

# Fish reduce habitat coupling by a waterbird: evidence from combined stable isotope and conventional dietary approaches

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**Abstract** Aquatic consumers can function as habitat couplers by using allochthonous subsidies of prey that migrate across ecosystem boundaries. We examined the relative use of allochthonous (invertebrates—terrestrial or living on littoral vegetation; immigrating amphibians) versus autochthonous (aquatic invertebrates, fish) resources by the red-necked grebe *Podiceps grisegena*, a generalist predator, on fishless ponds versus ponds stocked with common carp *Cyprinus carpio*. We combined conventional methods of diet estimation with stable carbon and nitrogen analyses of egg yolks and putative prey of grebes. Preying grebes were observed to take mainly adult amphibians

on fishless ponds and fish on stocked ponds. Alimentary tract analyses gave more weight to invertebrate prey, especially leaf beetles Donaciinae, apparently picked off water or emergent plants. Bayesian isotopic mixing models did not reveal predominance of a single food source but indicated that in the presence of fish grebes received relatively less energy for egg formation from amphibians and leaf beetles. Overall, our results show that grebes relied more on allochthonous resources (range of means 50–97 % of the biomass contribution estimated by different assessment methods) in the absence than in the presence of fish (8–23 %). We suggest that habitat coupling by waterbirds may be controlled by fish, which can suppress external prey subsidies, apart from being an attractive food for piscivorous birds.

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## Introduction

Freshwater ecosystems are coupled with the surrounding terrestrial matrix by reciprocal fluxes of materials or organisms transcending ecosystem borders (e.g. Polis et al. 1997; Loreau et al. 2003; Baxter et al. 2004). The effects of coupling/decoupling of cross-boundary subsidies could propagate throughout food

webs, and both the recipient and donor systems can be strongly affected. Consequently, there is a growing interest in factors regulating the exchange of biomass and energy between ecosystems (Wesner 2010; Greig et al. 2012). Waterbirds often participate in cross-habitat exchange of energy and nutrients by obtaining food in one system and depositing organic material in the form of faeces, prey remains and carcasses in another (Kameda et al. 2006; Sugiura and Ikeda 2013). Spatial flows of resources from outside the focal habitat can be particularly important energy pathways for consumers in shallow-water food webs of ponds and small lakes (Beaudoin et al. 2001; Solomon et al. 2011), where communities are driven by seasonal pulses of insect and amphibian migration in and out. Higher-level consumers, such as birds, even when confined to a single ecosystem during the breeding period, may take advantage of allochthonous subsidies of prey migrating across ecosystem boundaries (Strashevicius et al. 2013). Such habitat coupling is likely to be affected by fishes, which can influence migratory movements of insect and amphibian populations (Baxter et al. 2004; Wesner 2010; Greig et al. 2012). Fish influence on connectors across ecosystems adds a new dimension to the complex network of trophic and nontrophic interactions between fishes and birds (LeBourdais et al. 2009; Kloskowski 2011). Their understanding may require reconstruction of multiple food-web linkages, including those among ecosystems and communities.

The relative abundance of stable isotopes in organic matter derived from animal tissues has become an increasingly common source of information about freshwater food-web structure and dynamics. Stable isotopes of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ,  $\delta^{15}\text{N}$ ) are effective tracers of trophic relationships, as due to preferential excretion of  $^{14}\text{N}$  in nitrogenous waste an increase of 2–5 ‰ in  $\delta^{15}\text{N}$  per trophic step is commonly observed (Peterson and Fry 1987; McCutchan et al. 2003). Stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ,  $\delta^{13}\text{C}$ ) enable identification of the sources of the primary production within a food web, because tissues of consumers tend to be rather weakly enriched in  $^{13}\text{C}$  relative to their prey (France and Peters 1997; but see McCutchan et al. 2003). Stable isotope analysis (SIA) is successfully used to partition energy sources, contributing to the isotope signature of the consumer in dietary studies (Peterson and Fry 1987; Phillips and Gregg 2003; Parnell et al. 2010); thus, stable isotopes may also serve

as tracers of energy flow in food webs. However, ecologists using SIA should be aware of caveats related to the complexity of food webs and of metabolic processes (e.g. Hart and Lovvorn 2002; Bond and Jones 2009; Quevedo et al. 2009). Consequently, combining conventional and isotope approaches may yield broader insights into trophic interactions.

We explored the influence of fish on the relative contribution of allo- and autochthonous resources to diet (or energy allocated to egg formation) in the red-necked grebe *Podiceps grisegena* subspecies *grisegena* (hereafter, grebe), a medium-sized bird associated with shallow, eutrophic waterbodies. Fish ponds are the prime nesting habitat of the species in Central and Eastern Europe (Vlug 2002). Although grebes, as pursuit divers, can extensively feed upon fishes, they are generalist predators well adapted to foraging on invertebrates by gleaning them from the bottom and from aquatic vegetation (Fjeldså 1982; reviewed in Vlug 2002), and may even avoid fish-dominated habitats to reduce competition for shared food (Wagner and Hansson 1998). Here, we compared the dietary sources of grebes nesting on fishless ponds and ponds stocked with common carp *Cyprinus carpio*, an omnivorous fish capable of a strong impact on the prey resources of waterbirds (Haas et al. 2007; Kloskowski 2011). We estimated prelaying diet composition of grebes through observations of foraging birds and gut content analyses. To determine the sources of nutrients allocated to egg formation, putative prey samples and egg yolks of grebes were analysed for carbon and nitrogen stable isotope composition. The isotopic data were translated into estimates of food source proportions using mixing models in a Bayesian framework (Parnell et al. 2010). Given the importance of migrating pond-breeding amphibians and insects for pond communities, we predicted that grebes would be largely reliant on external prey subsidies and that fish would influence the use of external resources.

