The Effects of Structural Complexity on Ecological and Evolutionary Processes in Shallow Lake Ecosystems

DISSERTATION

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"No man is an island,
entire of itself,
every man is a piece of the continent,
a part of the main..."

-John Donne, Meditation XVII

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Table of contents

Summary

Most of the world's lakes are small and shallow. In these lakes, littoral zones are of great importance for aquatic food web dynamics as they comprise a large proportion of the whole lake area. Shallow lakes can have two alternative equilibria, either a macrophyte-dominated and clear-water state or a turbid, phytoplankton-dominated state. Littoral zones (defined as the areas colonized by macrophytes) may therefore differ between shallow lakes of alternative stable states in their extent and structural complexity.

Littoral zones act as ecological boundaries, connecting lake ecosystems with their terrestrial surrounding. Pulsed annual fluxes of terrestrial particulate organic carbon (tPOC) in the form of leaf litter enter temperate lake ecosystems at the littoral zone, and lake food webs may obtain a significant proportion of carbon via this allochthonous subsidy. So far, no study has followed the fate of tPOC on a natural temporal and spatial scale.

In the first part of my thesis, I present the results of whole-lake experiments mimicking leaf litterfall. We added maize leaves (*Zea mays*) as an isotopically distinct carbon tracer into one half of a turbid and one half of a clear-water shallow lake, each divided by a plastic curtain. During the subsequent year, carbon isotope values for benthic macroinvertebrates, omnivorous and piscivorous fish were significantly higher with maize additions than in the reference side of each lake, demonstrating experimentally that tPOC was incorporated up to higher trophic levels of the lake food webs. Although I hypothesized that a greater structural complexity in the littoral zone would improve the transfer and processing of tPOC, the food web of the clear-water lake with abundant submerged vegetation did not exhibit a higher reliance on tPOC. I furthermore established the existence of reciprocal fluxes of tPOC back into the terrestrial surroundings. Larval Chironomidae fed on the added maize leaves and after emergence, served as prey for spiders living in the riparian reed belt. These findings indicate a close functional coupling of aquatic ecosystems with the adjacent terrestrial habitats.

In the second part of my thesis, I consider the structural complexity provided by macrophytes as a selective agent, directly and indirectly shaping populations of freshwater fish. The structural complexity of near-shore habitats provides a more diverse set of resources compared to the open-water zones and a resource-driven polymorphism has been reported for numerous fish species along the littoral-pelagic axes of lakes. In my thesis, I studied whether a similar polymorphism occurs among lakes that vary in structural complexity. I further considered predation pressure as a selective trait known to alter the phenotype of an organism. I analyzed the body shape of omnivorous roach (*Rutlius rutilus*) from four shallow lakes, which differed in littoral structural complexity and predator abundance (pike *Esox lucius*). Body shape was divergent among lakes, suggesting that processes previously reported from within single lakes may also be operating at the scale of whole lakes. Roach from the lake with the highest predation pressure showed the most distinct body shape, characterized by a more streamlined body and caudally inserted dorsal fins; features that facilitate predator escape. Surprisingly, diet composition was not associated with morphology and I concluded that a variable morphotype facilitating the efficient uptake of a variety of spatially and temporarily scattered resources seems to be favored in these small aquatic systems.

Altogether, this thesis adds an important body of knowledge to our understanding of the importance of the littoral zone for species interaction and food web dynamics in shallow lake ecosystems.

Zusammenfassung

Die meisten Seen der Welt sind klein und flach. Das Litoral ist in diesen Seen für die aquatischen Nahrungsnetze von besonderer Bedeutung, da es einen großen Anteil an der gesamten Seefläche einnimmt. Flachseen kommen in zwei alternativen stabilen Zuständen vor: Sie sind entweder klar und von Makrophyten dominiert oder trüb und von Phytoplankton dominiert. Folglich unterscheidet sich das Litoral (definiert als die Zone, welche von Makrophyten besiedelt ist) in seiner Ausdehnung sowie strukturellen Komplexität zwischen Flachseen unterschiedlicher stabiler Zustände.

Das Litoral wirkt als ökologische Grenzzone, welche das Ökosystem des Sees mit dem terrestrischen Umland verbindet. In gemäßigten Breiten wird terrestrischer partikulärer organischer Kohlenstoff (tPOC) in Form von Blättern einmal jährlich als gepulster Flux über die Litoralzone in das Seeökosystem eingetragen. Die aquatischen Nahrungsnetze können somit einen signifikanten Anteil ihres Kohlenstoffs über diesen allochthonen Beitrag erhalten. Bisher gibt es noch keine Studie, welche den Weg von tPOC im Nahrungsnetz von Seen im räumlichen und zeitlichen Verlauf verfolgt hat.

Im ersten Teil meiner Doktorarbeit präsentiere ich die Ergebnisse von Großexperimenten zur Simulation des Laubfalls in Flachseen. Es wurden Maisblätter (*Zea mays*) mit im Vergleich zu aquatischen Primärproduzenten hoher Kohlenstoff-Isotopensignatur in jeweils eine Hälfte eines trüben und eines klaren Flachsees eingebracht, welche durch eine Plastikplane geteilt wurden. Im darauf folgenden Jahr waren die Kohlenstoff-Isotopensignaturen von benthischen Makroinvertebraten, omnivoren sowie piscivoren Fischen in den Seehälften der Maiszugabe im Vergleich zu den Referenzseiten signifikant erhöht. Damit wurde experimentell bewiesen, dass tPOC bis in die höheren trophischen Ebenen genutzt wird. Es konnte keine verstärkte tPOC-Nutzung im Nahrungsnetz des klaren Sees mit submersen Makrophyten nachgewiesen werden, obwohl von mir eine erhöhte Umsetzung und Verarbeitung des tPOCs in einer strukturell komplexeren Litoralzone postuliert wurde. Chironomidenlarven fraßen von den zugefügten Maisblättern und wurden nach ihrer Emergenz Beute von Spinnen, welche den ufernahen Schilfgürtel bewohnten. Diese Ergebnisse weisen auf eine enge funktionelle Kopplung von aquatischen Ökosystemen mit den angrenzenden terrestrischen Habitaten hin.

Im zweiten Teil meiner Doktorarbeit habe ich überprüft, ob die durch Makrophyten hervorgerufene strukturelle Komplexität eines Sees als Selektionsfaktor wirkt, der direkt und indirekt Fischpopulationen formen kann. Die ufernahen Habitate mit ihrer strukturellen Komplexität bietet im Vergleich zur Freiwasserzone ein diverseres Nahrungsangebot. Für zahlreiche Fischarten ist ein ressourcenbasierter Polymorphismus entlang der litoral-pelagischen Achse innerhalb von Seen bekannt. In meiner Arbeit habe ich getestet, ob ein ähnlicher Polymorphismus auch zwischen Seen mit unterschiedlicher struktureller Vielfalt auftritt. Des Weiteren habe ich den Prädationsdruck als selektiv wirkendes Merkmal für Änderungen im Phänotyp von Fischen betrachtet. Dazu habe ich die Körperform von omnivoren Rotaugen (*Rutilus rutilus*) aus vier Flachseen untersucht, welche sich in der strukturellen Vielfalt der Makrophyten sowie der Abundanz von räuberischen Hechten (*Esox lucius*) unterscheiden.

Die Körperform der Rotaugen divergierte signifikant zwischen den Seen, was darauf hindeutet, dass die strukturelle Komplexität auch als Selektionsfaktor zwischen Seen wirkt. Hoher Prädationsdruck führte außerdem zu einer starken Änderung der Körperform von Rotaugen hin zu einem stromlinienförmigem Körper und einer caudal angefügten Dorsalflosse, eine Körperform, die die Flucht erleichtert. Überraschenderweise war die Nahrungszusammensetzung nicht mit der Morphologie verknüpft. Offensichtlich wird in diesen kleinen aquatischen Systemen ein variabler Morphotypus gefördert, der die effektive Aufnahme einer Vielfalt von räumlich und zeitlich verteilten Ressourcen erleichtert.

Insgesamt trägt diese Dissertation zum Verständnis der Bedeutung des Litorals für die terrestrisch-aquatische Kopplung, die Interaktion von Arten, sowie die Dynamik von Nahrungsnetzen in Flachseen bei.

Thesis outline

This thesis contains three chapters, and each of the chapter consists of an independent manuscript that is either published (Chapter 3), under review (Chapter 1) or submitted to (Chapter 2) a peer-reviewed journal. Every chapter thus forms a stand-alone unit, including its own introduction, methods, results and discussion sections. In a general introduction section, I provide the overall context of the study and give the purpose of writing this thesis. A general discussion section connects my findings to previous works and provides an outlook for potential perspectives of future studies.

Chapter 1:

Scharnweber, K., J. Syväranta, S. Hilt, M. Brauns, M. J. Vanni, S. Brothers, J. Köhler, J. Knežević-Jarić, and T. Mehner. Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes.

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Chapter 2:

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Chapter 3:

Scharnweber K., K. Watanabe, J. Syväranta, T. Wanke, M. T. Monaghan, and T. Mehner. Effects of predation pressure and resource use on morphological divergence in omnivorous prey fish.

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Chapter 1:

KS carried out the stable isotope analyses, conducted the statistical analyses and drafted the manuscript. JS and MB helped carrying out the stable isotope study and helped with the isotopic mixing model. MJM provided discussion on contribution of tPOC. SB and JK compiled the carbon budget. JKJ and SH conducted the leaf litter bag experiment. TM and SH together with **KS** conceived of the study, participated in its design and coordination and helped to draft the manuscript. All coauthors revised the paper.

Chapter 2:

KS carried out the stable isotope analyses, conducted the statistical analyses and drafted the manuscript. MJV, SH and TM together with **KS** conceived of the study, participated in its design and coordination and helped to draft the manuscript. JD contributed to secondary production estimation. All coauthors revised the paper.

Chapter 3:

KS carried out the morphometric, molecular genetic and stable isotope analyses, conducted most of the statistical analyses and drafted the manuscript. KW established some of the molecular genetics protocol and helped carrying out the genetic study. JS helped carrying out the stable isotope study and helped with the isotopic mixing model. TW carried out the gut content analysis. MTM provided laboratory facilities and discussion on evolutionary processes. TM together with **KS** conceived of the study, participated in its design and coordination, carried out some statistical analysis and helped to draft the manuscript. All coauthors revised the paper.

General introduction

The littoral zone of small and shallow lakes

Historically, limnologists have studied deep lakes and the focus was overall on the pelagic zone (Vadeboncoeur et al. 2002). However, the most common global lake type is small (<1 km^2 surface area) and shallow (<10 m depth) (Downing et al. 2006). Due to the low depth, the entire water column of these lakes is frequently mixed, making them polymictic.

Shallow lakes can exhibit two alternative stable states, either macrophyte-dominated and with clear water, or turbid and phytoplankton-dominated (Scheffer et al. 1993). These alternative stable states are self-sustained by multiple feedback loops (Scheffer and Jeppesen 1998). On the one hand, leaves and stems serve as refugia for zooplankton to hide from predators (Timms and Moss 1984, Burks et al. 2001). For this reason, the biomass of planktonic grazers is increased in macrophyte-dominated lakes and the zooplankton community shifts to large-sized specimens (Schriver et al. 1995), further increasing grazing pressure on phytoplankton. Also, macrophytes and associated epiphytes suppress algal production via competition for nutrients and light, and by exuding allelochemicals (Sand-Jensen and Borum 1991, Hilt and Gross 2008). A further positive effect of vegetation on water clarity is due to the enhanced sedimentation and reduced resuspension of sediments (James and Barko 1990). Vegetation can thus stabilize the clear-water state in shallow lakes up to relatively high nutrient loading rates, before the lake ecosystem shifts to the turbid state (Scheffer et al. 1993). In shallow lakes, the littoral zone comprises a large proportion of the whole lake area (Wetzel 1990) and plays an important role for the whole lake ecosystem (Schindler and Scheuerell 2002). Within this thesis, I define the littoral zone as the area of macrophyte coverage. More specifically, this is the zone that is colonized by floating-leaved, emergent and submerged macrophytes. In shallow lakes, the spatial extent of the littoral zone varies greatly: in the clear-water, macrophyte-dominated state, the littoral zone is pronounced and often extends to the whole lake surface, whereas in the turbid, phytoplankton-dominated state, the littoral zone is mostly limited to a belt of emergent vegetation.

The littoral zone as an ecological boundary

The littoral zone is characterized by a high heterogeneity, associated with the physical structure provided by aquatic plants (Lodge et al. 1988). It acts as the land-water interface and thus as an ecological boundary (Naiman and Decamps 1997). Traditionally, ecological boundaries have been viewed as well defined, and food web dynamics of each compartment have been studied separately. This conceptualization has greatly helped advance our understanding of basic ecological processes within natural ecosystems (e.g. Levin and Paine 1974). However, ecosystem boundaries are often permeable and connect adjacent habitats via physical, chemical and biotic linkages (Polis et al. 1997). Allochthonous matter (i.e. externally produced matter, in contrast to autochthonous matter which is produced internally) thus enters a focal ecosystem, and can substantially affect its populations and food web dynamics (Polis et al. 1997, Leroux and Loreau 2008).

As lakes tend to lie in lowland areas and landscape depressions, one can aspect a strong gravity-driven flux from land-to-lake, primarily through physical transport paths such as run-off, wind and precipitation (Polis et al. 1996). However, fluxes in the opposite direction (from water to land) occur as well and as these fluxes have to overcome gravity, the transfer of material by mobile organisms plays an important role (Vander Zanden and Gratton 2011). Especially important to this process are organisms that have an aquatic-terrestrial life cycle, and thus different ontogenetic stages, occupying different ecosystems or habitats and consuming different resources. This means that amphibians and especially insects may be a potent vector of aquatic resources to the land (Schreiber and Rudolf 2008).

Within the context of global climate change, the role and fate of organic carbon (OC) is of special interest since lake ecosystems are active and important regulators in global carbon cycling (Tranvik et al. 2009). Freshwater systems receive terrestrial carbon in three different forms (Cole et al. 2006):

- as terrestrial dissolved organic carbon (tDOC)
- as terrestrial organisms (tPrey)
- as terrestrial particulate organic carbon (tPOC)

tDOC can enter lakes primarily via surface run-off from the catchment and can be channeled through the lake food web by the direct uptake of heterotrophic bacteria and the subsequent consumption of zooplankton via the microbial loop (Azam et al. 1983, Tranvik 1988, Karlsson et al. 2003, Kritzberg et al. 2004).

tPrey are small organisms (e.g. insects, amphibians and even small reptiles and mammals) that usually fall into the lake by accident, but are frequently ingested by consumers, and especially fish (Mehner et al. 2005, Francis and Schindler 2009).

The third OC form, tPOC, occurs primarily as an input of leaves. These leaves are ingested by zooplankton, but more often by benthic macroinvertebrates (Jones et al. 1999, Karlsson et al. 2003, Pace et al. 2007). Higher trophic levels such as fish and predatory macroinvertebrates can also receive this tPOC via predation (Solomon et al. 2008, Weidel et al. 2008). This input may act as a subsidy, which is defined as “a donor-controlled resource (prey, detritus, nutrients) from one habitat to a recipient (plant or consumer) from a second habitat which increases population productivity of the recipient” (Polis et al. 1997). Most of the benthic macroinvertebrates that are living in lakes are consumers with complex life cycles. When they reach maturity, they will emerge and may serve as prey for riparian predators including bats, birds and arthropods, such as spiders (Jonsson and Wardle 2009, Pope et al. 2009).

To my knowledge, the direct experimental subsidy of tPOC to the food webs of lentic systems is limited to a single study. Bartels et al. (2012) added tPOC as fine particles (cornstarch) to the sediment surface of lake enclosures. However, tPOC in nature enters lakes primarily as leaves (in the form of larger particles), and thus the contribution of tPOC to the food webs of lakes featuring alternative stable states, and its subsequent return to the terrestrial surroundings, has not been adequately traced and quantified.

tPOC predominantly enters lakes at the littoral zone (Wetzel 1992), where it is trapped by the structural complexity provided by submerged macrophytes (Vermaat et al. 2000). I assumed that this effect enhances the food availability for consumers in the littoral zone, as has been shown regarding the structural complexity provided by woody debris (Francis et al. 2007, Roth et al. 2007). However, when the littoral zone is limited in its extent, I assumed that tPOC would be transported into deeper waters by hydrodynamics and gravity, and thus less would be available in the littoral zone. Little is known about the tPOC reliance of consumers in macrophyte-dominated littoral zones of clear-water lakes compared to those living in near-shore zones of turbid lakes.

The littoral zone as a zone of adaptations

In littoral zones, physical structures create more microhabitat types and thus a greater niche space. The physical structures provide a substrate for epiphytic algae, which serve as a resource for herbivorous macroinvertebrates (Gresens 1995). Dead parts of plants also enter the food web via the detrital pathway (Neill and Cornwell 1992, Bunn and Boon 1993). Furthermore, the structural complexity created by macrophytes serves as a refuge for macroinvertebrates from fish predation (Gilinsky 1984, Keast 1984). Vegetated zones thus typically support a greater abundance, biomass, and diversity of macroinvertebrates (Pardue and Webb 1985, Beckett et al. 1992, Hargeby et al. 2007) compared to open waters. For this reason, I assumed that omnivorous fish would feed to a higher degree on macroinvertebrates in lakes where submerged macrophytes are present.

Next to resource availability, shallow lakes also may differ in another fundamental aspect shaping the ecology and evolution of organisms: clear-water lakes are known to have a higher ratio of piscivorous to planktivorous fishes (Persson et al. 1998, Jeppesen et al. 2000). This suggests that the predation pressure on omnivorous prey fish is presumably higher in a clear-water than in a turbid lake. This is because macrophytes play an important role in the life cycle of the Northern pike (*Esox lucius* L.), the top predator of most freshwater fish communities of the northern hemisphere. Macrophytes are especially important for spawning and provide a refuge for juvenile pike (Bry 1996, Grimm and Klinge 1996). Also, their “ambush” style of predation requires cover, for which aquatic macrophytes are preferred (Casselman and Lewis 1996).

Since complex littoral zones are characterized as species-rich habitats of high resource abundance (Lodge et al. 1988), it can reasonably be assumed that specific selective pressures are acting upon the fish living in the extended littoral zone of macrophyte-dominated lakes, whereas different pressures act upon fish in turbid lakes featuring more limited littoral zones. In freshwater fish, segregation in habitat and diet across alternative habitats is common in the early stage of population divergence (Streelman and Danley 2003, Hendry 2009). When differences in resource use are stable over time, natural selection may lead to morphological adaptations and polymorphism (Smith and Skúlason 1996, Puebla 2009).

Divergence of morphs is frequently shown along the pelagic-littoral habitat axis, e.g. arctic charr *Salvelinus alpinus* L. (Malmquist et al. 1992), three-spined sticklebacks *Gasterosteus aculeatus* L. (Schluter and McPhail 1992) and European whitefish *Coregonus lavaretus* L. (Kahilainen and Ostbye 2006). Foraging in the open water is associated with a high search rate for the widely distributed and conspicuous planktonic prey. To facilitate a high attack speed and reduce drag, a more streamlined body is expressed in these morphs. In contrast, foraging in the structurally complex littoral zones results in a lower search rate for the more cryptic benthic prey. Here, a deeper body is optimally adjusted for high manoeuvrability (Anderson 1984, Ehlinger 1989). However, the selective forces in littoral and pelagic habitats which induce the intralacustrine (i.e. within lake) divergence of populations may similarly create an interlacustrine (i.e. among lake) divergence in fish. Evidence for this has been found in three-spined sticklebacks, where littoral morphs of a deeper body form were present in shallow and structurally complex lakes, whereas a pelagic morph of a more streamlined body form was found in deeper lakes of low structural complexity (Aguirre 2009).

Another selective agent, known to alter the phenotype of an organism is predation. These predator-induced morphological defenses (Harvell 1990) have been investigated in numerous taxa, including aquatic invertebrates, such as cladocerans (Dzialowski et al. 2003) and mussels (Grant and Bayly 1981), but also vertebrates such as tadpoles (Relyea 2001). The pioneering work on the plastic morphological response of fish to predators was published by Brönmark and Miner (1992) who showed that Crucian carp (*Carassius carassius*, Nilsson) in lakes with fish predators (pike) develop a deeper body that increases the handling time by predators, and further enhances their escape performance due to a higher muscle mass (Domenici et al. 2008). Also, pumpkinseed sunfish (*Lepomis gibbosus*, L.) were found to increase body depth and the length of the dorsal spines when stimulated by a predation cue (Januszkiewicz and Robinson 2007), and similar predator-induced defenses have been described for perch as well (Persson et al. 1996).

Although several studies have shown the effects of either resource use or predation on the morphology of fishes, it remains poorly understood how these selective forces may act in combination on omnivorous fish between lakes that vary in structural complexity and predation pressure.

Aims of the thesis

The aim of my thesis was to evaluate structural complexity in the littoral zone of shallow lakes from two different perspectives. First, I used a more limnological approach to view the littoral zone as an ecological boundary, important for the processing and cycling of allochthonous and autochthonous carbon. I investigated the influence of littoral zones on the linkage of lake ecosystems and adjacent habitats in lakes featuring alternative stable states, either clear-water and macrophyte-dominated with a pronounced littoral zone or turbid and phytoplankton-dominated with a smaller littoral zone. In particular, I focused on the reciprocal cycle of tPOC between aquatic and terrestrial environments.

Furthermore, I adopted an evolutionary approach to study the small scale divergence of fish populations in small shallow lakes, as well as the selective pressures which are derived from the structural complexity of littoral zones and which act upon omnivorous prey fish.

Chapter 1:

The primary goal of this chapter was to follow the fate of terrestrial organic carbon entering the food webs of a clear-water and a turbid shallow lake at a natural temporal and spatial scale. Thus, as part of the interdisciplinary TERRALAC project, I conducted whole-lake experiments, adding maize leaves (*Zea mays* (L.)) as an isotopically distinct carbon tracer into one half of each lake, both divided by a curtain. I hypothesized that consumers in the clear-water lake with a pronounced littoral zone would be more reliant on tPOC than consumers in the turbid lake.

Chapter 2:

In this chapter, I focused on the subsequent transformation of the experimentally-added tPOC into terrestrial prey, as facilitated by consumers with an aquatic-terrestrial life cycle. I considered Chironomidae midges as vectors which fed as aquatic larvae on maize carbon, and became prey to terrestrial predators (spiders) after emergence. I predicted that a higher proportion of the maize carbon would be transported back to adjacent terrestrial habitats in the macrophyte-dominated lake, compared to the turbid lake where submerged macrophytes were absent.

Chapter 3:

Within this chapter, I focused on the selective pressures acting on an omnivorous fish species that occurs in many lakes and rivers in the European temperate zone, the cyprinid roach (*Rutilus rutilus* L.) (Kottelat and Freyhof 2007). For this fish species, resource polymorphism had previously been demonstrated in two Swedish lakes, where individuals inhabiting the pelagic zone had a more streamlined body and were feeding on zooplankton, while those living in the littoral zone had a deeper body and were feeding on benthic macroinvertebrates (Svanbäck et al. 2008). I thus assumed that similar interlacustrine adaptions would exist in roach populations from differently structured shallow lakes. Furthermore, I expected that morphological divergence would be enhanced by differences in predation pressure.

Chapter 1:

Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes

Scharnweber, K., J. Syväranta, S. Hilt, M. Brauns, M.J. Vanni, S. Brothers, J. Köhler, J. Knežević-Jarić, and T. Mehner

Abstract

Lake ecosystems are strongly linked to their terrestrial surroundings by material and energy fluxes across ecosystem boundaries. However, the contribution of terrestrial particulate organic carbon (tPOC) from annual leaf falls to the trophic dynamics of lake food webs has not yet been adequately traced and quantified. We hypothesized that food webs in clear-water lakes with abundant submerged vegetation would have a higher reliance on tPOC (hence, higher allochthony) than turbid lakes lacking submerged macrophytes, because structural complexity in littoral zones is assumed to facilitate the transfer and processing of tPOC.

We conducted whole-lake experiments in a clear-water and turbid shallow lake of similar eutrophic status. We divided each lake with a curtain, and added maize (*Zea mays*) leaves (as a tPOC load and an isotopically-distinct carbon tracer) into one half of each lake. Maize carbon additions were four-fold higher than the tPOC entering the lakes as natural leaf fall from alder (*Alnus glutinosa*) trees, but were low compared to the annual autochthonous carbon fixation, as estimated from lake carbon budgets. To quantify the reliance of consumers on terrestrial food sources, we measured the stable carbon isotope ratios at all trophic levels from both sides of each lake throughout the year following maize additions.

Carbon isotope values for benthic macroinvertebrates and omnivorous fish were significantly higher with maize additions than in the reference side of each lake, demonstrating experimentally for the first time that tPOC can be traced up to higher trophic levels of lake food webs. Whereas the relative contributions of tPOC from maize to benthic consumer diets were relatively low, the carbon contribution of alder to consumer diets was substantially higher than the contribution of alder to the overall lake carbon budgets. Contrary to our expectation, the overall allochthony of benthic consumers was similar in both lakes and independent of the coverage by submerged macrophytes. This suggests that tPOC may replace autochthonous carbon sources, but is not a subsidy to the food web. Our results support the notion that benthic habitats in small lakes are hotspots for carbon fixation and processing.

Introduction

Lakes have historically been considered ideal model ecosystems due to their well-defined boundaries with the surrounding terrestrial environment. However, recent evidence suggests a tight coupling with terrestrial ecosystems via reciprocal fluxes of energy and nutrients that can strongly subsidize the food webs of recipient habitats (Jones 1992, Cole et al. 2006, Jansson et al. 2007). For many small lakes, terrestrial subsidies may substantially complement autochthonous (internally produced) production (Schindler and Scheuerell 2002, Babler et al. 2011). Both the magnitude and quality of the allochthonous (externally produced) organic matter entering the lake may determine the degree of allochthony of the food web in recipient ecosystems (Marcarelli et al. 2011).

Within the context of global carbon cycling, the role and fate of organic carbon (OC) may be of particular interest because lakes are "hotspots" for C cycling (Tranvik et al. 2009). OC from adjacent landscapes can enter the aquatic food webs in different forms (Cole et al. 2006), for example as dissolved OC (DOC) or as terrestrial prey. The quantitative contribution of these OC-forms to lake food webs is relatively well studied (Karlsson et al. 2003, Kritzberg et al. 2004). The third OC form, terrestrial particulate organic carbon (tPOC), enters lakes primarily at the land-water interface, i.e., the littoral zone (Wetzel 1992), where it is mobilized by microbial communities or ingested by macroinvertebrate shredders or zooplankton (Jones et al. 1999, Karlsson et al. 2003, Pace et al. 2007). By consuming zooplankton and benthic macroinvertebrates, higher trophic levels (fish, predatory macroinvertebrates) may also derive a significant contribution of tPOC (Solomon et al. 2008, Weidel et al. 2008). Compared to the pelagic zone, near-shore habitats comprise a relatively large proportion of the surface area of small, shallow lakes (Wetzel 1992), which is the most common global lake type (Downing et al. 2006). Shallow lakes can exhibit two alternative stable regimes: a clear-water state typified by submerged macrophytes (and thus an extensive littoral zone), or a turbid state typified by a high phytoplankton biomass and a reduced littoral zone (Scheffer et al. 1993). Structural complexity in the littoral zone created by woody debris prevents OC transport by gravity and hydrodynamics to deeper waters, thus enhancing food availability for consumers in the littoral (Francis et al. 2007, Roth et al. 2007). Similarly, macrophytes promote structural complexity and trap tPOC in the littoral zone (Vermaat et al. 2000). It can thus reasonably be hypothesized that consumers in macrophyte-dominated littoral zones of clear-water lakes are more reliant on tPOC than those living in near-shore zones of turbid lakes.

Previous studies estimated the contribution of tPOC to lakes indirectly by using $NaH^{13}CO_3$ as a tracer to enrich the $\delta^{13}C$ of dissolved inorganic carbon used by photosynthetic organisms (Carpenter et al. 2005, Cole et al. 2006, Weidel et al. 2008). This method bears some uncertainty regarding the estimation of allochthony, because the contribution from ‘old’ unlabeled detritus cannot be split into autochthonous and terrestrial origin (Solomon et al. 2008, Weidel et al. 2008) and because addition of $NaH^{13}CO_3$ may not label phytoplankton in the metalimnion (Francis et al. 2011). A recent study included stable isotope ratios of hydrogen to overcome this problem and confirmed high allochthony of macroinvertebrates and intermediate levels of allochthony of fish (Solomon et al. 2011). In contrast, the direct experimental addition of tPOC to lentic systems is limited to a single study. Bartels et al. (2012) added tPOC as fine particles (cornstarch) to the sediment surface of lake enclosures. However, the major contribution of tPOC to temperate lakes is leaf litter, which enters lakes as relatively large particles and is processed via the detrital pathway by macroinvertebrate shredder species. The relative importance of this seasonally pulsed tPOC to benthic and pelagic food webs in lakes therefore remains unquantified. Moreover, to our knowledge no study has investigated whether a tPOC addition at the whole-lake scale can be traced to upper trophic levels such as fish.

In this study, we conducted whole-lake experiments to quantify the potential increase in allochthony in response to the artificial addition of tPOC to two shallow lakes. The study lakes differed with respect to water clarity and the occurrence of submerged macrophytes, and thus the extent of the littoral zone. The lakes were both classified as eutrophic due to comparable, elevated ambient nutrient concentrations (Table

1.1), and hence we expected only a moderate reliance of the food web on allochthonous carbon sources (Carpenter et al. 2005). Both lakes were divided, and tPOC with a distinct $\delta^{13}C$ signature (maize leaves, *Zea mays* (L.)) was added to one side of each lake in autumn. Simultaneously, the lakes received natural tPOC loads from the surrounding landscape. Our experiment therefore mirrored both the type of tPOC that naturally enters the lakes, as well as the spatial and temporal distribution of the natural entry. We hypothesized that the added tPOC could be traced up to higher trophic levels of lake food webs, as indicated by altered stable isotope signatures of benthic and pelagic consumers of lake sides with maize additions, compared to those in the untreated reference sides. Secondly, we compared the contribution of tPOC to the diet of consumers with the contribution of these carbon sources to a lake carbon budget. By this approach we aimed to demonstrate that allochthonous carbon in form of tPOC may be preferentially used relative to its availability in the lakes. Third, we predicted a greater reliance on tPOC, and hence higher allochthony, in the clear-water lake with extended coverage by submerged macrophytes, compared to the turbid lake.

Material and methods

Study sites and experimental design

Our experiment was conducted in two eutrophic, shallow lakes located in northeastern Germany (Table 1.1). Kleiner Gollinsee (hereafter referred to as Gollinsee) is turbid and dominated by phytoplankton, whereas Schulzensee has higher water clarity and approximately 22% of the lake area is colonized by submerged macrophytes (*Ceratophyllum submersum* L.). Both lakes are surrounded by alder trees (*Alnus glutinosa* (L.) Gaert.) and reed stands (*Phragmites australis* (Cav.) Trin. ex Steud.). Floating-leaved macrophytes (*Nymphaea alba* L. and *N. lutea* (L.)) grow in both lakes and cover 3% of the total lake area in Gollinsee and 12% in Schulzensee. In October 2010, we used plastic curtains to divide each lake into two approximately equally proportioned halves, based on their hydrology and morphology (Table 1.1). The curtains were sealed in the sediment and completely isolated the water volume of each half.

