

Assessing diet of the non-indigenous predatory cladoceran *Cercopagis pengoi* using stable isotopes

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Received July 29, 2011; accepted in principle January 25, 2012; accepted for publication January 31, 2012

Corresponding editor: Mark J. Gibbons

In the Baltic Sea, the predatory cladoceran *Cercopagis pengoi* is a non-indigenous species that has potential to compete for mesozooplankton with pelagic zooplanktivorous fish. To understand the extent of diet overlap with these fishes in a coastal area of the northern Baltic proper, we studied the feeding of *C. pengoi* using stable ^{13}C and ^{15}N isotope signatures of the predator and possible prey. Feasible combinations of sources were estimated in two ways: (i) with the IsoSource mixing model, and (ii) temporal-tracking analysis. Further, contribution of different prey was related to ambient zooplankton composition to gauge selectivity. The modelling results indicate that *C. pengoi* is an opportunistic generalist predator with a positive selection towards older copepodites (CIV–VI) of *Acartia* spp. and *Eurytemora affinis*, which also have the greatest contribution to its diet. Positive selection towards podonid Cladocera is also likely. In contrast, evidence for extensive feeding on microzooplankton was inconclusive, and bosminids were not found to be an important prey in the zooplankton assemblages studied. As the derived diet of *C. pengoi* overlaps greatly with that of zooplanktivorous fish, food competition between these zooplanktivores is possible.

KEYWORDS: mixing models; temporal-tracking analysis; selectivity; food web interactions; zooplankton

INTRODUCTION

Non-indigenous species can have detrimental effects upon the biodiversity and food web functioning in invaded ecosystems (Noonburg and Byers, 2005; Beardsley, 2006). In some cases, the impact on the recipient community has been catastrophic, as in the case with the invasion of the ctenophore *Mnemiopsis leidyi* into the Black and Caspian seas (e.g. Kideys, 2002; Roohi *et al.*, 2008). In other cases, however, no measurable impact (Gozlan, 2008) or even positive effects have been observed as, for example, with another ctenophore *Beroe*

ovata that is able to control populations of the earlier introduced *M. leidyi* by predation (Shiganova *et al.*, 2001). In general, to predict and evaluate the potential predatory impact of the newly introduced species, one must know the pre-existing trophic linkages in the food web as well as those that the invader establishes and to quantify its dietary composition and requirements.

Cercopagis pengoi, a predatory cladoceran originating from the Ponto-Caspian region (Rivier, 1998), was first recorded in 1992 in the Gulfs of Riga and Finland (Ojaveer and Lumberg, 1995; Krylov *et al.*, 1999). Later,

it spread to other parts of the Baltic Sea (Gorokhova *et al.*, 2000) and colonized the North American Great Lakes (Leppäkoski and Olenin, 2000; Therriault *et al.*, 2002). *Cercopagis pengoi* is a voracious predator that can reach high densities during summer (Baltic Sea: up to 1800 ind m⁻³, Uitto *et al.*, 1999; Lake Ontario: up to 2600 ind m⁻³, Ojaveer *et al.*, 2001; Lake Michigan: 700 ind m⁻³, Witt and Cáceres, 2004) and has a potential to alter native zooplankton populations (Ojaveer *et al.*, 2004; Kotta *et al.*, 2006). In pelagic food webs, it acts as both predator and prey and is recognized as a species with a large potential to affect food webs and fish feeding conditions in the invaded ecosystems (Leppäkoski *et al.*, 2002; Vanderploeg *et al.*, 2002).

Before the introduction of *C. pengoi* into the Baltic Sea, the diet of Baltic zooplanktivorous fish, such as herring (*Clupea harengus*) and sprat (*Sprattus sprattus*), was largely composed of copepods (*Acartia* spp. and *Eurytemora affinis*) and cladocerans (podonids and *Bosmina maritima*), with a preference for the copepods (Rudstam *et al.*, 1992; Mehner and Heerkloss, 1994; Arrhenius, 1996; Antsulevich and Välipakka, 2000). *Cercopagis* is a voracious predator, with a very peculiar feeding mode; it punctures the carapace and ingests soft tissues discarding the exoskeleton (Rivier, 1998). This feeding mode enables feeding on a broad size spectrum of prey from an order of magnitude below its own body mass (Pichlová-Ptáčnicková and Vanderploeg, 2009). Previous field and experimental studies in the Baltic Sea (Simm *et al.*, 2006; Lehtiniemi and Gorokhova, 2008) and the Laurentian Great Lakes (Laxson *et al.*, 2003; Pichlová-Ptáčnicková and Vanderploeg, 2009) have shown that *C. pengoi* feeds on copepods and cladocerans, and hence, it might compete with zooplanktivorous fish feeding on the same kind of prey. Evidence is, however, accumulating that *C. pengoi* can prey upon zooplankton that is too small for the adult fish to utilize efficiently, such as large ciliates, rotifers and small meroplanktonic larvae (Gorokhova *et al.*, 2005; Simm *et al.*, 2006; Lehtiniemi and Lindén, 2006). Because *C. pengoi* is also a prey for various Baltic fish, such as herring and sprat (Ojaveer and Lumberg, 1995; Antsulevich and Välipakka, 2000; Gorokhova *et al.*, 2004), it may channel energy from previously underutilized biomass produced at lower trophic levels to fish. Therefore, if microzooplankton contributes substantially to the diet of *Cercopagis*, the invasion may have a positive effect on fish feeding conditions in the Baltic Sea, particularly in August–September, when mesozooplankton decline (Johansson *et al.*, 1993; Adrian *et al.*, 1999) during the consumption peak by young-of-the-year fish (Rudstam *et al.*, 1992; Arrhenius and Hansson, 1993). During this period, the microzooplankton contribution to the total

zooplankton biomass increases and, simultaneously, *C. pengoi* reaches its abundance peak and may become an important food source for adult herring and sprat, but also contributes to the diets of the young-of-the-year fish (Gorokhova *et al.*, 2004).