## Methods

### Study system

This research, carried out between 2001 and 2012, was part of a larger study on trophic interactions between fish and waterbirds breeding on ponds used for extensive carp culture in eastern Poland (51°18'–

27°N; 22°16′–25°E). The ponds studied ( $n = 32$ , mean depth range 0.8–1.2 m, surface area 1–8 ha) mimicked natural temporary aquatic ecosystems. As they were artificially stocked with fish, the ponds provided an excellent opportunity to employ a large-scale manipulative design to examine the effects of fish on aquatic trophic webs (see Kloskowski 2011 for more details on the study system). Grebes, which spent the nonbreeding period mainly in coastal marine or estuarine waters (Vlug 2002), arrived at the study area in April, and the first clutches were initiated from late April to late May. As regards grebe laying phenology, the ponds used in this study could be divided into two types: (1) fishless ponds, filled with water from March through early May; they were usually intended to hold fish fry, but remained fishless until late May; (2) fish-containing ponds, stocked prior to arrival of grebes (from late March through April) with one-year-old carp (ca 11–15 cm total length) at a density of ca 5,000–6,000 individuals  $\text{ha}^{-1}$ , i.e. within biomass density ranges cited for carp in natural systems (Crivelli 1983). Carp at the lower end of the size range stocked could be preyed on by adult grebes (Kloskowski 2012).

#### Feeding observations and gut content analyses

From mid-April, the ponds were visited at least weekly to locate grebe pairs and to control nests. Breeding birds were the subject of a concerted ringing effort. Grebes are territorial during the breeding season, they cannot walk, and no flights to other ponds were observed during the 1–2 weeks prior to egg laying. Therefore, all food consumed during this period had to be obtained within the nesting pond. We termed prey taxa identified during observed feeds or present in grebe alimentary tracts ‘prey items’, while an individual of any prey species observed caught by grebes or represented in the alimentary tract was defined as ‘prey’ (cf. Bugoni and Vooren 2004).

Observations of feeding birds, each lasting 1–8 h, were made with 20 × 60 spotting scopes during daylight hours in the prelaying period (from mid-April to end of May). Focal watches of individual pairs spanned <2 weeks. Altogether, 17 pairs were watched on fishless ponds and 20 pairs on carp ponds. To avoid possible pseudoreplication, we targeted pairs with at least one colour-ringed adult, but the total sample contained six unringed pairs, included on the

assumption that the probability of entering the same pairs more than once into the analyses was low. For observations, we usually selected birds marked during the preceding years; to avoid abandonment of freshly settled territories by disturbed birds, no ringing attempt was done prior to clutch completion.

The relatively small ponds provided good watching conditions. Prey length was estimated visually using the bird’s bill length (defined as the distance from the bill tip to the corner of the mouth) as a reference. Bill length was measured in all captured adults. They were also molecularly sexed using PCR-based sex-specific genetic markers; therefore, for their uncaptured mates, the mean bill length in the given sex was applied (Kloskowski et al. 2006). While our focus was on feeding females, in some pairs, it was difficult to distinguish the mates during foraging bouts; in this case, we pooled the male and female feeding data and used the mean bill length calculated for both sexes to recalculate prey size. We assumed that this did not bias our estimates, because no significant differences in food composition between the sexes were recorded in the subsample of pairs where mates were well discriminated from each other, presumably because sexual dimorphism was slight (Kloskowski et al. 2006). Carp was easily recognisable due to its distinctive body shape and relatively large size. Other large- and medium-sized prey was usually identified to at least order level. However, the smallest prey (<1/4 bill length) was often difficult to identify; these were classified as invertebrates, because carp and adult amphibians occurring in the ponds were of larger size. For presentation, we assigned the prey to four categories: (1) invertebrates picked from above/off the water surface, (2) invertebrates obtained by diving, (3) adult amphibians and (4) carp.

Alimentary tract contents were obtained from four males and seven females collected shortly after sudden death (killed by predators, drowned in nets or colliding with power lines) in the prelaying period or the first week of incubation. The birds collected were territorial, and post-mortem analyses indicated that they were in good condition. Alimentary tract contents were individually deep-frozen for later analysis or immersed in 4 % formalin. Only grebes containing more than ten recognised prey individuals were considered in the analysis. Taxon-specific hard parts were used to identify prey to at least order level and to estimate the minimum number of prey individuals.

Diet data obtained by the two methods were expressed as the percentage contribution of the dry

mass (hereafter biomass) of a prey item to the total biomass of all food components. For prey taxa identified in grebe alimentary tracts, comparative material was collected and the specimens were weighed after drying to stable weight. For carp and invertebrates most commonly taken by grebes, exponential or linear regressions were developed to convert body length to biomass. For other prey items, 3–4 biomass classes were established. When taxonomic identity could not be precisely determined, biomass values for the most likely taxa were applied. For gut contents analysis, which yielded the highest degree of taxonomic accuracy, the percentage contribution by number (tallied by number of prey individuals) was used as well.

For all dietary analyses, we categorised sources as allochthonous or autochthonous. In pond, food webs such a distinction is not discrete, as some prey taxa, depending on the life stage, inhabit both freshwater bodies and nearby terrestrial habitats, or live in their ecotones. We classified adult amphibians and all invertebrates seen or assumed to be picked from above/off the water surface (Odonata and Lepidoptera imagos) as terrigenous sources. Adult leaf beetles Donaciinae were treated as representatives of the land–water ecotone (i.e. allochthonous sources as well), because their locally occurring adult forms typically live on emergent parts of semi-aquatic macrophytes (Otto 1985). All other prey items (including Donaciinae larvae, which live under water) were classified as autochthonous resources. Some emerging insects picked from the water surface could have been misclassified as terrestrial prey. On the other hand, insects of unknown origins retrieved from gut contents were assumed to be autochthonous, although some of them were likely to be terrestrial forms that fell on the water surface.

