ischnura* komen talrijk samen voor met *Daphnia*, waarop zij soms prederen. Aan de hand van verschillende laboratoriumexperimenten toonden we aan dat predatie door *Ischnura* op *Daphnia* 1.5 maal hoger was in open water dan in de waterplanten. Echter, doordat het voorkomen van *Ischnura* larven sterk gebonden is aan waterplanten, bleek het uiteindelijke predatierisico voor *Daphnia* tienmaal hoger in de waterplanten dan in open water. In een laatste experiment bestudeerden we de horizontale distributie van *Daphnia* in afwezigheid, aanwezigheid en bij aanwezigheid van enkel de geur van *Ischnura* larven. Na twee uur verbleven er ongeveer 10% minder *Daphnia* in de waterplanten in aanwezigheid van *Ischnura* of *Ischnura* geur, vergeleken met de situatie waarin *Ischnura* of *Ischnura* geur afwezig waren. We interpreteren deze resultaten als een antipredatie gedragsrespons van *Daphnia* op de aanwezigheid van *Ischnura* larven in de vegetatie die gemedieerd wordt via geurstoffen. Deze studie toont aan dat er een gedragslink bestaat tussen pelagiale *Daphnia* en littorale *Ischnura* larven waardoor het pelagiaal en het littoraal van vijvers gekoppeld zijn.

Hoofdstuk IV: Horizontaal migratie gedrag van *Daphnia* in respons op littorale en pelagiale invertebrate predators

De overgrote meerderheid van de studies die ruimtelijke distributiepatronen van prooien bestuderen in aanwezigheid van een predator, negeerden het feit dat onder natuurlijke omstandigheden vaak meerdere predators aanwezig zijn die bovendien onderling interageren en elk op zich een specifiek ruimtelijk distributiepatroon kunnen bezitten. Aan de hand van een reeks laboratoriumexperimenten onderzochten we horizontaal migratiegedrag van *Daphnia magna* tussen open water en waterplanten in aanwezigheid van twee belangrijke invertebrate predators met een kontrasterend ruimtelijk distributiepatroon: pelagiale *Chaoborus* en waterplant-geassocieerde *Ischnura*. Zoals verwacht was het predatierisico voor *Daphnia* door *Chaoborus* groter in open water dan in de waterplanten door een combinatie van hogere predatoraantallen en een efficiëntere predatie; het predatierisico voor *Daphnia* door *Ischnura* daarentegen was groter in de waterplanten, voornamelijk vanwege de sterke associatie tussen *Ischnura* en waterplanten. In overeenstemming met deze waarnemingen observeerden we dat

Daphnia naar de waterplanten migreerde in aanwezigheid van *Chaoborus*. Echter, *Daphnia* vertoonde geen ruimtelijke respons op de aanwezigheid van *Ischnura*. Dit is mogelijk het gevolg van een constitutief gedrag van *Daphnia* om het pelagiaal en pelagiale vissen te vermijden bij verhoogd predatierisico. In aanwezigheid van beide predatorsoorten migreerden de *Daphnia* naar het open water. Dit kan mogelijk verklaard worden doordat het predatierisico voor *Daphnia* het sterkst toenam in de waterplanten, doordat *Chaoborus* larven de predatie-efficiëntie van *Ischnura* op *Daphnia* verhoogden. De geobserveerde ruimtelijke respons van *Daphnia* verminderde echter naargelang de tijd vorderde, en *Daphnia* migreerde na verloop van tijd weer naar de waterplanten. Doordat de activiteit van *Ischnura* larven ook sterk afnam tijdens de duur van het experiment was het relatief predatierisico voor *Daphnia* in de waterplanten mogelijk weer lager dan in open water.

