This is to certify that the

thesis entitled

A SEASONAL INVESTIGATION OF ENERGY TRANSFER AND FOOD WEB STRUCTURE OF THE DEEPWATER SCULPIN (MYOXOCEPHALUS THOMPSONI) IN GRAND TRAVERSE BAY, LAKE MICHIGAN

presented by

Colleen Frances Masterson

has been accepted towards fulfillment of the requirements for

M.S. degree in Environmental Geosciences

Major professor

Date 3-14-01

A SEASONAL INVESTIGATION OF ENERGY TRANSFER AND FOOD WEB STRUCTURE OF THE DEEPWATER SCULPIN (*MYOXOCEPHALUS THOMPSONI*) IN GRAND TRAVERSE BAY, LAKE MICHIGAN

By

Colleen Frances Masterson

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Geological Sciences

2001

ABSTRACT

A SEASONAL INVESTIGATION OF ENERGY TRANSFER AND FOOD WEB STRUCTURE OF THE DEEPWATER SCULPIN (*MYOXOCEPHALUS THOMPSONI*) IN GRAND TRAVERSE BAY, LAKE MICHIGAN

By

Colleen Frances Masterson

This study attempts to elucidate the diet of the deepwater sculpin in Grand Traverse Bay, Lake Michigan using a combination of stomach content and stable isotope techniques.

Stomach contents revealed that the diet of the deepwater sculpin was composed primarily of the amphipod, *Diporeia hoyi*. Other major prey items were mysids (*Mysis relicta*) and chironomid larvae. The abundance of minor prey items in the deepwater sculpin stomachs varied seasonally, and isotope data implied that these minor prey items may have been more important than stomach content data suggested.

The importance of long-term seasonal sampling in food web studies was clearly supported. Seasonal variation in carbon and nitrogen isotope values was observed in the food web members of Grand Traverse Bay, especially at lower trophic levels. An isotopic mixing model was used to explore the relative importance of seston, sinking POM and sediments to the deepwater sculpin. The model results suggested that seston was an important primary organic source for the benthic food web and that atmospheric deposition may be a significant source of contaminants to the deepwater sculpin in Grand Traverse Bay. Difficulties in the application of isotopic mixing models in food web studies are discussed and recommendations for future research are made.

To those who have kept me strong
and encouraged me to persist.

ACKNOWLEDGMENTS

I would first like to thank my committee members, Nathaniel Ostrom, Peggy Ostrom, Don Hall and Tom Coon for their support and thoughtful comments throughout the progress of my research. I am grateful to John Skubinna for collecting the stomach content data included in this report, and equally important, for his guidance in the early days of my grad school career at MSU. This work would not have been possible without the support of Michigan SeaGrant and the US EPA or the hard work of the many folks involved in the field sampling in Grand Traverse Bay.

I would like to thank my fellow graduate students for their advice and friendship and for providing me with a great amount of amusement in the last couple of years. Eileen McCusker and Amy Macrellis introduced me to the lab and showed me the ropes when I was the new kid in town. Mary Russ was a good sounding board in times of frustration and always had something quirky to make me smile. I would like to extend a special thank you for the remaining 2 of the '3 musketeers', Tucker McNulty and Andery Calkins. We have had some grand times together, and my invitation to you both is to continue to build and enjoy these friendships regardless of how many miles are separating us. I would also like to thank Chris Harvey for his thoughtful advice and for the many emails that had me laughing out loud.

I would like to thank my boyfriend and best friend, Chris Bzdok, for the humour and love he has shared with me before, during and after my time at MSU. I can't imagine finishing this work without you by my side. You deserve part of this degree for keeping me strong, keeping me sane and keeping me smiling. I think you're spectacular.

I would like to thank my Mom and Dad for their never-ending support. I want to thank you for leading me to believe that I could become anything I wanted to be, and for encouraging me in every route I've chosen to pursue. I'd like to thank my sister, Karen, for lifting my spirits when I needed it most, and for being the one who always understands me. I'm lucky to have a best friend and a sister in the same amazing woman. I'd also like to thank my brothers, Tim and Pat for just being downright awesome. My family have made me who I am today and I know that with them behind me, I can do anything.

And finally, thanks to my lil' Emy Mahina for all the love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In this study we present a combination of stomach content and stable isotope analyses to elucidate the food web structure of a benthic forage fish, the deepwater sculpin (*Myoxocephalus thompsoni*), in Grand Traverse Bay, Lake Michigan. Understanding the food web structure of Grand Traverse Bay at the level of forage fishes may provide future insight into the relative importance of different exposure routes of organic contaminants to higher level consumers of great commercial and recreational importance in the Great Lakes.

There are three hypothesized exposure pathways by which organic contaminants enter the food web of Great Lakes fishes: (1) atmospheric deposition transferred through the pelagic food web; (2) atmospheric deposition transferred, *via* rapidly-settling particles through the benthic food web, and; (3) transfer from historically-contaminated, in place sediments through the benthic food web (Baker et al. 1996). The primary organic sources (POS) associated with each of these exposure routes are: seston for route (1); sinking particulate organic matter (POM) for route (2); and sediments for route (3). A thorough analysis of food web structure provides estimates of the relative importance of the three POS to upper level consumers. The combination of these data with knowledge of contaminant levels in the POS will allow predictions to be made about the relative importance of the 3 exposure routes of organic contaminants to upper level consumers.

Stomach content and stable isotope analyses supply distinct, yet complimentary information in food web investigations. While stomach content data provide a direct assessment of ingested material, there are inherent difficulties in identifying partially

digested food items, and there is a bias towards the identification of food types with slow digestion rates (Gu et al. 1996, Gould et al. 1997b, Jennings et al. 1997). Stomach content data provide an estimate of the food items ingested, but may be misleading if assimilation is not considered. Assimilation may be incomplete, and certain organisms or parts of organisms may be preferentially digested over others. Stable isotope data strongly indicate nutritional dependence, as the isotopic composition of an organism is directly related to the organic matter assimilated from its diet (Tieszen et al. 1983, Rosenfeld et al. 1992, Hesslein et al. 1993, Ostrom et al. 1996). In addition, stomach contents provide only a snapshot of recently ingested material, while the isotopic composition of a consumer is a reflection of its food resources assimilated over a longer period of time from weeks to months (Harrigan et al. 1989, Hobson and Welch 1992, Gould et al. 1997a,b). The combination of stomach content and stable isotope analyses in food web research therefore offers a unique approach to assess trophic relationships and food web structure.

The stable isotope technique uses natural differences in the ^{13}C:^{12}C and ^{15}N:^{14}N ratios of organisms (conventionally expressed as $\delta^{13}C$ and $\delta^{15}N$ [1]) to identify food sources of carbon and nitrogen which move with predictable isotopic alterations from one

---

[1] Stable isotope ratios are reported as $\delta^{13}C$ and $\delta^{15}N$ (‰), according to:

$$\delta^{I}E = [(R_{sample}/R_{standard})-1] * 1000$$

where I is the heavy isotope of element E, either carbon or nitrogen, and R is the abundance ratio of the heavy to light isotope. Internationally recognized standards are Vienna Pee Dee Belemnite for carbon, and atmospheric N_2 for nitrogen.

trophic level to the next (Goering et al. 1990). Trophic fractionation is a term that describes the difference in $\delta^{13}C$ values or $\delta^{15}N$ values between a consumer and its food that is due to discrimination against the heavy isotope during metabolism and excretion (Peterson and Fry 1987). Generally, $\delta^{13}C$ values change by 0-1 ‰ per trophic level (e.g. DeNiro and Epstein 1978, Fry and Parker 1979, Peterson and Fry 1987, Harrigan et al. 1989, Goering et al. 1990, Keough et al. 1996). Such small increases in $\delta^{13}C$ values with trophic level, relative to individual variations and the precision of analytical techniques, can make the determination of trophic position based on $\delta^{13}C$ values difficult. Carbon isotopes are more often used to identify sources of organic carbon and to trace the flow of these carbon sources through an ecosystem (Fry and Scherr 1984, Goering et al. 1990, Gearing 1991). In contrast to $\delta^{13}C$ values, greater increases in $\delta^{15}N$ values of 3-4 ‰ generally take place between each trophic level (e.g. DeNiro and Epstein 1981, Minagawa and Wada 1984, Fry 1988, Goering et al. 1990, Keough et al. 1996). For this reason, $\delta^{15}N$ values are often used as more robust indicators than $\delta^{13}C$ values in determining trophic positions of organisms. With reliable estimates of trophic fractionation of both $\delta^{13}C$ and $\delta^{15}N$ values, it is possible to trace each step of the food web back to the primary sources of organic matter. The use of both carbon and nitrogen isotope ratios can be instrumental in resolving complex food web relationships that depend on multiple sources of organic matter (Creach et al. 1997).

While many food web studies have employed the use of stable isotopes and stomach content analyses, relatively few of these studies have investigated seasonal variation in food web structure (Gearing et al. 1984, Goering et al. 1990, Toda and Wada 1990, Leggett 1998, Neilson et al. 1998). Factors such as the seasonal availability of

nutrients, succession of plankton communities and changes in the spatial distribution of aquatic organisms may greatly affect temporal food web dynamics. Temporal change in trophic interactions is still one of the least understood factors affecting the biogeochemical material flow in aquatic ecosystems (Yoshioka et al. 1994). Therefore, efforts to obtain seasonal stomach content and isotope data will make important contributions to the understanding of food web dynamics.

The present study not only combines stomach content and stable isotope techniques to investigate the food web dynamics of the deepwater sculpin, but also probes the nature of temporal variation in food web structure. Deepwater sculpin are abundant benthic-dwelling secondary consumers in the benthic food web of the upper Great Lakes, including Grand Traverse Bay. Based on previous stomach content studies, they are thought to utilise benthic food resources nearly exclusively as adults, feeding mainly on amphipods, and to a lesser extent on mysids (Kraft and Kitchell 1986, Wojcik et al. 1986, Selgeby 1988). They are also known to feed to a varying extent on other seasonally important food resources, such as chironomids, fish eggs, larval fish and terrestrial insects (Wojcik et al. 1986). Deepwater sculpin are important prey for higher level consumers in Lake Michigan, including burbot (*Lota lota*), lake trout (*Salvelinus namaycush*), steelhead (*Oncorhynchus mykiss*), coho salmon (*Oncorhynchus kisutch*) and other salmonines (Wojcik et al. 1986).

This study attempts to elucidate the diet of the deepwater sculpin in Grand Traverse Bay, Lake Michigan, using a combination of stable isotope and stomach content techniques, and to assess seasonal variation in the isotopic composition and diet of food web members. By improving our understanding of nutrient transfers and trophic

interactions, food web studies in the Great Lakes may provide valuable insight into the bioaccumulation of organic contaminants in fish.

MATERIALS AND METHODS

Study Location and Sample Acquisition

All samples were collected on approximately a monthly basis from April to September, 1997 and 1998 in the western arm of Grand Traverse Bay, Lake Michigan (Figure 1). In 1997, samples were collected from study sites GT1 and GT3, while in 1998, the sampling effort focused only on site GT3. Site GT1 has a depth of 98 m and site GT3 has a depth of 112 m.

Deepwater sculpin were collected with a 4.9 m otter trawl with 33 mm body mesh and 5 mm codend mesh. Tows of 10-20 minutes were made near sites GT1 and GT3 at depths greater than 80 m. Collected fish were sorted for stable isotope analysis or stomach content analysis, and the weight and total length of each individual was recorded. Fish intended for stable isotope analysis were frozen immediately after collection, while fish intended for stomach content analysis were preserved in formalin (10% formaldehyde solution).

Zooplankton were collected using daytime oblique tows from 80m to the surface using a 1m diameter plankton net with 505 μm mesh. Bulk zooplankton samples were filtered through pre-combusted (500°C, 1 hr) GF/F glass fibre filters (Whatman) and frozen for isotope analysis.

A benthic sled was employed to collect macroinvertebrates such as amphipods, mysids, oligochaetes and chironomids (design modified from Nesler 1981). The sled was equipped with a 90 cm x 60 cm rectangular net with 750 μm mesh, and was towed along the bottom near sites GT1 and GT3 for 10-20 minutes per sample. Samples were sieved

through 595, 833, 1400 and 2000 μm mesh screens, and taxa were hand sorted and frozen for stable isotope analysis.

Seston samples were obtained by filtering 3-5 L of water from each of five depth intervals at low pressure through pre-combusted (500°C, 1 hr) Whatman GF/F glass fiber filters (McCusker et al. 2000). Sediments were collected using a box core or ponar grab. Sinking POM samples were collected in biweekly intervals by multi-sequencing sediment traps (8 in. OD, 8:1 aspect ratio; Eadie et al. 1984), which remained at the sampling site throughout the season.

Stomach Content Analysis

John Skubinna (Ph.D. candidate, Michigan State University Department of Fisheries and Wildlife) completed the identification of all deepwater sculpin stomach contents. Prey taxa were identified using digestion resistant hard parts (Balcer et al. 1984, Merritt and Cummins 1996). Optimas™ image analysis software was used to measure prey length or key morphological features of each prey item obtained. Care was taken in avoiding duplicate counts of the same individual by choosing morphological features that are singular in the morphology of the prey (e.g. body length, head width, etc.) when possible. The linear measurements of length or hard part size were converted to estimates of dry weight biomass of whole individuals using length-weight or hard part size-weight relationships (e.g. Dumont et al. 1975, Smock 1980, Sell 1982, Culver et al. 1985). Specific conversion relationships used are described in detail in the dissertation of John Skubinna (*in prep.*).

