

# A combined approach to understand trophic interactions between *Cercopagis pengoi* (Cladocera: Onychopoda) and mysids in the Gulf of Finland

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## Abstract

We use molecular markers, feeding experiments, and stable isotopes to understand trophic interactions between native mysids, *Mysis mixta* and *Mysis relicta*, and a recent invader to the Baltic Sea, the cladoceran *Cercopagis pengoi*. In feeding experiments, both mysid species ingested *C. pengoi* at a maximum of 16 prey mysid<sup>-1</sup> d<sup>-1</sup> and a specific consumption rate of 0.15 d<sup>-1</sup>, similar to rates reported for predation on other prey species. The frequency of predation on *C. pengoi* assayed by molecular diet analysis of field-collected mysids varied between 5% and 43% depending on mysid size and species. Surprisingly, it was consistently higher in juveniles than in adults and in *M. mixta* than in *M. relicta*. The results of stable isotope analysis corroborate those of feeding experiments and molecular analysis and indicate a higher contribution of *C. pengoi* to the nutrition of juveniles and *M. mixta*. These ontogenetic and interspecific differences in mysid predation on *C. pengoi* are likely to reflect differences related to their different migratory behavior. Thus, despite its low relative abundance, *C. pengoi* is readily consumed by mysids; this may impact lower food web interactions and the nutrition of mysids in the invaded ecosystems.

Major questions in ecology concerning the dynamics of food webs, exploitation and management of marine living resources, and the assessment of the effects of human activities on ecosystems can only be answered through accurate quantitative assessment of trophic interactions within the food web. This depends critically upon the methods employed. Traditionally, the primary tools for detecting and quantifying diet and predation rates in aquatic invertebrates have been stomach content analysis and feeding experiments, i.e., methods based on direct observations. Although much of what we know about predator–prey interactions has come from such studies, in many situations prey choice and predation rates in situ cannot be quantified by relying solely on these methods because they rarely provide sufficient resolution and experience a variety of shortcomings. In gut content analysis, the identification of food items can be difficult with underrepresented soft-bodied or rapidly digested prey species; furthermore, it provides only snapshot information on recently ingested food (Båmstedt et al. 2000). Feeding

experiments are indispensable as a means of directly measuring consumption rates and selectivity, but can have numerous limitations (Boyd et al. 1984; Omori and Ikeda 1984), and it is rarely possible to simulate natural conditions and food assemblages in the laboratory experiments. Because of these technical challenges and to better understand predator–prey interactions, ecologists have coupled video technology and feeding trials (Viitasalo et al. 2001) and developed a variety of biomarkers and tracers, e.g., fatty acids, DNA markers, allozymes, stable isotopes, etc. (see reviews by Båmstedt et al. 2000, Hagen 2000, and Symondson 2002). In particular, there has been a rapid increase in the use of stable isotopes and DNA-based techniques in feeding biology studies. Although the feasibility of applying these approaches in this field has been demonstrated (Sheppard and Harwood 2005), few studies have integrated these methods with other techniques and conventional diet analysis to quantify trophic interactions in the field.

Animals that macerate their food beyond recognition in the gut or feed by fluid ingestion are particularly challenging for feeding studies. When they are omnivores, feeding on a wide range of different species, the task becomes even more daunting. For such predators, it has been suggested that molecular methods may be particularly useful in identifying prey (Symondson 2002; Harper et al. 2005). Combining species- or group-specific polymerase chain reaction (PCR) assays (i.e., genetic markers) with other biomarkers and biological tracers (e.g., such as stable isotopes and fatty acids) would facilitate the assessment of diet analysis, providing independent and complementary information. Indeed, whereas the molecular detection of prey in the gut content of predators describes predation events that have occurred recently, stable isotopes and fatty

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acids provide estimates based on actual food assimilation and integrate the diet for a longer time, i.e., a period that depends on the feeding rates, assimilation, tissue turnover, and growth (Peterson and Fry 1987; Post 2002). Thus, the fundamental difference between the stable isotope and the gut content approaches in diet studies is the time scale each method addresses. Stable isotope analysis (SIA) has been used to evaluate mysid diet in coastal pelagic ecosystems in the Baltic Sea (Hansson et al. 1997) and the Great Lakes (Branstrator et al. 2000; Johannsson et al. 2001). To facilitate its application to trophic interactions involving mysids, their carbon (C) and nitrogen (N) fractionation factors were determined experimentally for muscle tissue and chitin (Gorokhova and Hansson 1999), tissues that enable diet reconstruction across the last few weeks (chitin) or months (muscle).

*Cercopagis pengoi* (Ostroumov 1884), a cladoceran of Ponto-Caspian origin, was introduced in the 1990s to the Baltic Sea and the Laurentian Great Lakes, where it has spread rapidly (Leppäkoski et al. 2002; Therriault et al. 2002; ICES 2005). In the invaded ecosystems, *C. pengoi* has become an important prey for native fish (Antsulevich and Välipakka 2000; Bushnoe et al. 2003; Gorokhova et al. 2004); however, it was hypothesized that *C. pengoi* can compete with larval fish for zooplankton and can reduce fish recruitment to larger size classes (Vanderploeg et al. 2002). The significance of *C. pengoi* in the diets of other zooplanktivores in the invaded ecosystems is yet unclear. To detect *C. pengoi* remains in the guts of predators, a DNA-based method was developed and used to identify *C. pengoi* in the stomachs of *Neomysis integer*, a common Baltic mysid (Gorokhova 2006). This study contributed to a growing body of evidence that *C. pengoi* successfully integrates into food webs in the invaded ecosystems and represents a novel prey not only for fish but also for invertebrate zooplanktivores. Recent feeding experiments corroborated these findings (Lehtiniemi and Lindén, 2006). The fact that mysids are able to consume *C. pengoi* has significant implications for the consequences of this invasion in the Baltic Sea and possibly also in the Great Lakes, where mysids are also important zooplanktivores (Johannsson et al. 2001), co-occurring with increasingly abundant *C. pengoi* (Vander Zanden et al. 2004). If *C. pengoi* is an additional food source for mysids, then a predation pressure exerted by mysids might contribute to a control of *C. pengoi* abundance. However, assessing the magnitude of this predation is difficult because Baltic mysids have a broad omnivorous diet (Rudstam et al. 1989; Viherluoto et al. 2000) and macerate their prey; traditional gut content analysis often fails to identify prey species (Mauchline 1980).

Here we use a combination of the DNA-based prey analysis, feeding experiments, and SIA to assess the contribution of *C. pengoi* to the diet of *Mysis mixta* and *Mysis relicta*, the two most common pelagic mysids in the Baltic Sea (Köhn 1992). These methods were chosen because they provide complementary information about the trophic relationships for this particular predator-prey system. Three crucial aspects are covered: (1) *molecular diet analysis*: what is the in situ frequency of mysid predation on

*C. pengoi*; (2) *feeding experiments*: what are the predation rates of mysids of different sizes at environmentally realistic prey concentrations; and (3) *SIA*: what are the short- and long-term dietary contributions of *C. pengoi* to mysid diet?

## Materials and methods

**Collection**—The study was conducted onboard RV *Aranda* (Finnish Institute of Marine Research) in the Gulf of Finland on two occasions: August 2004 and August 2005. Details of sampling sites and methods are summarized in Table 1. Zooplankton, *C. pengoi*, and mysid shrimps were collected using WP2 plankton nets with different mesh sizes equipped with a cod-end (Table 1). In 2004 and 2005, acclimation, maintenance of live organisms, and all experiments were conducted in a climate chamber at 15°C (the average temperature in the mixed layer of the gulf). Live mysids intended for feeding trials were placed individually in 0.2- $\mu$ m-filtered seawater in 0.5-liter glass vials and left for ~12-h acclimation. Immediately after collection, plankton was transferred to a 30-liter plastic container supplied with aeration and stored at the same temperature; all planktonic organisms used for experiments/samples were kept in captivity for no longer than 24 h. When we refer to zooplankton in this article, we mean all mesozooplankton except *C. pengoi*.

