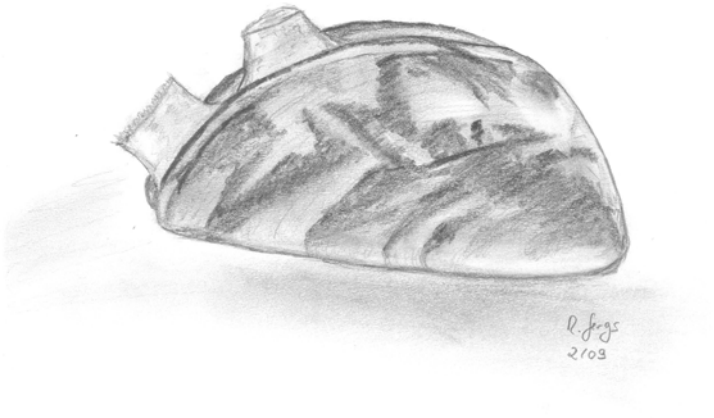
Dreissena polymorpha in Lake Constance: An example of a keystone engineer?



Dissertation

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„Es gibt keinen guten und erfolgreichen Biologen,
der nicht aus inniger Freude an den Schönheiten
der lebendigen Kreatur zu seinem Lebensberufe
gelangt wäre und dem das Wissen, das ihm aus
diesem Berufe zuwuchs, nicht auch wieder die
Freude an Natur und Arbeit vertieft hätte.“

Konrad Lorenz (1903 – 1989)

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Chapter I

General Introduction

The role of interactions for community structuring

Organisms can influence their environment in many ways. With respect to other organisms, these ways are often summarized as interaction, for example intra- and interspecific competition, predation, parasitism or symbiosis (Begon et al. 1998, Krebs 2001). Besides, organisms can modify their environment by creating and/or maintaining habitats. However, this organismic interaction is not studied systematically (Gurney and Lawton 1996). The direct or indirect provision of structures that modulate resource flow through physical alteration of habitats is called *ecosystem engineering* and the responsible organisms *ecosystem engineers* (Jones et al. 1994). It is important to note that the direct provision of a resource by the organism (e.g. the organism as resource) is not *ecosystem engineering*. In general, there are two possibilities for alteration of resource flow. Autogenic *engineers* change habitats directly by their own physical structure. Simple examples for autogenic *engineers* are trees. Although trees resemble a resource (and thus are no *ecosystem engineers*), they nevertheless provide a physical structure, which alters the environment (Fish 1983). Allogenic *engineers* change their environment indirectly by transforming living or dead material from one physical state into another. For example, in North America the local beaver (*Castor canadensis*) colonizes rivers and constructs dams by cutting trees and uses them as building material. This alters the hydrological regime and restructures the wetland from a river system to a lake-like system (Naiman et al. 1988).

Jones et al. (1994) described five different cases of *ecosystem engineering*. In the following two important cases are explained in more detail (Fig. I.1). Autogenic *engineering* occurs when an organism shifts from one state in

another, which modulates directly the resource flow. Submerged macrophytes grow and create weed beds, which attenuate light, enhance sedimentation and oxygenate the rhizosphere (Carpenter and Lodge 1986). Allogenic *engineering* occurs when an organism is responsible for the shift of living or non-living material from one state to another, which results directly in modulation of the resource flow. Zooplankton filters living, dead or inorganic material and form them into pellets, which are excreted. The sinking pellets enhance the vertical transport and alter therefore the resource flow (e.g. Wallace et al. 1981, Fowler and Knauer 1986).

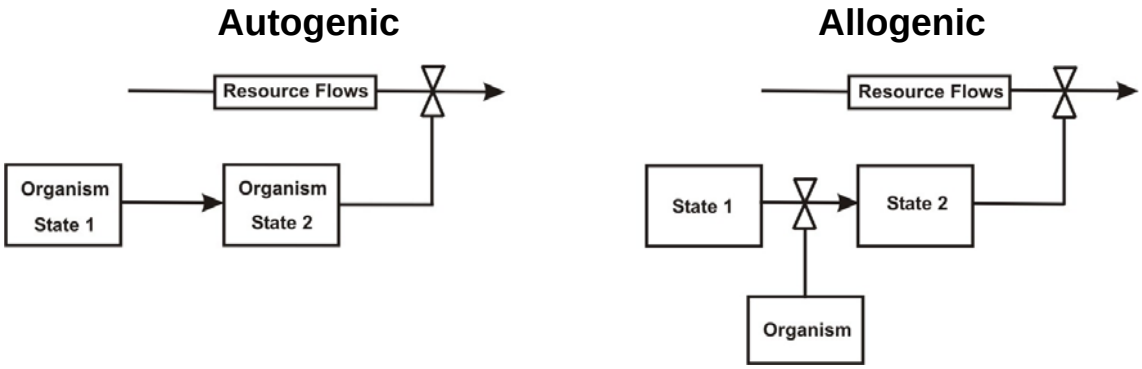

Figure I.1: Different cases of *ecosystem engineering* by organisms (modified after Jones et al. 1994). In the autogenic *engineering* the organisms modulate the resource flow by it their own physical structure; in the allogenic engineering the species transform living or non-living material from one physical state into a second state. Points of modulation are marked with the ⋈ symbol.

In comparison to the concept of *ecosystem engineering*, a *keystone species* usually plays an exceeding role in its surrounding food web. Removal of *keystone species*, e.g. a top-predator, would result in a change of species community composition (Paine 1969, Krebs 2001). A well known example is the North American starfish *Pisaster ochraceous* in rocky intertidal invertebrate communities (Paine 1974). The starfish feeds on the mussel *Mytilus californianus*, which outcompetes other invertebrates in absence of *P. ochraceous*. Since only small mussels are eaten by the starfish, the population of *Mytilus* is preserved by egg production of large mussels. In recent years the *keystone species* concept has been broadened and is not any more restricted to predators. Even resources can have a *keystone* function; important hallmarks for a *keystone species* are that their presence is crucial for maintaining structure

and diversity of their community and their exceptional importance in relation to the rest of the community (Mills et al. 1993).

The *keystone species* and *ecosystem engineer* concepts do not exclude each other, but can rather complement one another. A *keystone species* may influence the impact of an *ecosystem engineer*, with further modulation of resource flow and consequences for other species in the interaction web (Estes and Palmisano 1974). On the other hand, an *engineer species* can be a *keystone species* even though the species itself plays a minor role in the community food web (Jones et al. 1994). The authors mentioned that the role of *ecosystem engineers* as *keystone species* has often not been recognized, and hypothesize that *keystone engineers* occur in almost all habitats.

Impact of zebra mussels for aquatic ecosystems

The zebra mussel *Dreissena polymorpha* is one of the most successful invaders of the last two centuries (Kinzelbach 1995). Originally located in the region of the Black Sea and the Caspian Sea, the species is now common all over Europe and North America. The dispersion over Europe started in the early 19th century (Thienemann 1950). Because of the migration speed and occurring densities in invaded systems, Thienemann (1950) compared the spreading of the zebra mussel with the mass-invasion of the brown rat (*Rattus norvegicus*). In Central Europe, river systems were colonized first in regions near the estuaries in the first half of the 19th century; most upper regions of the river systems were invaded until the end of the 19th century. Zebra mussels can tolerate brackish water and were therefore also able to reach Scandinavia during this time (Thienemann 1950). For reproduction in spring and summer, zebra mussels release veliger larvae, which are pelagic for a period of one to five weeks. After this time the 200 µm small mussels attach to any hard substrate by their byssal threads (summarized by Sprung 1993). The planktonic larvae phase has a great influence in dispersing of this species. They attach to ships or are part of ballast waters, whereby even parts of a river system can be reached in upstream direction (Thienemann 1950, Kinzelbach 1995). The zebra mussel was not able to pass the waterfalls near Schaffhausen (Switzerland) and thus to colonize the upper Rhine system for a long time. Lake Constance, a pre-alpine lake belonging to the upper Rhine system, was invaded in the mid

1960s and the mussels colonized the entire lake within a few years (Siessegger 1969). Zebra mussels, attached to boats or ships are able to survive outside the water for some days (Martens and Grabow 2008); a carryover was supposedly the case for invading Lake Constance (Kinzelbach 1972). In the mid 1980s, zebra mussels reached the Laurentian Lakes in North America via ballast water of cargo ships (Hebert et al. 1989), where the species is now widespread and still spreading.

In successfully invaded ecosystems, the zebra mussels had great impacts not only on the ecosystem structure. Juvenile zebra mussels foul to any hard substrata, also on equipment of economic importance such as dock and canal walls, rudders and keels of ships, anchor chains, water pipes and many more (MacIsaac 1996). Removal or prevention of the mussel is often necessary to keep serviceability and causes therefore an immense economic damage. The financial burden of responding to the zebra mussel damage for the Monroe Waterworks (Michgigan, United States) was estimated at about more than $ 300,000 for a two year period from 1989 to 1991 (LePage 1993). Zebra mussels also foul organic structures such as macrophytes or crayfish and settle also in high numbers on native mussels (*Unionidaceae*), causing suffocation, starvation, and energetic stress leading to death. Loss of native mussel populations has increased dramatically if zebra mussels are present, particularly in the Great Lakes (Hunter and Bailey 1992, Ricciardi et al. 1996). Filtration by the mussels reduces the overall concentration of phytoplankton, which increases the water clarity and therefore the light transmittance (Holland 1993, Nicholls and Hopkins 1993). Increases in water clarity can lead in turn to stronger photosynthesis and proliferation by rooted aquatic macrophytes (Skubinna et al. 1995). The reduction of the phytoplankton in the pelagic zone results in an accumulation of organic material in the benthic zone by the excretion of digested faeces and undigested pseudofaeces, together described as biodeposited material (Klerks et al. 1996, Stewart et al. 1998). Hence, *D. polymorpha* can be considered as an allogenic *ecosystem engineer* according to Figure I.1, which transforms the phytoplankton from a pelagic to benthic state and alters therefore the resource availability (light, organic material). The establishment of zebra mussels as a biological filter was discussed, because in polluted systems toxic contaminants accumulate in the

biodeposited material (Reeders and Bij de Vaate 1992, Bruner et al. 1994). Feeding by zebra mussels is highly selective, mostly depending on the size of the particles with highest preference for particles between 7 and 50 µm, but also driven by food quality (Ten Winkel and Davids 1982, Sprung and Rose 1988, Naddafi et al. 2007). As a result of this selectivity mainly cryptophytes and small diatoms are ingested, whereas large chlorophytes and cyanobacteria, and pennate diatoms are rejected as pseudofaeces (Bastviken et al. 1998, Naddafi et al. 2007). This characteristic of the mussel is far-ranging, even promoting an algae bloom of the toxic cyanobacteria *Microcystis aeruginosa* in Lake Huron and Lake Erie, North America (Vanderploeg et al. 2001).

Regarding density and biomass, zebra mussels are the dominant macro-invertebrates in invaded benthic systems and have an impact on the benthic community structure. Many other invertebrate species also benefit from the invasion of the mussel and increased in abundance (e.g. Stewart and Haynes 1994, MacIsaac 1996). Stewart and Haynes (1994) additionally found an increase in benthic invertebrate diversity. The reaction of the macro-invertebrates was a response to two different factors of the zebra mussel. First, many invertebrate species, such as snails, mayflies or caddis flies, responded to the increased habitat structure provided by the mussel shells (Dermott et al. 1993, Griffiths 1993, Mörtl and Rothhaupt 2003). The mussel shells increase the surface area and create small cavities, which are used for protection from predators and wave effects. Thus, the alteration of the benthic surface area structure is a case of autogenic *ecosystem engineering* as shown in Figure I.1. Second, other invertebrate species, especially amphipods and chironomids, showed also an increase in abundance caused by the living mussel (Stewart and Haynes 1994, Mörtl and Rothhaupt 2003). By their filtration activity, zebra mussels mediate the benthic-pelagic coupling and enhance the resource flow to the benthos (Klerks et al. 1996, Ricciardi et al. 1997, Roditi et al. 1997). It is assumed that the increased amount of organic material by zebra mussels´ biodeposition is responsible for this effect and is considered to support a biodeposition-based food web (Stewart and Haynes 1994, Mitchell et al. 1996). Although first studies revealed that chironomids and amphipods are able to grow on zebra mussel biodeposited material (Izvekova and Lvova-Katchanova 1972, González and Burkart 2004, respectively), little is known about the quality

and utilization of this resource. The significance of the accumulation of zebra mussel faeces and pseudofaeces under natural conditions remains also largely unknown, although there is evidence that this food partly constitutes the diet of the amphipod *Gammarus fasciatus* (Limén et al. 2005).

As central theme of this thesis I hypothesize, that the resource flow to the benthos is enhanced by *D. polymorpha* and forms a biodeposition-based food web. As a result, the zebra mussel would be a *keystone species*, which influences the benthic macroinvertebrate community not only as an *ecosystem engineer*. Thus, the zebra mussel would be one of few examples for a *keystone engineer*, which are hypothesized to be common but often have been failed to recognize (Jones et al. 1994).

Aims of this study

My thesis assesses the responsibility of the biodeposited material to the increase in abundance of macroinvertebrates in presence of living zebra mussels. In a first step, I studied the impact of different resources provided by zebra mussels on the distribution of the two dominant amphipod species of Lake Constance, *Dikerogammarus villosus and Gammarus roeselii* (**Chapter II**).

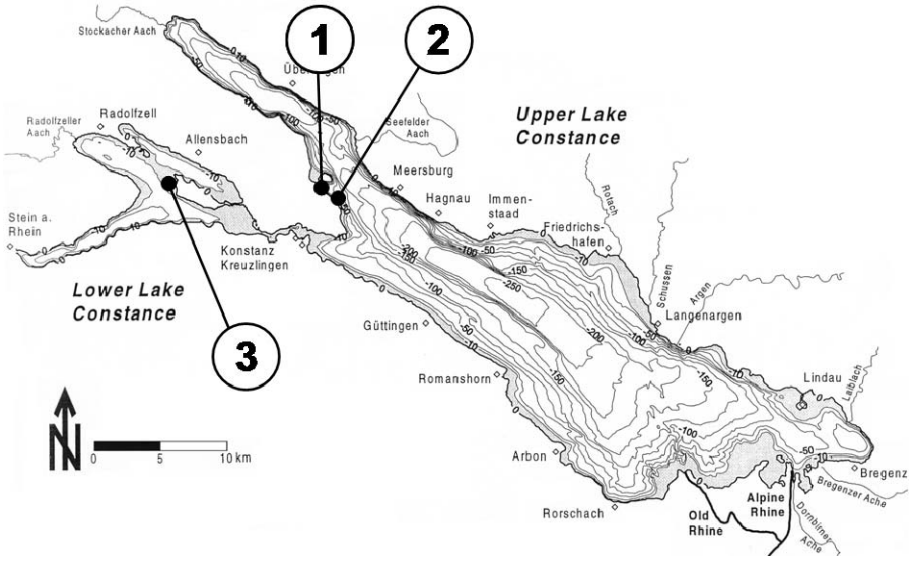

Figure I.2: Map of Lake Constance, modified after Wessels (1998). Black dots show the sampling sites of field experiments. At site **1**, estimation of zebra mussel biodeposition rate of Chapter IV was conducted. Site **2** is the routine sampling site at the Limnological Institute in Upper Lake Constance, the "Litoralgarten". Site **3** is in Lower Lake Constance at the west top of the peninsula Reichenau. Site **2** and **3** were sampled for benthic community structure and stable isotope analysis of Chapter V.

In the first experiment, the influence of structural complexity and food sources directly or indirectly provided by zebra mussels on the amphipods´ habitat-choice were compared. In a second experiment, I assessed the influence of *Dreissena* kairomones on the distribution of amphipods. In a second step, the importance of the zebra mussel biodeposited material as a diet for the amphipod species was investigated in more detail (**Chapter III**). In order to classify this food source, feeding, assimilation and growth rates were estimated for the same amphipod species used in the first chapter and additionally compared to a high and low quality food (dead animal material and conditioned alder leaves), respectively. In these laboratory experiments the focus was on amphipods, since the local amphipod in Lake Constance, *G. roeselli*, was found to increase in presence of living zebra mussel under natural conditions (Mörtl and Rothhaupt 2003). The invasive amphipod *D. villosus* was also included in these studies, because it invaded Lake Constance in 2002, and seems to displace the indigenous *G. roeselii* (Mürle et al. 2004).

Since usage of a food source in the laboratory is not necessarily linked with the relevance under natural conditions, two studies were designed to estimate the significance of these results under natural conditions. First, I measured the year-round biodeposition rate of zebra mussel in Lake Constance with modified sediment traps in order to assess the base of a potential food web; the experimental set-up was exposed monthly in the bay “Obere Güll” (Fig. I.2; point 1). Laboratory experiments with single alga species were performed in a flow-through system to investigate the mechanisms in the production of biodeposition material by zebra mussels (**Chapter IV**). Finally, I studied the contribution of zebra mussel biodeposited material to the diet of macro-invertebrates under natural conditions by Stable Isotope Analysis (**Chapter V**). A field sampling program was established with two sampling sites in Lake Constance (Fig. I.2) in autumn 2005 and 2006. I sampled quantitatively the benthic invertebrate community and primary resources. To investigate trophic relationships, macroinvertebrates and primary producers were also collected for stable isotope analyses at each sampling site. All these aspects might result in strong evidences for a *Dreissena* biodeposition-based food web and confirm the hypothesis of Stewart and Haynes (1994) and Mitchell et al. (1996).

Chapter II

Effects of zebra mussels on a native amphipod and the invasive *Dikerogammarus villosus*: the influence of biodeposition and structural complexity

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Abstract

In the last decades, zebra mussels (*Dreissena polymorpha*) have invaded many freshwater systems with severe consequences for entire communities. Most benthic macroinvertebrates, especially amphipods and chironomids, increase in abundance in the presence of zebra mussels. Increased structural complexity and an unknown biotic factor lead to this effect. *Dreissena*-associated factors that might influence populations of the native *Gammarus roeselii* and the invader *Dikerogammarus villosus* in Lake Constance, Central Europe, were investigated in laboratory experiments. These factors were: 1) increased structural complexity related to mussel shells, 2) *Dreissena* biodeposition, 3) chironomids, the presence of which is increased by biodeposited matter, and 4) *Dreissena* kairomones. In habitat-choice experiments, the native and omnivorous amphipod *G. roeselii* showed a preference for mussel shells with biodeposited material and for mussel shells with biodeposited material and chironomids, whereas the invasive and predatory amphipod *D. villosus* showed a preference only for mussel shells with biodeposited material and chironomids. In a kairomone y-maze experiment, both amphipods avoided zebra-mussel-conditioned lake water. These results indicate that habitat complexity and food availability, mediated directly or indirectly through biodeposited material, are the

factors by which amphipod abundances are increased in the presence of *Dreissena*. Thus, biodeposited material can form an important new food resource, translocated from the pelagic zone to the benthos by zebra mussel filtration, and this biodeposited material might support a new detritus-based food web in the benthos.

Keywords: biodeposition, zebra mussel, food web, *Gammarus roeselii*, *Dikerogammarus villosus*, habitat-choice, kairomone, preference.

Introduction

Zebra mussel invasions in Europe and North America have led to many changes in planktonic and benthic communities in invaded habitats (Stanczykowska et al. 1976, Griffiths 1993, Roditi et al. 1997, Mörtl and Rothhaupt 2003). Filtration by mussels reduces the overall concentration of phytoplankton (e.g., Holland 1993, Nicholls and Hopkins 1993) and alters the makeup of the phytoplankton community. The reduction in phytoplankton leads to higher light levels, which in turn, lead to the proliferation of submersed macrophytes (Skubinna et al. 1995).

Abundances of many benthic taxa, especially amphipods and chironomids, increase in response to zebra mussel invasion (Stewart and Haynes 1994, Mörtl and Rothhaupt 2003). Zebra mussels alter the benthic habitat by increasing the surface area and restructuring the substrate in the form of mussel shells. The mussels also appear to influence the benthic community by biodeposition, causing an accumulation of pelagic resources in the benthos (Stewart and Haynes 1994, Silver Botts et al. 1996, Ricciardi et al. 1997, Stewart et al. 1998). Zebra mussel density is controlled through predation by diving ducks in some habitats: a reduction of > 90% of the zebra mussel density has been observed in Lake Constance during winter (Cleven and Frenzel 1993, Werner et al. 2005). Control of zebra mussel density by waterfowl increases seasonal variation of the habitat structure and the amount, and therefore, the importance of biodeposited matter.

The omnivorous amphipod *Gammarus roeselii* (Bärlocher and Kendrick 1973, Pöckl 1992, 1993, Friberg and Jacobsen 1994) was the dominant species in the littoral zone of Lake Constance (Mörtl 2004) before the Ponto Caspian

amphipod *Dikerogammarus villosus* invaded the lake in 2002 (Mürle et al. 2004). Presence of the predatory *D. villosus* has been correlated with decreases in abundances of other benthic species in other invaded ecosystems (Dick and Platvoet 2000, Dick et al. 2002). Thus, post *D. villosus*-invasion decreases in abundances of many benthic species are expected in Lake Constance.

Biodeposition by zebra mussels could lead to the development of a biodeposit-based food web (Stewart and Haynes 1994, Mitchell et al. 1996). Macroinvertebrates can benefit from this resource directly by feeding on biodeposited material or indirectly by predation on other macroinvertebrates. Detritivorous chironomids, the abundances of which increase after zebra mussel invasions (Stewart and Haynes 1994, Mörtl and Rothhaupt 2003), might be a link to higher trophic levels. Zebra mussels also might influence the benthic community through kairomones, which might cause an increase in abundance of the olfactory sensitive amphipods (Wudkevich et al. 1997, Baumgärtner et al. 2002). A kairomone-based response would result in redistribution of amphipods in the habitat rather than an increase in total amphipod abundance.

We hypothesized that either the availability of additional food from biodeposition or zebra mussel kairomones are responsible for the increase in amphipod abundance near living zebra mussels. We tested these hypotheses by investigating the importance of biodeposited material, living chironomids, and kairomones produced by zebra mussels on the behavioural response of 2 amphipod species, the native and omnivorous *G. roeselii* and the invasive and predatory *D. villosus*. These results could help to elucidate the importance of zebra mussel biodeposition for the benthic food web of lake littoral zones (Walz 1978b).

Material and Methods

Origin and maintenance of the animals

The amphipod species *G. roeselii* and *D. villosus*, living zebra mussels, and mussel shells were obtained from the littoral zone of the pre-alpine and oligotrophic Lake Constance (Central Europe, on the border of Germany, Switzerland, and Austria). Water levels fluctuate annually within 2 m, depending largely on the unregulated alpine system of the Rhine River. The main basin of

Lake Constance has a mean depth of 100 m and covers 473 km^2 (Internationale Gewässerschutzkommission für den Bodensee 2004).

In the laboratory, the 2 amphipod species and the zebra mussels were kept separately in a 15 °C climate chamber with a diurnal light rhythm of 12 h:12 h (day:night). Habitat-choice and y-maze experiments also were conducted in this climate chamber. Zebra mussels and *G. roeselii* were maintained in tanks filled with water from Lake Constance. *Dikerogammarus villosus* was kept in a flow-through system with water from Lake Constance to minimize their mortality rate. Gravels of different grain sizes were provided for shelter for amphipods. Both amphipod species were fed commercially available frozen chironomids. In all experiments, only adult amphipods (0.7–1.7-mm length) of both sexes were used. Different amphipods were used in each replicate in both experiments.

Habitat-choice experiment

The attractiveness of food sources provided directly or indirectly by zebra mussels was examined in a 2-chamber habitat-choice arena (Fig. II.1). Each 9 × 9 × 7 cm chamber was constructed of gray polyvinyl chloride (PVC), and chambers were separated by a removable dividing wall (3.5 × 3 7 cm). Each chamber featured a notch to hold an unglazed 4.7 × 4.7 cm tile. The reference habitat for all tests was a tile with 15 mussel shells attached to the tile with superglue (UHU plus 2-components epoxy resin glue; UHU, Bühl, Germany).

Five different habitat treatments were tested against the reference habitat: 1) tiles with mussel shells (reference habitat = mussel shells), 2) bare tile, 3) tiles with living mussels (living mussels), 4) tiles with mussel shells and biodeposited material produced by zebra mussels (biodeposited material), and 5) tiles with mussel shells, biodeposited material, and 30 living chironomids (biodeposited material with chironomids). The number of mussel shells or living mussels in test habitats was the same as the number of mussel shells in the reference habitat. The 5 treatments were chosen to measure the attractiveness of factors that might influence the amphipods in their natural lake environment. Mussel shells acted as a control, and bare tile tested the habitat effect caused by the mussel shells. The remaining 3 treatments specified the biotic influences of the zebra mussel, i.e., the presence of living mussels without biodeposited material and the availability of different food sources. Each treatment was replicated 8×,

except for treatments 1, 2, and 3 for *G. roeselii*, which were replicated 16×. The higher number of replicates in these 3 treatments was necessary for better precision. All habitat-choice experiments were conduced in a period of 1.5 mo in August and September 2006. Four replicates from 2 different treatments (8 replicates in all) were run at the same time. Food treatments (4, 5) of each amphipod species were done within a single 1-wk period.