Table 1.1. Characteristics of the studied lakes.

Lake	**Stable state**	**Area (m^2)**	**Mean depth (m)**	**Volume of treatment side (m^3)**	**Volume of reference side (m^3)**	**Secchi depth (m)**	**TP (µg L^{-1})**	**Annual C-load by alder leaves (g C m^{-2} yr^{-1})**
Gollinsee	turbid, phytoplankton dominated	33202	1.7	23290	33843	1.1 ± 0.3	38.6 ± 8.1	7.2
Schulzensee	clear, macrophyte dominated	39132	2.3	41777	47793	1.7 ± 0.1	34.1 ± 6.4	6.4

At the beginning of the experiment (November 2010), we added roughly 2 t of coarsely shredded fresh maize leaves and stems (without cobs) into one half of Gollinsee (25 g C m^{-2} yr^{-1}, C content measured by a Vario EL CHNOS Element Analyzer, elementar Analysensysteme GmbH, Hanau, Germany) and 3 t to one half of Schulzensee (28 g C m^{-2} yr^{-1}) (subsequently referred to as treatment sides as compared to non-treated reference sides). The quantity of maize carbon added (per area) was approximately four times higher than the estimated lake-specific areal input of terrestrial carbon by alder leaves, which occurred at the same time as the addition of maize leaves (Table 1.1, Figure 1.1, see Appendix A for Material and Methods of the carbon budget). Maize is a C_4-plant which is naturally enriched in $\delta^{13}C$ (mean $\delta^{13}C$ -13.5 ± 1.8‰ (SD) compared to -28.4 ± 0.7‰ in alder leaves) (Smith and Epstein 1971). This difference enables the tracking of added tPOC which is incorporated into the food web (Bartels et al. 2012), which is useful because $\delta^{13}C$ ranges of autochthonous resources and terrestrial C_3-plants often overlap (Meyers and Lallier-Verges 1999). To demonstrate that maize leaves are processed at a rate comparable to that of alder and reed leaves, we conducted a litter bag experiment with maize leaves, and compared the rates obtained in the lakes with degradation rates from the literature (for a detailed description of methods, see Appendix B). Our results show that maize leaves degraded at rates comparable with those for reed (Gessner (2000)) and Green Alder (*Alnus viridis* (Chaix.) D.C) (Robinson et al. (1998). After nine months, only 20% in Gollinsee and 17% in Schulzensee of the initial dry mass remained in the litter bags (Appendix B).

To estimate the extent to which tPOC from alder and maize was used in the food web relative to other carbon sources, we calculated a carbon budget including littoral and pelagic autochthonous production, the input by tDOC, and the contributions from alder and maize (Figure 1.1, see Appendix A for Methods of the carbon budget). The relative proportions of tPOC were then compared between the lake carbon budget and the diet of consumers, as estimated from mixing models (see below).

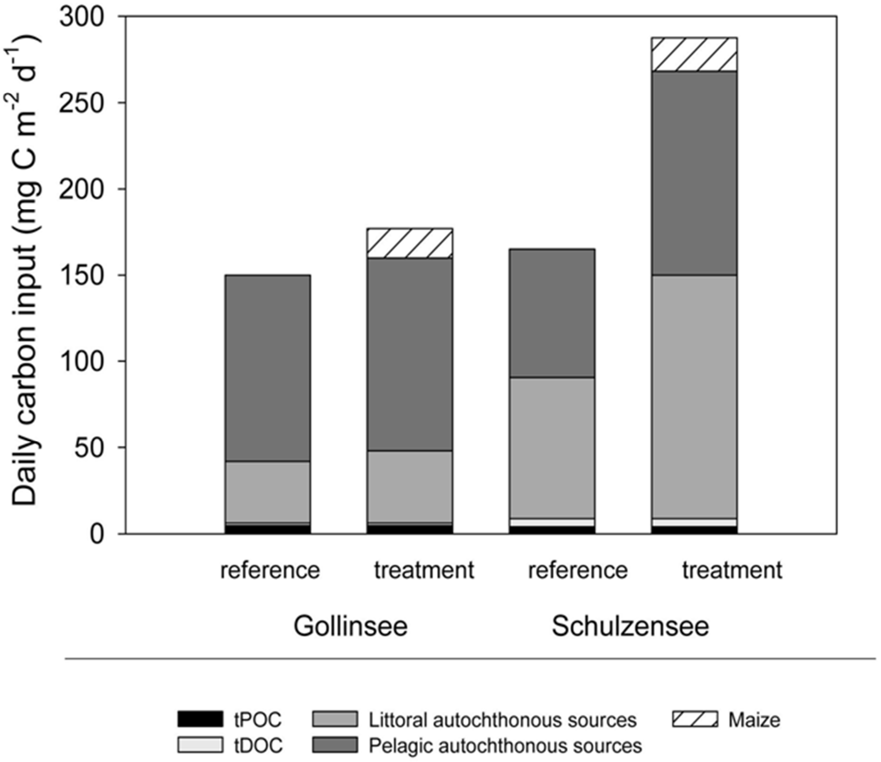

Figure 1.1. Daily carbon input (mg C m^2 d^{-1}) of Gollinsee and Schulzensee (reference and treatment sides). Depicted are values of carbon input as tPOC (from alder leaves in autumn), tDOC (via groundwater and precipitation), littoral autochthonous sources (from submerged macrophytes, only present in Schulzensee, emergent macrophytes, epiphyton, epipelon, *Aphanotece stagnina*, a blue-green algae present only in Schulzensee and phytoplankton), pelagic autochthonous sources (epipelon and phytoplankton) and experimentally added maize leaves on the treatment sides.

Sampling procedure and stable isotope analysis

In 2011 we sampled all primary resources (alder, maize, periphyton, seston) and consumer groups (zooplankton, macroinvertebrates, fish) for stable isotope analysis from treatment and reference sides in both lakes in spring (April), summer (June) and autumn (September). Leaves from macrophytes were collected in summer 2010. Macrophyte, alder and maize leaves were taken from the lakes and gently wiped clean of loosely attached biofilm. Periphyton was scraped from the dividing curtains at a depth of 0.1 m. Further periphyton samples were obtained from reed stems and artificial substrates made from transparent polypropylene sheets with a slightly textured surface, which had been placed in the lakes for four weeks at a depth of 1.2

m. Periphyton was brushed into a plastic container filled with non-chlorinated tap water and samples were allowed to settle.

We obtained seston samples by hauling 30 µm mesh nets vertically through the full water column and horizontally behind a boat to obtain sufficient material. Larger particles and visible animals were removed, and samples were allowed to settle. The sedimented green seston material was then removed and dried in an oven at 60°C. Zooplankton was sampled by hauling nets (mesh sizes 55 µm and 100 µm) through the whole water column. Zooplankton biomass consisted mainly of cyclopoid copepods (Ehrlich 2012, but see Appendix C for a species list). Samples were transferred to non-chlorinated tap water and stored in a refrigerator overnight to allow zooplankton individuals to void their guts. The next day, samples were filtered on 100 µm filters. Macroinvertebrate samples were collected with a kick net (mesh size 500 µm) from several eulittoral and sublittoral locations, whereas no macroinvertebrates were found in the deeper benthic zone devoid of macrophytes. Macroinvertebrates were sorted into major taxonomic groups (see Appendix C for a species list) and held in non-chlorinated tap water overnight to allow emptying of their guts. Additional macroinvertebrate samples (21% of total sample number) were taken from leaf litter bags of 1 cm mesh size filled with maize leaves, which were deployed at several locations in the treatment sides of both lakes simultaneously with the addition of maize leaves in autumn 2010. There was no difference in carbon isotope signatures between randomly collected individuals of Chironomidae, Trichoptera and Ephemeroptera larvae from the treatment side and individuals from leaf litter bags ($t \leq 1.175$, $P \geq 0.252$), but values differed for Isopoda (Gollinsee: $t_{13} = -3.218$, $P = 0.007$; Schulzensee: $t_{26} = -4.841$, $P < 0.001$). Therefore, we pooled the samples from different origin within benthic groups for subsequent analyses, but repeated the statistical tests (see Results) for Isopoda by excluding all individuals from the litter bags.

Fish were sampled by Nordic multi-mesh gill nets and electrofishing. Fish species included sunbleak (*Leucaspius delineatus,* Heckel), roach (*Rutilus rutilus* L.), rudd (*Scardinius erythrophthalmus* L.), tench (*Tinca tinca* L.), perch (*Perca fluviatilis* L.) and pike (*Esox lucius* L.). Fish were measured, weighed and immediately stored on ice. In the laboratory, a small amount of dorsal muscle tissue was removed and placed on aluminum foil.

All samples for stable isotope analysis were oven dried at 60°C for 48 hours, ground to a fine powder using a mortar and pestle and then weighed into tin capsules. For animal tissue, we used about 0.5 mg dry mass (dm), and for plant material a variable amount (≤ 6 mg dm), due to variable C:N ratios. Elemental and stable isotope analyses of carbon and nitrogen were conducted at the University of Jyväskylä, Finland, using a FlashEA 1112 elemental analyzer coupled to a Thermo Finnigan DELTA $^{\text{Plus}}$ Advantage mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA). Results are expressed in the δ notation, using ratios of samples and international standards (PeeDee Belemnite for δ^{13}C, atmospheric N for δ^{15}N). Analytical error (mean SD from in-house standard) for each run was always smaller than 0.3‰ for δ^{13}C and δ^{15}N.

Calculations and statistical analysis

Seston samples usually consist of phytoplankton, but may also contain large proportions of tPOC (Jones et al. 1999, Finlay 2004). Therefore, to achieve a more reliable carbon isotope value for phytoplankton, we corrected the seston δ^{13}C values by a two end-member mixing model (Bade et al. 2006, Taipale et al. 2007):

$$\delta^{13}C_{\text{phytoplankton}} = \frac{\left[\delta^{13}C_{\text{seston}}\text{-}(\delta^{13}C_{\text{terr}} \times \text{proportion of detritus})\right]}{\text{estimated proportion of phytoplankton}}$$

where the estimated proportion of phytoplankton in the particulate organic matter pool was calculated from the ratio between phytoplankton biomass and seston concentration, both expressed in µg C L^{-1}. To obtain phytoplankton biomass, we used chl *a* concentrations multiplied by an algae C/chl *a* ratio of 45, which was a good approximation for the two lakes in preliminary studies (Busse 2011). Seston carbon concentrations were measured by a Vario elemental analyzer. The residual POC was considered to be terrestrial detritus and was assumed to have the isotope signature of alder leaves (Gollinsee: -29.0 ± 0.1‰; Schulzensee: 29.2 ± 0.1‰), which agreed well with measured δ^{13}C of DOC (Gollinsee: -28.7 ± 0.2‰; Schulzensee: -30.0 ± 0.2‰).

For statistical analyses, we averaged the signatures across Ephemeroptera and Trichoptera taxa due to their very similar isotopic values. Predatory macroinvertebrates (Odonata, Hirudinea and Coleoptera) were pooled into one group. We grouped fish species into omnivorous (roach, rudd, tench and perch smaller than 15 cm), planktivorous (sunbleak and omnivorous fish smaller than 5 cm) and piscivorous (pike) feeding guilds. Due to a severe natural fish kill over the 2009/2010 winter season, no large piscivorous perch were caught and the sample size of pike was low.

Fish $\delta^{15}N$ signatures were log-transformed to obtain normally distributed data. Subsequently, assumptions of normal distribution and homogeneity of variances were met for all analyses. Isotopic data ($\delta^{13}C$ and $\delta^{15}N$) were analyzed using linear mixed models (RM LMM) with main factors lake, lakeside (maize added or reference) and season (as repeated measure), and all two-way interactions and compound symmetry as covariance type. For periphyton, zooplankton, and phytoplankton, only one sample per season was typically available. For these groups, univariate general linear models (GLM) with lake and lakeside, and their interaction, were calculated. For all significant main effects, estimated marginal means (EMM) were calculated to elucidate the differences between lakes, treatment and reference sides, or seasons. Statistical tests were performed using PASW for windows v. 17.0 (IBM Corporation, New York, U.S.A.). For details of all statistical tests, see Appendix D. There was no effect of maize addition on $\delta^{15}N$ signatures (Appendix D), as ambient values were very similar to $\delta^{15}N$ of maize. Therefore, we subsequently focus only on the differences in $\delta^{13}C$ signatures.

Isotopic mixing models

To calculate the proportion of different food resources in the diets of consumers, we used isotope mixing models as implemented in the SIAR (Stable Isotope Analysis in R) package (Parnell et al. 2010) in R 2.12.0 (R Development Core Team 2012). We focused the analyses on those consumer groups for which a significant treatment effect by maize addition was found and for which sufficient isotope values were available (Chironomidae, Isopoda, Ephemeroptera + Trichoptera and omnivorous fish; see Results). Because of strong seasonal differences in the treatment effect (see Results), we pooled the spring and summer data for the mixing models, but excluded autumn samples. We further pooled data of periphyton and reeds because of similar isotope signatures ($t \leq -1.549$, $P \geq 0.172$ for $\delta^{13}C$ and $t \leq 0.901$, $P \geq 0.418$ for $\delta^{15}N$; see Appendix E for biplots of data used for mixing models). We removed single individuals from each of the groups that did not fit into the isotopic mixing polygon

(8.1% of all individuals), as is common practice recommended by Phillips and Koch (2002) and Parnell et al. (2010). Source estimates were corrected for trophic fractionation using a fractionation factor of 0.4 ± 1.3 for δ^{13}C and 3.4 ± 1.0 for δ^{15}N (Post 2002). As different resources incorporated in the model varied in C:N ratios, we included concentration dependence (Phillips and Koch (2002).

The allochthony of zooplankton was estimated using a two end-member mixing model of the δ^{13}C signatures with only one autochthonous (phytoplankton) and one allochthonous source (alder). The position of maize in isotopic space suggested that it was unlikely to be a major carbon source for zooplankton (Appendix E). For omnivorous fish, we estimated the indirect contribution of primary resources via the consumption of benthic prey and zooplankton organisms using ratio calculations (Brauns et al. 2011). For all results of Bayesian isotope mixing models, we refer to trends in mean values and give 95% Bayesian credibility intervals in the figures, but do not refer to significant differences between means in the sense of frequentist statistics.

Results

Maize additions significantly elevated δ^{13}C in all benthic consumer groups except for predatory macroinvertebrates (Figure 1.2, Table 1.2), as demonstrated by the linear mixed model. Estimated marginal means (EMM) of δ^{13}C were higher in the treatment than in the reference sides of both lakes for most groups. For omnivorous and piscivorous fish, significant interactions of lakeside and season induced higher EMM in the reference than in the treatment sides, caused by a reversed signal in autumn relative to the spring and summer data (Table 1.2). In contrast, maize addition did not change δ^{13}C values within the pelagic food web (phytoplankton, zooplankton, and planktivorous fish) in either lake (Figure 1.2, Table 1.2).

There was a significant lake effect (Table 1.2), with δ^{13}C signatures being higher in turbid Gollinsee than in clear-water Schulzensee in six of eight consumer groups from both pelagic and benthic food webs. Furthermore, significant seasonal effects were found in most benthic consumer groups, including fish. EMM were usually highest in summer, whereas overall low δ^{13}C signatures were found in autumn samples (Figure 1.2), suggesting that the effects of the maize addition had disappeared by approximately one year after the additions.

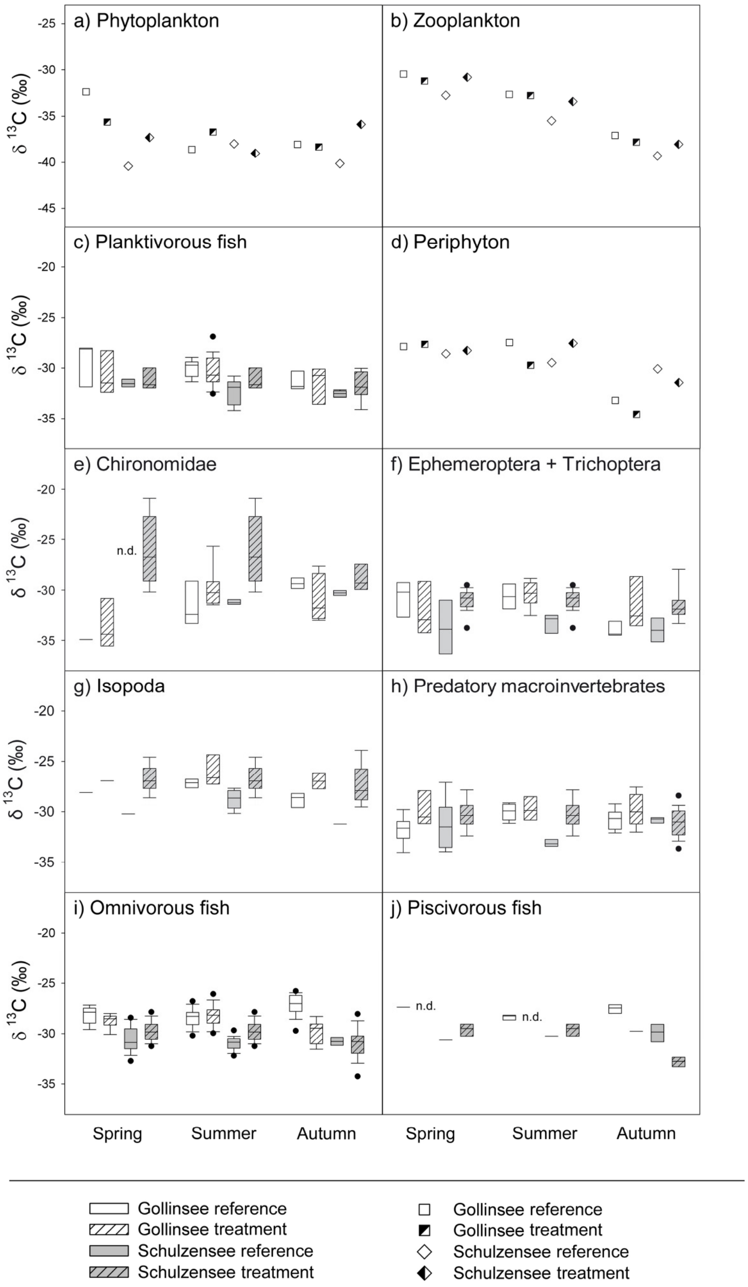

Figure 1.2. Boxplots of δ^{13}C signatures of selected taxa, measured from reference and treatment (maize addition) sides of Gollinsee (turbid and phytoplankton dominated) and Schulzensee (clear and macrophyte dominated) in spring, summer and autumn 2011. Boxplots with mean values and 25th and 75th percentile are shown, whiskers represent 10th and 90th percentiles and dots are 5th and 95th percentiles; n.d. represents no data. For six out of thirty-six occasions, when samples of phytoplankton, zooplankton and periphyton were taken more than once per season, mean values are shown.

Table 1.2. Results of repeated-measure linear mixed models (RM LMM) or univariate general linear models (GLM) with $\delta^{13}C$ as a dependent variable, and lake (G=Gollinsee, S=Schulzensee), treatment (r=reference side, t=treatment side (maize addition)) and season (sp=spring, su=summer, au=autumn) and their bivariate interactions as independent variables. *= *P*≤0.05, **= *P*≤0.01, ***= *P*≤0.001. For significant main effects, comparisons of estimated marginal means (EMM) are shown. Higher values of $\delta^{13}C$ indicate higher reliance on terrestrial material. *N*=number of samples processed.

			Factor								
Group	***N***	**Model type**	**Lake**		**Lakeside**		**Season**		**Lake × Lakeside**	**Lake × Season**	**Lakeside × Season**
			P	**EMM**	***P***	**EMM**	***P***	**EMM**	***P***	***P***	***P***
1. Pelagic food chain:											
Phytoplankton	12	GLM					not calculated			not calculated	not calculated
Zooplankton	18	GLM					not calculated			not calculated	not calculated
Planktivorous fish	104	RM LMM	***	G > S							*
2. Benthic food chain											
Periphyton	26	RM LMM					not calculated			not calculated	not calculated
Chironomidae	69	RM LMM			**	r < t	**	su > sp > au	**		*
Ephemeroptera + Trichoptera	102	RM LMM	*	G > S	**	r < t	*	su > sp > au	*		
Isopoda [a]	67	RM LMM	*	G > S	***	r < t	*	su > sp > au			
Isopoda [b]	51	RM LMM	***	G > S	***	r < t	***	su > sp > au	**		
Predatory macroinvertebrates	114	RM LMM	***	G > S			**	su > au > sp			**
Omnivorous fish	300	RM LMM	***	G > S	***	r > t	**	su > au > sp	***	*	***
Piscivorous fish	32	RM LMM	***	G > S	*	r > t	***	sp > su > au			***

[a] all individuals
[b] individuals from leaf litter bags excluded

Using the observed differences in $\delta^{13}C$ signatures of spring and summer in response to maize addition, we calculated the contribution of different food sources for zooplankton, Chironomidae, Isopoda, Ephemeroptera + Trichoptera and omnivorous fish. Zooplankton relied on both alder leaves and phytoplankton at about equal proportions, and the allochthonous contribution from alder was slightly higher in the reference sides of Gollinsee (56%) compared to Schulzensee (43%) (Appendix F). Macroinvertebrate taxa generally showed a strong reliance on littoral autochthonous sources (i.e., periphyton + reeds and macrophytes) (Figure 1.3a, b, c). On average, littoral autochthonous carbon contributed more to the diet of macroinvertebrates in Gollinsee (41%) than the contribution of littoral C to the lake carbon budget (24%, Figure 1.1). In Schulzensee, the proportions of littoral C were roughly similar in macroinvertebrate diets (44%) and in the carbon budget (50%, Figure 1.1). The contribution of pelagic autochthonous carbon sources (phytoplankton and epipelon) to macroinvertebrate diets was lower (Gollinsee 29%, Schulzensee 24%) than the relative contribution of pelagic C fixation to carbon budgets (Gollinsee 72%; Schulzensee 45%, Figure 1.1).

For mixing models of omnivorous fish, we used five potential resources according to previous gut content analyses (benthic macroinvertebrates, Isopoda, zooplankton, periphyton + reeds, and macrophytes) (Wanke 2011). We used Isopoda (only *Asellus aquaticus* L.) as a separate resource due to their distinct isotope signature (see Appendix E). Macroinvertebrates contributed most to the diet of omnivorous fish (in the reference sides: 44% in Gollinsee, 34% in Schulzensee, Figure 1.3d), suggesting that littoral autochthonous sources indirectly contributed 41% to the diet of fish diet in Gollinsee and 50% in Schulzensee (Appendix G).

Maize leaves contributed more to the diet of Isopoda (both lakes) and Chironomidae (Schulzensee only) than expected given the small proportional contribution of maize to the lake carbon budget (Figure 1.4). For all other consumer groups, carbon from maize was incorporated less than expected from its relative availability. In contrast, there was a high contribution of carbon from alder in the diet of consumers in Gollinsee (average of 35%) and Schulzensee (32%), despite the low contribution of alder to the lake carbon budget (Gollinsee 3%, Schulzensee 1%; Figures. 1.1, 1.4). Average allochthony across all five consumer groups (sum of contribution from alder and maize leaves in the treatment sides and alder leaves in the reference sides) was similar in both lakes (37% in Gollinsee and 34% in Schulzensee).

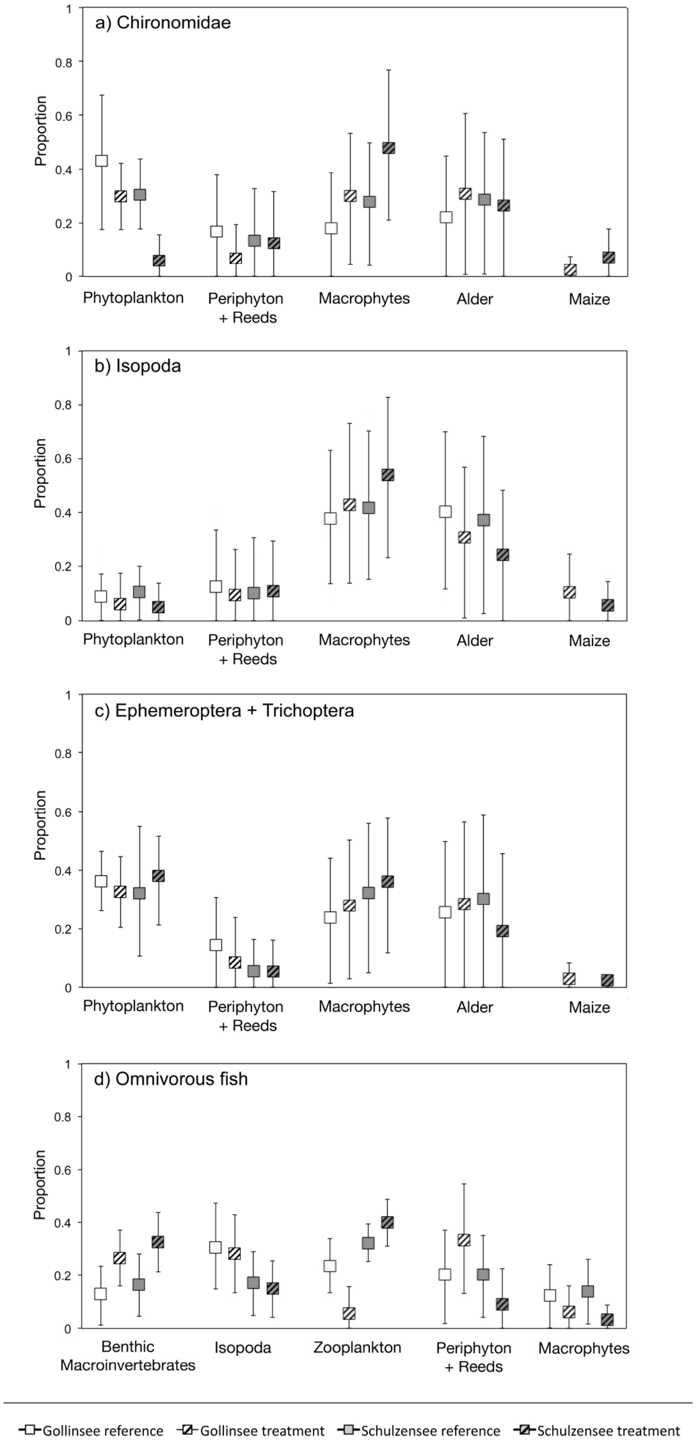

Figure 1.3. Comparison of mean diet contributions (range 0-1), as calculated by isotopic mixing model SIAR, to the four consumer groups (a) Chironomidae, (b) Isopoda (c) Ephemeroptera + Trichoptera and (d) omnivorous fish. Resources included were phytoplankton, periphyton, alder leaves, and maize leaves (for a, b, c); or benthic macroinvertebrates (Chironomidae, Trichoptera + Ephemeroptera), Isopoda, periphyton, and zooplankton (for d). White symbols represent Gollinsee, gray ones Schulzensee. Treatment sides (addition of maize leaves) are depicted by shaded patterns. Error bars represent 95% Bayesian credibility intervals.

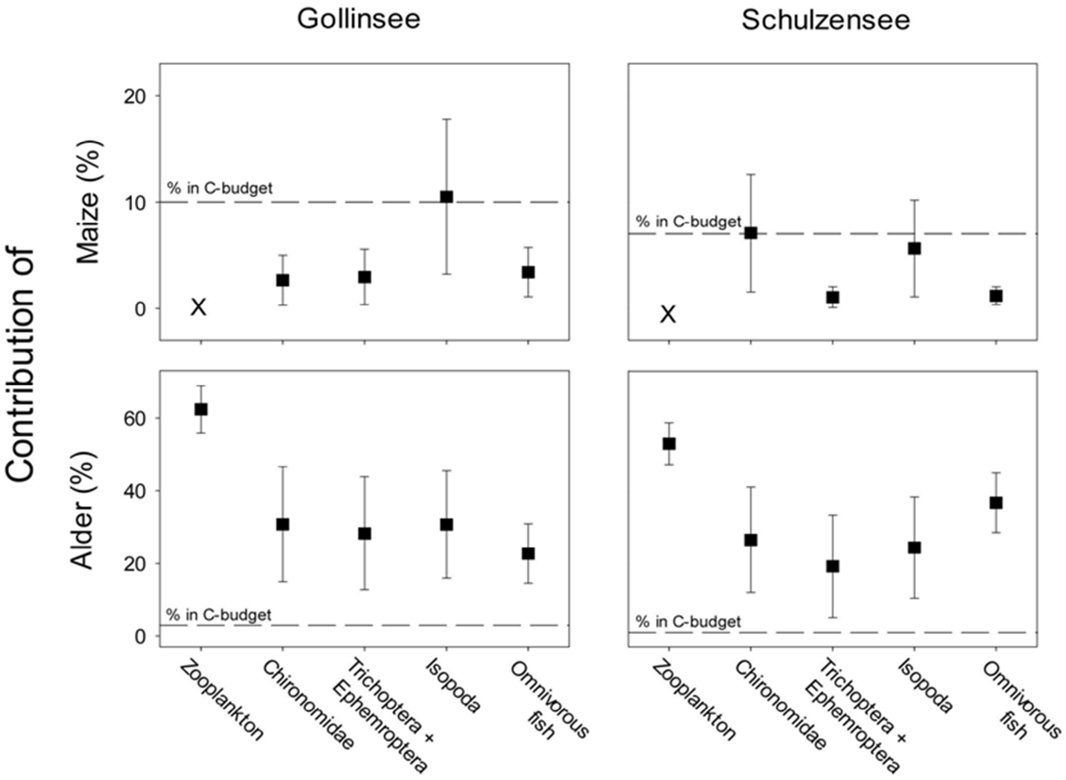

Figure 1.4. Contribution of tPOC to consumers' diet. Depicted is contribution of maize and alder in the treatment sides of Gollinsee and Schulzensee for zooplankton, Chironomidae, Trichoptera + Ephemeroptera, Isopoda and omnivorous fish. Dashed lines represent proportion of primary resource on total carbon budget.

Discussion

We demonstrated experimentally that tPOC can be traced up to higher trophic levels of lake food webs. Several macroinvertebrate taxa as well as omnivorous and piscivorous fish had significantly elevated $\delta^{13}C$ in response to tPOC addition through maize leaves. This is the first direct experimental evidence at a whole-lake scale that tPOC addition mimicking annual leaf fall is incorporated into benthic food webs of shallow lakes. Furthermore, by comparing lake carbon budgets and the contributions of carbon resources to the diet of consumers, we could show that terrestrial carbon, primarily from alder leaves, substantially contributes to the carbon stored in the entire food web of both lakes. Contrary to our expectations, the larger littoral zone composed of submerged macrophytes in Schulzensee did not increase allochthony relative to the turbid Gollinsee. In both lakes, the average allochthony as derived from the combined contributions of alder and maize was intermediate for macroinvertebrates (28-35%) and omnivorous fish (26-38%), whereas the main carbon for these organisms originated from autochthonous C-fixation in the littoral of both lakes.

