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### Carbon and nitrogen stable isotopes reveal the use of pelagic resources by the invasive Ponto-Caspian mysid *Limnomysis benedeni*

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## Short Communication

# Carbon and nitrogen stable isotopes reveal the use of pelagic resources by the invasive Ponto-Caspian mysid *Limnomysis benedeni*

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The Ponto-Caspian mysid shrimp *Limnomysis benedeni* has rapidly invaded freshwater systems throughout Europe and is now found in extremely high abundances in invaded systems. However, very little is known about the trophic ecology of this mysid in invaded ecosystems, in particular the relative degree of herbivory, carnivory and detritivory of this potentially omnivorous species and where it derives its energy, i.e. via carbon fixed by algae inhabiting benthic or pelagic habitats or through allochthonous inputs. Here, we investigate the trophic ecology of *L. benedeni* in a recently established population in North-Western Germany using stable carbon and nitrogen isotopes. Our results suggest that in contrast to its previous classifications as a benthic or benthopelagic herbivore, *L. benedeni* is an omnivorous species, which can derive the bulk of its carbon from pelagic resources. Its trophic niche in different invaded ecosystems will be determined by multiple, system-dependent factors which have to be considered in order to predict the mysids' invasion potential.

**Keywords:** carbon-13; food webs; invasive species; isotope ecology; lakes; *Limnomysis*; nitrogen-15; resource pools; shrimps; trophic ecology

## Introduction

There is ample evidence that invasive species can have (both direct and indirect) negative impacts on the food web integrity of aquatic ecosystems [1]. In order to assess how species invasions affect trophic interactions and ecosystem functioning, it is crucial to understand how invasive species are integrated into the invaded food webs, and knowledge on the trophic position of the invasive species is of paramount importance in any investigation on the ecosystem consequences of species invasions. In particular, the stable nitrogen isotope ratio  $^{15}\text{N}/^{14}\text{N}$  is an excellent metric of trophic position in food webs [2] and the analysis of stable carbon and nitrogen isotopes has proved to be an exceptionally useful tool to investigate trophic structure and consequences of species invasions in ecosystems [3,4].

The Ponto-Caspian mysid *Limnomysis benedeni* Czerniavsky, 1882, has recently invaded many European lake and river systems and is now found in mass occurrences throughout Europe [5,6]. Since then, a number of studies by Gergs et al. [6,7] and Hanselmann et al. [8–10] on the recently

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established population of *L. benedeni* in Lake Constance have demonstrated the importance of knowing population parameters of *L. benedeni* to understand its invasion and distribution potential, and Fink et al. [5] have shown that the invasion of *L. benedeni* can have profound and complex impacts on the community structure of the native zooplankton fauna. Nevertheless, there is somewhat conflicting evidence on the trophic ecology of *L. benedeni* in invaded lake ecosystems. In particular, the degree of herbivory [6], detritivory [7] or carnivory [5] of this mysid is unclear and *L. benedeni* was previously classified as omnivorous based on gut content analyses [11] or laboratory investigations on feeding behaviour [5] and gut fullness [6]. Very recently, two studies reported analyses of stable isotopes from *L. benedeni*: Hanselmann et al. [10] found *L. benedeni* to have slightly higher  $\delta^{15}\text{N}$  and intermediate  $\delta^{13}\text{C}$  compared with zooplankton (*Bosmina* spp.) and periphyton. Arbaciauskas et al. [12] estimated a trophic level of approximately two (i.e. herbivory) across three size classes of *L. benedeni*. Unfortunately, the data of Hanselmann et al. [10] were based on sampling of only two food web components besides *L. benedeni* and a very low number of replicates, and the paper of Arbaciauskas et al. [12] reported only trophic level estimates without further information on sampled organisms and the level of replication which limits the possibilities to generalise from these references. Hence, it is still unclear in how far these organisms rely on phytoplankton, periphyton, zooplankton, benthic invertebrates, leaf litter and/or detritus as resources and whether the resource use patterns differ between native and invaded habitats. In order to allow a rapid assessment of the trophic ecology of *L. benedeni* to support further studies, we sampled adult mysids in a recently (<10 year) invaded lake and investigated the mysids' nutrition and trophic position using the analysis of stable carbon and nitrogen isotopes of a more comprehensive set of samples compared with previous studies [10,12].