The aim of this study was to determine temporal changes in diet composition of *C. pengoi* in the northern Baltic proper. In particular, our objectives were to investigate (i) to what extent *C. pengoi* utilizes microzooplankton and (ii) if *C. pengoi* has a preference for copepods, i.e. preferred planktivorous fish prey. Using stable C and N isotope signatures of potential zooplankton prey and *C. pengoi*, feasible ranges of different prey contributions to *C. pengoi* nutrition were estimated using IsoSource mixing models (Phillips and Gregg, 2003). Stable isotope analysis (SIA) has been proved useful in determining trophic interactions in aquatic ecosystems (Zanden and Rasmussen, 2001; Post, 2002). This method enables diet analysis when traditional methods are not applicable; as when the study organisms, feeding habits make gut content analysis impossible. Such feeding habits include fluid feeding, as is the case with *C. pengoi*. Further, we related IsoSource-based estimates to ambient zooplankton community composition to derive prey preferences. In addition, we analysed relationships between the consumer and its potential prey on different sampling occasions and used significant positive relationships as an indication of strong trophic linkage, following a probabilistic approach proposed by Melville and Connolly (Melville and Connolly, 2003); the results were compared with the IsoSource modelling results. This allowed us to evaluate the selectivity of *C. pengoi* for various zooplankton prey organisms and their contribution to its diet.

METHOD

Study site and sampling

Himmerfjärden Bay is situated in the southern archipelago of Stockholm, north-western Baltic proper. It is ~30-km long, has a mean salinity of 6 (PSU) and a mean depth of 17 m. It receives discharge water from a municipal water treatment plant located at the bay-head. Zooplankton for SIA and population analysis were sampled at two stations in Himmerfjärden Bay on a fortnightly basis in June–September 2007. The sampling stations, H2 and H4 (Fig. 1), are situated at the mouth of the bay (H2) and half-way up the bay, closer to the water treatment plant (H4).

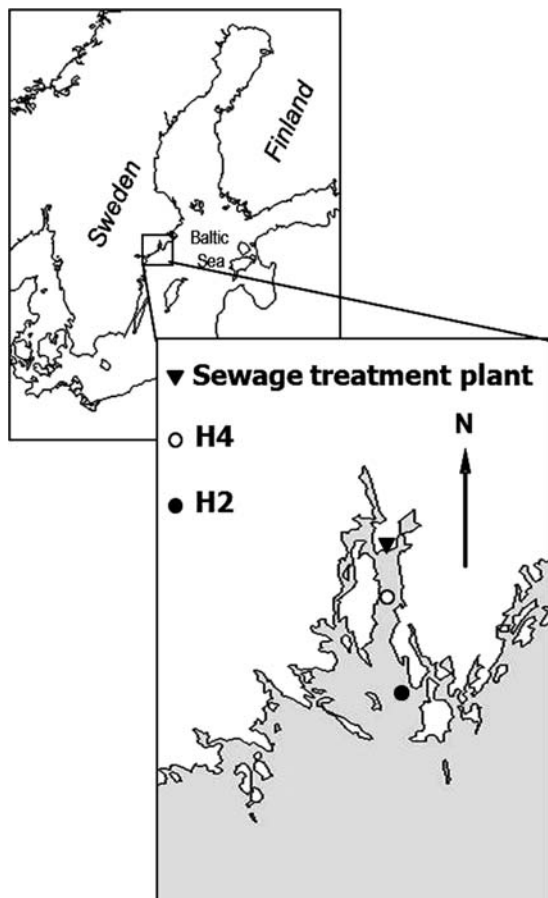


Fig. 1. Map of the study area, Himmerfjärden Bay, showing the locations of the sampling stations (H2: N 58° 50' 55", E 17° 47' 42" and H4: N 58° 59' 02", E 17° 43' 52") and the sewage treatment plant at the head of the bay.

Zooplankton population analysis

Zooplankton samples for population analysis were taken using vertical net tows (WP-2, 90 μm) from the bottom (~ 25 m) to the surface. The samples were preserved and analysed according to the standard protocol of the Baltic Monitoring Programme (HELCOM, 1988). Organisms were identified and counted in subsamples (Kott, 1953) of ≥ 500 specimens using an inverted microscope (Wild Leitz fluovert FS, Leica) at $\times 80$ magnification. All *C. pengoi* were removed from the samples prior to the subsampling and processed separately under a dissecting microscope ($\times 50$). Conventional methods of population analysis of Cercopagidae were employed (Rivier, 1998) and biomass (wet weight) of *C. pengoi* was calculated according to Svensson and Gorokhova (Svensson and Gorokhova, 2007). The biomass (w.w.) of other zooplankton was calculated according to Rosen (Rosen, 1981) and Hernroth (Hernroth, 1985).

Stable isotope analysis

Samples for SIA were taken from the upper 15 m of the water column using the same net as for the population analysis sampling. Immediately after collection, the zooplankton were separated from phytoplankton and cyanobacteria using a light trap (Larsson *et al.*, 1986). The samples were diluted in filtered seawater and kept in insulated containers for transportation back to the laboratory (3–4 h), which allowed time for the zooplankton to clear their guts.