Availability and quality of resources

Differences in $\delta^{13}C$ values between lake sides were seasonally variable and strongest during summer when warm temperatures induced high metabolic requirements and hence the highest feeding rates of consumers. These differences in $\delta^{13}C$ signatures had disappeared until autumn when the remains of maize leaves were no longer detectable, as confirmed by our leaf litter experiment (Appendix B). The rapid degradation of leaves, similarly reported from Green Alder (Robinson et al. 1998) and reeds (Gessner 2000), supports our approach that addition of tPOC as maize leaves was suitable to mimic the natural input by terrestrial carbon to lakes. Nevertheless, incorporation of alder and maize differed. A substantial contribution of maize to the diet of consumers was confined to Isopoda and Chironomidae. Although about four times more tPOC from maize than from alder leaf fall was added, the consumption of alder leaves by macroinvertebrates was higher (Gollinsee 30% and Schulzensee 23%) than the consumption of maize leaves (both lakes 5%).

There are two alternative explanations for this finding. Long-lived macroinvertebrates that are characterised by low tissue turnover rates may not have reached full equilibrium of tissue isotopes despite feeding partly on maize, and hence their $\delta^{13}C$ signal underestimated the uptake of maize carbon. The slightly elevated carbon signatures of Isopoda sampled directly in the maize leaf litter bags relative to individuals caught at other littoral locations support this conjecture. Alternatively, the higher quality indicated by a lower C:N ratio of alder (15.7 ± 4.2 (SD)) relative to maize (41.1 ± 0.9) may have induced a preference of macroinvertebrates for alder (del Rio et al. 2009). It must be mentioned, however, that the high C:N ratio in maize resembles that of common tree leaves of the temperate zone; e.g. European beech (*Fagus sylvatica* L.), European hornbeam (*Carpinus betulus* L.), and Norway maple (*Acer platanoides* L.) (Jacob et al. 2010).

Allochthony

Our estimates of allochthony (28-35% in macroinvertebrates and 26-38% in fish) of these two eutrophic lakes were lower than estimates from oligotrophic systems. Carpenter et al. (2005) used mass balance calculations from whole-lake ^{13}C additions and reported up to 84% allochthonous reliance in macroinvertebrates and up to 80% in fish in small dystrophic lakes. The inclusion of hydrogen stable isotope ratios confirmed that estimates can be even higher for macroinvertebrates, but lower for fish (Solomon et al. 2011). In contrast, Batt et al. (2012) studied a more productive lake (summer mean of phosphorus concentration 22.9 µg L^{-1}) and found allochthony estimates comparable to our study (up to 21% in macroinvertebrates and 23% in fish). Similarly, Babler et al. (2011) found an average of 35% reliance on terrestrial carbon by detritivorous fish (Gizzard shad *Dorosoma cepedianum*, Lesueur) in mostly productive reservoirs. There are some shortcomings with respect to the estimates of allochthony in lakes by the usual application of mixing models from stable isotopes. We estimated the contribution of tPOC as the unweighted average allochthony from the isotopic signatures of all benthic consumers. This calculation neglects differences in biomass and production rates between these groups, and differences in consumer communities between lakes. Therefore, a more reliable estimate of allochthony for a certain trophic level or for an entire food web requires sufficiently precise estimates of secondary production in lakes, a process which can be measured only by investing substantial sampling effort (Butkas et al. 2011). Combining estimates of allochthony from stable isotopes with carbon production estimates hence deserves further study.

Estimated allochthony was furthermore particularly high for zooplankton in both our study lakes, although similarly high estimates are reported by others (Matthews and Mazumder 2006, Cole et al. 2011, but see Brett et al. (2012) for evidence of high autochthonous reliance in zooplankton.). There are two processes by which tPOC can become a resource for pelagic zooplankton. First, zooplankton can feed on seston which contains tPOC (Cole et al. 2006, Brett et al. 2009, Brett et al. 2012). Second, zooplankton may have fed on bacteria or fungi which have used leachates from tPOC (Karlsson et al. 2003, Lennon and Pfaff 2005, Wurzbacher et al. 2010), as indicated by the high zooplankton $\delta^{13}C$ signatures relative to the lower values of phytoplankton in spring 2011. Isotopic signatures of pelagic bacteria could not be measured, but bacterial production increased for a few days immediately after maize addition in autumn 2010 in response to the higher carbon and nutrient availability from leachates (Attermeyer et al. 2013). However, this enhanced carbon availability did not persist long enough to shift the carbon signatures of zooplankton significantly relative to the reference sides, as evidenced by short tissue turnover rates in zooplankton (between 7.7 days in rotifers and 20.0 days in herbivorous crustaceans) (Straile 1998). Nevertheless, if bacteria, instead of leaf detritus, were the second carbon source of zooplankton (after phytoplankton), our estimates of zooplankton allochthony should not change much, because $\delta^{13}C$ signatures of DOC, as the main bacterial resource, are very similar to $\delta^{13}C$ of alder, suggesting that lake DOC is mostly of allochthonous origin.

The role of littoral vegetation in mediating allochthony

Autochthonous littoral carbon was the main source of benthic consumers in both lakes, although the lakes differed with respect to the extension of their littoral zones. Littoral carbon fixation dominated the carbon budgets only in Schulzensee (50%, Figure 1.1, reference side) but was low in Gollinsee (24%, Figure 1.1). The dominance of littoral carbon sources for benthic consumers in both lakes despite structural differences and differing carbon budgets suggests that carbon was not a limiting resource even in the small littoral zone of Gollinsee. Recent studies have similarly demonstrated a significant importance of littoral carbon for food webs in lakes of variable morphometry and productivity (Vadeboncoeur et al. 2002, Karlsson and Byström 2005, Batt et al. 2012). The largest uncertainty in the carbon budgets is associated with the contribution by littoral macrophytes that have been assumed to be only rarely consumed due to the presence of phenols (Lodge 1991, Newman 1991). However, as they lose these inhibitors during decomposition (Kok et al. 1992), littoral macrophytes can be considered a resource of high quality, and our mixing models suggest that they contributed substantial fractions to the diet of a few consumer groups.

The average allochthony was likewise very similar in both lakes, which is in opposition to our expectations. We had assumed that the larger and more complex littoral zone in Schulzensee may facilitate the processing of tPOC by trapping leaves within the macrophytes (Vermaat et al. 2000, see also Francis et al. 2007 and Roth et al. 2007 for similar processes caused by woody debris), thus causing higher allochthony in Schulzensee. This assumption was not confirmed. We cannot exclude that both lakes were structurally more similar than considered before due to a similar coverage by emergent macrophytes (*Phragmites* reeds) which dominated the near-shore zones in both lakes. However, it is more likely that tPOC trapping by structural complexity is not the primary process which determines the allochthony of littoral consumers. Total carbon availability from littoral and pelagic autochthonous sources and from allochthonous sources together exceeded the carbon demand of all consumers by an order of magnitude (unpublished data), as corroborated by a substantial carbon sedimentation rate in both lakes (Brothers et al. unpublished manuscript). Consequently, benthic consumers may choose their carbon source predominantly according to quality and not according to availability. Our study thus provides experimental evidence for the model findings of Jones et al. (2012) which suggest that tPOC may replace, but not subsidize, autochthonous carbon sources for macroinvertebrates in lakes.

In summary, our results provide the first direct experimental tracing of tPOC use in lake food webs at the ecosystem scale, and highlight the linkage of lentic freshwater systems with their terrestrial surroundings, similar to the strong aquatic-terrestrial coupling via tPOC repeatedly demonstrated for streams (Vannote et al. 1980, Nakano and Murakami 2001). We argue that for a more comprehensive understanding of food web dynamics and carbon cycling in lakes, an ecosystem approach that quantifies carbon from various autochthonous and allochthonous pathways needs to be established. Combining experimental additions of terrestrial organic C, carbon budgets, isotopic mixing models, and estimates of secondary production may result in a more reliable quantification of allochthony of lake food webs.

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Appendix A: Organic carbon budget material and methods

All organic carbon inputs were calculated for a period spanning November 1st, 2010 until June 30th, 2011. We here refer to all primary production occurring within the lakes, including littoral reeds growing at or immediately beyond the lakeshore, as autochthonous production. The allochthonous organic carbon load is therefore considered to constitute fallen leaves from alder trees and the net flux (import minus export) of groundwater dissolved organic carbon (DOC).

Gross primary production was calculated separately for each lake half and for littoral and pelagic habitats separately. We defined the littoral zone as the area where macrophytes grow. Hourly, gross phytoplankton production rates were calculated from photosynthesis light curves measured by a Phyto-PAM fluorometer (Walz, Effeltrich, Germany), following procedures outlined in Brothers et al. (in press), and applied to the mean depth of each habitat, adjusting for daily estimates of light attenuation through the water column.

Periphyton (attached algae) production was calculated from the monthly fluorescence and chl *a* concentrations of periphyton growing on plastic strip exposures at 1.2m in the pelagic zone of each lake half. For epiphyton (periphyton growing on submerged plant surfaces) production estimates, periphyton production values were applied to the estimated underwater macrophyte surface area within each lake. The underwater macrophyte surface area was calculated for each lakeside by applying the mean measured submerged surface areas of separate plant groups to the mapped surface area covered by the given group (described below). For epipelon (benthic algae growing on muddy sediments) production estimates of littoral and pelagic zones, monthly periphyton production values were multiplied by maximum (full-year) biomass values from plastic strips, and applied to the benthic morphometry of each lake, adjusting for daily estimates of water column light attenuation.

The gross primary productivity of benthic cyanobacteria (*Aphanothece stagnina* (Sprengel) A. Braun, only in Schulzensee) were measured directly from O_2 production in light and dark core and flask experiments, described in detail in Brothers et al. (in press). Submerged macrophyte (*Ceratophyllum submersum*) production (only in Schulzensee) was estimated using a modeled productivity-to-biomass ratio of 1.5 (Best 1982).

For productivity of floating-leaved macrophytes (*Nymphaea lutea* and *N. alba*), plant density was estimated per m^2, and the total surface area occupied was estimated by geographic information systems (GIS) mapping, using global positioning system (GPS) data by mapping the habitat boundary of each macrophyte group. Lake coverage estimates were also available from direct measurements taken in 2007 (A. Becker, unpublished data), and the mean value of both estimates is here applied. For the carbon contribution of emergent reeds (*Phragmites australis*), plants growing within and directly beyond the lake shore were included to account for loading during periods of high water levels or by leaching into the shoreline sediments and groundwater. Detailed measurements of reed area and density per m^2 were therefore made along the periphery of each lake. Maximum biomass estimates (made in mid-July) involved twenty random samples of floating-leaved and emergent macrophytes. Macrophytes were cut at the sediment surface, dried at 60°C for two days, and were subsequently measured for total dry weight. The mean carbon content of *Nymphaea* (38.3 ± 0.6%, both lakes combined) was measured by a Vario EL CHNOS Element Analyzer (elementar Analysensysteme GmbH, Hanau, Germany), and mean values for reeds (45.6 ± 1%) are applied from Mille-Lindblom et al. (2006). It was assumed that the maximum quantity of living organic carbon in emergent and floating-leaved macrophytes was equivalent to the annual quantity of carbon loaded into the aquatic environment by these groups. This differs from our assumption for submerged macrophytes, as respiratory losses from emergent and floating leaved plants were expected to pass directly to the non-aquatic environment.

Plant group-specific estimates of gross primary production were unavailable during the winter ice-cover period (approximately December 1st to March 15th). During this period, we therefore calculated daily oxygen (O_2) curves from under-ice YSI probes (Xylem Inc., Yellow Springs, OH, USA) installed at 1.2m in each lake center (described in Brothers et al. in press.). Gross primary production was here calculated as the difference between the net nighttime decrease in oxygen (gross respiration) and the net change in oxygen during the following daytime period (net production). These values were converted to carbon units applying a common respiratory quotient of 1. We here assume that the under-ice GPP rates are predominantly the result of pelagic and littoral phytoplankton productivity, as this plant group is expected to have the greatest access to light transmitted through the ice.

Groundwater DOC concentrations were measured every two to four weeks in the immediate vicinity of the lakes (4 to 6 m from the shore), in areas of groundwater input. Wells were created to determine the underwater hydrology and flow rates of groundwater to and from each lake (Rudnick 2011). Shallow groundwater tables were compared to changes in water levels, precipitation (recorded continuously at each lake), and estimates of evapotranspiration, providing monthly estimates of the gain and loss of groundwater for each lake. The organic carbon provided by the leaves of trees was estimated from floating leaf traps installed at multiple locations along the shore of each lake.

Productivity estimates for emergent reeds and floating-leaved water lilies may be considered net primary production (NPP), but all other productivity estimates are GPP. Our estimates of total autochthonous carbon availability may therefore be considered maximum values, and thus likely overestimates of the carbon available to consumers in each habitat. Although plant respiration rates differ between plant groups and conditions, NPP values for plant groups may feasibly be 20-50% lower than our GPP estimates suggest (e.g. Platt et al. 1991), in terms of the proportional significance of added maize carbon to the total quantity of available carbon, accounting for plant respiration rates would therefore result in a shift of approximately 1% in either lake system.

Appendix B: Leave degradation

Approach

A total of 36 litter bags (nine per site and lake), each containing 10 g of dried maize leaves were placed in the littoral zone of the studied lakes immediately after the addition of the maize leaves in November 2010. Bags were of 1 cm mesh size to allow macroinvertebrates entering the bags. Sampling was conducted in March, June and August 2011, with six replicates being retrieved at each time of sampling for the assessment of litter dry weight (drying at 60°C until weight constancy).

Here we show that degradation rate of maize leaves in our lakes was almost identical to rates obtained for degradation of alder or reed.

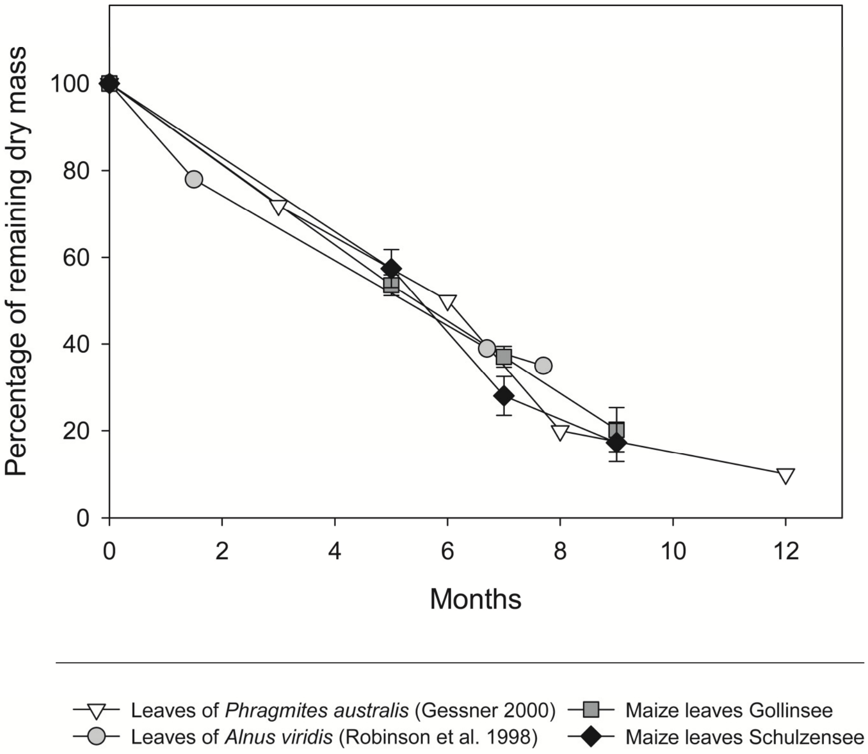

Figure 1.AppB. Break down of different leaves as demonstrated by leaf litter bag experiments. Triangular symbols represent results from experiments with leaves of reeds (*Phragmites australis* (Cav.) Trin. ex Steud.) (Gessner 2000), circles represent results from Green Alder (*Alnus viridis* (Chaix.) D.C) degradation (Robinson et al. 1998) and squared symbols (Gollinsee) as well as diamond symbols (Schulzensee) represent degradation of maize leaves within our own leaf litter bag experiments, including standard error bars.

Appendix C: Zooplankton and macroinvertebrate species list

Table 1.AppC. Species list

Group	Taxon	Species
Zooplankton	Rotifer	*Ascomorpha ecaudis* Perty 1850
		Asplanchna priodonta Gosse 1850
		Brachionus angularis Gosse 1851
		Brachionus calyciflorus Pallas 1776
		Brachionus quadridentatus Hermann 1783
		Brachionus urceolaris O. F. Müller 1773
		Colurella sp.
		Conochilus sp.
		Epiphanes pelagica (Jennings 1900)
		Euchlanis dilatata Ehrenberg 1832
		Filinia longiseta (Ehrenberg 1834)
		Hexarthra mira (Hudson 1871)
		Keratella cochlearis (Gosse 1851)
		Keratella quadrata (O.F. Müller 1786)
		Lecane sp.
		Platyas sp.
		Ploesoma hudsoni (Imhof 1891)
		Polyarthra dolichoptera Idelson 1925
		Polyarthra euryptera Wierzejski 1891
		Polyarthra vulgaris Carlin 1943
		Pompholyx complanata Gosse 1851
		Pompholyx sulcata Hudson 1885
		Scaridium longicaudum (O.F. Müller 1786)
		Synchaeta pectinata Ehrenberg 1832
		Trichocerca capucina (Wierzejski & Zacharias 1893)
		Trichocerca similis (Wierzejski 1893)
		Trichotria pocillum (O.F. Müller 1776)
	Cladocera	*Acroperus harpae* (Baird 1835)
		Alona guttata G.O. Sars 1862
		Alona quadrangularis (O.F Müller 1776)
		Alona weltneri Keilhack 1905
		Alonella excisa (Fischer 1854)
		Alonella nana (Baird 1843)
		Bosmina longirostris (O.F. Müller 1785)
		Chydorus sphaericus (O.F. Müller 1776)
		Daphnia cucullata G.O. Sars 1862

		Diaphanosoma brachyurum (Liévin 1848)
		Eurycercus lamellatus (O.F. Müller 1776)
		Graptoleberis testudinaria (Fischer 1884)
		Pleuroxus aduncus (Jurine 1820)
		Pleuroxus truncates (O.F. Müller 1785)
		Scapholeberis mucronata (O.F. Müller 1776)
		Sida crystallina (O.F. Müller 1776)
	Calanoid copepod	*Eudiaptomus gracilis* (G.O. Sars 1862)
	Cyclopoid copepod	*Simocephalus exspinosus* (Koch 1841)
		Cyclops vicinus Ulyanin 1875
		Eucyclops serrulatus (Fischer 1851)
		Thermocyclops sp.
	Harpacticoid copepod	*Canthocamptus staphylinus* (Jurine 1820)
Macroinvertebrates	Chironomidae	Chironominae
		Chironomidae sp.
		Tanypodinae
	Coleoptera	*Enochrus testaceus* (Fabricius 1801)
		Hydaticus seminiger (De Geer 1774)
		Hydrobius fuscipes (L. 1758)
		Hyphydrus ovatus (L. 1761)
		Laccophilus hyalinus (De Geer 1774)
		Rhantus exsoletus (Forster 1771)
	Ephemeroptera	*Cloeon dipterum* (L. 1761)
		Caenis horaria (L. 1758)
		Caenis robusta Eaton 1884
	Hirudinea	*Erpobdella octoculata* (L. 1758)
		Erpobdella testacea (Savigny 1822)
		Helobdella stagnalis (L. 1758)
		Glossiphonia complanata (L. 1758)
		Alboglossiphonia heteroclita (L. 1758)
	Isopoda	*Asellus aquaticus* (L. 1758)
	Odonata	*Aeshna cyanea* (O.F. Müller 1764)
		Aeshna grandis (L. 1758)
		Brachytron pratense (O.F Müller 1764)
		Coenagrion sp.
		Cordulia aenea (L. 1758)
		Epitheca bimaculata (Charpentier 1825)
		Erythromma najas (Hansemann 1823)
		Ischnura elegans Vander Linden 1820
		Libellula fulva O.F Müller 1764

		Libellula quadrimaculata L. 1758
		Orthetrum cancellatum L. 1758
		Platycnemis pennipes Pallas 1771
		Somatochlora metallica (Van der Linden 1825)
	Trichoptera	*Agrypnia pagetana* Curtis 1835
		Agrypnia varia Fabricius 1793
		Anabolia furcata Brauer 1857
		Athripsodes aterrimus (Stephens 1836)
		Ceraclea senilis (Burmeister 1839)
		Cyrnus crenaticornis (Kolenati 1859)
		Cyrnus flavidus McLachlan 1864
		Cyrnus trimaculatus (Curtis 1834)
		Ecnomus tenellus (Rambur 1842)
		Glyphotaelius pellucidus (Retzius 1783)
		Holocentropus picicornis (Stephens 1836)
		Leptocerus sp.
		Limnephilus binotatus Curtis 1834
		Limnephilus decipiens (Kolenati 1848)
		Limnephilus flavicornis (Fabricius 1787)
		Limnephilus marmoratus Curtis 1834
		Limnephilus nigriceps (Zetterstedt 1840)
		Limnephilus politus McLachlan 1865
		Limnephilus rhombicus (L. 1758)
		Limnephilus stigma Curtis 1834
		Oecetis furva (Rambur 1842)
		Orthotrichia sp.
		Phryganea bipunctata Retzius 1783
		Polycentropus irroratus (Pictet 1834)
	Gastropoda	*Acroloxus lacustris* (L. 1758)
		Anisus vortex (L. 1758)
		Bathyomphalus contortus (L. 1758)
		Bithynia leachii (Sheppard 1823)
		Bithynia tentaculata (L. 1758)
		Gyraulus albus (L. 1758)
		Gyraulus crista (L. 1758)
		Hippeutis complanatus (L. 1758)
		Planorbis carinatus (L. 1758)
		Radix balthica (L. 1758)
		Valvata cristata O.F.Müller 1774
		Valvata piscinalis O.F. Müller 1774
		Viviparus contectus Millet 1813

Appendix D: Details of statistical analyses to compare carbon and nitrogen isotope signatures between lakes, lakesides and seasons

Table 1.App.D. Model types are RM LMM (linear mixed model with factors lake, lakeside and season (as repeated measure) as well as all two-way interactions), and model type GLM (univariate general linear model with factors lake, lakeside and interactions of both) using $\delta^{13}C$ and$\delta^{15}N$ as dependent variable.

Dependent variable	Food chain	Trophic level	Model type	Effect	df	F	*P*
$\delta^{13}C$	Pelagic	Phytoplankton	GLM	Lake	1	2.283	0.169
				Lakeside	1	0.412	0.539
				Lake × Lakeside	1	1.182	0.309
				Error	8		
$\delta^{13}C$	Pelagic	Zooplankton	GLM	Lake	1	0.228	0.640
				Lakeside	1	0.613	0.447
				Lake × Lakeside	1	1.359	0.263
				Error	14		
$\delta^{13}C$	Pelagic	Planktivorous fish	RM LMM	Lake	1; 63.1	18.881	< 0.0001
				Lakeside	1; 63.4	1.021	0.316
				Season	2; 66.3	3.072	0.053
				Lake × Lakeside	1; 54.0	2.373	0.129
				Lake × Season	2; 70.0	1.617	0.206
				Lakeside × Season	2; 70.7	3.384	0.039
$\delta^{13}C$	Benthic	Periphyton	GLM	Lake	1	0.943	0.342
				Lakeside	1	0.048	0.828
				Lake × Lakeside	1	1.049	0.317

				Error	22		
$\delta^{13}C$	Benthic	Chironomidae	RM LMM	Lake	1; 45.5	1.724	0.196
				Lakeside	1; 42.8	7.991	0.007
				Season	2; 45.3	7.409	0.002
				Lake × Lakeside	1; 39.3	8.568	0.006
				Lake × Season	2; 49.9	0.877	0.422
				Lakeside × Season	2; 45.2	3.944	0.026
$\delta^{13}C$	Benthic	Ephemeroptera + Trichoptera	RM LMM	Lake	1; 44.2	4.759	0.035
				Lakeside	1; 45.8	9.503	0.003
				Season	2; 73.7	4.793	0.011
				Lake × Lakeside	1; 44.7	4.342	0.043
				Lake × Season	2; 74.7	0.769	0.467
				Lakeside × Season	2; 73.1	0.981	0.380
$\delta^{13}C$	Benthic	Isopoda, all individuals	RM LMM	Lake	1; 40.9	6.155	0.017
				Lakeside	1; 38.6	13.720	0.001
				Season	2; 44.1	4.812	0.013
				Lake × Lakeside	1; 38.3	2.284	0139
				Lake × Season	2; 43.2	0.075	0.927
				Lakeside × Season	2; 44.0	0.709	0.497
$\delta^{13}C$	Benthic	Isopoda, leaf litter bag individuals excluded	RM LMM	Lake	1; 19.1	14.224	0.001
				Lakeside	1; 18.1	16.464	0.001

				Season	2; 39.9	9.157	0.001
				Lake × Lakeside	1; 22.3	8.031	0.010
				Lake × Season	2; 39.8	2.519	0.093
				Lakeside × Season	2; 38.9	3.175	0.053
$\delta^{13}C$	Benthic	Predatory macro-invertebrates	RM LMM	Lake	1; 31.5	16.723	< 0.0001
				Lakeside	1; 30.8	0.2.495	0.124
				Season	2; 76.9	6.695	0.002
				Lake × Lakeside	1; 32.4	1.068	0.309
				Lake × Season	2; 75.8	1.579	0.213
				Lakeside × Season	2; 77.2	5.034	0.009
$\delta^{13}C$	Benthic	Omnivorous fish	RM LMM	Lake	1; 129.3	267.931	< 0.0001
				Lakeside	1; 128.4	27.254	< 0.0001
				Season	2; 206.1	4.767	0.009
				Lake × Lakeside	1; 134.4	12.062	0.001
				Lake × Season	2; 201.4	3.206	0.042
				Lakeside × Season	2; 211.2	39.112	< 0.0001
$\delta^{13}C$	Benthic	Piscivorous fish	RM LMM	Lake	1; 21.2	60.224	< 0.0001
				Lakeside	1; 20.7	6.820	0.016
				Season	2; 20.5	10.937	0.001
				Lake × Lakeside	1; 19.8	0.986	0.333
				Lake × Season	2; 20.6	0.851	0.441

				Lakeside × Season	2; 20.2	9.914	0.001
δ^{15}N	Pelagic	Phytoplankton	GLM	Lake	1	0.573	0.463
				Lakeside	1	0.030	0.865
				Lake × Lakeside	1	< 0.0001	0.987
				Error	8		
δ^{15}N	Pelagic	Zooplankton	GLM	Lake	1	3.109	0.100
				Lakeside	1	3.780	0.072
				Lake × Lakeside	1	0.029	0.867
				Error	14		
δ^{15}N	Pelagic	Planktivorous fish	RM LMM	Lake	1; 61.1	0.375	0.543
				Lakeside	1; 61.6	1.295	0.259
				Season	2; 67.6	31.459	< 0.0001
				Lake × Lakeside	1; 53.0	2.415	0.126
				Lake × Season	2; 71.6	0.067	0.935
				Lakeside × Season	2; 72.2	3.806	0.027
δ^{15}N	Benthic	Periphyton	GLM	Lake	1	0.404	0.532
				Lakeside	1	1.017	0.324
				Lake × Lakeside	1	1.512	0.232
				Error	22		
δ^{15}N	Benthic	Chironomidae	RM LMM	Lake	1; 43.9	30.315	≤ 0.0001
				Lakeside	1; 40.4	0.716	0.403
				Season	2; 40.7	0.938	0.400
				Lake × Lakeside	1; 34.3	0.133	0.717
				Lake × Season	2; 45.8	3.752	0.013

				Lakeside × Season	2; 40.6	0.907	0.412
$\delta^{15}N$	Benthic	Ephemeroptera + Trichoptera	RM LMM	Lake	1; 24.1	≤ 0.0001	0.986
				Lakeside	1; 25.1	0.535	0.471
				Season	2; 72.1	4.030	0.022
				Lake × Lakeside	1; 26.6	4.074	0.054
				Lake × Season	2; 74.7	7.549	0.001
				Lakeside × Season	2; 72.3	0.175	0.840
$\delta^{15}N$	Benthic	Isopoda, all individuals	RM LMM	Lake	1; 40.9	6.155	0.017
				Lakeside	1; 38.6	13.720	0.001
				Season	2; 44.1	4.812	0.013
				Lake × Lakeside	1; 38.3	2.284	0139
				Lake × Season	2; 43.2	0.075	0.927
				Lakeside × Season	2; 44.0	0.709	0.497
$\delta^{15}N$	Benthic	Isopoda, individuals from leaf litter bags excludes	RM LMM	Lake	1; 24.9	4.056	0.055
				Lakeside	1; 23.0	13.401	0.001
				Season	2; 28.1	10.937	< 0.0001
				Lake × Lakeside	1; 21.0	0.177	0.679
				Lake × Season	2; 27.5	1.310	0.286
				Lakeside × Season	2; 27.6	0.734	0.489
$\delta^{15}N$	Benthic	Predatory macro-invertebrates	RM LMM	Lake	1; 26.8	11.648	0.002

				Lakeside	1; 26.6	16.729	< 0.0001
				Season	2; 36.7	0.218	0.805
				Lake × Lakeside	1; 27.2	1.429	0.242
				Lake × Season	2; 62.5	4.363	0.017
				Lakeside × Season	2; 64.0	0.938	0.397
$\delta^{15}N$	Benthic	Omnivorous fish	RM LMM	Lake	1; 121.1	52.686	< 0.0001
				Lakeside	1; 121.3	5.664	0.019
				Season	2; 232.5	5.489	0.005
				Lake × Lakeside	1; 132.2	0.055	0.815
				Lake × Season	2; 237.2	4.914	0.008
				Lakeside × Season	2; 237.2	2.991	0.052
$\delta^{15}N$	Benthic	Piscivorous fish	RM LMM	Lake	1; 21.9	0.147	0.705
				Lakeside	1; 21.8	0.915	0.349
				Season	2; 14.1	0.062	0.940
				Lake × Lakeside	1; 20.9	1.055	0.316
				Lake × Season	2; 14.7	0.583	0.570
				Lakeside × Season	2; 13.9	2.173	0.151

Appendix E: Stable isotope biplots

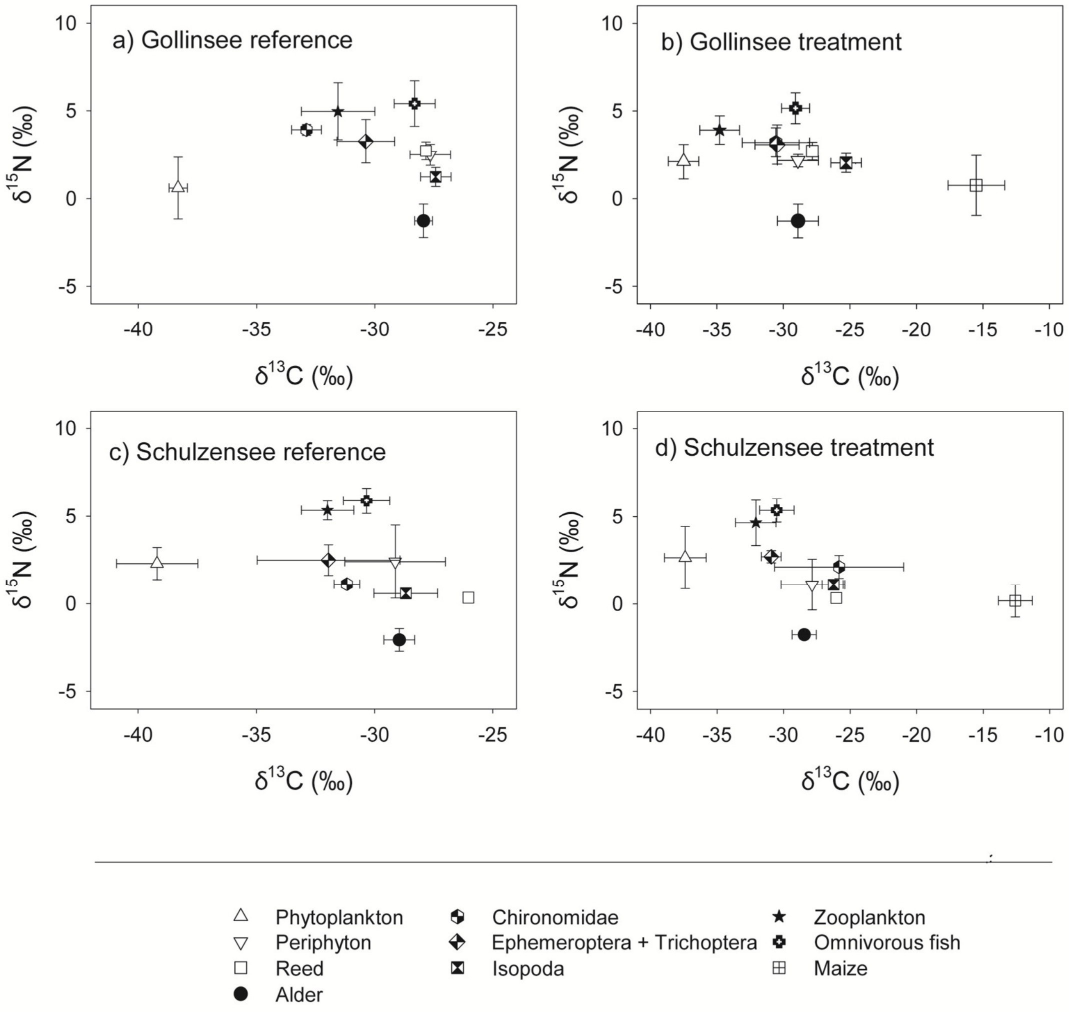

Figure 1.App.E. Stable nitrogen and carbon isotope ratios of groups used for isotopic mixing models of both lakes (Gollinsee and Schulzensee) in treatment and reference sides. Mean values (± SD) of samples taken in spring and summer are depicted.