**Feeding experiments**—The experimental details are summarized in Table 2. Instar II *C. pengoi* were picked from the plankton using forceps, needles, and a dissecting microscope and acclimated for 4–6 h at the experimental temperature. Instar I *C. pengoi* were collected from the jars in which ovigerous females were left overnight to give birth; the neonates were considered to be sufficiently acclimated. Acclimated *C. pengoi* individuals were added to each bottle containing a single mysid (*M. mixta* or *M. relicta*) and incubated in darkness (Gorokhova and Hansson 1997). Three control replicates contained the same number of *C. pengoi* and no mysids. At the end of the incubation, the remaining prey individuals were removed, fixed in acid Lugol's solution, and counted. The body length of mysids (BL, mm) was measured as a distance between the tip of the rostrum to the end of the telson, and mysid species were identified using a dissecting microscope. Mysid dry weights (DWs, mg) were calculated from the body length measurements according to the equations:  $DW = 0.0032 \times BL^{2.85}$  for *M. mixta* (Gorokhova and Hansson 2000) and  $DW = 0.0047 \times BL^{2.72}$  for *M. relicta* (Johannsson 1995). Prey recovery was 100% in all control incubations; therefore, predation rate (prey mysid<sup>-1</sup> day<sup>-1</sup>) was directly calculated as the difference between initial and final numbers of prey and scaled to 24 h. The specific consumption rate (g of prey DW eaten per g of predator DW per day; day<sup>-1</sup>) was calculated using mysid DW values and assumes the average individual weight of *C. pengoi* is 7  $\mu$ g for Instar I and 20  $\mu$ g for Instar II (Uitto et al. 1999). The predation rates presented include all *C. pengoi* individuals that were at least partially consumed. When counting remaining prey, we frequently observed that *Cercopagis* caudal process was not ingested in the experiments.

Table 1. Material analyzed, sampling methods, and sample preparation for molecular and stable isotope analyses (DNA and SIA, respectively) and usage of samples for different tests; EtOH, 95% ethanol; C<sup>+</sup>, positive control, C<sup>-</sup>, negative control; n/a, not applicable.

Species/group and material analyzed	Sampling location and bottom depth (m)	Mesh size of WP2 net and sampling depth (m)	Preservation and sample preparation method	Mean sample size for SIA (mg)	Analysis
Seston	59°35'51"N, 24°20'81"E (85)	100- $\mu$ m + 20- $\mu$ m sieve (20)	Drying	0.070	SIA
Mesozooplankton	59°35'51"N, 24°20'81"E (85)	200- $\mu$ m (80)	Drying	0.125	SIA
<i>C. pengoi</i> (mixed stages)	59°42'01"N, 24°01'81"E (69)	500 $\mu$ m (20)	Drying	0.148	SIA
<i>C. pengoi</i> (mixed stages)	59°50'79"N, 24°50'27"E (100)	500 $\mu$ m (20)	EtOH	n/a	DNA, C <sup>+</sup>
<i>M. mixta</i> , <i>M. relicta</i> , stomachs after feeding on <i>C. pengoi</i>	59°51'50"N, 24°28'50"E (55)	500 $\mu$ m (50)	EtOH	n/a	DNA, C <sup>+</sup>
<i>M. mixta</i> , <i>M. relicta</i> , pleopods	59°50'79"N, 24°50'27"E (100)	500 $\mu$ m (95)	EtOH	n/a	DNA, C <sup>-</sup>
<i>M. mixta</i> , whole body	59°50'79"N, 24°50'27"E (100)	500 $\mu$ m (95)	Drying	0.172	SIA,
	60°11'34"N, 19°08'55"E (285)	500 $\mu$ m (280)	EtOH + drying	0.208	
<i>M. mixta</i> , stomachs	59°50'31"N, 25°51'41"E (84)	500 $\mu$ m (79)	EtOH	n/a	DNA
	60°11'34"N, 19°08'55"E (285)	500 $\mu$ m (280)			
<i>M. mixta</i> , chitin	60°11'34"N, 19°08'55"E (285)	500 $\mu$ m (280)	EtOH + drying	0.062	SIA
<i>M. relicta</i> , whole body	59°50'79"N, 24°50'27"E (100)	500 $\mu$ m (95)	Drying; EtOH +drying	0.154	SIA, effect of preservation
	59°51'50"N, 24°28'50"E (55)	500 $\mu$ m (50)	Drying	0.138	
	60°11'34"N, 19°08'55"E (285)	500 $\mu$ m (280)	EtOH + drying	0.156	
<i>M. relicta</i> , stomachs	59°51'50"N, 24°28'50"E (55)	500 $\mu$ m (50)	EtOH	n/a	DNA
	60°11'34"N, 19°08'55"E (285)	500 $\mu$ m (280)			
<i>M. relicta</i> , chitin	60°11'34"N, 19°08'55"E (285)	500 $\mu$ m (280)	EtOH + drying	0.064	SIA

Table 2. *M. mixta*, *M. relicta*: Mysid body length (BL, mm, mean $\pm$ SD), prey developmental stage (Instar I or II), prey density (prey liter<sup>-1</sup>), and experimental conditions (container volume [liter] and duration of trial period [h]) in feeding experiments with mysids as predators and *C. pengoi* as a prey; n, number of replicate incubations. In 2005, the total number of prey in the experiments was adjusted to obtain prey densities similar to 2004.

Year	Mysid species	BL	Prey Instar	Prey density	Container volume	Trial period	n
2004	<i>M. mixta</i>	21.3 $\pm$ 0.2	II	10	1	2	15
	<i>M. mixta</i>	19.0 $\pm$ 0.6	I	5	1	2	3
	<i>M. relicta</i>	20.4 $\pm$ 0.7	II	10	1	2	5
2005	<i>M. mixta</i>	8.2 $\pm$ 1.0	II	4	0.5	4	3
	<i>M. relicta</i>	12.0 $\pm$ 3.5	II	4	0.5	4	11

*Sample preparation for DNA analysis and SIA*—Mysids were either sorted within 2 h after collection and preserved individually or preserved in bulk in 95% ethanol and sorted 3–4 weeks later before analyses (Table 1). Samples for DNA analysis included dissected mysid stomachs (test samples), *C. pengoi* (positive control), and mysid pleopods (negative controls). Samples for SIA included seston, mesozooplankton, *C. pengoi*, mysid muscle tissue (abdomen), and mysid chitin (Table 1). Each mysid used for any of the analyses was measured for length and identified to species.

*DNA samples*: Mysid stomachs for DNA analysis were dissected from the dorsal side under a dissecting microscope and either transferred to Eppendorf tubes containing 50  $\mu$ L of 95% ethanol for storage (mysids sorted within 2 h after collection) or used for DNA extraction directly after

dissection (those preserved in bulk). *C. pengoi* were preserved in bulk in 95% ethanol.

**SIA samples:** When preparing for SIA, seston was collected as a filtrate from a 100- $\mu\text{m}$ -filtered plankton sample and retained on a 20- $\mu\text{m}$  sieve. Using a dissecting microscope, zooplankters (small copepodites and nauplii) occasionally found in seston as well as large filaments of cyanobacteria were removed with pipette and forceps. Residual water was removed from underneath the sieve with paper tissue. Bulk samples of seston were then transferred to preweighed tin capsules, dried at 60°C for 24 h, and stored at -20°C until analysis. Zooplankton (mostly copepods *Eurytemora affinis* and *Acartia bifilosa*) and *C. pengoi* were cleaned from debris and filamentous cyanobacteria under a dissecting microscope and rinsed with 0.2- $\mu\text{m}$ -filtered seawater. Bulk animal samples were treated in the same way as seston samples. Samples of mysid muscle tissue were prepared using fresh or ethanol-preserved mysids (Table 1) that were processed individually by drying at 60°C to a constant weight. The abdomens (i.e., muscle tissue) were separated from the dried bodies, ground in a mortar, and transferred to preweighed tin capsules. In juvenile mysids smaller than 8 mm, the whole body was used for the analysis. Chitin samples were prepared using ethanol-preserved mysids (Table 1). No attempt was made to molt stage individual mysids as we are not aware of any evaluation of the effect of the molt stage on the isotopic composition of chitin. Chitin was extracted from composite samples of 3–10 individuals (depending of mysid size), purified, and used for SIA following the procedure outlined by Webb and coworkers (1998). The dry mass of the samples was determined to the nearest microgram using a Sartorius M3P microbalance.

