

## Measuring life-history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes

**Abstract**—*Mysis relicta* is a species of omnivorous zooplankton that has been implicated over the last several decades in important food-web processes such as the lengthening of food chains, the mobilization of benthic contaminants to the pelagic, and the restructuring of community dynamics. We used stable nitrogen isotopes ( $\delta^{15}\text{N}$ ) to quantify ontogenetic diet shifting of *M. relicta* in five North American lakes. Analyses indicated that *M. relicta* measuring  $>1.5$  cm were elevated, on average, 0.73 trophic levels above conspecifics measuring  $<1$ -cm length, and 0.57 trophic levels above conspecifics measuring  $>1$  but  $<1.5$ -cm length. This pattern suggests a trend toward increasing carnivory with maturity, which is consistent with the results of previous conventional approaches to diet analysis in this species. Our results should be useful to modelers concerned with quantifying the effects of *M. relicta* on energy transfer and contaminant bioaccumulation in food chains.

The opossum shrimp, *Mysis relicta*, has received considerable attention in recent studies that examine the effects of trophic structure on contaminant bioaccumulation in aquatic food chains (Evans et al. 1982; Rasmussen et al. 1990; Cabana and Rasmussen 1994; Bentzen et al. 1996; Vander Zanden and Rasmussen 1996). As a predator on zooplankton, *M. relicta* may represent up to one complete additional trophic level and thereby contribute significantly to higher contaminant loads in top predators such as lake trout. Aside from its importance in contaminant loading, *M. relicta* can have strong effects on the population dynamics and community structure of pelagic organisms. For example, in Lake Tahoe (Morgan et al. 1978) and Flathead Lake (Beattie and Clancey 1991) sizeable decreases in growth or abundance of adult kokanee (*Oncorhynchus nerka*), a predator on *M. relicta*, have been suggested to be linked to the strong competitive interaction between *M. relicta* and juvenile kokanee for herbivorous zooplankton.

*M. relicta* are broadly omnivorous (Grossnickle 1982) and this dietary flexibility has in part contributed to the difficult task of predicting their trophic linkages from one lake to the next. Previous studies based on experimentation and gut analyses have shown that mysids can consume algae but generally become more zooplanktivorous as they mature (life-history omnivory), which suggests that body size is a good predictor of diet composition (Cooper and Goldman 1980; Grossnickle 1982; Nero and Sprules 1986). As a means to better predict the effects of *M. relicta* on food-web processes, we used stable nitrogen isotopes ( $\delta^{15}\text{N}$ ) to quantify ontogenetic changes in trophic position of this species in nature (Peterson and Fry 1987; Kling et al. 1992; Vander Zanden and Rasmussen 1996).

Samples were collected in 1994 from Lac Achigan (central basin), Lake Memphremagog (central basin), and Lake Champlain (eastern side) (Table 1). Lake Huron and Lake

Michigan were both sampled in 1996, at the stations indicated in Table 1. *M. relicta* were collected at night from the epilimnion and metalimnion layers using a square-frame plankton net (1-m diameter opening, 1-mm aperture mesh). Live individuals were selected randomly and sorted into three length classes as  $<1$ -cm length (small), 1–1.5-cm length (intermediate), and  $>1.5$ -cm length (large). Length was determined as the distance from the center of the eyes to the base of the telson. Because *M. relicta* generally mature between 1 and 1.5-cm length, the small and large groups correspond well to juveniles and adults, respectively (Balcer et al. 1984). About 20 to 100 animals were picked for each size class to ensure a representative population sample for isotope determination.

Bulk zooplankton (rotifers and crustacean zooplankton) were collected by repeated vertical casts of a net (0.5-m diameter opening, 153- $\mu\text{m}$  aperture mesh) pulled through the mixed layer at night shortly after *M. relicta* were collected. Occasional *M. relicta* and *Diaporia hoyi* caught in the cod-end were discarded prior to drying the material for isotope analysis. This left the bulk zooplankton sample composed primarily of cladocerans and copepods. Rotifers comprised a minor portion of the biomass. In Lake Huron and Lake Michigan, all specimens of the large cladoceran predator, *Bythotrephes cederstroemi*, were removed from the samples and preserved separately as either instar 1, 2, or 3 based on the number of lateral barb pairs on the caudal spine (Yurista 1992).