## Methods

Samples were collected on 3 June and 22 July 2008 by dip net and water sampling in the littoral of Lake Reeserward II (51°47'20N and 6°20'2E), a gravel pit lake temporarily connected to the River Rhine near Rees-Grietherbusch, Germany (for details, see [5]). For *L. benedeni*, both adults (>5mm,  $n = 5$  in June and  $n = 10$  in July) and juveniles (<4.5 mm,  $n = 8$ , July only) were sampled separately. As representatives of the native zooplankton community, samples of *Daphnia* sp. ( $n = 4$ ) and copepods (both calanoids and cyclopoids,  $n = 4$ ) were collected in July (0.3–0.6 mg dry mass corresponding to 5–20 individuals per sample) in tin cups (HekaTech, Wegberg, Germany) and dried for 24 h at 60 °C. Furthermore, seston (lake phytoplankton <30  $\mu\text{m}$ ,  $n = 8$ ) and littoral filamentous algae ( $n = 8$ ) collected in July were filtered on pre-combusted GF/F filters (Whatman), which were also dried for >24 h at 60 °C prior to packing into tin capsules.

Subsequently, the dried samples packed in tin capsules were analysed for carbon and nitrogen stable isotope ratios by continuous flow isotope ratio mass spectrometry (Eurovector EuroEA 3000 Series Elemental Analyser coupled to a Micromass Isoprime Mass Spectrometer). Analysis of an internal *Rutilus rutilus* standard showed a precision of  $\pm 0.1\%$  for a single analysis of both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . A high variance was observed for the  $\delta^{15}\text{N}$  values of the seston samples: the standard deviation of the seston samples (1.6) was four times as high as the mean standard deviation of all other  $\delta^{15}\text{N}$  values (0.4). Since an inclusion of this data set would not have allowed the use of parametric statistics due to heteroscedasticity, we decided to exclude the seston samples from the statistical analysis for  $\delta^{15}\text{N}$ . All remaining data were tested for homogeneity of variances using Levene's test and compared via one-way analyses of variance (ANOVA) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  using the general linear model of SPSS® (Version 20, IBM®) followed by *post hoc* comparisons using Tukey's HSD test. Results with  $p < 0.05$  were considered significant.

## Results and discussion

ANOVA revealed highly significant differences between the sampled taxa both for  $\delta^{13}\text{C}$ :  $F_{(6,39)} = 330.5$ ;  $p < 0.001$  and for  $\delta^{15}\text{N}$ :  $F_{(5,32)} = 94.2$ ;  $p < 0.001$  (Figure 1). While the  $\delta^{15}\text{N}$  was found to be significantly different between all groups in the *post hoc* comparisons with one exception (adult and juvenile *L. benedeni* sampled in July,  $p = 0.885$ ), the picture was more complex with respect to  $\delta^{13}\text{C}$ . While the  $\delta^{13}\text{C}$  of *L. benedeni*, *Daphnia* sp. and copepods (all sampled in July) did not differ significantly, the  $\delta^{13}\text{C}$  of the seston (phytoplankton) and the filamentous green algae (phytobenthos) was significantly enriched compared with the other sample groups (Figure 1). In comparison with values typically found for the  $\delta^{13}\text{C}$  of food web components in lake ecosystems [4,13], our data largely matched expectations, at least for the primary producers. The filamentous green algae (mean  $\delta^{13}\text{C} \pm \text{SD} = -22.8 \pm 0.6\text{‰}$ ) show a typical  $^{13}\text{C}$  enriched 'littoral'  $\delta^{13}\text{C}$ , while the seston samples (mean  $\delta^{13}\text{C} \pm \text{SD} = -27.3 \pm 0.1\text{‰}$ ) exhibit  $^{13}\text{C}$  depleted  $\delta^{13}\text{C}$  values typical for pelagic primary production [4]. In general, the finding that the  $\delta^{13}\text{C}$  values of *L. benedeni* did not differ statistically from those of the other zooplankton groups (*Daphnia* sp. and copepods) indicates that all zooplankton groups investigated here rely on the same basic resource pool. Surprisingly, however, all members of the zooplankton community analysed here (*Daphnia* sp. mean  $\delta^{13}\text{C} \pm \text{SD} = -31.8 \pm 0.6\text{‰}$ , copepods  $-32.0 \pm 0.6\text{‰}$  and *L. benedeni*  $-31.3 \pm 0.6\text{‰}$ ) were strongly depleted in  $^{13}\text{C}$  compared with their presumed basal resources (littoral and pelagic algae, Figure 1). This could be due to various reasons: we have possibly failed to analyse the true primary producer community at the base of the zooplankton food web of Lake Reeserward II. For example, the offshore phytoplankton might be more depleted in  $^{13}\text{C}$  than the nearshore seston sampled here. In this case, the strong depletion in zooplankton (and particularly *L. benedeni*)  $\delta^{13}\text{C}$  could possibly be explained by diel horizontal migrations between predator avoidance in