Hoofdstuk V: Dispersie van lentische macroinvertebraten tussen vijvers via vijveroverlopen

Er is nog maar weinig geweten over dispersie van lentische macroinvertebraten. In deze studie kwantificeerden we de dispersie van lentische macroinvertebraten doorheen vijververbindingen in een systeem van sterk verbonden vijvers, en onderzochten we hoe deze dispersie beïnvloed werd door het tijdsstip van de bemonstering (dag/ nacht) en de fysische eigenschappen van de vijververbindingen. Daarnaast testten we ook of dispersie door vijververbindingen een toevalsproces of soortspecifiek is, door de samenstelling van de groep disperserende macroinvertebraten en de samenstelling van de gemeenschap macroinvertebraten in de bronvijvers onderling te vergelijken. Een groot aantal taxa disperseerde door de overlopen en dit aantal was sterk proportioneel aan het aantal taxa in de bronvijvers. Het aantal disperserende individuen daarentegen vertoonde geen relatie tot de densiteiten in de bronpopulatie, mogelijk doordat macroinvertebraten sterk gegroepeerd voorkomen en we slechts een relatief korte tijd bemonsterden. Baetidae, Chironomidae en Physidae vertoonden een hogere kans op dispersie dan zeven overige geselecteerde taxa dan verwacht op basis van hun talrijkheid in de bronvijvers. Geen enkele van de onderzochte eigenschappen van de overlopen had een significante invloed op dispersie van macroinvertebraten. Het aantal disperserende macroinvertebraten was groter ’s nachts dan overdag. Een meer gedetailleerde studie bij zeven taxa toonde aan dat enkel Chaoboridae en Chironomidae talrijker disperseerden tijdens de nacht, mogelijk doordat deze soorten verticaal migratie gedrag vertonen. De dispersie-intensiteit van actief en passief disperserende

macroinvertebraten was niet verschillend. Dispersie door overlopen kan hierdoor potentieel sterk interageren met dispersie over land, waarvan de intensiteit waarschijnlijk sterk verschilt tussen actieve en passieve disperseerders, bij het structureren van genetische en similariteitspatronen van populaties en gemeenschappen van actief en passief disperserende macroinvertebraten.

Hoofdstuk VI: Snelle herkolonisatie door lentische macroinvertebraten na drainagebeheer van ondiepe vijvers

Ondiepe vijvers over heel de wereld worden geregeld gedraineerd, hetzij voor het oogsten van vis in viskweekvijvers, hetzij als beheersmaatregel voor het herstellen van geeutrofieerde en vertroebelde vijvers. Het is welbekend dat de drainage van visvijvers de zooplankton- en waterplantengemeenschap sterk positief kan beïnvloeden, maar het effect op de macroinvertebratengemeenschap is nauwelijks bekend. In deze studie bestuderen we de veranderingen doorheen de tijd van de macroinvertebratengemeenschap na vijverdrainage in zes ondiepe, verbonden vijvers. We bekeken daarbij drainage-effecten op de gehele macroinvertebratengemeenschap op familieniveau, en voor Coleoptera, Heteroptera en Odonata op genus- of soortniveau. De macroinvertebratendiversiteit op familieniveau, en de diversiteit van Heteroptera en Odonata nam significant toe na de drainages. De soortenrijkdom nam significant toe voor Coleoptera, Heteroptera en Odonata. Binnen het eerste jaar na het hervullen van de vijvers herkoloniseerden de voormalige macroinvertebratengemeenschappen de vijvers, aangevuld met een groep taxa die voordien zeldzaam of niet voorkwamen in de vijvers. Veranderingen in de lokale omgevingsfactoren van de vijvers als gevolg van de drainages waren klein en voorbijgaand, uitgezonderd voor de visgemeenschap die sterk afnam. De snelle herkolonisatie door de taxa die voordien al in de vijvers voorkwamen wijten we aan de intense verbondenheid van deze vijvers met niet-gedraineerde vijvers. Dat ook extra nieuwe soorten de gedraineerde vijvers koloniseerden is vermoedelijk gerelateerd aan de daling in vispredatiedruk, wat suggereert dat vispredatie een belangrijke factor is in het structureren van de macroinvertebratengemeenschappen. Als besluit kunnen we stellen dat vijverdrainages een positieve invloed uitoefenen op de diversiteit en de soortenrijkdom van macroinvertebraten in ondiepe, verbonden vijvers. Dit positief effect is waarschijnlijk te wijten aan de combinatie van een sterk daling van de vispredatiedruk, en de hoge snelheid van herkolonisatie mede door de sterke onderlinge verbondenheid van dit systeem. Vijverdrainage is bijgevolg een zeer kost-efficiënte manier voor het herstel van ondiepe verbonden vijvers. Deze resultaten

