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Stable isotopes show food web changes after invasion by the predatory cladoceran *Cercopagis pengoi* in a Baltic Sea bay

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Abstract *Cercopagis pengoi*, a recent invader to the Baltic Sea and the Laurentian Great Lakes, is a potential competitor with fish for zooplankton prey. We used stable C and N isotope ratios to elucidate trophic relationships between *C. pengoi*, zooplankton (microzooplankton, 90–200 μm, mostly copepod nauplii and rotifers; mesozooplankton, >200 μm, mostly copepods), and zooplanktivorous fish (herring, size range 5–15 cm and sprat, 9–11 cm) in a coastal area of the northern Baltic Sea. The isotope ratios in *C. pengoi* and fish were much higher than those of zooplankton, showing general trends of enrichment with trophic level. Young-of-the-year (YOY) herring had a significantly higher $^{15}N/^{14}N$ ratio than *C. pengoi*, suggesting of a trophic linkage between the two species. To evaluate the possible relative importance of different food sources for *C. pengoi* and YOY herring, two-source isotope-mixing models for N were used, with micro- and mesozooplankton as prey for *C. pengoi* and mesozooplankton and *C. pengoi* as prey for YOY herring. These models indicate that mesozooplankton was the major food source of both species. However, microzooplankton may be important prey for young stages of *C. pengoi*. Comparative analyses of the herring trophic position before and after the invasion by *C. pengoi* showed a trophic level shift from 2.6 to 3.4, indicating substantial alterations in the food web structure. Our findings contribute to a growing body of evidence, showing that *C. pengoi* can modify food webs and trophic interactions in invaded ecosystems.

Keywords Diet · Effects of preservation and storage · Invasive species · Zooplankton · Zooplanktivorous fish

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Introduction

Cercopagis pengoi (Ostroumov 1884) is a pelagic cladoceran native to the Ponto-Caspian region. It appeared in the Baltic Sea in the 1990s and established permanent populations, first in the Gulfs of Finland and of Riga (Ojaveer and Lumberg 1995; Ojaveer et al. 1998) and later in the Baltic proper, including coastal waters of the Stockholm archipelago (Gorokhova et al. 2000), and in the Gulf of Gdansk (Bielecka et al. 2000). Shortly after its appearance in the Baltic Sea, *C. pengoi* invaded the North American Great Lakes (MacIsaac et al. 1999), where it is spreading rapidly (Therriault et al. 2002).

When *Bythotrephes longimanus* (Cercopagididae), a species of similar morphology and feeding habits, invaded North American lakes in the early 1980s (Lehman 1987), it severely affected native zooplankton communities by selective predation (Yan et al. 2002). It has been feared that the zooplanktivorous *C. pengoi* will have similar effects, competing for herbivorous zooplankton with young stages of planktivorous fish, while *C. pengoi* will themselves be protected from predation by their long caudal spine (Vanderploeg et al. 2002). Several lines of evidence indicate that *C. pengoi* may affect resident zooplankton communities by selective predation (Lake Ontario, Benoit et al. 2002; Gulf of Riga, Ojaveer et al. 1999, 2004; Gulf of Finland, Uitto et al. 1999). On the other hand, zooplanktivorous fish both in the Baltic (Ojaveer and Lumberg 1995; Antsulevich and Välipakka 2000; Gorokhova et al. 2004; Ojaveer et al. 2004) and in the Great Lakes (Bushnoe et al. 2003) have been reported to prey on *C. pengoi*. It is, however, difficult to study food competition between small fish and *C. pengoi* since there are no good methods for diet analysis applicable to cercopagids. Conventional methods, such as gut content analysis and feeding experiments, are difficult to apply as they: (1) shred their prey and ingest only soft tissues (Rivier 1998), giving an amorphous and mainly unidentifiable material in the gut content; and (2) are extremely sensitive to handling and experimental

manipulations and survive poorly in captivity (personal observations). Consequently, that *C. pengoi* is a carnivorous feeder is largely inferred from the morphology of its feeding appendages (i.e. raptorial, with no filtering devices). The experimental evidence of carnivorous feeding and its prey preference is, however, very limited for this species (however, see Laxson et al. 2003).

The stable isotopes of N (δ^{15}N) and C (δ^{13}C) provide useful tools in analyses of energy flow pathways and food web structures including pelagic systems (e.g. reviews by Fry and Sherr 1984; Owens 1987; Wada et al. 1991; Vander Zanden and Rasmussen 2001; Post 2002). The N isotope signatures indicate the trophic level of species (Owens 1987; Cabana and Rasmussen 1994; Vander Zanden and Rasmussen 2001), because δ^{15}N of an organism is enriched by ~3.4‰ (±1‰, SD) relative to its diet (Post 2002). The stable C isotope ratio (mean trophic fractionation 0.4 ± 1.3‰; Post 2002) can be used to trace the sources of organic C in food webs, provided that different sources have distinct isotopic signatures (Fry and Sherr 1984; Wada et al. 1991). These stable isotopes have also been used to detect changes in food web structure after biological invasions (Vander Zanden et al. 1999, 2004; Greenwood et al. 2001).