Plankton material that was subjected to  $\delta^{15}\text{N}$  analysis was either dried on the night of collection (60°C), or frozen and dried later. Samples were kept dry and dark in scintillation vials until analysis, at which time they were homogenized to a fine powder with a mortar and pestle. Sample amounts of about 0.5 to 2 mg were packaged into pressed tin capsules (Elemental Microanalysis). The samples from Lac Achigan, Lake Memphremagog, and Lake Champlain were measured by a Europa tracer mass spectrometer interfaced with a Roboprep-CN analyzer at McGill University (Cabana and Rasmussen 1994). The samples from Lake Michigan and Lake Huron were measured by a VG Micromass 602E mass spectrometer and a Dumas combustion system at Harvard University. The results are reported in reference to a dinitrogen standard by the equation

$$\delta^{15}\text{N}(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000, \quad (1)$$

where  $R = (^{15}\text{N}/^{14}\text{N})$ . The reproducibility of replicate analyses ranged between about 0.1 to 0.3‰. Algae at the base of food chains can vary considerably in  $\delta^{15}\text{N}$  between lakes (Cabana and Rasmussen 1996). Owing to the fact that this baseline variation is preserved at higher trophic levels, we were unable to compare directly the  $\delta^{15}\text{N}$  signatures of the

Table 1. Physical and biological characteristics of the study lakes. Sampling dates and locations are Lac Achigan—8 June, 27 July, 14 September, and 6 October of 1994; Lake Memphremagog—9 June, 28 June, 26 July, and 16 September of 1994; Lake Champlain—12 August 1994; Lake Michigan—(Sta. 1) 21 August 1996 at 45°19.3'N, 85°29.9'W ( $z = 100$  m), (Sta. 2) 22 August 1996 at 43°N, 86°40.0'W ( $z = 100$  m); and Lake Huron—(Sta. 1) 17 August 1996 at 44°10.0'N, 83°30.0'W ( $z = 60$  m), (Sta. 2) 20 August 1996 at 45°N, 82°45.0'W ( $z = 110$  m), and (Sta. 3) 20 August 1996 at 43°30.0'N, 82°14.0'W ( $z = 50$  m).

Lake	Surface area (km <sup>2</sup> )	$Z_{\max}$ (m)	Estimated summer Chl <i>a</i> ( $\mu\text{g L}^{-1}$ )	(Chl <i>a</i> reference)
Achigan	5	26	1.9	(Pinel-Alloul et al. 1996, table 1)
Memphremagog	90	108	6.8	(Duarte and Kalff 1990, table 1)
Champlain	1,270	122	4.0	(Effler et al. 1991)*
Michigan	57,850	265	1.1	(Rockwell et al. 1989)†
Huron	59,510	223	1.2	(Rockwell et al. 1989)†

\* Mean of all values reported in their table 2.

† Mean of values given in their table 25 for summer conditions in the north and south.

mysid populations. As a means to adjust for lake-specific variation in  $\delta^{15}\text{N}$  we used the index

$$\text{Trophic elevation} = [(\delta^{15}\text{N}_{M. relicta} - \delta^{15}\text{N}_{\text{bulk zooplankton}})/3.2] \quad (2)$$

where 3.2 represents one trophic level increment (e.g., the expected difference between *M. relicta* and its diet). Trophic elevation measures the relative number of trophic levels, or fractions thereof in most instances, that *M. relicta* are positioned above bulk zooplankton in the food chain. In the absence of data on nitrogen isotope fractionation for *M. relicta*, we used 3.2‰ following Toda and Wada (1990), who experimentally demonstrated this to be the  $\delta^{15}\text{N}$  enrichment factor of *Neomysis intermedia* raised in the laboratory on frozen cladoceran food. The value compares favorably with the average value of 3.4‰ reported for many other consumers (DeNiro and Epstein 1981; Minagawa and Wada 1984).

We found strong support in all five lakes for the hypothesis that *M. relicta* is a life-history omnivore that becomes more carnivorous as it matures (Grossnickle 1982; Sprules and Bowerman 1988). This was reflected by a progressive enrichment in  $\delta^{15}\text{N}$  (Table 2) and a corresponding increase in trophic elevation that paralleled body length (Table 2, Fig. 1). The results are consistent with previous studies that have shown that greater body size admits mysids to a wider range of particle sizes (Zaret 1980; Grossnickle 1982; Nero and Sprules 1986), including quicker prey taxa, such as cyclopoids and *Epischura*, that themselves feed high in the food chain (Cooper and Goldman 1980). Hecky (1984) recognized that pelagic omnivores that pursue a wider diet breadth almost inevitably become more carnivorous by virtue of the strong correlation that exists between body size and trophic position in pelagic food chains. Increasing carnivory in mysids appears to be a good example of this phenomenon.