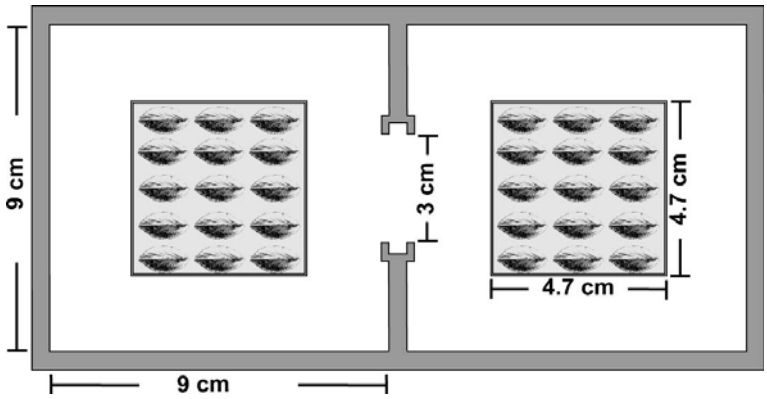

Figure II.1: Diagram of the arena used in the 2-chamber habitat-choice experiment. The passage between the chambers was closed with a removable dividing wall during the set-up phase of each trial. A tile was placed into a notch in each chamber. Fifteen mussels shells (MS) were glued to the surface of the reference tile in 1 chamber. One of 5 habitat treatments (MS, bare tile, living mussels, MS plus biodeposited material, or MS plus biodeposited material and chironomids) was presented on the surface of the tile in the 2nd chamber.

Mussels were measured with a digital sliding calliper (Preisser; Digi-Met, Gammertingen, Germany) to the nearest 0.01 mm. The mean length of empty mussel shells and living mussels was 14.99 ± 0.46 mm and 14.95 ± 0.49 mm, respectively. Shell length did not differ significantly between the empty mussel shells and living mussels (Mann–Whitney U-test, $p = 0.31$). The mussel shells and the living mussels were cleaned before use in the experiments.

Biodeposited material was collected in the lake using modified sediment traps. The sediment trap consisted of a tube of gray PVC (50 cm length; 10 cm diameter). A funnel and a 200 mL polyethylene terephthalate (PET) flask were fixed at the lower end of the tube to collect the settling sediment. A clamp was used to hold 2 tiles (4.7 × 4.7 cm) with 15 living mussels (14.91 ± 0.48 mm shell length) each in a vertical position above the upper opening of the sediment trap. Biodeposited material was collected twice between the end of August and the beginning of September 2006. Five traps were suspended at a depth of 2 m from a pontoon in the pelagic zone of Lake Constance for 7 d. The collected

material was centrifuged (1180 × *g*, 6 min), and the supernatant was replaced with enough pure water to bring the volume to 100 mL. Biodeposited material was stored at 4 °C in darkness. Ash-free dry mass (AFDM) of biodeposited material was determined on an aliquot of this material (combustion at 550 °C, 8 h). An amount of biodeposited material equivalent to 8.0 ± 0.9 mg AFDM was added to the tile of one chamber in each replicate of the treatments with added biodeposited material of the habitat-choice experiment. All animals were starved for 24 h over a mesh (1 mm-mesh size) before the test to allow them to empty their guts. Thus, the presence of feces in a chamber indicated food uptake by the amphipods during the test. Each replicate in all tests consisted of a single arena separated by the dividing wall into 2 chambers, each of which had 1 tile of the appropriate treatment and 0.5 L aerated lake water prefiltered through a 0.45 µm filter to prevent biodeposition by zebra mussels during the test. The treatments were randomly assigned to the left or right chamber. Arenas were set up with the dividing wall in place. Ten amphipods of one species were added to each chamber (20 amphipods of the same species per replicate). This quantity of amphipods was equivalent to a density of ~1200 individuals (ind.)/m², which is slightly higher than natural densities (Mörtl et al. 2005). To avoid stress for the amphipods, the experimental arena was shaded by a black curtain. Thirty minutes after the amphipods were added to the chambers, the dividing wall was removed. After 24 h, the dividing wall was re-inserted to inhibit crossing to the other chamber. The amphipods in each chamber were counted after the dividing wall was inserted.

Y-maze experiment

In September 2006, the influence of zebra mussel kairomones on both amphipod species was tested in a y-shaped flow-through system according to the protocol of Baumgärtner et al. (2002, 2003). The y-maze (length × width of root: 36 × 13 cm, and of each y-arm: 30 × 9 cm) consisted of gray PVC. Similar to the setup in Baumgärtner et al. (2002, 2003), the y-maze was shielded from the light in the climate chamber by a black curtain and was dimly illuminated with 2 fluorescent lamps (Osram Lumilux Plus, 18 W; Osram, München, Germany). Inflow of water took place near the surface at the ends of both y-arms. The outflow at the end of the mixing zone was covered by a 1 mm net so

that animals could not drift away. Inflow was provided from 2 basins with taps, and the inflow volume was adjusted to 0.5 L/min for each y-arm. In each replicate, 30-µm-filtered and aerated lake water was used as a reference. Fresh kairomone water was prepared on every experimental day by holding 1000 zebra mussels from a natural population (13.2 ± 1.6 mm in length) of zebra mussels in 60 L of lake water for 24 h. The conditioned water was purified through a 30-µm-size filter to remove potential food for the amphipods before it was used in the experiment.

At the beginning of each trial, the y-maze was filled with reference water, and the inflow was adjusted. After 1 min of preflow time, 20 individuals of one amphipod species were placed in the mixing zone near the outlet of the y-maze. Each replicate test lasted 30 min, and the number of the amphipods in each y-arm was determined every 2 min. Only the values from minute 10 to minute 30, the period when water in the root of the y-maze was fully mixed (Baumgärtner et al. 2002), were included in our calculations. Reference water was tested against reference water as a control to demonstrate that neither arm of the y-maze was preferred by the amphipods. The order of the replicates was random, and treated water and reference water were alternated between the y-arms for every replicate. Both treatments (control and kairomone water) were repeated 8×.

Statistical analysis

Habitat-choice experiment.—Some animals died during the habitat-choice experiments. Therefore, the difference in the number of amphipods in the test and in reference habitats relative to the number of amphipods in the reference habitat (% difference) at the end of the experiment was the dependent variable in the analysis. Percentages rather than actual counts were used so that replicates in which amphipods died during the experiment could be included in the analysis without introducing bias. Percent difference described the preference of amphipods for the test habitat treatment. The strength of the influence of the test habitat was assessed by ignoring the direction of habitat choice and comparing the absolute value of the % difference to 0 (equal numbers on both habitats). Percentages were arcsine ($\sqrt{x}$)-transformed to satisfy assumptions of normal distribution and homogeneity of the variances

(Underwood 2006). Homogeneity of variances was tested with a Levene test. Differences in preference of a single species between habitat treatments were evaluated using a 1-way analysis of variance (ANOVA) with subsequent Tukey's Honestly Significant Difference (HSD) *post-hoc* tests. Differences between species were evaluated using a 2-way ANOVA.

Y-maze experiment.—No animals died during these experiments. Therefore, the difference in the number of individuals between the 2 y-arms at each time point was used as the dependent variable in the analysis. Homogeneity of variances was tested with a Levene test. Differences in preference between species and kairomone treatments were analyzed with a repeated-measures ANOVA (rm-ANOVA). All analyses were done with Statistica software (version 6.1; StatSoft Inc., Tulsa, Oklahoma) as described in Baumgärtner et al. (2002, 2003).

Results

Habitat-choice experiment

Habitat preferences differed significantly between species (2-way ANOVA, $p = 0.048$; Table II.1). However, the species 3 habitat interaction term was not significant ($p = 0.13$). Preferences of *G. roeselii* differed significantly among the 5 treatments (ANOVA, $p = 0.002$; Table II.1). Amphipods were equally distributed when reference habitat and mussel shells and when reference habitat and living mussels were compared (Fig. II.2A). Preferences for mussel shells and living mussels over reference habitat did not differ significantly from preferences for bare tile or biodeposited material with or without chironomids over reference habitat. Preferences for biodeposited material and biodeposited material with chironomids over reference habitat were significantly greater than preferences for reference habitat over bare tile (no food). The color of feces of *G. roeselii* was similar to that of the food offered, indicating that amphipods ate the biodeposited material.

Preferences of *D. villosus* also differed significantly among the 5 habitat treatments (ANOVA, $p < 0.001$; Table II.1). Amphipods were equally distributed when reference habitat and mussel shells and when reference habitat and biodeposited material were compared (Fig. II.2B). Amphipods preferred

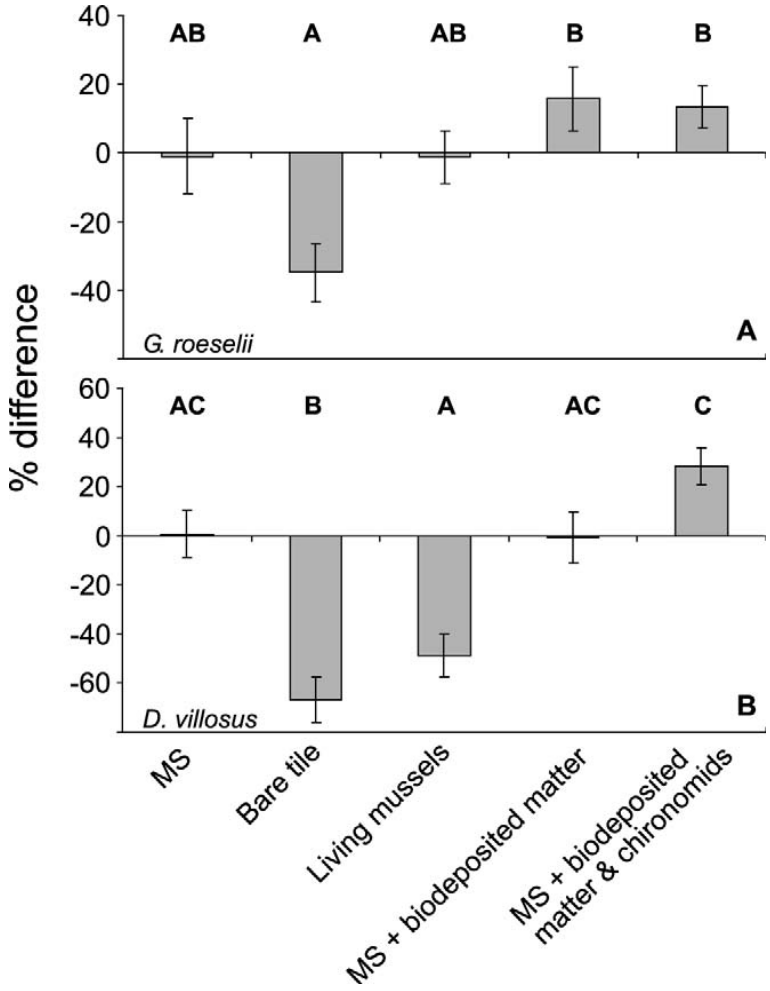

Figure II.2: Mean % difference (± 1 SE) of *Gammarus roeselii* (A) and *Dikerogammarus villosus* (B) for habitats in pairwise choice experiments comparing responses to 1 of 5 test habitat treatments (mussel shells [MS], bare tile, living mussels, MS plus biodeposited material from zebra mussels, and MS plus biodeposited material and 30 living chironomids) against a reference habitat (MS). Percent difference was calculated based on the distribution of amphipods at the end of the experiment. Negative values indicate a preference for the reference habitat; positive values indicate a preference for the test habitat. Bars with the same uppercase letter are not significantly different ($p > 0.05$).

reference habitat over bare tile. Preferences for reference habitat over living mussels and preferences for mussel shells and biodeposited material over reference habitat were not significantly different, and preferences for biodeposited material with and without chironomids over reference habitat were not significantly different. Preference for biodeposited material with chironomids over reference habitat was significantly greater than preference for reference habitat over living mussels and bare tile. Only a few *D. villosus* feces matched the color of the biodeposited material, indicating that amphipods ate little biodeposited material.

The bare tile treatment had a significantly stronger effect than the other treatments on the distribution of *D. villosus* (ANOVA, F = 9.159, p < 0.001). Treatment effects on *G. roeselii* differed significantly (ANOVA, F= 2.829, p = 0.032), but the Tukey HSD post-hoc test did not identify a specific treatment that differed significantly from the others. However, the effect of the bare tile treatment appeared to be stronger than the effect of other treatments because the bare tile effect did not differ between amphipod species (ANOVA, F = 3.679, p = 0.105), and the bare tile treatment did affect *D. villosus* significantly.

Table II.1: Analysis of variance results comparing responses to habitat treatments of 2 amphipod species, *Gammarus roeselii* and *Dikerogammarus villosus*, in the habitat-choice experiment. The dependent variable, % difference, was calculated between pairwise combinations of habitats on reference (15 mussel shell [MS]) and test (MS, bare tile, living mussels, MS with biodeposited material, or MS with biodeposited material and chironomids) tiles in 2-chamber arenas.

Factor	Species	*F*	df	*p*
Habitat	Both	15.893	4	<0.001
Habitat	*G. roeselii*	4.757	4	0.002
Habitat	*D. villosus*	13.260	4	<0.001
Species	Both	4.030	1	0.048
Species × habitat	Both	1.826	4	0.13

Y-maze experiment

The habitat-choice experiments revealed no preference for living mussels (*G. roeselii*; Fig. II.2A) or avoidance of living mussels (*D. villosus*; Fig. II.2B), but the elicitor of the avoidance reaction was unknown. The y-maze experiment tested whether kairomone produced by the zebra mussels was a possible elicitor. The presence of kairomones in water elicited significant negative responses from both amphipod species (Fig. II.3A, B). The mean difference in abundance of individuals between y-maze arms with reference and kairomone water was 16.2 ± 4.3 % for *G. roeselii* (rm-ANOVA, p < 0.001) and 10.4 ± 3.1 % for *D. villosus* (rm-ANOVA, p < 0.01).

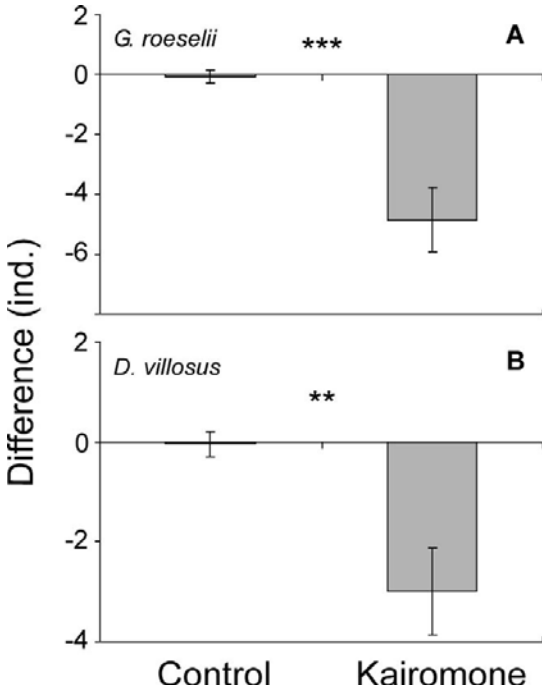

Figure II.3: Mean (± 1 SE) difference in the number of individuals (ind.) of *Gammarus roeselii* (A) and *Dikerogammarus villosus* (B) in 2 arms of a y-maze experiment testing responses to lake water conditioned with zebra mussels, i.e., containing kairomones, and unconditioned water. Values are the difference in the number of individuals between the arms of the y-maze, averaged at 10-min intervals from 10 to 30 min after initiating the experiment. Positive values indicate preference for the conditioned water; negative values indicate avoidance of the conditioned water. ** $p < 0.01$, *** $p < 0.001$.

Discussion

Zebra mussels increase the amount of benthic organic matter by filtration and biodeposition (e.g. Stanczykowska et al. 1976, Klerks et al. 1996, Ricciardi et al. 1997, Stewart et al. 1998). Benthic organic matter is an appropriate food source for detritivores and omnivores, and its increase often is hypothesized to be the direct biotic factor that causes abundances of detritivores and omnivores to increase in habitats that have been invaded by zebra mussels. Moreover, predators might benefit indirectly from organic matter deposition if it increases the abundance of detritivores, such as chironomids. Zebra mussels also increase the structural complexity of benthic habitats, and the additional structure is hypothesized to be an abiotic factor that causes abundances of benthic invertebrates to increase in habitats that have been invaded by zebra mussels. Our results support both of these hypotheses and indicate that the native *G. roeselii* and invasive *D. villosus* both responded (either directly or indirectly) to the increased food supply associated with biodeposited material in Lake Constance. Therefore, the biodeposition of invading zebra mussels could

lead to the formation of new food webs and increase secondary production in benthic systems.

The 2 species differed significantly in their habitat preferences (Table II.1), which indicated different responses to food sources. *Gammarus roeselii* responded positively to the structure associated with mussel shells and to any additional food source (biodeposited material with or without chironomids), as was evident from the feces at the end of the experiment. However, our data did not provide evidence that *G. roeselii* actually fed on chironomids. Moreover, chironomids were added only in combination with the biodeposited material, so a true preference for one food source over the other could not be determined. On the other hand, *D. villosus* responded positively to biodeposited material with chironomids but not to biodeposited material without chironomids. These results agree with the known feeding strategies of both amphipod species. *Dikerogammarus villosus* is a predator (Dick et al. 2002), and *Gammarus* is a shredder or omnivore (Bärlocher and Kendrick 1973, Pöckl 1992, 1993, Friberg and Jacobsen 1994).

Both species avoided bare tile. The response of *D. villosus* to bare tile was significantly stronger than its response to any other habitat treatment, and the response of *G. roeselii* to bare tile tended to be stronger than its response to any other treatment. Thus, the high structural complexity provided by the mussel shells was a clear driver of habitat choice for both species. This strong response might be associated with reduced predation risk with increasing habitat complexity (González and Downing 1999). It is not specific to amphipods, and the potential explanation can be applied to most benthic macroinvertebrates (Stewart et al. 1998).

Both amphipods species showed a significant negative response to lake water conditioned with zebra mussels in the y-maze experiments. *Dikerogammarus villosus* also avoided tiles with living mussels in the habitat-choice experiment. These results indicate that kairomones released by living zebra mussels cannot explain the increase in abundance of amphipods in the field and that kairomones might actually provoke an avoidance reaction. Zebra mussels excrete NH_4^+ as an end product of their metabolism, and NH_4^+ accumulates in the surrounding water (Heath et al. 1995). Amphipods are very sensitive to NH_4^+ contamination, which can be lethal at high concentrations (Williams et al.

1986, Berenzen et al. 2001). Thus, the amphipods in our experiments might have avoided living mussels because of NH_4^+ accumulation in the chamber.

Implications for the benthic community

Our experiments were carried out under standardized laboratory conditions and cannot reproduce the variability of environmental conditions in the lake. Under natural conditions, the habitat preference of amphipods varies seasonally, and high-complexity substrates are not preferred over low-complexity substrates throughout the year (González and Downing 1999). Living mussels are preferred by gammarids only in late summer, possibly because late-summer peaks in phytoplankton concentrations cause maximum biodeposition by zebra mussels and high food availability (Reeders and Bij de Vaate 1992). During winter and spring, abundances of zebra mussels are low in Lake Constance because of predation by diving ducks (Werner et al. 2005). Thus, structural complexity and biodeposition vary seasonally, and both are less important in winter and spring than in summer and autumn. The abundances of amphipods also are lowest during winter (Mörtl et al. 2005). Mörtl and Rothhaupt (2003) observed an increase in abundance of *G. roeselii* and chironomids on zebra mussels in July 1999, during a period when habitat complexity and biodeposition rate presumably were high. Our results suggest that this increase might be attributable to the structural complexity and additional food source from biodeposition by zebra mussels.

In Lake Constance, the benthic food web has been disturbed by the Ponto Caspian invader *D. villosus*, which has colonized the lake rapidly since 2002 (Mörtl et al. 2005). One consistent response in many freshwater systems during the establishment of *D. villosus* is the decline or extirpation of native amphipods because of predation by *D. villosus* (Ponyi 1956, Dick and Platvoet 2000, Kinzler and Maier 2003, Josens et al. 2005). Other benthic macroinvertebrates are also prey of *D. villosus*, and their abundances decrease after its invasion (Dick et al. 2002, Krisp and Maier 2005).

In conclusion, our results provide strong indications that amphipods are involved in a *Dreissena*-biodeposit-based food web. To our knowledge, ours is the first study that indicates how biodeposition by zebra mussels might influence habitat choice by benthic macroinvertebrates. The effects of zebra

mussels on amphipods differ between species. Omnivorous *G. roeselii* was attracted to biodeposited material and to mussel shells, whereas the predatory invader *D. villosus* was attracted to shells only when added animal prey was present. Therefore, the invasive amphipod species is influenced indirectly by zebra mussels through their effect on other species that feed on biodeposited material. The other species involved in this possible food web and the ways in which they are influenced by invading species, such as *D. villosus*, remain to be clarified.

Acknowledgements

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Chapter III

Feeding rates, assimilation efficiencies and growth of two amphipod species on biodeposited material from zebra mussels

René Gergs and Karl-Otto Rothhaupt

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Summary

1. Accumulation of organic material by the zebra mussel *Dreissena polymorpha* is assumed to be the source of a biodeposition-based food web. However, only little is known about the importance of the biodeposited material as a food source and its contribution to increased abundances of macroinvertebrates in the presence of *D. polymorpha*.

2. Feeding, assimilation and growth of the amphipods *Gammarus roeselii* and *Dikerogammarus villosus* on food sources directly and indirectly associated with *D. polymorpha* (biodeposited material and chironomids) and on conditioned alder leaves were measured. The stoichiometry of carbon, nitrogen and phosphorus of the diets was measured as an important determining factor of food quality.

3. Chironomids had the highest nitrogen and phosphorus contents, alder leaves were depleted in nitrogen and phosphorus, and the stoichiometry of biodeposited material was intermediate.

4. Both amphipod species had highest feeding rates and assimilation efficiencies on chironomids. *Gammarus roeselii* fed more on biodeposited material than on alder leaves, but assimilation efficiencies were similar; *D. villosus* also had similar feeding rates and assimilation efficiencies on the two diets.
5. Both amphipod species had highest growth rates on chironomids and lowest growth rates on alder leaves. Both grew at intermediate rates on biodeposited material of *D. polymorpha*. The growth rates of the amphipod species were related to food stoichiometry. Overall, the invasive *D. villosus* grew faster than the indigenous *G. roeselii*.
6. Food resources directly and indirectly associated with *D. polymorpha* are potential diets for amphipods, providing further evidence for a *D. polymorpha* biodeposition-based food web.

Keywords: *Dikerogammarus villosus*, feeding strategy, food quality, food web, *Gammarus roeselii*

Introduction

The growth and reproduction of many benthic macroinvertebrates depends on the quality and availability of potential food sources (Willoughby and Sutcliffe 1976, Fuller et al. 1988, Söderström 1988). An important determining factor of food quality is the stoichiometry of carbon, nitrogen and phosphorus in the food (Frost et al. 2002). A stoichiometric mismatch between diet and consumer, caused by a low food quality, can lead to lower growth rates of the consumer even under a saturated food quantity (Frost and Elser 2002). A compensatory feeding response to low-nutrient food is possible but cannot fully compensate food quality-related deficiencies in growth (Fink and von Elert 2006).
Allochthonous leaves are an important energy source in many streams, but are a low-quality food because of low phosphorus and nitrogen contents (Kaushik and Hynes 1971, Anderson and Cummins 1979, Friberg and Jacobsen 1994, Cross et al. 2005). Animal matter, in contrast, is a high-quality food source because of high phosphorus and nitrogen contents (Cross et al. 2005, Fink et al. 2006). Diet quality has often been estimated by assessing feeding rates and assimilation efficiencies (e.g. Bärlocher and Kendrick 1975, McCullough and

Minshall 1979, Graca et al. 2001). However, these parameters cannot be related directly to growth and the estimation of growth rates is also important (Fuller et al. 1988).

In many freshwater systems in Europe and North America, the littoral habitat and benthic energy flow have been modified by the invasion of the zebra mussel, *Dreissena polymorpha* (Pallas). Following the arrival of zebra mussels, the abundance of many benthic taxa, especially amphipods and chironomids, increases (Stewart and Haynes 1994, Stewart et al. 1998, Mörtl and Rothhaupt 2003). Zebra mussels alter the benthic habitat by increasing surface area and restructuring the substrate in the form of mussel shells. The mussels influenced the benthic community also by biodeposition, the excretion of faeces and pseudofaeces. This causes an accumulation of pelagic resources in the benthos (Stanczykowska et al. 1976, Klerks et al. 1996, Silver Botts et al. 1996, Ricciardi et al. 1997). It is assumed that the availability of this new food resource leads to a biodeposition-based food web (Stewart and Haynes 1994, Mitchell et al. 1996). The amphipods may benefit from the new resource directly by feeding on the biodeposited matter or indirectly by feeding on associated invertebrates (i.e. those that feed on the matter, such as chironomids). Gammarids are often classified as shredders, but it is usually not possible to classify them into a discrete functional feeding group because their feeding strategy has great plasticity (MacNeil et al. 1997). Hence, gammarid amphipods are best characterized as omnivores (Bärlocher and Kendrick 1973, Pöckl 1992).

Recent laboratory experiments have shown that the biodeposited material of zebra mussels is a food source and affects habitat choice of the native amphipod *Gammarus roeselii* Gervais, whereas the invasive amphipod *Dikerogammarus villosus* (Sowinsky), a predator (Dick and Platvoet 2000, Dick et al. 2002), is not attracted by biodeposited material but rather by the associated chironomids (Gergs and Rothhaupt 2008a). Although gammarid amphipods and chironomids can grow on faeces and pseudofaeces of zebra mussels (Izvekova and Lvova-Katchanova 1972, González and Burkart 2004), little is known about the quality and utilization of the biodeposited material as food. Since biodeposited matter and chironomids might be important in habitats dominated by zebra mussels, we investigated the feeding, assimilation and

growth of *G. roeselii* and *D. villosus* on these resources. We also compared these food sources to allochthonously introduced leaves, which are an important energy source in many aquatic systems (Minshall 1967, Kaushik and Hynes 1971, Webster and Benfield 1986) and a better food source for gammarid amphipods than decaying macrophytes or green algae (Pöckl 1995).