To assess the importance of each prey item in the deepwater sculpin diet, four indices of prey importance were calculated: (1) percent frequency of occurrence (% FO), the number of stomachs containing a single type of prey divided by the total number of stomachs containing organic matter; (2) percent of prey number (% PN), the number of items of a single prey taxon divided by the total number of all items in the sample; (3) percent of prey mass (% PM), the mass of a single prey taxon divided by the total mass of all material in the sample and; (4) percent prey index of relative importance (% IRI; Pinkas *et al.* 1971), the IRI of the food group divided by the IRI of the total diet, where IRI is calculated as:

$$IRI = \%\ FO\ (\%\ PN + \%\ PM) \qquad \text{(eq. 1)}$$

Monthly values of prey importance were obtained by pooling all prey items found in deepwater sculpin stomachs from that particular month. The seasonal mean was calculated for each index of prey importance, with each month weighted equally to account for unequal sample sizes and seasonal variation in prey importance. Each index of prey importance provides a unique perspective of diet with a different bias. Percent IRI incorporates the three traditional indices of prey importance, and is thought to minimize the biases of each, obtaining a more representative description of diet (Pinkas et al. 1971).

Stable Isotope Analysis

To provide a manageable sample, a subsample of each fish (~10 %) was obtained in preparation for isotope analysis. The isotopic signature of a subsample of this size was found to differ from that of whole fish by 0.07 ± 0.42 ‰ for $\delta^{15}N$ and 0.19 ± 0.39 ‰ for

$\delta^{13}C$ (n=5). The subsample was freeze dried, ground and lipid extracted for six hours in a soxhlet apparatus containing an azeotropic mixture of chloroform and methanol (87:13 v/v). After lipid extraction, the tissue was dried and homogenized with a Wig-L-Bug (Crescent Industries) mechanical mill. Bulk zooplankton and macroinvertebrate samples (bulk samples of many individuals) were prepared in the same manner as fish, with an additional step of acidification using 10 % HCl to remove carbonates prior to lipid extraction. Fish, zooplankton and macroinvertebrate samples were weighed (3-4 mg for $\delta^{15}N$ values; 0.4-0.5 mg for $\delta^{13}C$ values) into 6 mm x 4 mm ultra-light weight tin capsules (Elemental Microanalysis Ltd.), and analysed for isotopic abundances using a Carlo Erba NA 1500 nitrogen/carbon analyzer interfaced to a Micromass Prism mass spectrometer (Wong et al. 1992).

In preparation for stable isotope analysis, filters containing seston samples were dried (40°C), acidified with 10 % HCl to remove carbonates, and dried again. The seston sample was obtained by removing the surface layer of the filter containing the seston (McCusker et al. 2000). Sediments and sinking POM were freeze dried, acidified with 10 % HCl to remove carbonates, and dried again (40°C). Seston, sediments and sinking POM samples were placed in precombusted (500°C, 1hr) quartz tubes with excess precombusted CuO and Cu (approximately 3 g of each). The tubes were evacuated, sealed, and combusted at 850°C. The combustion products were separated cryogenically on a vacuum line and the isotopic composition of the purified carbon dioxide and nitrogen gas was determined on a Micromass Prism mass spectrometer.

Additional sediment trap samples are available for nitrogen and carbon isotope analysis, and modifications to the laboratory protocol (acidification with more

concentrated HCl and sample shaking to more thoroughly remove carbonates) may further improve the accuracy of the results. However, the results presented here are thought to be a representative subset of the available data.

Estimates of Trophic Fractionation

In diet analyses, mass balance equations can be used to estimate the relative contribution of different sources of carbon or nitrogen to a consumer, or if this is known, to estimate trophic fractionation. Stomach content analysis provided the relative importance of different prey items to the deepwater sculpin (based on the three indices of prey importance), and the average isotopic composition of the deepwater sculpin diet for each month was calculated as:

$$\delta^{15}N_{\text{sculpin diet}} = \sum_{i=1}^{n} f_i \delta^{15}N_i \quad \text{and} \quad \delta^{13}C_{\text{sculpin diet}} = \sum_{i=1}^{n} f_i \delta^{13}C_i \qquad \text{(eq. 2)}$$

where f is the fractional contribution of individual prey items to the deepwater sculpin (based on each index of prey importance) and n is the number of different prey items. When the isotopic composition of a prey item was not known for a particular month, isotope values were assumed to be equal to the nearest month with available data. Trophic fractionation factors for both $\delta^{15}N$ values (TF_n) and $\delta^{13}C$ values (TF_c) were then quantified by:

$$TF_n = \delta^{15}N_{\text{sculpin}} - \delta^{15}N_{\text{sculpin diet}} \quad \text{and} \quad TF_c = \delta^{13}C_{\text{sculpin}} - \delta^{13}C_{\text{sculpin diet}} \qquad \text{(eq. 3)}$$

Estimates of the Relative Contribution of Primary Organic Sources (POS) to Prey Items

To estimate the relative contribution of each POS to individual deepwater sculpin prey items, an isotopic mixing model of the following form (Harrigan et al. 1989) was used:

$$\delta^{15}N_{prey} - TF_n = a\delta^{15}N_{seston} + b\delta^{15}N_{sinkingPOM} + c\delta^{15}N_{sediments} \quad \text{(eq. 4)}$$

$$\delta^{13}C_{prey} - TF_c = a\delta^{13}C_{seston} + b\delta^{13}C_{sinkingPOM} + c\delta^{13}C_{sediments} \quad \text{(eq. 5)}$$

$$1 = a + b + c \quad \text{(eq. 6)}$$

where the terms a, b and c represent the percent contribution for nitrogen or carbon of each of three prey items, and TF_n and TF_c represent the difference in $\delta^{15}N$ or $\delta^{13}C$ values, respectively, between the consumer and its diet (trophic fractionation factors). This model requires that the three POS are isotopically distinct, which has been verified in previous studies (Takahashi et al. 1990, Yoshioka et al. 1994, Zohary et al. 1994, Ostrom et al. 1997).

Due to the lack of stomach content data from prey items of deepwater sculpin in this study, species-specific trophic fractionation factors for the mixing model were unavailable. Because trophic fractionation factors have been found to vary widely among taxa in previous studies, the mixing model was explored using both the trophic fractionation factors calculated in this study (between deepwater sculpin and sculpin diet), as well as trophic fractionation factors taken from the literature. The trophic fractionation factor adopted from the literature for $\delta^{15}N$ was 3.4 ‰ per trophic level, the widely cited average of Minagawa and Wada (1984). The trophic fractionation factor adopted from the literature for $\delta^{13}C$ values was 0.5 ‰ per trophic level, the midpoint of the commonly reported range of 0-1 ‰ (Peterson and Fry 1987).

Seasonal (6-month) averages of $\delta^{15}N$ and $\delta^{13}C$ values of deepwater sculpin prey items and POS were used in the mixing model to develop the best estimate of the long-term relative contributions of POS to the nutrition of the prey items. Because of the large seasonal variations observed in the isotopic compositions of deepwater sculpin prey items, each month was weighted equally in the seasonal mean to prevent a disproportionate contribution from months with larger sample sizes.

The amount of nitrogen or carbon assimilated from each POS by the deepwater sculpin was then determined by the relationship:

$$V_s = \sum_{i=1}^{n} f_i V_p \qquad \text{(eq. 7)}$$

where Vs is the percent contribution of a POS to the deepwater sculpin, f is the fractional contribution of the prey item to the deepwater sculpin, Vp is the fractional contribution of the POS to a prey item and n is the number of prey items. These techniques were used in an attempt to reach our ultimate goal of determining the relative importance of the three primary organic sources to higher trophic levels.

RESULTS

Stomach Content Analysis

Stomach content data were available from deepwater sculpin collected in April of 1997 and 1998, May, July, August and September of 1997 and June of 1998. An inter-annual comparison of deepwater sculpin stomach contents between April 1997 and April 1998 (John Skubinna, Department of Fisheries and Wildlife), found that the two years did not differ significantly in the distribution of biomass among the different prey items. A similar comparison using % IRI data found the relative proportions of prey items to differ by less than 1.5 % between the two years. Stomach content data from 1997 and 1998 were therefore combined to provide a full monthly data set from April through September.

For each month from April to September, sculpin stomach contents were summarized according to the 4 indices of prey importance, that included one measure of the frequency of occurrence of prey items in stomachs (% FO) and 3 measures of the relative abundance of prey items in stomachs (% PN, % PM and % IRI; Table 1). For each index of prey importance, the mean diet was calculated by taking the average of the indices for the six months.

Stomach content data indicated that the amphipod *Diporeia hoyi* was consistently the most abundant prey item in the deepwater sculpin diet, regardless of the month sampled (Table 1). On the basis of % FO, *D. hoyi* were present in 86.2 – 100.0 % of sculpin stomachs in each month and in 95.4 % of the stomachs on an annual basis. *D. hoyi* comprised 69.9 and 78.8 % of the annual sculpin diet based on % PN and % PM,

respectively. The index of prey relative importance (% IRI) also substantiated the importance of *D. hoyi* in the deepwater sculpin diet, with a prediction of 85.8 % of the annual diet.

Estimates of % FO indicated that opossum shrimp *(Mysis relicta)*, fish eggs and chironomid larvae were each present in over 38 % of deepwater sculpin stomachs annually, and estimates of % PN, % PM and % IRI indicated that each of these prey items comprised > 3 % of the annual sculpin diet. Minor prey items found in the stomachs of deepwater sculpin included chironomid pupae and adults, unidentified dipteran adults, annelids, microcrustacean zooplankton, aquatic and terrestrial beetles, and crayfish. Each of these minor prey items were found in fewer than 25 % of deepwater sculpin stomachs annually, and each comprised < 3 % (range 3.2-14.1 %) of the annual sculpin diet as estimated by % PN, % PM and % IRI.

While seasonal variation in the relative importance of most prey items to the deepwater sculpin over our six month sampling period was minor, notable variation was observed in the relative importance of some of the less abundant prey items in the deepwater sculpin stomachs. Fish eggs were most abundant in the sculpin stomachs during April and May (% FO of 89.7 and 100.0 %, % PN of 42.1 and 17.0 %, % PM of 10.4 and 3.5 %, and % IRI of 29.0 and 10.2 % for April and May, respectively), and declined in importance throughout the rest of the sampling season (% FO of 17.0 %, % PN of 3.0 %, % PM of 1.1 % and % IRI of 0.5 % by September). Beetles increased in importance in the sculpin diet later in the season, and peaked in abundance in July and September (% FO of 4.8 and 4.0 %, % PN of 0.2 and 0.0 %, % PM of 6.6 and 0.6 % and % IRI of 0.1 and 0.0 % for July and September, respectively).

The salient features of the deepwater sculpin stomach content data were reviewed here solely for the purpose of comparing indices of prey importance and as an introduction for further sections of this thesis combining stomach content and stable isotope analyses. A more extensive investigation of this stomach content analysis can be found in the Ph.D. dissertation of John Skubinna, Department of Fisheries and Wildlife (*in prep.*).

Stable Isotope Analysis

Isotopic compositions of food web members generally differed by less than 1 ‰ between 1997 and 1998 when monthly averages were compared, and yearly averages differed by less than 0.5 ‰. For this reason, data from the two years were pooled to include both sampling seasons and increase sample sizes. One exception was the isotopic composition of seston, which showed a dramatic enrichment in $\delta^{15}N$ values in 1998, with $\delta^{15}N$ values over 6 ‰ higher than those observed in 1997 (Table 2, Figure 2). Seston values from 1997 and 1998 are reported separately in Table 2 and Figure 2. Thus, while average isotope values of the two years combined were adopted for all other food web members, those analyses involving the isotopic composition of seston were repeated with 1997 seston values, 1998 seston values and the mean value of the two years combined.

No significant relationships were found between length of deepwater sculpin and $\delta^{15}N$ values ($R^2 = 0.0002$, Figure 3A) or $\delta^{13}C$ ($R^2 = 0.0029$, Figure 3B). Consequently, all sizes and ages of deepwater sculpin were combined for the remainder of the analyses and discussion.

Nitrogen isotope values generally increased with trophic level. For example, deepwater sculpin were enriched in ^{15}N relative to their major prey items, which were in turn enriched in ^{15}N relative to the three POS (Table 2, Figure 2). Trends in carbon isotope values were not as clear. Whereas the deepwater sculpin were enriched in ^{13}C relative to *D. hoyi* and *M. relicta*, they were depleted in ^{13}C relative to all other prey items (Table 2, Figure 2). *D. hoyi* and *M. relicta* were enriched in ^{13}C relative to only one of the POS (seston), while the remaining deepwater sculpin prey items were enriched in ^{13}C relative to all three POS (Table 2, Figure 2).

Seasonal Variation in $\delta^{15}N$ and $\delta^{13}C$ of Deepwater Sculpin and Average Diet

A unique feature of this study was the emphasis on seasonal trends in isotopic compositions. Seasonal variations were observed among lower trophic level food web members, with ranges of up to 8 ‰ in $\delta^{15}N$ values and 4 ‰ in $\delta^{13}C$ values (Table 2) between April and September. Much smaller ranges in $\delta^{15}N$ and $\delta^{13}C$ values were observed for the deepwater sculpin (0.6 ‰ range in $\delta^{15}N$ values and 0.4 ‰ range in $\delta^{13}C$ values; Table 2).

In contrast to the small range of $\delta^{15}N$ values of deepwater sculpin from April to September, the average deepwater sculpin diet exhibited considerable variation in $\delta^{15}N$ values over the sampling season, with an observed range of approximately 4 ‰ (Figure 4). The $\delta^{15}N$ value of the average diet decreased from April through June, peaked briefly in July, decreased to August and began to increase slightly in September (Figure 4). While estimates of the $\delta^{15}N$ value of the average diet derived from mass balance

equations using three different indices of prey importance differ slightly, they all display a general decline throughout the season (Figure 4).

Monthly estimates of trophic fractionation for $\delta^{15}N$ values were calculated by subtracting the $\delta^{15}N$ value of the average diet from the $\delta^{15}N$ value of the deepwater sculpin. The constant $\delta^{15}N$ values of the deepwater sculpin, combined with the seasonal decline in the $\delta^{15}N$ value of their average diet, resulted in a range of apparent trophic fractionation factors throughout the season. The trophic fractionation factor ranged from 0.9 to 4.4 ‰ based on % PN, from 1.9 to 5.2 ‰ based on % PM and from 1.5 to 4.9 ‰ based on % IRI. The average trophic fractionation between $\delta^{15}N$ values of the deepwater sculpin and its diet for the six-month sampling period was calculated by taking the mean trophic fractionation of all sampling months. The average trophic fractionation of $\delta^{15}N$ values was determined to be 3.3 ‰ based on % PN, 4.0 ‰ based on % PM and 3.9 ‰ based on % IRI (Table 3).