**DNA analysis**—For DNA extraction, stomachs were placed individually into extraction tubes, thoroughly crushed with a plastic pellet pestle (Kontes), and processed according to Asahida and coworkers (1997). The total DNA yield was 0.2–10.3  $\mu\text{g}$  stomach<sup>-1</sup>. As a first positive control, DNA from a single *C. pengoi* individual was extracted and used for amplification. As a second positive control, stomachs of mysids that were observed to consume *C. pengoi* in the feeding experiment were run in parallel with stomachs of field-collected mysids. As a negative control (i.e., to confirm that mysid DNA does not produce positive amplification), a dissected mysid pleopod was used with each set of PCR reactions; this body part was chosen to exclude the possibility of contamination with DNA that originated from the gut content. Amplifications were performed with an MJ Research MiniCycler according to Gorokhova (2006). Completed PCRs were separated on a 2% (w/v) agarose, 1  $\times$  TBE (tris-borate EDTA), 0.5 mg mL<sup>-1</sup> ethidium bromide gel with a 100 base pair (bp) ladder at 70 V. A sample was considered successful when a band was observed in the expected size range (i.e., ~150 bp); band intensity was not evaluated.

**Stable isotope analysis**—Concentrations of <sup>13</sup>C, <sup>12</sup>C, <sup>15</sup>N, and <sup>14</sup>N in the samples were determined using continuous-

flow isotope mass spectrometry provided in an automated NC analysis (ANCA) SL 20-20, PDZ Europa, at the Stable Isotope Facility, the University of California–Davis. The standard reference material for C was Vienna Pee Dee Belemnite (vPDB), and atmospheric N<sub>2</sub> was used for N. Isotope ratios were expressed as parts-per-thousands (‰) differences from the standard reference material (Peterson and Fry 1987). Repeated analyses of homogeneous material yielded standard deviations of <0.05‰ for both isotopes. The ‰C and ‰N data were used to calculate C:N atomic ratios.

Neither  $\delta^{13}\text{C}$  nor  $\delta^{15}\text{N}$  signatures were affected by ethanol preservation followed by a 3-week storage, as was indicated by a comparison of *M. relicta* samples collected at the same station and processed with and without ethanol preservation ( $\delta^{13}\text{C}$ : unpaired *t*-test,  $p > 0.3$ ,  $t = 1.008$ , degrees of freedom [df] = 8;  $\delta^{15}\text{N}$ : unpaired *t*-test,  $p > 0.8$ ,  $t = 0.1662$ , df = 8). Therefore, samples preserved in 95% ethanol before drying and those dried immediately after collection were pooled together for further comparisons.

Mysid  $\delta^{13}\text{C}$  is known to vary with C:N ratios (Gorokhova and Hansson 1999). Because fluctuations in carbon signature are more easily traced when the influence of high lipid content is removed, the muscle  $\delta^{13}\text{C}$  signatures of both mysid species were lipid corrected using C:N ratios. The lipid normalization procedure of McConaughy and McRoy (1979) was used to remove lipid isotope effects; the lipid-corrected values are expressed as  $\delta^{13}\text{C}'$ . This was not necessary for chitin, zooplankton, and *C. pengoi* samples, because (1) chitin is a polysaccharide and therefore does not contain lipids, (2) copepod species that were present at the time of sampling (i.e., *E. affinis* and *A. bifilosa*) do not accumulate large amounts of fat (Walve and Larsson 2000), and (3) the C:N ratio of *C. pengoi* was low ( $4.5 \pm 0.4$ ) and not significantly different from that in mesozooplankton (unpaired *t*-test,  $p > 0.2$ ,  $t = 1.212$ , df = 17), suggesting similarly low lipid content.

To determine the contribution of *C. pengoi* to a mysid diet and to explore size-based shifts in the diets, we applied a concentration-weighted mixing model for carbon and nitrogen isotopes (Phillips and Koch 2002) using seston, mesozooplankton, and *C. pengoi* as potential prey for large (adults and subadults, >14 mm) and small (juveniles, <11 mm) *M. mixta* and *M. relicta*. The choice of the end-members was based on earlier studies on mysid diet (seston and mesozooplankton: Rudstam et al. 1989; Viherluoto et al. 2000) and results of the molecular diet analysis obtained in this study (*C. pengoi*). Means of  $\delta^{13}\text{C}'$ ,  $\delta^{15}\text{N}$ , ‰C, and ‰N were used for data modeling. The diets were reconstructed separately using isotopic signatures of mysid muscle tissue (i.e., long-term diet) and chitin (i.e., relatively recent diet). The trophic fractionation was assumed to be constant, and the following fractionation factors were applied: 3.6‰ for <sup>15</sup>N in body and muscle tissue, -2.4‰ for <sup>15</sup>N in chitin as determined experimentally for *M. mixta* (Gorokhova and Hansson 1999), 0.3‰ for <sup>13</sup>C in body (McCutchan et al. 2003), and -0.4‰ for <sup>13</sup>C in chitin (Gorokhova and Hansson 1999). Our mixing model assumes that the categories seston and mesozooplankton are isotopically homogeneous and that mysids

feed unselectively within these categories; thus the results of this analysis should be considered as preliminary until more data on the species-specific isotope composition of plankton in the Baltic become available.

**Statistics**—Statistical tests were performed with GraphPad Prism 4.01 (GraphPad Software). Deviations from a Gaussian distribution were tested using the Kolmogorov-Smirnov test as Dallal and Wilkinson approximation to Lilliefors' method. When comparing two groups (i.e., *M. mixta* vs. *M. relicta*, etc.), the unpaired *t*-test was used, followed by the *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 with non-Gaussian distribution (i.e., mysid size groups in feeding experiments), Kruskal-Wallis (KW) analysis of variance (ANOVA) was used. When comparing size groups from two species (i.e., molecular data analysis, SIA), two-way ANOVA with a Bonferroni multiple comparison test was used. The calculation for the source proportions for the mixing model was performed according to Phillips and Koch (2002). Unless specified otherwise, data are presented as means with standard deviations; in all cases significance was accepted when  $p < 0.05$ .

## Results

**Feeding experiments**—Altogether, 43% of all mysids used in feeding experiments were found to prey on *C. pengoi*. Among mysids that were observed to consume the prey, the daily predation rate ranged from 1 prey mysid<sup>-1</sup> d<sup>-1</sup> to 16 prey mysid<sup>-1</sup> d<sup>-1</sup> (Fig. 1A,B), varying between the experiments conducted using different conditions, predator sizes, and prey densities (Table 2). The estimated maximal specific consumption rates were 5% and 15% of mysid body mass per day in *M. mixta* and *M. relicta*, respectively (Fig. 1C,D). In small juveniles (6–9 mm; 2005), specific consumption of *M. mixta* was significantly lower than that of *M. relicta* (unpaired *t*-test,  $p < 0.03$ ,  $t = 3.669$ ,  $df = 4$ ; Fig. 1C,D), although individual predation rates did not differ significantly (unpaired *t*-test,  $p > 0.3$ ,  $t = 1.138$ ,  $df = 4$ ; Fig. 1A,B). In experiments conducted in 2005, *M. relicta* was the only species that was available in a sufficiently wide size range to enable statistical evaluation of the effect of predator size on consumption rate. In this species, there was a significant decrease in both individual and specific consumption rates with increased mysid size (individual consumption rate: KW statistic = 6.747,  $p < 0.034$ ; specific consumption rate: KW statistic = 6.651,  $p < 0.036$ ; Fig. 1B,D). In *M. mixta*, a similar trend for specific consumption rate was observed when data from the two years were pooled (unpaired *t*-test comparing juveniles and adults,  $p < 0.031$ ,  $t = 3.261$ ,  $df = 4$ ; Fig. 1C). For individual consumption rate, however, the opposite trend was observed, with adults having approximately eight-fold higher predation rates than juveniles (Fig. 1A), albeit the difference was not significant because of the low number of observations and high within-