Material and Methods

Test animals: origin and maintenance

The experiments were conducted with the two dominant amphipod species of Lake Constance, the indigenous *Gammarus roeselii* and the invasive *Dikerogammarus villosus*. The species were obtained from the littoral of Lake Constance and kept separate in a 15 °C climate chamber with a diurnal light rhythm of 12 h : 12 h (day : night). *G. roeselii* was maintained in tanks filled with water from Lake Constance. *D. villosus* was kept in a flow-through system with water from Lake Constance to minimize their mortality rate. Both were fed on commercially available frozen chironomids. For shelter, a mixture of gravels of different grain sizes was provided. In the experiments, amphipods of both sexes were used randomly.

Food types

Three different food sources were tested: dead animal material (commercially available frozen chironomids), material biodeposited by zebra mussels (*D. polymorpha*) and conditioned alder leaves.

To estimate the quantity of chironomids at the beginning of the feeding experiments, a length–ash-free dry mass correlation was established. The chironomids were measured with a digital sliding calliper (Preisser; Digi-Met, Gammertingen, Germany) to the nearest 0.01 mm, and the ash-free dry mass was determined by drying the chironomids at 105 °C for 24 h, weighing, combusting at 550 °C for 8 h and weighing again for ash content.

Biodeposited material was collected in the lake using modified sediment traps consisting of a tube of grey PVC (50 cm length; Ø 10 cm) to which a funnel and a 200 mL PET flask were fixed at the lower end to collect the settling sediment. A clamp was used to hold two tiles (4.7 × 4.7 cm) with 15 living mussels (15.01 ± 0.40 mm shell length) each in a vertical position above the upper opening of

the sediment trap. The mussels were collected from the littoral of Lake Constance. Five traps were suspended at a depth of 2 m from a pontoon in the pelagic zone of Lake Constance for 7 days. The collected material was centrifuged (1180 *g*, 6 min), and the supernatant was replaced with enough distilled water to bring the volume to 100 mL. Biodeposited material was stored at 4 °C in darkness. An aliquot was filtered on pre-combusted glass fibre filters (GF/6, Ø 25 mm; Whatman/Schleicher & Schuell, Kent, U.K.), and the ash-free dry mass was determined to estimate the appropriate amount for the experiments.

The alder leaves were conditioned by exposing them for 3 weeks in the littoral of Lake Constance in 200-µm litterbags to exclude macroinvertebrates. From a sub-sample of 16 leaf discs (diameter 1 cm), the ash-free dry mass was determined to estimate the appropriate amount for the experiments.

The organic carbon, nitrogen and phosphorus content of the three food sources were estimated to assess food quality. Aliquots of the biodeposited material were filtered on pre-combusted glass fibre filters (GF/6, Ø 25 mm; Whatman/ Schleicher & Schuell). Sub-samples of the conditioned alder leaves and the chironomids were ground. The samples were dried at 55 °C for subsequent analysis of particulate organic carbon and particulate organic nitrogen with an NCS–2500 analyser (Carlo Erba Instruments, Milano, Italy). For determination of particulate phosphorus, aliquots of the biodeposited matter were filtered through acid-rinsed polysulfone membrane filters (0.2 µm pore size, Ø 45 mm; HT-200, Pall, Ann Arbor, MI, U.S.A.). For the conditioned alder leaves and the chironomids, sub-samples as described above were used. The samples were digested with a solution of 10% potassium peroxodisulfate and 1.5% sodium hydroxide at 121 °C for 60 min, and soluble reactive phosphorus was then determined using the molybdate-ascorbic acid method (Greenberg et al. 1985). Both analyses were replicated five times for each food type.

Feeding rates and assimilation efficiencies

To estimate feeding rates and assimilation efficiencies, single adult test animals (>10 mm body length) were fed a specific amount of a single food source. All food sources were provided in saturated quantity. Chironomid replicates each received seven chironomids (5.2 ± 0.4 mg ash-free dry mass), alder leaf

replicates received one leaf disc (4.8 ± 1.0 mg ash-free dry mass) and biodeposition replicates received an aliquot of 4.8 ± 0.1 mg ash-free dry mass.
The weight-specific feeding rate was determined as food ingested per day, being the difference between the ash-free dry mass of offered and remaining food per unit weight (ash-free dry mass) of animal. The assimilation efficiency was calculated as the percentage ratio between assimilated (ingested food – faeces) and ingested food.
The experiments were arranged in containers (10.5 × 10.5 × 3.5 cm) filled with 0.3 L of aerated lake water that had been filtered through a 0.45-µm filter to eliminate potential food for the amphipods. A stone approximately 2 cm in diameter was provided as a shelter. All amphipods used in the experiments were pre-fed on the tested food source for 24 h and pre-starved for another 24 h individually. After 24 h of feeding on the tested food source the remaining food and the faeces were collected separately. Faeces particles were identifiable easily by cylindrical pellets. Subsequent the feeding period, each individual was starved for 24 h to collect faeces. The accumulated faeces produced during the feeding and the post-experimental starving time was pooled for each individual. In every 24-h period described above a new container with new water was provided. For both starving periods, individuals were placed in a PVC cylinder (Ø 6 cm; 8 cm height) with a 1-mm gauze 0.5 cm above the ground, installed in a container filled with lake water. Since amphipods do not empty their gut completely (Bärlocher and Kendrick 1975), the pre-feeding and pre-starving were integrated into the experiment. We assumed that gut fullness at the start of the experiment equals gut fullness at the end of the starving period when faeces are collected. After 24 h of feeding, the remaining food of each replicate and the ash-free dry mass were determined. All faeces of each replicate were filtered on pre-combusted glass fibre filters (GF/6, Ø 25 mm; Whatman/ Schleicher & Schuell) and their weight determined. All experiments were conducted in April and May 2007. Each diet was replicated 15–16 times, depending on survival of the amphipods. Sixteen additional replicates of each food source without amphipods were installed as controls to estimate the weight decline of food during the experimental period.

Growth experiment

During the growth experiment, all amphipods (juveniles, *c*. 5 mm body length at the beginning of the experiment) were kept individually in 100-mL wide-necked flasks. Each flask was filled with 90 mL lake water (30-µm filtered, held at 17 °C) with a flow-through of approximately 6 mL min^{-1}. The outflow passed through a 1-mm net to avoid drift of animals. The same food sources as in the feeding experiments were offered *ad libitum*. Additionally, individuals of both species were kept without food as controls. The growth experiment lasted 8 weeks from June to August 2007. All food tests and controls were replicated 10 times for each species. Flasks were cleaned, new food was added and the survival of the amphipods was noted weekly. Body length, the distance between the anterior of the head and the posterior of the final abdominal segment (Baumgärtner and Rothhaupt 2003), was measured at the beginning of the experiment and then every 2 weeks. Length was determined from photographs taken under a stereomicroscope (Zeiss Stemi 2000-C, Jena, Germany) with an attached fire-wire camera (Imaging Source, Bremen, Germany) connected to a computer. Each amphipod was measured three times using a computer program developed by the electronics facility of the University of Konstanz (G. Heine, pers. comm.).The mean value of the three measurements was used for further analyses.

Statistical analysis

All statistical analyses were made using the statistical package SPSS (version 15.0／2006; SPSS Inc., Chicago, IL, U.S.A.).

Weight-specific feeding rates were calculated as the dry weight of food ingested per animal body mass and day. To homogenize variances, all values were logarithmically transformed [ln(x + 1)] and checked with the Levene test. Assimilation efficiency was calculated if the feeding rate was >0.05 mg food (mg amphipod)$^{-1}$ day^{-1}. At lower feeding rates, the systematic error in the determination of faeces and ingested food was a limiting factor. It was not necessary to transform the assimilation efficiency data to homogenize variances; the values were checked directly with the Levene test. Intraspecific differences between the food sources were analysed using a one-way ANOVA with subsequent Scheffe *post hoc* tests for unequal number of replicates.

Interspecific differences between *G. roeselii* and *D. villosus* were evaluated using a two-way ANOVA to test for food and species effects. The C : P and the C : N ratios were the factors used to determine food quality. Differences in stoichiometry were analysed using a one-way ANOVA with subsequent Tukey-HSD *post hoc* test.

To analyse time effects on body lengths measured in the growth experiment, two-way ANOVAs with the factors time and food for both amphipod species were conducted. The control treatment without food was excluded from the analyses because of the high mortality of both species. We calculated weekly growth rates [body length$_{(week\ n)}$–body length$_{(week\ n\ -\ 2)}$ ⁄ 2] for each food and species using amphipods that survived until the following measurement. Intraspecific differences in food resources were analysed using a repeated-measures ANOVA with subsequent Scheffe *post hoc* tests for unequal numbers of replicates. Interspecific differences between *G. roeselii* and *D. villosus* were evaluated using a two-way ANOVA to test for food and species effects. For all ANOVAs, all values were logarithmically transformed [ln(x + 1)] and homogeneity of variances was checked with the Levene test. Survival of the amphipods was recorded weekly and analysed using a nonparametric Gehan–Wilcoxon test for estimating survival distribution (Pyke and Thompson 1986). We tested for intraspecific differences among food tests and interspecific differences within each food test.

Results

Food stoichiometry

The organic carbon, nitrogen and phosphorus stoichiometry (C : N and C : P ratios) of the three food sources differed significantly (Fig. III.1; ANOVA; $p < 0.001$). Chironomids had the highest nitrogen content, as indicated by a low C : N ratio of approximately 5. The biodeposited material of zebra mussels contained less nitrogen (C : N ratio of 18), and conditioned alder leaves had the lowest nitrogen content (C : N ratio of 22). Chironomids also had the highest phosphorus content (C : P ratio of 120). The biodeposited material of zebra mussels contained less phosphorus (C : P ratio of 320), and conditioned alder leaves were severely depleted in phosphorus (C : P ratio of nearly 1000).

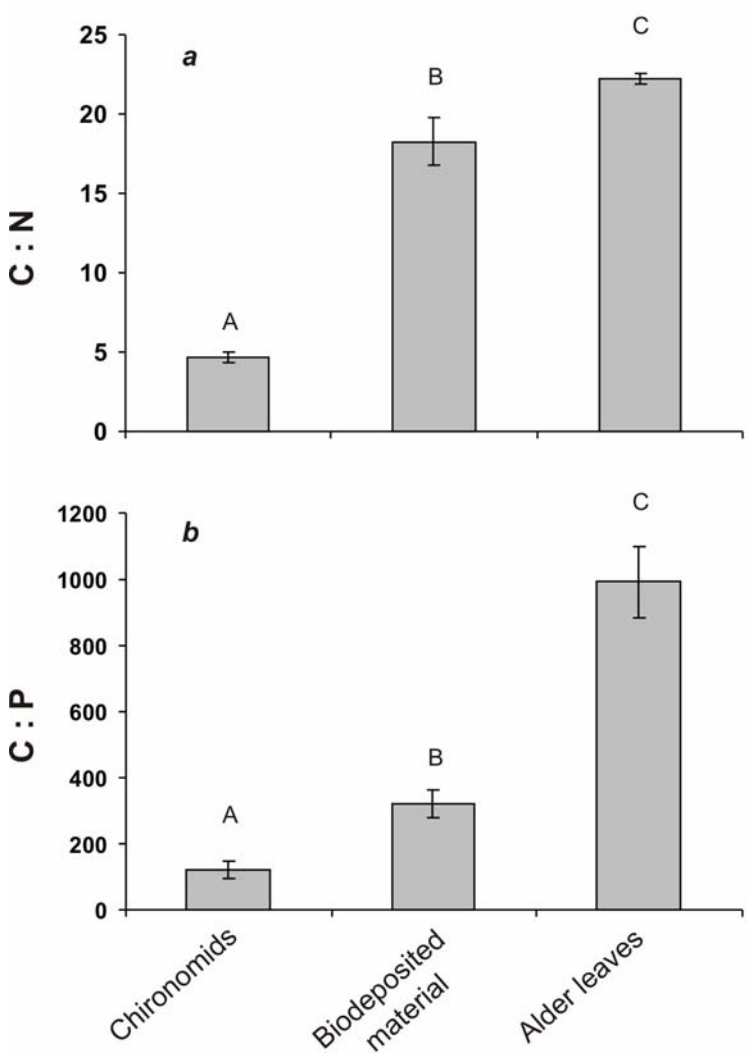

Figure III.1: Organic carbon, nitrogen and phosphorus stoichiometry of the three tested food sources, as indicated by (a) the C : N ratio and (b) the C : P ratio. Mean values ± SD are shown. Capital letters indicate homogenous subgroups detected by ANOVA with subsequent Tukey-HSD *post hoc* tests.

Feeding rates and assimilation efficiencies

The weight-specific feeding rates of *G. roeselii* on the three food sources differed significantly (ANOVA; $p < 0.001$; Table III.1). Chironomids resulted in the highest feeding rates; the feeding rates on biodeposited material of *D. polymorpha* were intermediate and lowest on conditioned alder (Fig. III.2a). The feeding rate of *D. villosus* was also highest on chironomids, and the rates on the other two food sources were lower, respectively (ANOVA; $p < 0.001$). The interspecific comparison showed significant differences between the feeding rates of the amphipod species (ANOVA; $p < 0.001$; Table III.1) and significant species × diet interactions (ANOVA; $p = 0.001$). The weight-specific feeding rate

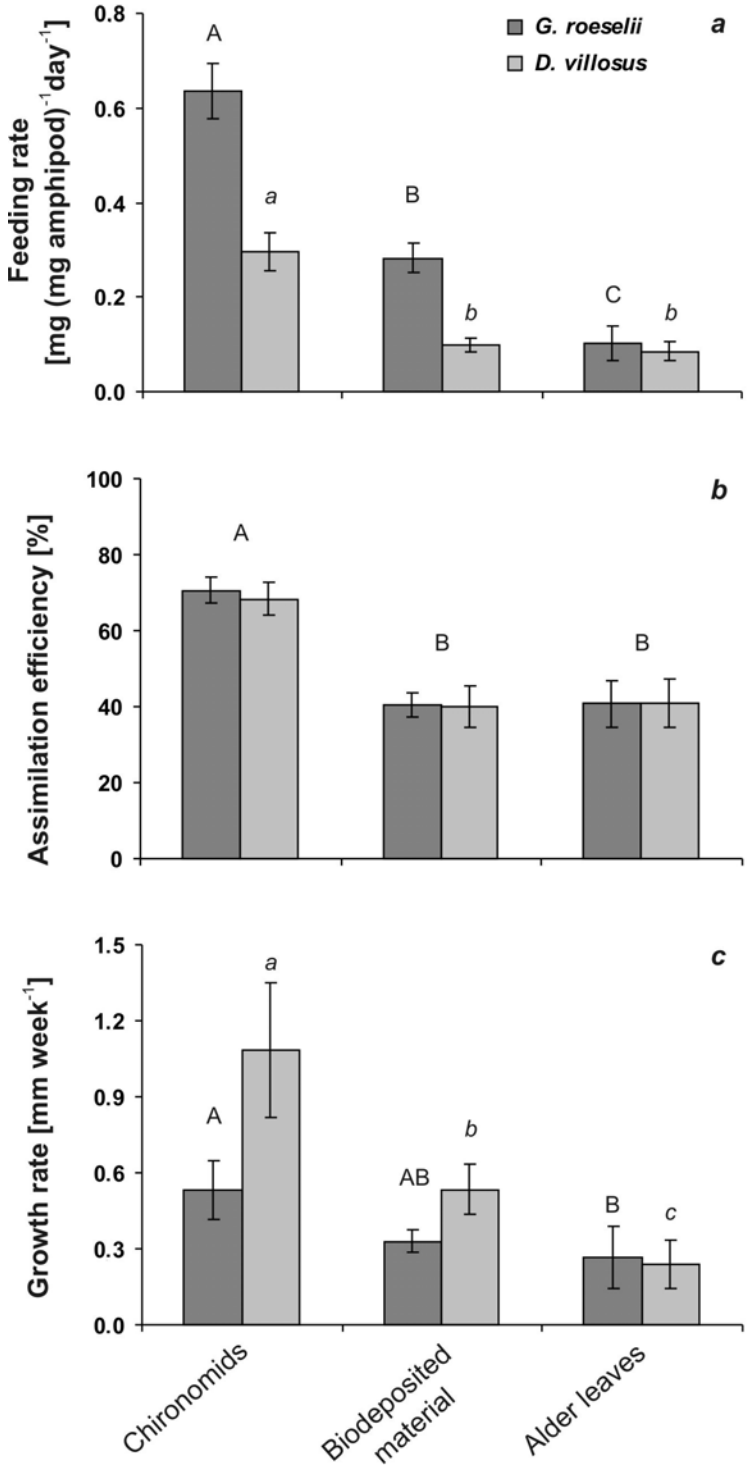

Figure III.2: (a) Feeding rate, (b) assimilation efficiency and (c) growth rate of the amphipod species *Gammarus roeselii* and *Dikerogammarus villosus* on three different food sources: chironomids, biodeposited material of zebra mussels, and conditioned alder leaves. Mean values ± SD are shown. In (a) and (c), upper case letters indicate homogenous subgroups for *G. roeselii* and lower case letters indicate homogenous subgroups for *D. villosus*, as detected by the ANOVA with subsequent Scheffe *post hoc* tests for unequal number of replicates. In (b), no significant differences were found between species and the upper case letters indicate homogenous subgroups for both species.

of *G. roeselii* on chironomids was two-fold higher than that of *D. villosus* and three-fold higher on biodeposited material. The feeding rates of the two amphipod species on conditioned alder leaves did not differ.

Table III.1: ANOVA results comparing feeding rates, assimilation efficiency and growth rates of the 2 amphipod species, *Gammarus roeselii* and *Dikerogammarus* villosus. The differences between the three food types, chironomids, material biodeposited by zebra mussels (*D. polymorpha*) and conditioned alder leaves were analysed.

Factor	Species	Effect	F	df	p-value
Feeding rate	*G. roeselii*	diet	43.8	2	< 0.001
	D. villosus	diet	11.2	2	< 0.001
	both	species	26.6	1	< 0.001
		diet	51.0	2	< 0.001
		species × diet	7.4	2	0.001
Assimilation efficiency	*G. roeselii*	diet	30.8	2	< 0.001
	D. villosus	diet	10.4	2	< 0.001
	both	species	0.2	1	0.69
		diet	32.0	2	< 0.001
		species × diet	0.1	2	0.87
Growth rate	*G. roeselii*	diet	5.0	2	0.03
	D. villosus	diet	61.2	2	< 0.001
	both	species	15.2	1	< 0.001
		diet	41.8	2	< 0.001
		species × diet	8.3	2	0.001

The assimilation efficiencies of the two amphipod species did not differ (ANOVA; $p = 0.69$; Table III.1) and no significant species × diet interactions (ANOVA; $p = 0.87$) were found. However, assimilation efficiencies on the food sources differed for *G. roeselii* and *D. villosus* (ANOVA; both species: $p < 0.001$). Assimilation efficiency was highest with chironomids as the food source (*c*. 70%) and lowest on biodeposited material of *D. polymorpha* and conditioned alder leaves (*c*. 40% each; Fig. III.2b).

Growth experiment

Both species increased in body length during the 8-week experiment on all food sources (Fig. III.3; ANOVA; both species: $p < 0.001$; Table III.2). Growth differed on the different food source (ANOVA; both species: $p < 0.001$) and significant diet × time interactions were found (ANOVA; both species: $p < 0.001$). The body length of *G. roeselii* and *D. villosus* increased the most when fed chironomids, followed by biodeposited material of *D. polymorpha*. The increase in body length was lowest when both species fed conditioned alder leaves.

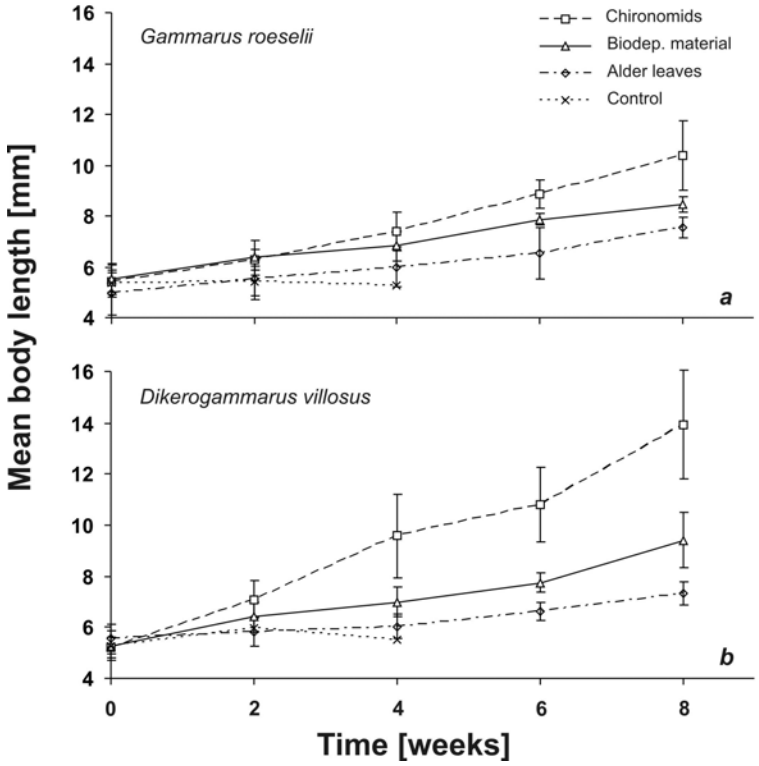

Figure III.3: Body length (mean ± SD) of (a) *Gammarus roeselii* and (b) *Dikero-gammarus villosus* fed chironomids, biodeposited material of zebra mussels, and conditioned alder leaves for 8 weeks and in the control without food.

Growth rates of both amphipod species on the different food sources differed significantly (Fig. III.2c; repeated-measures ANOVA; *G. roeselii*: $p = 0.03$; *D. villosus*: $p < 0.001$; Table III.1). The growth rate of *G. roeselii* fed chironomids twice as high as *G. roeselii* fed conditioned alder leaves; growth of *G. roeselii* fed biodeposited material was intermediate. The growth rate of *D. villosus* fed chironomids was twice and triply higher than *D. villosus* fed biodeposited material and conditioned alder leaves respectively. The growth of

the two species differed (ANOVA; $p < 0.001$), and there were significant species × diet interactions (ANOVA; $p = 0.001$). *Dikerogammarus villosus* fed chironomids and biodeposited material had higher growth than *G. roeselii* fed on the same diets respectively. The growth rates of the two species fed conditioned alder leaves did not differ.

Table III.2: ANOVA results comparing increase in mean body length of the 2 amphipod species, *Gammarus roeselii* and *Dikerogammarus villosus* for the duration of the growth experiment of 8 weeks. Differences depending on time and the three food source chironomids, material biodeposited by zebra mussels (*D. polymorpha*) and conditioned alder leaves were analysed.

Species	Effect	F	df	p
G. roeselii	time	29.813	4	< 0.001
	diet	23.906	2	< 0.001
	diet × time	14.145	8	< 0.001
D. villosus	time	17.16	4	< 0.001
	diet	62.148	2	< 0.001
	diet × time	30.001	8	< 0.001

The survival of the two species on the different food sources also differed (*G. roeselii*: Wilcoxon–Gehan statistic = 42.19, $p < 0.001$; *D. villosus*: Wilcoxon–Gehan statistic = 121.88, $p < 0.001$). In the controls lacking food, survival of both species was significantly lower than when food was available (Fig. III.4). Survival of both *G. roeselii* and *D. villosus* was highest when fed conditioned alder leaves. Survival of *G. roeselii* fed chironomids and biodeposited material was lower. For *D. villosus* the same order in survival as described for *G. roeselii* occurred among the three diets. Survival of the two species in the controls lacking food did not differ (Wilcoxon–Gehan statistic = 0.02, $p = 0.897$). The survival of *D. villosus* on all three food sources was higher than that of *G. roeselii* (chironomids: Wilcoxon–Gehan statistic = 9.31, $p = 0.002$; biodeposited material: Wilcoxon–Gehan statistic = 21.01, $p < 0.001$; conditioned alder leaves: Wilcoxon–Gehan statistic = 6.32, $p = 0.012$).

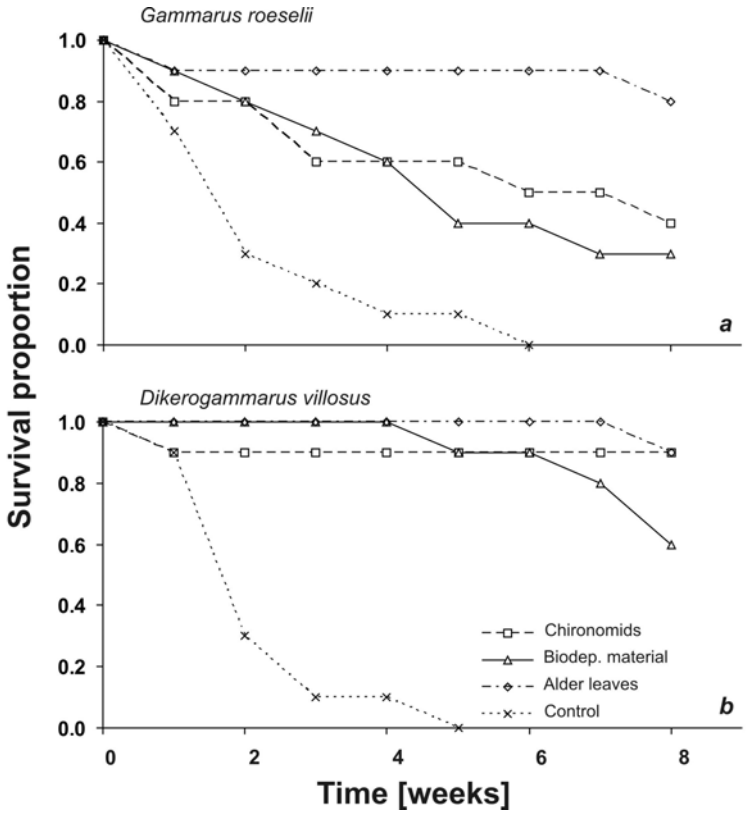

Figure III.4: Survival of (a) *Gammarus roeselii* and (b) *Dikerogammarus villosus* fed chironomids, biodeposited material of zebra mussels, and conditioned alder leaves for 8 weeks and in the control without food.