Stable isotope analysis (SIA) has been used to evaluate the food web structure of coastal pelagic ecosystems in the Baltic Sea (Hansson et al. 1997). The trophic structure determined from δ^{15}N in this study agreed well with that from gut content analyses and confirmed that herring (*Clupea harengus* L.) and sprat (*Sprattus sprattus* L.) are dominant zooplanktivores in this area (Rudstam et al. 1992). Before the *C. pengoi* invasion, the fish diets consisted mainly of calanoid copepods (*Acartia* spp., *Eurytemora affinis*, and *Temora longicornis*) and cladocerans (*Bosmina coregoni maritima* and *Pleopsis polyphemoides*), with some differences between the coastal and open sea areas and between northern and southern parts of the Baltic proper (Rudstam et al. 1992; Mehner and Heerkloss 1994; Arrhenius 1996). After its appearance in the Baltic, *C. pengoi* has become a significant part of the diet of both adult and young-of-the-year (YOY) herring (Ojaveer and Lumberg 1995; Ojaveer et al. 1998, 2004; Antsulevich and Välipakka 2000; Gorokhova et al. 2004), smelt (Ojaveer et al. 2004), and sprat (Gorokhova et al. 2004) in coastal waters. This cladoceran is generally present in the water column between 7 weeks and 24 weeks in the season, usually peaking in August–September (Gorokhova et al. 2000, 2004; Ojaveer et al. 2004) and thus coinciding with the peak in food consumption by clupeoids (Arrhenius and Hansson 1993). However, quantitative assessment of *C. pengoi* in fish diets based on stomach content analysis is difficult as the gut retention time may vary between species and body parts (Antsulevich and Välipakka 2000).

The invasion by *C. pengoi* necessitates a re-evaluation of the Baltic Sea food webs. The main purpose of this study was to determine relative trophic positions of *C. pengoi* and its importance to fish diets in a coastal area of the northern Baltic proper using stable isotopes. Moreover, we attempted to quantify differences in the trophic position of zooplanktivorous fish (YOY herring) before and after the *C. pengoi* invasion, which required a study of the effects of formalin preservation and storage time (6–24 months) on C and N isotope ratios in *C. pengoi*.

Materials and methods

The study was conducted during the summers of 2000 and 2002 in a 30-km-long inlet with a mean salinity of 6‰ and mean depth ~17 m (Himmerfjärden Bay, 58°59′N, 17°44′E), located in the northern Baltic proper and receiving discharges from a modern municipal sewage treatment plant. In 1997, *C. pengoi* was first found in the area (Gorokhova et al. 2000), where abundances are usually higher than in neighbouring coastal and offshore waters (personal observations). The bay is a spawning and nursery area for many fish species, including herring (Axenrot and Hansson 2004).

Collection and sample preparation

Details on sampling methods and sample preparation are summarized in Table 1. Sampling sites were located at 59°02′19″N, 17°43′40″E (station 1); 58°59′07″N, 17°43′60″E (station 2), and 58°56′04″N, 17°43′81″E (station 3). Fish were collected at stations 1, 2 and 3 (however no YOY fish were collected at station 3), while zooplankton and *C. pengoi* were collected at station 2 (see also Fig. 1 in Gorokhova et al. 2004).

Zooplankton

Zooplankton were sampled and prepared for SIA as specified in Table 1. For each sampling occasion, two parallel samples were taken, one of which was preserved in a 4% borax buffered formaldehyde for species identification and population analysis, following the standard protocol of the Baltic Monitoring Programme (HELCOM 1988); biomass was calculated according to Hernroth (1985). Animals for SIA were separated from filamentous cyanobacteria in a light trap—a black funnel illuminated from below, in which zooplankton were attracted to the illuminated funnel tip while the cyanobacteria floated up. Animals retained on a 200-μm sieve were defined as mesozooplankton, while those passing through this mesh size but caught on a 90-μm sieve were considered microzooplankton. The samples were cleaned from debris and filamentous cyanobacteria under a dissecting microscope, and rinsed with an isotonic NaCl (6‰) solution. Bulk samples of animals were then transferred to pre-weighed tin capsules, dried at 60°C, and stored at −20°C until analysed. When discussing zooplankton in this paper, we refer to all planktonic invertebrates except for *C. pengoi*.