### Stable isotopes

From late April through May of 2005–2012, putative food sources of grebes (all prey taxa present in more than one alimentary tract examined or identified during observed feeds in more than one grebe territory) were sampled using activity traps or dipnets. Data for conventional dietary analyses were collected from a larger area of ponds where grebe breeding performance was monitored (see Kloskowski 2012). Owing to expected inter-site differences in

baseline  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, we checked for isotopic variation at the base of the food web using snails *Lymnaea stagnatilis*, long-living primary consumers effective at representing isotopic signatures of organisms occupying low positions in littoral food webs (Post et al. 2000; Quevedo et al. 2009). We ultimately limited our samples for SIA to two clusters comprising 16 ponds altogether, dispersed at a distance of about 20 km, which did not significantly differ in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of snail tissues (Mann–Whitney test,  $U_{7,7} \geq 18$ , for both isotopes  $P > 0.4$ ). Consequently, the selected area was treated as one system, and the  $\delta^{15}\text{N}$  values of consumers were not baseline-adjusted. We pooled the data among years assuming that under the same fish culture regime basal resource signatures did not change in the ponds over the study period. We did not observe any between-year patterns in the isotopic data; however, the yearly samples were too small for statistical comparisons.

Sources other than carp were pooled into four groups according to their natural history and isotopic similarity (Table 1). Samples representing species-rich fishless ponds contained more taxa, i.e. some which were virtually absent from fish-containing ponds (cf. Kloskowski 2011). We collected eggs, estimated to be in the first week of incubation, salvaged from destroyed nests or pushed off the nest into the water. A single egg was sampled from each clutch; the laying sequence was often unknown, but a captivity study by Hobson (1995) indicated that isotopic routing between diet and eggs was not influenced by laying order (but see Ramírez et al. 2011). Grebes are income breeders, i.e. they do not utilise their body reserves for egg formation, but use nutrients acquired on the breeding grounds (Paszkowski et al. 2004; McParland et al. 2010; authors' unpubl. data). Also, pairs from which we collected eggs began laying at least 3 weeks after territory acquisition, so we assume that the physiological processes underlying yolk deposition (Williams 2012), which are strongly coupled to follicle development, were initiated in the breeding territories. To compare isotopic values, we used the nonparametric Mann–Whitney test.

Prey samples were deep-frozen after collection, divided into yolk and albumen, dried and ground to a fine powder. In vertebrates, white muscles were used for analyses because they are less variable in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than other tissues (Pinnegar and Polunin 1999). Invertebrates were utilised whole, but shells from

**Table 1** Stable isotope values (mean  $\pm$  SD) and elemental concentrations of red-necked grebe food sources and egg yolks used in this study

| Samples                   | Fishless ponds |                           |                           | Fish-containing ponds |                           |                           | %C   | %N   |
|---------------------------|----------------|---------------------------|---------------------------|-----------------------|---------------------------|---------------------------|------|------|
|                           | <i>n</i>       | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) | <i>n</i>              | $\delta^{13}\text{C}$ (‰) | $\delta^{15}\text{N}$ (‰) |      |      |
| Amphibians <sup>a</sup>   | 9              | $-26.0 \pm 0.9$           | $6.1 \pm 1.1$             | 9                     | $-26.0 \pm 0.9$           | $6.1 \pm 1.1$             | 12.0 | 37.7 |
| Carp                      |                |                           |                           | 11                    | $-27.2 \pm 1.2$           | $6.9 \pm 0.6$             | 14.5 | 44.9 |
| Leaf beetles <sup>a</sup> | 8              | $-23.8 \pm 1.5$           | $3.2 \pm 0.5$             | 8                     | $-23.8 \pm 1.5$           | $3.2 \pm 0.5$             | 8.4  | 49.0 |
| Small aquatic insects     | 14             | $-28.8 \pm 0.9$           | $5.3 \pm 1.0$             | 12                    | $-28.1 \pm 0.8$           | $5.8 \pm 0.8$             | 10.5 | 45.2 |
| Dytiscids                 | 5              | $-27.2 \pm 1.3$           | $7.3 \pm 0.3$             | 4                     | $-26.1 \pm 0.9$           | $7.4 \pm 0.3$             | 11.9 | 42.9 |
| Grebe yolks               | 7              | $-26.6 \pm 1.6$           | $9.0 \pm 1.1$             | 7                     | $-27.9 \pm 2.1$           | $11.2 \pm 1.2$            | 54.5 | 5.3  |

Amphibians were frogs *Hyla arborea*, *Rana temporaria* and *Pelophylax lessonae*. Leaf beetles were *Donacia* sp. common on cattail *Typha* sp. beds along the pond margins, mainly *D. cinerea*

'Small aquatic insects' were Coleoptera <1 cm body length, Hemiptera other than Donaciinae, and larval Odonata. For models for fishless ponds, this group also included Hydrophilidae

Dytiscids were imagos >3 cm

<sup>a</sup> Amphibians and leaf beetles, which were not confined to individual pond food webs, were entered into models for both fishless and fish-stocked ponds irrespective of where they were collected

snails were discarded. Owing to the low body weight of some insects, samples from more than one individual had to be combined to ensure a sufficient quantity of tissue for analysis.