Discussion

The growth and feeding of the amphipod species *G. roeselii* and *D. villosus* differed on the three food sources offered in our experiments. In general, however, both amphipod species responded according to the nutritional gradient proposed by Anderson and Cummins (1979), with animal material as a high-quality food and leaf litter as a low-quality food. The animal material food source, chironomids, led to the highest feeding rates and assimilation efficiencies of both amphipod species, resulting in high growth rates. The high nitrogen and phosphorus contents of the chironomids food source also indicate its high food quality. Similar C : N and C : P ratios have been found for *G. roeselii* in Lake Constance (Fink et al. 2006). The leaf litter food source, conditioned alder leaves, had the lowest food quality, and the quality of biodeposited material of zebra mussels as a food source was intermediate between that of the animal material and the leaf litter.

Gammarus roeselii fed at a higher rate on higher quality food sources, which resulted in faster growth. *Gammarus roeselii* had higher feeding rates than *D. villosus* on chironomids and biodeposited material at similar assimilation

efficiencies. This implies a higher assimilated food biomass per animal biomass for *G. roeselii*. However, the growth rate of *D. villosus* was higher, which indicated that energy allocation differed between the two amphipod species. Since the native amphipod *G. roeselii* has a higher activity than the invasive *D. villosus* (Kinzler and Maier 2006), *G. roeselii* probably requires a larger proportion of available energy for motility, whereas *D. villosus* possibly allocates more energy to somatic growth. The growth rate of *D. villosus* fed chironomids in our experiments corresponds well with the calculated field growth rates in spring and summer of Piscart et al. (2003). Compared to indigenous amphipod species, the invader *D. villosus* has rapid growth, a bigger brood size, earlier sexual maturity and a shorter egg development time (Piscart et al. 2003, Pöckl 2007). These ecological traits are important for successful invasion (Ricciardi and Rasmussen 1998, Kolar and Lodge 2001).

The preference of gammarid amphipods for various leaf litters and the role of aquatic fungi on leaves have been well investigated (e.g. Bärlocher and Kendrick 1973), but the predatory impact of gammarids has often been neglected until recently (Kelly et al. 2002b). In contrast to *G. roeselii*, the invader *D. villosus* is clearly a predator (Dick and Platvoet 2000, Dick et al. 2002), which may also explain its high growth rate on chironomids in our experiments. Although the feeding rates and assimilation efficiencies of *D. villosus* on biodeposited material of zebra mussels and conditioned alder leaves did not differ, the amphipods grew better on the biodeposited material. The higher growth rate on this food source may be attributed to the higher phosphorus content, which can enhance growth under limiting conditions (Frost and Elser 2002, Frost et al. 2005).

Food constituents can be assimilated with different efficiencies, and consumers limited by nutrients (N or P) have been shown to assimilate the limiting element with a higher efficiency than carbon (Rothhaupt 1995, Sterner and Elser 2002). Gross growth efficiencies and hence assimilation efficiencies can be close to 100% for elements that are deficient in the food (Sterner and Elser 2002). In our experiments, the C : P ratio of biodeposited material was 320 : 1, whereas that of alder leaves was about 1000 : 1. If we assume that the assimilation efficiencies that we determined for both food types (*c*. 40%) reflect the assimilation of carbon and that the deficient element phosphorus was

assimilated with a high efficiency of 90%, the resulting assimilated C : P ratios would be 140 : 1 for the biodeposited material and 440 : 1 for the alder leaves. A C : P ration of 140 : 1 is close to the body stoichiometry of *Gammarus* (Fink et al. 2006).

In the control lacking food, the survival of both amphipod species was lowest; only a few individuals survived after 3 weeks. The flow through of lake water in the flasks did not contain enough food for growth or survival of the amphipods. Hence, all growth effects observed in our experiments can be attributed to the offered food sources.

Both amphipod species had the highest survival and the lowest growth rate on conditioned alder leaves. This apparent paradox can be explained as follows. Individual growth of crustaceans requires moulting, and animals may be less resistant to toxicants in the post-moult stage, caused by high metabolic stress and the not yet hardened exoskeleton (Carlisle and Knowles 1959). The intermoult interval increases with decreasing growth rates caused by lower food quality (Willoughby and Sutcliffe 1976, Pöckl 1995). Therefore, at lower growth rates, fewer individuals are in the vulnerable moulting phase, and survival should increase, as we observed.

Our results indicate that biodeposited material of the zebra mussel *D. polymorpha* is a potential intermediate-quality food source for both *G. roeselii* and *D. villosus*. In line with this result, González and Burkart (2004) found that mussel faeces and pseudofaeces were a valuable food source for *Gammarus fasciatus* Say. These findings support the assumption of a biodeposition-based food web originating from food sources provided by the zebra mussel (Stewart and Haynes 1994, Mitchell et al. 1996). Furthermore, chironomids feed well on this food source (Izvekova and Lvova-Katchanova 1972). Hence, predators such as *D. villosus* can benefit indirectly from the biodeposition of the zebra mussel by predation on macroinvertebrates. Thus, the increase in abundance of many benthic taxa after the invasion of *D. polymorpha* might to some extent be an effect of increased food availability. However, the significance of the accumulation of organic matter by zebra mussels under natural conditions remains unknown although stable isotope analyses have indicated that this food source constitutes a part of the diet of the amphipod *G. fasciatus* (Limén et al. 2005). Our results on the feeding and growth of the two amphipods *G. roeselii*

and *D. villosus* on resources associated with zebra mussels provide further evidence for the importance of zebra mussel biodeposited material under natural conditions.

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Chapter IV

Zebra mussels mediate benthic–pelagic coupling by biodeposition and changes in detrital stoichiometry

René Gergs, Karsten Rinke and Karl-Otto Rothhaupt

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Summary

1. The zebra mussel (*Dreissena polymorpha*) is one of the most successful invasive species; it has colonized many aquatic systems in Europe and North America with strong impacts on various ecosystem processes. The effect of *D. polymorpha* filtration on pelagic seston concentrations has been quantified in several studies, but the magnitude and stoichiometry of the transfer of sestonic biomass into benthic detritus by *D. polymorpha* and the accompanying enrichment of the benthic habitat is still under-investigated.

2. We studied biodeposition by zebra mussels in two series of laboratory experiments with the food algae *Cryptomonas erosa* and *Scenedesmus obliquus*. We also measured the year-round biodeposition rate under natural conditions in the oligotrophic Lake Constance.

3. In all experiments, zebra mussel biodeposition was linearly related to seston concentration. In the field, the relationship changed with a seasonal shift in algal composition and lower biodeposition rates during the spring algal bloom.

4. For both algal species in laboratory experiments, biodeposited material was depleted in phosphorous at an algal concentration ≤0.6 mg ash-free dry mass L^{-1}, but not at higher concentrations. This effect was not observed in the field, probably because of high variation in C:N:P stoichiometry.

5. By mediating the transfer of pelagic resources into the benthos zebra mussels provide a sufficient amount of detritus for benthic invertebrates, especially during summer. Thus, material biodeposited by the mussels might increase benthic secondary production from pelagic resources, and zebra mussels are important mediators of this flux of organic matter from the pelagic zone into the benthos.

Keywords: *Dreissena polymorpha*, pseudofaeces, organic matter, food web, invasive species

Introduction

The zebra mussel *Dreissena polymorpha* (PALLAS) plays an important role in altering the structure of aquatic ecosystems because the species usually develops large densities in invaded systems. Their filtration activity reduces the overall concentration of phytoplankton and depends on seston concentration; the rate is constant and maximal at food concentrations greater than 2 mg C L^{-1} and declines exponentially at higher food concentrations (Walz 1978a, Sprung and Rose 1988). When food concentration is sufficiently high, not all filtered material is ingested and agglomerated aggregates are rejected. These pseudofaeces consist of undigested algae and detritus bound in mucus and are periodically expelled by the inhalant siphon of the mussel (Stanczykowska et al. 1975). Pseudofaeces are produced at food concentrations above 0.2 mg C L^{-1}, and their production increases rapidly with an increasing food supply (Walz 1978a).

The removal of algal cells and other seston components from the open water by *D. polymorpha* is an important mechanism in benthic–pelagic coupling (e.g. Boegman et al. 2008), whereas the biodeposition by *D. polymorpha* constitutes a mechanism that potentially supports benthic food webs. The flux of organic matter from the pelagic into the benthic zone is greatly increased by the continuous conversion of seston into faeces and pseudofaeces by *D. polymorpha*. This material is considered to support a biodeposition-based food web (Stewart and Haynes 1994, Mitchell et al. 1996) and affects the structure of benthic communities. The abundance of many benthic taxa, especially amphipods and chironomids, increases following the arrival of zebra

mussels (Stewart and Haynes 1994, Stewart et al. 1998, Mörtl and Rothhaupt 2003). This is attributable to the high structural complexity of mussel shells, the accumulation of pelagic resources, and the increase in organic matter in the benthos (Stanczykowska et al. 1976, Klerks et al. 1996, Silver Botts et al. 1996, Gergs and Rothhaupt 2008a). The organic matter is an additional food source for benthic invertebrates (Izvekova and Lvova-Katchanova 1972, González and Burkart 2004, Gergs and Rothhaupt 2008b). From these observations, we conclude that *D. polymorpha* is a prominent mediator of benthic-pelagic coupling with consequences for both the pelagic and the benthic community. Moreover, we would expect the invasion of *D. polymorpha* into previously uncolonized habitats to induce a major change in the interaction between benthic and pelagic communities.

D. polymorpha is highly selective in the food it ingests, and the selection is mostly dependent on particle size; the highest preference is for particles between 7 and 50 µm (Ten Winkel and Davids 1982, Sprung and Rose 1988, Naddafi et al. 2007). As a consequence, cryptophytes and small diatoms are ingested at high rates, whereas most large chlorophytes, cyanobacteria and pennate diatoms are rejected as pseudofaeces (Ten Winkel and Davids 1982, Holland 1993, Bastviken et al. 1998, Naddafi et al. 2007). Less evidence is available about whether filtration selectivity is affected by food quality characteristics other than cell size (Ten Winkel and Davids 1982, Vanderploeg et al. 2001). Quality of ingested small algae can vary in terms of biochemical composition. Thus, cryptomonads are rich in polyunsaturated fatty acids, whereas green algae such as *Scenedesmus obliquus* (TURBIN) are poor in these substances (Wacker and Von Elert 2004). These fatty acids can control growth and reproduction of zebra mussels (Wacker and Von Elert 2002, 2003).

The effect of biodeposition by *D. polymorpha* on the species composition and biomass of benthic communities has been intensively studied, but little is known about the year-round availability and quality (e.g. the stoichiometry) of this food source. Whereas biodeposition rate is linearly correlated to the seston concentration (Reeders and Bij de Vaate 1992, Klerks et al. 1996, Roditi et al. 1997), pseudofaeces production appears to be restricted to high seston concentrations. At low seston concentrations, ingestion rate is not saturated and the deposited material consists mainly of faeces (MacIsaac and Rocha 1995,

Roditi et al. 1997). Furthermore, elemental components of food can be assimilated with different efficiencies. For example, consumers limited by phosphorous, the most frequently limiting factor in oligotrophic freshwater systems (Lampert and Sommer 1999), are able to assimilate this limiting element with a higher efficiency than carbon (Rothhaupt 1995, Sterner and Elser 2002).

In this study we focused on the transfer magnitude of seston from the pelagic compartment into benthic detritus mediated by *D. polymorpha*. Since ingestion of algae by *D. polymorpha* is mainly driven by particle size, we hypothesized that biodeposition rate would not depend on the quality of the algal food and would also be mainly driven by food particle size under field conditions. Because at low food concentrations the biodeposited material consists entirely of faces, while with increasing seston concentration the relative importance of pseudofaces increases, we predicted an interaction between seston concentration and C:N:P stoichiometry. Accordingly, we hypothesized that biodeposited material would be depleted in phosphorous at low seston levels. To investigate these two hypotheses, we conducted laboratory experiments with a high and a low quality algal species (a cryptomonad and a green algae, respectively) at concentrations around the threshold of pseudofaeces production. We also investigated the year-round biodeposition rate of zebra mussels under natural conditions in the oligotrophic Lake Constance. Our results could also help to elucidate the importance of zebra mussels in benthic-pelagic coupling within oligotrophic aquatic systems since most studies performed to date have been in more eutrophic systems.

Material and Methods

Collection and maintenance of zebra mussels

Zebra mussels (*D. polymorpha*) for the field and laboratory experiments were obtained from the littoral zone of Lake Constance. *D. polymorpha* colonized the Rhine in the 1850s, but did not invade Lake Constance, through which the river flows near its southern end, until the 1960s (Siessegger 1969). At the time of the introduction of this invasive species to the lake, intensive eutrophication began and continued until the late 1970s; since then, the lake has been subjected to strong re-oligotrophication. Today, Lake Constance is a pre-alpine

lake with a relatively low seston concentration (Internationale Gewässerschutz-kommission für den Bodensee 2004).

Collected mussels were pre-sorted for the required size class (see below) and kept in a 15°C climate chamber with a light cycle of 12 h:12 h (day:night). To minimize culturing effects, mussels were replaced by freshly collected mussels monthly. Mussels were cultured in a tank (approximately 90 L) in a flow-through system (0.5 L min^{-1}) with filtered water (30 µm) from Lake Constance. Stones from the lake were provided for mussel attachment. The mussels were fed *ad libitum* on the green algae *Scenedesmus obliquus* (SAG 276-3a, Sammlung von Algenkulturen Göttingen, Germany). The algae were grown in batch cultures in Cyano medium (Jüttner et al. 1983).

Laboratory experiments

In the laboratory, we estimated *D. polymorpha* biodeposition rates when fed on the green algae *Scenedesmus obliquus* and when fed on the algal flagellate *Cryptomonas erosa* EHRENB. (Max-Planck Institute for Limnology, Plön, Germany), each at five different concentrations. *S. obliquus* was grown in Cyano medium (Jüttner et al. 1983), and *C. erosa* was grown in modified Woods Hole (WC) medium containing vitamins (Guillard 1975). Both algal species were cultured semi-continuously at 20 °C with an illumination of 120 µmol m^{-2} s^{-1} in sterile, aerated 5-l vessels. Part (25%) of the culture was harvested each day and replaced with freshly prepared medium, resulting in a dilution rate of 0.28 d^{-1}. Both algal species were harvested by centrifugation (*S. obliquus:* 1890 × *g,* 15 min; *C. erosa:* 1180 × *g,* 6 min), and cells were resuspended in fresh medium. A defined carbon concentration was achieved by adjusting the resuspended cells to an optical density of 0.8 (photometric light extinction: *S. obliquus,* 480 nm; *C. erosa,* 800 nm). The final concentration of *S. obliquus* and *C. erosa* was 0.25 and 0.32 mg C ml^{-1}, respectively.

To estimate biodeposition rates, a flow-through system was used to keep the algal concentration constant. Lake water was filtered (0.45 µm), aerated and kept at 16 °C. The flow-through rate of this pre-treated lake water was adjusted to 200 ml min^{-1}. A calculated volume of prepared algal suspension was added continuously by a flexible-tube pump (Gilson, MiniPlus3; Villiers Le Bel, France). The experimental basin was 34 × 40 × 7.5 cm (width × depth × height), with a

water level of 6 cm, resulting in a water volume of 8 L. To minimize sedimentation of the algae, the water in the basin was gently aerated.

In both sets of laboratory experiments, 15 living mussels (14–16 mm in length) were glued (UHU plus, 2-component epoxy resin glue; UHU, Bühl, Germany) to an unglazed tile (4.7 × 4.7 cm), and their biodeposited material was estimated. A bare tile served as control. Each tile was placed in a mini-container (5 × 5 × 2.5 cm) and the containers with and without attached mussels were randomly placed in two rows within the basin. The experiments with *S. obliquus* at five concentrations ranging from 0.2 to 2.0 mg ash-free dry mass (AFDM) L^{-1} ran consecutively from July until September 2005. The concentration of algae in the inflow was determined at the end of each experiment. The treatment with attached mussels was replicated six times; the control treatment and the estimation of algal concentration were replicated three times. The experiments with *C. erosa* at five concentrations ranging from 0.3 to 1.3 mg AFDM L^{-1} ran consecutively from March until April 2006. The treatment with attached mussels, the control treatment, and the estimation of the algal concentration were replicated six times. Each experiment lasted three days. At the end of each experiment, the material deposited in the containers was transferred to a beaker, and pure water was added to 100 ml. Subsequently, analyses of the measured parameters (see below) were performed.

Field experiments

Biodeposited material was collected in Lake Constance using modified sediment traps. Each sediment trap consisted of a tube of grey PVC (50 cm length; Ø 10 cm) with a funnel and a PET flask (200 ml) fixed at the lower end of the tube to collect the settling sediment. At the upper end of the sediment trap, a clamp for two tiles (4.7 × 4.7 cm) was fixed vertically. This clamp held the tiles in the centre above the opening of the sediment trap, ensuring that the faeces and pseudofaeces of the zebra mussels were collected in the trap. Fifteen living mussels (14–16 mm in length) were glued on each tile. Bare tiles served as a control. Traps were exposed for 7 days each month from August 2005 until September 2006 and additionally during the spring bloom from March until May 2007.

Five traps with attached mussels and five with bare tiles were exposed to the pelagial of Lake Constance on a pontoon at a depth of 2 m. At the end of the exposure, the material in each funnel and PET flask was collected and filtered through a 200 µm net to exclude zooplankton. Pure water was then added to a defined volume. Subsequently, analyses of the measured parameters (see below) were performed. At the beginning and the end of each exposure, seston at a depth of 2 m was sampled with a horizontal water sampler. Seston data from the beginning and end of each exposure period were pooled.

Measured parameters

The lengths of the zebra mussel shells were measured with a digital sliding calliper (Preisser; Digi-Met, Gammertingen, Germany) to the nearest 0.01 mm.

The AFDM of each sample from the field and from the laboratory experiments was determined as follows. An aliquot of each sample was filtered on pre-combusted glass-fibre filter (Schleicher & Schuell; GF/6; Ø 25 mm, Dassel, Germany) and dried at 105 °C for 24 h. The filter was weighed (dry mass), combusted at 550 °C for 8 h, and then weighed again (ash content). The AFDM was calculated as the difference of the dry mass and the ash content. In both field and laboratory experiments, the biodeposition rate per zebra mussel and day was calculated as the difference in AFDM between the samples from attached mussels and the corresponding control.

To assess the nutrient content in samples from all experiments, we estimated the organic carbon, nitrogen and phosphorus stoichiometries of the deposited material. Aliquots of the samples were filtered on pre-combusted glass-fibre filters (Whatman GF/F; Ø 25 mm, Kent, England). The samples were dried at 55 °C, and then particulate organic carbon and particulate organic nitrogen was analysed with an NCS–2500 analyser (Carlo Erba Instruments, Milano, Italy). To determine particulate phosphorus, aliquots of the samples were filtered through acid-rinsed polysulfone membrane filters (HT-200, Pall; Ø 45 mm, Ann Arbor, Mich., USA). The samples were digested with 10% potassium peroxodisulfate and 1.5% sodium hydroxide at 121°C for 60 min, and soluble reactive phosphorus was determined using the molybdate-ascorbic acid method (Greenberg et al. 1985).

Statistical analyses

All statistical analyses were done using the statistical software package R (R Development Core Team 2006).

The dependence of biodeposition rate on seston concentration in both field and laboratory experiments was calculated by regression analyses. The data from laboratory experiments for each algal species were analysed separately. Differences between the regressions for laboratory data were analysed with ANCOVA. For the field data, a number of multiple linear models were calculated and compared using the Akaike Information Criterion (AIC). Based on maximum-likelihood estimates and the number of model parameters, the AIC provides a measure for selecting among competing models of a given data set. The model having the lowest AIC is chosen as the best model because it provides the best compromise between predictive power and model complexity (see Johnson and Omland 2004). Seasonal differences in biodeposition rate were detected using a one-way ANOVA with a subsequent Tukey HSD post-hoc test. The sampling dates can be denoted as independent, because factors that might influence zebra mussel biodeposition rate, seston concentration and phytoplankton community composition can change greatly within this time scale (Gaedke 1998).

To determine stoichiometry, molar C:N, C:P, and N:P ratios were considered as dependent variables. Each field and laboratory experiment was analysed separately. Differences in stoichiometry between seston, control and biodeposited material were analysed with ANCOVA and a subsequent Tukey HSD post-hoc test. All stoichiometry data were 1/(x)-transformed, and homogeneity of variances was checked with the F-test. For the field data, the homogeneity of variances was not necessary because large, balanced experiments do not cause problems for the interpretation of an analysis with heterogeneous variances (Underwood 2006).

Results

Laboratory experiments

We found significant linear, positive relationships between zebra mussel biodeposition rate and algal concentration in laboratory experiments with *S. obliquus* and *C. erosa* (Fig. IV.1; Table IV.1). Overall, biodeposition rates were not

Table IV.1: Linear regressions between the ambient seston concentration and the biodeposition rate of *Dreissena polymorpha* in the laboratory and in the field. Asterisks indicate a significant regression. Note that the table provides regressions for the ash-free dry mass (AFDM) and carbon content of the biodeposited material dependent on the seston concentration.

			Intercept ± SE	Slope ± SE	R^2	p
Laboratory	*C. erosa*	AFDM	–0.005 ± 0.013	0.089 ± 0.017	0.90	0.013*
		Carbon	–0.002 ± 0.004	0.034 ± 0.005	0.95	0.005*
	S. obliquus	AFDM	0.004 ± 0.007	0.055 ± 0.007	0.95	0.005*
		Carbon	0.004 ± 0.006	0.021 ± 0.006	0.83	0.032*
Field	Summer to Winter	AFDM	–0.026 ± 0.026	0.165 ± 0.034	0.73	0.001*
		Carbon	–0.030 ± 0.027	0.130 ± 0.035	0.61	0.005*
	Spring bloom	AFDM	–0.007 ± 0.011	0.050 ± 0.012	0.81	0.015*
		Carbon	–0.006 ± 0.008	0.320 ± 0.008	0.80	0.016*

significantly different between the algal species (ANCOVA, F = 0.98, p = 0.33). However, biodeposition rate increased more steeply with increasing algal concentration when the mussels were fed *C. erosa* than *S. obliquus* (ANCOVA,

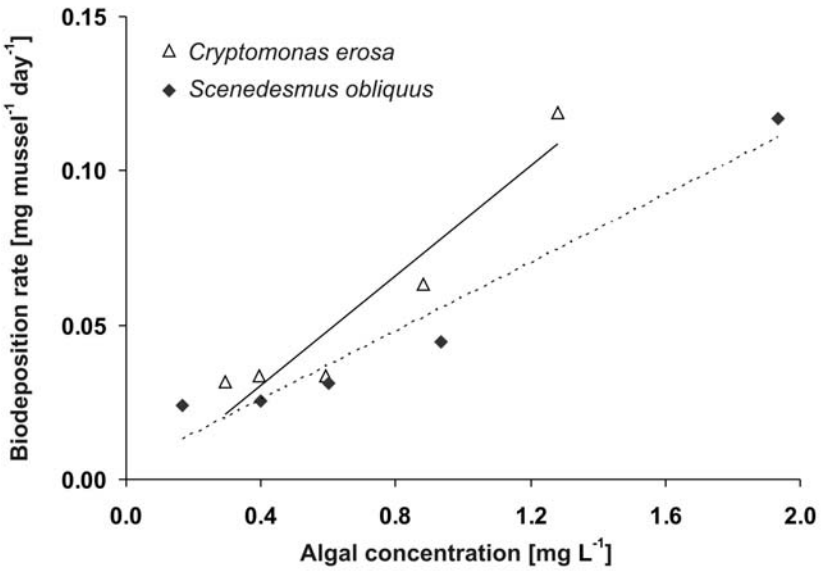

Figure IV.1: Biodeposition rates in laboratory experiments with different algal concentrations. The algal mass unit is mg ash-free dry mass. Lines represent linear regressions for both algal species; for statistics see Table IV.1.