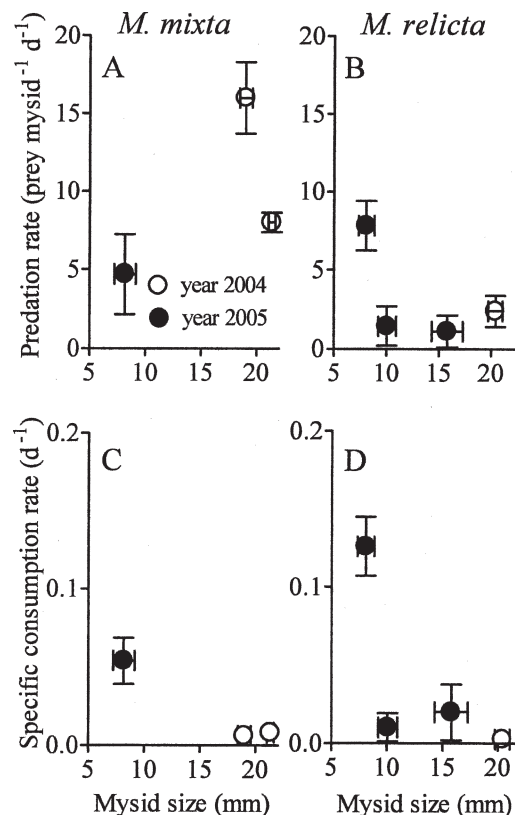


Fig. 1. (A, C) *M. mixta*, (B, D) *M. relicta*. (A, B) Individual and (C, D) specific consumption rates (mean  $\pm$  SE) on *C. pengoi* in feeding experiments with different prey densities: 5–10 ind. L<sup>-1</sup> (2004) and 4 ind. L<sup>-1</sup> (2005).

treatment variability (unpaired *t*-test,  $p > 0.3$ ,  $t = 1.300$ ,  $df = 4$ ). In part, this discrepancy could be explained by different experimental conditions (i.e., shorter incubation time and larger container size in 2004; Table 2) and, therefore, overestimated daily consumption rate (Gorokhova and Hansson 1997) and by higher prey densities in 2004 (Table 2).

**Molecular diet analysis**—The PCR products generated from the stomachs of both wild-caught mysids and those used in feeding experiments were the same size as the 16S rDNA fragment from *C. pengoi*, indicating that target DNA was present in the stomachs. In 2005, all mysids that were observed to consume *C. pengoi* in the feeding experiment contained its DNA in their stomachs. For wild-caught mysids, 23 out of 107 stomachs produced positive amplifications, implying that on average 22% of mysids used for the analysis contained *C. pengoi* DNA in their stomachs (Fig. 2). In all size classes, *M. mixta* appeared to produce positive amplifications at a higher frequency than *M. relicta*, although the difference was only marginally significant (two-way ANOVA:  $df = 2, 1$ ; size,  $p < 0.047$ ; species,  $p > 0.079$ ; interaction,  $p > 0.05$ ). However, *C. pengoi* was significantly more frequent in the stomachs of smaller individuals (43% in *M. mixta* and 28% in *M. relicta*; Fig. 2) as compared to larger ones. The difference was consistent within each species, with the

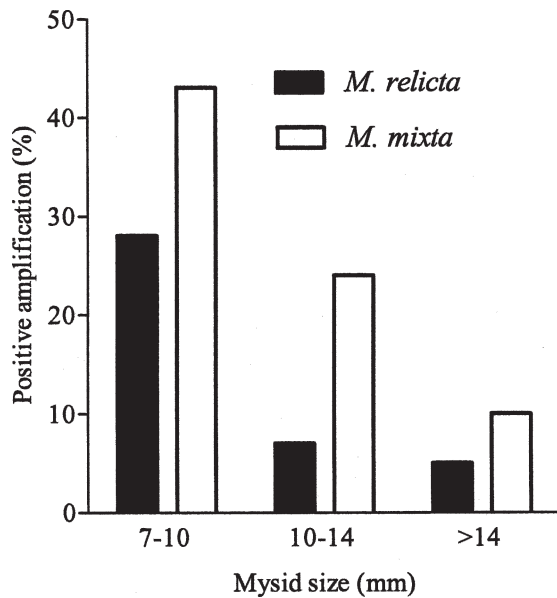


Fig. 2. *M. mixta*, *M. relicta*: molecular gut analysis of field-collected mysids. The frequency of mysid predation on *C. pengoi* in each species/size category is determined as a percent of stomachs in this category that produced positive amplification of *C. pengoi* mitochondrial 16S rDNA.

frequency of predation on *C. pengoi* by juveniles (7–10 mm) being  $\sim 5\times$  higher than that in adults (>14 mm).

**Stable isotopes**—The  $\delta^{13}\text{C}'$  and  $\delta^{15}\text{N}$  values and elemental concentrations in mysids, seston, mesozooplankton, and *C. pengoi* are given in Table 3. There was a considerable variation between the isotopic signatures of the mysids. Two-way ANOVA with species (i.e., *M. mixta* vs. *M. relicta*) and age (juveniles vs. adults) as factors and  $\delta^{13}\text{C}'$  and  $\delta^{15}\text{N}$  values in muscle tissue and chitin as response variables revealed significant effects of both factors on muscle  $\delta^{15}\text{N}$ , with higher values in *M. mixta*

than in *M. relicta* and in adults than in juveniles (Table 4). For chitin  $\delta^{15}\text{N}$  values, there was a significant species effect and also a significant interaction between the factors (Table 4). Unpaired *t*-tests were conducted as follow-up tests because of the significant interaction. Whereas in *M. mixta* adults had significantly higher  $\delta^{15}\text{N}$  values than did juveniles ( $p < 0.003$ ,  $t = 4.216$ ,  $df = 8$ ,  $r^2 = 0.69$ ), this difference was only marginally significant in *M. relicta* ( $p > 0.06$ ,  $t = 2.739$ ,  $df = 4$ ,  $r^2 = 0.61$ ). By contrast, the species effect was not significant for  $\delta^{13}\text{C}'$  values in either tissue, whereas the age effect was: muscle tissue of adults was significantly enriched in  $^{13}\text{C}$  as compared to the juveniles (Table 4). Thus, the stable isotope data suggest shifts in the food source as a function of age as well as differences between the two species.

Both isotope compositions and elemental concentrations differed considerably between the diet sources (Table 3), which confirms applicability of the concentration-weighted mixing model (Phillips and Koch 2002). For juvenile mysids, the mixing model based on muscle isotopic signatures predicted seston, mesozooplankton, and *C. pengoi* proportions to be about 60:30:10 and 70:25:5 for *M. mixta* and *M. relicta*, respectively (Fig. 3A,B). Mixing models for adult mysids derived from muscle tissue resulted in negative *C. pengoi* proportions (Fig. 3C,D), suggesting that some aspect of the model has been violated (i.e., incorrect fractionation and/or poorly constrained dietary sources). Relative prey contributions based on chitin isotopic signatures predicted seston, mesozooplankton, and *C. pengoi* proportions of about 30:60:10 and 40:55:5 for juvenile *M. mixta* and *M. relicta*, respectively (Fig. 4A,B). Chitin isotopic composition of adults indicated that 4% to 9% of their biomass synthesized during the preceding 2–4 weeks (i.e., the last molting cycle; Gorokhova and Hansson 1999; Gorokhova 2002) were derived from *C. pengoi* (Fig. 4C,D). The primary sources for this age group were mesozooplankton and seston, contributing about 80% and 10%, respectively, to the diet of *M. mixta* and 50% and 45% to that of *M. relicta*.