In the laboratory, the samples were sequentially filtered through different sieves (500, 250 and 35 μm), to get a rough size separation. The samples were then snap-frozen on a mesh at -80°C and stored for up to 10 days before sorting. Mesozooplankton samples were thawed and sorted by taxa and life stage under a dissecting microscope (Wild Heerbrugg, $< \times 50$) into the following groups: (i) copepodites CI–III (younger copepodites, stage I–III), *Acartia* spp. (*A. bifilosa*, *A. tonsa* and *A. longiremis*) and *E. affinis*; (ii) *Acartia* spp. CIV–VI (older copepodites, stage IV–VI); (iii) *E. affinis* CIV–VI; (iv) podonids (*Podon leuckartii*, *P. intermedius* and *Pleopsis polyphemoides*); (v) *B. maritima*; (vi) juvenile *C. pengoi* (barb stage I) and (vii) adult *C. pengoi* (barb stages II and III; these were pooled due to their similar body sizes; Uitto *et al.*, 1999). Sorted individuals were placed in pre-weighed tin capsules and dried at 60°C for 72 h. Three replicates were taken for each prey group, with an average sample dry weight of 0.13 mg and typically consisted of three to seven individuals for *C. pengoi* or > 50 individuals for the copepodites and smaller cladocerans. To obtain samples of microzooplankton for SIA, zooplankton retained on the 35- μm sieve were sieved once more through a 200- μm sieve to remove residual mesozooplankton. The zooplankton fraction retained on the 35- μm sieve was considered microzooplankton; and were mainly composed by rotifers (*Keratella* spp. and *Synchaeta* spp.) and juvenile copepods (mainly nauplii of *Acartia* spp. and *E. affinis*). Using a dissecting microscope, the animals were pipetted onto pre-combusted (5 h at 500°C) and pre-weighed Whatman filters (47 mm) and dried at 60°C for 72 h. Once dry, four discs of 4-mm diameter were cut from the filters and placed in pre-weighed tin capsules; these samples had an average dry weight of 0.20 mg, consisting of several hundred individuals. All samples were stored in desiccators until shipping for analysis. The SIA was conducted by continuous-flow mass spectrometry with an automated CN analyzer SL 20-20, PDZ Europa at the Stable Isotope Facility, University of California at Davis, USA. Ratios of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ were expressed as the relative per mil (δ , ‰) difference between samples and

conventional standards (Vienna Pee Dee belemnite for C and atmospheric N₂ for N).

Effects of freezing on $\delta^{15}\text{N}$ values

Snap freezing has been reported to increase $\delta^{15}\text{N}$ values in cyclopoid copepods and cladocerans (Feuchtmayr and Grey, 2003). Therefore, the effect of freezing on $\delta^{15}\text{N}$ values in our zooplankton samples was tested by taking an additional five replicate samples of *Acartia* spp. CIV–VI that were processed directly after sampling, rather than freezing them. The results from these samples were then compared with five replicates of frozen samples from the same sampling occasion using an unpaired *t*-test. As no significant difference in $\delta^{15}\text{N}$ values was found between the frozen and fresh samples (unpaired *t*-test: $t_8 = 0.88$, $P > 0.4$), the freezing was considered an adequate storage method allowing the sampling schedule to be followed and all samples to be processed uniformly.

Mixing models

To determine the source contributions to the diet of *C. pengroi*, we estimated the relative contribution of each prey with the IsoSource mixing model (Phillips and Gregg, 2003) implemented in the SISUS platform (<http://statacumen.com/sisus/>). Only $\delta^{15}\text{N}$ values of potential prey and predators were used because relative uniformity of $\delta^{13}\text{C}$ values among the prey and predators limited the utility of these data. The model examines all possible combinations of each prey potential contribution (0–100%) in small increments (here 1%). Combinations that summed to within 0.01‰ of *C. pengroi* signature were considered feasible solutions. The results are presented as histograms to demonstrate the frequency distribution of dietary biomass contributions (%). In the Baltic Sea, *C. pengroi* has a generation time of 14–17 days (Svensson and Gorokhova, 2007), and hence, its isotopic signature should reflect the diet during that period. Therefore, to obtain prey isotopic composition, which is representative of the consumer signature, food sources were averaged for a 2-week period, i.e. between the neighbouring sampling occasions. To be used in the mixing model, food sources are required to be significantly different from each other; they were compared pairwise prior to being used as end-members in SISUS with an unpaired *t*-test ($P < 0.05$ in all cases). Two ^{15}N fractionation factors were tested for the model calculations: 2.4‰ (calculated using the model for invertebrates suggested by Caut *et al.* (Caut *et al.*, 2009) and data for H2 as the least affected by the sewage effluents) and 3.4‰ (the average

fractionation factor for $\delta^{15}\text{N}$ recommended by Post, 2002). As the latter fractionation factor resulted in non-converging models (in 13 out of 37 cases, data not shown), whereas all models using the fractionation factor 2.4‰ converged, the results presented here are based on models using the 2.4‰ fractionation factor. All statistical analyses were performed using GraphPad Prism 4.01 (GraphPad Software, USA); significance was accepted when $P < 0.05$.

Temporal-tracking analysis

To determine whether temporal-tracking occurred, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *C. pengroi* and potential prey on each sampling occasion and station were used as Cartesian coordinates, and Euclidean distances were calculated between the value for *C. pengroi* and a prey on all dates when they both occurred. These distances were averaged (D_0) to produce a measure of correlation in a two-dimensional space (tracking). To obtain a distribution of predator/potential prey distances, sampling date labels of prey groups were changed and Euclidean distances were recalculated. The observed D_0 of the predator/prey combination was then compared with this distribution of possible D values, giving a probabilistic significance test (Melville and Connolly, 2003). If the D_0 value was small relative to the distribution of possible values, then *C. pengroi* was said to be tracking that particular prey. This was done for all possible combinations of prey against the observed *C. pengroi* data.

RESULTS

Seasonal dynamics of zooplankton communities

The total biomass of zooplankton communities varied at both stations over the season, with ~2-fold higher values at H4 than at H2 (Fig. 2). At both stations, two biomass peaks occurred, one at the end of July (940 and 560 mg m⁻³ at H4 and H2, respectively) and the other in September (690 and 270 mg m⁻³ at H4 and H2, respectively). The zooplankton community structure differed between the two sites: *Acartia* spp., *E. affinis* and microzooplankton contributed most to the zooplankton stocks at station H2, while *B. maritima* and *E. affinis* dominated at station H4. Throughout the season, *C. pengroi* biomass was relatively low at both stations, contributing up to 2 and 5% to the total zooplankton biomass, at H2 and H4, respectively, and with a 10-fold difference between the sites (≤ 5 and ≤ 46 mg m⁻³ at H2 and H4, respectively; Fig. 2).