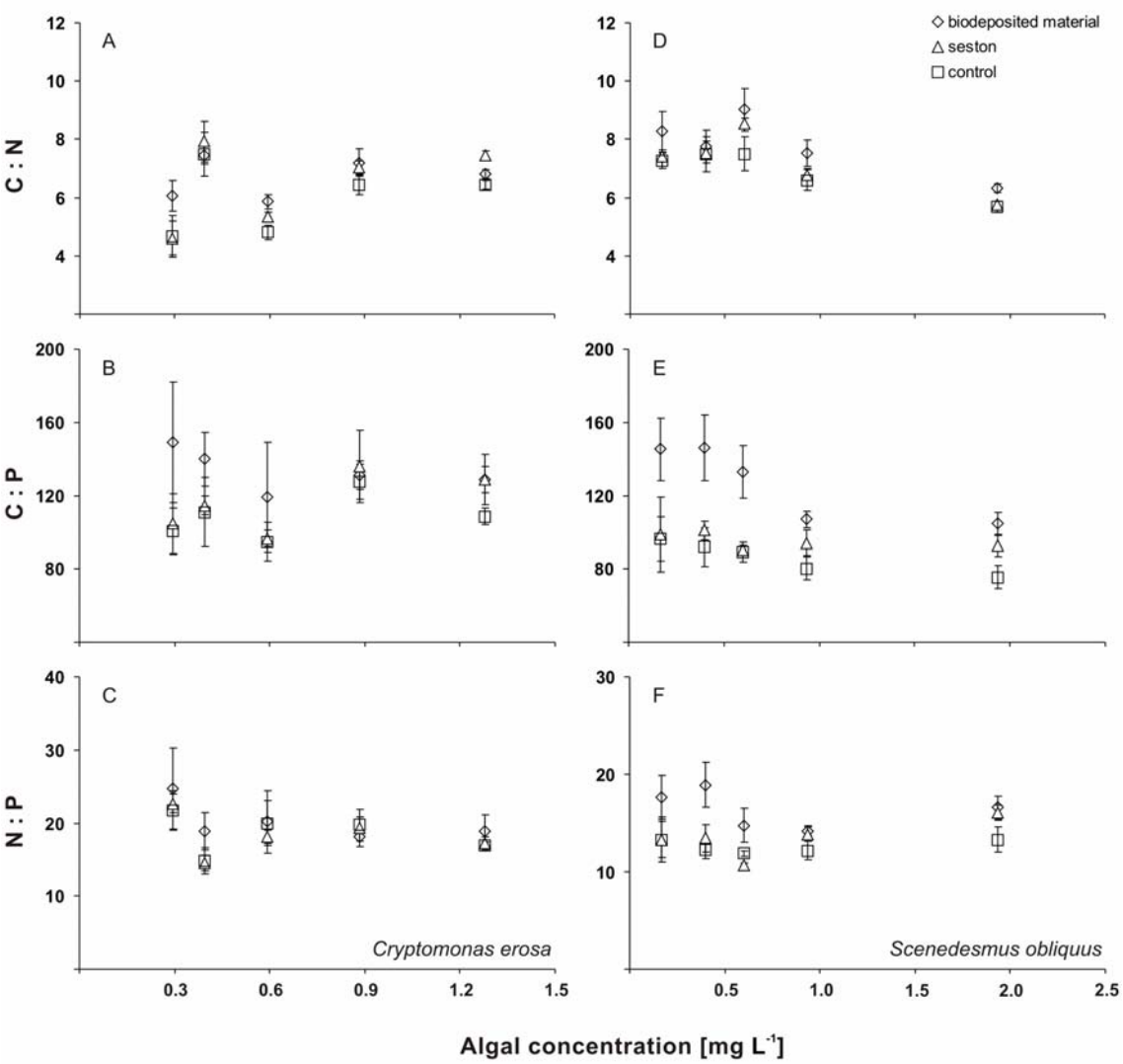

Figure IV.2: Stochiometric composition in the experiment using *Cryptomonas erosa* and *Scenedesmus obliquus* as food. Elemental ratios for (A/D) C:N, (B/E) C:P, and (C/F) N:P of biodeposited material from *Dreissena polymorpha*, and of seston and the control are shown. The algal mass unit is mg ash-free dry mass.

algal species × seston concentration, F = 8.53, p = 0.006). At low algal concentrations, the amount of biodeposited material was similar in the two sets of experiments.

In the experiments with both algal species, post-hoc tests revealed that C:P and C:N ratios of the biodeposited material differed from those of the seston and the controls, which were quite similar to each other (see Table IV.2). With either alga, when seston concentrations were low the C:P ratio of biodeposited material was markedly higher than that of the control and of seston. At higher seston concentrations, however, this effect disappeared. This pattern was demonstrated by a significant compartment × seston interaction term (compartments: biodeposited material, seston, control; ANCOVA: *C. erosa*,

p = 0.011; *S. obliquus*, p = 0.009; Table IV.2; Fig. IV.2). For both algal foods, the shift from higher C:P ratios in the biodeposited material compared to that of control and seston to similar C:P ratios consistently took place at a seston concentration of 0.6–0.9 mg AFDM L^{-1}. The same phenomenon was found for the N:P ratio in the experiments with *S. obliquus* (shown by a significant compartment × seston interaction term for the N:P ratio: ANCOVA, p = 0.595; Fig. IV.2). In the experiments with *C. erosa,* the N:P ratio did not differ in the different compartments (ANCOVA, p = 0.099). With either alga, the C:N ratios of the biodeposited material and the seston differed, but that of the control did not differ from the other compartments (ANCOVA, *C. erosa*: p = 0.013, *S. obliquus*: p < 0.001; Table IV.2; Fig. IV.2). However, there was no significant compartment × seston interaction (ANCOVA: *C. erosa,* p = 0.153; *S. obliquus*, p = 0.566).

Table IV.2: Comparison of the C:N:P stoichiometry of *Dreissena polymorpha* biodeposited material (BM), the control, and the seston. The results were analysed by ANCOVA with the seston concentration as the covariate. Asterisks indicate a significant ANCOVA result, and capital letters indicate homogenous subgroups detected by the subsequent Tukey-HSD post-hoc tests.

Variable		Factor	F-value	df	p	Compartment		
						BM	Control	Seston
C. erosa	C:N	Seston	14.92	1	< 0.001*			
		Compartment	4.56	2	0.013*	A	AB	B
		Compartment×Seston	1.92	2	0.153			
	C:P	Seston	5.53	1	0.021*			
		Compartment	13.95	2	< 0.001*	A	B	B
		Compartment×Seston	4.78	2	0.011*			
	N:P	Seston	2.33	1	0.131			
		Compartment	2.38	2	0.099	A	A	A
		Compartment×Seston	0.52	2	0.595			
S. obliquus	C:N	Seston	101.7	1	< 0.001*			
		Compartment	9.48	2	< 0.001*	A	AB	B
		Compartment×Seston	0.57	2	0.566			
	C:P	Seston	41.88	1	< 0.001*			
		Compartment	103.5	2	< 0.001*	A	B	C
		Compartment×Seston	5.13	2	0.009*			
	N:P	Seston	2.23	1	0.141			
		Compartment	31.19	2	< 0.001*	A	B	B
		Compartment×Seston	4.55	2	0.014*			
Field experiment	C:N	Seston	1.46	1	0.228			
		Compartment	169.1	2	< 0.001*	A	A	B
		Compartment×Seston	4.74	2	0.009*			
	C:P	Seston	3.20	1	0.075			
		Compartment	0.94	2	0.392	A	A	A
		Compartment×Seston	16.22	2	< 0.001*			
	N:P	Seston	7.99	1	0.005*			
		Compartment	86.79	2	< 0.001*	A	A	B
		Compartment×Seston	7.68	2	< 0.001*			

Field experiments

Sedimentation in the presence of zebra mussels was generally twice as high as in the control on all field sampling occasions, indicating that *D. polymorpha* markedly enhances the flux of organic matter from the pelagic to the benthic zone. In August 2005, for example, the deposited biomass in the presence of zebra mussels was approximately 40 mg per trap compared to approximately 20 mg per trap in the control (Fig. IV.3; t-test, p = 0.002).

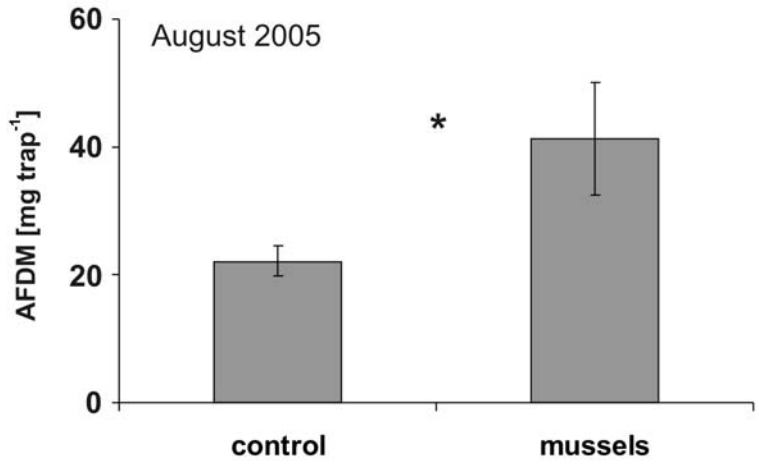

Figure IV.3: Comparison of accumulated ash-free dry-mass (AFDM) in the two compartments "control" and "mussels" after exposure of sediment traps for 7 days (n = 5). Shown are the values for the experiment in August 2005. The asterisk indicates the significant different between the compartments revealed by a t-test.

The biodeposition rate of zebra mussels varied seasonally (ANOVA, p < 0.001; Fig. IV.4) and was higher in August–October 2005 and July–September 2006 than in December 2005, and January, March, and April 2006 (post-hoc tests), when biodeposition rate was lowest. In general, high biodeposition rates were reached in summer, when temperatures and seston concentrations were high. However, there was a deviation from this pattern. The biodeposition rate was relatively low during the spring algal mass development (defined as the time from the onset of phytoplankton growth to the clear-water phase), when seston concentration was maximal. When the data from August 2005 to September 2006 (Fig. IV.4), and data collected in spring 2007 (not shown in Fig. IV.4) were pooled, we found only a weak relationship between biodeposition rate and seston concentration (Table IV.3, model 1.1). We hypothesized that either lower water temperature or a different algal community composition during spring was

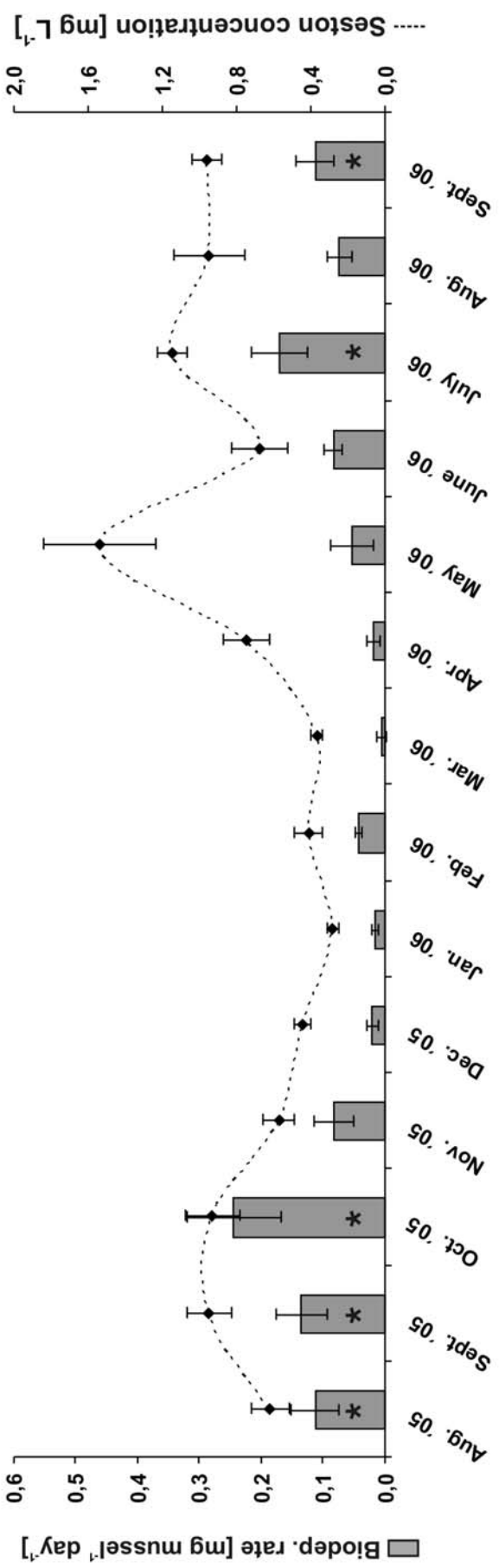

Figure IV.4: Monthly biodeposition rate of *Dreissena polymorpha* and the seston concentration in Lake Constance from August 2005 to September 2006. The mass unit is mg ash-free dry mass. Asterisks indicate significantly higher rates than in December, January, March, and April, detected by one-way ANOVA with a subsequent Tukey-HSD post-hoc test.

Table IV.3: Multiple linear models with biodeposition rate as the dependent variable and different independent variables: SC, seston concentration; SP, spring; WT, water temperature. Note that SC and WT are continuous variables, whereas SP is a categorical variable (spring/not spring). Interactions between variables are indicated by an asterisk between the respective variables. Models were compared by applying the Akaike information criterion (AIC). Asterisks indicate a significant result.

Model	Independent variables	R^2	F-value	df	p	AIC
1.1	SC	0.26	5.30	1, 15	0.004*	–51.88
1.2	SC + SP	0.69	15.89	2, 14	< 0.001*	–64.88
1.3	SC + SP + SC*SP	0.81	18.50	3, 13	< 0.001*	–70.99
2.1	WT	0.64	26.39	1, 15	< 0.001*	–63.99
2.2	SC + WT	0.65	13.06	2, 14	< 0.001*	–62.64
2.3	SC + WT + SC*WT	0.67	8.81	3, 13	0.002*	–61.69

responsible for these relatively low biodeposition rates. To determine which of these two factors best accounts for the observations, we calculated different multiple linear regression models (Table IV.3) and used the Akaike Information Criterion (AIC) to find the model with the highest predictive power. The different algal community composition during spring explained as much as 81% of the observed variation in the biodeposition rates. The alternative model with water temperature as a covariate explained only 67%. The best model, as indicated by AIC, is model 1.3 (Table IV.3), which assumes that the slope between biodeposition rate and seston concentration for spring samples is different from that of samples from other seasons.

Accordingly, two different significant regressions between seston concentration and biodeposition rate emerged: one for the spring samples and one for the samples from other seasons (Fig. IV.5, Table IV.1). The regression line for the algal spring bloom (March–May 2006 and 2007) was significantly lower than the regression for all other sampling dates (Table IV.3). During spring, the biodeposition rate increased less with increasing seston concentration than during the rest of the year.

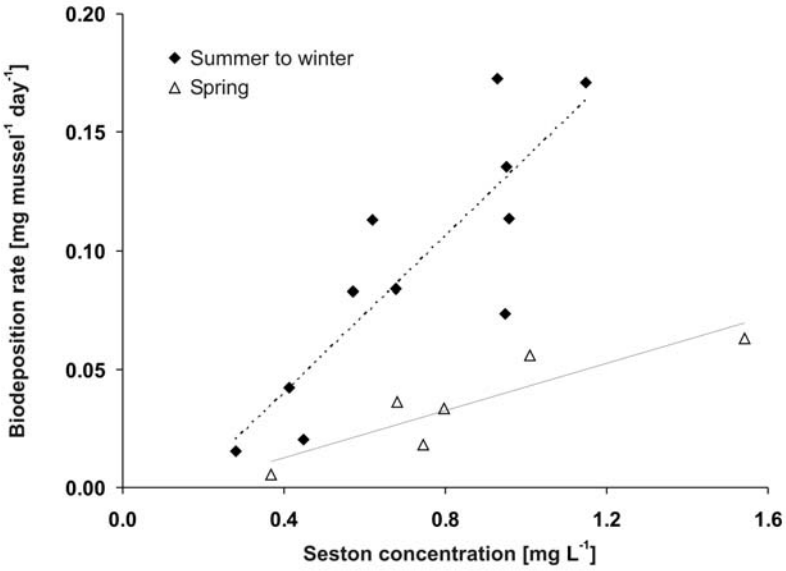

Figure IV.5: Biodeposition rates and lake seston concentration in field experiments. The mass unit is mg ash-free dry mass. Lines represent linear regressions for the two seasons caused by different algae compositions; for statistics, see Tables IV.1 and IV.3.

The CNP stoichiometry of the biodeposited material was similar to that of the control (Fig. IV.6, Table IV.2). Significant differences were found between seston CNP stoichiometry and that of the control and the biodeposited material. The C:N ratio of the seston ranged from 6 to 12 and was lower (ANCOVA, $p < 0.001$) than that of the biodeposited material and the control, ranging from 9 to 28. The N:P ratio of the seston ranged from 15 to 50 and was higher (ANCOVA, $p < 0.001$) than that of the biodeposited material and the control, ranging from 10 to 25. The C:P ratios of biodeposited material, seston and control did not differ (ANCOVA, $p = 0.392$), but varied strongly, ranging overall from 100 to 500. The trends in CNP stoichiometry for biodeposited material and the control to depend on seston concentration were similar (Fig. IV.6). This indicates that similar processes were operating in the treatment with and without zebra mussel, and that the effect of zebra mussels is not relevant for these effects. Both differed from the trend for CNP stoichiometry of seston, as shown by the significant compartement × seston interaction for all three measured ratios (ANCOVA: C:N, $p = 0.009$; C:P/N:P, $p < 0.001$).

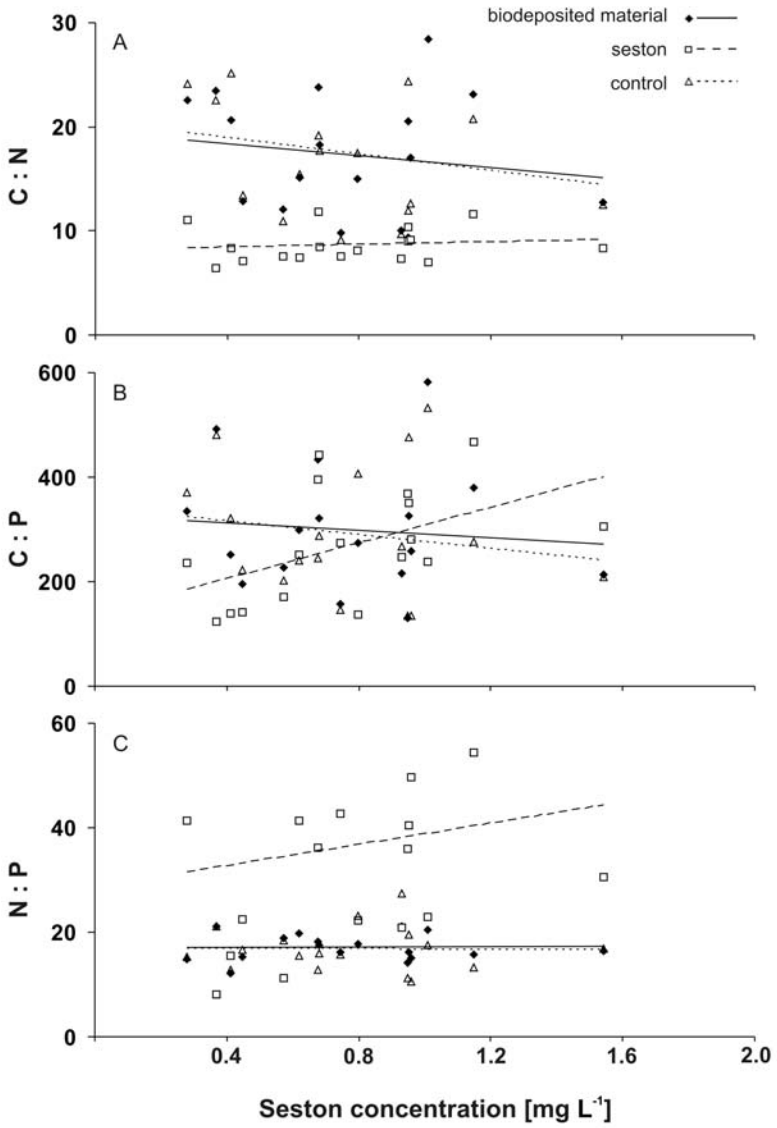

Figure IV.6: Stochiometric composition in the field experiment at natural food conditions. Elemental ratios for (A) C:N, (B) C:P, and (C) N:P of biodeposited material from *Dreissena polymorpha* and of seston and the control are shown. The seston mass unit is mg ash-free dry mass.

Discussion

Our results indicate that biodeposition by zebra mussels represents an important process of benthic–pelagic coupling that leads to an increased flow of organic matter from the pelagic zone into the benthos. The carbon flux from the pelagial into the benthos was twice as high in the presence of mussels than in the control without mussels. We found a positive relationship between zebra mussel biodeposition and seston concentration in an oligotrophic lake. Such relationships have also been found in more eutrophic systems with higher seston concentrations than in our study (Reeders and Bij de Vaate 1992, Klerks et al. 1996). However, the rate of biodeposition can vary depending on

environmental factors (e.g. algal community composition) and the quality of biodeposited material can also be affected by zebra mussels.

The biodeposition rates of mussels fed on two algal species in the laboratory differed only slightly, particularly in comparison to biodeposition rates observed in the field. The biodeposition rate with the algae cryptomonad *C. erosa* was slightly higher than that with the green algae *S. obliquus*. This contrasts with other studies where zebra mussels had higher clearance rates on cryptomonads than on green algae (Bastviken et al. 1998, Naddafi et al. 2007). A high preference for cryptomonads can be explained by the higher food quality of these alga, due to their C:N:P stoichiometry and high concentrations of long-chained polyunsaturated fatty acids (Vanderploeg et al. 1996, Wacker and Von Elert 2004). However, the similar biodeposition rates when fed contrasting alga indicates that differences in food quality are of less importance for biodeposition. Both algal species are in the size range preferred by *D. polymorpha* (between 7 and 50 µm) and are therefore ingested at high rates, resulting in lower biodeposition rates (Ten Winkel and Davids 1982, Naddafi et al. 2007).

Under natural conditions, the biodeposition rate of zebra mussels was also related to seston concentration, but seston concentration only explained a small proportion of biodeposition rate variation. A low dependence of zebra mussel biodeposition rate on seston concentration was also found by Reeders and Bij de Vaate (1992). A second important factor was season, which had a higher predictive power than temperature. We observed a seasonal difference in biodeposition rate, with lower rates during the spring algal bloom compared to the rest of the year (Fig. IV.5). Similarly, Stanczykowska et al. (1975) found that zebra mussels had higher consumption and assimilation rates in spring than in summer, as a result of a seasonal shift in phytoplankton composition, with small diatoms in spring and large dinoflagellates (mainly *Ceratium hirundinella* (O.F. MÜLLER)) in summer. In Lake Constance, similar seasonal dynamics take place in the phytoplankton community. The spring bloom is dominated by cryptomonads and small diatoms (e.g. *Stephanodiscus* sp.), but after the clear-water phase the community is dominated by large pennate diatoms (e.g. *Asterionella* sp., *Fragilaria* sp.) and other large-sized taxa (e.g. *Dinobryon* sp. and *Ceratium* sp.) (Gaedke 1998, Gege 1998, Kümmerlin 1998). Algal

groups dominating in spring are small and are therefore ingested at high rates by zebra mussels, resulting in low pseudofaeces production and low biodeposition. Algae dominating in summer are larger and are therefore ingested at lower rates or not at all, resulting in a high biodeposition (Ten Winkel and Davids 1982, Heath et al. 1995, Bastviken et al. 1998, Naddafi et al. 2007). The rate at which zebra mussels deposit organic matter in spring agrees well with the rates we observed in the laboratory for both *C. erosa* and *S. obliquus*. This is not unexpected because both algae are as small as those that dominate lake phytoplankton in spring.

Zebra mussels had a clear impact on the stoichiometry of biodeposited material at low concentrations of either *C. erosa* or *S. obliquus* in laboratory experiments. Elemental components of food can be assimilated with different efficiencies and consumers are able to assimilate the limiting elements with a higher efficiency than carbon (Rothhaupt 1995, Sterner and Elser 2002). Since phosphorous is the most limiting factor, especially in oligotrophic freshwater systems (Lampert and Sommer 1999), it would be reasonable for zebra mussels to assimilate phosphorous better than nitrogen, as was shown in our laboratory experiments. This differential phosphorus assimilation resulted in an increased C:P ratio in the biodeposited material, potentially leading to further stoichiometric constraints for consumers of this biodeposited material. It is important to note that this altered C:P ratio in the biodeposited material was only found when seston concentrations were low, when biodeposited material consists mostly of faeces. As seston content increases, the production of pseudofaeces becomes the dominant process for biodeposition and stoichiometric composition cannot change further. A significant depletion in phosphorous was only observed up to a concentration of 0.6 mg AFDM L^{-1} (approximately 0.35 mg C L^{-1}). This concentration is slightly above the threshold of pseudofaeces production of 0.2 mg C L^{-1} (Walz 1978a).

In our laboratory experiments, the biodeposited material of zebra mussels was depleted in phosphorous at low algal concentrations. This effect, however, was not observed in the field where the C:P ratio of biodeposited material did not differ markedly from that of the natural seston and the deposited material in the sediment traps was depleted in nitrogen, indicating bacterial activity. There are two possible explanations for these results. First, the C:P ratio of the deposited

material could have been affected by occasional advection and sedimentation of resuspended littoral sediments. Although the sediment traps were placed in the pelagial and had no direct contact to the littoral, resuspended material from the littoral could have been transported to the experimental site during rough weather conditions and hence collected in the sediment traps. Secondly, the field experiments lasted 7 days, more than twice as long as the laboratory experiments. Microbial activity and microbial density in biodeposited material from zebra mussel can increase within a few days and accelerate its degradation (Izvekova and Lvova-Katchanova 1972, Roditi et al. 1997, Strayer et al. 1999). Simultaneously, an assemblage of ciliates, nematodes and rotifers could develop (Walz 1978b). The shorter exposure of sediment traps in the field could potentially solve this problem in future field studies. Although we were not able to provide evidence for changing C:N:P stoichiometric composition of biodeposited material in the field, our laboratory experiments consistently showed such an effect at low food concentrations. We therefore hypothesize that this process has relevance also *in situ*, particularly in oligotrophic systems where stoichiometric constraints are most prominent (Hessen 2006).