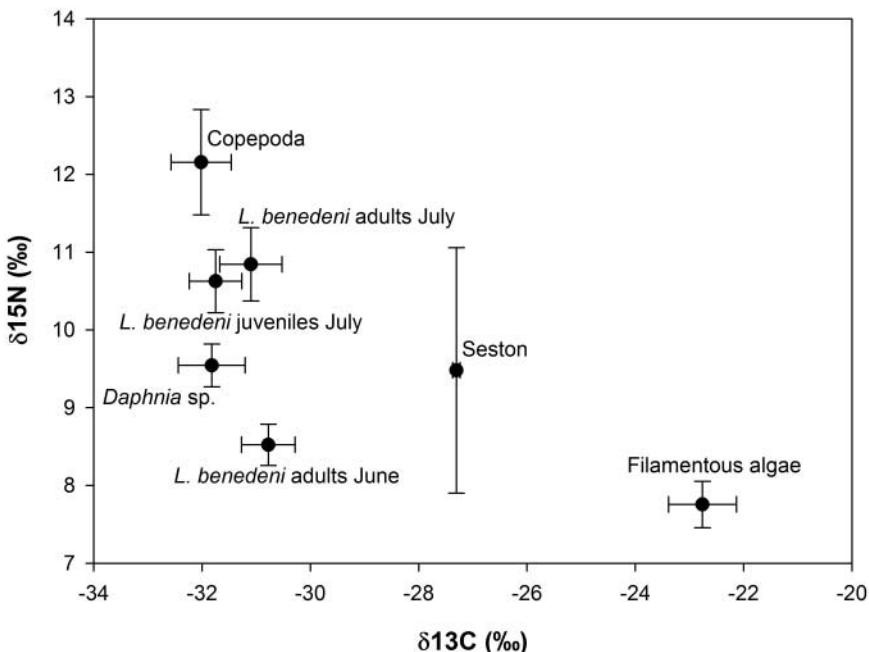


Figure 1. Isotope biplot (means  $\pm$  SD) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Limnomysis benedeni* and their potential resources sampled in Lake Reeserward II; adult ( $n = 10$ ) and juvenile ( $n = 8$ ) *L. benedeni* sampled in July 2008, adult *L. benedeni* ( $n = 5$ ) sampled in June 2008, *Daphnia* sp. ( $n = 4$ ), copepods ( $n = 4$ ), littoral seston (phytoplankton,  $n = 7$ ) and filamentous green algae ( $n = 8$ ).

the littoral during the day (where the animals were sampled) and feeding during night in the pelagic zone. Such plastic predator avoidance behaviours have already been described for other zooplankton taxa [14]. While we do not exclude this possibility, it seems unlikely to be the sole explanation for the observed zooplankton  $\delta^{13}\text{C}$  values considering the marked differences to  $\delta^{13}\text{C}$  values commonly reported for lake primary producers [4] and those reported here for the littoral phytoplankton and phyto-benthos.

A more plausible explanation for the strongly  $^{13}\text{C}$ -depleted zooplankton taxa, however, is that detrital particles and detritus-associated heterotrophic and/or methanotrophic bacteria (which were unfortunately not analysed here) have contributed significantly to the nutrition of both the daphnids and the mysids [15,16]. This would not only explain the quite low  $\delta^{13}\text{C}$  values, but also the relatively high  $\delta^{15}\text{N}$  values of all zooplankton taxa investigated here and give further support for the previously proposed detritivory of *L. benedeni* [11]. In this context of detritivory, another important aspect is a possible contribution of carbon derived from leaf litter to the mysids' diet [6,7]. Unfortunately, leaf litter was not directly sampled in this study. However, if *L. benedeni* were feeding on leaf litter, their  $\delta^{13}\text{C}$  values should be expected to be around  $-28$  or  $-27\text{‰}$ , which are typical values for  $\text{C}_3$  plants such as the willows on the lake's shorelines [13]. While this is remarkably close to our seston  $\delta^{13}\text{C}$  value (which thus could have been a mixture of phytoplankton and fine particulate leaf litter), these seston (and presumed leaf)  $\delta^{13}\text{C}$  values are considerably different from those found for *L. benedeni* in Lake Reeserward II (Figure 1).