Table 1 Material analysed, sampling methods, sample preparation for stable isotope analysis (*SIA*), and usage of samples for different tests and comparisons. Ø Diameter

Species/group	Month year; number of sampling occasions	Sampling gear and depth	Preservation method	Number of replicates for SIA; sample size (mg)	Test
Microzooplankton (90–200 µm)	June–September 2000 (every second week); 6	90-µm plankton net (Ø 50 cm); upper 10 m	Formaldehyde for species composition; frozen, −20°C for SIA	2; 0.2–0.6	Relative trophic position
Mesozooplankton (> 200 µm)				4; 0.3–1.2	
Cercopagis (mixed stages)	June–August 2000; 2	100-µm plankton net (Ø 30 cm); upper 5–10 m	Frozen, −20°C	9; 0.8–1.4	Relative trophic position; effects of preservation and storage period (reference point)
Cercopagis (separate stages)	August 2002; 2	60-µm plankton net (Ø 23 cm); upper 5–10 m	Fresh (sorted within 2–4 h of capture)	3; 0.5–1.1	Relative trophic position;effects of preservation and storage (reference point);ontogenetic differences
Cercopagis (mixed stages)	June–September 2000 and 2002 (every 2nd week); 6	90-µm WP-2 net (Ø 57 cm); bottom to surface	Formaldehyde, storage 6–24 months at 4–6°C	9; 0.5–1.2 9; 0.7–1.4	Effects of preservation and storage period
Herring (5–10 and 10–15 cm) and sprat (9–11 cm)	July–September 2002; 5	Gill nets, mesh sizes 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, and 29 mm	Frozen, −18°C	8–20 for each species/size class;1.1–2.7	Relative trophic position

Fig. 1 Effects of formaldehyde preservation and storage time on N (**a**) and C (**b**) isotope composition in *Cercopagis pengoi*. Initial concentrations were obtained using fresh (2002) and frozen (2000) samples. Values shown as means along with SDs. *The same letters* indicate absence of significant differences between the values within a graph

Cercopagis

Cercopagis were sampled in several ways (Table 1) and used either fresh, frozen in bulk, or preserved in 4% borax buffered formaldehyde. To evaluate different preparation methods and the effects of storage in formalin, we compared the isotope composition of samples treated in different ways. When preparing them for SIA, *C. pengoi* individuals were removed from either fresh or preserved samples (frozen samples was first thawed on ice) and sorted under a dissecting microscope. To study ontogenetic variations in isotopic composition, 15–40 individuals of the same developmental stage [barb stage (BS) I, BS II or BS III, according to Rivier 1998] for each replicate sample were transferred to pre-weighed tin capsules. Other samples were prepared without stage determination. All samples were processed in the same way as the zooplankton samples.

Fish

Fish specimens for SIA came from the collection used for stomach content analysis in an earlier study (Gorokhova

et al. 2004), thus allowing direct comparison of these two diet analysis techniques. Herring and sprat were collected on five occasions in July–September 2002 (Table 1). Most stable isotope analyses were made on fish from August and September, when *C. pengoi* was most abundant. The fish were kept frozen (−18°C) until analysis; since this sometimes damaged the caudal fin we used the distance (centimetres) from the tip of the nose to the beginning of the caudal fin as length. Two size groups of herring (5–10 cm and 10–15 cm) were analysed for stable isotope ratios. Herring < 10 cm (range: 5.0–9.2 cm) were mainly YOY fish produced from spawning in the study area (Arrhenius and Hansson 1996). Herring > 10 cm (10.4–14.7 cm) and sprat (8.9–10.6 cm) were mainly > 2-year-old fish, and they had probably not stayed in the area all their life (Hansson et al. 1997). White muscle tissues from above the lateral line were dissected for SIA, since white muscle tends to be less variable in terms of $\delta^{13}C$ and $\delta^{15}N$ than other tissues (Pinnegar and Polunin 1999). For the smallest herring the entire filet was used, while for larger fish a 2- to 3-cm-long piece was taken just behind the head. Samples were dried at 60°C for 24 h and ground in a mortar. The resulting powder was transferred to pre-weighed tin capsules, which were further dried to constant weight and stored in a desiccator until SIA.

Stable isotope ratio and data analyses

Concentrations of ^{13}C, ^{12}C, ^{15}N, and ^{14}N in the samples were determined using continuous-flow isotope mass spectrometry provided by automated NC analysis SL 20-20, PDZ Europa at the Stable Isotope Facility, University of California at Davis, California. The standard reference material for C was Vienna Pee Dee belemnite with atmospheric N_2 used for N. Isotope ratios were expressed as parts-per-thousands (‰) differences from the standard reference material according to Peterson and Fry (1987). Repeated analyses of homogeneous material yielded SD of < 0.05% for both isotopes.