The seasonal trends observed in the $\delta^{13}C$ values of deepwater sculpin and their diet closely resembled the variation in $\delta^{15}N$ values, although the magnitude of seasonal variation differed between the two measures. The $\delta^{13}C$ values of deepwater sculpin displayed little seasonal variation, with an overall range in $\delta^{13}C$ values of only 0.4 ‰ (Figure 5). The $\delta^{13}C$ values of the average diet showed a substantially greater amount of seasonal variation than the deepwater sculpin, with a range of approximately 2 ‰ (Figure 5). According to all three indices of prey importance, the $\delta^{13}C$ values of the average diet declined from April through August, followed by a slight increase in September (Figure 5).

Monthly estimates of trophic fractionation for $\delta^{13}C$ values were calculated by subtracting the $\delta^{13}C$ value of the average diet from the $\delta^{13}C$ value of the deepwater sculpin. The constant $\delta^{13}C$ values of deepwater sculpin combined with the seasonal variation in the $\delta^{13}C$ values of the average diet again resulted in a range of apparent trophic fractionations throughout the season. The trophic fractionation in $\delta^{13}C$ values ranged from 0.2 ‰ to 2.4 ‰ based on % PN, from 1.4 to 2.7 ‰ based on % PM and from 0.7 to 2.7 ‰ based on % IRI. The average trophic fractionation of $\delta^{13}C$ was calculated to be 1.5 ‰ based on % PN, 1.9 ‰ based on % PM and 2.0 ‰ based on % IRI (Table 3).

Annual estimates of trophic fractionation for both $\delta^{15}N$ and $\delta^{13}C$ were calculated using linear regressions between months with available data to estimate isotope values of food web members in months where data were lacking. While seasonal variation undoubtedly occurs during the winter months, the estimated annual trophic fractionation factors differed from the calculated 6-month seasonal averages by less than 0.6 ‰ for $\delta^{15}N$ and 0.2 ‰ for $\delta^{13}C$.

Estimates of the Relative Contribution of POS to Prey Items

An isotopic mixing model was used to determine the relative contribution of carbon and nitrogen from each of the three POS to the deepwater sculpin prey items. The isotopic compositions of the average *D. hoyi* and *M. relicta* diets were calculated by correcting the actual $\delta^{15}N$ and $\delta^{13}C$ of *D. hoyi* and *M. relicta* for trophic fractionation. Due to the lack of stomach content data from prey items of deepwater sculpin in this study, and since trophic fractionation factors have been found to vary widely among taxa

in previous studies, the mixing model was explored using trophic fractionation factors calculated between deepwater sculpin and sculpin diet (Table 3), as well as trophic fractionation factors taken from the literature. The adopted literature trophic fractionation factors were 3.4 ‰ for $\delta^{15}N$ values (Minagawa and Wada 1984) and 0.5 ‰ for $\delta^{13}C$ values (midpoint of range reported by Peterson and Fry 1987). In addition, while yearly differences in the isotope values of most food web members were negligible and average values of the two years combined were adopted for the mixing models, the model was repeated with 1997 seston values, 1998 seston values and the mean value of the two years combined because of the large inter-annual discrepancy in isotopic composition of this single food web member.

In the application of the isotopic mixing model, it was assumed that *D. hoyi* were positioned one trophic level above the POS, since they are generally classified as detritivores and are thought to rely primarily on organic particles that settle from the water column to the sediments for their nutrition (Gardner et al. 1985, Quigley 1988, Gauvin et al. 1989). It was assumed that *M. relicta* were positioned at a trophic level 1.5 above that of the three POS. The trophic level of 1.5 above the three POS was chosen because bulk *M. relicta* samples included individuals of varying life stages. *M. relicta* are known to exhibit ontogenetic shifts in feeding behaviour, from herbivory as juveniles to increasing carnivory with maturity (Grossnickle 1982, Nero and Sprules 1986). While larger size classes tended to have higher isotope values when size classes of *M. relicta* were analysed separately in this study, sample sizes were low, and the ability to make conclusions based on size class effects on isotope values was restricted. All data were subsequently combined in the remainder of the analyses.

Table 4 outlines the results of the mixing model attempts used to determine the relative contribution of carbon and nitrogen from each of the three POS to *D. hoyi* and *M. relicta*. All mixing model attempts using the trophic fractionation factors calculated between deepwater sculpin and sculpin diet (from Table 3) failed to yield plausible solutions (Table 4). Two of the mixing model attempts using literature values of trophic fractionation resulted in plausible solutions (Table 4). Mixing model attempts using the estimated annual isotope values of food web members calculated by linear regressions between months with available data failed to provide any plausible results.

A graphical representation of the isotopic mixing model of *D. hoyi* and the three POS (using literature values of trophic fractionation) is shown in Figure 6, in which the area within each triangle represents isotopic compositions of the *D. hoyi* diet that would yield plausible solutions. The isotopic composition of the average *D. hoyi* diet did not lie within a triangle created with the isotope values of the three POS based on overall averages for 1997 and 1998 seston or 1998 seston values, but did provide plausible solutions based on 1997 seston values alone. The mixing model predicted that in 1997, 73.1 % of the nutrition of *D. hoyi* (in the form of carbon and nitrogen) was derived from seston, 19.7 % was derived from sediment, and only 7.2 % was derived from sinking POM (Figure 6). Because the mixing model did not provide plausible solutions when seston isotope values were used from 1998 or from the two years combined, it was not possible to determine the relative importance of the 3 POS to *D. hoyi* in 1998 using this analysis.

To further examine the prediction of the mixing model that seston was the primary POS that contributed nitrogen to *D. hoyi* in 1997, the $\delta^{15}N$ monthly averages of

D. hoyi throughout the season were plotted with the $\delta^{15}N$ values of seston and sinking POM (Figure 7). The seasonal trend in $\delta^{15}N$ values of *D. hoyi* resembled that observed in the 1997 seston nearly perfectly from April through August. The seasonal trend in the $\delta^{15}N$ values of *D. hoyi* was less similar to that observed in the sinking POM, particularly in the month of June (Figure 7). This may be further support that *D. hoyi* were indeed relying to a greater extent on seston than sinking POM in this system.

A graphical representation of the isotopic mixing model was also created for *M. relicta* and the three POS (Figure 8). While the mixing model for *M. relicta* was explored using the same suite of trophic fractionation factors used in the *D. hoyi* model (Table 4), the graphical representation again shows only results of the model using literature values of trophic fractionation. It was assumed that *M. relicta* were positioned at a trophic level 1.5 above that of the three POS. The isotopic composition of the average *M. relicta* diet was located within the triangle formed by the isotope values of the three POS when overall averages for 1997 and 1998 were used for all components (Figure 8). The isotopic mixing model predicted that 43.4 % of the nutrition of the *M. relicta* diet (in the form of carbon and nitrogen) was ultimately derived from seston, 3.7 % was derived from sinking POM, and 52.9 % was derived from sedimentary sources. The mixing model could not be used to determine the relative importance of the three POS to *M. relicta* using 1997 or 1998 seston isotope values alone.

Estimating the relative importance of the three POS as sources of carbon and nitrogen to fish eggs and chironomid larvae, the other main prey items of the deepwater sculpin, was more difficult. Fish eggs do not directly utilize any of the three POS. Thus, a mixing model analysis with fish eggs and these three endmembers would be

inappropriate. In addition, the isotopic mixing model of chironomid larvae and the three POS did not provide any plausible solutions using the trophic fractionation factors chosen and trophic levels between 1 and 1.5. The mixing model was also not applied to the minor prey items of the deepwater sculpin because seston, sinking POM and sediments were inappropriate endmembers for trophic relationships involving some of these prey items, and provide implausible solutions for others.

The relative contribution of carbon and nitrogen from the three POS to the deepwater sculpin was determined based on the results of the isotopic mixing models of *D. hoyi* and *M. relicta* alone. *D. hoyi* and *M. relicta* comprised the vast majority of the deepwater sculpin diet. Together, they comprised 74.6 % of the deepwater sculpin diet based on % PN, 90.3 % of the diet based on % PM and 89.6 % of the diet based on % IRI. Given that we had accounted for this large proportion of the diet, errors in the estimates of relative importance of POS to the deepwater sculpin were expected to be minimal. Under these circumstances, seston, sinking POM and sediments were discovered to be the ultimate source of 69.3-71.8 %, 6.8-7.1 % and 21.1-23.9 %, respectively, of the carbon and nitrogen of the deepwater sculpin (Figure 9). It is important to note that the mixing model of the deepwater sculpin actually had four endmembers contributing carbon and nitrogen using this method of calculation: 1997 seston (from the *D. hoyi* mixing model results), average 1997-1998 seston (from the *M. relicta* mixing model results), and average 1997-1998 sinking POM and sediments (from both the *D. hoyi* and *M. relicta* model results). While the overall results of the deepwater sculpin mixing model suggested that seston was the ultimate source of 69.3-71.8 % of the carbon and nitrogen for the deepwater sculpin (Figure 9), 1997 seston

actually represented 63.8-70.0 % of this overall contribution of seston, while average 1997-1998 seston represented only 1.8-5.5 % of the total.

DISCUSSION

Stomach Content Analysis

The four indices of prey importance explored in this study may each provide a unique perspective of prey importance owing to inherently different biases (Pinkas et al. 1971, Gould et al. 1997a). Sampling error, particularly in the case of low sample size, may bias % FO to a greater extent than the other indices. Percent PN may allow numerous small prey items to overshadow the importance of fewer large prey items, while % PM may be biased by the occurrence of a few large prey items that are not representative of the normal diet. Percent IRI incorporates the three previously described indices in an attempt to minimize the biases of each and obtain a more representative description of diet (Pinkas et al. 1971). An ideal index of prey importance would also incorporate a measurement of nutrition, such as calories per unit volume (Pinkas et al. 1971), but such analyses were beyond the scope of this study.

In this study, the four indices of prey importance differed in their diet assessment in each month as expected, with % IRI integrating the suggested significance of each prey item from % FO, % PN and % PM. The importance of *D. hoyi* to the deepwater sculpin diet was supported by the highest seasonal mean value of relative importance according to all four indices (Table 1). Large prey items, such as *M. relicta*, were deemed to comprise a large portion of the deepwater sculpin diet according to the traditional measure of % PM, while numerous small prey items like fish eggs were suggested to have a higher significance according to % FO and % PN (Table 1). The seasonal means of % PN, for example, suggested that *M. relicta* comprised 4.7 % of the deepwater

sculpin diet and fish eggs comprised 14.1 %. In contrast, the seasonal means of % PM suggested that *M. relicta* comprised 11.5 % of the deepwater sculpin diet while fish eggs comprised only 3.2 %. The seasonal means of % IRI provided a balance between the means of % PN and % PM, with *M. relicta* comprising 3.7 % of the deepwater sculpin diet and fish eggs comprising 6.5 %.

The most notable example of potential bias in stomach content data based on a single index of prey importance was evident in the comparison of results for the September deepwater sculpin diet. In this month, a single crayfish was found in a single deepwater sculpin stomach. Because of its large biomass relative to more common prey items, however, % PM suggested that crayfish comprised 19.1 % of the deepwater sculpin diet in September, and 3.2 % of the annual deepwater sculpin diet. Percent IRI, in contrast, suggested that crayfish comprised only 0.1 % of the September diet, and 0.0 % of the seasonal average. In any event, this prey item was removed from further analyses because of the lack of analogous stable isotope data. While it is important to take into consideration the potential biases of each index of prey importance, each index provided a unique perspective regarding the relative importance of each prey item to the deepwater sculpin in this study.

Seasonal Variation in $\delta^{15}N$ and $\delta^{13}C$ of Deepwater Sculpin and Average Diet

Seasonal variation in the $\delta^{15}N$ and $\delta^{13}C$ values of food web members was prevalent in Grand Traverse Bay. While differing amounts of seasonal variation were observed in the isotopic composition of the many food web members, the range of $\delta^{15}N$ values throughout the sampling season was always much higher than that observed in

$\delta^{13}C$ values. In fact, the seasonal variation of $\delta^{15}N$ values was consistently about twice that observed in $\delta^{13}C$ values, regardless of food web member examined. Although seasonal variability may be observed in both the $\delta^{15}N$ values of inorganic nitrogen and the $\delta^{13}C$ values of inorganic carbon, variation in $\delta^{15}N$ values at the base of the food web may be accentuated by shifts in phytoplankton uptake of the two inorganic nitrogen pools (NO_3 and NH_4), which differ isotopically by more than 10 ‰ (McCusker et al. 2000). This large amount of isotopic variation in nitrogen at the base of the food web may then be transferred through the food web.

The $\delta^{15}N$ and $\delta^{13}C$ values of the deepwater sculpin in Grand Traverse Bay exhibited very little seasonal variation compared with lower trophic level food web members (Figures 4 and 5). Higher trophic level organisms differ in growth and physiology from smaller, lower trophic level organisms, and have slower tissue turnover rates (Brett et al. 1969, Kitchell and Stewart 1977, Hansen and Christoffersen 1995, Dittel et al. 1997, Herzka and Holt 2000). Thus, the constant $\delta^{15}N$ and $\delta^{13}C$ values of the deepwater sculpin relative to its diet are not surprising, as strong seasonal signals observed at lower trophic levels are dampened out or minimized as trophic level increases (Cabana and Rasmussen 1996, Harvey and Kitchell 2000). Several other studies have also noted increasing isotopic variability at lower trophic levels (Bun and Boon 1993, Yoshioka et al. 1994, Zohary et al. 1994). The isotopic signature of growing broad whitefish (*Coregonus nasus*) was found to integrate the isotopic signature of its diet over a period greater than 1 year (Hesslein et al. 1993). Slow metabolic rates would be expected for organisms such as deepwater sculpin that live in extremely cold water ($\leq$ 4°C). Therefore, turnover times are likely greater than the growing broad whitefish.