On average, small and intermediate mysids were elevated 0.11 and 0.27 trophic levels, respectively, above bulk zooplankton, which implies that these mysid size classes had diets similar to the bulk zooplankton. By comparison, the average trophic elevation of large mysids was 0.84, and always  $\geq 0.64$ , which implies that most of their assimilated diet derived from the bulk zooplankton. Mann-Whitney *U*-

tests (SYSTAT 6.0) revealed that mean trophic elevation (Table 2) in large mysids (0.84) was significantly greater than in either intermediate mysids (0.27) ( $P = 0.09$ ) or small mysids (0.11) ( $P = 0.009$ ) but that mean trophic elevation was not significantly different between the small and intermediate mysids ( $P = 0.141$ ). The lack of significance between the small and intermediate mysids was influenced strongly by the values in Lake Champlain and Lake Michigan, which depressed the mean of the intermediate size class (Table 2). *M. relicta* measuring  $>1.5$ -cm length were feeding, on average, 0.73 (0.84–0.11) trophic levels above conspecifics measuring  $<1$ -cm length and 0.57 (0.84–0.27) trophic levels above conspecifics measuring  $>1$  but  $<1.5$ -cm length.

Measurements of  $\delta^{15}\text{N}$  in the predator *Bythotrephes cederstroemi* provided a reference for characterizing the diets of mysids in Lake Michigan and Lake Huron. We reasoned that since *B. cederstroemi* feeds predominantly on daphnid prey (Lehman and Cáceres 1993; Lehman and Branstrator 1995), its tissues ought to theoretically reflect the signature of a primary carnivore in the system. Comparing data in Tables 2 and 3 shows that the small and intermediate mysids were generally less enriched than *B. cederstroemi*, which implies that these two mysid size classes were partially herbivorous. On the other hand, large mysids were more enriched than *B. cederstroemi*, which indicates that they were more carnivorous than a primary carnivore. In Lake Michigan and Lake Champlain, *M. relicta* appear to prolong a transition to carnivory, in comparison to mysids in the other lakes (Fig. 1), which may in the case of Lake Michigan owe to the abundance of large diatoms (*Melosira* and *Fragilaria*) available in the spring (Grossnickle 1978; Bowers and Grossnickle 1978). Considering  $\delta^{15}\text{N}$  signatures in *B. cederstroemi*, it is noteworthy that they were relatively constant among the three developmental instars (Table 3), which implies a lack of life-history omnivory in this species.

Our numerical estimates of trophic separation between mysid size classes (Table 2, Fig. 1) should be useful to studies concerned with predicting the levels of contaminant bioaccumulation transmitted through food chains that contain

Table 2.  $\delta^{15}\text{N}$  values of bulk zooplankton and *M. relicta* in the five study lakes, including mean seasonal values for Lac Achigan and Lake Memphremagog, station mean values for Lake Michigan and Lake Huron, and all lakes means. Estimated values for trophic elevation are based on Eq. 2. *M. relicta* were grouped by body length as small (<1.0 cm length), intermediate (1–1.5 cm length), and large (>1.5 cm length).