Lipid extraction reduces variability in the samples resulting from discrimination against  $^{13}\text{C}$  in favour of the lighter  $^{12}\text{C}$  during lipid synthesis (DeNiro and Epstein 1977; Hobson 1995), but may lead to an increase in  $\delta^{15}\text{N}$  (Pinnegar and Polunin 1999; Oppel et al. 2010). Therefore, individual samples were divided into two subsamples after grinding. Lipids were removed from samples for  $\delta^{13}\text{C}$  analyses using a Soxhlet apparatus with chloroform as the solvent, while those for  $\delta^{15}\text{N}$  were analysed in bulk form. A few samples were analysed whole for  $\delta^{13}\text{C}$ ; their values were corrected by adding a constant determined by analysing  $\delta^{13}\text{C}$  in both lipid-extracted and nonextracted tissues in a subset of samples (cf. Oppel et al. 2010).

Carbon SIA was conducted using a dual-inlet system triple-collector mass spectrometer at the Mass Spectrometry Laboratory of Maria Curie-Skłodowska University (Lublin, Poland). Nitrogen stable isotope ratios of the samples were analysed by Elemental Analysis-Isotope Ratio Mass Spectrometry (EA-IRMS) at Iso-Analytical Ltd (Cheshire, UK). Samples (0.5–1.0 mg dry tissue) were weighed into Pyrex glass ampoules (carbon SIA) or tin capsules (nitrogen SIA). The  $\delta$  notation was used to express stable isotope ratios relative to internationally recognised standards (VPDB for carbon and AIR for nitrogen). Replicate

assays of standards indicated analytical errors of 0.2 ‰ for both carbon and nitrogen.

The proportional contributions of the different food sources to the isotopic composition of grebe egg components were estimated in a Bayesian mixing model (Stable Isotopes Analysis in R, SIAR v4.1.3; Parnell et al. 2010). The models incorporated concentrations of carbon and nitrogen. Species-specific diet-tissue fractionation factors for grebes were unavailable, so we used estimates taken from the literature. We assumed that lipid-free yolk was not enriched in  $^{13}\text{C}$  (SD = 0.5 ‰), and enriched in  $^{15}\text{N}$  by  $3.5 \pm 0.3$  ‰ (mean  $\pm$  SD) over females' diet (Hobson 1995). As mixing models are sensitive to the discrimination factors used (Bond and Diamond 2011), our model robustness to variation in trophic enrichment between grebe eggs and the food sources was tested by changing each discrimination factor by one standard deviation; the differences from initial estimates of dietary proportions were <5 %.

## Results

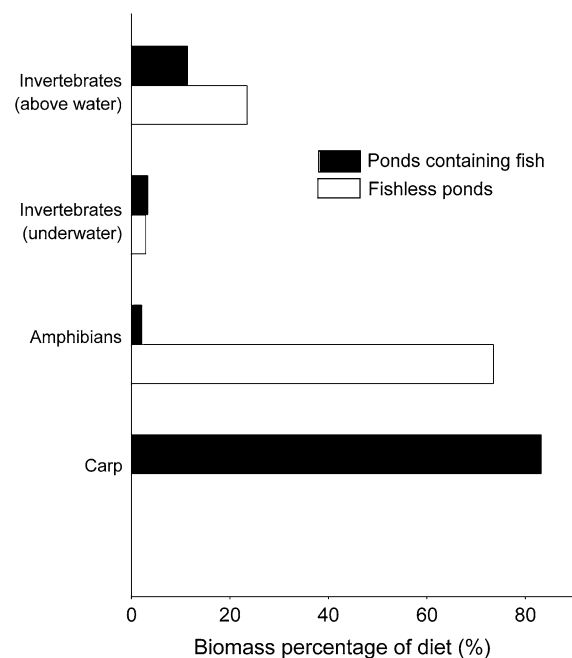
### Observations of feeding habits

A total of 146 h of observations were carried out. On fish-stocked ponds, carp was the dominant prey item (on average 83.5 % of the estimated mass eaten). All fish observed ingested were relatively large individuals of

ca. 7.5–12.5 cm total length). Amphibians were rarely taken (2 % by mass; Fig. 1). By contrast, in the absence of fish, grebes were observed to eat mostly amphibians: *Rana* sp. and *Bombina bombina* were identified, and occasionally newts *Lissotriton vulgaris* (together 73 % of the estimated total biomass consumed). Especially on fishless ponds, a significant proportion of prey was not obtained by diving, but by picking floating and water-striding insects off the water, low-flying insects from the air, and plant-dwelling arthropods from emergent vegetation (Fig. 1).

#### Gut content analyses

Altogether, 832 prey were identified. Leaf beetles, present in all guts examined, were the most frequently recorded prey item (Table 2). Various developmental stages and sizes of Coleoptera were also well represented, together comprising 33.3 and 16.8 % of the estimated prey biomass on fishless and fish-containing ponds, respectively. Carp remains were infrequently retrieved; however, with total lengths (reconstructed from pharyngeal bone and vomer size) from 11.5 to 12.5 cm, preyed carps were substantially larger than



**Fig. 1** Mean percentage contribution by weight of prey groups seen to be ingested by red-necked grebes. Invertebrates were classified as obtained by diving underwater and picked from above or off the water surface

the invertebrate prey and constituted the bulk of the estimated food biomass in the guts where they occurred. Except for one stomach containing anuran bones, alimentary tract contents from fishless ponds consisted entirely of invertebrates (Table 2). All stomachs contained detritus, presumably accidentally ingested while foraging on epibenthic prey.