Appendix F: Contribution of resources to zooplankton diet

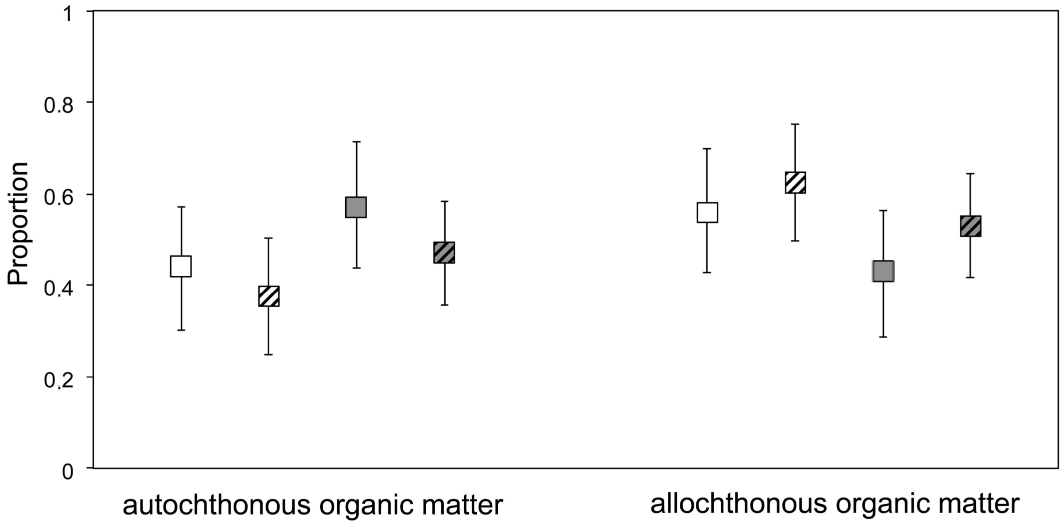

Figure 1.AppF. Comparison of mean diet contribution (range 0-1), as calculated by isotopic mixing model SIAR to zooplankton. We included autochthonous (phytoplankton) and allochthonous organic matter (alder leaves) as potential resources (see discussion on potential pathways of allochthonous matter contribution to zooplankton diet). White symbols represent Gollinsee, gray ones Schulzensee. Treatment sides (addition of maize leaves) are depicted by shaded patterns. Error bars represent 95% Bayesian credibility intervals.

Appendix G: Contribution of primary resources to diet of omnivorous fish

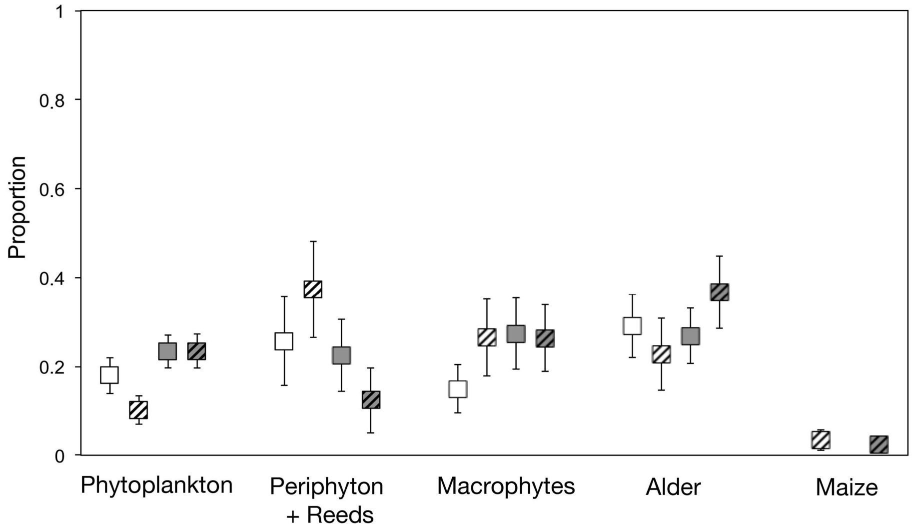

Figure 1.AppG. Contributions were calculated using isotopic mixing model SIAR and ratio calculations. Indirect contribution (via feeding on macroinvertebrates and zooplankton) from phytoplankton, periphyton and reeds, macrophytes, alder leaves, and maize leaves. Periphyton and reeds and macrophytes were also ingested directly. White symbols represent Gollinsee, gray ones Schulzensee. Treatment sides (addition of maize leaves) are depicted by shaded patterns. Depicted are global mean values (± SDs).

Chapter 2:

Boomerang ecosystem subsidies:

Organic carbon inputs from land to lakes are returned to terrestrial food webs via aquatic insects

Scharnweber, K., M. J. Vanni, S. Hilt, J. Diekmann, and T. Mehner

Abstract

Many ecosystems are subsidized by inputs of matter derived from adjacent ecosystems. For instance, the reciprocal transfer of organic matter between streams and riparian ecosystems has repeatedly been demonstrated. It has been assumed that land-water coupling with lakes is relatively weak, due to their concave shape within terrestrial landscapes. We experimentally demonstrated fluxes of terrestrial carbon to lakes and their return to terrestrial surroundings. The main vectors were aquatic insect larvae (Chironomidae) that fed on experimentally added terrestrial particulate organic carbon (tPOC), and became prey to terrestrial predators (spiders) after emergence. Carbon transfer was quantified using isotopically distinct maize (*Zea mays*) leaves in two experimentally divided lakes. The isotopic signals of Chironomids and spiders were significantly enhanced in the lake treatment sides compared to reference sides. Flux estimates indicated that only a minor fraction of added tPOC returned to spiders. Furthermore, extended coverage by submerged macrophytes in one lake facilitated a higher Chironomid biomass, and hence a four-fold greater carbon transfer to spiders. The "boomerang" carbon flux between land and lakes challenges the distinction between autochthonous and allochthonous carbon sources and signals a strong functional coupling of terrestrial and aquatic ecosystems.

Introduction

Historically, lake ecosystems were viewed as isolated “habitat islands in a sea of lands” (Forbes 1887). However, recent evidence suggests that adjacent terrestrial habitats can be coupled to lakes via multiple linkages and bi-directional fluxes of carbon and nutrients (Polis et al. 1997, Vander Zanden and Gratton 2011). Terrestrial organic matter can enter lakes in different forms (Cole et al. 2006), for example as terrestrial organisms (tPrey) which may be preyed upon by aquatic predators. Alternatively, allochthonous (externally produced) organic matter can enter the lake as either terrestrial dissolved organic carbon (tDOC) or terrestrial particulate organic carbon (tPOC). One of the most important land-to-lake linkages is provided by annual leaf litterfall, when a pulsed flux of tPOC enters lakes primarily at the land-water interface via the littoral zone (Wetzel 1992). Within this structured habitat, tPOC is processed by microbial communities or ingested by macroinvertebrate shredders or zooplankton (Jones et al. 1999, Karlsson et al. 2003, Pace et al. 2007). A potential return of organic matter from a lake back to land may occur via animals with a life cycle that includes both an aquatic and a terrestrial stage. Such complex life cycles have evolved in numerous taxa, for example in amphibians and insects (Knight et al. 2005, Schreiber and Rudolf 2008). Aquatic insects are particularly potent vectors across ecosystem boundaries (Lundberg and Moberg 2003, Gratton et al. 2008, Kraus and Vonesh 2012) and may act as “mobile resources” (sensu Kraus et al. 2011). During their larval (aquatic) stage, they feed on both aquatic and terrestrial resources and can receive a high proportion of their dietary energy from the latter (Carpenter et al. 2005, Cole et al. 2006, Solomon et al. 2011). When they reach maturity and emerge from the aquatic environment, they serve as prey for terrestrial predators inhabiting riparian zones, including bats, birds and arthropods (e.g. spiders) (Sabo and Power 2002b, Jonsson and Wardle 2009, Pope et al. 2009).

Most studies of reciprocal aquatic-terrestrial flux linkages have been conducted in streams and rivers (Nakano and Murakami 2001, Baxter et al. 2005, Iwata 2007). However, a few studies show that organisms can link lakes and terrestrial ecosystems in a similar way (Mehner et al. 2005, Gratton et al. 2008, Hoekman et al. 2011), and calculations suggest that small lakes with a high perimeter-to-area ratio may exhibit the highest fluxes of organic matter across habitat boundaries (Mehner et al. 2007). Small and shallow lakes are the world's most common lakes type (Downing et al. 2006). However, the presence of a structured littoral habitat in shallow lakes can be strongly linked to nutrient loading (Brothers et al. unpublished manuscript), and may furthermore be relevant for reciprocal fluxes of organic matter. Shallow lakes can either be turbid, with a high biomass of phytoplankton and absence of submerged macrophytes, or clear with a dominance of submerged macrophytes (Scheffer et al. 1993). When present, macrophytes provide structural complexity and may trap tPOC entering the lake (Vermaat et al. 2000), thus enhancing food availability for littoral consumers. This mechanism has also been suggested for structural complexity provided by woody debris (Francis et al. 2007, Roth et al. 2007). Lakes with and without extensive macrophyte beds may thus differ in terms of their benthic invertebrate biomass and species composition (Pardue and Webb 1985, Beckett et al. 1992, Hargeby et al. 2007), which may affect fluxes from lakes to land via emergence of terrestrial life stages.

In this study, we aimed to demonstrate and quantify the complete and reciprocal cycling of organic matter between terrestrial environments and small, shallow lakes, thereby building on previous approaches which examined the unidirectional flows of organic matter between lakes and land (Gratton et al. 2008, Jonsson and Wardle 2009, Hoekman et al. 2011, Earl and Semlitsch 2012). We conducted whole-lake experiments by tracing artificially added tPOC across ecosystem boundaries. We divided two eutrophic lakes which differed in littoral zone size and complexity, and added maize (*Zea mays* (L.)) leaves to one side of each lake to mimic a pulsed tPOC load during autumn leaf litterfall. Maize leaves are a suitable tracer due to their distinct carbon stable isotope signature relative to aquatic primary producers and tree leaves (Chapter 1). Using this study design, we wanted to address two hypotheses. First, we hypothesized that terrestrial organic carbon entering lakes in particulate form is transformed within the lakes into prey for terrestrial predators. This transformation is facilitated by consumers with aquatic-terrestrial life cycles. In this way, the tPOC entering the lake is reciprocally cycled by these consumers back into the ecosystem from which it originated. Second, we hypothesized that a structurally complex littoral zone facilitates a higher processing capacity of tPOC, and hence a greater proportion of the tPOC experimentally added to the lakes would return to the terrestrial environment from the lake with a more complex littoral habitat.

Materials and methods

Study sites and experimental design

We conducted whole-lake experiments in two eutrophic, shallow lakes located in northeast Germany (Table 1.1). The two lakes differ with respect to water clarity, as Kleiner Gollinsee (hereafter referred to as Gollinsee) is turbid and dominated by phytoplankton, whereas Schulzensee has clearer water and contains submerged macrophytes (*Ceratophyllum submersum* L.) Both lakes are surrounded by alder trees (*Alnus glutinosa* (L.) Gaert.) and reed stands (*Phragmites australis* (Cav.) Trin. ex Steud.), whereas floating-leaved macrophytes (*Nymphaea alba* L.) cover a total area of only 3% in Gollinsee but 12% in Schulzensee. In total, the littoral area in Schulzensee covers a larger surface area (32%) than that of Gollinsee (18%), and provides a considerably more complex underwater habitat by the presence of submerged macrophytes. In October 2010, each lake was divided by plastic curtains into two approximately equally-proportioned halves (Table 1.1). As curtains were sealed into the sediment, they completely isolated the water volume of each half. To mimic an allochthonous tPOC subsidy, we added a carbon source with a distinctly elevated $\delta^{13}C$ signature, outside the overlapping $\delta^{13}C$ range of autochthonous resources and terrestrial C_3-plants (Smith and Epstein 1971, Meyers and Lallier-Verges 1999). We thus used maize leaves, which have a mean $\delta^{13}C$ of -13.5 ± 1.8‰ (standard deviation (SD)), compared to -28.4 ± 0.7‰ in alder leaves. In a complementary study, we showed that this source has been successfully used to trace the fate of tPOC in lake food webs by stable isotope analysis (Chapter 1). In November 2010, we added roughly 2 t of coarsely shredded fresh maize leaves and stems (without cobs) into one half of Gollinsee (25×10^3 mg C m^{-2} yr^{-1}) and 3 t to one half of Schulzensee (28×10^3 mg C m^{-2} yr^{-1}) (subsequently referred to as treatment sides as compared to non-treated reference sides). The quantity of maize carbon added (per area) was approximately four times greater than the estimated lake-specific areal input of terrestrial carbon by alder leaves, which occurred at the same time as the addition of maize leaves (Table 1.1). Simultaneously with the maize addition, we started a litter-bag experiment to quantify maize degradation over nine months, suggesting that maize leaves degrade at rates similar to reed or alder leaves (Chapter 1).

Sampling and estimation of secondary production

To collect adult Chironomidae, we used emergence traps similar to the WEEK design from LeSage and Harrison (1979). A wooden frame of 0.6 × 0.6 m covered a surface area of 1.2 m^2 and supported a collection tent covered with fine mesh (0.05 - 0.2 mm). The tent opening, 0.45 m above the water surface, was covered with a collector made from an acrylic glass cylinder (diameter 10 cm, 10 cm height), where an upside-down funnel was glued in place. The collector was filled with water in which recently emerged individuals accumulated. Some individuals obtained from the collector were alive, but others were dead when collected. However, we only used individuals that showed no signs of decomposition. We placed four emergence traps in each lake, two on the reference sides and two on the treatment sides. Traps were sampled between June and July 2011, approximately once per week. Terrestrial (adult) stages of Chironomidae were identified to the finest taxonomic resolution possible (see Table 2.1 for a list).

Table 2.1. Species list of Chironomidae

Sub-family	Tribe	Species
Tanypodinae		*Tanypus punctipennis* (Meigen, 1818)
Chironominae	Chironomini	*Chironomus plumosus* (Linnaeus, 1758)
		Cryptotendipes pseudotener (Goetghebuer, 1922)
		Glyptotendipes pallens (Meigen, 1804)
		Glyptotendipes paripe/ (Edwards, 1929)
		Polypedilum sordens (van der Wulp, 1874)
		Polypedilum (cf) tritum (Walker, 1856)
		Tribelos intextus (Walker, 1856)
	Tanytarsini	*Tanytarsus* sp.

Spiders were collected by hand picking from their webs in the reed belt surrounding each lake. This was carried out at night, when the spiders were most active and could be easily spotted with a flashlight. Spiders were sampled on two occasions (June 6th and July 5th, 2011). Spiders were collected in plastic bags and killed by freezing. In the lab, samples were sorted and either dried at 60°C for stable isotope analysis or placed in ethanol for further species determination. We focused our analyses on the most abundant spider family, the long-jawed orb weavers (Tetragnathidae). Three species of Tetragnathidae were sampled: *Tetragnatha pinicola* (L. Koch, 1870), *T. montana* (Simon, 1874) and *T. shoshone* (Levi, 1981). Spiders of the family Tetragnathidae are typically found in near-shore vegetation (e.g. reeds), where they build delicate, short-lived and orb-shaped webs of sticky silk (Gillespie 1987). Prior isotope studies revealed that they feed extensively on aquatic resources (Collier et al. 2002, Kato et al. 2003, Sanzone et al. 2003).

To calculate the secondary production of aquatic Chironomidae, benthic macroinvertebrates were sampled monthly between April and November 2011. Samples were taken along transects across each lake half, thus covering three different habitat zones, the eulittoral (0-1 m depth), sublittoral (1-2 m depth) and profundal zones (> 2 m depth). Chironomidae were rare in the profundal zones, and hence we restricted all subsequent analyses to the eu- and sublittoral zones. After sampling a 0.6 m^2 area using a kick net of 250 µm mesh size, the entire sample including all substrates was fixed in ethanol and sorted in the laboratory, with macroinvertebrate individuals being stored in ethanol. The dry mass (DM) of Chironomidae was obtained from the wet mass (WM) of ethanol-fixed samples by correcting for mass loss during fixation in ethanol (26% loss for WM, 34% of DM, Leuven et al. 1985). To increase the number of Chironomidae individuals for stable isotope analyses, additional samples were collected from several littoral locations of the four lake sides in June 2011, using a kick net (500 µm mesh size). The organisms were transferred to containers with tap water and held in the refrigerator for one night to allow animals to void their guts. Species of larval Chironomidae could not be determined and hence all individuals were grouped together for subsequent calculations.

To facilitate the calculation of secondary production according to the length-increment method of cohorts, all Chironomidae from samples taken in April, June and November 2011 were individually measured using an ocular micrometer (Nikon SMZ 1500, Nikon Instruments Europe B.V., Amsterdam, Netherlands). However, an inspection of length-frequency distributions from these months revealed that distinct cohorts could not unequivocally be determined in the samples. Therefore, the annual production (P, g DM m^{-2} yr^{-1}) of Chironomidae in the littoral zone was estimated using the allometry-based approach of Plante and Downing (1989):

$$\mathrm{Log}\ (P)=0.06+0.79\times \mathrm{Log}\ (B)-0.16\times \mathrm{Log}\ (M_{\mathrm{max}})+0.05\times T,$$

where B is the mean annual biomass (g DM m^{-2} averaged across the eight sampling months) and M_{max} is the maximum individual mass (mg DM $\mathrm{individual}^{-1}$) for each habitat on each lake side (estimated from the three sampling occasions during which individuals were measured). Because calculations based on single maximum-sized individuals may overestimate production, we used the average length of the largest 10% of individuals of pooled Chironomidae for M_{max} (compare Bergtold and Traunspurger 2005). T is the annual water temperature (13.6°C in Gollinsee, 14.3°C in Schulzensee; measured using a stationary weather station at each lake). We assumed that the secondary production of Chironomidae from April to July (four months) equaled about 50% of the annual production of Chironomidae, as estimated from our data for the eight-month period from April to November. To convert Chironomidae biomass to carbon (C) units (to facilitate comparison of carbon fluxes from maize leaves), we multiplied their DM by 0.45 (Peters 1983, Wetzel 2001). To disentangle potentially enhanced secondary production in the treatment sides via a tPOC subsidy from an indirect effect of higher biomass of Chironomidae in more complex habitats on production estimates, we calculated production to biomass (P/B) ratios for all lake halves. Higher P/B ratios in the treatment sides would suggest that Chironomidae grew quicker and to larger sizes than in the reference sides as a direct effect of a tPOC subsidy, whereas similar or lower P/B values in the treatment sides may indicate that differences in production are due to differences in Chironomidae biomass.

For logistical reasons, we could deploy only two emergence traps per lakeside, and therefore we refrained from using catch data as reliable quantitative estimates of emergence fluxes. We used the following formula to estimate the flux of maize carbon (F_{maize}; g C m^{-2} $(4\ \mathrm{months})^{-1}$) via larval and adult Chironomidae to land and ultimately to the spiders:

$$\mathrm{F}_{\mathrm{maize}}=P\times \mathrm{E/P}\times D_{\mathrm{maize}}\times M_{\mathrm{spider}},$$

where P is the estimated production of Chironomidae between April and July (in C units), $\mathrm{E/P}$ is the emergence to production ratio, which is on average 0.3 for lakes (Gratton and Vander Zanden 2009) and D_{maize} is the mean contribution of maize carbon to the diet of larval Chironomidae, as calculated by mixing models using stable isotope data from these lakes (Chapter 1). According to these estimates, maize carbon contributed, on average, 3% and 7% to the diet of Chironomidae (i.e., D_{maize} = 0.03 and 0.07) in Gollinsee and Schulzensee, respectively (Chapter 1). We lacked reliable estimates of spider abundances; therefore we assumed a mortality rate of emerged adult Chironomidae imposed by spiders (M_{spider}) of 0.0105 (i.e. 1.05% of Chironomidae were preyed upon by spiders), averaged from literature data on the mortality rates of aquatic insects captured by terrestrial spiders: in studies of capture rates of damselflies, Gribbin and Thompson (Gribbin and Thompson 1990) estimated the mortality from spiders in *Pyrrhosoma nymphula* (Sulzer) as 1.7%, and for *Calopteryx haemorrhoidalis* (Vander Linden) mortality rate was estimated as 0.9% for females and 1.4% for males (Rehfeldt 1992)]. In the study of Williams et al. (Williams et al. 1995), the minimum estimate of the proportion of total insect emergence that is captured by two Tetragnathid species was 0.2%. We recognize that our estimates of C flux to spiders are based on the assumptions that rates of emergence flux and spider-induced mortality are equal to mean rates from the literature. However, this approach allows us to derive at least a rough estimate of the magnitude of this lake-to-land subsidy.

Stable isotope and statistical analyses

Samples for stable isotope analyses of larval and adult Chironomidae and spiders were ground to a fine powder and weighed in tin capsules. Elemental and stable isotope analyses were conducted using a FLASH EA 1112 elemental analyzer coupled to a Thermo Finnigan DELTA $^{\mathrm{Plus}}$ Advantage mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA) at the University of Jyväskylä, Finland. Results are expressed in the δ notation using ratios of samples and international standards (PeeDee Belemnite for $\delta^{13}C$, atmospheric N for $\delta^{15}N$).

For larval Chironomidae, the stable isotope data presented here reflect a pooled sample from undetermined species. Species determination for adult Chironomidae was possible but sample sizes for single species were usually too low for separate analyses, except for *Polypedium sordens* in Gollinsee. We thus pooled all species and obtained the average value of stable isotopes for this group. In the same way, we pooled the isotopic data for the three species of Tetragnathid spiders.

The assumptions of normal distribution and homogeneity of variances were met for all statistical analyses. Isotopic data of larval Chironomidae, which were sampled only once, were analyzed using a univariate general linear model (GLM) with main factors lake, lake side (treatment or reference) and the two-way interaction as independent variables. For adult Chironomidae and spiders, we used linear mixed models (RM LMM) with main factors lake, lakeside (treatment or reference) and all two-way interactions and compound symmetry as covariance type. We further included month and the two-way interactions with month as a repeated measure, as adult Chironomidae and spiders were sampled during June and July. Estimated marginal means (EMM) were calculated for all significant main effects to indicate whether isotope signatures differed between lakes, treatment and reference sides, or months. Statistical tests were performed using PASW for windows v. 17.0 (IBM Corporation, New York, U.S.A.). As the δ^{15}N of maize was very similar to the δ^{15}N values of aquatic primary producers, there was no effect of maize addition on δ^{15}N signatures of aquatic consumers and hence we focus subsequently only on the differences in δ^{13}C signatures between lakes, lakesides, and months.

Results

There was a significant enhancement of δ^{13}C values in treatment sides relative to reference sides for larval Chironomidae, adult Chironomidae and spiders in both lakes (Figure 2.1, Table 2.2). A significant lake effect was found only for spiders, with their δ^{13}C values being higher on the shore of Gollinsee than on the shore of Schulzensee (Table 2.2). June δ^{13}C values were significantly higher than those in July for adult Chironomidae and spiders (not calculated for larval Chironomidae, Table 2.2). The significant interaction term of lake x treatment in adult Chironomidae (*P*= 0.002) and the similar trend in spiders (*P*= 0.054) indicate that the treatment by maize addition affected the lakes differently (Figure 2.1, Table 2.2).

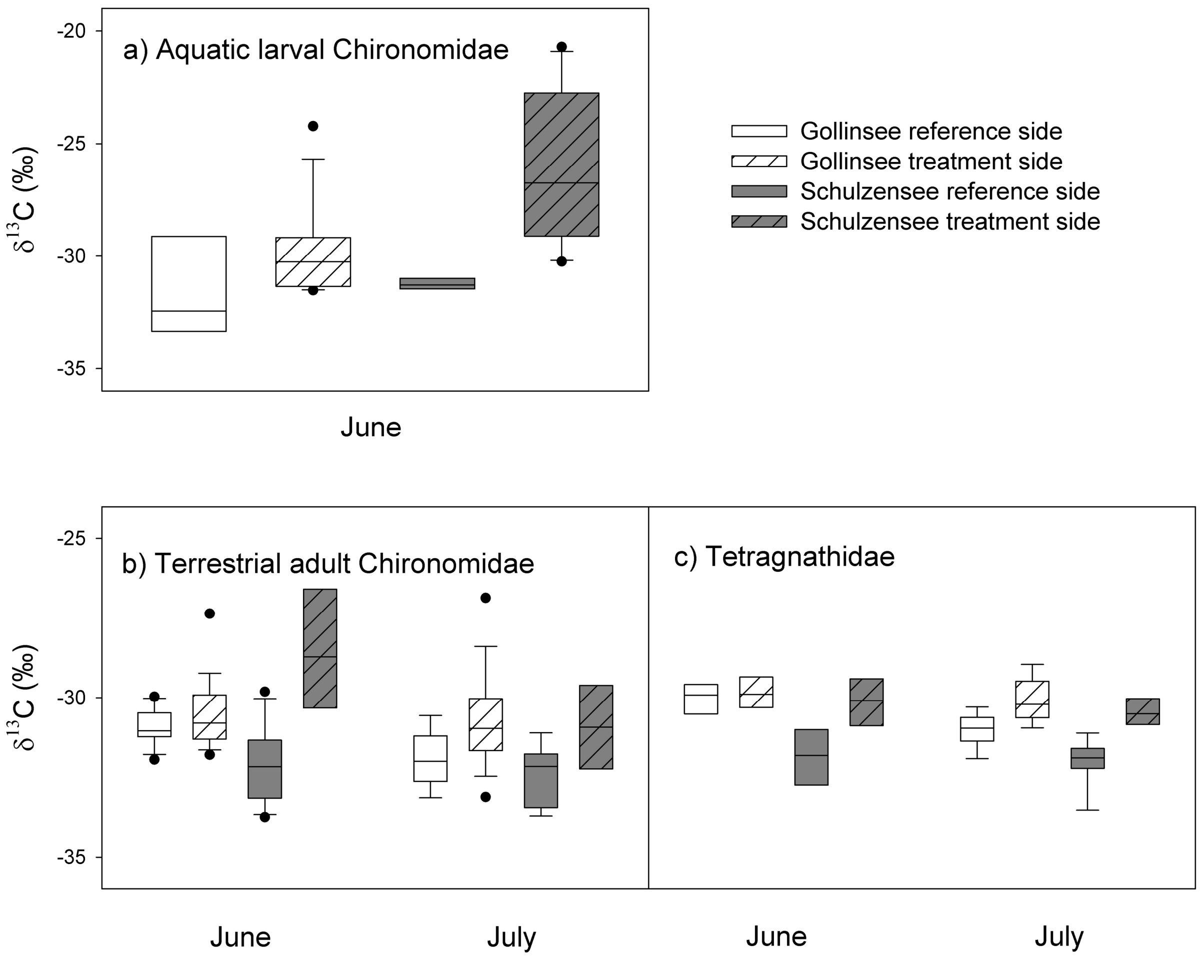

Figure 2.1. $\delta^{13}C$ signatures of a) aquatic larval Chironomidae (sampled in June only), b) terrestrial adult Chironomidae and c) Tetragnathid spiders (sampled in June and July) of reference and treatment (maize added) sides of Gollinsee (turbid and phytoplankton dominated) and Schulzensee (clear and with macrophytes). Boxplots with mean values and 25th and 75th percentiles are shown, whiskers represent 10th and 90th percentiles and dots are 5th and 95th percentiles.

For the single species of adult Chironomidae (*P. sordens*) for which sample size was high enough to facilitate an individual comparison, a *t*-test confirmed elevated $\delta^{13}C$ values in the treatment relative to the reference side in Gollinsee (t_{16} = -2.760; *P* = 0.014). The secondary production of Chironomidae from April to July 2011 was higher in Schulzensee (448 mg C m^{-2} (4 months)$^{-1}$) than in Gollinsee (278 mg C m^{-2} (4 months)$^{-1}$; weighted means from differently sized treatment and reference lake sides). However, the average biomass of Chironomid individuals was significantly larger in Schulzensee than in Gollinsee (median Gollinsee: 0.44 mg DW, Schulzensee 0.53 mg DW; Mann-Whitney *U*= 1498543; *P*= 0.001).

Table 2.2. Results of univariate general linear models (GLM) for larval Chironomidae with $\delta^{13}C$ as a dependent variable, and lake (G= Gollinsee, S= Schulzensee), treatment (r= reference side, t= treatment side (maize addition)) and bivariate interactions as independent variables, or repeated-measure linear mixed models (RM LMM) for adult Chironomidae and Tetragnathid spiders with $\delta^{13}C$ as a dependent variable, and lake, treatment, month and their bivariate interactions as independent variables. For significant main effects, comparisons of estimated marginal means (EMM) are shown. Higher values of $\delta^{13}C$ indicate higher reliance on terrestrial material.