With such slow tissue turnover, the invariable $\delta^{15}N$ and $\delta^{13}C$ values of the deepwater sculpin over the time span of this study are not surprising.

Previous studies have noted high $\delta^{15}N$ values of lower trophic level food web members in the spring, followed by a decline throughout the summer months (Goering et al. 1990, Toda and Wada 1990, Bun and Boon 1993, Yoshioka et al. 1994). The observed seasonal decline in the $\delta^{15}N$ values of the average sculpin diet (Figure 4) was likely due to a combination of several factors. The $\delta^{15}N$ values of the major prey items of the deepwater sculpin may have been tracking a similar seasonal decline in $\delta^{15}N$ values observed in two of the primary organic sources at the base of the food web in Grand Traverse Bay: seston and sinking POM (Table 2). In addition, prey items with high $\delta^{15}N$ values (i.e. fish eggs) were more important to the sculpin diet in the spring, while prey items with particularly low $\delta^{15}N$ values (i.e. beetles) became more important to the sculpin diet as the season progressed (Table 1; Figure 2). Although fish eggs and beetles comprised a relatively small portion of the overall deepwater sculpin diet, their isotopic compositions were quite unique relative to more common prey items. A combination of system-wide spring enrichment and the availability or selection of prey items with unique isotopic signatures was most likely controlling the seasonal decline in $\delta^{15}N$ values of the average deepwater sculpin diet in Grand Traverse Bay.

The relatively constant $\delta^{15}N$ and $\delta^{13}C$ values of the deepwater sculpin, combined with the observed variation in the monthly estimates of the average isotopic composition of the sculpin diet, resulted in a large range of trophic fractionation factors for both $\delta^{15}N$ and $\delta^{13}C$ values. Consequently, it was difficult to define an accurate single estimate of trophic fractionation of $\delta^{15}N$ and $\delta^{13}C$ values for the deepwater sculpin in Grand Traverse

Bay. Previous studies have suggested that trophic fractionation is better represented by seasonal averages of $\delta^{13}C$ and $\delta^{15}N$ values than by instantaneous values (Neilson et al. 1998), and given the large range in fractionation factors in this study, the best estimate of fractionation was calculated here as a 6-month seasonal average. It is recognized that such averages are simply estimates and that because seasonal variation likely continues during the winter months, additional months of data would further refine these trophic fractionation factors. Our results may be biased by the lack of sampling during the winter months, and this stresses the importance of sampling throughout the entire year.

Trophic fractionation factors calculated between deepwater sculpin and sculpin diet (ranging from 3.3 ‰ to 4 ‰) were within the commonly reported range of 3-4 ‰ (e.g. DeNiro and Epstein 1981, Minagawa and Wada 1984, Fry 1988, Harrigan et al. 1989, Goering et al. 1990, Keough et al. 1996, Gorokhava and Hansson 1999, Roth and Hobson 2000). There is a range of trophic fractionation factors in $\delta^{15}N$ values among taxa, and fractionation factors well below 3‰ (Dittel et al. 1997, Tatrai et al. 1999, Adams and Sterner 2000, Herzka and Holt 2000) and as high as 5 or 6 ‰ (Estep and Vigg 1985, Adams and Sterner 2000) have been recorded. The trophic fractionation of $\delta^{13}C$ values between deepwater sculpin and sculpin diet (ranging from 1.5 ‰ to 2 ‰) was quite high relative to other studies, which often cite fractionation factors of 1 ‰ or less (e.g. DeNiro and Epstein 1978, Fry and Parker 1979, Peterson and Fry 1987, Goering et al. 1990, Keough et al. 1996, Dittel et al. 1997). However, a large range of trophic fractionation in $\delta^{13}C$ values has also been reported among taxa in the literature, ranging from negative trophic fractionation factors (DeNiro and Epstein 1978, Hesslein et al. 1991, Zohary et al. 1994, Kiriluk et al. 1995) to those greater than 2 ‰ (DeNiro and

Epstein 1978, Yoshioka and Wada 1994, Gu et al. 1996, Tatrai et al. 1999, Roth and Hobson 2000).

Our somewhat high estimates of carbon trophic fractionation relative to those in the literature may have been a result of a bias in our stomach content analysis. Literature values of trophic fractionation determined with methods other than stomach content analysis (ex. laboratory feeding experiments) would not be affected by such biases. While stomach content analyses were exhaustive, there are inherent biases in identifying only those food items that are still distinguishable after partial digestion and those food items with slow rates of digestion (Gu et al. 1996, Gould et al. 1997b, Jennings et al. 1997). Small, relatively soft-bodied prey items like chironomids and fish eggs may have been under-represented in the results of stomach content analyses, while large-bodied prey with protective carapaces like mysids and amphipods may have been over-represented. Because some of the minor prey, such as chironomid larvae, fish eggs and beetles, had very unique carbon isotopic compositions relative to more common prey items (Figure 2), a shift in diet to include a larger proportion of these minor prey could dramatically affect the isotope values of deepwater sculpin. In addition, variations in the isotopic composition of the minor prey items may not have been taken sufficiently into account because of the small number of minor prey samples collected. The application of more common literature values of trophic fractionation in this study (~ 3 ‰ for $\delta^{15}N$ values and ~ 1 ‰ for $\delta^{13}C$ values) would imply a much larger proportion of minor prey items in the deepwater sculpin diet (Figure 2). Calculating trophic fractionation factors in this manner, based on a combination of stomach content and stable isotope analyses (equations 2 and 3), assumes that ingestion and assimilation are equal. The model is, in

part, based on stomach content analysis and is therefore not entirely free of error associated with differential digestion. Differential digestion may actually play a significant role in the nutrition of the deepwater sculpin. Our trophic fractionation factors may have, therefore, been biased somewhat by the stomach content analysis, and minor prey items may play a larger role in the nutrition of deepwater sculpin than previously thought.

In summary, large seasonal variations in trophic fractionation were observed in this study. Relatively high estimates of mean trophic fractionation factors may indicate not only the importance of sampling throughout an annual cycle, but also the increased importance of minor prey items to the deepwater sculpin diet. Trophic fractionation may vary among organisms and environments, and although our estimates were at the upper limit or above most commonly reported values, they were not outside the range of previously observed results. There is clearly a strong seasonal influence in Grand Traverse Bay, and it is easy to recognize the importance of long-term data in isotopic food web studies upon reviewing these results.

The models described below are particularly sensitive to estimates of trophic fractionation. We have made a best attempt to investigate results of the models with our calculated trophic fractionation factors as well as literature values of trophic fractionation, but but admit that errors in estimates of fractionation may greatly alter the model results.

Estimates of the Relative Contribution of POS to Prey Items

The isotopic mixing model assumed that seasonal averages of isotopic compositions were the best estimates of long-term assimilation for the study organisms in Grand Traverse Bay, and that trophic relationships could be explored using these seasonal averages. The mixing model was used to predict the relative contribution of carbon and nitrogen from each of the three POS to the prey items of the deepwater sculpin. This was an important step leading to the ultimate determination of the relative importance of the three POS to the deepwater sculpin of Grand Traverse Bay.

D. hoyi use benthic habitats almost exclusively, and are thought to rely primarily on organic particles that settle from the water column to the sediments (Gardner et al. 1985, Quigley 1988, Gauvin et al. 1989). The mixing model indicated that seston was the most important source of carbon and nitrogen for *D. hoyi* in 1997. Seasonal isotope values supported this idea, as the $\delta^{15}N$ values of *D. hoyi* exhibited nearly identical seasonal trends to those observed in the seston; trends that were less similar to those observed in the $\delta^{15}N$ of sinking POM (Figure 7). This heavy reliance of *D. hoyi* on seston throughout the season is inconsistent with previous assertions that amphipods depend on sinking POM for their sustenance in the spring, and rely on lipids gained during this period throughout the remainder of the year (Gardner et al. 1985, Gauvin et al. 1989).

Several factors may be responsible for the observation that *D. hoyi* relied primarily on carbon and nitrogen from seston in 1997. *D. hoyi* in Grand Traverse Bay may have consumed primarily sinking POM, but may have assimilated only the portion of sinking POM that was most similar to the seston, isotopically. That is, they may have

been selectively feeding or selectively assimilating the freshest and most nutritious portion, or the smallest size fraction, of the sinking POM. If fresh algal material in the sinking POM was available for a large portion of the year, then *D. hoyi* would be expected to continue feeding, rather than resort to their lipid stores for sustenance during this time period. In fact, *D. hoyi* collected from Grand Traverse Bay were not found to have significantly higher lipid stores in the spring than later in the sampling season (Stapleton et al. *in prep.*). This is consistent with previous observations of intense feeding by *D. hoyi* on fresh settling algal particles when available (Gardner 1985, Gauvin et al. 1989).

The physical structure of Grand Traverse Bay in 1997 may also have facilitated the direct use of seston by *D. hoyi*. In 1997, a subthermocline chlorophyll maximum was present in Grand Traverse Bay (Macrellis 1999). There was, therefore, no structural barrier to mixing between algal production and the direct utilization of this nutritious resource by *D. hoyi*. In contrast, the chlorophyll maximum was above the thermocline for most of the year in 1998 (Macrellis 1999, McCusker et al. 1999), which may have limited the availability of recently produced algal particles to *D. hoyi*.

It remains unclear what POS *D. hoyi* were utilising in 1998. Selective feeding may again have played a role, but it is clear that seston, sinking POM and sediments were not suitable endmembers for the isotopic mixing model investigating the trophic relationships of *D. hoyi* in 1998 (Figure 6). Selective feeding or assimilation of particular fractions of the POS may pose difficulties in the selection of appropriate endmembers for an isotopic mixing model of this form. The actual POS that contributed to the base of the food web may have been masked in the analysis because of selective feeding by *D. hoyi*,

and may be a combination of organic sources including uncommon material at times like partially decomposed fish corpses. In addition, organisms may assimilate nitrogen and carbon from different food sources (Kikuchi and Wada 1996, Liden and Angerbjorn 1999), introducing the possibility that *D. hoyi* did indeed rely on sinking POM as a major source of nutrition in the form of nitrogen, but relied on seston or another nutritional source for carbon. Such de-coupling of nitrogen and carbon resources would present additional difficulties in interpreting mixing model results. Finally, despite efforts to preserve sediment trap material, it may possible that the sinking POM collected by the sediment traps in Grand Traverse Bay were altered somewhat by partial decomposition and rendered isotopically distinct from the sinking POM that actually arrived at the lake bottom. The sinking POM delivered to the bottom of the bay would likely have been relatively fresh and unaltered by microbes at the time of delivery and ingestion by *D. hoyi*, relative to the sediment trap samples analysed for isotopic composition.

The role of *M. relicta* in the food webs of freshwater lakes is often difficult to characterize (Leggett 1998). *M. relicta* are omnivorous crustaceans which exhibit diet vertical migrations and ontogenetic shifts in feeding behaviour (Rudstam et al. 1989, Leggett 1998, Rudstam et al. 1998, Branstrator et al. 2000). The results of the isotopic mixing model suggested that approximately half of the carbon and nitrogen of the *M. relicta* diet was ultimately derived from each of seston and sedimentary sources, while sinking POM was minor. Although the considerable role of sediment in the nutrition of *M. relicta* may be somewhat surprising, selective feeding on the surface detritus of the sediment layer during the day by *M. relicta* directly, or by their zooplankton prey, may have resulted in this observation. Large numbers of calanoid copepods and mysids in

contact with the sediment surface have previously been observed using a video camera mounted on an ROV in central Lake Michigan (Lee and Hall, personal communication).

The isotopic mixing model of *M. relicta* and the three POS provided plausible results only when average 1997 and 1998 seston isotope values were utilized, and not when 1997 or 1998 seston values were used alone. However, the 1997 seston values resulted in a triangle in Figure 8 that nearly provided plausible solutions for the diet of *M. relicta*. Based on the discussion of the *D. hoyi* mixing model, the 1997 seston may be more likely to influence the Grand Traverse Bay food web because of the subthermocline chlorophyll maximum present in that year. In contrast, the chlorophyll maximum was above the thermocline for much of the year in 1998. The thermocline likely acted as a barrier for mixing the seston to greater depths. *M. relicta* may have been less influenced by this barrier to mixing in 1998 (resulting in a plausible model including both 1997 and 1998 seston) than *D. hoyi* because of their diel vertical migrations from the benthos to the metalimnion in pursuit of food (Lasenby and Langford 1973). These vertical migrations may have allowed *M. relicta* to take advantage of seston particles in the metalimnion directly and it may have provided access to zooplankton prey that feed on seston particles in the epilimnion and metalimnion.

The mixing model of *M. relicta* and the three POS may have been biased by the difficulty of assigning of a trophic level for *M. relicta*. *M. relicta* may both prey on and compete with zooplankton for food (Johannsson et al. 1994). The assumption that *M. relicta* was positioned 1.5 trophic levels above the POS was based on a parsimonious estimate between a juvenile herbivore which would be positioned 1 trophic level above the POS, and an omnivorous or carnivorous adult which may be positioned 1.5-3 trophic

levels above the POS. However, studies have often found mysids to rely nearly exclusively on zooplankton for their sustenance (Lasenby and Langford 1973, Leggett 1998). The complexity of the food web at the level of bulk zooplankton alone makes it difficult to accurately assess the diet of *M. relicta* using this type of model. A thorough stomach content analysis on the *M. relicta* collected in this study may have provided additional insight into the feeding habits of *M. relicta* and a more accurate trophic level could have been assigned. A previous attempt at determining trophic fractionation through stomach content analysis of *M. relicta* found a large range of fractionation factors, and the author resorted to literature values of fractionation as the most reliable estimates available (Leggett 1998). The estimates of trophic fractionation in this study may have been an additional source of error to the *M. relicta* isotope model.