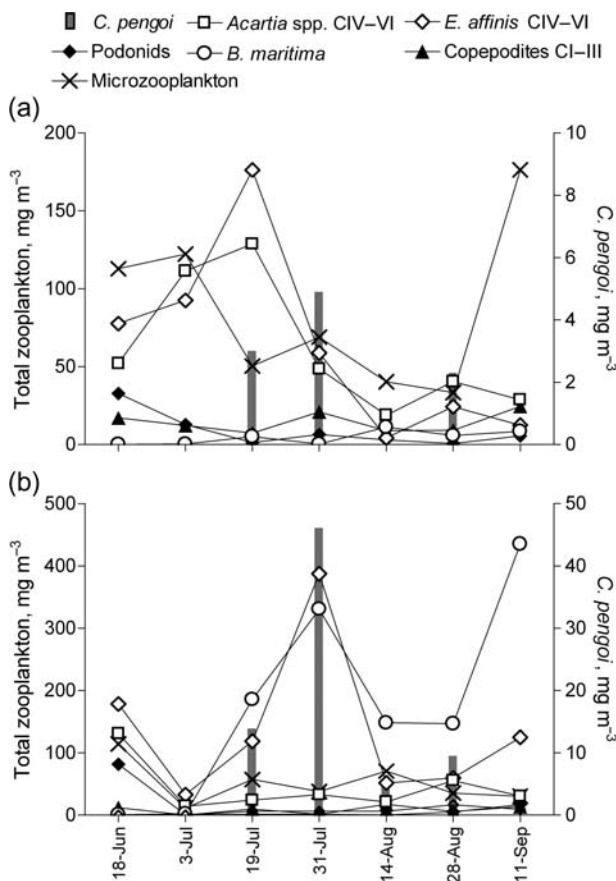


Fig. 2. Seasonal dynamics of potential prey and *Cercopagis pengoi* populations at station H2 (a) and H4 (b). Note the difference in y-axes scales between the panels. Connecting lines are a visual aid for reading the figure, not interpolation of results between dates. On August 14 (at H2), *C. pengoi* abundance was not determined because of the high abundance of filamentous cyanobacteria that formed inseparable aggregates with the cladoceran and precluded microscopic analysis.

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the predator and prey

The $\delta^{15}\text{N}$ values varied from 6 to 10‰ and from 10 to 14‰ in samples collected at H2 and H4, respectively; while $\delta^{13}\text{C}$ values were more similar between the stations: -25 to -21 and -25 to -19 ‰ at H2 and H4, respectively (Fig. 3). There were considerable differences in the isotopic composition of zooplankton between the two stations (Fig. 3). At H4, zooplankton $\delta^{15}\text{N}$ values were 3–4‰ higher compared with those at H2; the differences were significant for all prey groups considered (unpaired *t*-test; $P < 0.05$ in all cases). In most groups, the $\delta^{13}\text{C}$ values followed the same trend, being higher at H4 compared with H2; the exception were podonids ($P > 0.10$) and *C. pengoi* ($P > 0.15$) that did not differ between the stations. Since there were significant differences in isotopic composition of zooplankton between

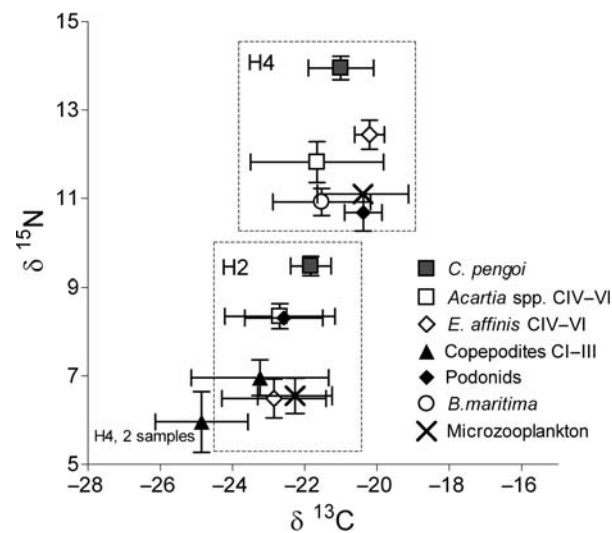


Fig. 3. Zooplankton stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) at stations H2 and H4 averaged over the study period (mean \pm SD).

the stations, the data from H2 and H4 were used separately in the mixing models and temporal-tracking analysis.

When values were averaged over the season, no significant differences in $\delta^{13}\text{C}$ values were found between prey groups at either station ($P > 0.05$ in all cases), whereas differences in $\delta^{15}\text{N}$ were significant ($P < 0.05$ in all cases). At both sites, *C. pengoi* occupied the highest trophic position among the zooplankton tested, as indicated by its $\delta^{15}\text{N}$ values (9.47 and 13.95‰ for H2 and H4, respectively; Fig. 3). There were no significant differences in either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values between juvenile and adult *C. pengoi* (paired *t*-test; $\delta^{15}\text{N}$: $t = 1.744$, $df = 8$, $P > 0.12$; $\delta^{13}\text{C}$: $t = 1.626$, $df = 8$, $P > 0.14$), and therefore, all samples for this species were pooled before use in IsoSource models and temporal-tracking analysis. A high level of observed within-station variability was found, and this was mostly due to changes in isotopic concentrations over the season, with particularly pronounced decrease in $\delta^{15}\text{N}$ in nearly all prey groups during the end of July–August (data not shown). These seasonal changes made it necessary to construct separate mixing models for each sampling occasion.

Source contributions estimated by IsoSource mixing models

Several prey groups contributed substantially to the diet of *C. pengoi* (Fig. 4), with large copepodites (CIV–VI) having the greatest contribution on a seasonal basis. At station H2, dominant contributors were *Acartia* spp.