Group	***N***	**Model type**	**Factor**								
			Lake		**Treatment**		**Month**		**Lake × Treatment**	**Lake × Month**	**Treatment × Month**
			P	**EMM**	***P***	**EMM**	***P***	**EMM**	***P***	***P***	***P***
Aquatic larval Chironomidae	33	GLM	0.193		0.004	r < t	0.196	not calculated	0.196	not calculated	not calculated
Terrestrial adult Chironomidae	85	RM LMM	0.922		<0.0001	r < t	0.015	June > July	0.002	0.453	0.947
Tetragnathidae	61	RM LMM	0.0001	G > S	<0.0001	r < t	0.021	June > July	0.054	0.474	0.726

In both lakes, production was higher in the treatment than in the references sides (+41% in Schulzensee, +18% in Gollinsee; Figure 2.2a). Higher production in the treatment sides was most strongly expressed in the eulittoral habitat in Schulzensee, where production was about twice as high as in the similar habitat of the reference side (Figure 2.2a). Production to biomass ratios (P/B values) were lower in the treatment than in the reference sides in both Schulzensee (reference: P/B = 5.5; treatment: P/B = 4.1) and Gollinsee (reference: P/B = 5.7; treatment: P/B = 5.6), suggesting that the higher secondary production was primarily caused by a higher total larval Chironomidae biomass (Figure 2.2b), and not by higher growth rates and higher maximum individual lengths in the treatment sides.

From April to July, the flux of maize carbon from the lake treatment sides to the land via emergence of Chironomidae was estimated to be 2.77 mg C m^{-2} $(4\ months)^{-1}$ for Gollinsee (0.0111% of added maize C) and 12.17 mg C m^{-2} $(4\ months)^{-1}$ for Schulzensee (0.0435% of added maize C) (Figure 2.3). By assuming a spider predation rate of 1.05% of total emergence, the maize carbon consumed by riparian spiders was 0.03 mg C m^{-2} $(4\ months)^{-1}$ for Gollinsee and 0.13 mg C m^{-2} $(4\ months)^{-1}$ for Schulzensee (Figure 2.3). Therefore, about 0.00012% in Gollinsee and 0.00046% in Schulzensee of the tPOC entering the treatment sides of lakes as maize leaves was ultimately consumed by the terrestrial spiders, meaning that Schulzensee exported approximately 3.92 times more C relative to C input by maize leaves than Gollinsee.

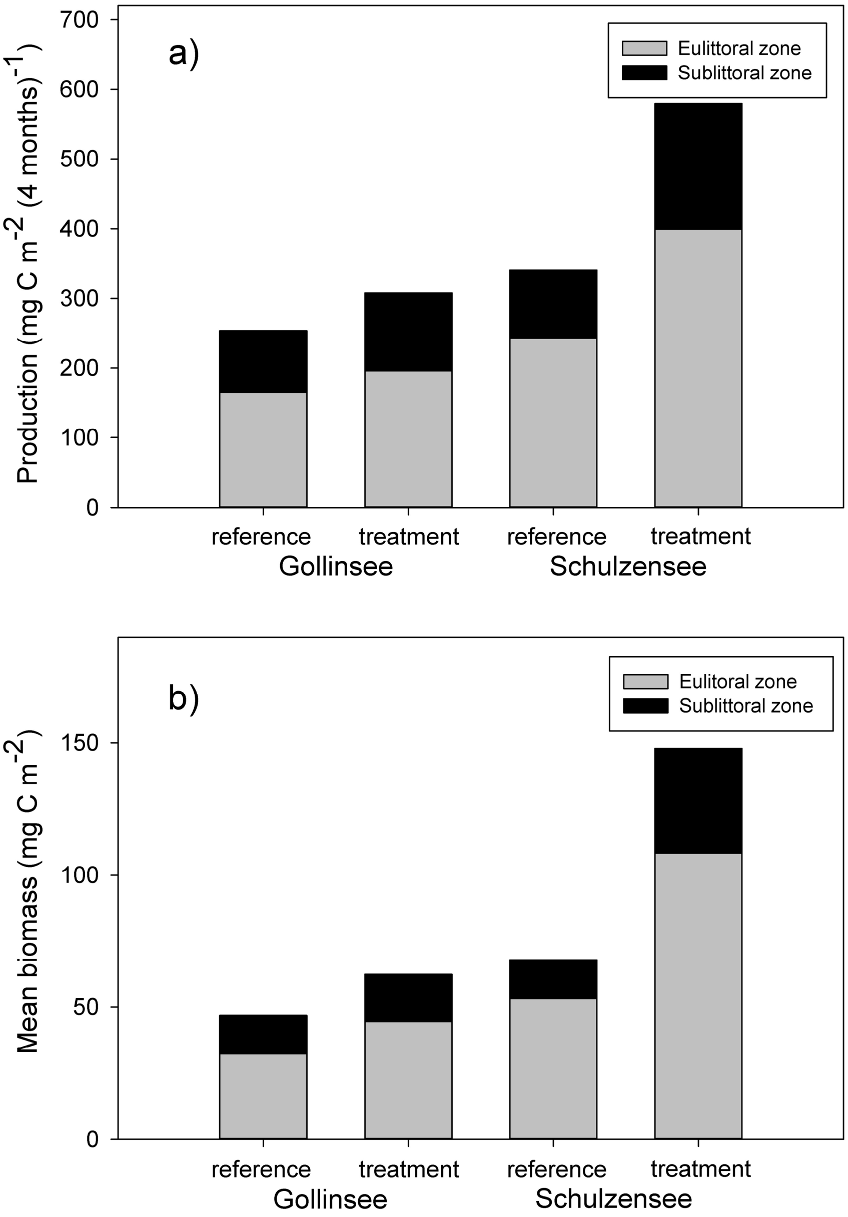

Figure 2.2. a) Secondary production of Chironomidae (mg C m^{-2} (4 months)$^{-1}$) estimated by the model of Plante and Downing (1989) and b) mean biomass in the eulittoral (0-1 m depth) and sublittoral (1-2 m depth) zones of reference and treatment sides of Gollinsee and Schulzensee.

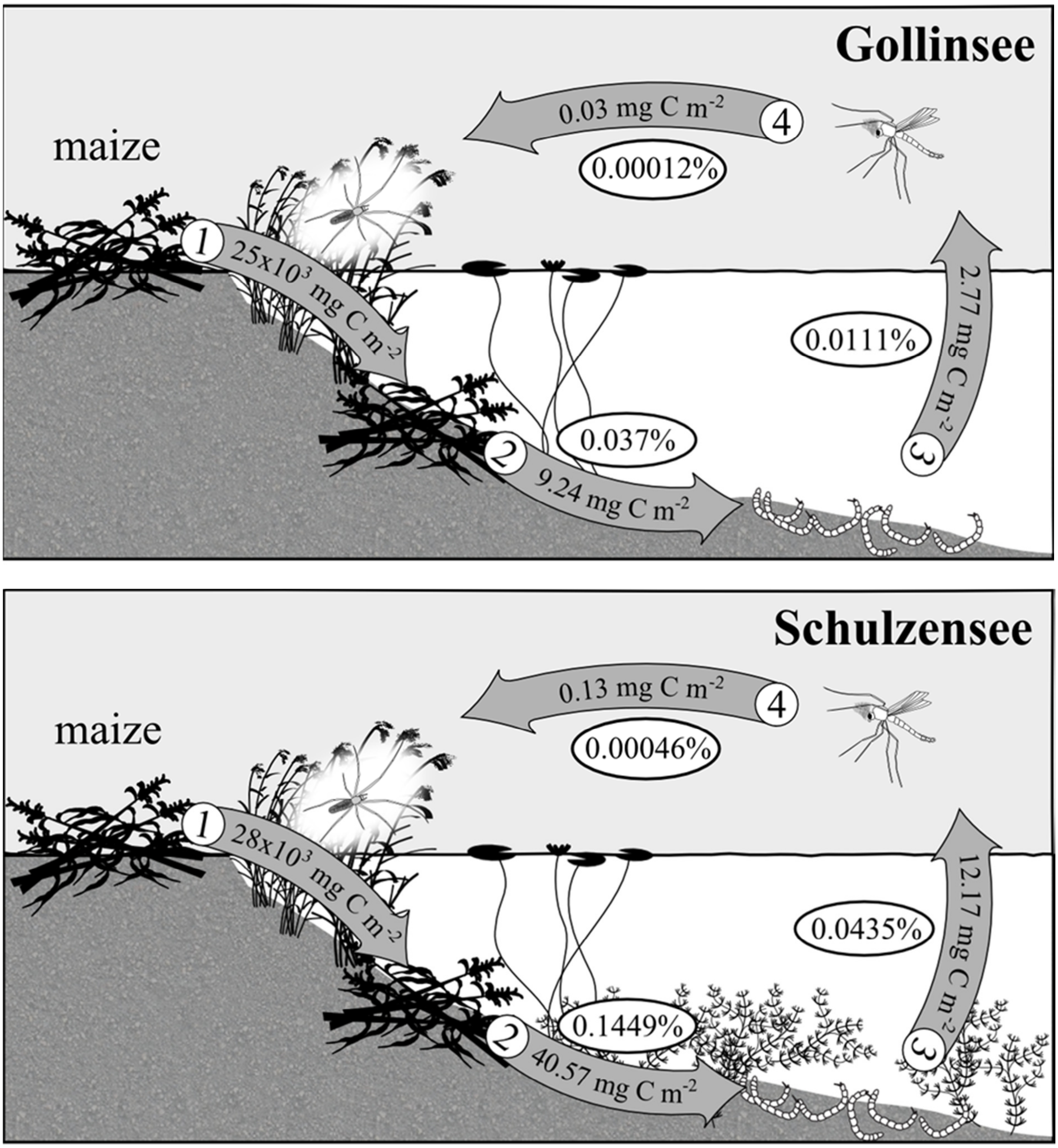

Figure 2.3. Boomerang pathway of experimentally added terrestrial particulate organic carbon (tPOC) between aquatic and terrestrial ecosystems in turbid Gollinsee and macrophyte-dominated Schulzensee. 1) Addition of tPOC as maize leaves; 2) Flux of maize carbon to larval Chironomidae, considering 3% (Gollinsee) and 7% (Schulzensee) diet contribution from maize from isotope mixing model; 3) Flux of maize carbon across the ecosystem boundary via adult (emerging) Chironomidae, considering an emergence rate of 30%; 4) Flux of maize carbon to riparian predators via predation of Tetragnathid spiders on adult Chironomidae, considering a predation rate of 1.05%. Flux 1) is related to the area (m^2) of the lake sides; all other fluxes are related to the area (m^2) of littoral zones.

Discussion

Our results showed that artificially added tPOC in the form of maize leaves was processed and transformed in two small, shallow lakes, and that a fraction of this carbon returned to the terrestrial ecosystem via riparian predators. This return was facilitated by Chironomidae, insects with complex life cycles which include both aquatic and terrestrial stages. Absolute carbon fluxes back to the land were estimated to be very small relative to the experimental input into the lake, but the stable isotope signatures of terrestrial spiders on the shores of both lakes were significantly enriched by maize additions. Both absolute and relative C fluxes back to the land were almost four times higher in the lake with submerged macrophytes, and thus a greater structural complexity in the near-shore zone (Schulzensee), than in the lake without submerged macrophytes (Gollinsee). Elevated C fluxes were caused by the higher secondary production of Chironomidae in Schulzensee, which itself was closely coupled with higher biomasses of larval Chironomidae in this lake. Accordingly, the structural complexity created by submerged macrophytes may facilitate higher densities of benthic macroinvertebrates, and thus higher rates of allochthonous carbon processing.

Back-cycling of tPOC additions

Our results reveal strong linkages between small, shallow lakes and their terrestrial surroundings via consumers that have aquatic-terrestrial life cycles and serve as prey for riparian spiders. Based on stable isotope analyses, Collier et al. (2002) found that 58% of the diet of riparian spiders originated from stream invertebrates. Similarly, Sanzone et al. (2003) showed that spiders living close to streams exhibited a strong reliance on stream invertebrates (up to 100%), characterized by a very high production of Diptera and Ephemeroptera. Although data are scarce, it appears that the emergence of insects from lakes can likewise be a major resource for consumers in adjacent terrestrial habitats. This is particularly well documented for the mass-emergence of Chironomidae from Icelandic lakes, which are used by terrestrial arthropod communities (Gratton et al. 2008, Hoekman et al. 2011). Furthermore, both lizards (Sabo and Power 2002a) and spiders have shown a numerical response to aquatic resource abundances, and were most abundant on small lake islands with high insect biomasses (Jonsson and Wardle 2009). As spiders themselves serve as prey for larger riparian predators like birds and bats (Jackson and Fisher 1986, Iwata 2007), they facilitate the transfer of energy and nutrients further into the terrestrial environment. In turn, terrestrial insects may serve as resources for aquatic predators, as shown for a variety of fish predators in lakes (Mehner et al. 2005, Vander Zanden et al. 2006, Weidel et al. 2008). Reciprocal subsidies between land and water have thus been documented several times, but experimental evidence for a complete land-water-land cycle of POC has heretofore been missing.

The novelty of our approach in this study is that we traced terrestrial carbon added experimentally to the lakes back into the terrestrial system. Thus, because the experiments were run in duplicate, we could reliably confirm a complete two-way flux of POC across the land-water ecotone. One may argue that the fluxes we have calculated are quantitatively unimportant for the recipient terrestrial environment, because they comprise only a very minor fraction of the carbon added to the lakes in form of maize leaves. We could not apply mixing models to quantify carbon sources for the spiders because we did not sample all their potential prey sources. Hence, we are unable to determine whether spiders totally relied on aquatic prey during the emergence of Chironomidae in these lakes.

The estimate of the total flux of carbon from maize leaves to land is critically dependent on the proportional contribution of maize carbon to the diet of Chironomidae larvae, the fraction of larvae that emerge as adults, and the proportion of emerging insects actually captured by the spiders. For all these fractions, we have applied rather conservative figures. For example, the mean contribution of maize to the diet of Chironomidae was only 7% for Schulzensee and 3% for Gollinsee, as estimated from mixing models on stable isotopes (Chapter 1). In contrast, allochthonous carbon from alder leaves contributed 27% in Schulzensee and 31% in Gollinsee in the treatment sides to the diet of Chironomidae larvae. The emergence rate (emergence/production) was assumed to be 0.3, the mean value obtained from a synthesis of 18 lakes, but it varies from 0.05-0.75 among lakes (Gratton and Vander Zanden 2009). Finally, we assumed that spiders captured only about 1% of all emerging Chironomidae. Even if this is a realistic fraction, most of the remaining 99% of adult insects likewise transport allochthonous carbon back to terrestrial environment by being prey for other predators (Gray 1993, Henschel et al. 2001, Nakano and Murakami 2001) or as detritus after natural mortality (Gratton et al. 2008).

When we converted the lake-wide abundances of Chironomidae larvae (Gollinsee 6,686,096 individuals; Schulzensee 8,236,297 individuals) into numbers of emerging adult insects, we estimate that 2,005,829 and 2,470,889 adult Chironomidae emerged from the treatment halves of Gollinsee and Schulzensee in 60 days, respectively (assuming an emergence/production ratio of 0.3, as mentioned above). Considering that each spider must consume at least 11 Chironomidae per day (Williams et al. 1995), approximately 3039 (3744) spiders would have received enough energy from emerging Chironomidae alone over a 60-day emergence period in Gollinsee (Schulzensee). By considering that maize and alder together comprised about 34% of the diet of Chironomidae larvae (see above), roughly 1033 to 1273 spiders were totally fuelled over two months in both lakes by terrestrial carbon which had been processed in the lake by aquatic Chironomidae larvae and then returned back to the terrestrial system via emerging Chironomidae. With a shoreline length of about 314 to 402 m for the treatment sides of both lakes, roughly 3 spiders per meter of shoreline may have received all of their carbon and energy from terrestrial sources, which in the interim had been transformed in the lakes.

Structural complexity facilitates tPOC processing

We had predicted that added maize leaves would be more intensely trapped and processed in the structured littoral zones in Schulzensee than in Gollinsee. This was confirmed, as 3.92 times more of the tPOC experimentally added to the lakes returned to the terrestrial environment from Schulzensee than from Gollinsee via emerging Chironomidae. The higher C flux calculated for Schulzensee can be separated into two contributions. First, the proportion of C originating from maize was higher in the diet of Chironomidae larvae in Schulzensee (7%) than in Gollinsee (3%), as estimated from stable isotope mixing models (Chapter 1). This difference suggests that the higher structural complexity in Schulzensee relative to Gollinsee may have facilitated a higher availability of maize carbon to Chironomidae larvae. Second, the higher carbon flux return to land is also attributable to the greater secondary production of Chironomidae in the littoral areas of Schulzensee (448 mg C m^{-2} (4 months)$^{-1}$) than in Gollinsee (278 mg C m^{-2} (4 months)$^{-1}$). This difference between the lakes is, however, primarily attributable to differences in treatment sides, whereas the mean secondary production in the reference sides was only slightly higher in Schulzensee than in Gollinsee. A higher secondary production in response to allochthonous carbon additions suggested that the quantity of autochthonous resources can limit secondary production, and this limitation was alleviated more in Schulzensee.

We can exclude the hypothesis that a faster growth and hence greater maximum size of Chironomidae larvae in response to tPOC subsidies accounted for the increased secondary production. The indirect calculation of secondary production as applied here was based on the maximum length of Chironomidae and average temperatures, neither of which differed substantially between lakes (Mann-Whitney U = 65312.5; P = 0.840), or between sides. Similarly, the P/B-values did not differ substantially between all lake halves, thus likewise suggesting that there were no differences in Chironomidae growth rates. On the other hand, Chironomidae biomasses were substantially greater in the treatment than in the reference sides of both lakes, and higher in Schulzensee than in Gollinsee. The correspondence between biomasses and production rates across all four lake halves suggests that differing biomasses of Chironomidae larvae were the main cause of production differences. A numerical response of the Chironomidae populations to the added maize leaves is not possible because the experiment was started in November, after Chironomidae eggs had already been deposited into the lakes. However, the tPOC subsidy may reduce the overall mortality of Chironomidae larvae through relaxed density dependence, and hence may result in higher biomasses. Experiments showed decreased growth and development rates when larval densities increased (Rasmussen 1985, Ristola et al. 1999). This process may explain higher biomasses in treatment than in references sides of both lakes.

As a complementary explanation, biomass differences may also reflect the direct effect of structural complexity on benthic macroinvertebrate densities, suggesting that habitat availability, but not carbon, is the limiting resource for benthic macroinvertebrates. There are a number of studies that have shown higher abundances of benthic macroinvertebrates, including Chironomidae, in areas with high complexity in comparison with lakeshore areas devoid of macrophytes or woody debris (Beckett et al. 1992, Hargeby et al. 1994). A habitat effect would explain the higher biomasses of Chironomidae in the more structured Schulzensee as compared to Gollinsee, a difference that was strongly expressed in the comparison of the treatment sides. This is supported by the observation that even in the reference sides, production and biomass of Chironomidae were higher in Schulzensee than in Gollinsee. Furthermore, macrophyte coverage provides a refuge against fish predation, and hence a reduced size-specific fish predation on macroinvertebrates (Bechara et al. 1993, Leppä et al. 2003) may have caused the higher average size of Chironomidae in Schulzensee. Overall, these alternative explanations do not allow for firm conclusions on the mechanisms of a potential subsidy effect by tPOC on secondary production. Clearly, more experimental studies crossing gradients of carbon and habitat availability are needed to elucidate in which way tPOC subsidy is facilitated by structural complexity in nearshore lake zones.

Conclusion

The complete carbon cycling via consumers with aquatic-terrestrial life cycle as demonstrated here challenges the usual distinction between autochthonous and allochthonous carbon sources. The maize carbon produced in terrestrial ecosystems is technically autochthonous to spiders, although it is provided via emerging Chironomidae whose secondary production occurred in aquatic habitats, and hence may also be considered allochthonous to spiders. Therefore, allochthonous subsidies to terrestrial predators may have been overestimated in previous studies (Collier et al. 2002, Kato et al. 2004, Iwata 2007) because some of the production of insects emerging from aquatic systems was in fact supported by terrestrial production, as shown by our results. In turn, terrestrial insects which often are a major prey source of aquatic predators such as fishes likely contain a fraction of carbon which was originally produced in the aquatic system in which the fishes live, and subsequently may have been translocated to the terrestrial system via multiple vectors. The start of such a second carbon loop could easily be demonstrated if terrestrial predators feeding on emerging insects would again become prey for aquatic predators from lakes (Ingley et al. 2012). Therefore, while lakes may appear to be "habitat islands in a sea of lands," (Forbes 1887) a viewpoint of such isolation does not reflect the continued back-and-forth "boomerang" cycling of organic matter and nutrients across the borders of terrestrial and aquatic systems.

Acknowledgements

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Chapter 3:

Effects of predation pressure and resource use on morphological divergence in omnivorous prey fish

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Abstract

Background

Body shape is one of the most variable traits of organisms and responds to a broad array of local selective forces. In freshwater fish, divergent body shapes within single species have been repeatedly observed along the littoral-pelagic axes of lakes, where the structural complexity of near shore habitats provides a more diverse set of resources compared to the open-water zones. It remains poorly understood whether similar resource-driven polymorphism occurs among lakes that vary in structural complexity and predation pressure, and whether this variation is heritable. Here, we analyzed body shape in four populations of omnivorous roach (*Rutilus rutilus*) inhabiting shallow lakes. We tested the relationship between body shape, gradients of resources, predation pressure, and, in a subset of two lakes, diet composition. We used genome scans of 331 polymorphic AFLP markers to test whether there was a heritable component to the observed morphological diversification.

Results

Body shape differed among lakes and was significantly correlated to differences in predation pressure. Roach from the lake with highest predation pressure were most divergent from the average body shape of all populations, characterized by a more streamlined body and caudally inserted dorsal fins; features that facilitate predator escape. Surprisingly, diet composition was not associated with morphology. AFLP analysis revealed weak genetic differentiation among lakes and no isolation by distance (IBD). Outlier analysis detected three loci under positive selection with

differing frequencies in the four populations. General linear models did not support an association of lake-specific genotypes with morphological variation.

Conclusion

Body shape was divergent among lakes, suggesting that processes previously reported from within single lakes may also be operating at the scale of whole lakes. We found no evidence for body shape being heritable, although sample size was small in these natural populations. Rather than habitat structure and diet, we conclude that predation had a stronger effect on the prevalence of local morphotypes. A variable morphotype facilitating the efficient uptake of a variety of spatially and temporarily scattered resources seems to be favored in these small aquatic systems.

Background

Populations can exhibit divergent body morphologies in response to environmental cues and these differences may have a significant influence on the performance of individuals (Koehl 1996). A common form of intraspecific divergence in body morphology is resource polymorphism, where morphological variation is associated with segregation in habitat and diet (Robinson and Wilson 1994, Skúlason and Smith 1995). In freshwater fish, divergence of morphs has been frequently shown along the pelagic-littoral habitat axis of lakes (i.e., from the near shore to open-water habitats) (Skúlason and Smith 1995). Foraging in the open water is associated with a high search rate for widely distributed and conspicuous planktonic prey. A more streamlined body occurs in these morphs to facilitate a high attack speed. In contrast, foraging in the structurally complex littoral zones results in a lower search rate for more cryptic benthic prey. Thus, a deeper body supports higher manoeuvrability (Anderson 1984, Ehlinger 1989). Fish species with coexisting pelagic and benthic morphs include three-spined stickleback (*Gasterosteus aculeatus*) (McPhail 1984, Schluter and McPhail 1992, Cresko and Baker 1996), Eurasian perch (*Perca fluviatilis*) (Svanbäck and Eklöv 2002, 2003, Olsson and Eklöv 2005) and Arctic charr (*Salvelinus alpinus*) (Skúlason et al. 1989, Malmquist et al. 1992, Alekseyev et al. 2002).

Predation pressure is another potentially selective force affecting the morphology of organisms. “Inducible defenses” (Harvell 1990) have been intensively studied in cladocerans (Grant and Bayly 1981, Dzialowski et al. 2003) and mussels (Cote 1995) and are known from a range of fish species. In the presence of predators, Crucian carp (*Carassius carassius*) develop a deeper body that increases the handling time by predators (Brönmark and Miner 1992). Pumpkinseed sunfish (*Lepomis gibbosus*) increase body depth and dorsal spine length when stimulated by permanent predation cues of walleye (*Sander vitreus*) (Januszkiewicz and Robinson 2007). Similar predator-induced responses have been described for perch (Persson et al. 1996).

Phenotypic variation can result from phenotypic plasticity (Day et al. 1994, Agrawal 2001) or from heritable adaptations to local selective forces (Smith and Skúlason 1996, Losos 2000, Rundell and Price 2009, Lundsgaard-Hansen et al. 2013). To distinguish between phenotypically plastic and inherited variation, population genomics are widely used to test for the genetic basis of adaptive divergence (Nosil et al. 2009). Genome scans are an appropriate method to compare natural populations of non-model organisms that are nonetheless ecologically important, allowing simultaneous screening of many loci throughout the genome and the identification of loci that are putatively under selection (Rice et al. 2011). As an example, genome scans were used to identify loci associated with morphological variation in European minnows (*Phoxinus phoxinus*) living in different habitat types (Collin and Fumagalli 2011).

In the present study, we focused on a fish species for which we hypothesized substantial morphological variation due to a variety of locally specific selective forces. The omnivorous cyprinid roach (*Rutilus rutilus*) occurs in many lakes and rivers of the European temperate zone (Kottelat and Freyhof 2007). Resource polymorphism in roach was recently demonstrated in two lakes (Svanbäck et al. 2008): individuals inhabiting the pelagic zone were streamlined and fed on zooplankton, whereas those living in the littoral zone had a deeper body and were feeding on benthic invertebrates (Svanbäck et al. 2008). The dominant feature of near-shore littoral habitats is their structural complexity, created by aquatic macrophytes or woody debris that accumulates in shallow water zones. Structural complexity enhances the biomass of prey organisms for omnivorous fish, as the physical structures provide a substrate for epiphytic algae, a primary food source for the invertebrate prey (Pardue and Webb 1985, Beckett et al. 1992, Gresens 1995, Hargeby et al. 2007).

Much previous work has focused on structural complexity within lakes, but complexity also varies among lakes, with high complexity being a typical feature of shallow lakes in particular (Scheffer et al. 1993). Shallow lakes with low phytoplankton abundance ("clear" lakes) can contain extended zones of structural complexity because the clear water allows light penetration and facilitates the growth of submerged macrophytes. In contrast, shallow lakes with an abundant phytoplankton community ("turbid" lakes) can have low structural complexity because the lack of light prevents macrophyte establishment. As a result, the differential selective forces operating in littoral and pelagic habitats within single lakes and inducing intralacustrine (i.e. within lake) divergence may also operate in an interlacustrine (i.e. among lake) comparison of populations from differently structured shallow lakes.

Here we examined morphology (geometric morphometrics), diet (gut content and stable isotopes) and genetic variation (AFLP) of roach in four shallow lakes in order to assess ecomorphological divergence and local adaptation among lakes. Two lakes were clear and had extensive macrophyte cover while two were turbid and had almost no macrophyte growth. We predicted that roach in clear lakes would feed mainly on benthic macroinvertebrates and have a correspondingly deeper body shape, and that roach from turbid lakes would be more planktivorous and exhibit a more slender shape. We also expected that morphological divergence would be further enhanced by exposure to different predators. The four lakes exhibited a gradient of predation pressure, measured as the proportion of piscivorous fish biomass in total fish assemblages, thus providing a basis to test for an association of body morphology with relative predation pressure. Finally, we tested whether the roach populations differed genetically between the lakes, and whether genetic markers were associated with the interlacustrine morphological divergence.

Results

Morphometric analysis

Morphometric data were obtained from 119 individual roach: 43 from Kleiner Gollinsee (a turbid lake, hereafter referred to as Gollinsee), 38 from Schulzensee (clear), 18 from Globsowsee (turbid), and 20 from Kleiner Döllnsee (clear, hereafter referred to as Döllnsee). Fish from the four lakes differed significantly in their morphology, as revealed by permutation tests of Mahalanobis distances (Table 3.1). As visualized by graphical output of MorphoJ, landmarks 1 (anterior tip of snout), 7 (posterior insertion of dorsal fin) and 13 (insertion of pelvic fin) were most divergent among the four populations. As revealed from Canonical Variate Analyses (CVA), shape variations of canonical variate 1 (CV1, 74.9% of variance explained) were associated with the position of landmark 1 and body depth. CV2 (19.9% of variance explained) was associated with the location of the dorsal (landmarks 7 and 8) and pelvic fins (landmark 13) (Figure 3.1a). Roach from Döllnsee (clear) formed a distinct cluster in morphospace, located along the positive values of CV1. As visualized by warped outline drawings, roach from Döllnsee had a more elongated and fusiform body shape (Figure 3.1b). Furthermore, the dorsal fin was in a more posterior position relative to the average shape of roach. Variation in morphospace on CV2 was less pronounced and was driven by roach from Globsowsee (turbid, located along the positive values of CV2), characterized by a contraction of the dorsal fin and a shift of the pelvic fin toward the anterior part of the body.

Table 3.1. Pairwise distances between the group means in shape space. Results of DFA on size-corrected data are shown.