Table 3. *M. mixta* and *M. relicta*: mysid body length (BL, mm), isotopic composition (as  $\delta^{13}\text{C}$ ,  $\delta^{13}\text{C}'$ , and  $\delta^{15}\text{N}$ ), and carbon (C) and nitrogen (N) content (%) of muscle tissue and chitin in juveniles and adults, seston, zooplankton, and *C. pengoi*. All data are presented as mean  $\pm$  SD; *n*, number of samples analyzed.

Material analyzed	<i>n</i>	BL	$\delta^{13}\text{C}$	$\delta^{13}\text{C}'$	$\delta^{15}\text{N}$	C	N
<i>M. mixta</i>							
Juvenile, muscle tissue	10	8.8 $\pm$ 1.8	-22.4 $\pm$ 0.8	-20.6 $\pm$ 0.8	9.9 $\pm$ 0.6	44.2 $\pm$ 1.2	10.1 $\pm$ 0.8
Adult, muscle tissue	10	15.0 $\pm$ 0.8	-23.4 $\pm$ 0.8	-20.2 $\pm$ 0.8	10.6 $\pm$ 0.7	49.8 $\pm$ 1.4	8.1 $\pm$ 0.9
Juvenile, chitin	5	9.2 $\pm$ 1.1	-21.7 $\pm$ 1.4	—	4.6 $\pm$ 0.3	41.8 $\pm$ 0.3	7.1 $\pm$ 0.2
Adult, chitin	5	15.4 $\pm$ 0.9	-21.5 $\pm$ 1.1	—	5.7 $\pm$ 0.7	40.5 $\pm$ 0.3	6.7 $\pm$ 0.1
<i>M. relicta</i>							
Juvenile, muscle tissue	10	9.0 $\pm$ 1.2	-22.8 $\pm$ 1.0	-20.8 $\pm$ 1.0	9.3 $\pm$ 0.6	45.4 $\pm$ 1.6	9.9 $\pm$ 1.3
Adult, muscle tissue	10	14.3 $\pm$ 0.5	-23.6 $\pm$ 0.9	-20.4 $\pm$ 0.9	10.2 $\pm$ 1.1	50.1 $\pm$ 2.1	8.2 $\pm$ 1.5
Juvenile, chitin	5	8.7 $\pm$ 1.5	-21.8 $\pm$ 1.0	—	4.3 $\pm$ 0.3	40.2 $\pm$ 0.4	6.8 $\pm$ 0.2
Adult, chitin	5	14.4 $\pm$ 1.7	-21.9 $\pm$ 0.2	—	4.1 $\pm$ 0.2	41.1 $\pm$ 0.3	6.9 $\pm$ 0.7
Seston (>20 $\mu\text{m}$ )	5	—	-21.4 $\pm$ 0.3	—	4.2 $\pm$ 0.4	45.5 $\pm$ 0.5	5.7 $\pm$ 0.9
Zooplankton (>100 $\mu\text{m}$ )	7	—	-20.4 $\pm$ 0.2	—	7.5 $\pm$ 0.6	48.2 $\pm$ 0.7	10.6 $\pm$ 0.8
<i>Cercopagis</i> , mixed stages	7	—	-20.6 $\pm$ 0.4	—	8.8 $\pm$ 0.5	44.7 $\pm$ 2.1	10.2 $\pm$ 0.7

Table 4. *M. mixta*, *M. relicta*: two-way ANOVA for  $\delta^{13}\text{C}$ ,  $\delta^{13}\text{C}'$ , and  $\delta^{15}\text{N}$  values in muscle tissue and chitin of juvenile and adult mysids. Significant effects are indicated with asterisks (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). Abbreviations: df, degrees of freedom; SS, sum of squares; MS, mean square;  $F$ ,  $F$  statistic.

Variables and sources of variation	df	SS	MS	$F$
<b>Muscle tissue</b>				
$\delta^{13}\text{C}'$ (‰)				
Interaction	1	0.004000	0.004000	0.005303
Species	1	0.1000	0.1000	0.1326
Age	1	3.249	3.249	4.308*
Error	36	27.15	0.7542	
$\delta^{15}\text{N}$ (‰)				
Interaction	1	0.1092	0.1092	0.1776
Species	1	2.545	2.545	4.140*
Age	1	5.860	5.860	9.532**
Error	36	22.13	0.6148	
<b>Chitin</b>				
$\delta^{13}\text{C}$ (‰)				
Interaction	1	0.09113	0.09113	0.08942
Species	1	0.2101	0.2101	0.2062
Age	1	0.001125	0.001125	0.001104
Error	16	16.30	1.019	
$\delta^{15}\text{N}$ (‰)				
Interaction	1	0.6125	0.6125	23.36***
Species	1	2.112	2.112	80.56***
Age	1	0.01250	0.01250	0.4767
Error	16	0.4196	0.02622	

## Discussion

We attempted to clarify the role of *C. pengoi*, a new species in the Baltic Sea, as a food source for two common mysid species, *M. mixta* and *M. relicta*. To do this, we applied a combination of three different methods—feeding experiments, molecular identification of *C. pengoi* in mysid stomachs, and SIA. There was general agreement and complementarities between the data obtained by different methods, providing a surprisingly consistent picture of the dietary links between *C. pengoi* and mysids. The results from the feeding experiments revealed that both mysid species readily preyed on *C. pengoi*. The maximal individual and specific predation rates of mysids on *C. pengoi* (16 prey - mysid<sup>-1</sup> d<sup>-1</sup> and 0.15 d<sup>-1</sup>, respectively) are in the range of commonly observed predation rates of mysids on other zooplankton prey in laboratory experiments, i.e., copepods (*M. relicta*: Cooper and Goldman 1982; Spencer et al. 1999; *M. mixta*: Mohammadian et al. 1997; Viherluoto and Viitasalo 2001) and cladocerans (*M. relicta*: Johannsson et al. 1994; *M. mixta*: Mohammadian et al. 1997; Gorokhova and Hansson 1999). However, the fact that predation occurred in the controlled laboratory conditions with no alternative prey in the system does not imply that it does occur in nature. Indeed, in the Baltic Sea the average abundance of *C. pengoi* varies between 60 ind. m<sup>-3</sup> and 350 ind. m<sup>-3</sup> (Krylov et al. 1999; Gorokhova et al. 2000; Ojaveer et al. 2004), which is usually <5% (and often <1%) of total zooplankton abundance. At such low relative