#### Stable isotopes

The major food sources used for the SIAR models showed a separation in  $\delta^{13}\text{C}$  values (fishless ponds, ANOVA  $F_{3,32} = 42.97$ , stocked ponds,  $F_{4,39} = 27.56$ ; both  $P < 0.001$ ). However, post hoc Tukey HSD with unequal  $n$  (Spjøtvoll–Stoline) tests revealed that for both types of ponds, leaf beetles were the only group significantly different (more  $^{13}\text{C}$  enriched) from all other sources; also, amphibians and carp had significantly higher  $\delta^{13}\text{C}$  than small invertebrates ( $P$  set at 0.05).

The  $^{13}\text{C}$  depletion of grebe yolks did not differ between the two pond types (Mann–Whitney test,  $U_{7,7} = 14$ ,  $P = 0.180$ ); however, yolks were more enriched in  $^{15}\text{N}$  on carp-stocked than fishless ponds ( $U_{7,7} = 0$ ,  $P < 0.002$ ; Fig. 2).

The C and N isotope biplot showed that most yolks (corrected for using tissue-diet discrimination factors) lay within the isotope space delineated by the main food sources, although there was large variability in yolk values (Fig. 2). SIAR outputs indicated that no one source predominated in contribution to grebe eggs (Fig. 3). Despite large credibility intervals, the models showed that on fishless ponds amphibians and leaf beetles were more important contributors (means 28 and 26 %) than on fish-stocked ponds, where carp (27 %) replaced the allochthonous resources (Fig. 3).

Overall, by all the diet assessment techniques used, prelaying grebes relied to a considerably higher extent on allochthonous prey (total means: 97 % by watching feeding grebes, 65 % by examining gut contents and 50 % by SIA) on fishless ponds than on fish-containing ponds (13, 23 and 8 %, respectively).

## Discussion

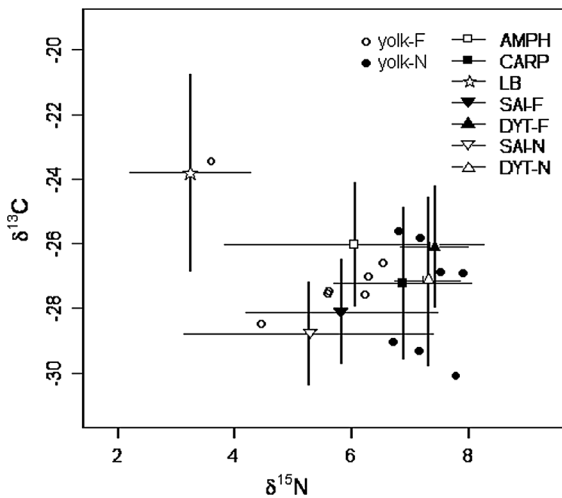
### Main results and methodological issues

Grebe diet estimates differed to some extent between the methodologies applied. Observations of foraging

**Table 2** Mean per cent abundance of prey items recorded in alimentary tracts of grebes collected from fishless and fish-containing ponds

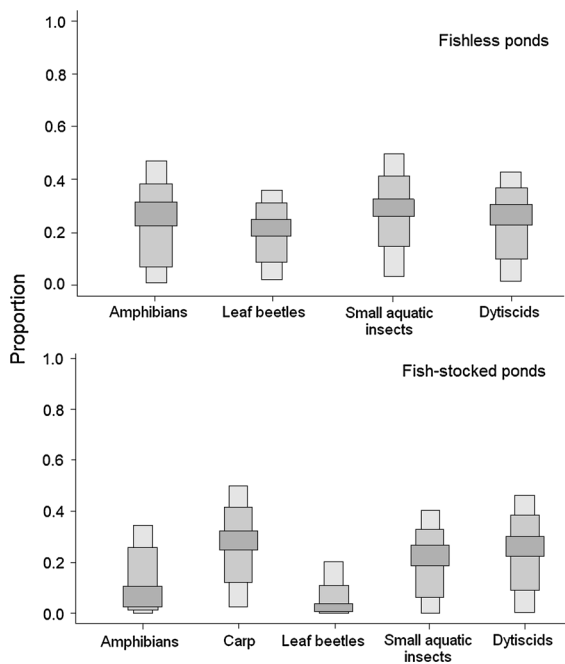
| Food category               | Fishless ponds ( <i>n</i> = 7) |                  | Fish-stocked ponds ( <i>n</i> = 4) |                  |
|-----------------------------|--------------------------------|------------------|------------------------------------|------------------|
|                             | % Individuals                  | % Biomass        | % Individuals                      | % Biomass        |
| Lymnaeidae                  | 2.30                           | 5.54             |                                    |                  |
| Larval Trichoptera          | 0.99                           | 0.24             |                                    |                  |
| Large Dytiscidae (>30 mm)   | 1.00                           | 21.01            | 0.76                               | 8.69             |
| Larval Dytiscidae           | 14.80                          | 3.56             |                                    |                  |
| Other Coleoptera            | 16.45                          | 8.70             | 43.91                              | 8.10             |
| Lepidoptera                 | 0.30                           | 0.79             |                                    |                  |
| Donaciinae                  | 43.42                          | 41.78            | 49.24                              | 23.07            |
| Larval Donaciinae           |                                |                  | 2.27                               | 0.88             |
| <i>Ilyocoris cimicoides</i> |                                |                  | 0.19                               | 0.10             |
| Corixidae                   | 18.42                          | 1.33             | 2.46                               | 0.10             |
| <i>Notonecta</i> sp.        |                                |                  | 0.19                               | 0.10             |
| Odonata                     | 1.00                           | 0.99             |                                    |                  |
| Larval Odonata              | 0.99                           | 0.24             | 0.38                               | 0.05             |
| Amphibia                    | 0.33                           | 15.82            |                                    |                  |
| <i>Cyprinus carpio</i>      |                                |                  | 0.60                               | 58.91            |
|                             | 304 prey                       | 12.64 g dry mass | 528 prey                           | 40.74 g dry mass |