Factor	**Group comparison**	**Mahalanobis distance *D***	***P*-value**
Lake	Gollinsee-Schulzensee	1.7470	0.0339
	Gollinsee-Globsowsee	4.1565	< 0.0001
	Gollinsee-Döllnsee	6.8997	< 0.0001
	Schulzensee-Globsowsee	4.1778	< 0.0001
	Schulzensee-Döllnsee	6.5990	< 0.0001
	Globsowsee-Döllnsee	9.4319	< 0.0001

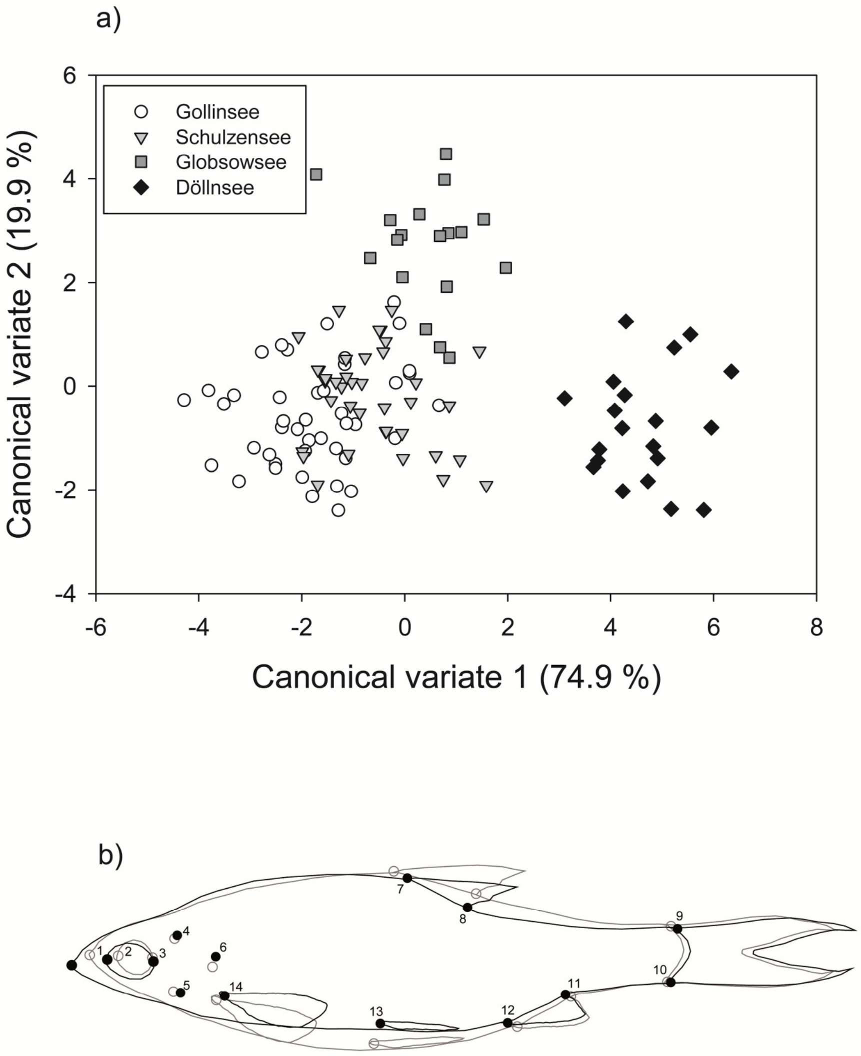

Figure 3.1. Shape differences among populations of the four study lakes. a) Results of CVA using geometric morphometric data to test for body shape differences among populations. Bivariate plot show variation along the first two major axes of shape variation (canonical variates 1 and 2, % of predicted variance in brackets). b) Warped outline drawing (derived from thin-plate spline method, amplified by 250% to facilitate visualization) depict shape differences of fish from Döllnsee (black line), compared to the average shape of all fish (grey line). Positions of the 14 digitized landmarks used in geometric morphometric shape analysis are shown. 1: anterior tip of snout; 2: anterior margin of eye; 3: posterior margin of the eye; 4: dorsal margin of opercular (principal opercular bone); 5: ventral margin of opercular (principal opercular bone); 6: posterior margin of operculum; 7: posterior insertion of dorsal fin; 8: anterior insertion of dorsal fin; 9: superior insertion of caudal fin; 10: inferior insertion of caudal fin; 11: anterior insertion of anal fin; 12: posterior insertion of anal fin; 13: insertion of pelvic fin; 14: anterior insertion of pectoral fin.

Genetic (AFLP) analysis

AFLP genotypes were obtained for 183 roach (69 from Gollinsee, 70 from Schulzensee, 23 from Globsowsee and 21 from Döllnsee). We scored 447 loci, of which 331 (74%) were polymorphic over the four populations (Table 3.2). The percentage of polymorphic loci varied from 58.5 to 85.9% within the four populations (Table 3.2). Gene diversity (h_s), as calculated by HICKORY, varied only slightly among lakes, ranging from 0.263 to 0.279 (Table 3.2). Bayesian statistics revealed a small but significant genetic differentiation among all four lakes (θ (HICKORY) = 0.03; 0.0246-0.0417 95% CI). Pairwise differentiation among the lake populations ranged from 0.0092 – 0.0702 with highest differentiation between populations of Globsowsee and Döllnsee and lowest between Schulzensee and Döllnsee.

Table 3.2. Genetic diversity of roach from four lakes of different structural complexity. *N*= number of individuals analyzed, % polymorphic loci refers to the total of 447 loci analyzed, h_s= gene diversity with credibility intervals calculated by a Bayesian approach as implemented in HICKORY, H_s= the mean within-population expected heterozygosity.

Lake	***N***	**% polymorphic loci**	**h_s**	**95% credibility interval of h_s**	
				lower	**upper**
Gollinsee	69	62.0	0.275	0.268	0.280
Schulzensee	70	58.8	0.263	0.257	0.269
Globsowsee	23	85.9	0.279	0.270	0.286
Döllnsee	21	85.9	0.266	0.258	0.273
	Σ= 183	Total= 77.6	H_s= 0.271		

Outlier analysis using BAYESCAN classified three loci (loci 31, 48 and 155; 0.91% of polymorphic loci examined) as outliers under divergent selection (probability *P*> 0.97 that the locus-specific component of F_{ST}, α, is different from zero, corresponding to Posterior Odds> 32, estimated false discovery rate= 0.0084). Probabilities for the remaining 328 loci ranged from 0.064 to 0.572. The three outlier loci were present in all four populations, but with differing frequencies. Locus 48 dominated in Gollinsee (97%) and Globsowsee (88%), but was rarer in Schulzensee (28%) and Döllnsee (15%). In contrast, locus 155 dominated in Döllnsee (95%) and Schulzensee (78%), but was rare in Globsowsee (19%) and Gollinsee (11%). Locus 31 was highly frequent in Döllnsee (100%), Gollinsee (97%) and Schulzensee (91%), but less frequent in Globsowsee (31%). Linkage disequilibrium (LD; ARLEQUIN) was estimated for all possible pairs of the three outlier loci, but among the 12 (4 lakes x 3 pairs) pair-wise tests, only one locus pair in Gollinsee showed a significant linkage (*P*= 0.009, all other *P*> 0.23).

Association of shape with predation pressure and genetic markers

Predation risk was highest for roach in Döllnsee (Figure 3.2d), because predator density was highest and roach were small in this lake. Lowest risk was in Schulzensee (Figure 3.2a), with intermediate risks in Gollinsee and Globsowsee (Figure 3.2b,c).

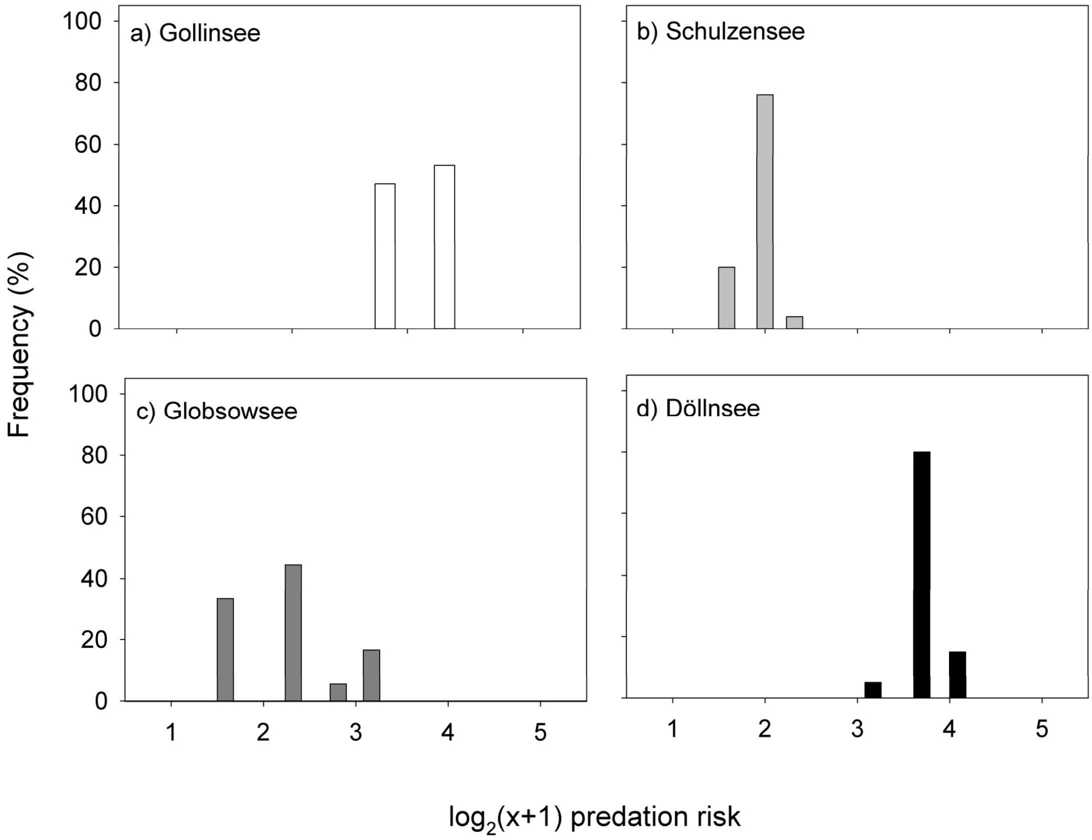

Figure 3.2. Predation risk in roach of the four lakes studied. Frequency distribution of individual predation risk (from 1= low to 5= very high) of roach in the four lakes studied (a-d). Predation score is a $\log_2$ (x+1)-transformed composite of individual fish size and lake-wide piscivory.

Across the four lakes, shape and estimated predation pressure were significantly associated (two-block partial least square analyses (PLS), RV= 0.0533; permutation test against the null hypothesis of independence with 1000 randomization runs: *P*= 0.0153) (Figure 3.3). In lakes with higher predation pressure, the deviation in shape was attributable to body form and fin insertion.

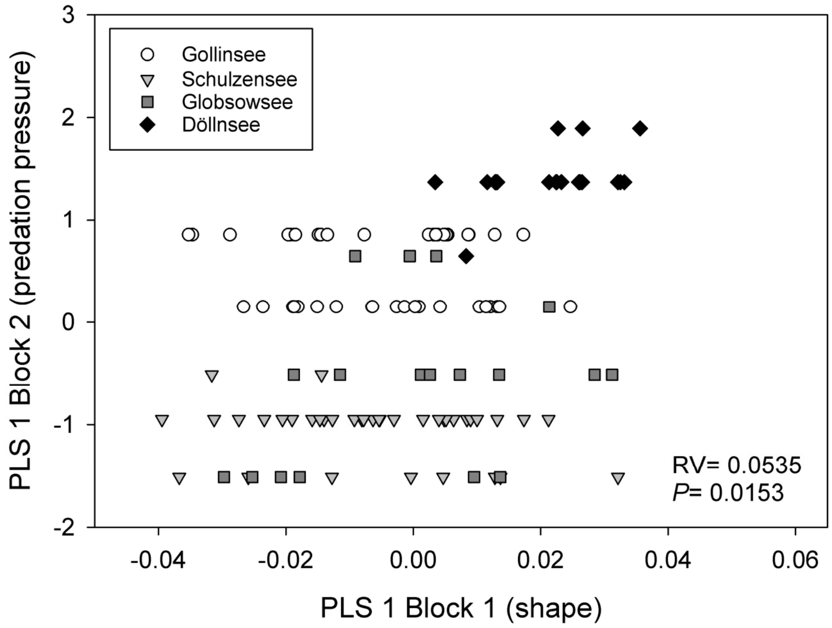

Figure 3.3. Correlation of shape and predation pressure of four lakes of different structural complexity. Results from PLS using size-corrected Mahalanobis distances as first block and predation pressure as second block are shown. *P*-value is obtained from permutation test against the null hypothesis of independence with 1000 randomization rounds.

Principal coordinate analysis (PCoA) of the 331 polymorphic AFLP loci aggregated 58.8% of variation along PCoA axis 1 (26.2%), PCoA2 (17.7%) and PCoA3 (14.9%). CV1 of shape analysis was significantly smaller in Globsowsee, Schulzensee and Gollinsee than in Döllnsee (GLM, $F_{7,85}$= 75.0, adjusted R^2= 0.85, P<0.0001, coefficients of lake contrasts: t< -7.1, P< 0.0001), but aggregated genotypes (PCoA1) did not contribute to shape variations (coefficient for PCoA1, t= 0.36, P=0.72). Interactions between PCoA1 and the lakes were likewise not significant (coefficients of interactions, t> 0.22, P> 0.83). Similarly, PCoA2 of genotypes and its interactions with lakes were not significant predictors of shape (CV1) (coefficient of PCoA2, t= -0.36, P= 0.34), but CV1 again significantly differed between Döllnsee and the other three lakes in this analysis (GLM, $F_{7,85}$= 78.5, adjusted R^2= 0.86, P< 0.0001; coefficients of lake contrasts, t< -7.1, P< 0.0001). General linear models on CV1 as the dependent dominant shape variable and lake and each of the three outlier loci as predictors confirmed that the genotype had no effect on shape of fish, because the effect of the outlier loci was not significant in any case (GLMs, $F_{7,85}$> 75.0, adj. R^2> 0.85, P< 0.0001; coefficients of the single outlier loci: locus 31, t= -0.27, P= 0.79; locus 48, t= 0.58, P= 0.56; locus 155, t= 0.43, P= 0.67).

Roach diet and association with morphological data

Diet composition (gut content, GC, and stable isotope analysis, SIA) was analyzed from Gollinsee (turbid) and Schulzensee (clear). Roach from Gollinsee fed almost exclusively on benthic prey (Figure 3.4), as indicated by GC (mean 99% of prey) and the isotopic mixing model (mean 94%). In Schulzensee, the contribution of benthic and pelagic food sources was similar (Figure 3.4), although GC indicated a slightly higher use of prey from benthic habitats (mean 65%) than did the isotopic mixing model (mean 52%). The proportions of ingested benthic and pelagic prey items (as shown from GC) differed significantly between both lakes (Mann Whitney *U*= -5.033; *P*< 0.001).

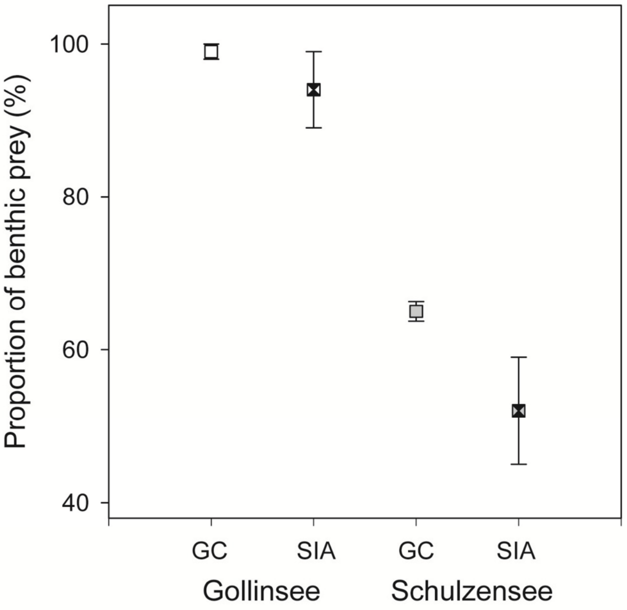

Figure 3.4. Contribution of benthic prey to diet of roach. Comparison of benthic contribution in diets of roach, revealed by gut content analysis (open symbols; depicted are mean values ± 95% confidence interval) and stable isotope analysis (shaded symbols; depicted are mean values ± 95% bayesian credibility interval) for roach from Gollinsee and Schulzensee.

PLS analysis of shape as the first block and gut contents of roach from Gollinsee and Schulzensee as the second block revealed no correlation of shape with diet composition (RV= 0.0475; permutation test with 1000 randomization runs: *P*= 0.66). Similarly, PLS with shape as the first block and isotopic ratios as second block did not show a significant correlation (RV= 0.0205; permutation test with 1000 randomization runs: *P*= 0.70).

Discussion

Body morphologies differed in all four studied roach populations in shallow lakes, in particular with respect to overall body form and anterior-posterior location of the fins. Our analyses suggest that these morphological differences reflect a gradient in exposure to predation risk among the lakes, as shape and predation pressure were significantly correlated. Contrary to our predictions, fish morphology was not correlated with resource use. Therefore, we suggest that the strong morphological divergence among lakes in roach was primarily driven by differences in predation risk. The four populations exhibited small genetic differences. However, we did not find evidence that genetic differentiation and morphological variation among lakes were associated; suggesting that shape variation in roach did not have a heritable component detectable in our analysis.

Roach body shapes were most distinct in Döllnsee, the lake characterized by highest predator abundance. There are several examples for induced morphological defenses in fish, including a plastic response to achieve a size refuge against predators limited by gape-size (Brönmark and Miner 1992, Persson et al. 1996, Januszkiewicz and Robinson 2007). Here, we observed a more slender body and a more caudal position of the dorsal fin. In contrast to morphological changes that prevent handling of prey by predators (Brönmark and Miner 1992, Persson et al. 1996, Januszkiewicz and Robinson 2007) we consider this body form to be advantageous for flight. To escape a predator attack, most fish show a similar fast-start behavior of sudden, high-energy swimming bursts, called a “C-start” (Weihs 1973, Webb 1984, Domenici and Blake 1997). During a C-start, fish bend into a “C” shape (stage 1) and then produce a propulsive stroke of the caudal region in the opposite direction (stage 2). This is facilitated by a slender body form and a relatively shallow anterior body and head region, as these contribute minimally to drag (Weihs 1989, Walker 1997, Langerhans et al. 2004). Thrust can be increased if the dorsal fin is in a caudal position (Walker 1997); however, Domenici et al. (Domenici et al. 2008) demonstrated higher speed and acceleration in Crucian carp of deeper body forms, attributable to a high percentage of muscle mass. Therefore, there seems to be a trade-off between a streamlined body that reduces drag and a deeper body of high muscle mass that increases burst.

A previous study compared morphology of one-year-old roach raised under experimental conditions in the presence and absence of pike (*Esox*) predators (Eklöv and Jonsson 2007). The main differences were changes of dorsal fin displacement and morphological responses similar to our findings. The authors suggested that these morphological changes affect swimming speed and manoeuvrability in roach (Eklöv and Jonsson 2007). Contrary to our study, they detected a wider anal fin in roach exposed to predators. Adaptations to faster swimming seem advantageous, as roach were found to increase activity and even show the tactic of jumping out of the water when threatened by a predator (Christensen and Persson 1993). Comparable adaptations to faster predator escape as a response to high predator abundances were also found in Western mosquitofish (*Gambusia affinis*) (Langerhans et al. 2004) and in *Galaxias platei* (Milano et al. 2002), where caudal regions were enlarged to enhance burst performance. A recent review (Bourdeau and Johansson 2012) concluded that most of the observed predator-induced morphological defenses are purely a by-product of swimming activity. Prey swim less when predators are present, resulting in lower feeding rates or decreased metabolism, leading to reduced growth. We cannot exclude that variation in roach morphology between the lakes reflects differences in swimming activity (in response to differing predation risk); however, roach from Döllnsee, where predation risk was highest, had morphologies that appear better adapted to rapid and extended swimming, which contrasts the conclusion that predation risk always induces reduced swimming activity of prey.

Body morphology was not associated with structural complexity in the lakes created by macrophyte coverage, with little evidence for an association of morphology and diet composition in this omnivorous species. Gut content and stable isotopes both suggested that benthic invertebrates dominated the diet of roach in the two lakes analyzed, whereas shape data significantly differed between these populations. Similar studies of fishes mainly focused on intralacustrine (within-lake) morphological divergence and resource polymorphism, with specialization on benthic food usually associated with a preference for littoral habitats with high structural complexity. Intralacustrine resource polymorphism driven by intraspecific competition has been studied in roach of two Swedish lakes (Svanbäck et al. 2008). Benthivorous individuals from the littoral zone had a deeper body compared to planktivorous individuals from the pelagic zone. Similarly, pumpkinseed sunfish produce molluscivorous and a planktivorous morphotypes to decrease intraspecific competition (Robinson et al. 1993, Gerry et al. 2013). Ours is the first study of interlacustrine (among-lake) divergence of body morphology in roach of which we are aware, but similar morphological divergence between lakes has been reported for populations of three-spined sticklebacks in Alaska, USA (Aguirre 2009). Very shallow lakes, where most of the stickleback habitat is structurally complex, were inhabited by individuals of the benthic form, characterized by a deeper body that favors maneuverability, whereas the limnetic form with a narrower body that reduces drag during prolonged swimming was found in deeper lakes with large areas of open waters (Walker 1997).

A possible explanation for the lack of correlation between resource use and body shape here could be the spatial dimension of the systems studied. The four lakes we studied were small (< 0.2 km²) and relatively shallow. Roach may be able to easily exploit the resources from all habitats because near shore and offshore sites are usually less than 100 m apart. Furthermore, seasonal and annual changes in resource abundance require flexibility in resource use, perhaps counteracting any selective advantage gained by specialization. A high diet flexibility of roach is supported by the fact that guts of roach in the lake with almost no macrophytes (Gollinsee) contained even more benthic prey than those from fish in the lake with intermediate macrophyte coverage (Schulzensee). A more balanced diet composition with resources from both littoral and pelagic origin was obtained by applying the mixing model based on stable isotope analysis. Therefore, in systems where resources are spatially dispersed and temporally variable, selection may favor morphological shapes that support omnivory rather than discrete morphotypes specialized on a particular prey and habitat type.

Many previous studies found morphological changes to be phenotypically plastic (Brönmark and Miner 1992, Frommen et al. 2011), although a few studies have demonstrated a heritable component (O'Steen et al. 2002, Ghalambor et al. 2004, Langerhans et al. 2004). In our dataset, we did not find any statistical association of shape differences with aggregated or outlier-specific genotypes among the lakes. However, the three outlier loci occurred in all populations, although with differing frequencies, suggesting that the allele frequencies of these loci are not the result of neutral genetic drift. There is some inherent uncertainty in AFLP studies, such as false scoring of alleles due to differences in PCR efficiency (false positives) or peak overlap (false negatives) (Bonin et al. 2004). Furthermore, reduced precision during sample preparation due to sampling mix-up, pipetting or contamination may cause genotyping errors (Pompanon et al. 2005). Nevertheless, we have no indication that false positives had a strong influence on our results, because the reproducibility was 98% within the randomly chosen repeated 17.4% of samples.

The proportion of outlier loci (0.91%) fits within the range of reported outliers of former studies (0.4 – 24.5%) (Nosil et al. 2009). In a comparison of three available statistical programs, BAYESCAN (the method we used here) showed the lowest rates of false positives (Perez-Figueroa et al. 2010), and hence we consider the three loci found in our study to be reliable (see also the very low false discovery rate estimated by BAYESCAN for our data). AFLP outlier loci have been linked to morphological diversification in other studies of fish, including European minnows in lake and stream habitats (Collin and Fumagalli 2011) or in sympatric lake whitefish (*Coregonus clupeaformis*) ecotypes (Campbell and Bernatchez 2004). A similarly strong association between morphotype and genotype could not be confirmed for roach in our study. It is possible that our AFLP markers simply failed to include genomic regions that are associated with the shape variations between the lakes. Alternatively, the lack of association between genotype and phenotype may suggest a plastic response to predation pressure. Compared to fixed genetic controls, plastic responses to environmental pressures are advantageous when predatory regimes are variable (Miner et al. 2005). Strong interannual variability of predation pressure is likely in the communities analyzed here because these small and shallow lakes are characterized by frequent fish kills during strong winters that can substantially reduce predator abundance (Ruuhijärvi et al. 2010).

Overall, the extent of genetic divergence between populations is determined through a mixture of adaptive and neutral processes (Storfer et al. 2010). Local adaptation is achieved through environmental filtering of genotypes (Manel et al. 2010), whereas neutral processes include drift-migration equilibrium wherein landscape isolation plays a role in reducing gene flow (e.g., isolation by distance, IBD) (Wright 1943). We did not find evidence for a dominance of neutral processes and IBD in our data. The geographical distance between the four lakes is very limited (< 35 km), and the two lakes situated only 4 km apart (Gollinsee and Döllnsee) exhibited intermediate genetic divergence (θ= 0.0266). Thus, we conclude that interlacustrine morphological diversification of the four roach populations is mainly led through processes related to local environments of each lake (in particular predator abundance) rather than neutral process due to geographic isolation between the lakes.

Conclusions

We observed divergent morphologies in omnivorous fish populations among four shallow lakes. Our results support previous studies suggesting that predation risk may play a more important role than habitat and diet in shaping body morphology of this prey species. In the case of roach inhabiting small and shallow lakes, we consider these morphological changes advantageous for fast escapes and thus directly cued by predation. Furthermore, we did not find evidence that the observed morphological divergence had a heritable component.

Methods

Study areas and sampling

The four lakes are situated in northeast Germany; about 100 km north of Berlin (see Table 3.3 for more details). All lakes are small and shallow, and characterized as either clear, structurally complex lakes with some coverage by submerged macrophytes (Schulzensee and Döllnsee) or turbid, phytoplankton-dominated lakes without submerged macrophytes (Gollinsee and Globsowsee). Geographical distances between the lakes range from 4 - 35 km. They are not connected by waterways and the small size of their inlets and outlets allows dispersal only in years with extremely high water levels.

Table 3.3. Characteristics of the four lakes included in the study

Lake	Area (km²)	Mean depth (m)	No. fish species	Coverage of submerged macrophytes (%)	Relative abundance of piscivorous fish (%)
Gollinsee	0.033	1.7	7	0	3
Schulzensee	0.039	2.2	5	22	0.3
Globsowsee	0.148	2.8	7	< 1	12
Döllnsee	0.250	4.1	12	51	23

Fish were caught using Nordic multi-mesh gill nets (length 30 m, height 1.5 m; 12 mesh sizes from 5 - 55 mm; Lundgrens Fiskredskapsfrabrik AB) or electrofishing with 200 - 400 V DC current (4-8 electrofishing aggregates EFG 4000, EFGI 4000; Bretschneider Spezialelektronik, Breitenbrunn, Germany) with anodic handnets (4 -

4.5 m long, 40 cm diameter, 6 mm mesh size) during summer and early autumn 2010. After capture, fish were measured (mm total length, TL) and weighed (g wet mass, wm) and subsequently stored on ice. In total, 185 roach (69 from Gollinsee, 70 from Schulzensee, 25 from Globsowsee and 21 from Döllnsee) were processed. Due to methodological problems with some individuals, the number of fish per analysis was slightly lower.

Morphometric analysis

Body morphology of individual roach was examined using a landmark-based geometric morphometric method (Bookstein 1991). Fish were photographed from the left side in a standardized way using a Nikon DX40 and a 28 mm lens, which represents a normal lens when considering the sensor size of the camera. Optical distortions were minimized by using a normal lens, a moderate distance to the specimen, and an aperture close to the critical aperture. Individuals were placed in a bowl of fine white gravel to obtain a straight position and the fins were stretched out to make the fin base visible. A few specimens with injuries on the left side were photographed from the right side and then the photographs were digitally flipped horizontally prior to analysis. After taking the photo, individuals were cut open to determine the sex. The shape data of the two sexes did not differ significantly (Mahalanobis *D*= 1.0761; *P*= 0.53), therefore sexes were pooled for further analyses. All roach were at least two summers old, an age where roach in lakes at this latitude are sexually mature (Lappalainen et al. 2008).

Digital photographs were transferred to TPSdig2 (all TPS- programs http://life.bio.sunysb.edu/morph) and 14 landmarks were digitized. To diminish measurement error, digitizing was always performed by the same person. Haphazardly chosen individuals were repeated and results were compared to determine reliability of the results. We checked for outliers with the “Find outliers” function of the software MorphoJ. TPSSmall was used to determine whether the amount of variation in shape in our data set was small enough to permit statistical analyses in the linear tangent space approximated to the non-linear Kendall's shape space. For all further analyses, MorphoJ was used. Landmark configurations were aligned by Procrustes Superimposition (Rohlf and Slice 1990) to minimize effects of translation, rotation and scaling. Shape data were size-corrected using a regression of shape (i.e. Procrustes coordinates) on size (i.e. log centroid size) for each lake separately. Residuals obtained from this regression were used for all further analyses. A Discriminant Function Analysis (DFA) and a Canonical Variate Analysis (CVA) were used to assess significance of shape differences between groups. A pairwise comparison between all four lakes was conducted (Globsowsee vs. Döllnses vs. Gollinsee vs. Schulzensee).

Genetic (AFLP) analysis

AFLP analysis followed Vos et al. (Vos et al. 1995) with some exceptions noted below. Total genomic DNA was extracted from dorsal fin tissue using DNeasy Tissue Kits (Qiagen, Hilden, Germany) following the manufacturer’s protocol. DNA quality and concentration were inspected using a spectrophotometer (Nano Drop 1000, Thermo scientific, Wilmington, USA). DNA (250 ng per sample) was digested for 6 h at 37°C using 2U MseI and 10U EcoRI (all enzymes from New England Biolabs, Ipswich, USA), 2 µg BSA, 2 µL EcoRI buffer, and 16 µL sterilized dH_20. Ligation was then performed by adding 2U T4 DNA-Ligase, 1 µL MseI adapter, 1 µL EcoRI adapter, 4 µL T4 DNA ligase buffer (10x), 4 µL ATP and 7.5 µL sterilized dH_20 at 16 °C overnight. Preselective amplification was carried out using one additional base on each primer (MseI+C and EcoRI+A, oligonucleotides from Metabion International AG, Martiensried, Germany) and a thermocycler (VWR DuoCycler, VWR International GmbH, Darmstadt, Germany) programmed with a denaturation at 92 °C for 20 seconds, an annealing at 56 °C for 30 seconds, and an elongation at 72 °C for two minutes for a total of 20 cycles, followed by a final extension for 30 minutes at 60 °C. Selective amplification was conducted under the same thermal protocol using four primer pairs where three additional bases were added at 3’-end of each primer. On EcoRI-primers, four different fluorescent dye labels were attached (6FAM™-labeled primers from Metabion; VIC®-, NED™- and PET®-labeled ones from Applied Biosystems, Life Technologies, Darmstadt, Germany). Initially, 20 different primer-pair combinations were tested, and four gave best results (Table 3.4). Fragments of selective amplification were denatured at 95°C for 5 min and then cooled on ice for 10 min, before they were separated on an ABI 3500xl capillary sequencer (Applied Biosystems) with an internal size standard (GeneScan-600 LIZ®; Applied

Biosystems). Correct fit of the size standard was visually inspected for all electropherograms.

Table 3.4. Primer combinations of selective amplification. All primers had 3 additional base pairs at the 3'end. EcoRI-Primers were also labeled by a fluorescent dye at the 5'end.

Primer combination	MseI-Primer	EcoRI-Primer	Number of loci
1	MseI+CAG	EcoRI+ACT+6FAM™	133
2	MseI+CAG	EcoRI+ACA+VIC®	139
3	MseI+CAG	EcoRI+AAG+NED™	107
4	MseI+CTA	EcoRI+AAG+PET®	68

Signal processing and binning was conducted using Genemapper™ V.4.1 (Applied Biosystems). Presence (1) or absence (0) of fragments were scored between 50 and 450 bp using a peak intensity threshold > 100 relative florescent units. A bin width of 1 bp and a max peak of 1.5 bp yielded optimization and fragments were scored between 50 to 450 bp. A complete repetition of a random 17.4% of the samples yielded 97.8% reproducibility.