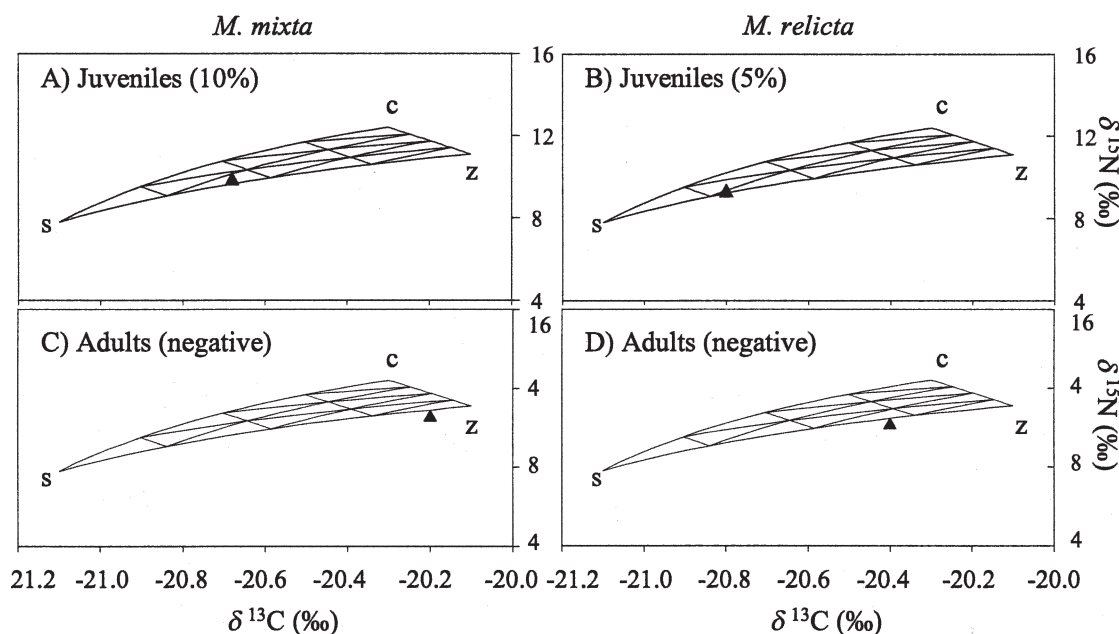


Fig. 3. (A, C) *M. mixta*, (B, D) *M. relicta*, muscle tissue. Concentration-weighted mixing model for carbon and nitrogen isotopes in mysid muscle tissue. Seston (s), mesozooplankton (z), and *C. pengoi* (c) are considered as diet sources of (A, B) juveniles and (C, D) adults. Isotopic values for monodiets at the vertices of each dietary mixing triangle have been corrected for trophic fractionation (see Materials and methods). The lines connecting the vertices of the triangle are simple, two-source mixing lines; lines within the triangle are iso-diet lines, along which the proportion of one dietary component is invariant. The filled triangle shows estimated dietary proportions of the three sources. The contribution of *C. pengoi* in mysid diet calculated by the model for each species/size group is shown as a percentage. In adult mysids of both species, isotopic signatures fall outside the region constrained by the source isotopic signatures, resulting in nonsensical negative *Cercopagis* proportions in the diet.

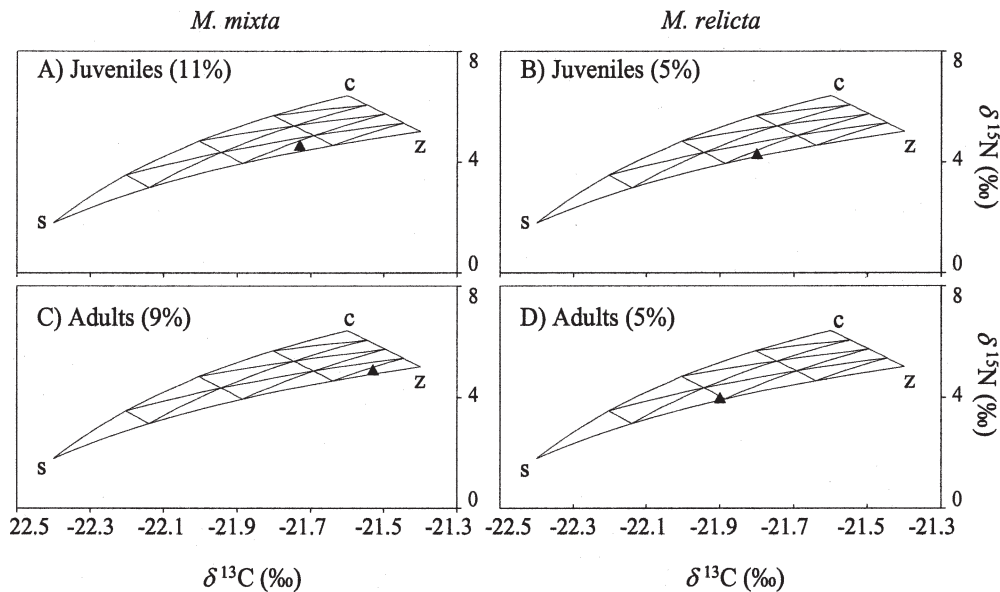


Fig. 4. (A, C) *M. mixta* and (B, D) *M. relicta* chitin. The concentration-weighted mixing model for carbon and nitrogen isotopes in chitin of (A, B) juveniles and (C, D) adults. The contribution of *C. pengoi* in mysid diet calculated by the model for each species/size group is shown as percentage. Diet sources are denoted as in Fig. 3.

abundances, the encounter rate might be extremely low resulting in a very weak or virtually nonexistent trophic interaction between mysids and *C. pengoi*. Nevertheless, the in situ predation on *C. pengoi* in the field was confirmed by molecular diet analysis; *Cercopagis* 16S rDNA was identified in 5% to 43% of the stomachs of field-collected mysids depending on size and species. Interestingly, these frequencies of predation are comparable with the frequency of feeding incidence observed in the feeding experiments (43%). Thus, despite its low relative abundance, *C. pengoi* was consumed with a relatively high frequency by mysids, indicating that this prey is accessible to a significant part of the mysid population and that positive selection toward feeding on *C. pengoi* is likely to occur.

Surprisingly, the frequency of predation on *C. pengoi* decreased with increasing mysid size (Fig. 2), although *C. pengoi* is a large prey that should be easier to handle for larger mysids. Most probably, this is related to a greater spatiotemporal overlap between juvenile mysids and *C. pengoi* resulting in higher encounter rates. Indeed, mysid shoals in the northern Baltic Sea tend to stay near the bottom during the day and feed in the pelagic zone at night (Salemaa et al. 1986; Rudstam et al. 1989). In both species, juveniles were found to rise higher than adults (Salemaa et al. 1986) and stay longer above the thermocline (Rudstam et al. 1989), where *Cercopagis* resides (Krylov et al. 1999). Thus, juvenile mysids and *C. pengoi* spend more time in the same strata, and this translates into tighter trophic linkage between the organisms. In support of this idea was the observation that small *M. relicta* individuals had significantly higher consumption rate than large ones in feeding experiments (Fig. 1B), suggesting that juveniles, being exposed to *C. pengoi* more frequently than adults, might have developed a learned response to consume this prey. Further, the frequency of predation on *C. pengoi* was

consistently higher in *M. mixta* as compared to *M. relicta* (Fig. 2), suggesting dietary differences between the two species, although in the feeding experiments their consumption rates were similar (Fig. 1). Again, when migrating, *M. mixta* was found to rise higher than *M. relicta* (Salemaa et al. 1986), and therefore they might have a better access to *C. pengoi*, resulting in a higher predation frequency. Thus, the ontogenetic and interspecific differences in relative frequencies of *C. pengoi* occurrence in mysid stomachs are likely to reflect differences in predation rates related to their different migratory behavior.

SIA of mysids and other pelagic components adds further insights not only into *C. pengoi* significance for mysid feeding, but also into the mysid diets in general. The dietary estimates derived from the mixing models agree with those obtained by gut content analysis in earlier studies (Rudstam et al. 1989; Viherluoto et al. 2000). In particular, SIA and mixing models suggest a substantial overlap between the diets of the two species in the Baltic; although there are differences in relative proportions of phyto- and zooplankton, with *M. mixta* being more carnivorous than *M. relicta* (Figs 3, 4). This is consistent with previous observations of Viherluoto and coworkers (2000), who reported that in August (i.e., the same time of the season as in the present study), zooplankton contribution to the mysid diet was around 60% and 50% for *M. mixta* ( $12.6 \pm 1.9$  mm) and *M. relicta* ( $7.4 \pm 1.4$  mm), respectively. As indicated by the isotopic composition of chitin, reflecting a more recent diet, the degree of zooplanktivory in *M. mixta* increases with size from 60% in juveniles to 80% in adults (*C. pengoi* included). These estimates are higher than those obtained by gut content analysis (Viherluoto et al. 2000), most likely because of the fact that conventional dietary analysis might have underestimated the nutritional importance of zooplankton for