Estimating the relative importance of the three POS to prey items other than *D. hoyi* and *M. relicta* was more difficult. Many of the prey items of the deepwater sculpin were not expected to depend on the three POS measured in this study. Fish eggs do not directly utilize any of the three POS. Chironomids do not feed during the pupal stage of their life cycle. In fact, the isotopic composition of chironomid pupae was quite high compared to chironomid larvae, possible evidence of the effects of starvation and enrichment of $\delta^{15}N$ and $\delta^{13}C$ values (Figure 2). In addition, the isotopic composition of chironomid adults, unidentified dipteran adults and terrestrial beetles are undoubtedly heavily influenced by terrestrial sources of carbon and nitrogen. While many of these minor prey items had unique isotopic signatures capable of affecting the $\delta^{15}N$ and $\delta^{13}C$ values of the deepwater sculpin, they comprised a very small amount of the total prey consumed. Assuming minor prey items were not grossly underestimated in the stomach

content analyses, they were unlikely to significantly bias the interpretation of the relative importance of seston, sinking POM and sediments as sources of carbon or nitrogen to the deepwater sculpin.

Based on the results of the isotopic mixing models of *D. hoyi* and *M. relicta*, the relative importance of each of the three POS as sources of carbon and nitrogen to the deepwater sculpin was determined. Seston was found to be, by far, the most important POS to the deepwater sculpin, followed by sediments and sinking POM (Figure 9). Finding an exclusively benthic feeder like the deepwater sculpin to ultimately derive ~ 70 % of its carbon and nitrogen from seston was somewhat unexpected. The heavy reliance on seston was the result of the fact that the majority of the deepwater sculpin diet consisted of *D. hoyi*, and *D. hoyi* were found to rely primarily on seston. Again, the true mechanism of nutrient transport may have been a direct route from seston through *D. hoyi* to the deepwater sculpin, or an indirect route from sinking POM with selective feeding by *D. hoyi* on a fraction of the sinking POM to the deepwater sculpin. It is important to keep in mind that these results are based on *M. relicta* and *D. hoyi* only, and exclude any influence of minor prey items on the relative proportions of the three POS to the deepwater sculpin.

The isotopic mixing models thus predicted that the majority of the carbon and nitrogen of deepwater sculpin in Grand Traverse Bay was ultimately derived from seston. Because seston is the POS associated with an exposure route of organic contaminants based on atmospheric deposition, these results imply that atmospheric deposition may in fact be a very important source of organic contaminants to the deepwater sculpin. However, these data must be used in conjunction with contaminant concentration data to

accurately determine the relative importance of the three hypothesized exposure routes of organic contaminants to the deepwater sculpin.

Sources of Error and Recommendations for Future Research

It is clear upon completion of this study that there are many challenges in food web research based on stable isotope analysis. Temporal variation in isotope values of food web members, particularly at lower trophic levels, made it impossible to derive an accurate single estimate of trophic fractionation between deepwater sculpin and sculpin diet. Seasonal averages were used as a best estimate of trophic fractionation, but seasonal variation continues during winter months when samples were not collected, and additional months of data may further refine such estimates. Calculating trophic fractionation factors by assuming that stomach contents accurately define the assimilated diet of the deepwater sculpin also provided a source of error to our analysis, as ingestion may not be equal to assimilation.

Difficulties also arose in assuming a trophic fractionation factor for the mixing model used to determine the relative importance of the three POS to *D. hoyi* and *M. relicta*. Trophic fractionation factors have been shown to vary greatly among species, diets and tissue types (ex. DeNiro and Epstein 1978, Tieszen et al. 1983, Minagawa and Wada 1984, Estep and Vigg 1985, Hobson and Clark 1992, Gu et al. 1996, Hogberg 1997, Focken and Becker 1998, Fantle et al. 1999, Gorokhova and Hansson 1999, Adams and Sterner 2000, Roth and Hobson 2000). The mixing models of *D. hoyi* and *M. relicta* were explored using the fractionation factors calculated with the deepwater sculpin and sculpin diet, as well as the most commonly reported literature values. However, given

that fractionation factors have been shown to vary even between similar types of organisms fed known diets, in the case of two species of fish (tilapia and common carp; Focken and Becker 1998) and two species of mysids (Gorokhova and Hansson 1999) in laboratory experiments, it is very problematic to apply fractionation factors among taxa. The first step in resolving such difficulties would be to perform laboratory feeding experiments with *D. hoyi* and *M. relicta* with known diets to accurately determine species-specific trophic fractionation factors. The effect of ontogenetic feeding shifts of *M. relicta* on isotopic composition should also be explored in more detail in future research, to more accurately define the trophic position of M. relicta above the POS.

The many difficulties that arose with the mixing model efforts in this study suggest that a more complex model may need to be developed to accurately assess trophic relationships with stable isotopes. Even with a more extensive temporal scale, a simple time lag approach to the current mixing model is not likely to provide accurate results, as only a portion of the tissue of a consumer would be affected by short-term feeding behaviour. A dynamic modeling approach incorporating temporal variations in isotopic signatures and the growth, metabolism and tissue turnover of food web members may increase the ability to test hypotheses and build inferences about food web linkages in aquatic ecosystems (Harvey and Kitchell 2000).

Given the great potential value of stable isotopes in food web studies, surprisingly little attention has been paid to the underlying physiological and biochemical mechanisms that account for trophic enrichment (Adams and Sterner 2000). Recent suggestions that organisms may disproportionately assimilate carbon and nitrogen from different food sources (Kikuchi and Wada 1996, Fantle et al. 1999) may actually prevent

the reliable use of mixing models in food web studies altogether. To thoroughly understand the implications of isotopic food web studies, scientists may need delay large ecosystem research for a time and first investigate trophic relationships through controlled laboratory investigations of the isotopic compositions of consumers and their diets. Studies combining such investigations with those in natural ecosystems could provide considerable new insight, and result in a more solid foundation from which future food web research could evolve.

SUMMARY AND CONCLUSIONS

This study was an attempt to elucidate the diet of the deepwater sculpin in Grand Traverse Bay, Lake Michigan using a combination of stomach content and stable isotope techniques. Stomach content analysis revealed that the diet of the deepwater sculpin was composed primarily of the amphipod, *D. hoyi*. Seasonal variation was observed in the abundance of minor prey items in the deepwater sculpin stomachs, and isotope data implied that these minor prey items may have been more important than stomach content data suggested. Four different indices of prey importance were used to summarize the stomach content data, and each provided a unique perspective of deepwater sculpin diet. The importance of long-term seasonal sampling was clearly supported by our study. Seasonal variations in isotopic composition were observed in the food web members of Grand Traverse Bay, and were particularly noteworthy at lower trophic levels. An isotopic mixing model was applied to the data in an attempt to determine the relative importance of the three POS to consumers. The mixing model results revealed that seston was an important POS of the benthic food web, suggesting that atmospheric deposition may be an significant source of contaminants to the deepwater sculpin in Grand Traverse Bay. Difficulties in the application of isotopic mixing models in food web studies were discussed and recommendations for future research were made.

TABLES

Table 1. Summary of Grand Traverse Bay deepwater sculpin stomach contents*, expressed as percent frequency of occurrence (% FO), percent prey number (% PN), percent prey mass (%PM) and percent index of relative importance (% IRI).

Food Item	April	May	June	July	August	September	Mean
Diporeia hoyi							
% FO	86.2	100.0	100.0	98.1	94.4	94.0	95.4
% PN	42.1	82.5	79.3	70.2	75.6	69.9	69.9
% PM	72.4	96.5	86.3	71.5	76.6	67.1	78.8
% IRI	60.8	89.7	90.2	82.0	84.9	84.4	85.8
Mysis relicta							
% FO	55.2	0.0	37.6	53.8	49.4	34.0	38.4
% PN	7.8	0.0	1.5	8.3	6.4	4.0	4.7
% PM	15.2	0.0	7.6	16.6	18.1	11.8	11.5
% IRI	7.8	0.0	1.9	7.9	7.1	3.3	3.7
Fish Eggs							
% FO	89.7	100.0	55.3	63.5	42.7	17.0	61.4
% PN	42.1	17.0	9.3	8.2	4.7	3.0	14.1
% PM	10.4	3.5	2.5	0.6	0.9	1.1	3.2
% IRI	29.0	10.2	3.6	3.3	1.4	0.5	6.5
Chironomidae larvae							
% FO	50.0	0.0	68.2	70.2	70.8	64.0	53.9
% PN	4.0	0.0	7.8	11.5	11.0	12.5	7.8
% PM	1.8	0.0	3.0	4.5	4.3	5.9	3.2
% IRI	1.8	0.0	4.0	6.6	6.4	8.0	3.6
Chironomidae pupae							
% FO	0.0	0.0	18.8	6.7	1.1	3.0	4.9
% PN	0.0	0.0	0.6	0.3	0.0	0.2	0.2
% PM	0.0	0.0	0.3	0.1	0.0	0.1	0.1
% IRI	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Chironomidae adults							
% FO	0.0	0.0	0.0	0.0	1.1	1.0	0.4
% PN	0.0	0.0	0.0	0.0	0.0	0.1	0.0
% PM	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% IRI	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 1 (cont'd)

Food Item	April	May	June	July	August	September	Mean
Unidentified Dipteran Adults							
% FO	0.0	0.0	0.0	0.0	0.0	1.0	0.2
% PN	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% PM	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% IRI	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Annelids							
% FO	12.1	33.3	25.9	18.3	21.3	27.0	23.0
% PN	0.5	0.6	1.2	0.8	0.8	2.0	1.0
% PM	0.0	0.1	0.1	0.1	0.0	0.2	0.1
% IRI	0.0	0.1	0.2	0.1	0.1	0.4	0.1
Copepoda							
% FO	31.0	0.0	3.5	7.7	10.1	14.0	11.1
% PN	3.2	0.0	0.1	0.3	1.1	0.9	0.9
% PM	0.1	0.0	0.0	0.0	0.0	0.0	0.0
% IRI	0.6	0.0	0.0	0.0	0.1	0.1	0.1
Bythotrephes cederstroemi							
% FO	5.2	0.0	3.5	2.9	6.7	57.0	12.6
% PN	0.2	0.0	0.1	0.2	0.2	6.7	1.2
% PM	0.0	0.0	0.0	0.0	0.0	0.7	0.1
% IRI	0.0	0.0	0.0	0.0	0.0	3.0	0.1
Beetles **							
% FO	1.7	0.0	2.4	4.8	1.1	4.0	2.3
% PN	0.0	0.0	0.1	0.2	0.0	0.0	0.2
% PM	0.0	0.0	0.2	6.6	0.0	0.6	3.0
% IRI	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Number of stomachs analysed	58	3	85	104	89	100	439

* stomach content analyses completed by PhD candidate John Skubinna, Department of Fisheries and Wildlife.

** includes aquatic Hemiptera and aquatic and terrestrial Coleoptera

Table 2. Nitrogen and carbon isotope values of food web members collected from Grand Traverse Bay, Lake Michigan. Isotope values shown are averages for each month for the years 1997 and 1998 combined. Values are reported as averages ± 1 S.D. ND = no data.

Food Web Member	April	May	June	July	August	September	Mean
Primary Organic Sources:							
Seston (>0.45um) *							
1997 $\delta^{15}N$ (‰)	6.6	6.1	4.8	5.0	3.6	7.4	5.6
1997 $\delta^{13}C$ (‰)	-28.9	-29.2	-29.3	-30.7	-27.5	-26.2	-28.6
1998 $\delta^{15}N$ (‰)	13.7	12.4	13.0	ND	8.8	12.5	11.9
1998 $\delta^{13}C$ (‰)	-28.5	-28.4	-28.0	ND	-26.5	-24.1	-27.1
Sinking POM							
$\delta^{15}N$ (‰)	9.4	8.9	9.4	7.3	4.9	5.7	7.6
$\delta^{13}C$ (‰)	-22.7	-24.9	-24.1	-25.0	-23.7	-22.0	-23.7
Sediment							
$\delta^{15}N$ (‰)	ND	5.1	ND	ND	ND	ND	5.1
$\delta^{13}C$ (‰)	ND	-25.6	ND	ND	ND	ND	-25.6
Invertebrates: **							
Diporeia hoyi							
$\delta^{15}N$ (‰)	11.2 ± 0.3	9.9 ± 0.8	9.5 ± 0.7	9.8 ± 0.7	7.6 ± 0.1	8.2 ± 0.6	9.4
$\delta^{13}C$ (‰)	-26.2 ± 0.6	-26.8 ± 0.4	-27.0 ± 0.4	-27.7 ± 0.2	-27.3 ± 0.2	-26.6 ± 1.0	-26.9
n	5	7	4	4	3	4	27
Mysis relicta							
$\delta^{15}N$ (‰)	12.6 ± 0.8	12.7 ± 1.1	11.4 ± 1.0	12.3	11.6	10.7 ± 0.5	11.9
$\delta^{13}C$ (‰)	-26.1 ± 0.3	-25.7 ± 0.8	-25.8 ± 0.5	-26.2	-26.4	-24.4 ± 1.9	-25.8
n	4	5	3	2	2	4	20

Table 2 (cont.)