Lake and sample date	$\delta^{15}\text{N}$ zoo-plank-ton	<i>M. relicta</i>		
		Small	Inter-mediate	Large
<b>Achigan</b>				
8 Jun 94	5.85	4.42	7.19	8.15
27 Jul 94	3.28	5.38	5.88	6.08
14 Sep 94	3.83	4.52	4.49	6.61
6 Oct 94	4.41	4.33	4.75	4.75
Seasonal mean	4.34	4.66	5.58	6.40
Trophic elevation*		0.01	0.39	0.64
<b>Memphremagog</b>				
9 Jun 94	9.05	7.75	10.81	10.23
28 Jun 94	4.40	7.18	9.41	10.58
26 Jul 94	7.20	7.25	7.60	9.24
16 Sep 94	8.78	10.67	8.83	10.28
Seasonal mean	7.36	8.21	9.16	10.08
Trophic elevation*		0.26	0.56	0.85
<b>Champlain</b>				
12 Aug 94	8.17	8.87	8.31	11.50
Trophic elevation		0.22	0.04	1.04
<b>Michigan</b>				
21 Aug 96 (1)	5.76	6.06	6.01	7.50
22 Aug 96 (2)	4.24	4.32	4.39	7.60
Station mean	5.00	5.19	5.20	7.55
Trophic elevation†		0.06	0.06	0.80
<b>Huron</b>				
17 Aug 96 (1)	5.18	5.43	6.36	8.97
20 Aug 96 (2)	6.00	5.25	6.40	7.93
20 Aug 96 (3)	4.75	5.32	5.98	§
Station mean	5.31	5.33	6.25	8.45
Trophic elevation†		0.01	0.29	0.89
<b>All lakes mean</b>				
Trophic elevation‡		0.11	0.27	0.84

\* Seasonal means were used.

† Station means were used.

‡ Seasonal means for Lac Achigan and Lake Memphremagog, but station means for Lake Michigan and Lake Huron were used.

§ Zero *M. relicta* >1.5 cm length were found in this sample.

mysids. Fishes that are positively size selective on adult mysids (Janssen and Brandt 1980) may be expected to accumulate much higher loads of contaminants (Evans et al. 1982) in comparison to fishes foraging on either juvenile mysids or the bulk zooplankton. Consequently, careful consideration should be taken in assigning trophic positions to consumers in food chains where mysids are an important link.

The trends reported here between body length and increasing carnivory in *M. relicta* were generally similar among the lakes despite variation in lake size and Chl *a* (Table 1). If

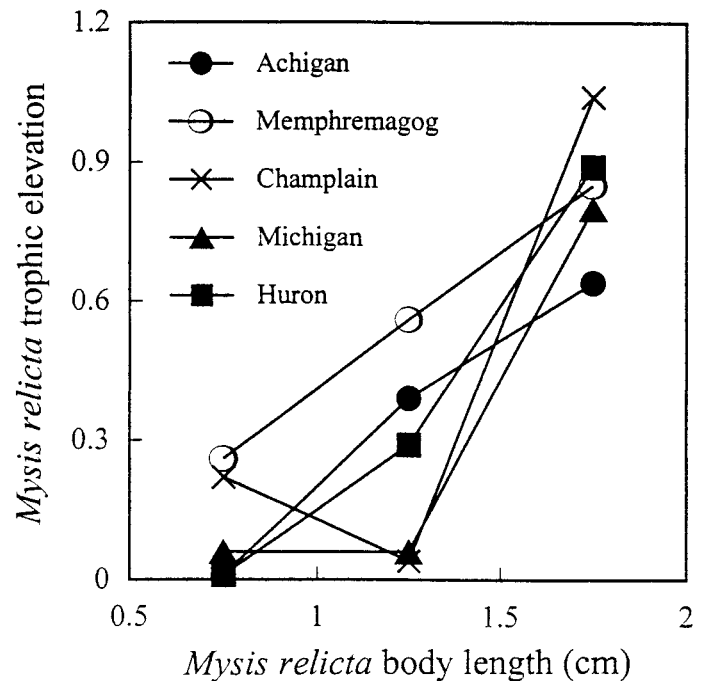


Fig. 1. Relationship between trophic elevation of *Mysis relicta*, based on Eq. 2, and body length. Body lengths were assigned as the midpoints of the ranges of pooled lengths, where small (<1 cm) = 0.75 cm, intermediate (1 to 1.5 cm) = 1.25 cm, and large (>1.5 cm) = 1.75 cm.

this pattern is broadly representative of mysids, it suggests that variation in algae biomass within the range of 1.1 to 6.8  $\mu\text{g L}^{-1}$  Chl *a* would have little effect on mysid diet composition. This is interesting in light of recent experimental manipulations of Lake Kootenay, British Columbia, where nitrogen and phosphorus have been added to stimulate algae and enhance crustacean prey for fish populations (Ashley et al. 1997).

Like many other studies on diet (Werner and Gilliam 1984; Polis 1991; Polis and Strong 1996), our results cast considerable doubt on the validity of the assumption of clas-

Table 3.  $\delta^{15}\text{N}$  values of *Bythotrephes cederstroemi* from Lake Michigan and Lake Huron. Animals were pooled by instar based on the number of lateral barb pairs on the caudal spine, notated as one-barb, two-barb, or three-barb (Yurista 1992). More than 10 individuals were combined for each sample. See Table 1 for the locations of the sampling stations.