To compare the feeding ecology of *C. pengoi* and fish within the same system, we determined the relative importance of food sources of *C. pengoi* and YOY herring with a N isotope two-source mixing model using micro- and mesozooplankton as potential prey for *C. pengoi* and mesozooplankton and *C. pengoi* as prey for YOY herring:

$$\delta^{15}N_M = f_X\left(\delta^{15}N_X + \Delta^{15}N\right) + f_Y\left(\delta^{15}N_Y + \Delta^{15}N\right);$$
$$1 = f_X + f_Y, \quad (1)$$

where X, Y, and M represent two food sources and the mixture (i.e. the consumer), f represents the proportions of N from each food source in the consumer's diet, $\Delta\,^{15}N$ is the assumed trophic fractionation, i.e. change in $\delta^{15}N$ over one trophic step, from a prey to its predator (Phillips and Gregg 2001; Post 2002). The trophic fractionation was assumed to be constant and either 3.4‰ (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002) or 2.4‰ (as determined in an earlier study in this area, Hansson et al. 1997). The choice of the end-members was based on gut content analyses of the fish from the same collections (Gorokhova et al. 2004). Due to the absence of empirical data, our mixing model assumes that the categories micro- and mesozooplankton are isotopically homogeneous and that *C. pengoi* feeds unselectively; thus the results of this analysis should be considered as preliminary until more data on species-specific isotope composition of zooplankton in the Baltic and *C. pengoi* feeding preferences become available.

The consumer's (cons) trophic position (TP_{cons}) was estimated according to Vander Zanden et al. (1999):

$$TP_{cons} = \frac{\left(\delta^{15}N_{cons} - \delta^{15}N_{zoopl}\right)}{\Delta^{15}N} + 2 \quad (2)$$

where $\delta^{15}N_{cons}$ is the consumer's isotopic signature and $\delta^{15}N_{zoopl}$ is the signature in zooplankton. $\Delta\,^{15}N$ is the trophic fractionation, which in this case was assumed to be 3.4‰; 2 is added as zooplankton were assumed to be entirely herbivorous and to constitute trophic level number 2 (i.e. primary consumers). Trophic position estimates and isotope-mixing model results for YOY herring 2002 rely on the 2000 zooplankton and *C. pengoi* isotopic composition averaged over 2000–2002, while all the data for *C. pengoi* models are from year 2000 (Table 1).

Statistics

Statistical tests were performed with GraphPad Prism 4.01 (GraphPad Software). Deviations from Gaussian distribution were tested using the Kolmogorov-Smirnov test as Dallal and Wilkinson approximation to Lilliefors' method. When comparing two groups (i.e. micro- and mesozooplankton, large and YOY herring, between-year comparisons for *C. pengoi*, etc.), the unpaired *t*-test was used followed by *F*-test to compare variances. If sample variances were significantly different, a Welch's *t*-test adjusted for unequal variances was used. When comparing three groups, Kruskal-Wallis (KW) ANOVA with Dunn's multiple comparison test were used. The effects of ontogeny and preservation were investigated by performing a one-way ANOVA on the stable isotope data obtained for *C. pengoi*; Student Neuman Keuls (SNK) multiple comparison procedures were carried out to further investigate significant differences. The calculation for the source proportions (Eq. 1), their variances, and SEs for the two-source mixing model were performed according to Phillips and Gregg (2001). Unless specified otherwise, data are presented as means with SDs; in all cases significance was accepted when $P < 0.05$.

Results

Zooplankton community structure

Microzooplankton were dominated by rotifers (*Keratella cochlearis* and *Synchaeta* spp., 3–33% of microzooplankton biomass, mean value 22%) and copepods (mostly nauplii

and early copepodites of *Acartia bifilosa* and *E. affinis*, 19–77%, mean value 42%). Mesozooplankton comprised older copepodites (C III to adults, 11–94% of mesozooplankton biomass) of *A. bifilosa* (3–80%) and *E. affinis* (2–49%). Cladocerans (*B. coregoni maritima*, *Pleopis polyphemoides*, and *Podon intermedius*) contributed at most 18% to the mesozooplankton biomass, but usually $<10\%$. Cladocerans and copepods were abundant in July, decreasing during August–September, while the rotifers showed a very drastic decline after mid August (see Fig. 2 in Gorokhova et al. 2004). Densities of *C. pengoi* were always low (≤ 64 individuals m^{-3}), comprising only $<0.1\%$ of the total zooplankton abundance and 0.3–1.2% of the biomass.

Effects of preservation and storage on stable isotope ratios

C. pengoi showed no significant differences in either $\delta^{15}N$ or $\delta^{13}C$ between 2000 and 2002, based on frozen and fresh samples respectively ($\delta^{13}C$, $t=0.8470$, $df=16$, $P>0.4$; $\delta^{15}N$, $t=1.339$, $df=16$, $P>0.2$; Fig. 1). Storage in formaldehyde tended to increase $\delta^{15}N$ values slightly, although not significantly (year 2000, $F_{2,24}=0.4974$, $r^2=0.04$, $P>0.6$; year 2002, $F_{2,24}=2.560$, $r^2=0.18$, $P>0.1$; Fig. 1a). Thus ^{15}N measurements obtained using fresh, frozen and formaldehyde preserved samples are directly comparable.