Food Web Member	April	May	June	July	August	September	Mean
Fish Eggs							
$\delta^{15}N$ (‰)	ND	14.9	13.6	ND	ND	ND	14.3
$\delta^{13}C$ (‰)	ND	-22.0	-23.0	ND	ND	ND	-22.5
n	ND	1	1	ND	ND	ND	2
Chironomidae larvae							
$\delta^{15}N$ (‰)	13.2 ± 1.1	12.0	12.5	14.8	12.5	13.2 ± 1.7	13.0
$\delta^{13}C$ (‰)	-23.5 ± 0.3	-24.2	-21.8	-23.4	-24.8	-22.9 ± 1.0	-23.4
n	3	2	1	2	2	3	13
Chironomidae pupae							
$\delta^{15}N$ (‰)	ND	14.0	14.3	14.2	ND	14.7	14.3
$\delta^{13}C$ (‰)	ND	-22.8	-18.7	-21.7	ND	-19.7	-20.7
n	ND	2	1	2	ND	2	7
Chironomidae adults							
$\delta^{15}N$ (‰)	ND	6.7	ND	ND	ND	ND	6.7
$\delta^{13}C$ (‰)	ND	-18.7	ND	ND	ND	ND	-18.7
n	ND	2	ND	ND	ND	ND	2
Unidentified Dipteran Adults							
$\delta^{15}N$ (‰)	ND	6.1	ND	ND	ND	ND	6.1
$\delta^{13}C$ (‰)	ND	-20.7	ND	ND	ND	ND	-20.7
n	ND	1	ND	ND	ND	ND	1
Annelids							
$\delta^{15}N$ (‰)	13.8	13.6	9.4	ND	10.7	10.9 ± 0.7	11.6
$\delta^{13}C$ (‰)	-24.2	-23.6	-23.1	ND	-22.6	-23.6 ± 1.2	-23.4
n	1	1	1	ND	2	3	8

Table 2 (cont.)

Food Web Member	April	May	June	July	August	September	Mean
Bulk Zooplankton (>505um)							
$\delta^{15}N$ (‰)	13.7	11.8 ± 1.6	10.3	9.5	6.4 ± 0.3	5.8	9.6
$\delta^{13}C$ (‰)	-27.5	-28.3 ± 0.7	-28.0	-28.1	-27.4 ± 0.8	-27.3	-27.8
n	2	5	1	2	3	2	15
Bythotrephes cederstroemi							
$\delta^{15}N$ (‰)	ND	ND	ND	ND	ND	10.2	10.2
$\delta^{13}C$ (‰)	ND	ND	ND	ND	ND	-22.9	-22.9
n	ND	ND	ND	ND	ND	2	2
Terrestrial Beetles							
$\delta^{15}N$ (‰)	ND	4.7 ± 0.5	ND	ND	ND	ND	4.7
$\delta^{13}C$ (‰)	ND	-21.2 ± 1.6	ND	ND	ND	ND	-21.2
n	ND	3	ND	ND	ND	ND	3
Fish:							
Deepwater Sculpin							
$\delta^{15}N$ (‰)	13.7 ± 0.4	13.9	14.1 ± 0.3	13.6 ± 0.4	13.5 ± 0.4	13.9 ± 0.4	13.8
$\delta^{13}C$ (‰)	-24.3 ± 0.2	-24.6	-24.4 ± 0.3	-24.5 ± 0.3	-24.2 ± 0.4	-24.5 ± 0.3	-24.4
n	13	1	15	42	15	25	111

* isotope values of seston are concentration weighted averages of 5 depths for each month (McCusker et al. 1999)
** all isotope values of invertebrates were determined from bulk composite samples of many individuals

Table 3. Trophic fractionation factors for $\delta^{15}N$ and $\delta^{13}C$ between deepwater sculpin and average sculpin diet (calculated using 6 month seasonal averages). Trophic fractionation factors were calculated with stomach content data expressed as percent prey number (% PN), percent prey mass (% PM) and percent index of relative importance (% IRI).

	$\delta^{15}N$	$\delta^{13}C$
% PN	3.3	1.5
% PM	4.0	1.9
% IRI	3.9	2.0

Table 4. Results of isotopic mixing model attempts, predicting the relative importance of seston, sinking POM and sediments as sources of nitrogen and carbon to *D. hoyi* and *M. relicta*. "ns" indicates that there was no solution to the mixing model.

A. Calculated fractionation (sculpin % PN*): TFn** = 3.3 ‰, TFc** = 1.5 ‰

	D. hoyi			*M. relicta*		
Relative Proportion of:	97S***	98S***	9798S***	97S	98S	9798S
Seston	ns	ns	ns	ns	ns	ns
Sinking POM	ns	ns	ns	ns	ns	ns
Sediments	ns	ns	ns	ns	ns	ns

B. Calculated fractionation (sculpin % PM*): TFn = 4.0 ‰, TFc = 1.9 ‰

	D. hoyi			*M. relicta*		
Relative Proportion of:	97S	98S	9798S	97S	98S	9798S
Seston	ns	ns	ns	ns	ns	ns
Sinking POM	ns	ns	ns	ns	ns	ns
Sediments	ns	ns	ns	ns	ns	ns

C. Calculated fractionation (sculpin % IRI*): TFn = 3.9 ‰, TFc = 2.0 ‰

	D. hoyi			*M. relicta*		
Relative Proportion of:	97S	98S	9798S	97S	98S	9798S
Seston	ns	ns	ns	ns	ns	ns
Sinking POM	ns	ns	ns	ns	ns	ns
Sediments	ns	ns	ns	ns	ns	ns

D. Literature estimates of fractionation: TFn = 3.4‰, TFc = 0.5 ‰

	D. hoyi			*M. relicta*		
Relative Proportion of:	97S	98S	9798S	97S	98S	9798S
Seston	**73.1**	ns	ns	ns	ns	**43.4**
Sinking POM	**19.7**	ns	ns	ns	ns	**3.7**
Sediments	**7.2**	ns	ns	ns	ns	**52.9**

* PN represents prey number, PM represents prey mass and IRI represents prey index of relative importance

** TFn denotes the trophic fractionation for $\delta^{15}N$, TFc denotes the trophic fractionation for $\delta^{13}C$

*** 97S indicates mixing model results using 1997 seston isotope values, 98S indicates mixing model results using 1998 seston isotope values and 9798S indicates mixing model results using average seston isotope values of the two years combined

FIGURES

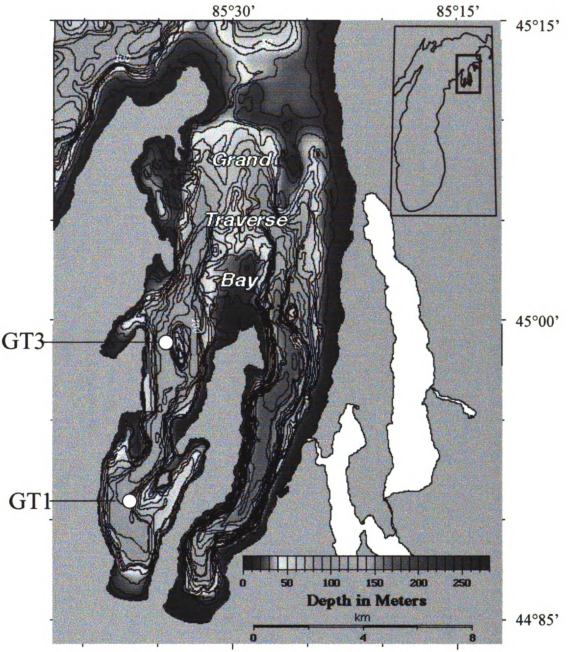

Figure 1. Location of the 2 study sites GT1 and GT3 in Grand Traverse Bay, Lake Michigan (http://www.glerl.noaa.gov).

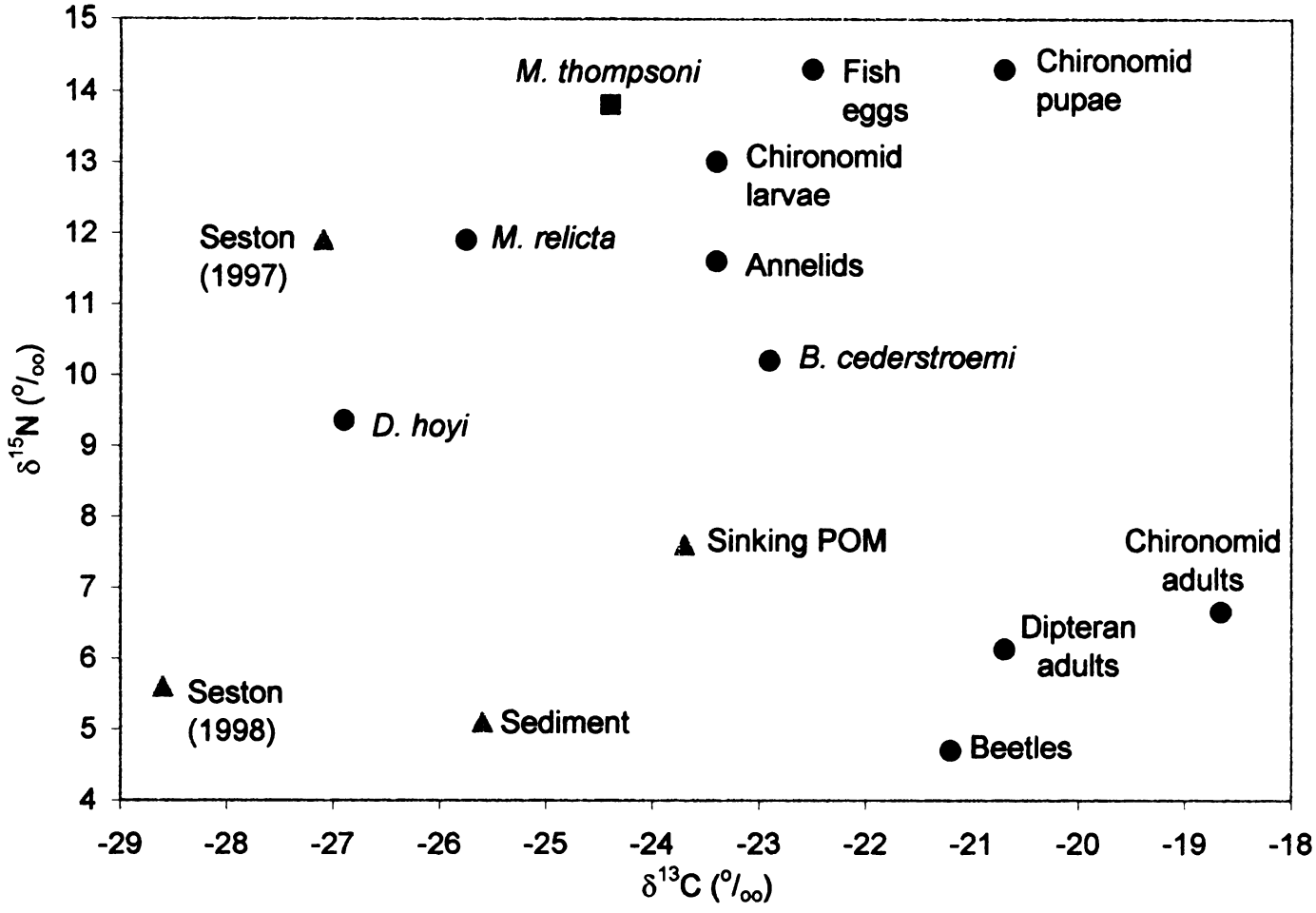

Figure 2. Nitrogen and carbon isotope values of food web members of Grand Traverse Bay, Lake Michigan in 1997 and 1998. Values shown are seasonal averages. Squares represent the isotope values of the deepwater sculpin, circles represent the isotope values of the deepwater sculpin prey items and triangles represent the isotope values of the three POS.

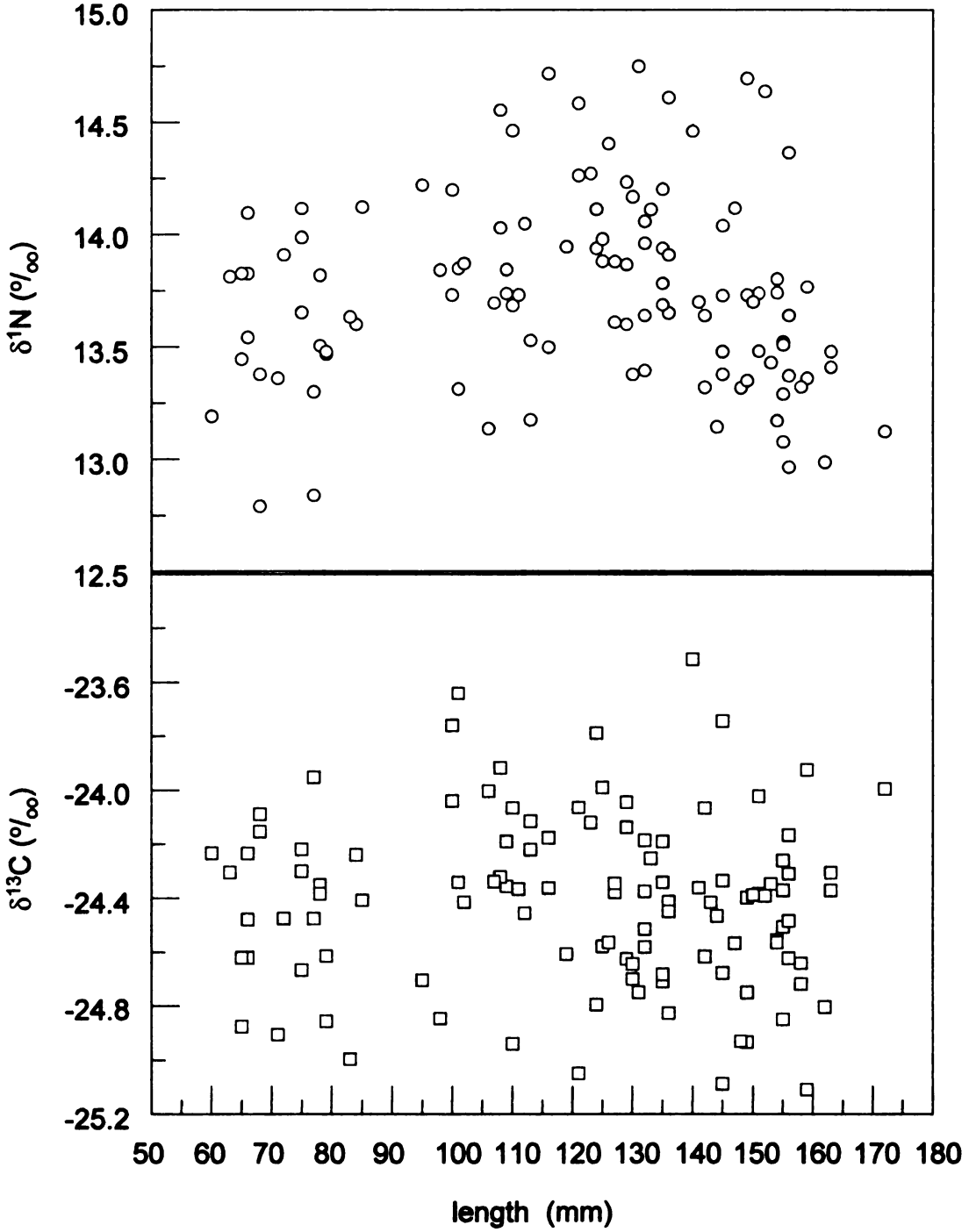

Figure 3. $\delta^{15}N$ (A) and $\delta^{13}C$ (B) distributions of deepwater sculpin as a function of length in Grand Traverse Bay, 1997-1998.