kunnen echter niet zonder meer veralgemeend worden naar systemen van geïsoleerde vijvers, doordat herkolonisatie hier potentieel sterk belemmerd kan zijn.

Hoofdstuk VII: Metagemeenschapsstructuur van actief en passief disperserende lentische macroinvertebraten

Macroinvertebraten kunnen volgens hun dispersievermogen ingedeeld worden in actieve en passieve disperseerders. Dit verschil in dispersievermogen vertaalt zich doorgaans ook in een verschillende dispersie-intensiteit tussen beide groepen, en mogelijk zelfs in verschillen in het ruimtelijke patroon van gemeenschapsstructuur en –samenstelling. De verschillen in het ruimtelijk patroon van gemeenschapstructuur zijn naar verwachting het opvallendst in systemen waarbij vijvers onderling verbonden zijn. Bij passieve disperseerders, waar dispersie mogelijk frequenter door de vijververbindingen verloopt dan over land, verwachten we dat vijvergemeenschappen immers meer gelijkaardig zijn volgens het ruimtelijk netwerk van verbindingen tussen de vijvers. Bij actieve disperseerders, waar dispersie waarschijnlijk het meest frequent over land plaatsvindt, verwachten we dat de in vogelvlucht meer nabijgelegen vijvers (kortste Euclidische afstand) een meer gelijkende gemeenschapsstructuur hebben en dat de vijverconnecties (vrijwel) geen effect hierop hebben. Onze bevindingen kwamen verrassend goed overeen met deze verwachtingen: de metagemeenschapsstructuur van passieve disperseerders maar niet van actieve disperseerders vertoonde gelijkenissen met het patroon van vijververbindingen. Zowel de metagemeenschap van de actieve als de passieve disperseerders vertoonde bovendien een structuurcomponent gelijkaardig aan de kortste Euclidische afstand tussen de vijvers, wat mogelijk suggereert dat bij actieve maar ook bij passieve disperseerders dispersie over land een relatief belangrijke rol speelt. Deze resultaten bevestigen dat habitatconnectiviteit in belangrijke mate de dynamiek en structuur van metagemeenschappen kan bepalen, en tonen bovendien aan dat het netto effect van connectiviteit sterk kan verschillen tussen gemeenschappen van organismen met een verschillende manier en vermogen tot dispersie.

Hoofdstuk VIII: Habitat selectie als drijvende kracht van de veranderingen in gemeenschapsstructuur langsheen de hydroperiode gradiënt

De vaak sterke veranderingen in gemeenschapsstructuur langsheen de hydroperiode gradiënt worden meestal verklaard aan de hand van sterke lokale selectiemechanismen zoals abiotische limitering en soortselectie, waarbij de mogelijkheid van habitat selectie meestal genegeerd wordt. In deze studie vonden we dat de samenstelling van de groep

macroinvertebraten die kleine, visloze habitatten koloniseerde sterk verschilde van de samenstelling van de gemeenschap macroinvertebraten in een uitgebreide set nabijgelegen visvijvers. Aan de hand van afstand-similariteitsanalyses konden we aantonen dat deze vismeren niet als brongemeenschappen fungeerden voor de kolonisatie van de visloze habitatten. Noch abiotische limitatie, noch soortselectie door predators (vis in de vismeren, invertebrate predators in de visloze habitatten) waren een afdoende verklaring voor de gevonden verschillen. Onze resultaten tonen aan dat habitat selectie bijdraagt aan de vorming van patronen van verandering in gemeenschapsstructuur langsheen de hydroperiode.