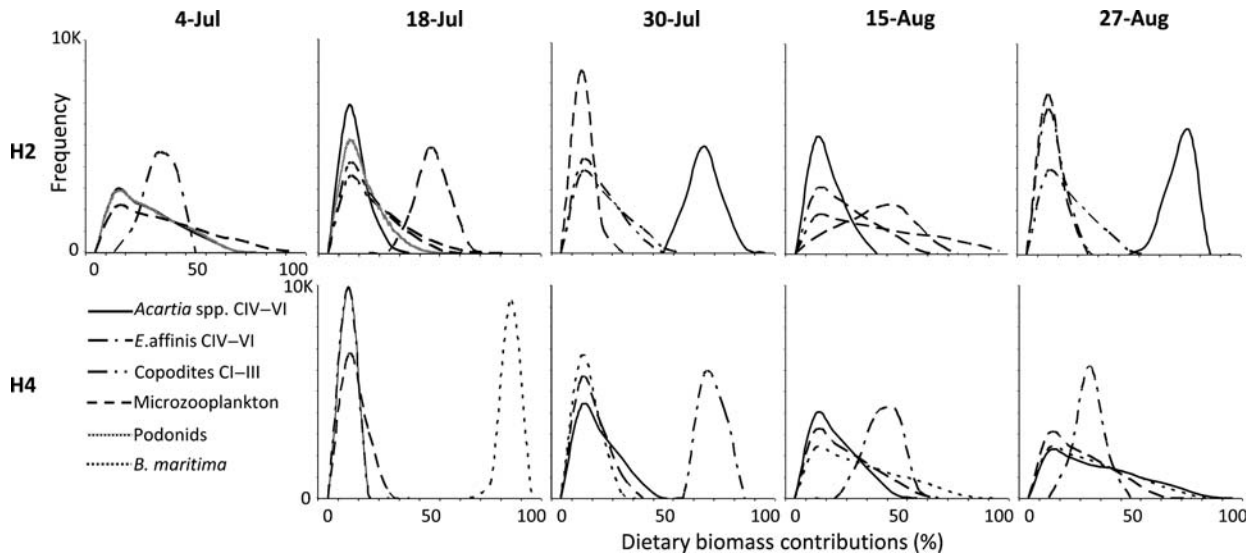


Fig. 4. Dietary biomass contributions (%) of different prey for *Cercopagis pengroi* on different sampling occasions at stations H2 and H4 estimated by the IsoSource mixing model using $\delta^{15}\text{N}$ values of the consumer and its potential prey: *Acartia* spp. CIV–VI, copepodites CI–III, *Eurytemora affinis* CIV–VI, podonids, *Bosmina maritima* and microzooplankton. The contributions are calculated for all model iterations and expressed as the percent frequency of all possible solutions. Owing to lack of material for samples, no stable isotope data were available for copepodites CI–III on the 4/7 at H2 and any occasion at H4, podonids on the 30/7, 15/8 and 27/8 at H2 and any occasion at H4 and bosminids for any occasion at H2.

CIV–VI (8–71%), followed by microzooplankton (5–47%), *E. affinis* CIV–VI (8–35%), podonids (12–22%) and copepodites CI–III (13–20%). At station H4, *B. maritima* (8–85%) and *E. affinis* CIV–VI (4–69%) dominated, whereas *Acartia* CIV–VI (4–29%) and microzooplankton (8–20%) contributed less. During the period when *C. pengroi* was present in the water column, abundances of *B. maritima* and podonids at station H2 and of podonids and copepodites CI–III at station H4 were very low (Fig. 2). As a result, it was not always possible to collect enough sample material for SIA of these groups and hence they were not included in models for all sampling occasions/stations (Fig. 4).

Prey-tracking analysis

Those prey that were more closely tracked by *C. pengroi* ($P < 0.05$) were well separated from those less closely tracked (Table I). Old copepodites (CIV–VI), particularly *E. affinis*, were most closely tracked, while *B. maritima* and microzooplankton were not. Tracking of podonids was not possible to evaluate, due to the fact that this prey nearly disappeared from the water column when the *C. pengroi* population started to increase.

Prey selectivity

A positive selection by *C. pengroi* was assumed to occur, if the average contribution of a prey species in

Table I: Temporal tracking of prey by Cercopagis pengroi at stations H2 and H4

Potential prey group	H2		H4	
	P%	P-value	P%	P-value
Copepodites CI–III	0	0.0243	nd	—
<i>Acartia</i> spp. CIV–VI	0	0.0218	67	0.4767
<i>E. affinis</i> CIV–VI	0	0.0220	0	0.0235
Podonids	50	na	nd	—
<i>B. maritima</i>	nd	—	0	0.1386
Microzooplankton	0	0.1184	33	0.4864

P%, the percentage of possible D values smaller than observed D_0 , and corresponding P -values (one-sample t -test) are shown. Low values indicate tracking in time of prey isotope signatures by the predator; na, prey occurred at insufficient occasions ($n < 4$) when the consumer was present; nd, no stable isotope data available. Significant values ($P < 0.05$) are in bold face.

the diet, estimated with the IsoSource mixing model, exceeded its contribution to the ambient zooplankton community. At station H2 (Fig. 5), this occurred for podonids prior to their abundance dropping to very low levels, and occasionally for the older copepodites (CIV–VI) of *Acartia* spp. and *E. affinis*. At H4 (Fig. 5), the old copepodites were the preferred groups; positive selection was also shown towards *B. maritima* on one occasion in July. At both stations (Fig. 5), positive or neutral selection was also observed for microzooplankton on several sampling occasions.