Lake Date (station)	$\delta^{15}\text{N}$			Popula-tion mean $\delta^{15}\text{N}$
	one-barb	two-barb	three-barb	
<b>Michigan</b>				
22 Aug 96 (2)	6.57	6.79	6.50	6.62
<b>Huron</b>				
17 Aug 96 (1)	5.06	5.70	5.67	5.48
20 Aug 96 (3)	6.51	6.12	6.38	6.34

sic food-chain models that species recognize single, discrete trophic levels in nature (Hairston et al. 1960; Oksanen et al. 1981).

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## Optical discrimination of a phytoplankton species in natural mixed populations

### Abstract

Developing optical detection techniques for discriminating particular phytoplankton species in mixed assemblages has long been a goal of aquatic scientists. Previously, a processing algorithm for phytoplankton absorption spectra was reported that suggested detection of the red tide dinoflagellate *Gymnodinium breve* was possible. The algorithm evaluated the fourth derivative of the particulate absorption spectrum of an unknown sample and compared it to a standard fourth derivative spectrum for *G. breve* using a similarity index. We report here the first-time application of this technique to the detection of *G. breve* in natural, mixed phytoplankton communities. Pigment and spectral absorption data were collected from natural blooms of *G. breve* in the eastern Gulf of Mexico. This dinoflagellate is the only species of phytoplankton in the Eastern Gulf of Mexico observed to contain the pigment gyroxanthin-diester, and it appears in constant proportion to cellular chlorophyll *a* (Chl *a*) in *G. breve*. The in vitro absorption spectrum of gyroxanthin-diester is nearly identical to other xanthophylls (including diadinoxanthin, lutein, and 19'-hexanoyloxyfucoxanthin) and is not singularly responsible for imparting a unique absorption signature. Quantifying gyroxanthin-diester and Chl *a* allowed us to estimate the fraction of the biomass in mixed populations associated with *G. breve*. Subsequent regression of the *G. breve* similarity indexes to the *G. breve* biomass fractions yielded a significant linear correlation. Finally, the liquid waveguide capillary cell appears to be a promising technology for automating this technique.

Harmful algal blooms pose a threat that requires efforts to reduce or eliminate their negative impacts and consequences (Boesch et al. 1996). In the Gulf of Mexico, toxic blooms of the dinoflagellate *Gymnodinium breve* Davis regularly lead to untimely restrictions on commercial and recreational shellfish harvesting and deleterious effects on tourism and public health (Steidinger et al. 1973). Mitigation of

some harmful effects from these toxic blooms of *G. breve* can be achieved by early detection of this species in mixed phytoplankton communities. Currently, microscope examination of discrete water samples is the principal detection method for *G. breve*. Unfortunately, this method is slow, labor intensive, and intermittent. Significant benefits can be gained from an automated detection method, and optical methods hold promise for such applications (Cullen et al. 1997).

Over the past two decades, oceanographers have developed optical instrumentation that can collect data in a non-intrusive manner. Optical techniques are amenable to a variety of platforms (satellites, aircrafts, mooring, and profiling instrumentation), allowing researchers to design multiplatform sampling networks capable of collecting data over ecologically relevant scales (Smith et al. 1987; Dickey 1993). Many integrated observing systems currently are under development by the oceanographic community (Glenn et al. 1998). Although promising, optical approaches have been criticized because they provide only bulk composite signals for a given water mass, and the signatures for distinct phytoplankton species are difficult to discriminate (Garver et al. 1994).

Laboratory work suggests that partial discrimination of algal species from cellular absorption is possible. For example, Johnsen et al. (1994), using stepwise discriminant analyses to classify absorption spectra among 31 bloom-forming phytoplankton (representing the four main groups of phytoplankton with respect to accessory chlorophylls; i.e., chlorophyll *b*, chlorophyll *c*<sub>1</sub> and/or *c*<sub>2</sub>, chlorophyll *c*<sub>3</sub>, and no accessory chlorophyll), differentiated toxic chlorophyll *c*<sub>3</sub>-containing dinoflagellates and prymnesiophytes from taxa not having this pigment. However, problematic and toxic taxa could not be further separated from other chlorophyll