By contrast, as a result of formaldehyde preservation, $\delta^{13}C$ in *Cercopagis* decreased significantly (year 2000, $F_{2,24}=36.11$, $r^2=0.75$, $P<0.0001$; year 2002, $F_{2,24}=57.20$, $r^2=0.83$, $P<0.0001$; Fig. 1b). There were, however, no significant differences among samples preserved in formaldehyde and stored for different periods ($F_{2,24}=2.378$, $r^2=0.18$, $P>0.1$; Fig. 1b). The mean difference in $\delta^{13}C$ values between the fresh/frozen and preserved samples was 0.6‰. This decrease is close to that found by Kaehler and Pakhomov (2001) who tested temporal changes in formaldehyde-preserved fish, octopus, and kelp over 3 months. Since $\delta^{13}C$ step-wise trophic enrichment is frequently $<1\%$, formaldehyde-preserved samples appear to be of limited use. There was, however, no trend of changes in $\delta^{13}C$ values with duration of preservation, and variations in the magnitude of the effect were low (2.3–3.3% depletion after formaldehyde preservation, Fig. 1b) and predictable. Therefore, it might be possible to use a correction factor when analysing archival samples.

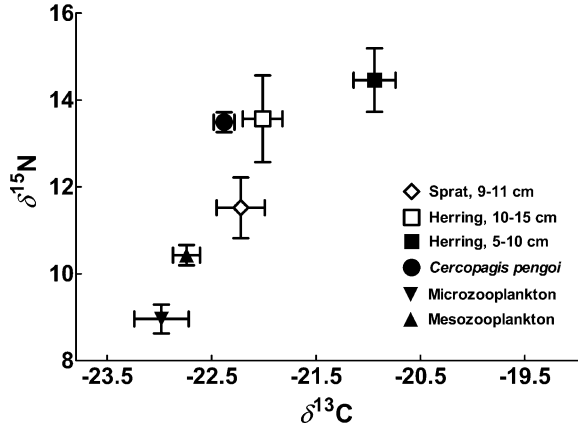

Fig. 2 C and N isotope composition of zooplankton (micro- and mesozooplankton), *C. pengoi* (fresh and frozen samples combined), and fish (sprat and herring of different size-classes) in Himmerfjärden Bay, northern Baltic Sea proper. Values shown as means with SEs

Ontogenetic variations in stable isotope ratios of C. pengoi

There were no significant differences in the isotopic ratios of the different instars ($\delta^{13}C$, $F_{2,6}=2.003$, $r^2=0.4$, $P>0.2$; $\delta^{15}N$, $F_{2,6}=1.395$, $r^2=0.3$, $P>0.3$), although the youngest individuals (BS I) had slightly lower $\delta^{15}N$ values than the older stages (mean difference of 0.4‰; Table 2). When $\delta^{15}N$ values for BS II and BS III were pooled together and compared to those for BS I, the difference became almost significant ($t=2.323$, $df=7$, $P<0.06$). Nevertheless, the differences were slight and we pooled data from different stages as well as from assorted samples in our mixing model to evaluate the food sources for herring. However, when estimating the relative contribution of micro- and mesozooplankton to the diet of *C. pengoi*, the calculations were performed both for mixed populations and for separate instars (BS I vs BS II + III; Table 3; Eq. 2).

Stable isotope ratios of predators and their prey

Zooplankton showed no significant difference in $\delta^{13}C$ between size fractions ($t=0.9155$, $df=30$, $P>0.3$). By contrast, a significant difference in $\delta^{15}N$ was found

Table 2 Isotopic composition of different groups of zooplankton, barb stages(BS) I–III (BS I–BS III) and mixed stages of *C. pengoi*, and fish

Species and groups	Number of samples analysed	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$
Microzooplankton (90–200 μm)	10	−23.0 ± 0.7	9.0 ± 1.1
Mesozooplankton (> 200 μm)	24	−22.7 ± 0.6	10.4 ± 1.1
C. pengoi, BS I, fresh	3	−22.2 ± 0.2	13.1 ± 0.3
C. pengoi, BS II, fresh	3	−22.3 ± 0.1	13.4 ± 0.4
C. pengoi, BS III, fresh	3	−22.5 ± 0.2	13.5 ± 0.3
C. pengoi, mixed stages, frozen	9	−22.5 ± 0.2	13.5 ± 0.4
C. pengoi, mixed stages, formalin[a]	36	−22.9 ± 0.2[b]	13.6 ± 0.2
Sprat	8	−22.2 ± 0.7	11.5 ± 0.7
Herring, 5–10 cm	23	−20.9 ± 0.9	14.5 ± 0.7
Herring, 10–15 cm	15	−22.0 ± 0.7	13.6 ± 1.0

[a]Average for all storage periods and years
[b]Not corrected for preservation and storage effects

Table 3 Relative contributions of N derived from the diet sources to the predator species and size/age groups as calculated with ^{15}N two-source mixing model (mean ± SE) and based on two alternative fractionation factors (2.4‰ and 3.4‰, see Materials and methods). *NA* Not applicable (denotes cases where isotopic signatures for the mixture fall outside the region constrained by the source isotopic signatures resulting in negative source proportions)