Relevance for benthic communities in oligotrophic lakes

The *in situ* biodeposition rate of zebra mussels was highest in summer and lowest in winter, due to seasonal variation in seston concentration and composition. Consequently, the impact of zebra mussel biodeposition on the benthos is highest during summer, a time when benthic communities are most active. The contrast between seasons is amplified by winter losses of zebra mussels through consumption by overwintering diving ducks, which can prey heavily on zebra mussels. A reduction of more than 90 % of zebra mussel biomass has been observed in Lake Constance during winter (Cleven and Frenzel 1993, Werner et al. 2005). In the following spring and summer, the population recovers by reproduction of the remaining young mussels and again reaches average densities of 10,000 adult individuals m^{-2} with a mean size of 15 mm on littoral hard substrates (Mörtl 2004, Werner et al. 2005). With a biodeposition rate of 0.15 mg AFDM $mussel^{-1}$ day^{-1} (cf. Fig. IV.4), a biomass of 1.5 g m^{-2} can be deposited by zebra mussels each day; this represents a significant benthic food source in a poorly productive system. For example, the

amphipod *Gammarus roeselii* GERVAIS has a feeding rate of 0.3 mg AFDM individual^{-1} day^{-1} on the biodeposited material (Gergs and Rothhaupt 2008b). Hence, the material deposited just by zebra mussel filtration would be enough to sustain 5,000 individuals m^{-2} of this *G. roeselii* in Lake Constance. In comparison, the abundance of *G. roeselii* in the littoral of Lake Constance is only up to approximately 500 individuals m^{-2} (Mörtl 2004). Moreover, our estimate of potential daily biodeposition by *D. polymorpha* can account for the frequently observed year-round pattern of increase of organic matter in the benthos associated with the invasion of *D. polymorpha* (Stanczykowska et al. 1976, Klerks et al. 1996, Silver Botts et al. 1996, Ricciardi et al. 1997). Furthermore, these results support the hypothesis that zebra mussels are responsible for the well-documented increase in abundance of many benthic taxa (e.g. Stewart and Haynes 1994, Stewart et al. 1998, Mörtl and Rothhaupt 2003). The subsequent microbial colonization of biodeposited material may additionally increase its nutritional value; chironomids show high growth rates on material deposited by *D. polymorpha* and assimilation rate increases with time since deposition (Izvekova and Lvova-Katchanova 1972). These results indicate that material deposited by *D. polymorpha* substantially contributes to food availability for benthic communities and thereby increases overall benthic secondary production and macroinvertebrate density. These findings provide evidence for the existence of a *Dreissena*-biodeposition-based food web.

If we extrapolate the number of zebra mussels above the mouth area of the sediment trap used, the resulting mussel density in the benthic zone amounts to 1,000 individuals m^{-2}. In our field experiments, this mussel density led to sediment deposition rates twice as high as the control without mussels. The same enhancement of sediment deposition was found by Klerks et al. (1996), with a comparable mussel density of 1,180 individuals m^{-2}. Since the zebra mussel density in Lake Constance is ten-fold higher, the transfer of pelagic resources into the benthic zone is predominantly caused by the mussels.

While the importance of *Dreissena* biodeposition for benthic communities is very obvious, the empirical evidence for the importance to the pelagic habitat is questionable, especially in deep lakes. A study by Walz (1978b) estimated consumption by *D. polymorpha* to be only 2–3% of the pelagic primary production of Lake Constance. However, at that time, the density of

D. polymorpha was much lower than today and mussel impact might now be higher; further studies are needed for verification. A completely different picture can, of course, be drawn for shallow lakes, where zebra mussels may have strong impacts on pelagic seston concentrations (Boegman et al. 2008).

Acknowledgments

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Chapter V

Zebra mussels (*Dreissena polymorpha*) as keystone species for benthic food web structure and macroinvertebrate community

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In preparation

Abstract

Stable isotope analysis is increasingly applied to investigate trophic relationships in aquatic food webs. Here we combined stable isotope analysis with quantitative benthos sampling to investigate the influence of zebra mussel density and biodeposition of organic material on the benthic macroinvertebrate community structure in *Dreissena*-dominated habitats of Lake Constance, Germany. The increased surface area provided by mussel shells and the accumulation of organic material excreted by *Dreissena* (faeces and pseudofaeces) are assumed to implement a biodeposition based food web. By means of stable isotope analyses, we found that carbon signature and trophic position of amphipods is positively related to the availability of zebra mussel biodeposition in *Dreissena*-dominated habitats. At high zebra mussel densities the benthic community in Lower Lake Constance showed a shift towards higher densities of the amphipod *Gammarus roeselii*, the mayfly *Caenis* spp., *Chironominae* and the caddisfly *Ecnomus tenellus*. The comparison between a *Dreissena*-habitat and a stonewort (*Chara* spp.)-habitat revealed similar stable isotope signatures of many macroinvertebrate groups. We assume that *Chara* spp.-habitats are affected by the production of zebra mussel biodeposits in surrounding *Dreissena*-habitats by wave-induced relocation of organic matter (i.e. biodeposited material) from sediments. Our results show that organic

matter biodeposited by zebra mussels is of great relevance for benthic communities and corroborate the hypothesis that zebra mussel biodeposited material is the base of a detrital food web.

Keywords: stable isotope analysis, trophic position, biodeposition, amphipods, invasive species, food web, baseline organism

Introduction

Trophic relationships significantly affect structure of aquatic communities (e.g. Rieman and Falter 1981, Langeland et al. 1991, e.g. Vander Zanden et al. 1999). A single species playing an outstanding role in its surrounding food web is called *keystone species*. Removal of the *keystone species*, for example a top-predator, would result in a change of the community's species composition (Paine 1969). In recent years this concept has been broadened and is not restricted to predators anymore. Prey or even resources can have a *keystone* function; important hallmarks for a *keystone species* are that their presence is crucial for maintaining community structure and diversity and their exceptional importance in relation to the rest of the community (Mills et al. 1993).

In recent years, stable carbon and nitrogen isotope measurements were increasingly used to investigate trophic relationships. The isotope ratios $\delta^{13}C$ and $\delta^{15}N$ isotopes change in a different manner when organic matter is transferred to higher trophic positions. The stable nitrogen isotope (^{15}N) is enriched by approximately 3.4 ‰ when transferred from one trophic level to the next, which makes it possible to determine the trophic position of organisms within the food web (Peterson and Fry 1987, Vander Zanden and Rasmussen 1999, Post 2002). To determine the trophic position of species, a lake-specific baseline of long-living primary consumers is recommended, which integrates the seasonal variations of primary producers (Cabana and Rasmussen 1996, Vander Zanden et al. 1997). In a consumer the carbon isotope ratio ($\delta^{13}C$) is only marginally enriched in comparison to the ratio of its diet; usually less than one per mill (Peterson and Fry 1987, Post 2002). Since pelagic primary producers are depleted in the ^{13}C isotope compared to benthic primary producers, the origin of carbon in a consumer can be determined (France

1995b, 1995a). Consumers feeding on resources of different origin have mixed carbon signatures, reflecting the relative contribution of each resource.

The invasion of the zebra mussel *Dreissena polymorpha* in North America and Europe was associated with changes in the structure of benthic habitats of many freshwater ecosystems. Habitat changes were due to an increase of surface area and thus structural complexity by mussel shells and presumably to the filtration activity of the mussels, which deposit organic material to the benthos (Stanczykowska et al. 1976, Klerks et al. 1996, Silver Botts et al. 1996, Ricciardi et al. 1997). Increased structural complexity due to the presence of living mussels and empty shells has been shown to affect the abundance of macroinvertebrates, especially amphipods and chironomids (Stewart and Haynes 1994, Stewart et al. 1998, Mörtl and Rothhaupt 2003). It is assumed that the availability of zebra mussel biodeposited material as a new food source leads to a biodeposition-based food web (Stewart and Haynes 1994, Mitchell et al. 1996). In laboratory experiments, the biodeposited material was characterized as a suitable food source for amphipods and chironomids (Izvekova and Lvova-Katchanova 1972, González and Burkart 2004, Gergs and Rothhaupt 2008b). In the field, amphipods may benefit from the new resource directly by feeding on the biodeposited material or indirectly by feeding on associated invertebrates. There is some evidence that the natural diet of the amphipod *Gammarus fasciatus* contains biodeposited material of zebra mussel (Limén et al. 2005), but only little is known about the relevance of these biodeposits under field conditions and its effects on the benthic macroinvertebrate community.

We hypothesize that the zebra mussels couple the pelagic primary production to the benthic food web by biodeposition and thus can be pronounced as a *keystone species*. During two years, we quantitatively studied the benthic community in *Dreissena*-habitats (the predominant habitat in the littoral of Lake Constance) at two sites. Additionally, we sampled macroinvertebrates and primary producers for stable isotope analyses at each site and in each year. A comparison with a stonewort (*Chara* spp.)-habitat, the second most frequent habitat in the littoral of Lake Constance, might help to elucidate the relevance of organic matter biodeposited by *Dreissena* as a food source for benthic invertebrates.

Material and Methods

Study area and sites

The present study was conducted in Lake Constance, a pre-alpine oligotrophic lake of the unregulated alpine system of the River Rhine, bordering Germany, Switzerland and Austria. Lake Constance is divided into two major basins, which are connected by a river-like part. The major basin, Upper Lake Constance (ULC), has a mean depth of approx. 100 m and covers a large area of 473 km², whereas the Lower Lake Constance (LLC) is more shallow (mean depth of approx. 13 m) and smaller (63 km²) (Internationale Gewässerschutz-kommission für den Bodensee 2004). Our two study sites were located in the western part of ULC (N 47° 41.5'; E 9° 12.2') and in the central part of the Lower Lake Constance, near a peninsula (N 47° 42.1'; E 9° 2.4'). All samples were collected in a depth of one meter below mean low-water level (MLL -1 m). At each site the two dominant habitats of the lake littoral were sampled, the *Dreissena* dominated stone substrate and the *Chara* spp. dominated soft substrate, were sampled. In the *Chara* spp.-habitat, stonewort species of the genus *Chara* were dominant, but may also contain other stonewort. Distance between the two habitats was approximately 300 m for both sampling sites. Sampling took place in mid of October in 2005 and 2006.

Benthic samplings

Quantitative macroinvertebrate samples were collected by scuba divers using an infralittoral suction sampler, which covered a sampling area of 625 cm^2 (Baumgärtner 2004, Mörtl 2004). Five replicates were taken at each of the two sites. In the laboratory, zebra mussels were detached; other macroinvertebrates were brushed from rocks or segregated from inorganic sediments and collected with a 200 µm sieve. The organic fraction was fixed in 95 % ethanol. All invertebrates were identified to the lowest taxonomic level possible (to species or genus level) and counted under a stereomicroscope (Zeiss Stemi 2000-C, Jena, Germany). Zebra mussels >5 mm were counted and each measured to the nearest 0.01 mm using an electronic caliper (Digi-Met, Preisser Messtechnik GmbH & CoKG, Gammertingen, Germany). All mussels <5 mm are young of the year and were counted as newly settled juveniles (Cleven and Frenzel 1993).

In the Dreissena-habitat, the amount of periphyton on stones was measured with a brush sampler as described by Peters et al. (2005). Five replicates were taken at each sampling date and site. For further analyses the sampled epilithon was resuspended and adjusted to a defined volume with distilled water. Additionally, seston in a depth of two meters below MLL was sampled with a horizontal water sampler, approximately 300 m distance from the shoreline. To exclude particles not relevant for zebra mussel feeding, lake water was prefiltered through a 200 µm net before further processing (Stanczykowska et al. 1975, Ten Winkel and Davids 1982). Five sub-samples of seston and periphyton were filtered on precombusted glass fibre filters (Schleicher & Schuell GF6, ∅ 25 mm, Dassel, Germany) for ash free dry mass (AFDM) and chlorophyll a analysis, respectively. For AFDM estimation, samples were dried at 105 °C for 24 h. After weighing (dry mass), the filters were combusted at 550 °C for 8 h and weighed again for the ash content. The AFDM was calculated as the difference between the dry mass and the ash content. The chlorophyll *a* concentration was measured spectrophotometrically after extraction of the filters in 90 % ethanol for 12 h in dark at 4 °C, correcting the values for pheopigment content (Lorenzen 1967, Marker et al. 1980, Nusch 1980).

Stable isotope analyses

Macroinvertebrates for stable isotope analysis were collected by scuba divers subsequently to the quantitative macroinvertebrate sampling described above. Additionally, zebra mussels were sampled from a surface marker buoy for shipping navigation in each lake basin in October 2006, to use mussels as baseline organism, which had only contact to pelagic primary production. Since mussels integrate temporal variations in primary producer $\delta^{15}N$, they are suitable baseline organisms to calculate the trophic position of all sampled macroinvertebrate species (Kling et al. 1992, Cabana and Rasmussen 1996). All invertebrates were maintained alive individually over a gaze to allow gut clearance for 24 h. Whole organisms were included in stable isotope analyses of invertebrates except for zebra mussels, where only the soft body was used. Single individuals were analysed whenever possible by size of species. For chironomids 5 to 15 individuals and for *Centroptilum luteolum* 5 individuals were pooled to get an appropriate amount. Periphyton samples for stable isotope

analyses were obtained from the brush samples. Seston was filtered on a pre-combusted glass fibre filter (Whatman GF/F; Ø 25mm, Kent, England). Samples of primary producers were split into two parts. One part was acidified (1 M HCl for approx. 2 h) to remove carbonate; the second part was used without acidification for stable nitrogen analysis. All samples were dried at 55 °C, grinded and weighed into a tin cup (0.4 – 0.7 mg for animal material and seston, and 1.5 – 3.0 mg for *Chara* spp. and periphyton). All samples were replicated five times, if no variation is explicitly noted. Isotope ratios were measured using a ThermoFinnigan Delta Plus Isotope Ratio Mass Spectrometer and given in the δ notation per mill (‰). The international standards Vienna PeeDee belemnite for carbon and atmospheric N_2 for nitrogen were used as reference.

Data processing and statistical analyses

Similarity of benthic macroinvertebrate communities between different sampling sites and dates were analyzed by nonmetric multidimensional scaling (nMDS) with PRIMER 5 (version 5.2.8). We analyzed Bray–Curtis similarities between the communities, which compare ranked similarities for differences between defined groups. Statistical differences between the communities at the sampling sites and years were analysed using analyses of similarity (ANOSIM; PRIMER 5). Percentage contribution of single species to the differences between benthic community structures was estimated using SIMPER (PRIMER 5) analyses. Changes in density of amphipods between sampling years were analysed by t-tests using the statistical package SPSS (version 15.0/2006; SPSS Inc., Chicago, USA).

We estimated daily production rates of periphyton and material biodeposited by zebra mussels as the potential available primary resources to the benthos in the *Dreissena*-dominated habitat. Periphyton production was calculated from its chlorophyll *a* concentration and local water temperature (Morin et al. 1999). Periphyton was derived from stone surfaces and thus, a correction factor for the area-specific production was necessary. At each sampling site in the *Dreissena*-dominated habitats, stones in a sampling area of 625 cm² (depth: MLL -1) were collected in four replicates. Proportion of stone surface to total area was calculated as an oval by the length and width of each stone per sample. Since biodeposited material is seston transformed by the zebra mussels, it is not a

primary resource per definition. However, the biodeposited material is also not assimilated by zebra mussels and therefore will be pronounced as a primary resource in the following. Zebra mussel biodeposition production was estimated from the seston biomass concentration (Gergs et al. 2009) and the zebra mussel density from the quantitative macroinvertebrate samples. In Lake Constance zebra mussel densities are reduced during late fall and winter by diving ducks (Cleven and Frenzel 1993, Werner et al. 2005). In October 2005, the diving ducks arrived earlier for over wintering at Lake Constance and zebra mussels were already strongly reduced at the sampling date (R. Gergs, personal observation). In September 2005 at the same sampling site, zebra mussel density was about six fold higher than during our sampling in October 2005, whereas in 2006 the density in September was not higher than in October (C. Fiek; routine sampling of the Limnological Institute, Konstanz, Germany; unpublished data). For a better representation of the available food resources responsible for stable isotope signatures of sampled macroinvertebrates – stable isotope signatures of macroinvertebrates represents food uptake with a time delay (Vizzini and Mazzola 2003) – , we adjusted the zebra mussel density of our sampling in October 2005 to the density in September 2005. Differences between primary resources, sampling year and sampling site were analysed using a three-way ANOVA. Data were ln(x) transformed to homogenise variances, checked by the Levene-test.

Trophic position of species was calculated using the formula of Vander Zanden et al. (1997). The trophic baseline (trophic position: 2 = primary consumers) was estimated for both, the Upper and the Lower Lake Constance, with respect to potential variations between lakes (Vander Zanden et al. 1997). Trophic position and carbon signature of macroinvertebrates and primary producers for each sampling site and habitat were compared between years using t-tests with *Bonferroni* correction of the significance level. The dependence of trophic position and carbon signature of sampled macroinvertebrates on the zebra mussel biodeposition and periphyton production were calculated by regression analyses, respectively.

Results

Dreissena-habitat

In both lakes isotopic signatures of seston and periphyton differed between sampling years, respectively. Nevertheless, there was a clear separation between pelagic and benthic primary production for both sampling sites and years with a more depleted carbon signature of the pelagic primary production (Fig. V.1; Table V.1). In ULC the carbon signature of chironomids was approximately −26 ‰ in both years (Fig. V.1a & b; Table V.1); trophic positions differed significantly, but were also in the same range in both years. (Table V.1; 2005: 2.5 and 2006: 2.3). In LLC the carbon signature of chironomids decreased significantly from −20.1 ‰ in 2005 to −21.3 ‰ in 2006 (Table V.1). The trophic position was 2.7 – 2.8 for both years. Overall, chironomids occupied a trophic position slightly above the baseline of primary consumers, and the carbon signature was between the pelagic seston- and the benthic periphyton-signature in the ULC in 2005 and near the periphyton carbon signature in the LLC. The carbon signature of zebra mussels was in the same range as the seston at both

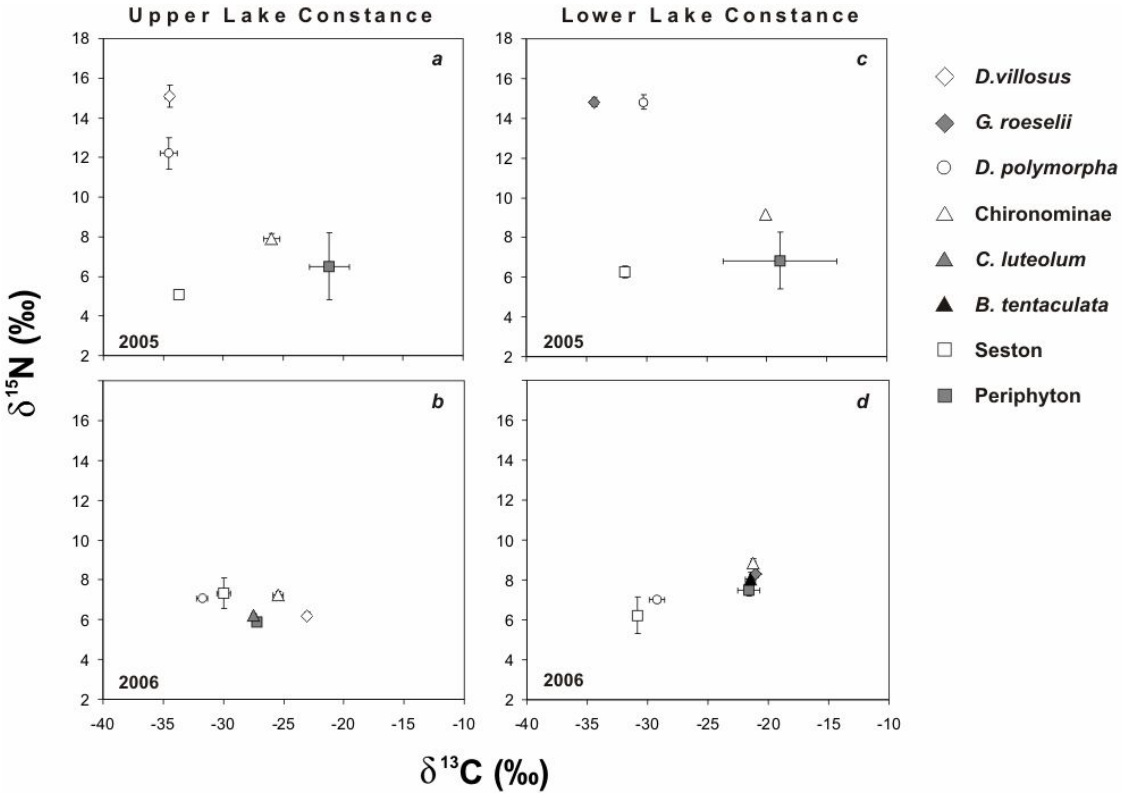

Figure V.1: Isotopic composition (relationship between $\delta^{15}N$ and $\delta^{13}C$) of collected macroinvertebrate species and primary producers in the *Dreissena*-habitat of both sites in fall 2005 and 2006. Errors bars in horizontal and vertical direction indicate the standard deviation.

Table V.1: Comparison of carbon signatures and trophic positions in the *Dreissena*-dominated habitat at two sites in Upper (ULC) and Lower Lake Constance (LLC) between sampling years; differences were analysed by t-tests and significance levels were adjusted after *Bonferroni* for each site. Asterisks indicate significant differences after correction. All periphyton samples and seston samples from ULC were replicated four times, seston samples from LLC three times.

		$\delta^{13}C$			Trophic position		
Site		2005	2006	p-value	2005	2006	p-value
ULC	*D. villosus*	-34.5 ± 0.3	-23.0 ± 0.2	< 0.001*	4.6 ± 0.2	1.9 ± 0.1	< 0.001*
	D. polymorpha	-34.5 ± 0.7	-31.8 ± 0.5	< 0.001*	3.7 ± 0.2	2.2 ± 0.0	< 0.001*
	Chironominae	-26.0 ± 0.7	-25.4 ± 0.4	0.19	2.5 ± 0.1	2.3 ± 0.1	0.001*
	Periphyton	-21.2 ± 1.7	-27.2 ± 0.0	0.006*	2.0 ± 0.5	1.9 ± 0.0	0.49
	Seston	-33.7 ± 0.1	-30.0 ± 0.5	0.001*	1.6 ± 0.0	2.3 ± 0.2	0.009*
LLC	*G. roeselii*	-34.4 ± 0.1	-21.1 ± 0.3	< 0.001*	4.5 ± 0.1	2.5 ± 0.1	< 0.001*
	D. polymorpha	-30.3 ± 0.3	-29.2 ± 0.6	< 0.001*	4.5 ± 0.1	2.2 ± 0.0	< 0.001*
	Chironominae	-20.1 ± 0.1	-21.3 ± 0.2	0.003*	2.8 ± 0.0	2.7 ± 0.1	0.024
	Periphyton	-18.9 ± 4.7	-21.6 ± 0.9	0.24	2.1 ± 0.4	2.3 ± 0.1	0.42
	Seston	-31.8 ± 0.1	-30.9 ± 0.3	0.001*	2.0 ± 0.1	1.9 ± 0.3	0.91

sampling sites and years, with a comparable increase in carbon signature from 2005 to 2006 (Table V.1). The trophic position of zebra mussels strongly decreased from high values in 2005 (ULC, 3.7 and LLC, 4.5) to a primary consumer level for both sites in 2006 (Table V.1). The carbon signatures of amphipods and their trophic position changed significantly between years. In the ULC, the local dominant amphipod *D. villosus* was ^{13}C depleted ($\delta^{13}C$ = −34.5 ‰) similar to seston in 2005, whereas the carbon signature was near the periphyton in 2006 ($\delta^{13}C$ = −23.0 ‰). The trophic position of *D. villosus* decreased from 4.6 in 2005 to 1.9 in 2006, which is equivalent to a change in trophic position from a top-predator to a primary consumer. In the LLC, the locally dominant amphipod *G. roeselii* showed a comparable change in carbon signature and trophic position as *D. villosus* in the ULC between years (Fig. V.1; Table V.1).

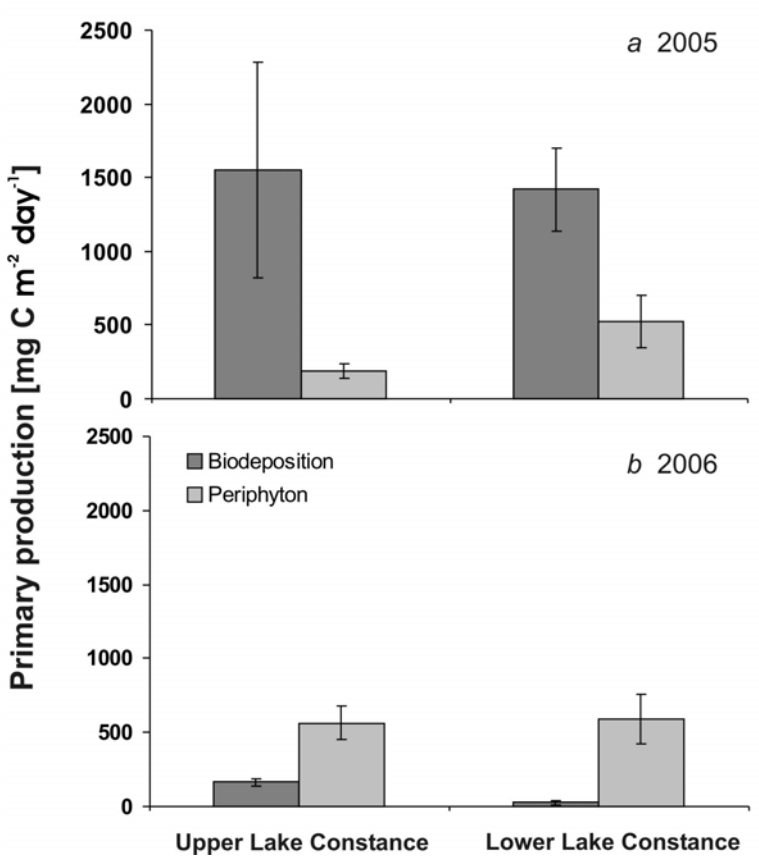

Figure V.2: Daily production rates of the two main available benthic resources, zebra mussel biodeposition and periphyton, in the *Dreissena*-habitat. Values were estimated according to the current conditions at both sites during the sampling in fall 2005 and 2006.