Amphibian and invertebrate taxa were represented by adult forms except where noted as larval. ‘Other Coleoptera’ included small Dytiscidae (<15 mm), Haliplidae, Hydrophilidae and Chrysomelidae other than Donaciinae



**Fig. 2** Isotope biplot of individual  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of grebe yolks and means ( $\pm$ SD) of potential prey. AMPH (amphibians), CARP (carp), LB (leaf beetles), SAI-F (small aquatic insects from fish-stocked ponds), DYT-F (dytiscids from fish-stocked ponds), SAI-N (small aquatic insects from fishless ponds), DYT-N (dytiscids from fishless ponds). Yolks from fish-stocked ponds are shown with a filled circle, yolks from fishless ponds with an empty circle. Yolk values are corrected for trophic fractionation. For sample sizes, see Table 2

birds indicated prevalence of vertebrate prey items in the diet: carp on stocked ponds and amphibians on fishless ponds. The alimentary tract contents suggested a higher share of invertebrates while isotopic mixing models showed that no single prey category predominated among sources of nutrients allocated for reproduction by grebe females. These differences are likely to reflect certain limitations on the use of particular techniques. Direct feeding observations were the most straightforward, noninvasive approach to collecting large data sets over an extended period of time. Large and struggling prey such as fishes and amphibians, which grebes had to bring to the surface, was obviously overestimated, because an unknown fraction of captured small prey (i.e. invertebrates) was swallowed by grebes diving underwater or gleaned from emergent plants amid stands of emergent vegetation, i.e. out of sight of observers. The appeal of gut content analysis is the taxonomic detail achieved, which was helpful in revealing the heterogeneity of the grebe diet. However, the technique is generally biased by its ‘snapshot’ character, differential digestibility, and, if the samples are collected opportunistically



**Fig. 3** Proportion contributions of different food sources to grebe yolks on fishless and fish-stocked ponds. The bars show 25, 75 and 95 % credibility intervals given by SIAR. See the main text for more detailed information on taxa aggregated in the four prey groups

from carcasses, by post-mortem digestion (Swanson and Bartonek 1970; Hornung and Foote 2008).

The advantage of SIA over traditional methods in obtaining trophic information is that isotopic estimates reveal what the animal has assimilated on an integrated time scale and not merely ingested (Phillips et al. 2014). However, the discriminatory power of isotopic models declines with increasing multiplicity of dietary sources and complexity of trophic pathways (e.g. Bond and Jones 2009; Solomon et al. 2011; Silva-Costa and Bugoni 2013). In fact, exploitation of prey originating in different food webs, when not recognised, can considerably obscure assessment of source apportionment for consumers, e.g. by leading to amalgamation of disparate  $^{15}\text{N}$  enrichment pathways (cf. Layman et al. 2007). Terrestrial and land–water ecotone sources have relatively less depleted  $\delta^{13}\text{C}$  values than typical aquatic sources (Peterson and Fry 1987), but the  $\delta^{13}\text{C}$  signatures are not necessarily distinct enough to be fully efficient in resolving energy sources of different origin (Beaudoin et al. 2001; Doucett et al. 2007; Dekar et al. 2012). In our study, leaf beetles representing the land–water ecotone were

clearly the least  $^{13}\text{C}$ -depleted source; frogs, however, most of which live in terrestrial environments (except the reproductive period), did not significantly differ from higher-level aquatic consumers such as dytiscids. While the outcomes of our models confirm high diversity of grebe diet composition, the wide credibility intervals around the estimates do not allow a conclusive discrimination between sources. The uncertainty in source partitioning may be further attributed to the apparent shortness and complexity of food chains in the ponds (Post et al. 2000) and the consequent necessity to pool the numerous prey taxa into source groups due to overlapping isotopic values. Moreover, optimally, stable isotope data should be collected and interpreted within a single system and not averaged for several interconnected subsystems (even with similar isotopic baselines due to sharing the same water supply or isotopically similar ones) like the ponds in our study. However, this approach proves impractical for territorial top predators, which usually occur at very low densities (in grebes, a single breeding pair can monopolise a pond).

#### Habitat coupling by grebes in relation to fish presence

Although our results need to be interpreted with caution because of the above-mentioned sampling and analysis biases, the three methods are fairly consistent in showing that in the absence of fish prelaying grebes used the allochthonous sources to a larger extent than did birds on fish-containing ponds. Leaf beetles, whose palatability to avian predators remains controversial (Otto 1985), were an important subsidy to grebe prelaying diet according to stomach contents. Further research is warranted to determine whether gathering of relatively small-sized insects above/off the water surface is a response to scarcity of aquatic food resources in the early breeding season or results from temporary abundance of Donaciinae. Breeding grebes often settle in freshly flooded areas or otherwise poorly predictable environments, so the use of variable foraging modes can be an adaptation to cope with uncertain food supplies (McParland et al. 2010). Our study results, indicating that grebes are flexible predators feeding upon multiple prey items from different trophic levels, including terrestrial insects, are in agreement with the findings of Paszkowski et al. (2004) on the North American *holboelli*