mysids because of the inability to determine dietary components assimilated and incorporated into mysid tissues. It is also possible that the availability of zooplankton compared to phytoplankton was different between the two years. The differences between the chitin-derived (60–70% of zooplankton food, *C. pengoi* included) and muscle-derived (30–40%) prey proportions in the diet of juvenile mysids also indicate dietary change with increased relative importance of mesozooplankton in the recent diet. However, in contrast to *M. mixta*, the degree of zooplanktivory in the recent diet of *M. relicta* derived from chitin did not show any substantial ontogenetic shifts, with phytoplankton and zooplankton contributing nearly equally to both juvenile and adult diets (Fig. 4). This, together with muscle-derived diet estimates, indicates that in the Baltic this species shifts to the more carnivorous diet earlier in the juvenile life, when mysids are smaller than those used for SIA in our study ( $9.0 \pm 0.7$  mm). In the North American lakes, *M. relicta* was found to become more carnivorous in conjunction with maturation ( $>10$  mm), however, the time of the shift varied from one lake to another, which was suggested to be related to the size spectra of phytoplankton in these lakes (Branstrator et al. 2000). The earlier shift observed in the Baltic *M. relicta* could be explained by the high relative abundance of microzooplankton, with rotifers, nauplii, and early copepodites contributing up to 70% of the total zooplankton biomass in July–August (Johansson et al. 2004); this might have enabled juvenile mysids to become carnivorous at a smaller size. In concert with the increasing zooplanktivory in larger mysids, one would expect an increased consumption of *C. pengoi*; however, this was not the case—the contribution of *C. pengoi* to the recent diet was 5–11% in juveniles and 4–9% in adults with slightly higher values for *M. mixta* as compared to *M. relicta* (Fig. 4). Thus, SIA results corroborate the results of feeding experiments and molecular diet analysis and support the conclusion that *C. pengoi* is a new food source for Baltic mysids that contributes more to the nutrition of juvenile mysids than to the nutrition of adults.

It has been suggested that an ecosystem-based approach to fisheries management can be used to suppress the invader by managing its predators. This approach was taken in Lake Michigan to control alewife and rainbow smelt (Rand and Stewart, 1998). It has also been discussed as a potential control measure for *C. pengoi* abundance in the Baltic Sea (Gorokhova et al. 2004). Indeed, identifying predator species with high capacities to control their prey as a possible means of mitigating risks and ecosystem impacts and incorporating this information into an ecosystem approach for fish management would make a valuable contribution to the search for the best management strategy. In the Baltic, the two dominant zooplanktivorous fish are herring and sprat, the populations of which are strongly influenced by the fishery and for which annual catch quotas are set by the International Baltic Sea Fisheries Commission. Both of these species feed on *C. pengoi* (Antsulevich and Välipakka 2000; Gorokhova et al. 2004; Peltonen et al. 2004), whereas the diet of adult herring also includes mysids (Möllmann et al. 2004). This may need to be accounted for in the management of the

fishery if we would like to reduce the abundance of *C. pengoi*. From the multiple-enemy perspective, there are well documented mechanisms whereby predator diversity can be both beneficial and detrimental to the overall rate of predation (Polis et al. 1989; Sih et al. 1998). For example, intraguild predation or behavioral interference can result in diminished enemy impacts on prey species, given a sufficient compensatory response by that prey, and can dampen cascading effects on basal resources (Finke and Denno 2004). Moreover, when native and exotic consumers share a predator, predation on the exotic species can have a negative indirect effect on the native (i.e., apparent competition; Noonburg and Byers 2005). Thus, the net effects of predation on this exotic species will be determined by multiple direct and indirect interactions with positive and negative effects that must be assessed to predict the effects of management options.

This study has shown the value of using multiple independent methods in combination in assessing the trophic relationships between the different components of a pelagic ecosystem. It is important to use several complementary and independent methods, because each method has its systematic errors, advantages, and drawbacks. No single analysis would have provided as complete an assessment as did the combination of the techniques used in this study. For example, feeding experiments were informative concerning the capability of mysids to handle this large prey and to determine possible (although not necessarily realized) consumption rates. To determine the magnitude of this predation in the field, molecular diet analysis was useful to identify frequencies of *C. pengoi* occurrence in mysid stomachs and to justify the inclusion of *C. pengoi* as a diet item in the stable isotope mixing model. The model has further confirmed the significance of *C. pengoi* in mysid nutrition and estimated the contribution of this prey to assimilated biomass on a short- and a long-term basis. There are errors and biases inherent in any method. A weak point of the molecular diet analysis is varying detectability of prey DNA in a predator stomach, which is a function of digestion time and DNA fragment size (Symondson 2002). Similarly, SIA may not always be accurate in reconstructing diets because of uncertainties in fractionation factors, turnover rates, and temporal variations in isotopic signatures of both muscle and chitin, including molt cycle-related changes (Webb et al. 1998). However, the concordant results between SIA, molecular diet analysis, and feeding experiments gave us confidence in each individual method. As data continue to indicate successful integration and significance of *C. pengoi* (and other introduced species) in the invaded ecosystems, it is increasingly important to use a suite of techniques to reconstruct an accurate picture of multiple direct and indirect interactions with positive and negative effects when making decisions about control measures.

## References

- ANTSULEVICH, A., AND P. VÄLIPAKKA. 2000. *Cercopagis pengoi*—New important food object of the Baltic herring in the Gulf of Finland. *Internat. Rev. Hydrobiol.* **85**: 609–619.