Hence, our data suggest a primary reliance of *L. benedeni* on pelagic and detrital (recycled) carbon. This does not correspond to the strong importance of littoral periphyton for the nutrition of *L. benedeni* recently postulated by Gergs et al. [6] and Hanselmann et al. [10] or the predominant herbivory indicated by the trophic level estimate of Arbaciauskas et al. [12] However, it has to be noted that the oligotrophic, pre-alpine Lake Constance, where the studies of Gergs et al. [6] and Hanselmann et al. [10] were conducted and the Curonian Lagoon investigated by Arbaciauskas et al. [12] differ considerably from the habitat investigated here: Lake Reeserward II is a highly turbid and eutrophic gravel pit lake temporarily connected to the large lowland river Rhine. Hence, the availability of benthic primary production is probably much lower in the turbid Lake Reeserward II [5] than in the transparent pre-alpine lake [6,10] and the Baltic lagoon [12], which might explain the observed differences in the behaviour and resource use of *L. benedeni* in these recently invaded lake ecosystems and further demonstrates the extremely high flexibility in niche use of this highly invasive species.

The  $\delta^{15}\text{N}$  values of all food web components in this study were relatively high compared with those commonly described from lake ecosystems [4], which gives further support for an important role of matter cycling in this ecosystem. Pairwise statistical comparisons of the  $\delta^{15}\text{N}$  values of the different food web components analysed here showed significant differences between all groups with copepods ( $\delta^{15}\text{N} \pm \text{SD} = 12.2 \pm 0.7\text{‰}$ ) exhibiting the highest and filamentous green algae the lowest  $\delta^{15}\text{N}$  ( $7.8 \pm 0.3\text{‰}$ , Figure 1). This relatively high mean  $\delta^{15}\text{N}$  and the high  $\delta^{15}\text{N}$  variance indicate that the copepod community (not determined to species level) probably consisted of both herbivorous and predatory species.  $\delta^{15}\text{N}$  did not differ between adult ( $10.6 \pm 0.4\text{‰}$ ) and juvenile ( $10.8 \pm 0.5\text{‰}$ ) *L. benedeni* (collected in July), but was significantly higher than the  $\delta^{15}\text{N}$  of the daphnids ( $9.5 \pm 0.3\text{‰}$ , Figure 1). This indicates that *L. benedeni* is not exclusively herbivorous, but supports the detritivory hypothesis [7,11]. Furthermore, it could indicate some degree of predatory behaviour, which supports the recent findings of Fink et al., [5] although the differences in  $\delta^{15}\text{N}$  between mysids and other zooplankton were much lower than the  $3.4\text{‰}$  typically considered as indicative of a trophic level step [2,12].

Adult *L. benedeni* were found to be more depleted in  $^{13}\text{C}$  and enriched in  $^{15}\text{N}$  in July compared with adults sampled in June (Figure 1), while there were no significant differences (neither in  $\delta^{13}\text{C}$  nor in  $\delta^{15}\text{N}$ ) between adults and juveniles sampled in July. This appears to be in contrast to the commonly reported ontogenetic niche shift from juvenile herbivory to adult carnivory in other

mysid species [17], but suggests a seasonal variability in resource use indicated by the different  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of adults in June and July. These differences found in the isotopic composition of *L. benedeni* at the two different sampling dates give a first indication of seasonal dynamics in the resource use of this invasive mysid (Figure 1). This is further supported by seasonal fluctuations in the fatty acid composition of *L. benedeni* in the field. There, a sharp increase in the mysids' content of the essential eicosapentaenoic acid (C20:5  $\omega$ 3) was observed between June and July 2008, which might be an indicator of a diet switch of *L. benedeni* [18]. The seasonal and ontogenetic dynamics of the resource use of *L. benedeni* clearly merit further attention in the future, since the 7 weeks between the first (early June) and the second (late July) sampling in this study were insufficient to allow detailed insights in the seasonal dynamics of the resource use in this invasive mysid.

In conclusion, our results suggest that *L. benedeni* is an omnivorous species, similar to other mysids [17], but in contrast to its previous classification as a specialised benthic [6] or bento-pelagic [10,12] herbivore. Rather, detritivory [7,11] and carnivory [5] seem to be more important, at least in eutrophic and turbid lakes with low benthic primary production. The trophic ecology of *L. benedeni* in the invaded ecosystems is therefore dependent on multiple factors such as lake trophic state, water transparency and resource availability which play important roles in determining its resource use and invasion success and therefore need detailed attention in further studies. Hence, these multiple factors have to be considered in order to predict the invasion potential not only of *L. benedeni*, but of omnivorous mysids in general.

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