subspecies (see also Stout and Nuechterlein 1999). Owing to its presumably different ecological niches, the larger-bodied and larger-billed *holboelli* is assumed to be more piscivorous than its European counterpart (Stout and Nuechterlein 1999); in Alberta, Canada, grebes breeding on wetlands containing fish occupied higher trophic positions (indicating fish consumption) than birds nesting on fishless wetlands, as shown by differential  $\delta^{15}\text{N}$  enrichment of egg tissues (McParland et al. 2010). *Holboelli*, however, even on lakes with fishes, has been reported to rely on a mixed diet of macroinvertebrates and fish (Paszowski et al. 2004; see also Newbrey et al. 2012). Similarly, in our study area, *griseogena* did not avoid ponds dominated by fish (contra Wagner and Hansson 1998) when the stocked carp was available as prey (Kloskowski 2012); although grebes were capable of preying on relatively large-bodied carp, on the ponds with plentiful fish insects formed a significant proportion of the diet (see also Onno 1960; Fjelds  1982). Addition of fish, a large-sized and energetically highly profitable prey, to the ponds might help prelaying grebes to maximise their net energy gain (McParland and Paszowski 2006). On the other hand, insects and amphibians migrating to the ponds can either be eliminated by fish predation or avoid oviposition in waterbodies containing fish, nontrophic effects playing an important role in the reduction of the allochthonous subsidies (Vonesh et al. 2009; Kloskowski 2011). Thus, the relative importance of allo- versus autochthonous resources (as well as of fish vs. nonfish resources) for grebes' reproductive effort is likely to depend on both the fish impact on the alternative resources and susceptibility of fish to avian predation.

In conclusion, our findings suggest that fish stocking can lead to a dramatic decline in the use of allochthonous resources by waterbirds and presumably also direct reduction of the external subsidies, thus limiting food-web connectivity. As cross-ecosystem movements of invertebrates and amphibians are essential for the trophic structure of temporary, shallow, aquatic ecosystems, possible fish interactions with organisms immigrating from adjacent ecosystems should be factored into conservation initiatives and considered in fish-stocking policy. To our knowledge, our study is the first to concurrently use conventional methods and SIA to check the effects of fish on habitat coupling by a waterbird. However, the utility of SIA to infer the degree of food-web

coupling decreases as the number of resource pools increases (Phillips and Gregg 2003; Phillips et al. 2014), while potentially effective habitat couplers are typically opportunist foragers using multiple energy channels (Polis et al. 1997). Multiple sources originating in tangled food webs may produce isotopically indistinguishable signals due to trophic intricacy and linkages between ecosystems (cf. Hart and Lovvorn 2002; Bond and Jones 2009). The most recent developments to enhance separation of allochthonous and autochthonous sources by using deuterium isotope ratios ( $\delta^2\text{H}$ ) are promising, but still little is known of trophic fractionation of  $^2\text{H}$  for aquatic consumers and the contribution of environmental water to consumer signatures (Doucett et al. 2007; Jardine et al. 2009; Dekar et al. 2012). While SIA can be a valuable method to partition energy flow in freshwater ecosystems, our study confirms that it should be complemented by conventional techniques both to ensure that all important resources are included in the end-member selection and to provide independent evidence (e.g. Hart and Lovvorn 2002; Tarvainen et al. 2008).

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## References

- Baxter CV, Fausch KD, Murakami M, Chapman PL (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology* 85: 2656–2663
- Beaudoin CP, Prepas EE, Tonn WM, Wassenaar LI, Kotak BG (2001) A stable carbon and nitrogen isotope study of lake food webs in Canada's Boreal Plain. *Freshw Biol* 46:465–477
- Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol Appl* 21:1017–1023
- Bond AL, Jones IL (2009) A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. *Mar Ornithol* 37:183–188