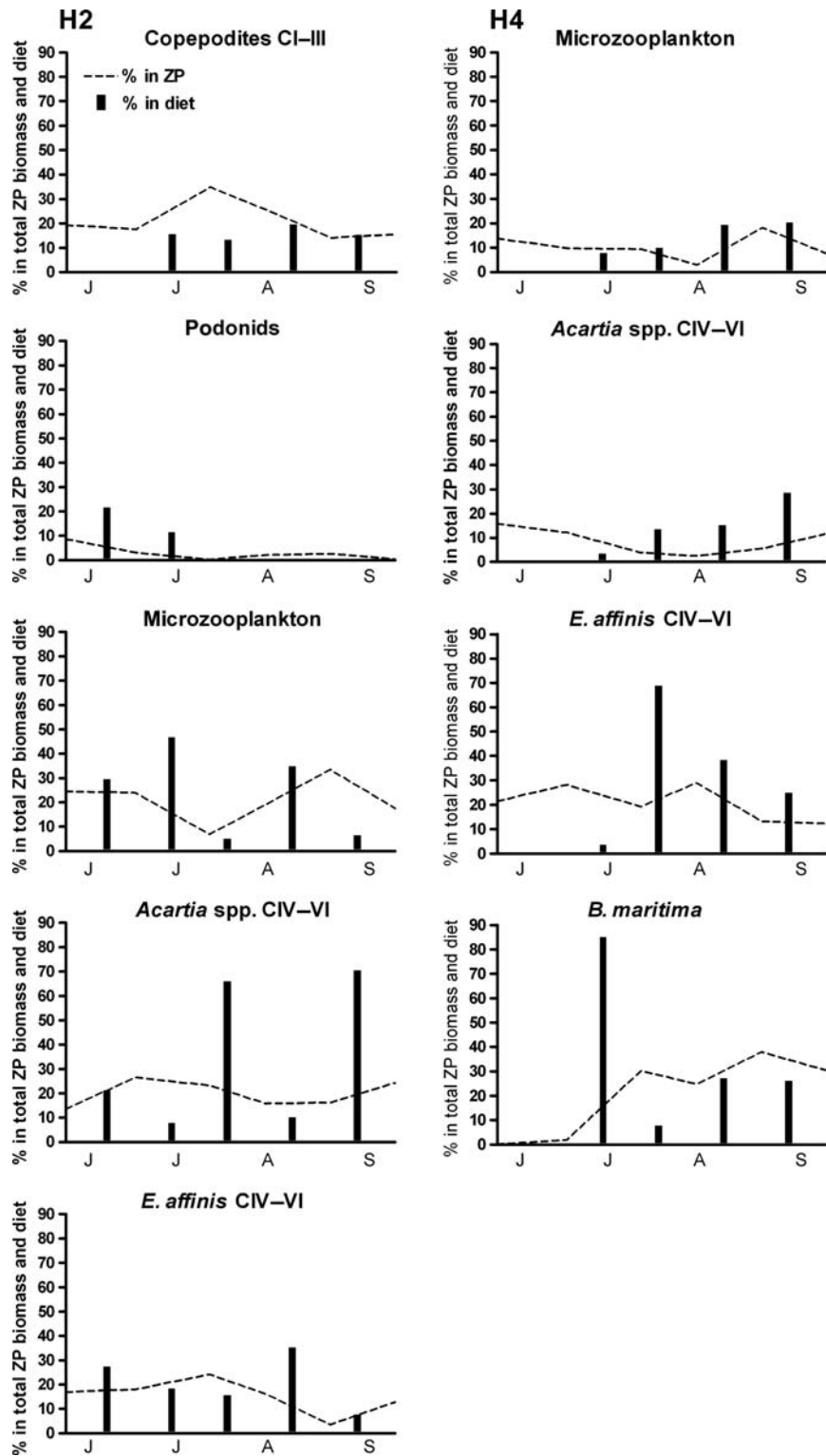


Fig. 5. Contribution (%) of different prey groups to total zooplankton community biomass and to the diet of *Cercopagis pengoi* (average %, estimated by the IsoSource mixing model using $\delta^{15}\text{N}$ values) at stations H2 and H4 (in columns). Capital letters on the x-axis represents the months June to September. Higher percentage of prey in the diet than in the ambient zooplankton community indicates a positive selection for this prey by *C. pengoi*. No diet contribution estimate was possible for *B. maritima* at H2 due to the lack of material for SIA, which was also the case for podonids at H4.

DISCUSSION

According to the IsoSource model outputs, relative contributions of different prey vary during the season and at different stations, consistent with the view that *C. pengoi* is a generalist predator (Pichlová-Ptáčnicková and Vanderploeg, 2009). Both methods employed (i.e. IsoSource mixing model and temporal tracking of prey) indicated older copepodites as both the dominant and the preferred prey of *C. pengoi*. This agrees with experimental studies showing that *C. pengoi* feed on copepods, a prey with relatively fast escape abilities (Pichlová-Ptáčnicková and Vanderploeg, 2009), including *E. affinis* (Simm *et al.*, 2006; Lehtiniemi and Gorokhova, 2008). Moreover, if individual body weight are taken into account to ensure a more direct comparison with the isotopic representation, the consumption rates observed by Simm *et al.* (Simm *et al.*, 2006) for copepodites of *E. affinis* would be >2-fold higher compared with those for *B. maritima* and copepod nauplii. Also, as copepods dominated the zooplankton community, our study is in line with earlier studies both from the Baltic Sea (Simm *et al.*, 2006) and The Laurentian Great Lakes (Laxson *et al.*, 2003) that suggested that zooplankton groups dominating the ambient community tend to contribute more to the diet of *Cercopagis*; this being indicative of opportunistic feeding. Evidence for the importance of microzooplankton, however, is inconclusive. While this prey had a low to high likelihood of contribution based on the IsoSource modelling (maximal values 16–90%) and in most cases was found to be positively selected for (Fig. 5), no evidence for the association between this source and *C. pengoi* nutrition was found in the temporal-tracking analysis. This is considered as a more robust analysis, as it does not involve any assumptions regarding trophic shift values (Melville and Connolly, 2003). As this fraction was taxonomically heterogeneous consisting of different species of rotifers, copepods and barnacle nauplii, it is likely that there was a differential predation on these taxa. This might have resulted in varying estimates of the dietary contribution, depending on the proportion of different taxa in the ambient plankton as was indeed indicated by the IsoSource mixing model results (Table I). Another explanation for the difference between the IsoSource output and temporal-tracking analysis could be related to the fact that the 90- μm mesh of the WP2 net has lower sampling efficiency for microzooplankton (Johansson, 1992). This may result in underestimation of the standing stocks and therefore possible overestimation of selectivity. Experimental studies have shown that *C. pengoi* can feed on these small zooplankters (Lehtiniemi and Lindén, 2006; Pichlová-Ptáčnicková and