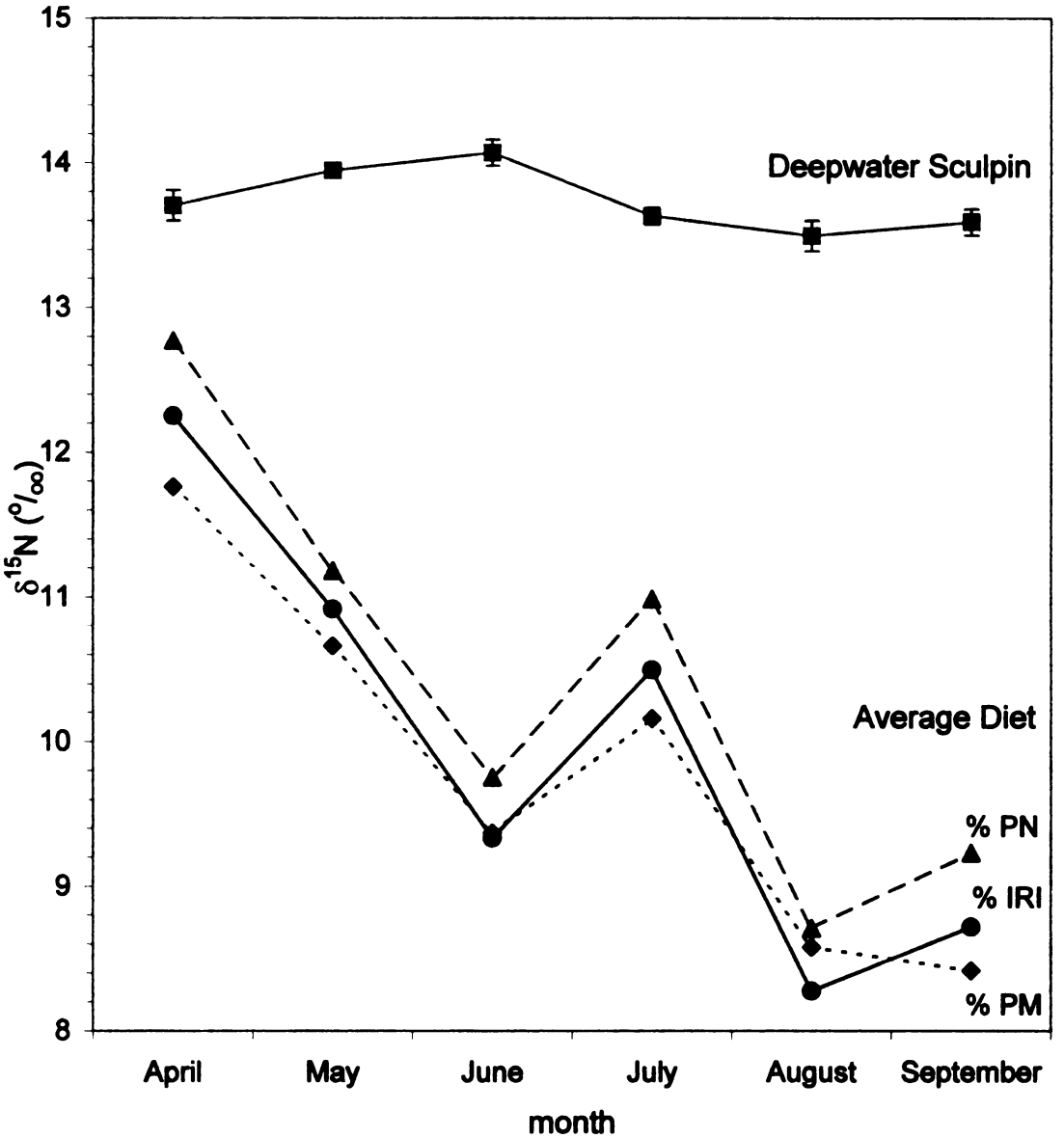

Figure 4. Monthly nitrogen isotope values for deepwater sculpin and average deepwater sculpin diet. Average diet was calculated based on stomach contents expressed as percent prey number (% PN), percent prey mass (% PM) and percent prey index of relative importance (% IRI). $\delta^{15}N$ values for deepwater sculpin are shown as averages +/- 1 S.E.

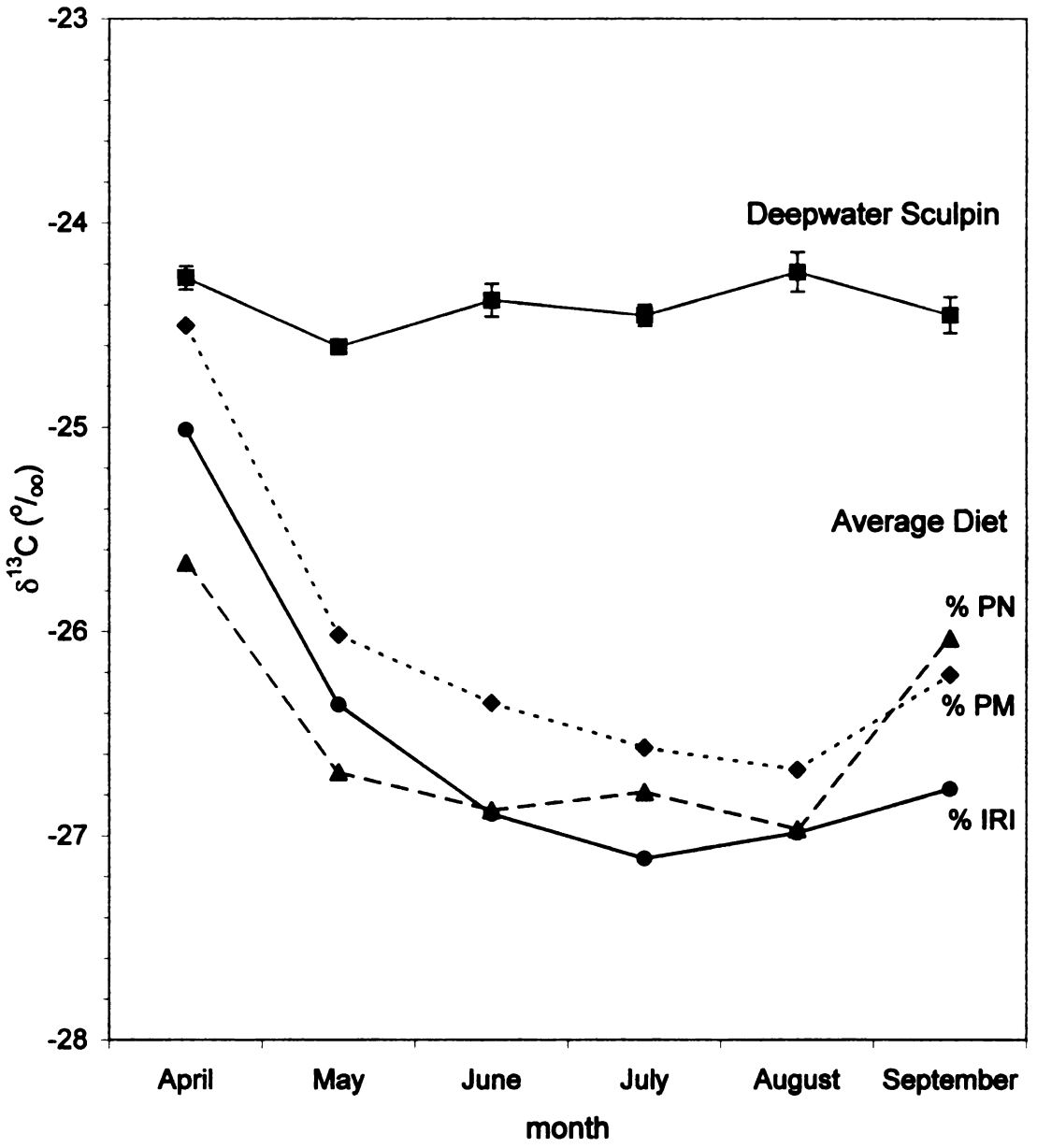

Figure 5. Monthly carbon isotope values for deepwater sculpin and average deepwater sculpin diet. Average diet was calculated based on stomach contents expressed as percent prey number (% PN), percent prey mass (% PM) and percent prey index of relative importance (% IRI). $\delta^{15}N$ values for deepwater sculpin are shown as averages +/- 1 S.E.

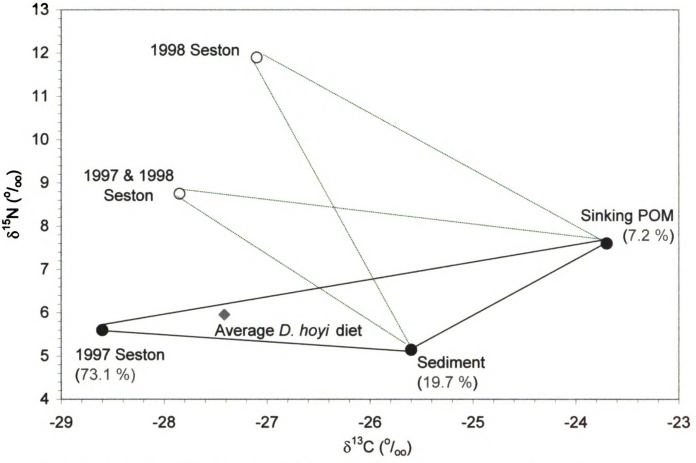

Figure 6. Graphical representation of the mixing model (Equations 4-6) for *Diporeia hoyi* and the three POS. Average *D. hoyi* diet was calculated by correcting the $\delta^{13}C$ and $\delta^{15}N$ values by trophic fractionation (0.5 °/∞ for $\delta^{13}C$ and 3.4 °/∞ for $\delta^{15}N$). *D. hoyi* was assumed to be 1 trophic level above the POS. Grey values in parentheses indicate relative proportions based on the mixing model.

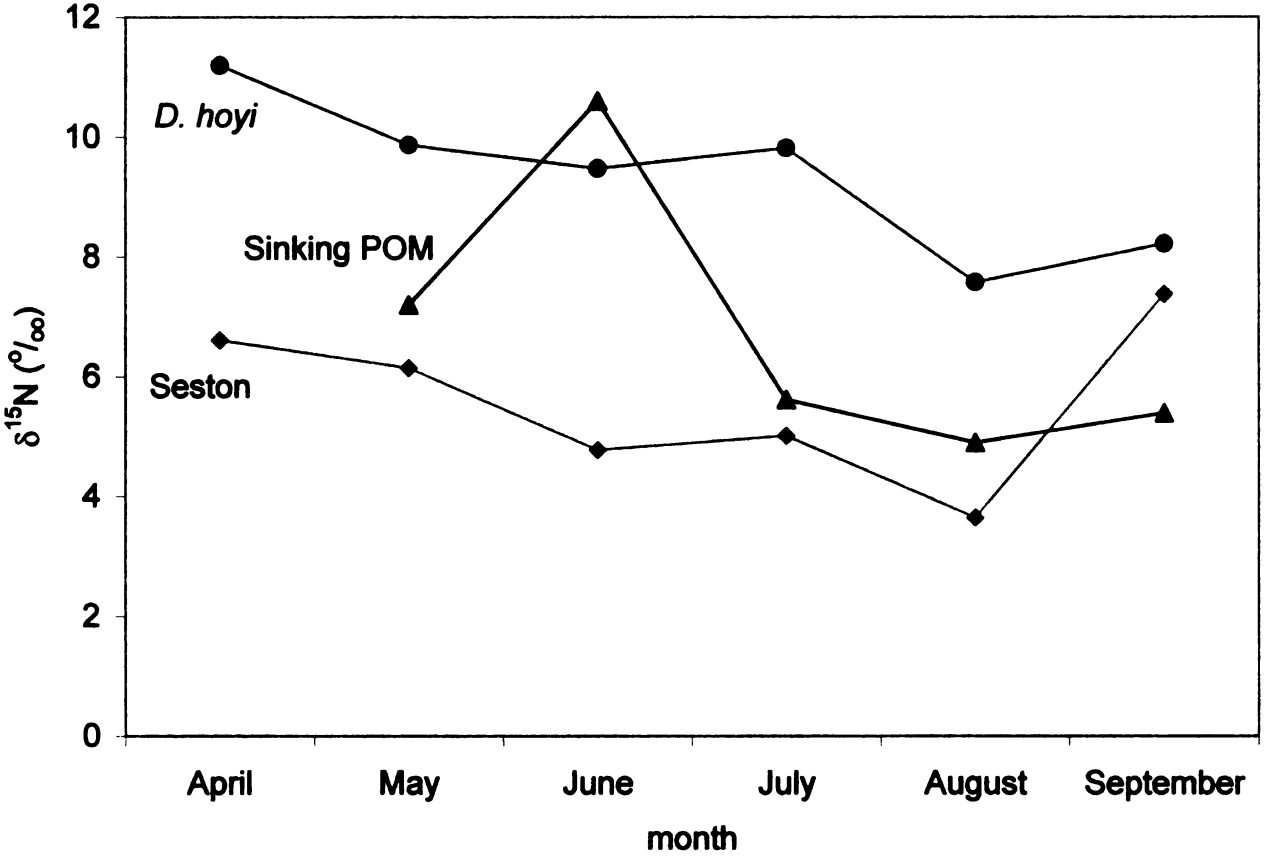

Figure 7. Monthly nitrogen isotope values of *D. hoyi*, seston and sinking POM in Grand Traverse Bay, Lake Michigan, 1997.