Table V.2: Results of analysis of variance (ANOVA) comparing type of production (biodeposition and periphyton), sampling site and sampling year; differences depending on these factors and their interactions were analysed. Asterisks indicate significant differences.

Effect	F	df	P
production	11.4	1	0.002*
sampling site	5.3	1	0.03*
sampling year	170.5	1	< 0.001*
production * site	62.1	1	< 0.001*
production * year	385.5	1	< 0.001*
site * year	57.1	1	< 0.001*
production * site * year	5.5	1	0.03*

The availability of the two important primary resources, zebra mussel biodeposits and periphyton, differed between the two years (Fig. V.2, Table V.2). ANOVA indicated a significant difference between the two sampling sites, but the pattern between years was similar for Upper and Lower Lake Constance. In fall 2005, the calculated production of biodeposition material by zebra mussels was 3 to 5 times higher than the periphyton production (Fig. V.2a), which points out that most of the available primary resources were provided by zebra mussel biodeposition. In fall 2006, production of biodeposition material was considerably lower at both sampling sites than the year before. Periphyton production was more than three times higher than the biodeposition production, which suggests that most of the available primary resources were provided by the periphyton (Fig. V.2b). Regression analysis revealed no significant correlation between biodeposition or periphyton production and the carbon signature or trophic position of chironomids and *D. polymorpha*, respectively. The carbon signature and trophic position of amphipods was positively related to the production of zebra mussel biodeposits, but not to periphyton production (Table V.3).

Table V.3: Linear regressions between trophic position / carbon signature of amphipods and zebra mussel biodeposition production / periphyton production. Asterisks indicate a significant regression.

	Biodeposition [mg C m^{-2} day^{-1}]			Periphyton [mg C m^{-2} day^{-1}]		
Dependent variable	regression coefficient	R^2	p-value	regression coefficient	R^2	p-value
Carbon signature	-0.99	0.99	0.003*	0.69	0.47	0.31
Trophic position	0.97	0.94	0.03*	-0.68	0.46	0.32

The macroinvertebrate community in the *Dreissena*-habitat differed between sampling sites and years (ANOSIM, p = 0.001), whereas the five replicates within one year and site were similar (Fig. V.3). There were strong differences in benthic macroinvertebrate communities between Upper and Lower Lake Constance (dissimilarity ~ 40 % for both years; Table V.4). Responsible for

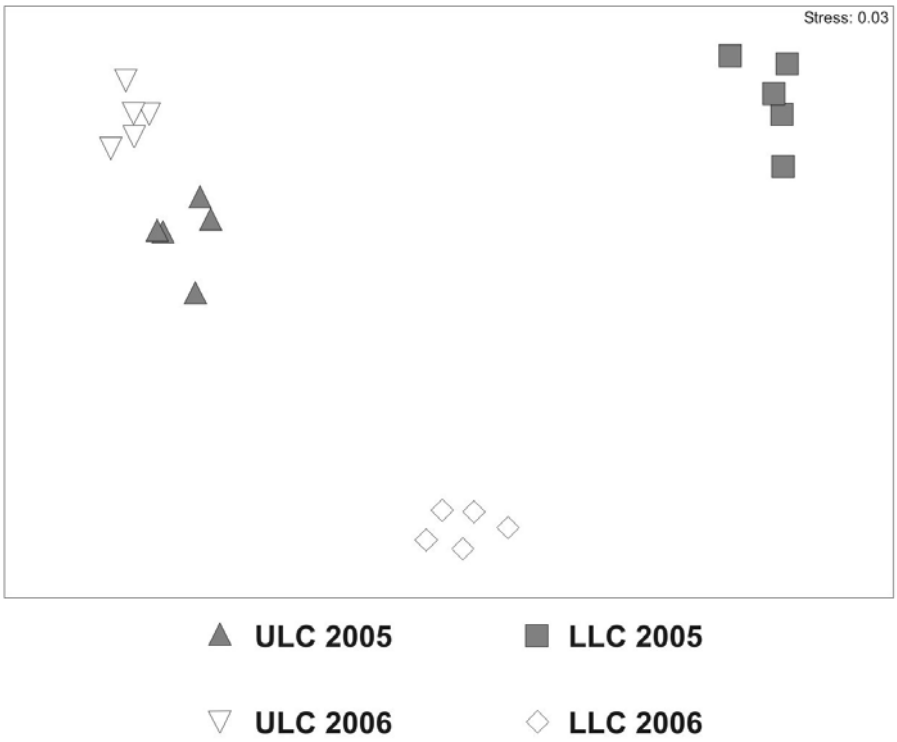

Figure V.3: Non-metric multidimensional scaling (nMDS) ordination plot of invertebrate densities, representing the benthic community structure (without *Dreissena polymorpha*) in the *Dreissena*-habitat at both sampling sites and years.

these differences were mainly chironomids (*Chironominae*) and the caddisfly *Ecnomus tenellus* in fall 2005 and the mayfly *Caenis* spp. in fall 2006 (Table V.4). For the ULC the difference between 2005 and 2006 was lower (dissimilarity ~ 29 %) in comparison to the LLC (dissimilarity ~ 35 %). In the LLC differences between years were mainly caused by the *Chironominae* and *E. tenellus*, in the ULC based on *Caenis* spp. (Table V.4). The amphipod densities showed a similar pattern as the community between the years. In the ULC the density of the dominant amphipod *D. villosus* was approximately 250 ± 100 individuals m^{-2} (mean ± SD; t-test, p = 0.56; Fig. V.4a). *G. roeselii* was not abundant in the *Dreissena*-habitat of the ULC. In the LLC *G. roeselii* decreased from 700 ± 200 individuals m^{-2} in fall 2005 to 90 ± 25 individuals m^{-2} in fall 2006 (t-test, p < 0,001; Fig. V.4b); *D. villosus* was only abundant in single individuals (10 ± 9 individuals m^{-2}).

Table V.4: Contribution of single macroinvertebrate species/groups to the dissimilarity between Upper Lake Constance (ULC) and Lower Lake Constance (LLC) or sampling years revealed by SIMPER analyses. Only species with a contribution > 10 % were included in the table to refer to the most important macroinvertebrates. Arrows describe higher (↑) or lower (↓) density in the mentioned year or site.

Site	Year	Dissimilarity	Species	Contribution	Density
ULC	2005 vs. 2006	28.8 %	*Caenis* spp.	41.9 %	2006 ↑
			Chironominae	22.5 %	2006 ↑
LLC	2005 vs. 2006	35.4 %	*Chironominae*	40.2 %	2006 ↓
			E. tenellus	16.5 %	2006 ↓
			Caenis spp.	14.7 %	2006 ↓
ULC vs. LLC	2005	41.7 %	*Chironominae*	50.2 %	LLC ↑
			E. tenellus	14.8 %	LLC ↑
	2006	39.0 %	*Caenis* spp.	51.6 %	LLC ↓

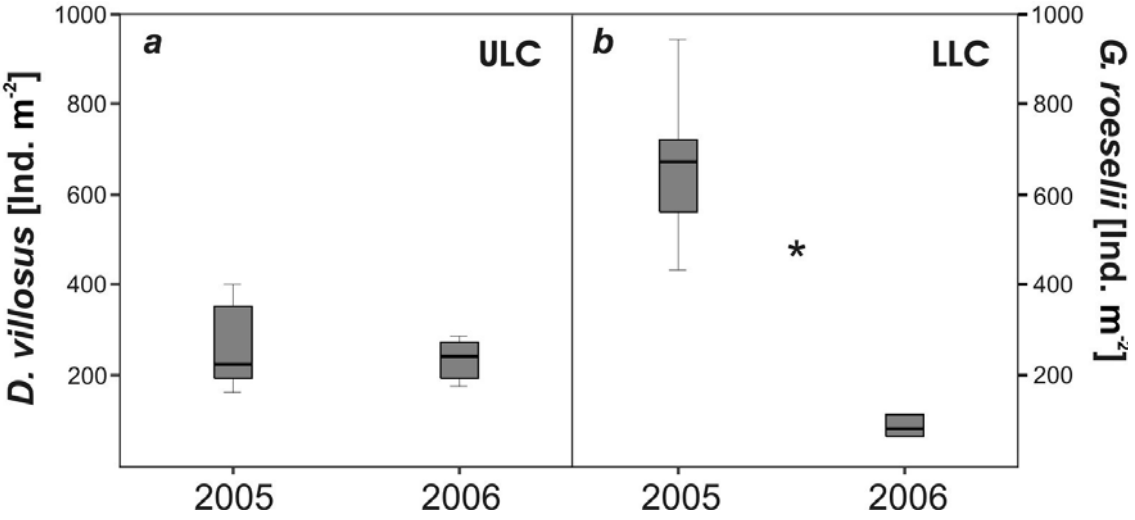

Figure V.4: Comparison of *D. villosus* density in Upper Lake Constance (ULC) and *G. roeselii* in Lower Lake Constance (LLC) between sampling years. Shown are the median densities ± the upper and lower quartile. Asterisk indicates a significant difference revealed by a t-test.

Chara spp.-habitat

In both lake basins the $\delta^{13}C$ and the $\delta^{15}N$ (i.e. trophic position) signature of the primary producer *Chara* spp. differed not significantly between 2005 and 2006 (Fig. V.5; Table V.5). The carbon signatures were around −19 ‰ and −16 ‰, respectively; and the $\delta^{15}N$ values were around 6 ‰ at both sampling sites in both years. Chironomids had a similar carbon signature (ULC: −26.6 ‰ (2005) to −25.6 ‰ (2006) & Lower Lake Constance −23 ‰) and trophic position signature (Upper Lake Constance: 2.3 & Lower Lake Constance 2.6 – 2.7) between years. Overall, chironomids occupied a trophic position slightly above the baseline of primary consumers, and the $\delta^{13}C$ signature was clearly higher than the benthic *Chara* spp.-signature. Between years, both carbon signatures and trophic position in the *Chara* spp.-habitat of the dominant amphipod species in the ULC, *D. villosus*, decreased from −35 ‰ to −24 ‰ and 3.7 to 2.3, respectively. Hence, the feeding strategy of *D. villosus* changed from a diet on pelagic resources and a top-predator in 2005 to a more benthic diet and almost

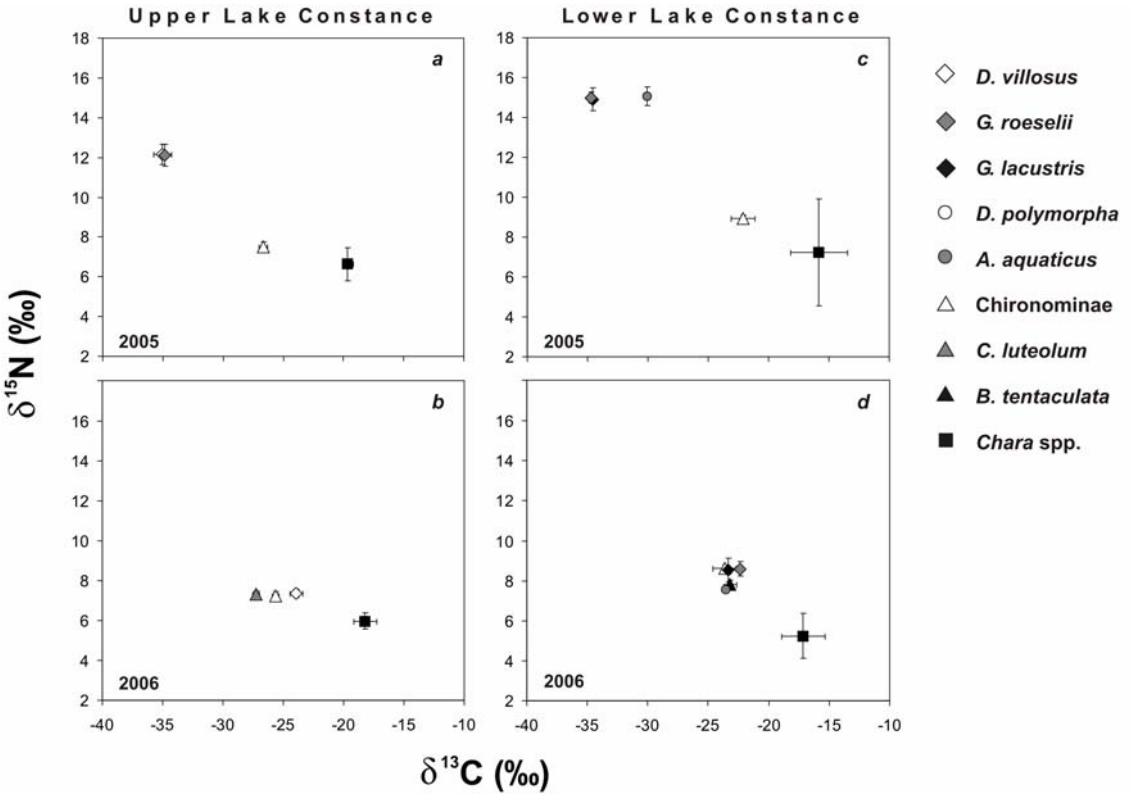

Figure V.5: Isotopic composition (relationship between $\delta^{15}N$ and $\delta^{13}C$) of collected macroinvertebrate species and primary producers in the *Chara* spp.-habitat of the Upper and Lower Lake Constance in fall 2005 and 2006. Errors bars in horizontal and vertical direction indicate the standard deviation of

replicates.

a primary consumer in 2006. In Lower Lake Constance, the dominant amphipod species *G. roeselii*, a second amphipod species *G. lacustris* and the isopod *A. aquaticus* were sampled. Both, carbon signature and trophic position decreased significantly for these three species between fall 2005 and 2006. In 2005 the carbon signature was similar to the seston value in the *Dreissena*-habitat (*G. roeselii* and *G. lacustris*, approx. −35 ‰; *A. aquaticus*, −30 ‰; Table V.5). All three species had a similar carbon signature as the chironomids ($\delta^{13}C$ approximately −23 ‰) in 2006. *G. roeselii*, *G. lacustris* and *A. aquaticus* occupied a trophic position of 4.5 in fall 2005, and were similar to the trophic position (2.3 – 2.6) of chironomids in fall 2006.

Table V.5: Comparison of carbon signatures and trophic positions in the C*hara*-dominated habitats between Upper Lake Constance (ULC) and Lower Lake Constance (LLC), and between sampling years; differences were analysed by t-tests and significance level was corrected to *Bonferroni* for each site. Asterisks indicate significant differences after correction.

		$\delta^{13}C$			Trophic position		
Site		2005	2006	p-value	2005	2006	p-value
ULC	*D. villosus*	-35.0 ± 0.7	-23.9 ± 0.5	< 0.001*	3.7 ± .02	2.3 ± .01	< 0.001*
	Chironominae	-26.6 ± 0.4	-25.6 ± 0.3	0.001*	2.3 ± 0.1	2.3 ± 0.1	0.10
	Chara spp.	-19.6 ± 0.4	-18.2 ± 0.9	0.02	2.1 ± 0.3	1.9 ± 0.1	0.15
LLC	*G. roeselii*	-34.7 ± 0.2	-22.4 ± 0.2	< 0.001*	4.5 ± 0.1	2.6 ± 0.1	< 0.001*
	G. lacustris	-34.6 ± 0.4	-23.3 ± 0.3	< 0.001*	4.5 ± 0.2	2.6 ± 0.2	< 0.001*
	A. aquaticus	-30.1 ± 0.2	-23.6 ± 0.2	< 0.001*	4.5 ± 0.1	2.3 ± 0.0	< 0.001*
	Chironominae	-22.1 ± 1.0	-23.6 ± 1.0	0.04	2.7 ± 0.1	2.6 ± 0.1	0.03
	Chara spp.	-15.8 ± 2.3	-17.2 ± 1.8	0.27	2.2 ± 0.8	1.6 ± 0.3	0.18

Comparison of the Dreissena- and Chara spp.-habitat

Carbon signatures and trophic positions within one year and changes between sampling years were rather similar comparing the two habitats, in spite of significant differences of some species between the two sampled habitats (cp. Fig. V.1 and Fig. V.5). In ULC stable isotope signatures (carbon signatures and trophic positions) of chironomids did not differ between the *Dreissena*- and the *Chara* spp.-habitat in both years (Table V.6). In LLC, the trophic position of chironomids did not differ between years. The carbon signatures of chironomids in both years were with 2 ‰ more depleted in the *Chara* spp.-habitat than in the *Dreissena*-habitat. The same pattern occurred for the isopod *A. aquaticus* in the LLC in fall 2006. The carbon signature of the isopod was 2 ‰ more depleted in

Table V.6: Comparison of carbon signature and trophic position at the two sites Upper Lake Constance (ULC) and Lower Lake Constance (LLC) between habitats; differences were analysed by t-tests and significance levels were adjusted after *Bonferroni* for each site. Asterisks indicate significant differences after correction. Note variation in replicates for *Centroptilum luteolum* (*Dreissena*-habitat, n = 3).

		$\delta^{13}C$		Trophic position	
Site		Sampling year	p-value	Sampling year	p-value
ULC	*D. villosus*	2005	0.18	2005	<0.001*
		2006	0.008*	2006	<0.001*
	Chironominae	2005	0.09	2005	0.04
		2006	0.47	2006	0.96
	C. luteolum	2006	0.32	2006	<0.001*
LLC	*G. roeselii*	2005	0.03	2005	0.31
		2006	<0.001*	2006	0.11
	Chironominae	2005	0.002*	2005	0.03
		2006	0.001*	2006	0.08
	B. tentaculata	2006	0.001*	2006	0.17

A. aquaticus	2006	0.001*	2006	0.17

the *Chara* spp.-habitat and the trophic position did not differ. *D. villosus* had a similar carbon signature between habitats in both years (ULC) and only in 2006 the difference (less than 1 ‰) was significant. The trophic position for *D. villosus* differed between habitats (Table V.6). In 2005 the amphipod was one trophic position higher in the *Dreissena*-habitat than in the *Chara* spp.-habitat, but in 2006 the trophic position of *D. villosus* was near the trophic baseline of a primary consumer in both habitats (ULC). However, differences between years were stronger than between habitats (see above). *G. roeselii* in LLC had almost the same signatures in both habitats for both years. Only in 2006 the carbon signature in the *Chara* spp.-habitat was significant depleted by 1 ‰ in comparison to the *Dreissena*-habitat. In the sampling season 2006, additional lake-specific macroinvertebrate species were sampled in both habitats. In the Upper Lake, the grazing mayfly *C. luteolum* was found in both habitats (cp. Fig V.1b and V.5b; Table V.6). The carbon signature of the mayfly did not differ between habitats ($\delta^{13}C$ approximately −27.5 ‰) and the trophic position was the same as for a primary consumer in the *Dreissena*-habitat (2.0 in mean) and just slightly higher in the *Chara* spp.-habitat (2.3 in mean). In LLC, the grazing snail *Bithynia tentaculata* was sampled in both habitats (cp. Fig V.1d and V.5d; Table V.6) and the trophic position was around 2.5 in both habitats, which is slightly above the primary consumer level. Carbon signature of the snails differed between habitats and was 2 ‰ more depleted in the *Chara* spp.-habitat than in the *Dreissena*-habitat, but were close to the benthic periphyton signature of the *Dreissena*-habitat (LLC).

Discussion

Food web structure in the Dreissena-habitat

Filtration of zebra mussels increases the amount of organic matter in the benthic zone, which is often hypothesized to be responsible in leading to higher abundance of benthic macroinvertebrates (Stanczykowska et al. 1976, Stewart and Haynes 1994, Stewart et al. 1998). We hypothesized the benthic food web structure to depend on zebra mussel density and organic matter biodeposited by zebra mussels as a major resource for benthic invertebrates in *Dreissena*-

dominated habitats. Carbon signature and trophic position of amphipods were positive related to available zebra mussel biodeposits. The amphipods seem to benefit indirectly from zebra mussel biodeposited material by feeding on other macroinvertebrates. Hence, the zebra mussel can be pronounced as *keystone species* for the benthic food web by shifting of pelagic resources to the benthic zone.

The diet of most sampled invertebrate species differed between years as shown by the stable isotope data, indicating a strong variation in food web structure. Especially the diet of amphipods (*D. villosus* and *G. roeselii*) is significantly based on the production of biodeposits by zebra mussels, and therefore on zebra mussel density. Previous studies demonstrated that zebra mussel biodeposition material affects habitat choice and growth of amphipods (González and Burkart 2004, Gergs and Rothhaupt 2008a, 2008b). In the field, the presence of zebra mussels is often associated with an increase of macroinvertebrates which benefit from the increased structural substrate complexity and from the activity of mussels (Stewart and Haynes 1994, Silver Botts et al. 1996, Mörtl and Rothhaupt 2003). It has been shown that the diet of the amphipod *G. fasciatus* is, at least partly, based on *Dreissena* biodeposition material (Limén et al. 2005). This is in agreement with our stable isotope data showing that organic matter biodeposited by *D. polymorpha* contributed to the diet of amphipods under natural conditions, dependent on the zebra mussel density. Both amphipod species also showed a clear diet shift in respect to the trophic position. *D. villosus* and *G. roeselii* were top-predators in 2005, and shifted to primary consumers in 2006. This can be explained as follows: High biodeposition rates of organic material increase the availability of resources for benthic invertebrates, resulting in higher secondary production. Subsequently in the benthic food web, higher abundances of predators are found in the presence of zebra mussels (Dusoge 1966). The estimation of the trophic level of amphipods gives new insights in their feeding strategy under field conditions. Especially the shift of the invasive *D. villous* to a primary consumer in autumn 2006 is in disagreement with other studies, where this species is characterized as a predator (Dick and Platvoet 2000, Dick et al. 2002, Kinzler and Maier 2003).

However, the prey organisms of the predacious amphipods are still unclear, because no other benthic primary consumers with a pelagic carbon signature were found in autumn 2005. Further investigations are needed to identify the trophic link from zebra mussel biodeposited material to the trophic position of predators. Like amphipods, chironomids are often pronounced to benefit from zebra mussel invasion and might provide a link to higher trophic levels (Mitchell et al. 1996, Silver Botts et al. 1996). Our stable isotope results show that pelagic resources contribute approximately 20 to 50% to the diet of chironomids. This indicates little dependence on zebra mussel biodeposition material in autumn, but we assume that it might vary seasonally and be highest during summer, when zebra mussel biodeposition rate is highest (Gergs et al. 2009). Mörtl and Rothhaupt (2003) found an increased chironomid density on substrates with living zebra mussels in comparison to substrates with empty mussel shells in a field experiment during summer. In Lower Lake Constance, the differences in the benthic macroinvertebrate structure were mainly caused by *Chironominae*, the mayfly *Caenis* spp. and the caddisfly *E. tenellus* with higher abundances in 2005 than 2006. This might be a hint for a connection of the may- and caddisfly to a *Dreissena*-biodeposition based food web and the trophic link to the amphipods.

Stable isotope analyses of zebra mussels revealed an unexpected high nitrogen signature in the *Dreissena*-habitat in fall 2005. The diet of zebra mussels can contain considerable amounts (>50%) of detritus beside the filtered seston (Garton et al. 2005), which can lead to an ingestion of processed material. For example, microbial activity and bacterial density in biodeposited material of zebra mussel can increase within a few days and accelerate its degradation (Izvekova and Lvova-Katchanova 1972, Roditi et al. 1997, Strayer et al. 1999). Simultaneously, a microbiocenosis consisting of ciliates, nematodes and rotifers could develop (Walz 1978b), which might increase nitrogen signature of animals feeding on the biodeposited material.

Differences between lakes

The density of gammarid amphipods is often positively related to the density of zebra mussels (e.g. Wisenden and Bailey 1995). In our study, the density of the dominant amphipod *G. roeselii* was also related to the production of zebra

mussel biodeposits in Lower Lake Constance (Fig. V.2 and V.4), whereas in the Upper Lake Constance, the density of the invasive amphipod *D. villosus* was constant and not connected to the production of zebra mussel biodeposits. Similar to the amphipod density, changes in the whole macroinvertebrate community were observed (Fig. V.3 and V.4; Table V.4). In comparison to LLC, the differences between sampling years 2005 and 2006 were low in ULC. Zebra mussel density was similar for both sites within one sampling year, as indicated from the estimated biodeposition production. Besides the impact of zebra mussels, other factors seem to be important in determining community structures in the two lake basins. The sampling site in the ULC is less wind-exposed with a north-west shore. The sampling site in the LLC is more wind-exposed at an east-shore, and therefore more turbulent, which influences the benthic community structure (Scheifhacken et al. 2007). Zebra mussels can tolerate and stabilize turbulent conditions, which may facilitate settlement of other invertebrates (Dusoge 1966, Wisenden and Bailey 1995). Due to zebra mussel stabilization, the strong differences in the benthic community in LLC might be an interaction of the zebra mussel density and wind-exposition, whereas in the more sheltered sampling place in the Upper Lake the stabilizing effect of zebra mussel might be of less importance.

Differences between habitats

Stable isotope signatures of many macroinvertebrate groups in the *Chara* spp.-habitat were comparable to those in the *Dreissena*-habitat in both years. We found a diet shift of amphipods from high to low trophic positions and a shift between a pelagic carbon signature to a more benthic signature in both habitats between 2005 and 2006. We assume that the *Chara* spp.-habitats are affected by zebra mussel biodeposition material of surrounding *Dreissena*-habitats. Macrophytes (like *Chara* spp.) form areas with low turbulence and therewith enhanced sedimentation of suspended material (Schulz et al. 2003). The wave conditions at the sampling site in Upper Lake Constance are affected by ship-induced (especially during summer) and wind-induced waves (Hofmann et al. 2008). Water velocities caused by waves exceeded the threshold for sediment re-suspension at 33% of the time; ship- and wind-waves contribute 58% and 42% of this re-suspension, respectively (Hofmann 2007). In LLC, the influence

of ship-waves might be lower, but the east shore at the sampling site is more susceptible to wind-waves. Therefore, the availability of resources in the *Chara* spp.-habitat and associated effects on the benthic community structure might be significantly affected by re-suspension of zebra mussel biodeposition material and re-sedimentation in areas adjacent to *Dreissena*-habitats.