Prey groups	Predators			
	C. pengoi, BS I	*C. pengoi*, BS II + III	*C. pengoi*, mixed stages	Herring, 5–10 cm
2.4‰				
Microzooplankton	NA	NA	NA	
Mesozooplankton	NA	NA	NA	0.48 ± 0.06
C. pengoi [a]				0.52 ± 0.06
3.4‰				
Microzooplankton	0.51 ± 0.14	0.14 ± 0.18	0.21 ± 0.17	
Mesozooplankton	0.49 ± 0.14	0.86 ± 0.17	0.79 ± 0.17	0.80 ± 0.08
C. pengoi [a]				0.20 ± 0.08

[a]Mixed stages, pooled fresh and frozen samples

between the micro- and mesozooplankton samples ($t = 3.505$, $df = 31$, $P < 0.002$) with microzooplankton being lower in ^{15}N (mean difference of 1.4‰; Table 2, Fig. 2).

The isotope ratios of fish (herring and sprat, both known zooplanktivores) were much higher than those of zooplankton. Moreover, herring showed significant ontogenetic differences in isotopic ratios and, in the case of $\delta^{15}N$, significantly different variances ($\delta^{13}C$, $t = 3.579$, $df = 75$, $P < 0.0006$; $\delta^{15}N$, $t = 3.270$, $df = 40$, $P < 0.002$, $F_{30,45} = 3.777$, $P < 0.0001$), with lower $\delta^{13}C$ and $\delta^{15}N$ values in larger herring (mean differences of 1.1 and 0.9‰, respectively; Table 2, Fig. 2). The lower $\delta^{13}C$ value of sprat and larger herring may, at least to some extent, be explained by a higher lipid content (cf. Pinnegar and Polunin 1999; Vander Zanden and Rasmussen 2001). Indeed, significantly higher C:N ratios were observed in the larger herring and sprat compared to those of YOY herring (KW statistic = 47.78, $P < 0.0001$), indicating greater lipid reserves (Lesage et al. 2001). For each fish species/size group, no significant differences between either the sampling stations or sampling dates were observed (KW statistics, $P > 0.05$ in all cases).

C. pengoi were significantly more enriched in both ^{13}C and ^{15}N than zooplankton (Table 2, Fig. 2), having $\delta^{13}C$ values 0.6‰ above those of microzooplankton ($t = 2.246$, $df = 9$, $P < 0.05$; $F_{9,41} = 49.80$, $P < 0.0001$) and 0.4‰ above those of mesozooplankton ($t = 2.246$, $df = 33$, $P < 0.05$; $F_{23,17} = 4.944$, $P < 0.0001$); corresponding values for $\delta^{15}N$ were 4.5‰ ($t = 12.51$, $df = 9$, $P < 0.0001$; $F_{9,41} = 4.428$, $P < 0.0004$) and 3.0‰ ($t = 12.07$, $df = 28$, $P < 0.0001$; $F_{9,41} = 49.80$, $P < 0.0001$), respectively. Whilst *C. pengoi*, sprat and larger herring could not be statistically differentiated on the basis of their $\delta^{13}C$ values (KW statistic = 4.302, $P > 0.1$), this was not the case for YOY herring. These fish had significantly higher $\delta^{13}C$ than *C. pengoi* ($t = 6.783$, $df = 53$, $P < 0.0001$; $F_{45,17} = 23.06$, $P < 0.0001$), the mean difference being 1.4‰. With respect to ^{15}N, *C. pengoi* differed significantly from sprat and herring (5–10 cm) (KW statistic = 65.99, $P < 0.0001$; Dunn's test, sprat < *C. pengoi* < herring, $P < 0.001$ in all cases; Fig. 2). Thus YOY herring, which we consider sedentary in the study area during their first summer, appeared to be at higher trophic level than *C. pengoi* (mean $\delta^{15}N$ difference 1.0‰).

Discussion

N isotope measurements can be used to infer diets and define realized trophic levels provided that the different organisms receive their N from the same ultimate source. While similar $\delta^{13}C$ signatures in zooplankton and *C. pengoi* suggest a single primary C source for these groups, the $\delta^{13}C$ signature in YOY herring is higher than the average increase in ^{13}C ratios per trophic level of 0.4‰ calculated by Post (2002). This might indicate that either an important food source has not been included or that this fish fractionates C at higher than average proportions. Most diet studies argue against the former explanation, emphasizing zooplankton as a single food source of YOY herring (Rudstam et al. 1992; Mehner and Heerkloss 1994; Arrhenius 1996), and no food items other than zooplankton were identified in the stomachs of the fish from the same collection (Gorokhova et al. 2004). On the other hand, C fractionation is known to be somewhat variable and species-specific and our values were within the range observed in other animals (Post 2002) including herring collected in the Baltic proper [i.e. 1.8‰ relative to mesozooplankton in this study versus 2.0‰ determined by Rolff and Elmgren (2000) relative to 200–500 μm zooplankton]. Therefore, we assume that there is a common ultimate source of N for zooplankton, *C. pengoi* and YOY herring, and this discussion will focus primarily on the composition of N isotopes, as these show stronger fractionation in the food web. Furthermore, we will merge data from 2000 and 2002, as the isotope signatures of *C. pengoi* were very similar in these years (Fig. 1, storage time 0 months). Also, as no geographical component was detected in the $\delta^{15}N$ signatures within the species/group during the study period, we assume that these variations are negligible. Indeed, the areal influence of sewage does not allow us to distinguish between the two northernmost stations