- Bugoni L, Vooren CM (2004) Feeding ecology of the Common Tern *Sterna hirundo* in a wintering area in southern Brazil. *Ibis* 146:438–453
- Crivelli AJ (1983) The destruction of aquatic vegetation by carp. *Hydrobiologia* 106:37–41
- Dekar MP, King RS, Beck JA, Whigham DF, Walker CM (2012) Allochthonous subsidies from grass-dominated wetlands support juvenile salmonids in headwater streams: evidence from stable isotopes of carbon, hydrogen, and nitrogen. *Freshw Sci* 31:121–132
- DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261–263
- Doucett RR, Marks JC, Blinn DW, Caron M, Hungate BA (2007) Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* 88:1587–1592
- Fjeldså J (1982) The adaptive significance of local variations in the bill and jaw anatomy of North European red-necked grebes *Podiceps griseigena*. *Ornis Fenn* 59:84–98
- France RL, Peters RH (1997) Ecosystem differences in the trophic enrichment of  $^{13}\text{C}$  in aquatic food webs. *Can J Fish Aquat Sci* 54:1255–1258
- Greig HS, Kratina P, Thompson PL, Palen WJ, Richardson JS, Shurin JB (2012) Warming, eutrophication, and predator loss amplify subsidies between aquatic and terrestrial ecosystems. *Glob Change Biol* 18:504–514
- Haas K, Kohler U, Diehl S, Kohler P, Dietrich S, Holler S, Jaensch A, Niedermaier M (2007) Influence of fish on habitat choice of water birds: a whole system experiment. *Ecology* 88:2915–2925
- Hart EA, Lovvorn JR (2002) Interpreting stable isotopes from macroinvertebrate foodwebs in saline wetlands. *Limnol Oceanogr* 47:580–584
- Hobson KA (1995) Reconstructing avian diets using stable-carbon and nitrogen isotope analysis of egg components: patterns of isotopic fractionation and turnover. *Condor* 97:752–762
- Hornung JP, Foote AL (2008) Comparing dietary preferences of Bufflehead ducklings in Western Canada through gut content and stable isotope analysis. *Aquat Ecol* 42:61–70
- Jardine TD, Kidd KA, Cunjak RA (2009) An evaluation of deuterium as a food source tracer in temperate streams of eastern Canada. *J N Am Benthol Soc* 28:885–893
- Kameda K, Koba K, Hobara S, Osono T, Terai M (2006) Pattern of natural  $^{15}\text{N}$  abundance in lakeside forest ecosystem affected by cormorant-derived nitrogen. *Hydrobiologia* 567:69–86
- Kloskowski J (2011) Consequences of the size structure of fish populations for their effects on a generalist avian predator. *Oecologia* 166:517–530
- Kloskowski J (2012) Fish stocking creates an ecological trap for an avian predator via effects on prey availability. *Oikos* 121:1567–1576
- Kloskowski J, Grela P, Krogulec J, Gąska M, Tchorzewski M (2006) Sexing red-necked Grebes *Podiceps griseigena* by molecular techniques and morphology. *Acta Ornithol* 41:176–180
- Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide quantitative measures of trophic diversity within food webs? *Ecology* 88:42–48
- LeBourdais SV, Ydenberg RC, Esler D (2009) Fish and harlequin ducks compete on breeding streams. *Can J Zool* 87:31–40
- Loreau M, Mouquet N, Holt RD (2003) Meta-ecosystems: a theoretical framework for a spatial ecosystem ecology. *Ecol Lett* 6:673–679
- McCutchan JH Jr, Lewis WM Jr, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378–390
- McParland CE, Paszkowski CA (2006) Effects of small-bodied fish on invertebrate prey and foraging patterns of waterbirds in Aspen Parkland wetlands. *Hydrobiologia* 567:43–55
- McParland CE, Paszkowski CA, Newbrey JL (2010) Trophic relationships of breeding red-necked Grebes (*Podiceps griseigena*) on wetlands with and without fish in the Aspen Parkland. *Can J Zool* 88:186–194
- Newbrey JL, Paszkowski CA, Gingras BA (2012) Trophic relationships of two species of grebe on a prairie lake based on stable isotope analysis. *Hydrobiologia* 697:73–84
- Onno S (1960) Zur Ökologie der Lappentaucher (*Podiceps cristatus*, *griseigena* und *auritus*) in Estland. XII International Ornithological Congress, vol 2, Helsinki, pp 577–582
- Oppel S, Federer RN, O'Brien DM, Powell AN, Hollmén TE (2010) Effects of lipid extraction on stable isotope ratios in avian egg yolk: is arithmetic correction a reliable alternative? *Auk* 127:72–78
- Otto C (1985) Effects of temporal and spatial variations in food availability on life cycle and palatability of a chrysomelid beetle (*Donacia cinerea*). *Aquat Insects* 7:19–28
- Parnell A, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5:e9672
- Paszkowski CA, Gingras BA, Wilcox K, Klatt PH, Tonn WM (2004) Stable isotope analysis of trophic relations of the red-necked grebe on lakes in the western boreal forest. *Condor* 108:638–651
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18:293–320
- Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261–269
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food web studies. *Can J Zool* 92:823–835
- Pinnegar JK, Polunin NVC (1999) Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Funct Ecol* 13:225–231
- Polis GA, Anderson WB, Holt RD (1997) Toward integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Ann Rev Ecol Syst* 28:289–316
- Post DM, Pace ML, Hairston NG (2000) Ecosystem size determines food-chain length in lakes. *Nature* 405:1047–1049
- Quevedo M, Svanbäck R, Eklöv P (2009) Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology* 90:2263–2274

- Ramírez F, Ramos R, Carrasco JL, Sanpera C, Jover L, Ruiz X (2011) Intra-clutch pattern of albumen  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in yellow-legged gulls *Larus michahellis*: female dietary shift or resource allocation strategy? *J Avian Biol* 42:239–246
- Silva-Costa A, Bugoni L (2013) Feeding ecology of Kelp Gulls (*Larus dominicanus*) in marine and limnetic environments. *Aquat Ecol* 47:211–224
- Solomon CT, Carpenter SR, Clayton MK, Cole JJ, Coloso JJ, Pace ML, Vander Zanden MJ, Weidel BC (2011) Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. *Ecology* 92:1115–1125
- Stout BE, Nuechterlein GL (1999) Red-necked Grebe (*Podiceps grisegena*). In: Poole A, Gill F (eds) *The Birds of North America*, No. 465. The Bird of North America, Inc, Philadelphia
- Strasevicius D, Jonsson M, Nyholm NEI, Malmqvist B (2013) Reduced breeding success of pied flycatchers *Ficedula hypoleuca* along regulated rivers. *Ibis* 155:348–356
- Sugiura S, Ikeda H (2013) Which insect species numerically respond to allochthonous inputs? *Naturwissenschaften* 100:749–759
- Swanson GA, Bartonek JC (1970) Bias associated with food analysis in gizzards of blue-winged teal. *J Wildl Manage* 34:739–746
- Tarvainen M, Vuorio K, Sarvala J (2008) The diet of ruffe *Gymnocephalus cernuus* (L.) in northern lakes: new insights from stable isotope analyses. *J Fish Biol* 72:1720–1735
- Vlug JJ (2002) Red-necked grebe. *BWP Update* 4:139–179
- Vonesh JR, Kraus JM, Rosenberg JS, Chase JM (2009) Predator effects on aquatic community assembly: disentangling the roles of habitat selection and post-colonization processes. *Oikos* 118:1219–1229
- Wagner BMA, Hansson L-A (1998) Food competition and niche separation between fish and the red-necked grebe *Podiceps grisegena* (Boddaert, 1783). *Hydrobiologia* 368:75–81
- Wesner JS (2010) Aquatic predation alters a terrestrial prey subsidy. *Ecology* 91:1435–1444
- Williams TD (2012) *Physiological adaptations for breeding in birds*. Princeton University Press, Princeton