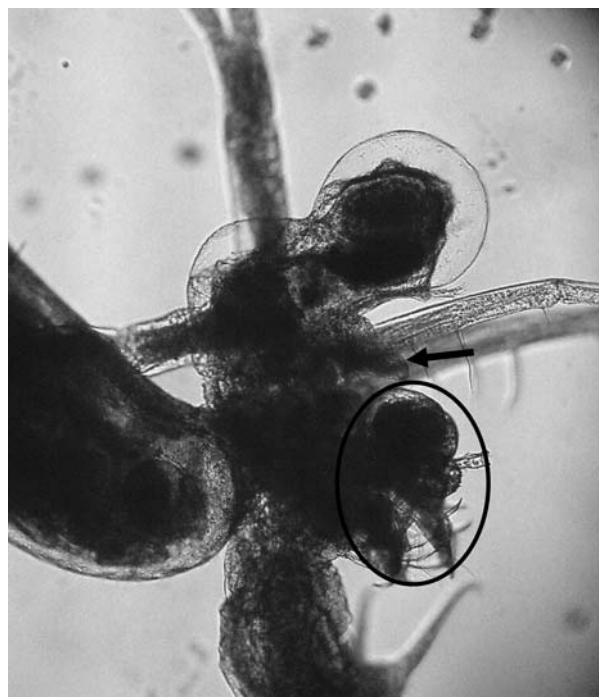


Fig. 6. Photograph of *Cercopagis pengoi* feeding on a small podonid (circled); the arrow indicates the predators' mouthparts.

Vanderploeg, 2009), but to what extent this occurs in nature is not clear. We also expected that young *C. pengoi* would have lower $\delta^{15}\text{N}$ values due to a higher contribution of microzooplankton in their diet compared with adults. However, the isotopic differences between the age groups were not significant, most probably due to the high variability in body size of Instar II (see Table I in Grigorovich *et al.*, 2000 and Fig. 6 in Uitto *et al.*, 1999) that comprised 58–90% of the *C. pengoi* population during the study period and, therefore, was most probably overrepresented in the stable isotope samples of adults. This high natural variability in instar body size precluded evaluating diet differences related to age. On the other hand, the lack of isotopic difference between ages may reflect overlapping diets in similarly sized *C. pengoi* regardless of their developmental stage, which is the most plausible explanation. Overall, the discrepancy between the IsoSource model output and temporal prey-tracking analysis indicates that different microzooplankton species should be treated separately and that size-, rather than, instar-specific differences in *C. pengoi* should be considered to find out what microzooplankton prey, and when, is actually consumed by *C. pengoi*. However, it is also likely that rotifers and nauplii would have similar isotopic signatures, as they feed on similar prey (mostly nanoplankton), and this may hamper their use as separate prey

groups in mixing models. IsoSource results indicated that cladocerans had low to medium (podonids, station H2 only) and low to high (*B. maritima*, station H4 only) contribution to the diet of *C. pengoi*. When these estimates were compared with the proportions of these prey groups in the ambient zooplankton communities, it became apparent that *C. pengoi* showed a positive selection towards podonids on every occasion SIA samples were available. However, podonids were only found in the water column until the beginning of July, comprising not >10% of the total zooplankton biomass (Fig. 2), resulting in the fact that there were only two sampling occasions when SIA samples for both *C. pengoi* and podonids could be collected. This precluded temporal-tracking analysis for this prey. However, tangible evidence for *C. pengoi* feeding on podonids was found while sorting samples for SIA (Fig. 6). IsoSource modelling results for the other cladoceran prey, *B. maritima*, suggest highest contribution to the diet and positive selection only at the beginning of the summer, when its share in the total zooplankton biomass was extremely low (<1%). This low abundance of *B. maritima* makes it highly unlikely that it could have been an important prey during this time. Consistent with this, the temporal-tracking analysis results do not indicate a significant association of this prey with *C. pengoi*. Field observations support this conclusion: a positive correlation between abundances of *B. maritima* and *C. pengoi* has been observed in the Gulf of Finland and was suggested to be a result of *C. pengoi* preying on other species that compete with *B. maritima*, such as *Pleopsis polyphemoides* and *Evadne nordmannii* (Pöllumäe and Kotta, 2007). Also, to meet the energy requirements of *C. pengoi*, feeding rates on bosminids were not sufficient, whereas those on larger prey were (Laxson *et al.*, 2003). However, considering the high diet contribution range of *B. maritima* during the period when this prey was abundant (Table 1) and the evidence from predation experiments suggesting this cladoceran is a readily consumed prey (Laxson *et al.*, 2003; Simm *et al.*, 2006; Pichlová-Ptáčnicková and Vanderploeg, 2009), it is possible that *C. pengoi* preys actively on bosminids when they dominate zooplankton (Laxson *et al.*, 2003). In the studied area, the prey that contribute most to the diet of *C. pengoi* are the dominant copepodites of *Acartia* spp. and *E. affinis*, while the contribution of other prey is less pronounced (podonids and microzooplankton) or not important (*B. maritima*).

When using the IsoSource mixing model, the diet composition and prey preference estimates differed between the two sites, with relative contributions of *Acartia* spp. and *E. affinis* differing between the stations (*Acartia* spp.: 35 ± 27 and $15 \pm 9\%$; *E. affinis*: 21 ± 9

and $34 \pm 4\%$; grand mean values \pm SD for the contributions to the diet at stations H2 and H4, respectively). These differences can be explained by (i) the differences in community composition and prey abundances (Fig. 2; note the higher abundance and proportion of *Acartia* spp. and *E. affinis* at stations H2 and H4, respectively) presenting the predator with different food choices, and (ii) the lack of SIA samples for certain prey, which might have resulted in errors in mixing model calculations. Indeed, the low abundance of young copepodites (CI–III) and podonids at H4 and of *B. maritima* and podonids at H2 made it impossible to get estimates for these prey groups and, consequently, the estimated proportions of other prey may be biased.