where the YOY fish were collected (i.e. stations 1 and 2 and corresponding stations H5 and H4 at http://130.237.170.4/dbhfj/Hfjsmall.htm); neither zooplankton isotopic signatures vary significantly between the samples collected near the outfall of the sewage (station 1) and station 2 (H5 and H4 in H. Höglander and U. Larsson, in preparation). Moreover, as no consistent pattern in fish diet during the study period was observed (Gorokhova et al. 2004), the contribution of different prey items to the fish diet was assumed to be relatively constant.

Mesozooplankton had significantly higher δ^{15}N signatures than microzooplankton (difference 1.4‰), which is probably explained by the different feeding preferences of these groups. In particular, unlike nauplii and rotifers, larger copepods are usually not strict herbivores, and *Acartia* species are known to prefer ciliates to phytoplankton (Stoecker and Sanders 1985; White and Roman 1992). The isotope signatures of *C. pengoi* were much higher (3–4‰) than those of zooplankton (Table 2; Fig. 2), supporting the accepted view that this cladoceran is zooplanktivorous. Further, the YOY herring (5–10 cm), which are known to be sedentary in the study area (Hansson et al. 1997), had higher δ^{15}N signatures than *C. pengoi* (difference ~1‰), which is consistent with a diet that includes both zooplankton and *C. pengoi* (Gorokhova et al. 2004). Larger herring (known to feed on both zooplankton and larger invertebrates; Möllmann and Köster 1999) and sprat (a true zooplanktivore, Möllmann and Köster 1999) showed δ^{15}N values that were similar or lower than those of *C. pengoi*. Similar results reported from this study area by Hansson et al. (1997) were explained as a result of the migration of these fish, between coastal and offshore habitats with different isotopic environments. Because of this, and taking into account that isotopic signatures of fish older than 1 year were likely to reflect feeding during the *Cercopagis*-free period, these fish were excluded from the stable isotope-based food web analysis discussed below.

To estimate diet compositions (Eq. 1), *C. pengoi* was assumed to prey on micro- and mesozooplankton while YOY herring were assumed to feed on mesozooplankton and *C. pengoi*. Diet models for *C. pengoi*, based on the relative enrichment of 2.4‰, resulted in negative source proportions and it was impossible, therefore, with this assumption to derive a relevant *C. pengoi* diet (Table 3). With a fractionation of 3.4‰, the mixing model predicted micro- and mesozooplankton proportions of about 50:50 and 15:85 for the younger (BS I) and older (BS II–BS III) instars of *C. pengoi*, respectively (Table 3). An alternative to a diet shift as an explanation for the isotope difference between BS I and BS II–BS III of *C. pengoi* is a difference in growth patterns and tissue turnover rates during ontogeny (Tieszen et al. 1983; Gorokhova and Hansson 1999). For YOY herring, the estimate based on an enrichment of 2.4‰ suggests diet proportions of mesozooplankton and *C. pengoi* to be about 50:50, while corresponding values with a fractionation of 3.4‰ are 80:20 (Table 3). The relative trophic positions of *C. pengoi* and YOY herring were estimated to be 3.1 and 3.4, respectively, using Eq. 2, assuming fractionation of 3.4‰, and setting zooplankton δ^{15}N to 9.7‰—the average of micro- and mesozooplankton values (Fig. 3b).

The diet predictions for YOY herring from the mixing model are reasonably similar to the results from gut content analyses (Gorokhova et al. 2004). These analyses showed that *C. pengoi* occurred in >60% of the analysed individuals, although copepods (copepodites of *Acartia* and *Eurytemora*) and cladocerans (mostly *Bosmina*) were the dominant prey. The mass of *C. pengoi* which contributed to the diet of YOY herring was on average 6.2 ± 10.6%, lower than predicted from the mixing model (Gorokhova et al. 2004). This discrepancy could be explained by limitations both of gut content analyses (e.g. difficulties in quantifying prey remains due to partial digestion, differential retention time for different species and body parts, high intra-population variability, etc.) and SIA. In particular, the choice of trophic fractionation value considerably influences the result (Post 2002) and it should be recalled that the commonly used fractionation of 3.4‰ is the average of reported values between 1.3‰ and 5.3‰ (Minagawa and Wada 1984; Post 2002). This is illustrated by our results of mixing model analyses estimating the proportion of *C. pengoi* in the YOY herring diet. The lower degree of