- ASAHIDA, T., Y. YAMASHITA, AND T. KOBAYASHI. 1997. Identification of consumed stone flounder, *Kareius bicoloratus* (Basilewsky), from the stomach contents of sand shrimp, *Crangon affinis* (De Haan) using mitochondrial DNA analysis. *J. Exp. Mar. Biol. Ecol.* **217**: 153–163.
- BÄMSTEDT, U., D. J. GIFFORD, X. IRIGOEN, A. ATKINSON, AND M. ROMAN. 2000. Feeding, p. 279–399. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjoldal and M. Huntley [eds.], ICES zooplankton methodology manual. Academic Press.
- BRANSTRATOR, D. K., G. CABANA, A. MAZUMDER, AND J. B. RASMUSSEN. 2000. Measuring life-history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes. *Limnol. Oceanogr.* **45**: 463–467.
- BOYD, C. M., M. HEYRAUD, AND C. N. BOYD. 1984. Feeding of the Antarctic krill, *Euphausia superba*. *J. Crust. Biol.* **4**: 123–141.
- BUSHNOE, T. M., D. M. WARNER, L. G. RUDSTAM, AND E. L. MILLS. 2003. *Cercopagis pengoi* as a new prey item for alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) in Lake Ontario. *J. Great Lakes Res.* **29**: 205–212.
- GOROKHOVA, E. 2002. Moults cycle and its chronology in *Mysis mixta* and *Neomysis integer* (Crustacea, Mysidacea): Implications for growth assessment. *J. Exp. Mar. Biol. Ecol.* **278**: 179–194.
- . 2006. Molecular identification of the invasive cladoceran *Cercopagis pengoi* (Cladocera: Onychopoda) in stomachs of predators. *Limnol. Oceanogr. Methods* **4**: 1–6.
- , T. FAGERBERG, AND S. HANSSON. 2004. Predation by herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) on *Cercopagis pengoi* in a western Baltic Sea bay. *ICES J. Mar. Sci.* **61**: 959–965.
- , AND S. HANSSON. 1997. Effects of experimental conditions on the feeding rate of *Mysis mixta* (Crustacea, Mysidacea). *Hydrobiologia* **355**: 167–172.
- , AND ———. 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. *Can. J. Fish. Aquat. Sci.* **56**: 2203–2210.
- , AND ———. 2000. Elemental composition of *Mysis mixta* (Crustacea, Mysidacea) and energy costs of reproduction and embryogenesis under laboratory conditions. *J. Exp. Mar. Biol. Ecol.* **246**: 103–123.
- HAGEN, W. 2000. Lipids, p. 113–119. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjoldal and M. Huntley [eds.], ICES zooplankton methodology manual. Academic Press.
- HANSSON, S., J. E. HOBBIIE, R. ELMGREN, U. LARSSON, B. FRY, AND S. JOHANSSON. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* **78**: 2249–2257.
- HARPER, G. L., R. A. KING, C. S. DODD, J. D. HARWOOD, D. M. GLEN, M. W. BRUFORD, AND W. O. C. SYMONDSON. 2005. Rapid screening of invertebrate predators for multiple prey DNA target. *Mol. Ecol.* **14**: 819–827.
- JOHANSSON, M., E. GOROKHOVA, AND U. LARSSON. 2004. Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. *J. Plankton Res.* **26**: 67–80.
- JOHANSSON, O. E. 1995. Response of *Mysis relicta* population dynamics and productivity to spatial and seasonal gradients in Lake Ontario. *Can. J. Fish. Aquat. Sci.* **52**: 1509–1522.
- , L. G. RUDSTAM, AND D. C. LASENBY. 1994. *Mysis relicta*—assessment of metalimnetic feeding and implications for competition with fish in lakes Ontario and Michigan. *Can. J. Fish. Aquat. Sci.* **51**: 2591–2602.
- , AND OTHERS. 2001. Diet of *Mysis relicta* in Lake Ontario as revealed by stable isotope and gut content analysis. *Can. J. Fish. Aquat. Sci.* **58**: 1975–1986.
- ICES. 2005. Report of the Working Group on Introductions and Transfers of Marine Organisms (WGITMO), by correspondence. ICES CM 2005/ACME:05 Ref. E: 1–173.
- KÖHN, J. 1992. Mysidacea of the Baltic Sea-State of the art, p. 5–24. *In* V. J. Köhn, M. B. Jones and A. M. Moffat [eds.], Taxonomy, biology and ecology of (Baltic) mysids (Mysidacea: Crustacea). International Expert Conference, Hiddensee, Germany. Rostock University Press.
- KRYLOV, P. I., D. E. BYCHENKOV, V. E. PANOVA, N. V. RODIONOVA, AND I. V. TELESH. 1999. Distribution and seasonal dynamics of the Ponto-Caspian invader *Cercopagis pengoi* (Crustacea, Cladocera) in the Neva Estuary (Gulf of Finland). *Hydrobiologia* **393**: 227–232.
- LEHTINIEMI, M., AND E. LINDÉN. 2006. *Cercopagis pengoi* and *Mysis* spp. alter their feeding rate and prey selection under predation risk of herring (*Clupea harengus membras*). *Mar. Biol.* **149**: 845–854. doi:10.1007/s00227-006-0243-2.
- LEPPÄKOSKI, E., S. GOLLASCH, P. GRUSZKA, H. OJAVEER, S. OLENIN, AND V. PANOVA. 2002. The Baltic—a sea of invaders. *Can. J. Fish. Aquat. Sci.* **59**: 1175–1188.
- MAUCLINE, J. 1980. The biology of mysids and euphausiids. *Adv. Mar. Biol.* **18**: 1–369.
- MCCONNAUGHEY T., AND C. P. MCROY. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar. Biol.* **53**: 257–262.
- MCCUTCHAN J. H., W. M. LEWIS, C. KENDALL, AND C. C. MCGRATH. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**: 378–390.
- MOHAMMADIAN, M. A., S. HANSSON, AND B. T. DE STASIO. 1997. Are marine planktonic invertebrates food limited? The functional response of *Mysis mixta* (Crustacea, Mysidacea) in the Baltic Sea. *Mar. Ecol. Prog. Ser.* **150**: 113–119.
- MÖLLMANN, C., G. KORNILOVS, M. FETTER, AND F. W. KOSTER. 2004. Feeding ecology of central Baltic Sea herring and sprat. *J. Fish Biol.* **65**: 1563–1581.
- NOONBURG, E. G., AND J. E. BYERS. 2005. More harm than good: When invader vulnerability to predators enhances impact on native species. *Ecology* **86**: 2555–2560.
- OMORI, M., AND T. IKEDA. 1984. Methods in marine zooplankton ecology. Wiley.
- PELTONEN, H., M. VINNI, A. LAPPALAINEN, AND J. PÖNNI. 2004. Spatial distribution patterns of herring (*Clupea harengus* L.), sprat (*Sprattus sprattus* L.) and the three-spined stickleback (*Gasterosteus aculeatus* L.) in the Gulf of Finland, Baltic Sea. *ICES J. Mar. Sci.* **61**: 966–971.
- PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* **18**: 293–320.
- PHILLIPS, D. L., AND P. L. KOCH. 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia* **130**: 114–125.
- POLIS, G. A., C. A. MYERS, AND R. D. HOLT. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Ann. Rev. Ecol. Syst.* **20**: 297–330.
- POST, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83**: 703–718.
- RAND, P. S., AND D. J. STEWART. 1998. Prey fish exploitation, salmonine production, and pelagic food web efficiency in Lake Ontario. *Can. J. Fish. Aquat. Sci.* **55**: 318–327.
- RUDSTAM, L. G., K. DANIELSSON, S. HANSSON, AND S. JOHANSSON. 1989. Diel vertical migration and feeding patterns of *Mysis mixta* (Crustacea, Mysidacea) in the Baltic Sea. *Mar. Biol.* **101**: 43–52.

- , S. HANSSON, S. JOHANSSON, AND U. LARSSON. 1992. Dynamics of planktivory in a coastal area of the northern Baltic Sea. *Mar. Ecol. Prog. Ser.* **80**: 159–173.
- SALEMAA, H., K. TYYSTJÄRVI-MUURONEN, AND E. ARO. 1986. Life histories, distribution and abundance of *Mysis mixta* and *Mysis relicta* in the northern Baltic Sea. *Ophelia Suppl.* **4**: 239–247.
- SYMONDSON, W. O. C. 2002. Molecular identification of prey in predator diets. *Mol. Ecol.* **11**: 627–641.
- SHEPPARD, S. K., AND J. D. HARWOOD. 2005. Advances in molecular ecology: Tracking trophic links through predator–prey food-webs. *Func. Ecol.* **19**: 751–762.
- TERRIAULT, T. W., I. A. GRIGOROVICH, D. D. KANE, E. M. HAAS, D. A. CULVER, AND H. J. MACISAAC. 2002. Range expansion of the exotic zooplankter *Cercopagis pengoi* (Ostroumov) into western Lake Erie and Muskegon Lake. *J. Great Lakes Res.* **28**: 698–701.
- VANDERPLOEG, H. A., AND OTHERS. 2002. Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Can. J. Fish. Aquat. Sci.* **59**: 1209–1228.
- VANDER ZANDEN, M. J., K. A. WILSON, J. M. CASSELMAN, AND N. D. YAN. 2004. Species introductions and their impacts in North American Shield lakes, p. 239–263. *In* J. M. Gunn, R. A. Ryder and R. J. Steedman [eds.], *Boreal shield watersheds: Lake trout ecosystems in a changing environment*. CRC Press.
- VIHERLUOTO, M., H. KUOSA, J. FLINKMAN, AND M. VIITASALO. 2000. Food utilisation of pelagic mysids, *Mysis mixta* and *M. relicta*, during their growing season in the northern Baltic Sea. *Mar. Biol.* **136**: 553–559.
- , AND M. VIITASALO. 2001. Effect of light on the feeding rates of pelagic and littoral mysid shrimps: A trade-off between feeding success and predation avoidance. *J. Exp. Mar. Biol. Ecol.* **261**: 237–244.
- VIITASALO, M., J. FLINKMAN, AND M. VIHERLUOTO. 2001. Zooplanktivory in the Baltic Sea: a comparison of prey selectivity by *Clupea harengus* and *Mysis mixta*, with reference to prey escape reactions. *Mar. Ecol. Prog. Ser.* **216**: 191–200.
- WALVE, J., AND U. LARSSON. 1999. Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. *J. Plankton Res.* **21**: 2309–2321.
- WEBB, S., R. E. M. HEDGES, AND S. J. SIMPSON. 1998. Diet quality influences the delta 13C and delta 15N of locusts and their biochemical components. *J. Exp. Biol.* **201**: 2903–2911.

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