The observed differences in isotopic composition between the two sites could depend on several factors, none of which is mutually exclusive. Seston at H4 is isotopically heavier than at H2 due to the greater terrestrial influence and the closer proximity to the water treatment plant (Höglander, 2005). Savage (Savage, 2005) found the strongest influence of the ^{15}N -enriched effluent to be within 10 km of the outfall, which encompasses H4 but not H2, a concentration gradient that may also be strengthened by the limited water exchange in the bay (Savage, 2005). In addition, the concentration of ^{15}N at H2 may be reduced due to the greater contribution of isotopically light diazotrophic cyanobacteria in the outer part of the bay (pers. com. Dr S. Hajdu, Systems Ecology, Stockholm University). Understanding causes of variability in isotopic composition and processes of isotope fractionation is important for interpretation of stable isotope data when using SIA for studying food web structure and functioning. Experimental studies show that fractionation is governed by many variables, such as temperature and feeding activity (Barnes *et al.*, 2007), type of food (Crawley *et al.*, 2007), diet isotopic ratios (Caut *et al.*, 2008), C:N ratio of the primary food source and availability of N (Adams and Sterner, 2000), making fractionation factors vary both geographically and temporally. Strictly speaking, the percentages of the prey contributions in the diet should always be treated with caution as exact diet- and temperature-specific fractionation factors are rarely known, particularly for omnivores living in fluctuating environments and/or nutrient gradients, such as the Himmerfjärden Bay exposed to sewage discharge. This is why it is important to use an independent line of evidence, such as temporal-tracking analysis of differences between sampling occasions, which does not rely on diet fractionation (Melville and Connolly, 2003). Insights into diet composition obtained with other methods, such as feeding experiments, biochemical tracers (e.g. fatty acids), DNA-based and compound-specific isotopic

analysis, could further facilitate interpretation and increase the reliability of SIA for feeding and food web studies (Ben-David and Schell, 2001; Gorokhova and Lehtiniemi, 2007).

With larger copepodites (CIV–VI) making up the main part of the *C. pengroi* diet (Fig. 4), there is a significant diet overlap with zooplanktivorous fish in the Baltic Sea (Rudstam *et al.*, 1992; Mehner and Heerkloss, 1994; Arrhenius, 1996). This should not present a problem as long as the copepod populations remain high and zooplanktivores are therefore not food limited. However, prior to the invasion of *Cercopagis*, young-of-the-year herring in areas close to Himmerfjärden were suggested to be food limited (Arrhenius and Hansson, 1996), indicating conditions of a possible food competition. On the other hand, since *C. pengroi* has been found to contribute substantially to the diet of Baltic Sea zooplanktivorous fish (Ojaveer and Lumberg, 1995; Antsulevich and Välipakka, 2000; Gorokhova *et al.*, 2004, 2005; Lankov *et al.*, 2010), the risk of competition may be reduced, particularly if adult herring are abundant. Moreover, given the low stocks of *C. pengroi* observed in our study (<5% of the total zooplankton biomass), it is unlikely to exert a heavy predation pressure on the rest of the zooplankton community. Nevertheless, *C. pengroi* does add an additional predation pressure on copepods and therefore might promote increased competition among zooplanktivores, particularly when abundant (Uitto *et al.*, 1999). In Lake Ontario, an increased predation on juvenile copepods has been implicated in causing a decline in the copepod populations (Benoit *et al.*, 2002). This may also occur in the Baltic Sea as suggested by decreased *E. affinis* stocks following *C. pengroi* invasion in the Gulf of Finland (Lehtiniemi and Gorokhova, 2008). With a reduction in copepod stocks, food availability for fish would decrease leading to a possible reduction in fish stocks. On the other hand, post-larval fish are not very efficient in preying upon microzooplankton that *C. pengroi* is able to feed upon (Lehtiniemi and Lindén, 2006; Pichlová-Ptáčnicková and Vanderploeg, 2009); to some extent this feeding was also supported by the IsoSource model outputs (Fig. 4). Therefore, as *C. pengroi* is preyed upon by zooplanktivorous fish (Gorokhova *et al.*, 2004; Lankov *et al.*, 2010), the energy from microzooplankton, channelled through *C. pengroi*, may give fish a better access to previously underutilized energy. However, limited dependence of *C. pengroi* on microzooplankton (Table I) together with differences in food quality between the copepods and microzooplankton makes it unlikely that the consumption of the microzooplankton compensates for the possible competition with fish for copepods. Furthermore, *C. pengroi* may not

represent a beneficial food source, as the indigestible tails occupy space in fish stomachs preventing more prey from being ingested (Lankov *et al.*, 2010).

To conclude, using SIA for *in situ* diet assessment, we found that *C. pengroi* behaves as an opportunistic generalist predator, with the main prey being copepods that dominated the zooplankton community in the study area. These characteristics of the predator agree with previously reported laboratory observations for *Cercopagis*-fed zooplankton typical for freshwaters (Pichlová-Ptáčnicková and Vanderploeg, 2009). Both IsoSource modelling and temporal-tracking analysis indicate that older copepodites of *Acartia* spp. and *E. affinis* are the dominant prey of *C. pengroi*. Additionally, IsoSource results implicate this prey as consistently preferred by *Cercopagis*. In contrast, evidence for rotifers (*Keratella* spp. and *Synchaeta* spp.) and nauplii (*Acartia* substantially to *Cercopagis* nutrition was inconclusive. To understand patterns of *C. pengroi* impacts on the food webs in the invaded ecosystems, cross-system comparative studies applying an array of methods are needed. In particular, studies investigating mechanisms by which opportunistic feeding is manifest in *Cercopagis* populations, the vulnerability of specific zooplankton communities and the effects this predator has on the trophic dynamics within and across ecosystems will be particularly important in light of increasing invasions.

ACKNOWLEDGEMENTS

We thank S. Svensson, B. Abrahamsson and L. Lundgren (Systems Ecology, Stockholm University, Sweden) for help in collecting zooplankton samples.

FUNDING

This research was supported by the Swedish National Monitoring program and grants from The Swedish Research Council for Environment, Agricultural Sciences, and Spatial Planning (Formas), the Swedish Environmental Protection Agency (Naturvårdsverket) and the foundation Baltic Sea 2020.

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