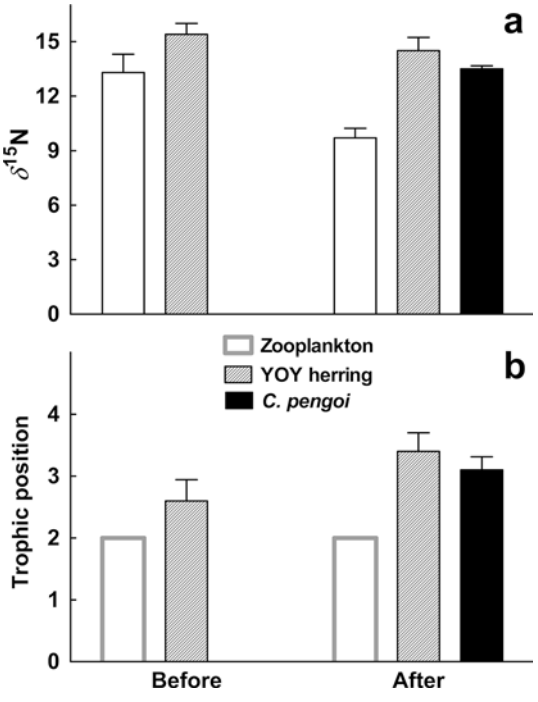

Fig. 3 N isotopes composition (**a**) and trophic positions (**b**) of zooplankton, young-of-the-year (*YOY*) herring, and *C. pengoi* in Himmerfjärden Bay before (1988, data are redrawn from Fig. 3 in Hansson et al. 1997, areas 2 and 3) and after the invasion of *C. pengoi* (this study). The relatively high zooplankton δ^{15}N values in 1988 were due to discharges from the sewage treatment plant. When estimating trophic positions, zooplankton as a baseline was set to an arbitrary value of 2; trophic fractionation was assumed to be 3.4‰. Data are presented as means with SDs

fractionation (2.4‰) produced a twofold higher proportion of *C. pengoi* in the diet than a fractionation of 3.4‰.

During the past 15 years, nutrient discharge from a sewage treatment plant into Himmerfjärden has varied considerably (Elmgren and Larsson 2001). In 1988, discharges from the treatment plant were responsible for significantly elevated δ^{15}N values in pelagic organisms, from phytoplankton to piscivorous fish (Hansson et al. 1997). Since then, enhanced denitrification in the treatment plant (~85% N removal from the wastewater) has greatly decreased the total N load and these changes in N source are likely to result in isotopic shifts in biota. In the macrophyte *Fucus vesiculosus*, δ^{15}N dropped ~4‰ between 1989 and 2002 (Savage and Elmgren 2004), which can be compared to the 3.6‰ drop in δ^{15}N in zooplankton (the difference between 9.7‰—average of micro- and mesozooplankton in this study and 13.3‰—average for the same area in 1988; Fig. 3a). However, in contrast to these decreases, δ^{15}N in YOY herring dropped only 0.8‰ (the difference between 14.5‰ in this study and 15.3‰ in 1988; Fig. 3a). One possible explanation for this apparent lack of response at higher levels might be the inclusion of *C. pengoi* in the diet of herring. The estimated trophic position of YOY herring (Eq. 2; Fig. 3b) has then increased from 2.6 before the invasion (i.e. in 1988) to 3.4 after the invasion (this study). With the central role of herring in the pelagic food web (cf. Rudstam et al. 1992), this implies that the invasion by *C. pengoi* has resulted in a general food web change.

Despite its rarity in plankton (< 0.1% of total zooplankton abundance), *C. pengoi* was ingested at a high frequency by fish as small as metamorphosed YOY herring (Gorokhova et al. 2004). This and supporting evidence from SIA suggest that *C. pengoi* has become an important item in the fish diet at least in some areas. Thus, at the same time that *C. pengoi* may compete for zooplankton prey, they may also be a valuable new food resource. In this coastal area, other groups of zooplankton decline rapidly in August (Johansson et al. 1993; Adrian et al. 1999; this study), while *C. pengoi* is present from July to October and is most abundant in August–September (Gorokhova et al. 2000; this study). This indicates that *C. pengoi* can be a particularly important food source for zooplanktivorous fishes during late summer and autumn, when the consumption by fish peaks (Rudstam et al. 1992; Arrhenius and Hansson 1993). However, as both the dominant pelagic fishes in the Baltic (herring and sprat, Thurow 1997) and *C. pengoi* depend largely on the same food source, a drastic increase in the *C. pengoi* population may result in a decreased fish production. Probably the only measure that can be taken to prevent this from happening is to manage herring and sprat stocks (or corresponding zooplanktivorous species in the North American lakes) in such a way that they are large enough to exert a substantial predation pressure on *C. pengoi*.

In conclusion, our study shows that *C. pengoi* is a true zooplanktivore and that its invasion of the study area appears to have changed the pelagic food web structure substantially. It is a potential competitor with pelagic fish, but these fish also exert a significant predation pressure on *C. pengoi*. The net trophic outcome of this invading species, seen from the perspective of higher trophic levels, is thus complex and presently difficult to evaluate.

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