The input data files for statistical analyses were prepared by AFLPDAT (Ehrich 2006). AFLP-SURV (Vekemans 2002) was used to calculate the frequency of polymorphic fragments. To obtain information on genetic structure within and among all four populations, genetic differentiation was calculated by using a Bayesian approach (θ) as implemented in HICKORY (Holsinger and Lewis 2007), using 20,000 burn-in iterations and 100,000 final iterations. AFLP loci potentially under selection (outlier loci) were identified using the BAYESCAN software (Foll and Gaggiotti 2008) by assuming that loci influenced by directional selection show larger genetic differentiation than neutral loci, and loci that have been subjected to balancing selection show a lower genetic differentiation. This software uses a Bayesian likelihood method by reversible jump Monte Carlo Markov Chains. From F_{ST} calculations, posterior probabilities of two models are estimated: one model including selection on a certain locus (the locus-specific component of F_{ST}, α, is different from zero), the other one not. The Posterior Odds (ratio between these probabilities) provides a detection level for a locus to be under selection. We retained loci with $P >$ 0.97 (Posterior Odds>32) as outliers presumably under directional selection. These calculations were run only with the 331 polymorphic AFLP loci. We further used

ARLEQUIN 3.5 to test for pairwise linkage disequilibrium (LD) of the outlier loci, using 1000 steps in the Markov chain and a dememorization of 1000 steps.

Predation pressure

To approximate the predation pressure by piscivores on roach for each lake, lake-specific abundances of piscivorous fish were multiplied with the individual, size-dependent predation risk of roach from that lake. For Globsowsee, Schulzensee and Gollinsee, relative abundances of piscivorous fish were obtained from standardized gill net catches (6 - 8 benthic multimesh gill nets set over night in autumn). We included perch (> 15 cm TL) (see Persson 1986 for a definition of piscivory in perch) and pike as piscivorous fish species. In the fourth lake (Döllnsee), destructive gill-net fishing could not be conducted because the lake is equipped with a 3D-telemetry system for *in-situ* studies of fish behavior. Therefore, we used information on fish assemblage composition from gill-net samplings conducted in previous years (Radke 1998, Pagel 2009). The proportion of piscivores in Döllnsee calculated from earlier samplings is conservative because the lake has been heavily stocked with pike during the last years, and hence roach presumably experience an even greater predation pressure. The lake-specific relative abundances of piscivorous fish were ranked from highest (predator abundance= 4) to lowest (predator abundance= 1) (Figure 3.2). Predation risk of prey fish is size-dependent due to gape limitation of piscivores (Persson et al. 1996). Therefore, predation risk of individual roach was ranked according to total length, with TL ≥ 25cm: predation risk= 0; TL≥ 20-25cm: predation risk= 1; TL≥ 15-20cm: predation risk= 2; TL≥ 10-15cm: predation risk= 3; TL< 10cm: predation risk= 4 (Figure 3.2). The individual predation risk was ultimately multiplied with the rank among lakes with respect to relative abundances of piscivores. Therefore, the smallest roach in Döllnsee had the highest relative risk (4 x 4= 16), whereas all roach > 25 cm TL had a risk of zero, independent of lake of origin. For all subsequent analyses, the obtained product for predation risk ranging from 0 to 16 was $\log_2(x+1)$-transformed to achieve a near-linear distribution.

Gut content and stable isotope analysis

Detailed data on roach diet composition was obtained from roach in Gollinsee (*N*= 42) and Schulzensee (*N*= 42). In the laboratory, roach guts were removed and stored in 5% formaldehyde for subsequent GC. Individuals with empty stomachs were removed and the anterior third of the gut was examined under a stereo microscope and the volume proportion (equivalent to area proportion at uniform width) of each prey category observed in the sample was estimated to the nearest 10%, adapted from Windell (Windell 1968). The observed prey items were grouped into two categories, namely benthic prey (Trichoptera, Gastropods, Isopods, Chironomid larvae, Bryozoans, algae and detritus) and pelagic prey (Cladocerans, Copepods, water mites, Ostracods, Rotifers, and Chaoborid larvae). Eleven fish with empty guts were removed prior to analysis. A non-parametric Mann-Whitney *U* test was conducted to compare the percentage of benthic and pelagic prey between the two lakes.

For SIA of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) from roach (*N*= 46 from Schulzensee and *N*= 47 from Gollinsee, including fish with empty guts), a small sample of dorsal muscle tissue was excised from each fish. To obtain baseline values of potential diet groups for the omnivorous roach, macroinvertebrate samples for SIA were collected in April and June from various sites in the littoral zone using a sweep net. Zooplankton samples for SIA were taken by hauling zooplankton nets (55 µm and 100 µm) vertically through the entire water column several times during summer. Samples were taken to the laboratory and transferred to clean tap water to allow the animals to void their guts overnight. Macroinvertebrates were then sorted into taxon groups and zooplankton samples were filtered (100 µm filters). All samples were dried at 60°C for 48 hours, then ground to a fine powder using a mortar and pestle and material of about 0.5 mg dry mass was loaded into tin cups. Carbon (%C) and nitrogen (%N) content and stable isotope ratios of C ($\delta^{13}C$) and N ($\delta^{15}N$) were analyzed on a FlashEA 1112 elemental analyzer coupled to a Thermo Finnigan DELTA ^{Plus} Advantage mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA) at the University of Jyväskylä, Finland. Stable isotope data are expressed in the conventional delta notation as the relative difference between ratios of samples and international standards (PeeDee Belemnite for $\delta^{13}C$, atmospheric N for $\delta^{15}N$). Analytical precision (mean SD from in-house standard) for each run was always better than 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$.

To calculate relative contribution of benthic and pelagic food sources to the diet of roach in both lakes, the Bayesian isotope mixing model package SIAR (Stable Isotope Analysis in R) (Parnell et al. 2010) was used in R version 2.12.0 (R Development Core Team 2012). Fractionation factors were set to 0.4 ± 1.3 ‰ for $\delta^{13}C$ and 3.4 ± 1.0‰ for $\delta^{15}N$, as suggested in a previous study (Post 2002). The mean isotopic value of the zooplankton samples of each lake was taken as an end point for pelagic $\delta^{13}C$ and $\delta^{15}N$ values. Littoral $\delta^{13}C$ and $\delta^{15}N$ end points were calculated from the mean value of the macroinvertebrates, containing Trichoptera, Isopoda, Chironomidae and Gastropoda. These taxa represent the most abundant littoral macroinvertebrates consumed by roach.

Association of shape data with genetic data and ecological variables

To reduce the dimensionality of the genotype information from 331 polymorphic AFLP loci, we performed Principal Coordinate Analysis (PCoA) by applying the standardized covariance method. Genetic distance between two individuals was calculated as the sum of loci with different character states, a method that automatically discards monomorphic loci. These analyses were conducted in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). To examine association of shape with genotype, general linear models (GLM) between the dominant canonical variate (CV1) of shape data as dependent variable and lake as group variable were calculated. We used contrasts between Döllnsee (the lake with the most deviating shape) and the other three lakes. The first two axes of PCoA on AFLP-data and their potential interactions with lake origin were then used as continuous predictors. Using these GLMs, we tested whether the shape of roach differed among the four lakes, and whether the aggregated lake-specific genotype contributed to this differentiation. In a second step, we used only the three outlier loci and their interactions with lake origin as binary predictors of shape. With these GLMs, we tested whether the genotype outliers were directly associated with the differences in shape between the lakes. GLM analyses were conducted in R version 2.15.1. (R Development Core Team 2012).

A two-block partial least square analysis (PLS), as implemented in MorphoJ, was used to study the association of shape and ecological variables at the level of individual roach. In all calculations, only those individuals for which all the respective variables were available to facilitate paired comparisons were included. All variables used in the second blocks were adjusted to standard deviate b= $(x_{ij}-\bar{x})/s_i$; where s_i is the standard deviation of row or column i. To study correlation with predation pressure, shape was used as a first block and relative predation pressure as second block. To correlate shape with diet composition, PLS were calculated using percentage of ingested diet items obtained from GC or isotope ratios. A permutation test against the null hypothesis of independence (1000 randomization runs) was used for all PLS, as implemented in the software MorphoJ.

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General discussion

Rationale

Early limnologists viewed lakes as self-contained entities (Forbes 1887) and a “pelagiocentric paradigm” (as dubbed by Vander Zanden and Gratton 2011) dictated the overall focus of lake research. Recent evidence suggests, however, that lakes are tightly linked to their adjacent habitats and fluxes may occur across habitat boundaries.

Also, it is increasingly recognized that benthic and littoral habitats may play an important role in lake food webs (Schindler and Scheuerell 2002, Vadeboncoeur et al. 2002). From a more evolutionary viewpoint, structural complexity provided by aquatic plants may directly (i.e. mediated by resource availability) and indirectly (i.e. mediated by predator abundance) shape the phenotypes of omnivorous fish, resulting in population divergence between more or less structured lakes.

Littoral zones can thus be viewed as “hot spots” of consumer and resource interactions and food web processes. This is especially the case in small and shallow lakes, where littoral habitats further comprise a large proportion of the whole lake area due to the high perimeter to area ratio.

The littoral zone as an ecological boundary

Littoral zones act as bridges between aquatic ecosystems and their terrestrial surroundings. In temperate systems, tPOC in the form of leaf litter enters a lake ecosystem every autumn in a pulsed flux. The importance and contribution of tPOC to lake food webs is the subject of a lively debate (Carpenter et al. 2005, Cole et al. 2006, Bartels et al. 2012). To my knowledge, no study has been conducted on the whole lake scale, taking into account the spatial and temporal complexity of natural ecosystems. Within this thesis, I provide evidence for a first experimental tracing of tPOC up to the higher trophic level of lake food webs (**Chapter 1,** Figure Gd.1). Maize leaves, an isotopically distinct carbon tracer were added to mimic the tPOC input, occurring naturally as the annual leaf litterfall. Another study of these lakes found that a substantial proportion of the carbon loaded into the lakes is permanently buried in the sediments (Brother et al. unpublished manuscript).

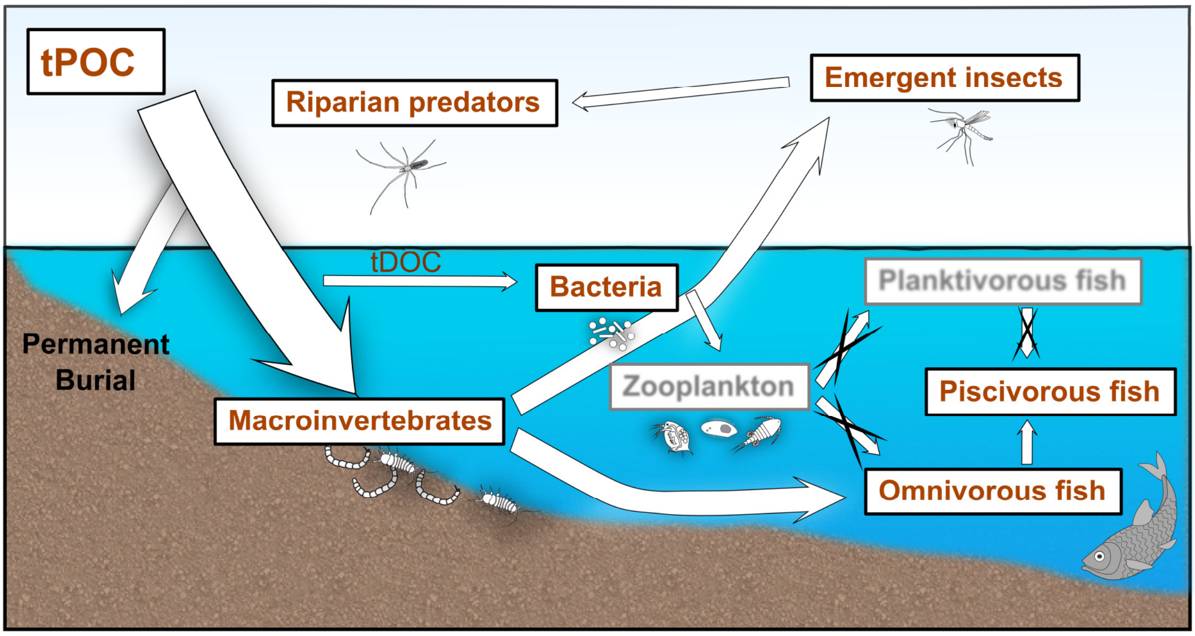

Figure Gd.1 Pathways of experimentally added terrestrial particulate organic carbon. Organisms where the maize signal could not be traced are depicted in gray.

This was apparently linked to low benthic mineralization rates (especially the case in the turbid lake), suggesting that a substantial proportion of the carbon entering the lakes is not incorporated to higher trophic levels of the food webs. Heterotrophic bacteria receive tPOC as leached tDOC and are subsequently consumed by zooplankton via the microbial loop (Azam et al. 1983, Tranvik 1988, Karlsson et al. 2003, Kritzberg et al. 2004). In our study, the isotopic signatures of bacteria could not be measured, but bacterial production displayed only a short-term response to the pulse in autumn (Attermeyer et al. 2013). This enhanced carbon availability did not persist long enough to significantly shift the carbon signatures of zooplankton relative to the reference sides, as evidenced by short tissue turnover rates in zooplankton (between 7.7 days in rotifers and 20.0 days in herbivorous crustaceans) (Straile 1998). Because of this, we were also unable to trace the maize signal in the planktivorous fish in the pelagic zone (**Chapter 1**, Figure Gd.1). Omnivorous fish have also been known to feed on zooplankton; yet we identified macroinvertebrates as the dominant food source in our lakes (**Chapter 1 & 3**). The dominant pathway for leaf carbon to enter the food webs was via the direct consumption of macroinvertebrate shredders, which served as prey for omnivorous fish, which in turn were consumed by piscivorous fish (**Chapter 1 & 3**, Figure Gd.1). Macroinvertebrates also emerge, serving as prey for riparian predators (**Chapter 2**).

With this experimental set-up I was therefore able to demonstrate the full cycling of terrestrial carbon back to the terrestrial surroundings (**Chapter 2**; Figure Gd.1).

The maize signal was traceable in Chironomidae larvae and adults, as well as in the riparian spiders feeding on this prey. As established in the dietary studies of spiders colonizing the riparian zone of streams, these predators showed a strong reliance on aquatic invertebrates (Collier et al. 2002, Sanzone et al. 2003). Emerging invertebrates are reported to be an important resource for the consumers in the terrestrial habitats surrounding lakes, as recorded for Chironomidae mass-emergence from Icelandic lakes (Gratton et al. 2008, Hoekman et al. 2011). In the study lake featuring a pronounced macrophyte belt, about four times more carbon was transported to the spiders colonizing the riparian zone compared to the lake where macrophytes were absent **(Chapter 2).** I identified two possible reasons for these findings: First, stable isotope mixing models revealed that the contribution of maize to the diet of Chironomidae was slightly greater in the more structured lake (7%) than in the lake where submerged macrophytes were absent (3%). This suggests that the high structural complexity facilitated a higher availability of maize carbon to the larvae. Secondly, the structurally complex lake supported a higher biomass of Chironomidae, and a greater overall secondary production. As Production to biomass (P/B) ratios were similar in all lake sides, we could exclude the possibility that the addition of tPOC had boosted the production of Chironomidae. We could therefore find no subsidy effect (sensu Polis et al. 1997) of tPOC.

We added about four times more maize than was provided naturally by alder leaves, but the macroinvertebrate diet together reflected a much higher degree (up to 30%) of alder leave consumption, compared to only 5% for maize leaves (**Chapter 1**). I conclude that consumers may choose their resources according to quality (maize has a high C:N ratio) rather than availability. Contrary to our predictions, the overall allochthony (i.e. reliance on tPOC) of consumers did not differ between the lakes of different macrophyte colonization and the extent of the littoral zone may not be the dominant factor to determine allochthony (**Chapter 1**). The findings provide direct experimental evidence for the model findings of Jones et al. (2012) which suggest that tPOC may replace autochthonous sources, but does not act as a subsidy.

Overall, my results add to the growing knowledge on the importance of benthic habitats for carbon degradation and cycling in lake ecosystems, and further provide an insight into the tight coupling of aquatic and terrestrial ecosystems.

The littoral zone as a zone of adaptation

Resource polymorphism (i.e. the segregation in habitat and diet) in freshwater fish has been frequently reported along the pelagic-littoral axis, with deep bodied morphs feeding on macroinvertebrates in the littoral zone, and more streamlined morphs feeding on zooplankton in the pelagic zone (Smith and Skúlason 1996). I assumed that a similar pattern may induce interlacustrine divergence in roach between lakes of different structural complexity, as it has been shown for three-spined stickleback (Aguirre 2009). Furthermore, I hypothesized that roach phenotypes would also be affected by predation pressure. Within my thesis, I found different morphologies in roach from four different lakes. Shape and predation pressure were significantly correlated, suggesting that changes in body shape of roach may facilitate predator escape **(Chapter 3)**. Fish from the lake with the highest predation pressure featured the most distinct morphology, with a more streamlined body and a more caudally inserted dorsal fin **(Chapter 3)**. In contrast to morphological changes that prevent the handling of prey by predators (e.g. to achieve a size refuge from gape-size limited predators) (Brönmark and Miner 1992, Persson et al. 1996, Januszkiewicz and Robinson 2007), I consider this body form to be advantageous for flight. To escape a predator attack, fish show a characteristically fast-start behavior, called a “C-start” (Weihs 1973, Webb 1984, Domenici and Blake 1997). During a C-start, fish bend into a “C” shape (stage 1) and then produce a propulsive stroke of the caudal region in the opposite direction (stage 2). The high-energy burst is facilitated by a slender body form and a relatively shallow anterior body and head region, as these are attributes which contribute minimally to drag (Weihs 1989, Walker 1997, Langerhans et al. 2004). Thrust can be further increased if the dorsal fin is caudally inserted (Walker 1997). Our findings support a previous study, where roach had been raised under laboratory conditions in the absence or presence of pike, showing similar changes in morphology (Eklöv and Jonsson 2007).

Gut content as well as stable isotope analyses showed a preference for benthic invertebrates in the diet of roach from lakes of different structural complexity. Contrary to my prediction fish morphology was not correlated with resource use **(Chapter 3)**. These findings suggest that fish were able to exploit all resources in these spatially-limited systems. I further assumed that annual and seasonal changes in resource availability may favor a variable and flexible morphotype.

Many previous studies found morphological changes to be phenotypically plastic (Brönmark and Miner 1992, Frommen et al. 2011), although a few studies have demonstrated a heritable component (O'Steen et al. 2002, Ghalambor et al. 2004, Langerhans et al. 2004). In my study, no association of genetic differentiation and morphological variation among lakes could be found, and I assumed that shape variation in roach is not heritable. Such plastic responses are advantageous when predatory regimes are variable (Miner et al. 2005), as is the case in these small and shallow aquatic systems where strong winters may produce a fish kill which reduces the abundance of predators (Ruuhijärvi et al. 2010).

Conclusions

The major findings of my thesis can be summarized as follows:

- Maize leaves are a suitable tracer to follow the pathway of tPOC up to the highest trophic levels of aquatic food webs (Chapter 1 & 2)
- tPOC plays an important role for food web dynamics of small and shallow lakes, but tPOC does not act as a subsidy (sensu Polis et al. 1997). Instead it replaces autochthonous sources (Chapter 1 & 2)
- Due to a strong aquatic-terrestrial coupling and translocation and recycling processes of carbon in recipient habitats, a distinction between allochthonous and autochthonous sources is challenging (Chapter 2)
- Predation risk may play a more important role than habitat and diet in shaping body morphology of omnivorous fish species (Chapter 3)

Future perspectives

The study of feeding relationships plays a central role in ecology. Although a rough schematic description of “who eats whom” can be relatively easily produced, comprehensive descriptions of food web interactions or energy pathways are more challenging to produce. In TERRALAC, we used a broad array of methods and expertise to better understand food web dynamics and carbon cycling in lakes. I argue that such an ecosystem approach that combines various analytical methods (e.g. experimental additions of terrestrial organic carbon, carbon budgets, isotopic mixing models, and estimates of secondary production) must be established for future studies.

Still, we were not able to answer all questions, and thus areas for future research have arisen. We found that macrophytes are an important resource for the lake food web, as previously shown in a study of 19 lakes in South America (Mendonca et al. 2013). Plant detritus and its implication as a food source have in particular been highly neglected in the literature, and represent an exciting field of research for the future. The inclusion of stable isotope analyses of hydrogen as a third stable isotope will provide additional power to resolve the use of this resource (Syväranta et al. in preparation).

I could identify littoral zones of shallow lakes as an active area of carbon degradation and processing. Due to the high number of small lakes worldwide (Downing et al. 2006), littoral zones constitute one of the major components of the global carbon cycling and have important implications for our climate (Cole et al. 2007). However, aquatic ecosystems are severely altered by human activities (Revenga et al. 2005). The loss of macrophytes, predominantly due to eutrophication has been identified as a general pattern (Sand-Jensen et al. 2000, Vermaire et al. 2012) and human shoreline alteration is known to be an increasing threat to lake ecosystems (Carpenter et al. 2007). Furthermore, regime shifts are becoming increasingly widespread (Barnosky et al. 2012), and therefore a shift from a macrophyte-dominated to a phytoplankton-dominated state in shallow lakes may be more likely to occur in the future. The tight linkage between aquatic and terrestrial habitats is ultimately coupled in vulnerability to habitat degradation. Therefore, the, degradation of aquatic habitats and the loss of macrophytes could alter the flux of aquatic prey to riparian consumers via emergence with potentially severe implications for adjacent habitats. Management strategies to conserve the integrity of littoral zones need to be established.

Furthermore, most previous food web studies have considered the components of the food web as fixed entities, neglecting to recognize them as the products and agents of a continuous evolutionary change. Species interactions such as competition and predation are strong selective forces, and thus evolutionary changes can be expected in populations. I thus suggest including a more darwinistic approach into ecological studies.

For further insights into the adaptations of organisms to variable predatory regimes, it would be also beneficial to conduct further research into avian predation pressures on lake prey fish. Furthermore, individuals within populations may vary substantially in their behavioral personalities (Reale et al. 2007), for example in risk-taking behavior (Magnhagen 2007, Webster et al. 2009). I propose that the connection between such differences in personalities with fitness differences would improve our understanding of the role for phenotypic divergence due to predation pressures.

References

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science **294**:321-326.

Aguirre, W. E. 2009. Microgeographical diversification of threespine stickleback: body shape-habitat correlations in a small, ecologically diverse Alaskan drainage. Biological Journal of the Linnean Society **98**:139-151.

Alekseyev, S. S., V. P. Samusenok, A. N. Matveev, and M. Y. Pichugin. 2002. Diversification, sympatric speciation, and trophic polymorphism of Arctic charr, *Salvelinus alpinus* complex, in Transbaikalia. Environmental Biology of Fishes **64**:97-114.

Anderson, O. 1984. Optimal foraging by largemouth bass in structured environments. Ecology **65**:851-861.

Attermeyer, K., K. Premke, T. Hornick, S. Hilt, and H.-P. Grossart. 2013. Ecosystem-level studies of terrestrial carbon reveal contrasting bacterial metabolism in different aquatic habitats. Ecology **preprint**.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyerreil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series **10**:257-263.

Babler, A. L., A. Pilati, and M. Vanni. 2011. Terrestrial support of detrivorous fish populations decreases with watershed size. Ecosphere **2**:1-23.

Bade, D. L., M. L. Pace, J. J. Cole, and S. R. Carpenter. 2006. Can algal photosynthetic inorganic carbon isotope fractionation be predicted in lakes using existing models? Aquatic Sciences **68**:142-153.

Barnosky, A. D., E. A. Hadly, J. Bascompte, E. L. Berlow, J. H. Brown, M. Fortelius, W. M. Getz, J. Harte, A. Hastings, P. A. Marquet, N. D. Martinez, A. Mooers, P. Roopnarine, G. Vermeij, J. W. Williams, R. Gillespie, J. Kitzes, C. Marshall, N. Matzke, D. P. Mindell, E. Revilla, and A. B. Smith. 2012. Approaching a state shift in Earth's biosphere. Nature **486**:52-58.

Bartels, P., J. Cucherousset, C. Gudasz, M. Jansson, J. Karlsson, L. Persson, K. Premke, A. Rubach, K. Steger, L. Tranvik, and P. Eklöv. 2012. Terrestrial subsidies to lake food webs: an experimental approach. Oecologia **186**:807-818.

Batt, R. D., S. R. Carpenter, J. J. Cole, M. L. Pace, T. J. Cline, R. A. Johnson, and D. A. Seekell. 2012. Resources supporting the food web of a naturally productive lake. Limnology and Oceanography **57**:1443-1452.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology **50**:201-220.

Bechara, J. A., G. Moreau, and L. Hare. 1993. The impact of Brook trout (*Salvenius fontinalis*) on an experimental stream benthic community - the role of spatial and size refugia. Journal of Animal Ecology **62**:451-464.

Beckett, D. C., T. P. Aartila, and A. C. Miller. 1992. Contrasts in density of benthic invertebrates between macrophyte beds and open littoral patches in Eau-Galle Lake, Wisconsin. American Midland Naturalist **127**:77-90.

Bergtold, M. and W. Traunspurger. 2005. Benthic production by micro-, meio-, and macrobenthos in the profundal zone of an oligotrophic lake. Journal of the North American Benthological Society **24**:321-329.

Best, E. P. H. 1982. The aquatic macrophytes of Lake Vechten - Species composition, spatial distribution and production. Hydrobiologia **95**:65-77.

Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetics studies. Molecular Ecology **13**:3261-3273.

Bookstein, F. L. 1991. Morphometric tools for landmark data. Cambridge University Press, New York.

Bourdeau, P. E. and F. Johansson. 2012. Predator-induced morphological defences as by-products of prey behaviour: a review and prospectus. Oikos **121**:1175-1190.

Brauns, M., B. Gucker, C. Wagner, X. F. Garcia, N. Walz, and M. T. Pusch. 2011. Human lakeshore development alters the structure and trophic basis of littoral food webs. Journal of Applied Ecology **48**:916-925.

Brett, M., G. Arhonditis, S. Chandra, and M. Kainz. 2012. Mass flux calculations show strong allochthonous support of freshwater zooplankton production is unlikely. Plos One **7**:e39508. doi:39510.31371/journal.pone.0039508.

Brett, M., M. Kainz, S. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production.

Proceedings of the National Academy of Sciences of the United States of America **106**:21197-21201.

Brönmark, C. and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in Crucian carp. Science **258**:1348-1350.

Bry, C. 1996. Role of vegetation in the life cycle of pike. Pages 45-67 *in* J. Craig, editor. Pike-Biology and exploitation. Chapman & Hall, London.

Bunn, S. E. and P. I. Boon. 1993. What Sources of Organic-Carbon Drive Food Webs in Billabongs - A Study Based on Stable-Isotope Analysis. Oecologia **96**:85-94.

Burks, R. L., E. Jeppesen, and D. M. Lodge. 2001. Littoral zone structures as Daphnia refugia against fish predators. Limnology and Oceanography **46**:230-237.

Busse, A. 2011. Funktionelles Verständnis der Regulation von Nahrungsnetzen in Flachseen mittels Datenanalyse. Bachelor Thesis, University of Potsdam, Germany.

Butkas, K. J., Y. Vadeboncoeur, and M. J. Vander Zanden. 2011. Estimating benthic invertebrate production in lakes: a comparison of methods and scaling from individual taxa to the whole-lake level. Aquatic Sciences **73**:153-169.

Campbell, D. and L. Bernatchez. 2004. Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. Molecular Biology and Evolution **21**:945-956.

Carpenter, S. R., B. J. Benson, R. Biggs, J. W. Chipman, J. A. Foley, S. A. Golding, R. B. Hammer, P. C. Hanson, P. T. J. Johnson, A. M. Kamarainen, T. K. Kratz, R. C. Lathrop, K. D. McMahon, B. Provencher, J. A. Rusak, C. T. Solomon, E. H. Stanley, M. G. Turner, M. J. Vander Zanden, C. H. Wu, and H. L. Yuan. 2007. Understanding regional change: A comparison of two lake districts. Bioscience **57**:323-335.

Carpenter, S. R., J. J. Cole, M. L. Pace, M. Van de Bogert, D. L. Bade, D. Bastviken, C. M. Gille, J. R. Hodgson, J. F. Kitchell, and E. S. Kritzberg. 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from C-13 addition to contrasting lakes. Ecology **86**:2737-2750.

Casselman, J. M. and C. A. Lewis. 1996. Habitat requirements of northern pike (*Esox lucius*). Canadian Journal of Fisheries and Aquatic Sciences **53**:161-174.

Christensen, B. and L. Persson. 1993. Species-specific antipredatory behaviors - effects on prey choice in different habitats. Behavioral Ecology and Sociobiology **32**:1-9.

Cole, J. J., S. R. Carpenter, J. Kitchell, M. L. Pace, C. T. Solomon, and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proceedings of the National Academy of Sciences of the United States of America **108**:1975-1980.

Cole, J. J., S. R. Carpenter, M. L. Pace, M. C. Van de Bogert, J. L. Kitchell, and J. R. Hodgson. 2006. Differential support of lake food webs by three types of terrestrial organic carbon. Ecology Letters **9**:558-568.

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems **10**:171-184.

Collier, K. J., S. Bury, and M. Gibbs. 2002. A stable isotope study of linkages between stream and terrestrial food webs through spider predation. Freshwater Biology **47**:1651-1659.

Collin, H. and L. Fumagalli. 2011. Evidence for morphological and adaptive genetic divergence between lake and stream habitats in European minnows (*Phoxinus phoxinus*, Cyprinidae). Molecular Ecology **20**:4490-4502.

Cote, I. M. 1995. Effects of predatory crab effluent on byssus production in mussels. Journal of Experimental Marine Biology and Ecology **188**:233-241.

Cresko, W. A. and J. A. Baker. 1996. Two morphotypes of lacustrine threespine stickleback, *Gasterosteus aculeatus*, in Benka Lake, Alaska. Environmental Biology of Fishes **45**:343-350.

Day, T., J. Pritchard, and D. Schluter. 1994. A comparison of two sticklebacks. Evolution **48**:1723-1734.

del Rio, C. M., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews **84**:91-111.

Domenici, P. and R. W. Blake. 1997. The kinematics and performance of fish fast-start swimming. Journal of Experimental Biology **200**:1165-1178.

Domenici, P., H. Turesson, J. Brodersen, and C. Brönmark. 2008. Predator-induced morphology enhances escape locomotion in crucian carp. Proceedings of the Royal Society B-Biological Sciences **275**:195-201.

Downing, J. A., Y. T. Prairie, J. J. Cole, C. M. Duarte, L. J. Tranvik, R. G. Striegl, W. H. McDowell, P. Kortelainen, N. F. Caraco, J. M. Melack, and J. J. Middelburg. 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnology and Oceanography **51**:2388-2397.

Dzialowski, A. R., J. T. Lennon, W. J. O'Brien, and V. H. Smith. 2003. Predator-induced phenotypic plasticity in the exotic cladoceran *Daphnia lumholtzi*. Freshwater Biology **48**:1593-1602.

Earl, J. E. and R. D. Semlitsch. 2012. Reciprocal subsidies in ponds: does leaf input increase frog biomass export? Oecologia **170**:1077-1087.

Ehlinger, T. J. 1989. Learning and individual variation in bluegill foraging - habitat-specific techniques. Animal Behaviour **38**:643-658.

Ehrich, D. 2006. AFLPDAT: a collection of R functions for convenient handling of AFLP data. Molecular Ecology Notes **6**:603-604.

Ehrlich, E. 2012. Einfluss von Fischsterben und allochthonem Material auf die Dynamik funktioneller Gruppen im Plankton von eutrophen Flachseen. Bachelor Thesis. University of Potsdam, Germany.