In conclusion, our study reveals the significance of organic matter deposited by zebra mussels under natural conditions for the benthic food web structure and benthic invertebrate community. In the *Dreissena*-habitat, the diet of amphipods was significantly related to the availability of zebra mussel biodeposits. In the *Dreissena*-rich year (2005), we found high trophic positions and pelagic carbon signatures of the amphipods. Additionally, at the wind-exposed sampling site in Lower Lake Constance, the density of the amphipod *G. roeselii* was related to the production of biodeposits by the zebra mussel and the whole benthic community showed a clear shift to less *Caenis* spp., *Chironominae* and *E. tenellus* with less *Dreissena* biodeposits. The impact of zebra mussel biodeposits was also found in the *Chara* spp.-dominated habitat indicating also an influence of *Dreissena*-habitats on surrounding areas. Our results support the hypothesis that the filtration activity of *Dreissena polymorpha* and the associated biodeposition of organic matter provide a basis of a benthic food web.

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Chapter VI

General Discussion and Perspectives

In the littoral of many lakes which were invaded by *Dreissena polymorpha*, the zebra mussel became the most abundant macroinvertebrate within few years after their introduction (Siessegger 1969, Stewart and Haynes 1994). The pelagic larvae of the mussel attach to all potential hard substrata and hence, colonize and alter a wide range of habitats. Changes in benthic community structure in presence of the zebra mussel are well documented. Increase in abundance of many macroinvertebrate species is attributed to *ecosystem engineering* by the habitat structuring effect of the mussel shells. As outlined in the introduction, only little is known about the trophic impact of the biodeposition activity of the zebra mussel and therefore its role as a *keystone species* for the benthic food web. With this thesis, I show that the shift of organic material by the filtration and biodeposition activity of zebra mussels (*D. polymorpha*) to the benthic habitat strongly affects the food web structure and marcoinvertebrate communities. Combining the concepts of *keystone species* and *ecosystem engineering*, the presence zebra mussel is crucial for the formation of the benthic community and can be called a *keystone engineer*. Furthermore, these results might not only be relevant for Lake Constance but also for many other aquatic systems invaded by the zebra mussel.

As proxies for macroinvertebrates, which might benefit from zebra mussel biodeposition, I chose the two dominant amphipod species in Lake Constance, *Gammarus roeselii* and *Dikerogammarus villosus.* Amphipods are known to benefit from the presence of zebra mussels (Griffiths 1993, Stewart and Haynes 1994) and in case of biomass, they are one of the most important species groups in the littoral zone of Lake Constance after zebra mussels (Baumgärtner

2004, Mörtl 2004). In the last years, the amphipod dominance in the Upper Lake shifted from the native *G. roeselii* to the invasive *D. villosus.* The invasive species did not colonize the Lower Lake in that degree yet.

As an initial step, the first laboratory experiments (**Chapter II**) were designed to gain knowledge about effects caused by the presence of *D. polymorpha*. These experiments showed the importance of the increased structural complexity provided by the mussel shells, but also the positive effect of the biodeposited material or/and associated chironomids on the habitat-choice of both amphipod species. In contrast, kairomones released by the mussels were not responsible for the attractiveness of living zebra mussels, as elucidated in y-maze experiments. These results were the first hints supporting the *Dreissena*-biodeposition based food web, which lead me to investigate the role of the zebra mussel biodeposits as food source for amphipods (**Chapter III**). To estimate the quality of this diet, two other potential diets were tested additionally. Zebra mussel biodeposited material had an intermediate quality for both amphipod species compared to the high-quality food chironomids (as proxy for animal material) and the low-quality food conditioned alder leaves, as revealed by the feeding rate, assimilation efficiency and growth rate. Overall, there were no crucial differences between the two amphipod species regarding the suitability of the three diets. The habitat-choice experiment revealed no evidence for a carnivorous feeding strategy of the indigenous *G. roeselii*, but both amphipod species clearly showed a preference to the animal food resource in the feeding and growth experiments. Gammarid amphipods were classified as shredders for a long time (e.g. Bärlocher and Kendrick 1973, Cummins and Klug 1979). Their potential predatory impact only got more into focus within the last years, and might also be important under natural conditions (Kelly et al. 2002a). This is supported by our stable nitrogen isotope results. With these data a predator trophic position was estimated for the amphipod *G. roeselii* in autumn 2005 (**Chapter V**). The invasive amphipod *D. villosus* is known as a strong predator, which is able to displace other invertebrates (Dick and Platvoet 2000, Dick et al. 2002) and can have a trophic position comparable to some predatory fish species (Marguillier 1998). *D. villosus* reacted to the presence of chironomids, but not only to the detritus food source in the habitat-choice experiments. Our feeding and growth experiments revealed inconsitent results. Even if animal

material was a better food resource for *D. villosus*, the invasive amphipod can feed, survive and even grow on a non-animal diet. Since the experiments were performed in a no-choice approach, they might be devaluated as artificial results. *D. villous* was a primary consumer under field conditions in autumn 2006 indeed, although the benthic habitat provided potential macroinvertebrate prey (**Chapter V**). It seems to be almost impossible to classify amphipods into discrete functional feeding groups, since their feeding strategy is depending on several factors and therefore shows a great plasticity (MacNeil et al. 1997). The question arises, which factors affect the current feeding strategy? These factors might not be only important for the two amphipod species, tested in this thesis, but also for a wide species range of the benthic community. For example, the amphipod *Gammarus pulex* has a reduced feeding rate under stress by toxicants or parasitic infestation (McCohan et al. 1991). Such stress situations might also lead to shifts in the feeding strategy. The invasive amphipod *D. villosus* has a lower predatory impact on the indigenous amphipod *G. roeselii* at low water temperatures (John Hesselschwerdt, personal communication). However, it is still under investigation, what causes the plasticity of invertebrate feeding strategies and further laboratory experiments, studying single potential factors, are needed.

Although the zebra mussel biodeposition material is of importance for amphiods under standardized laboratory experiments and would give explanations for the huge impact of zebra mussels, these results cannot be transferred to the field directly. It was important to estimate the seasonal amount and quality of the zebra mussel biodeposits in a field experiment. To evaluate the mechanism of the field biodeposition production, additionally two laboratory experimental series were conducted (**Chapter IV**). We found that the zebra mussel biodeposition rate is dependent on the seston concentration as also found in short-time experiments of other studies in more eutrophic systems (Reeders and Bij de Vaate 1992, Roditi et al. 1997). The second important factor affecting biodeposition rates under natural conditions was the season and therefore the phytoplankton community composition. Since digestibility of different algae is related to zebra mussel biodeposition rate (Bastviken et al. 1998, Naddafi et al. 2007), the trophic status of an aquatic system might be important besides the seasonal differences causing the amount of zebra mussel biodeposits. The field

estimation of biodeposition rates demonstrates that zebra mussels alter strongly the resource flow in aqutic systems. The mussels provide a huge amount of a suitable resource for benthic macroinvertebrates, which are able to use this resource, as shown for amphipods (**Chapter III**) and chironomids (Izvekova and Lvova-Katchanova 1972). Seasonal variations in zebra mussel biodeposition rates should cause seasonal variation in the importance of this food source for benthic invertebrates. During winter not only the seston amount is low, but also the density of zebra mussels, which is reduced by waterfowl predation (Cleven and Frenzel 1993, Werner et al. 2005). In spring, seston concentration increases, and small algae dominate (Gaedke 1998, Kümmerlin 1998) which are of high preference and well digestable for zebra mussels (Bastviken et al. 1998, Naddafi et al. 2007), resulting again in lower biodeposition rates. Importance should be greatest especially during summer and early autumn, because of the high biodeposition rates and density of zebra mussels.

While quality of the biodeposited material (i.e. phosphorous content) was statistically equal to the seston in the field experiment, a significant phosphorus depletion of the biodeposited material was found in the laboratory experiments at low algae concentrations, where faeces are the dominant part of the zebra mussel excretions (Walz 1978a). The field results were presumably caused by environmental factors and the long exposition time of the traps. To investigate wether the stoichiometric changes of the laboratory experiments can be transfered to the field conditions, it is important to reduce the exposition time. Furthermore, traps should be exposed at a sampling site with less contact to littoral zones. Resuspended material of the littoral zone distorts the measurement of potential stoichiometric effects of zebra mussels on the biodeposits. Food quality is not only determined by the C:N:P-stoichiometry. Especially polyunsaturated fatty acids (Wacker and von Elert 2001, 2004) and sterols (Martin-Creuzburg et al. 2005) are important factors determining food quality. To my knowledge, the role of fatty acids and sterols in zebra mussel biodeposits is largely unknown. It is also not known if differences in biodeposits quality affect invertebrate growth. This might be a valuable perspective in understanding the importance of this resource for benthic communities.

To discover if the shift of pelagic sources to the benthic habitat by the zebra mussels filtration and excretion affects the benthic invertebrate food web, I designed a field sampling program in early autumn. We used a quantitative sampling method (Baumgärtner 2004, Mörtl 2004) to characterize the benthic community and the stable carbon and nitrogen isotope signature to reveal trophic relationships (**Chapter V**). Isotopic signatures arise with a time-delay to the food-uptake (Vizzini and Mazzola 2003). Attending this effect, sampling was performed in early autumn, near the end of the period with highest biodeposition rates of *D. polymorpha*. The late sampling date enabled a longer feeding time and increased the probability to detect a dependence on zebra mussel biodeposits for macroinvertebrates. For the two sampling years 2005 and 2006, the contribution of pelagic resources to the diet and trophic position of the amphipods was significantly related to the production of zebra mussel biodeposits, but not to the periphyton production. This leads to the conclusion that presence of zebra mussels strongly affect the benthic food web structure, with amphipods as predators in a food web based on zebra mussel biodeposits. To the current state of knowledge, the link to higher trophic positions is still unknown. Since chironomids are known to benefit from zebra mussel biodeposits (Izvekova and Lvova-Katchanova 1972), I hypothesized that they feed directly on food resources supplied by the filtration and excretion of zebra mussels under natural conditions. The stable carbon isotope signature revealed that the diet of sampled chironomids contains only up to 50% of pelagic resources and was not significantly related to production of zebra mussel biodeposition material. Since the sampling was done only in autumn, no statement is possible, to what extend chironomids feed on zebra mussel biodeposits throughout the year. A shorter time scale between the samplings would be needed to investigate the seasonal changes of the benthic community in dependence on zebra mussels. Additionally, a broader sampling of macro-invertebrate species for stable isotope analysis would be necessary to reconstruct a food web based on the biodeposited material. Stable isotope signatures cannot show single prey species of a predator and gut content analysis might be necessary, which is a common method in fish ecology (Murphy and Willis 1996). In contrast to fishes, invertebrate predators (for example amphipods) chop their prey into small pieces before ingestion, which

disables the classification of prey species (personal observation). For future work, genetic gut-content analyses might be a possibility to investigate trophic relationships between macroinvertebrate species in addition to stable isotope analyses. This method was already successfully used to identify the diet of whale sharks (*Rhincodon typus*), where morphological analysis had failed to make a prey species identification (Jarman and Wilson 2004).

This thesis clearly shows that the zebra mussel (*Dreissena polymorpha*) is a *keystone engineer*. Mussel shells increase structural benthic habitat, which is case of autogenic *ecosystem engineering*. Food availability, mediated directly or indirectly through biodeposited material, is a factor by which amphipod abundances are increased in the presence of *Dreissena* (**Chapter II**) and provides also a suitable food source for amphipods (**Chapter III**). Furthermore, the filtration of zebra mussels shifts pelagic primary production to the benthos and is a frequent resource for benthic macroinvertebrates under natural conditions (**Chapter IV**). As a result, the food web structure is depending on the presence of zebra mussels (**Chapter V**), and is therefore a *keystone species* for benthic communities. In summary, this thesis confirms the hypothesis of a *Dreissena*-biodeposition based food web (Stewart and Haynes 1994, Mitchell et al. 1996).

As implied in **Chapter IV** and **V**, the impact of *D. polymorpha* on the benthic community varies strongly throughout the year. In Lake Constance, zebra mussel density is controlled through predation by diving ducks: a reduction of >90% of the zebra mussel biomass has been observed during winter (Cleven and Frenzel 1993, Werner et al. 2005). In response, the waterbird population increased by three- to four-fold since the invasion of the zebra mussel in the early 1960s (Stark et al. 1999). Remaining juvenile mussels can get adult until next summer, which reproduce again (Walz 1973). Control of zebra mussel density by waterfowl increases seasonal variation of the habitat structure and the amount, and therefore, the importance of biodeposited matter. The *ecosystem engineering* by modulating the benthic habitat is low during winter and increases until summer (Fig. VI.1). The *keystone* function of zebra mussels is a product of mussel density and size, and phytoplankton concentration and amount (as discussed above), with high importance in summer, indermediate in spring, and low food supply for benthic invertebrates during winter. This might lead to a change in the seasonality of the benthic community structure in

dependence of zebra mussels. However, the dimensions of the seasonal variations and the consequences for the benthic community and food web are understudied.

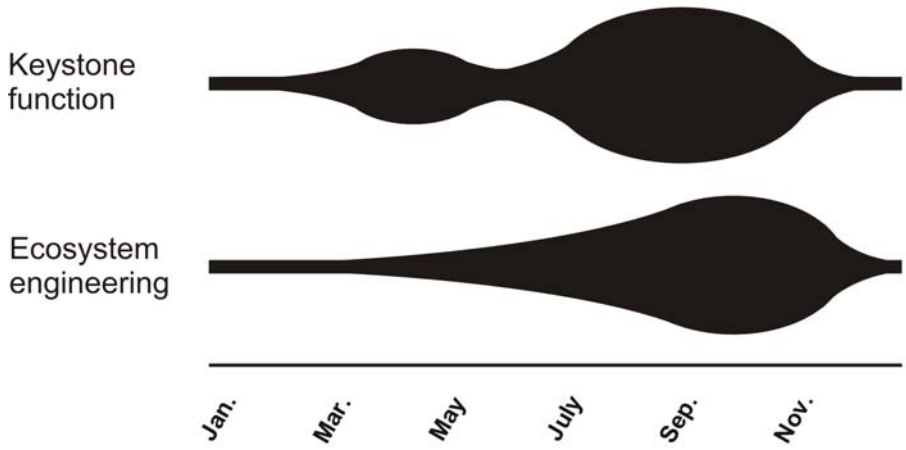

Figure VI.1: Schematic depiction of seasonal variation of importance in autogenic *ecosystem engineering* and *keystone* function of the zebra mussel (*D. polymorpha*). Increased structural complexity in the benthic habitat by the mussel shells is a case of autogenic *ecosystem engineering*. Biodeposition of organic material by the filtration of zebra mussels describes the *keystone* function.

In the last decades, a vast number of studies investigated the benthic effects of zebra mussels, with a consistent positive effect on macoinvertebrates (e.g. Dermott et al. 1993, Griffiths 1993). But a detailed look also shows inconsistent results. For example, contradictionary results occured for amphipods, which were mostly thought only to benefit from the increased structural complexity (Silver Botts et al. 1996, Ricciardi et al. 1997). In Lake Constance, the amphipod *G. roeselii* increased in abundance not only in presence of mussel shells, but also of living mussel (Mörtl and Rothhaupt 2003) and in Lake Erie the amphipod density tended to be higher on living mussel than on mussel shells (Stewart et al. 1998). Chironomids were denoted to profit from the accumulation of organic material by the filtration of zebra mussels (Griffiths 1993, Ricciardi et al. 1997, Mörtl and Rothhaupt 2003). In contrast, our results showed that the chironomids´ diet consists only up to 50 % of zebra mussel biodeposits. Perhaps, certain species are affected by zebra mussels in dependence from other factors (e.g. season, lake trophication) influencing the strength of the *ecosystem engineering* and the function as a *keystone species*.

Thus, the understanding of mechanisms how invading species act and associated species react remains an interesting and exciting field of research. This thesis provided an additional piece of the puzzle mapping of these mechanisms in case for benthic invertebrate community invaded by the zebra mussel (*Dreissena polymorpha*).

Summary

The zebra mussel (*Dreissena polymorpha*) is important for structuring the benthic habitats. As a result of increased structural complexity by mussel shells (*ecosystem engineering*), the benthic habitat is altered with positive consequences for most benthic macroinvertebrate species. However, often not only the increased structural complexity was responsible for the increase in abundance of certain species, but also living zebra mussels had an additionally effect. It is hypothesized that *D. polymorpha* builds a biodeposition-based detritus food web. In this thesis I showed that zebra mussels couple the pelagic primary production to the benthic food web and is therefore also a *keystone species* for benthic habitats. This would be one of few examples for a *keystone engineer*.

Laboratory experiments were conducted to investigate the potential impact of zebra mussels' associated resources on the indigenous amphipod *Gammarus roeselii* and the invasive *Dikerogammarus villosus*. Amphipods were used in the experiments, because these macroinvertbrates are known to benfit from the presence of zebra mussels. Habitat choice experiments showed the importance of the increased structural complexity provided by the mussel shells, but also the effect of the biodeposited material and *Dreissena*-associated chironomids on both amphipod species. *G. roeselii* showed a preference for mussel shells with biodeposited material, and for mussel shells with biodeposited material and chironomids. The invasive and predatory amphipod *D. villosus* showed a preference only for mussel shells with biodeposited material and chironomids. Kairomones released by the mussels were not responsible for the attractiveness of living zebra mussels, as elucidated from the y-maze experiments. The role of the zebra mussel biodeposits as a food source was the starting point of further investigation. To estimate the nutritional value of this diet, two other food sources were tested additionally. Chironomids (as proxy for animal material) had the highest nitrogen and phosphorus contents, alder leaves were depleted in nitrogen and phosphorus, and the stoichiometry of biodeposited material was in between. Both amphipod species showed an intermediate feeding rate, assimilation efficiency and growth rate on zebra mussel biodeposits compared

to the high-quality food chironomids and the low-quality food conditioned alder leaves. There were no crucial differences between the two amphipod species regarding the suitability of the three diets. Not only the pronounced predator *D. villosus*, but also the omnivorous *G. roeselii* clearly showed a preference to the animal food resource in the feeding and growth experiments.

The laboratory experiments showed the potential impact of zebra mussel biodeposited material on both amphipod species, but these results cannot be transferred to the field directly. Until this point of knowledge, neither the seasonal variations in amount and quality of the zebra mussel biodeposits under field conditions, nor the contribution of this resource to the diet of benthic macroinvertebrates was well-known. Subsequently, estimation of biodeposition rates in the field and laboratory were done, demonstrating that zebra mussels provide a suitable resource for benthic macroinvertebrates. By mediating the transfer of pelagic resources into the benthos, zebra mussels provide a high amount of detritus, especially during summer and early autumn, when highest biodeposition rates occurred. Thus, zebra mussel biodeposits might increase benthic secondary production, and are important mediators of the flux of organic matter from the pelagic zone into the benthos. Finally, a combination of stable isotope analysis with quantitative benthos sampling was performed to investigate the influence of zebra mussels to the benthic food web and macroinvertebrate community structure in a *Dreissena*-dominated habitat. The contribution of pelagic resources to the diet and trophic position of the amphipods was positive correlated to the production of zebra mussel biodeposits. This leads to the conclusion that amphipods are the top of a food chain based on zebra mussel biodeposits. Hence, the zebra mussel can be pronounced as *keystone species* for the benthic food web by shifting of pelagic resources to the benthic zone.

In conclusion, zebra mussels influences the benthic macroinvertebrate community not only as an autogenic *ecosystem engineer* by its shells, but also alter the food web structure by biodeposition of pelagic resources to the benthic habitat, and can therefore be also pronounced a *keystone engineer*.

Zusammenfassung

Die Zebramuschel (*Dreissena polymorpha*) ist bedeutend für den Aufbau von benthischen Habitaten. Durch die Muschelschalen wird die strukturelle Diversität des Habitats erhöht (*Ecosystem Engineering*), mit positiven Folgen für die meisten benthischen Makroinvertebraten. Oft ist aber nicht nur eine Strukturveränderung für eine erhöhte Makroinvertebraten-Abundanz verantwortlich, auch die lebenden Muscheln an sich haben einen Effekt. Es wird vermutet, dass *D. polymorpha* durch die Ablagerung (Biodeposition) von organischem Material ein Detritus-Nahrungsnetz bildet. In dieser Arbeit zeigte ich, dass die Zebramuschel die pelagische Primärproduktion an das benthische Nahrungsnetz koppelt und somit eine *Keystone-Art* für das Benthos darstellt. Damit wäre *D. polymorpha* eines der wenigen Beispiele eines *Keystone Engineers*.

Um den Einfluss der einzelnen mit *D. polymorpha* assoziierten Ressourcen auf das Makrozoobenthos des Bodensees zu untersuchen, wurden Laborversuche mit Amphipoda-Arten durchgeführt. Dafür wurden der einheimische *Gammarus roeselii* und der eingewanderte *Dikerogammarus villosus* ausgewählt, da von Amphipoden bekannt ist, von der der Anwesenheit von *D. polymorpha* profitieren zu können. In Habitatwahlversuchen wurde die Bedeutung der Muschelschalen deutlich, wie auch der Einfluss des Biodepositionsmaterials und der mit *D. polymorpha* assoziierten Chironomiden. *G. roeselii* zeigte eine Präferenz zu Muschelschalen mit Biodepositionsmaterial und zu solchen mit zusätzlich gegebenen Chironomiden, während der räuberische *D. villosus* nur die Schalen mit zusätzlich gegebenen Chironomiden präferierte. Von den lebenden Muscheln abgegebene Kairomone hingegen sind nicht für deren Attraktivität verantwortlich, wie sich in Versuchen mit einer Y-Rinne herausstellte. Die Rolle des Biodepositionsmaterials von *D. polymorpha* als Futter wurde dann in weiteren Versuchen genauer untersucht. Um die Qualität dieser Nahrung besser beurteilen zu können, wurden zum Vergleich zwei weitere Futtersorten mit in die Versuche einbezogen. Chironomiden (als Stellvertreter für tierische Nahrung) hatten den höchsten Stickstoff- und Phosphorgehalt, während konditioniertes Erlenlaub den geringsten Gehalt dieser Elemente hatte. Die Stöchiometrie des Biodepositionsmaterials lag dazwischen. Beide Amphipoda-Arten zeigten auf

Biodepositionsmaterial im Vergleich zu Chironomiden als gute und Erlenlaub als schlechte Nahrungsquelle eine mittlere Fraßrate, Assimilationseffizienz und Wachstumsrate. Insgesamt gab es keine grundlegenden Unterschiede zwischen den beiden Arten in der Verwertbarkeit der unterschiedlichen Futtersorten und zeigten beide eine klare Präferenz für die tierische Nahrungsquelle in den Futter- und Wachstumsversuchen.

Die Laborversuche zeigten also, dass das Biodepositionsmaterial der Zebramuschel eine potenzielle Nahrungsquelle für Amphipoda darstellen kann, doch können diese Ergebnisse alleine nicht direkt auf das Freiland übertragen werden. Weder saisonale Veränderungen in Menge und Qualität der Ressource, noch ihr Anteil an der Ernährung von benthischen Makroinvertebraten im Freiland waren bisher untersucht worden. Darauf hin wurde die Biodepositionsrate im Freiland und im Labor quantifiziert. Die Ergebnisse zeigen, dass die Zebramuschel auch im Freiland eine für benthische Makroinvertebraten verwendbare Ressource bereitstellt. Dies gilt insbesondere im Sommer und frühen Herbst, dem Zeitraum mit den höchsten Biodepositionsraten. *D. polymorpha* verstärkt den Transfer von organischem Material aus der pelagischen Primärproduktion hin zum Benthos und könnte also durch die Biodeposition die benthische Sekundärproduktion steigern. Um dies zu überprüfen wurde mit einer Kombination aus Stabile-Isotopen-Analyse und quantitativer Benthosprobenahme der Einfluss der Biodeposition auf das benthische Nahrungsnetz und die Makroinvertebraten-Gemeinschaft in einem *Dreissena*-dominierten Habitat untersucht. Dabei zeigte sich, dass der Anteil an pelagischen Ressourcen in der Ernährung und die trophische Position der Amphipoda positiv mit der Biodepositionsmaterial-Produktion korrelieren. Die Amphipoda profitieren somit indirekt von der Biodeposition und stellen die oberste Ebene eines Nahrungsnetzes dar, indem sie sich von Invertebraten ernähren, die direkt das Biodepositionsmaterial konsumiert. Die Zebramuschel kann somit berechtigt als eine *Keystone Art* für das benthische Nahrungsnetz bezeichnet werden, die pelagische Ressourcen zum Benthos verlagert und dort verfügbar macht.

Zusammenfassend kann gesagt werden, dass die Zebramuscheln benthische Invertebraten nicht nur als autogener *Ecosystem Engineer* beeinflusst, sondern auch das benthische Nahrungsnetz durch ihre Biodeposition erweitert. Damit kann *Dreissena polymorpha* als ein *Keystone Engineer* bezeichnet werden.

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Record of achivement / Abgrenzung der Eigenleistung

All Chapters were exclusively written by myself and contribution of the co-authors is mainly based on improvements and amendment statements with regard of content.

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Chapter 4	Design, field sampling and sample processing were exclusively performed by myself and I contributed to statistical analyses of the data.
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