Eklöv, P. and P. Jonsson. 2007. Pike predators induce morphological changes in young perch and roach. Journal of Fish Biology **70**:155-164.

Finlay, J. C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. Limnology and Oceanography **49**:850-861.

Foll, M. and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant Markers: A Bayesian perspective. Genetics **180**:977-993.

Forbes, S. A. 1887. The lake as a microcosm. Bulletin of the Peoria Scientific Association:77-87.

Francis, T. B. and D. E. Schindler. 2009. Shoreline urbanization reduces terrestrial insect subsidies to fishes in North American lakes. Oikos **118**:1872-1882.

Francis, T. B., D. E. Schindler, J. M. Fox, and E. Seminet-Reneau. 2007. Effects of urbanization on the dynamics of organic sediments in temperate lakes. Ecosystems **10**:1057-1068.

Francis, T. B., D. E. Schindler, G. W. Holtgrieve, E. R. Larson, M. D. Scheuerell, B. X. Semmens, and E. J. Ward. 2011. Habitat structure determines resource use by zooplankton in temperate lakes. Ecology Letters **14**:364-372.

Frommen, J. G., F. Herder, L. Engqvist, M. Mehlis, T. C. M. Bakker, J. Schwarzer, and T. Thunken. 2011. Costly plastic morphological responses to predator specific odour cues in three-spined sticklebacks (*Gasterosteus aculeatus*). Evolutionary Ecology **25**:641-656.

Gerry, S. P., M. Vogelzang, J. M. Ascher, and D. J. Ellerby. 2013. Variation in the diet and feeding morphology of polyphenic Lepomis macrochirus. Journal of Fish Biology **82**:338-346.

Gessner, M. O. 2000. Breakdown and nutrient dynamics of submerged *Phragmites* shoots in the littoral zone of a temperate hardwater lake. Aquatic Botany **66**:9-20.

Ghalambor, C. K., D. N. Reznick, and J. A. Walker. 2004. Constraints on adaptive evolution: The functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). American Naturalist **164**:38-50.

Gilinsky, E. 1984. The role of fish predation and spatial heterogeneity in determining benthic community structure. Ecology **65**:455-468.

Gillespie, R. G. 1987. The mechanism of habitat selection in the long-jawed orb-weaving spider *Tetragnatha elongata* (Araneae, Tetragnathidae) Journal of Arachnology **15**:81-90.

Grant, J. W. G. and I. A. E. Bayly. 1981. Predator induction of crests in morphs of the *Daphnia carinata* King complex. Limnology and Oceanography **26**:201-218.

Gratton, C., J. Donaldson, and M. J. Vander Zanden. 2008. Ecosystem linkages between lakes and the surrounding terrestrial landscape in northeast Iceland. Ecosystems **11**:764-774.

Gratton, C. and M. J. Vander Zanden. 2009. Flux of aquatic insect productivity to land: comparison of lentic and lotic ecosystems. Ecology **90**:2689-2699.

Gray, L. J. 1993. Response of insectivorous bird to emerging aquatic insects in riparian habitats of a tallgrass prairie stream. American Midland Naturalist **129**:288-300.

Gresens, S. E. 1995. Grazer diversity, competition and the response of the periphyton community. Oikos **73**:336-346.

Gribbin, S. D. and D. J. Thompson. 1990. A quantitative study of mortality at emergence in the damselfly *Pyrrhosoma nymphula* (Sulzer) (Zygoptera, Coenagrionidae). Freshwater Biology **24**:295-302.

Grimm, M. and M. Klinge. 1996. Pike and some aspects of its dependence on vegetation. Pages 125-156 *in* J. Craig, editor. Pike-Biology and exploitation. Chapman & Hall, London.

Hargeby, A., G. Andersson, I. Blindow, and S. Johansson. 1994. Trophic web structure in a shallow eutrophic lake during a dominance shift from phytoplankton to submerged macrophytes. Hydrobiologia **279**:83-90.

Hargeby, A., I. Blindow, and G. Andersson. 2007. Long-term patterns of shifts between clear and turbid states in Lake Krankesjon and Lake Takern. Ecosystems **10**:28-35.

Harvell, C. D. 1990. The ecology and evolution of inducible defenses. Quarterly Review of Biology **65**:323-340.

Hendry, A. P. 2009. Ecological speciation! Or the lack thereof? Canadian Journal of Fisheries and Aquatic Sciences **66**:1383-1398.

Henschel, J. R., D. Mahsberg, and H. Stumpf. 2001. Allochthonous aquatic insects increase predation and decrease herbivory in river shore food webs. Oikos **93**:429-438.

Hilt, S. and E. M. Gross. 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? Basic and Applied Ecology **9**:422-432.

Hoekman, D., J. Dreyer, R. D. Jackson, P. A. Townsend, and C. Gratton. 2011. Lake to land subsidies: Experimental addition of aquatic insects increases terrestrial arthropod densities. Ecology **92**:2063-2072.

Holsinger, K. E. and P. O. Lewis. Distributed by the author: Department of Ecology and Evolutionary Biology, University of Conneticut, Storrs. 2007. Hickory: a package for analysis of population genetic data v 1.1.

Ingley, S. J., S. M. Bybee, K. J. Tennessen, M. F. Whiting, and M. A. Branham. 2012. Life on the fly: phylogenetics and evolution of the helicopter damselflies (Odonata, Pseudostigmatidae). Zoologica Scripta **41**:637-650.

Iwata, T. 2007. Linking stream habitats and spider distribution: spatial variations in trophic transfer across a forest-stream boundary. Ecological Research **22**:619-628.

Jackson, J. K. and S. G. Fisher. 1986. Secondary production, emergence, and export of aquatic insects of a Sonoran desert stream. Ecology **67**:629-638.

Jacob, M., K. Viedenz, A. Polle, and F. M. Thomas. 2010. Leaf litter decomposition in temperate deciduous forest stands with a decreasing fraction of beech (*Fagus sylvatica*). Oecologia **164**:1083-1094.

James, W. F. and J. W. Barko. 1990. Macrophyte influence on the zonation of sediment accretion and composition in a north-temperate reservoir. Archiv fur Hydrobiologie **120**:129-142.

Jansson, M., L. Persson, A. M. De Roos, R. I. Jones, and L. J. Tranvik. 2007. Terrestrial carbon and intraspecific size-variation shape lake ecosystems. Trends in Ecology & Evolution **22**:316-322.

Januszkiewicz, A. J. and B. W. Robinson. 2007. Divergent walleye (*Sander vitreus*)-mediated inducible defenses in the centrarchid pumpkinseed sunfish (*Lepomis gibbosus*). Biological Journal of the Linnean Society **90**:25-36.

Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen, and F. Landkildehus. 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. Freshwater Biology **45**:201-218.

Jones, R. I. 1992. The influence of humic substances on lacustrine planktonic food-chains. Hydrobiologia **229**:73-91.

Jones, R. I., J. Grey, D. Sleep, and L. Arvola. 1999. Stable isotope analysis of zooplankton carbon nutrition in humic lakes. Oikos **86**:97-104.

Jones, S. E., C. Solomon, and B. C. Weidel. 2012. Subsidy or substraction: how do terrestrial inputs influence consumer production in lakes? Freshwater Reviews **5**:37-49.

Jonsson, M. and D. A. Wardle. 2009. The influence of freshwater-lake subsidies on invertebrates occupying terrestrial vegetation. Acta Oecologica-International Journal of Ecology **35**:698-704.

Kahilainen, K. and K. Ostbye. 2006. Morphological differentiation and resource polymorphism in three sympatric whitefish *Coregonus lavaretus* (L.) forms in a subarctic lake. Journal of Fish Biology **68**:63-79.

Karlsson, J. and P. Byström. 2005. Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. Limnology and Oceanography **50**:538-543.

Karlsson, J., A. Jonsson, M. Meili, and M. Jansson. 2003. Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. Limnology and Oceanography **48**:269-276.

Kato, C., T. Iwata, S. Nakano, and D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos **103**:113-120.

Kato, C., T. Iwata, and E. Wada. 2004. Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. Ecological Research **19**:633-643.

Keast, A. 1984. The introduced aquatic macrophyte, *Myriophyllum spicatum*, as habitat for fish and their invertebrate prey. Canadian Journal of Zoology-Revue Canadienne de Zoologie **62**:1289-1303.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature **437**:880-883.

Koehl, M. A. R. 1996. When does morphology matter? Annual Review of Ecology and Systematics **27**:501-542.

Kok, C. J., C. H. J. Hof, J. P. M. Lenssen, and G. Vandervelde. 1992. The influence of pH on concentrations of protein and phenolics and resource quality of decomposing floating leaf material of *Nympahe alba* L: (Nymphaeaceae) for the detritivore *Asellus aquaticus* (L.) Oecologia **91**:229-234.

Kottelat, M. and J. Freyhof. 2007. Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland.

Kraus, J. M., L. T. Pletcher, and J. R. Vonesh. 2011. Variation in active and passive resource inputs to experimental pools: mechanisms and possible consequences for food webs. Freshwater Biology **56**:491-502.

Kraus, J. M. and J. R. Vonesh. 2012. Fluxes of terrestrial and aquatic carbon by emergent mosquitoes: a test of controls and implications for cross-ecosystem linkages. Oecologia **170**:1111-1122.

Kritzberg, E. S., J. J. Cole, M. L. Pace, W. Graneli, and D. L. Bade. 2004. Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake C-13 addition experiments. Limnology and Oceanography **49**:588-596.

Langerhans, R. B., C. A. Layman, A. M. Shokrollahi, and T. J. DeWitt. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution **58**:2305-2318.

Lappalainen, J., A. S. Tarkan, and C. Harrod. 2008. A meta-analysis of latitudinal variations in life-history traits of roach, Rutilus rutilus, over its geographical range: linear or non-linear relationships? Freshwater Biology **53**:1491-1501.

Lennon, J. T. and L. E. Pfaff. 2005. Source and supply of terrestrial organic matter affects aquatic microbial metabolism. Aquatic Microbial Ecology **39**:107-119.

Leppä, M., H. Hämäläinen, and J. Karjalainen. 2003. The response of benthic macroinvertebrates to whole-lake biomanipulation. Hydrobiologia **498**:97-105.

Leroux, S. J. and M. Loreau. 2008. Subsidy hypothesis and strength of trophic cascades across ecosystems. Ecology Letters **11**:1147-1156.

LeSage, L. and A. D. Harrison. 1979. Improved traps and techniques for the study of emerging aquatic insects. Entomological News **90**:65-78.

Leuven, R. S. E. W., T. C. M. Brock, and H. A. M. Vandruten. 1985. Effects of the preservation on dry- and ash-free dry weight biomass of some common aquatic macro-invertebrates Hydrobiologia **127**:151-159.

Levin, S. A. and R. T. Paine. 1974. Disturbance, patch information, and community structure. Proceedings of the National Academy of Sciences of the United States of America **71**:2744-2747.

Lodge, D. M. 1991. Herbivory on freshwater macrophytes. Aquatic Botany **41**:195-224.

Lodge, D. M., J. W. Rapporteur, C. Barko, D. Strayer, J. M. Melack, G. G. Mittelbach, R. W. Howarth, B. Menge, and J. E. Titus. 1988. Spatial heterogeneity and habitat interactions in lake communities. Pages 181-208 *in* S. Carpenter, editor. Complex interactions in lake communities. Springer-Verlag New York, Berlin, Heidelberg, London, Paris Tokkyo.

Losos, J. B. 2000. Ecological character displacement and the study of adaption. Proceedings of the National Acadamy of Sciences of the United States of America **97**:5693-5695.

Lundberg, J. and F. Moberg. 2003. Mobile link organisms and ecosystem functioning: Implications for ecosystem resilience and management. Ecosystems **6**:87-98.

Lundsgaard-Hansen, B., B. Matthews, P. Vonlanthen, A. Taverna, and O. Seehausen. 2013. Adaptive plasticity and genetic divergence in feeding efficiency during parallel adaptive radiation of whitefish (*Coregonus* spp.). Journal of Evolutionary Biology **26**:483-498.

Magnhagen, C. 2007. Social influence on the correlation between behaviours in young-of-the-year perch. Behavioral Ecology and Sociobiology **61**:525-531.

Malmquist, H. J., S. S. Snorrason, S. Skúlason, B. Jonsson, O. T. Sandlund, and P. M. Jonasson. 1992. Diet differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. Journal of Animal Ecology **61**:21-35.

Manel, S., S. Joost, B. K. Epperson, R. Holderegger, A. Storfer, M. S. Rosenberg, K. T. Scribner, A. Bonin, and M. J. Fortin. 2010. Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. Molecular Ecology **19**:3760-3772.

Marcarelli, A. M., C. V. Baxter, M. M. Mineau, and R. O. Hall. 2011. Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. Ecology **92**:1215-1225.

Matthews, B. and A. Mazumder. 2006. Habitat specialization and the exploitation of allochthonous carbon by zooplankton. Ecology **87**:2800-2812.

McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) - morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology-Revue Canadienne de Zoologie **62**:1402-1408.

Mehner, T., J. Ihlau, H. Dörner, and F. Hölker. 2005. Can feeding of fish on terrestrial insects subsidize the nutrient pool of lakes? Limnology and Oceanography **50**:2022-2031.

Mehner, T., J. Ihlau, H. Dorner, M. Hupfer, and F. Holker. 2007. The role of insectivorous fish in fostering the allochthonous subsidy of lakes. Limnology and Oceanography **52**:2718-2721.

Mendonca, R., S. Kosten, G. Lacerot, N. Mazzeo, F. Roland, J. P. Ometto, E. A. Paz, C. P. Bove, N. C. Bueno, J. H. C. Gomes, and M. Scheffer. 2013. Bimodality in stable isotope composition facilitates the tracing of carbon transfer from macrophytes to higher trophic levels. Hydrobiologia **710**:205-218.

Meyers, P. A. and E. Lallier-Verges. 1999. Lacustrine sedimentary organic matter records of Late Quaternary paleoclimates. Journal of Paleolimnology **21**:345-372.

Milano, D., V. E. Cussac, P. J. Macchi, D. E. Ruzzante, M. F. Alonso, P. H. Vigliano, and M. A. Denegri. 2002. Predator associated morphology in *Galaxias platei* in Patagonian lakes. Journal of Fish Biology **61**:138-156.

Mille-Lindblom, C., H. Fischer, and L. J. Tranvik. 2006. Litter-associated bacteria and fungi - a comparison of biomass and communities across lakes and plant species. Freshwater Biology **51**:730-741.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology & Evolution **20**:685-692.

Naiman, R. J. and H. Decamps. 1997. The ecology of interfaces: Riparian zones. Annual Review of Ecology and Systematics **28**:621-658.

Nakano, S. and M. Murakami. 2001. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America **98**:166-170.

Neill, C. and J. C. Cornwell. 1992. Stable carbon, nitrogen, and sulfur isotopes in a prairie marsh food web. Wetlands **12**:217-224.

Newman, R. M. 1991. Herbivory and detrivory on freshwater macrophytes by invertebrates: a review. Journal of the North American Benthological Society **10**:89-114.

Nosil, P., D. J. Funk, and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology **18**:375-402.

O'Steen, S., A. J. Cullum, and A. F. Bennett. 2002. Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). Evolution **56**:776-784.

Olsson, J. and P. Eklöv. 2005. Habitat structure, feeding mode and morphological reversibility: factors influencing phenotypic plasticity in perch. Evolutionary Ecology Research **7**:1109-1123.

Pace, M. L., S. R. Carpenter, J. J. Cole, J. J. Coloso, J. F. Kitchell, J. R. Hodgson, J. J. Middelburg, N. D. Preston, C. T. Solomon, and B. C. Weidel. 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? Limnology and Oceanography **52**:2177-2189.

Pagel, T. 2009. Determination of individual reproductive success in natural pike (*Esox lucius*) population: a DNA-based parentage assignment approach. Master Thesis. Humboldt-University of Berlin, Germany.

Pardue, W. J. and D. H. Webb. 1985. A comparison of aquatic macroinvertebrates occurring in association with Eurasian watermilfoil (*Myriophyllum spicatum* L.) with those found in the open littoral-zone. Journal of Freshwater Ecology **3**:69-79.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. Plos One **5**:e9672 9610.1371/journal.pone.0009672.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Peakall, R. and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics **28**:2537-2539.

Perez-Figueroa, A., M. J. Garcia-Pereira, M. Saura, E. Rolan-Alvarez, and A. Caballero. 2010. Comparing three different methods to detect selective loci using dominant markers. Journal of Evolutionary Biology **23**:2267-2276.

Persson, L. 1986. Effects of reduced interspecific competition on resource utilization in perch (*Perca fluviatilis*). Ecology **67**:355-364.

Persson, L., G. Andersson, S. Hamrin, and L. Johansson. 1998. Predation regulation and primary production along the productivity gradient of temperate lake ecosystems. Pages 45-65 *in* S. Carpenter, editor. Complex interactions in Lake Communities. Springer Verlag, New York.

Persson, L., J. Andersson, E. Wahlstrom, and P. Eklöv. 1996. Size-specific interactions in lake systems: predator gape limitation and prey growth rate and mortality. Ecology **77**:900-911.

Peters, R. H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge, England.

Phillips, D. L. and P. L. Koch. 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia **130**:114-125.

Plante, C. and J. A. Downing. 1989. Production of fresh-water invertebrate populations in lakes. Canadian Journal of Fisheries and Aquatic Sciences **46**:1489-1498.

Platt, T., D. F. Bird, and S. Sathyendranath. 1991. Critical depth and marine primary production. Proceedings of the Royal Society B-Biological Sciences **246**:205-217.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Polis, G. A., R. D. Holt, B. Menge, and K. O. Winemiller. 1996. Time, space, and life history: influences on food webs. Pages 435-460 *in* G. A. Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics Chapman & Hall, New York.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: Causes, consequences and solutions. Nature Reviews Genetics **6**:847-859.

Pope, K. L., J. Piovia-Scott, and S. P. Lawler. 2009. Changes in aquatic insect emergence in response to whole-lake experimental manipulations of introduced trout. Freshwater Biology **54**:982-993.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

Puebla, O. 2009. Ecological speciation in marine v. freshwater fishes. Journal of Fish Biology **75**:960-996.

R Development Core Team. 2012. R: A language and environment for statistical computing. R foundation for statistical Computing, Vienna, Austria.

Radke, R. J. 1998. Strukturbildende Prozesse in Fischartengemeinschaften mesotropher Seen des nordostdeutschen Tieflandes. Doctoral Thesis. University of Konstanz, Germany.

Rasmussen, J. B. 1985. Effects of density and microdetritus enrichment on the growth of chironomid larvae in a small pond. Canadian Journal of Fisheries and Aquatic Sciences **42**:1418-1422.

Reale, D., S. M. Reader, D. Sol, P. T. McDougall, and N. J. Dingemanse. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews **82**:291-318.

Rehfeldt, G. 1992. Impact of predation by spiders on a terretorial damselfly (Odonata, Calopterygidae) Oecologia **89**:550-556.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82**:523-540.

Revenga, C., I. Campbell, R. Abell, P. de Villiers, and M. Bryer. 2005. Prospects for monitoring freshwater ecosystems towards the 2010 targets. Philosophical Transactions of the Royal Society B-Biological Sciences **360**:397-413.

Rice, A. M., A. Rudh, H. Ellegren, and A. Qvarnstrom. 2011. A guide to the genomics of ecological speciation in natural animal populations. Ecology Letters **14**:9-18.

Ristola, T., J. Pellinen, M. Ruokolainen, A. Kostamo, and J. V. K. Kukkonen. 1999. Effect of sediment type, feeding level, and larval density on growth and development of a midge (*Chironomus riparius*). Environmental Toxicology and Chemistry **18**:756-764.

Robinson, B. W. and D. S. Wilson. 1994. Character release and displacement in fishes - a neglected literature. American Naturalist **144**:596-627.

Robinson, B. W., D. S. Wilson, A. S. Margosian, and P. T. Lotito. 1993. Ecological and morphological differentiation of pumpinseed sunfish in lakes without bluegill sunfish. Evolutionary Ecology **7**:451-464.

Robinson, C. T., M. O. Gessner, and J. V. Ward. 1998. Leaf breakdown and associated macroinvertebrates in alpine glacial streams. Freshwater Biology **40**:215-228.

Rohlf, F. J. and D. Slice. 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. Systematic Zoology **39**:40-59.

Roth, B. M., I. C. Kaplan, G. G. Sass, P. T. Johnson, A. E. Marburg, A. C. Yannarell, T. D. Havlicek, T. V. Willis, M. G. Turner, and S. R. Carpenter. 2007. Linking terrestrial and aquatic ecosystems: The role of woody habitat in lake food webs. Ecological Modelling **203**:439-452.

Rudnick, S. 2011. Charakterisierung der Oberflächenwasser-Grundwasser-Wechselwirkung zweier eutropher Flachseen in Brandenburg. Diploma Thesis, University of Bayreuth, Germany.

Rundell, R. J. and T. D. Price. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. Trends in Ecology & Evolution **24**:394-399.

Ruuhijärvi, J., M. Rask, S. Vesala, A. Westermark, M. Olin, J. Keskitalo, and A. Lehtovaara. 2010. Recovery of the fish community and changes in the lower trophic levels in a eutrophic lake after a winter kill of fish. Hydrobiologia **646**:145-158.

Sabo, J. L. and M. E. Power. 2002a. Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. Ecology **83**:3023-3036.

Sabo, J. L. and M. E. Power. 2002b. River-watershed exchange: Effects of riverine subsidies on riparian lizards and their terrestrial prey. Ecology **83**:1860-1869.

Sand-Jensen, K. and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate fresh-waters and estuaries Aquatic Botany **41**:137-175.

Sand-Jensen, K., T. Riis, O. Vestergaard, and S. E. Larsen. 2000. Macrophyte decline in Danish lakes and streams over the past 100 years. Journal of Ecology **88**:1030-1040.

Sanzone, D. M., J. L. Meyer, E. Marti, E. P. Gardiner, J. L. Tank, and N. B. Grimm. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia **134**:238-250.

Scheffer, M., S. H. Hosper, M. L. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shallow lakes. Trends in Ecology & Evolution **8**:275-279.

Scheffer, M. and E. Jeppesen. 1998. Alternative stable states. Pages 397-406 *in* E. Jeppesen, M. Søndergaard, M. Søndergaard, and K. Christoffersen, editors. The structuring role of submerged macrophytes in lakes. Springer Verlag New York.

Schindler, D. E. and M. D. Scheuerell. 2002. Habitat coupling in lake ecosystems. Oikos **98**:177-189.

Schluter, D. and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist **140**:85-108.

Schreiber, S. and V. H. W. Rudolf. 2008. Crossing habitat boundaries: coupling dynamics of ecosystems through complex life cycles. Ecology Letters **11**:576-587.

Schriver, P., J. Bøgestrand, E. Jeppesen, and M. Søndergaard. 1995. Impact of Submerged Macrophytes on Fish-Zooplankton-Phytoplankton Interactions - Large-Scale Enclosure Experiments in A Shallow Eutrophic Lake. Freshwater Biology **33**:255-270.

Skúlason, S., D. L. G. Noakes, and S. S. Snorrason. 1989. Ontogeny of trophic morphology in 4 sympatric morphs of Arctic charr *Salvenius alpinus* in Thingvallavatn, Iceland. Biological Journal of the Linnean Society **38**:281-301.

Skúlason, S. and T. B. Smith. 1995. Resource polymorphism in vertebrates. Trends in Ecology & Evolution **10**:366-370.

Smith, B. and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiology **47**:380-384.

Smith, T. B. and S. Skúlason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematics **27**:111-133.

Solomon, C. T., S. R. Carpenter, M. K. Clayton, J. J. Cole, J. J. Coloso, M. L. Pace, M. J. Vander Zanden, and B. C. Weidel. 2011. Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. Ecology **92**:1115-1125.

Solomon, C. T., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2008. Support of benthic invertebrates by detrital resources and current autochthonous primary production: results from a whole-lake C-13 addition. Freshwater Biology **53**:42-54.

Storfer, A., M. A. Murphy, S. F. Spear, R. Holderegger, and L. P. Waits. 2010. Landscape genetics: where are we now? Molecular Ecology **19**:3496-3514.

Straile, D. 1998. Biomass allocation and carbon flow in the pelagic food web of Lake Constance. Advances in Limnology **53**:545-563.

Streelman, J. T. and P. D. Danley. 2003. The stages of vertebrate evolutionary radiation. Trends in Ecology & Evolution **18**:126-131.

Svanbäck, R. and P. Eklöv. 2002. Effects of habitat and food resources on morphology and ontogenetic growth trajectories in perch. Oecologia **131**:61-70.

Svanbäck, R. and P. Eklöv. 2003. Morphology dependent foraging efficiency in perch: a trade-off for ecological specialization? Oikos **102**:273-284.

Svanbäck, R., P. Eklöv, R. Fransson, and K. Holmgren. 2008. Intraspecific competition drives multiple species resource polymorphism in fish communities. Oikos **117**:114-124.

Taipale, S., P. Kankaala, and R. I. Jones. 2007. Contributions of different organic carbon sources to *Daphnia* in the pelagic foodweb of a small polyhumic lake: Results from mesocosm (DIC)-C-13-additions. Ecosystems **10**:757-772.

Timms, R. M. and B. Moss. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland eosystem. Limnology and Oceanography **29**:472-486.

Tranvik, L. J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of different humic content. Microbial Ecology **16**:311-322.

Tranvik, L. J., J. A. Downing, J. B. Cotner, S. A. Loiselle, R. G. Striegl, T. J. Ballatore, P. Dillon, K. Finlay, K. Fortino, L. B. Knoll, P. L. Kortelainen, T. Kutser, S. Larsen, I. Laurion, D. M. Leech, S. L. McCallister, D. M. McKnight, J. M. Melack, E. Overholt, J. A. Porter, Y. Prairie, W. H. Renwick, F. Roland, B. S. Sherman, D. W. Schindler, S. Sobek, A. Tremblay, M. J. Vanni, A. M. Verschoor, E. von Wachenfeldt, and G. A. Weyhenmeyer. 2009. Lakes and reservoirs as regulators of carbon cycling and climate. Limnology and Oceanography **54**:2298-2314.

Vadeboncoeur, Y., M. J. Vander Zanden, and D. M. Lodge. 2002. Putting the lake back together: Reintegrating benthic pathways into lake food web models. Bioscience **52**:44-54.

Vander Zanden, M. J., S. Chandra, S. K. Park, Y. Vadeboncoeur, and C. R. Goldman. 2006. Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. Canadian Journal of Fisheries and Aquatic Sciences **63**:2608-2620.

Vander Zanden, M. J. and C. Gratton. 2011. Blowin' in the wind: reciprocal airborne carbon fluxes between lakes and land. Canadian Journal of Fisheries and Aquatic Sciences **68**:170-182.

Vannote, R., G. Minshall, K. Cummins, J. Sedell, and C. Cushing. 1980. The river continuum concept. Candian Journal of Fisheries and Aquatic Sciences **37**:130-137.

Vekemans, X. 2002. AFLP-SURV version 1.0. Distributed by the author. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Belgium.

Vermaat, J. E., L. Santamaria, and P. J. Roos. 2000. Water flow across and sediment trapping in submerged macrophyte beds of contrasting growth form. Archiv fur Hydrobiologie **148**:549-562.

Vermaire, J. C., Y. T. Prairie, and I. Gregory-Eaves. 2012. Diatom-inferred decline of macrophyte abundance in lakes of southern Quebec, Canada. Canadian Journal of Fisheries and Aquatic Sciences **69**:511-524.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP - A new technique for DNA-fingerprinting. Nucleic Acids Research **23**:4407-4414.

Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L (Gasterosteidae) body shape. Biological Journal of the Linnean Society **61**:3-50.

Wanke, T. 2011. Community structure, diet and growth of fish in two shallow lakes of different macrophyte colonization. Diploma Thesis. University of Freiburg, Germany.

Webb, P. W. 1984. Body form, locomotion and foraging in aquatic vertebrates. American Zoologist **24**:107-120.

Webster, M. M., A. J. W. Ward, and P. J. B. Hart. 2009. Individual boldness affects interspecific interactions in sticklebacks. Behavioral Ecology and Sociobiology **63**:511-520.

Weidel, B., S. Carpenter, J. Cole, J. Hodgson, J. Kitchell, M. Pace, and C. Solomon. 2008. Carbon sources supporting fish growth in a north temperate lake. Aquatic Sciences **70**:446-458.

Weihs, D. 1973. The mechanisms of rapid starting of slender fish. Biorheology **10**:343-350.

Weihs, D. 1989. Design-features and meachanics of axial locomotion in fish. American Zoologist **29**:151-160.

Wetzel, R. 1990. Land-water interfaces: Metabolic and limnological regulators. . Internationale Vereinigung für theoretische und angewandte Limnologie, verhandlungen **24**:6-24.

Wetzel, R. G. 1992. Gradient-dominated ecosystems - sources and regulatory functions of dissolved organic-matter in fresh-water ecosystems. Hydrobiologia **229**:181-198.

Wetzel, R. G. 2001. Limnology. Third edition. Elsevier Academic Press, London, England.

Williams, D. D., L. G. Ambrose, and L. N. Browning. 1995. Trophic dynamics of two sympatric species of riparian spider (Araneae, Tetragnathidae). Canadian Journal of Zoology-Revue Canadienne de Zoologie **73**:1545-1553.

Windell, J. T. 1968. Food analysis and rate of digestion. Pages 197-203 *in* W. E. Ricker, editor. Methods for assessment of fish production in fresh water. Blackwell Scientific Publications, Oxford.

Wright, S. 1943. Isolation by distance. Genetics **28**:114-138.

Wurzbacher, C. M., F. Bärlocher, and H. P. Grossart. 2010. Fungi in lake ecosystems. Aquatic Microbial Ecology **59**:125-149.

Statement of academic integrity

I hereby declare, that the dissertation entitled “The Effects of Structural Complexity on Ecological and Evolutionary Processes in Shallow Lake Ecosystems” is my own work. No sources other than those indicated have been used. All collaboration that has taken place with other researchers is indicated and I have clearly stated my own personal share in those investigations in the thesis outline. This thesis has not been submitted for a doctoral degree at any other institution. I am aware of the underlying doctorate regulation of the Department this thesis is submitted to, the Department of Biology, Chemistry, Pharmacy of Freie Universität Berlin.

Hiermit erkläre ich, dass die Dissertation mit dem Titel “The Effects of Structural Complexity on Ecological and Evolutionary Processes in Shallow Lake Ecosystems” meine eigene Arbeit ist. Sie wurde nur unter der Verwendung der angegebenen Hilfen und Hilfsmittel angefertigt. Kooperationen mit anderen Wissenschaftlern wurden angegeben und ich habe meinen eigenen Anteil an den einzelnen Manuskripten klar im Teil „Thesis outline“ aufgezeigt. Diese Dissertation wurde an keiner anderen Universität eingereicht. Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung des Fachbereichs Biologie, Chemie, Pharmazie der Freien Universität Berlin.

Berlin, 11.07.2013

Kristin Scharnweber

Curriculum vitae

For reasons of data protection,
the *curriculum vitae* is not included in the online version