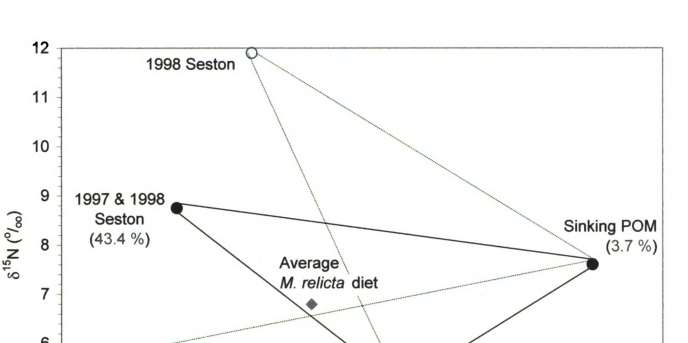

Figure 8. Graphical representation of the mixing model (Equations 4-6) for *Mysis relicta* and the three POS. Average *M. relicta* diet was calculated by correcting the $\delta^{13}C$ and $\delta^{15}N$ values by trophic fractionation (0.5 $^{o}/_{oo}$ for $\delta^{13}C$ and 3.4 $^{o}/_{oo}$ for $\delta^{15}N$). *M. relicta* was assumed to be 1.5 trophic levels above the POS. Grey values in parentheses indicate relative proportions based on the mixing model.

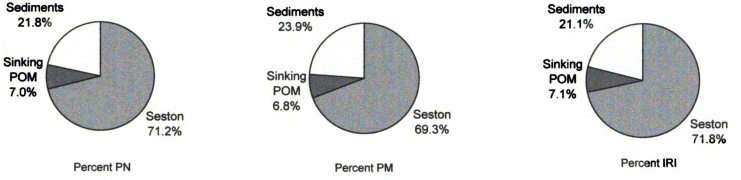

Figure 9. Relative importance of the three POS to deepwater sculpin in Grand Traverse Bay, 1997-1998 according to the isotopic mixing model (Equations 4-7). Plots shown represent results based on stomach content data expressed as prey number (PN), prey mass (PM) and prey index of relative importance (IRI).

LITERATURE CITED

Adams, T.S. and R.W. Sterner. 2000. The effect of dietary nitrogen content on trophic level 15N enrichment. Limnology and Oceanography 45(3):601-607.

Baker, J.E., N.E. Ostrom, P.H. Ostrom, B.J. Eadie, D.S. Lee and D. Hall. 1996. Trophic transfer of atmospheric and sedimentary contaminants into Great Lakes fish: controls on ecosystem-scale response times. Proposal to U.S. Environmental Protection Agency. 31pp.

Balcer, M.D., N.L. Korda and L.I. Dodson. 1984. Zooplankton of the Great Lakes. University of Wisconsin Press. Madison, WI.

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. Limnology and Oceanography 463-467.

Brett, J.F., J.E. Shelborn and C.T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and body size. Journal of the Fisheries Research Board of Canada 26:2363-2394.

Bunn, S.E. and P.I. Boon. 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96:85-94.

Cabana, G. and J.B. Rasmussen. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 372:255-257.

Creach, V., M.T. Schricke, G. Bertru and A. Mariotti. 1997. Stable isotopes and gut analyses to determine feeding relationships in saltmarsh macroconsumers. Estuarine, Coastal and Shelf Science 44:599-611.

Culver, D.A., M.M. Boucherle, D.J. Bean and J.W. Fletcher. 1985. Biomass of freshwater crustacean zooplankton from length-weight regressions. Canadian Journal of Fisheries and Aquatic Sciences 42:1380-1390.

DeNiro, M.J. and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495-506.

DeNiro, M.J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341-353.

Dittel, A.I., C.E. Epifanio, L.A. Cifuentes and D.L. Kirchman. 1997. Carbon and nitrogen sources for shrimp postlarvae fed natural diets from a tropical mangrove system. Estuarine Coastal and Shelf Science 45(5):629-637.

Dumont, H.J., I. Van de Velde and S. Dumont. 1975. The dry weight estimate of biomass in a selection of cladocera, copepoda and rotifera from the ploankton, periphyton and benthos of continental waters. Oecologia 19:75-97.

Eadie, B.J., R.L. Chambers, W.S. Gardner and G.L. Bell. 1984. Sediment trap studies in Lake Michigan: resuspension and chemical fluxes in the southern basin. Journal of Great Lakes Research 10:307-321.

Estep, M.L.F. and S. Vigg. 1985. Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan Lake system, Nevada. Canadian Journal of Fisheries and Aquatic Sciences 42:1712-1719.

Fantle, M.S., A.I. Dittel, S.M. Schwalm, C.E. Epifanio and M.L. Fogel. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120(3):416-426.

Focken, U. and K. Becker. 1998. Metabolic fractionation of stable carbon isotopes: Implications of different proximate compositions for studies of the aquatic food webs using delta 13C data. Oecologia 115(3)337-343.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnology and Oceanography 33(5):1182-1190.

Fry, B. and P.L. Parker. 1979. Animal diet in Texas seagrass meadows: ^{13}C evidence for the importance of benthic plants. Estuarine, Coastal and Marine Science 8:499-509.

Fry, B. and E. Sherr. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions Marine Science 27:13-47.

Gardner, W.S., T.F. Nalepa, W.A. Frez, E.A. Cuichocki and P.F. Landrum. 1985. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. Canadian Journal of Fisheries and Aquatic Sciences 42:1827-1832.

Gauvin, J.M., W.S. Gardner and M.A. Quigley. 1989. Effects of food removal on nutrient release rates and lipid content of Lake Michigan *Pontoporeia hoyi*. Canadian Journal of Fisheries and Aquatic Sciences 46:1125-1130.

Gearing, J.N. 1991. The study of diet and trophic relationships through natural abundance ^{13}C. Chapter 13 *in* Carbon Isotope Techniques, Academic Press Inc. pp.201-217.

Gearing, J.N., P.J. Gearing, D.T. Rudnick, A.G. Requejo and M.J. Hutchins. 1984. Isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. Geochimica et Cosmochimica Acta 48:1089-1098.

Goering, J., V. Alexander and N. Haubenstock. 1990. Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific Bay. Estuarine, Coastal and Shelf Science 30:239-260.

Gould, P., P. Ostrom and W. Walker. 1997a. Trophic relationships of albatrosses associated with squid and large-mesh drift-net fisheries in the North Pacific Ocean. Canadian Journal of Zoology 75:549-562.

Gould, P., P. Ostrom and W. Walker. 1997b. Food of flesh-footed shearwaters *Puffinus carneipes* associated with high-seas driftnets in the central North Pacific Ocean. EMU 97:168-173.

Gorokhova, E. and S. Hansson. 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. Canadian Journal of Fisheries and Aquatic Sciences 56(11):2203-2210.

Grossnickle, N.E. 1982. Feeding habits of *Mysis relicta* – an overview. Hydrobiologia 93:101-107.

Gu, B., C.L. Schelske and M.V. Hoyer. 1996. Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. Journal of Fish Biology 49:1233-1243.

Hansen, B. and K. Christoffersen, 1995. Specific growth rates of heterotrophic plankton organisms in a eutrophic lake during a spring bloom. Journal of Plankton Research 17(2):413-430.

Harrigan, P., J.C. Zieman and S.A. Macko. 1989. The base of nutritional support for the gray snapper (*Lutjanus griseus*): and evaluation based on a combined stomach content and stable isotope analysis. Bulletin of Marine Science 44(1):65-77.

Harvey, C.J. and J.F. Kitchell. 2000. A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. Canadian Journal of Fisheries and Aquatic Sciences 57:1395-1403.

Herzka, S.Z. and J.G. Holt. 2000. Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: Potential applications to settlement studies. Canadian Journal of Fisheries and Aquatic Sciences 57(1):137-147.

Hesslein, R.H., M.J. Capel, D.E. Fox and K.A. Hallard. 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River Basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences 48:2258-2265.

Hesslein, R.H., K.A. Hallard and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (Coregonus nasus) is response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$. Canadian Journal of Fisheries and Aquatic Sciences 50:2071-2076.

Hobson, K.A. and R.G. Clark. 1992. Assessing avian diets using stable isotopes: II. Factors influencing diet-tissue fractionation. Condor 94(1):189-197.

Hobson, K.A. and H.E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series 84:9-18.

Hogberg, P. 1997. Tansley review no. 96 15N natural abundance in soil-plant systems. New Phytologist 137(2):179-203.

Jacoby, C. 1953. Notes on the life history of the deepwater sculpin, *Myoxocephalus quadricornis* L,in Lake Superior. M.S. thesis. Univ. Mich., Ann Arbor, Mich.

Jennings, S., O. Renones, B. Morales-Nin, N.V.C. Polunin, J. Moranta and J. Coll. 1997. Spatial variation in the ^{15}N and ^{13}C stable isotope composition of plants, invertebrates and fishes on Mediterranean reefs: implications for the study of trophic pathways. Marine Ecology Progress Series 146:109-116.

Johannsson, O.E., Rudstam, L.G. and D.C. Lasenby. 1994. *Mysis relicta*: assessment of metalimnetic feeding and implications for competition with fish in Lakes Ontario and Michigan. Canadian Journal of Fisheries and Aquatic Sciences 51:2591-2602.

Keough, J.R., M.E. Sierszen and C.A. Hagley. 1996. Analysis of a Lake Superior coastal food web with stable isotope techniques. Limnology and Oceanography 41(1):136-146.

Kikuchi, E. and E. Wada. 1996. Carbon and nitrogen stable isotope ratios of deposit-feeding polychaetes in the Nanakita River Estuary, Japan. Hyropbiologia 321(1):69-75.

Kiriluk, R.M., M.R. Servos, D.M. Whittle, G. Cabana and J.B. Rasmussen. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex and PCB in a Lake Ontario pelagic food web. Canadian Journal of Fisheries and Aquatic Sciences 52:2660-2674.

Kitchell, J.F. and D.J. Stewart. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of the Fisheries Research Board of Canada 34:1922-1935.

Kraft, C.E. 1977. Comparative trophic ecology of deepwater sculpins (*Myosocephalus quadricornis*) and slimy sulpins (*Cottus cognatus*) in Lake Michigan. M.S. thesis, Oceanography and Limnology, Univ. Wisconsin-Madison, Madison, Wisc.

Kraft, C.E. and J.F. Kitchell. 1986. Partitioning of food resources by sculpins in Lake Michigan. Environmental Biology of Fishes 16(4):309-316.

Lasenby, D.C. and R.R. Langford. 1973. Feeding and assimilation of *Mysis relicta*. Limnology and Oceanography. 18:280-285.

Leggett, M.F. 1998. Food-web dynamics of Lake Ontario as determined by carbon and nitrogen stable isotope analysis. Ph.D. Dissertation, University of Waterloo. Waterloo, ON. 266 pp.

Liden, K. and A. Angerbjorn. 1999. Dietary change and stable isotopes: A model of growth and dormancy in cave bears. Proceedings of the Royal Society Biological Sciences Series B 266(1430):1779-1783.

Macrellis, A. 1999. Geochemical and isotope dynamics of dissolved inorganic nitrogen in Grand Traverse Bay, Lake Michigan. Masters Thesis, Michigan State University. East Lansing, MI. 42 pp.

McCusker, E.M., P.H. Ostrom, N.E. Ostrom, J.D. Jeremiason and J.E. Baker. 1999. Seasonal variation in the biogeochemical cycling of seston in Grand Traverse Bay, Lake Michigan. Organic Geochemistry 30:1543-1557.

Merritt, R.W. and K.W. Cummins, eds. 1996. An introduction to the aquatic insects of North America, 3rd ed. Kendall/Hunt Publishing Company. Dubuque, IA.

Minagawa, M. and E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48:1135-1140.

Mozley, S.C. an M.H. Winnell. 1975. Macrobenthic species assemblages of southeastern Lake Michigan. Verh. Internat. Verein. Limnol. 19:922-931.

Neilson, R., D. Hamilton, J. Wishart, C.A. Marriott, B. Boag, L.L. Handley, C.M. Scrimgeour, J.W. McNicol and D. Robinson. 1998. Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. Soil biology and biochemistry 30(13):1773-1782.

Nero, R.W. and W.G. Sprules 1986. Predation by three glacial opportunists on natural zooplankton communities. Canadian Journal of Zoology 64:57-64.

Nesler, T.P. 1981. The relative abundance of opossum shrimp, *Mysis relicta*, in Twin Lakes, Colorado using a benthic trawl. Engineering and Research Center Joint Report with Colorado Division of Wildlife. 22pp.

Ostrom, P.H., M. Colunga-Garcia and S.H. Gage. 1996. Establishing pathways of energy flow for insect predators using stable isotope ratios: field and laboratory evidence. Provisorische Seitenzahlen 6p.

Ostrom, N.E., S.A. Macko, D. Deibel and R.J. Thompson. 1997. Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean environment. Geochimica et Cosmochimica Acta 61(14)2929-2942.

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18:293-320

Pinkas, L., M.S. Oliphant and I.L.K. Iverson. 1971. Food habits of albacore, bluefin tuna, and bonito in California waters. California Fish and Game Fish Bulletin No. 152.

Quigley, M.A. and J.A. Robbins. 1984. Silica regeneration processes in nearshore southern Lake Michigan. Journal of Great Lakes Research 10:383-392.

Roth, J.D. and K.A. Hobson. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: Implications for dietary reconstruction. Canadian Journal of Zoology 78(5):848-852.

Rosenfeld, J.S. and J.C. Roff. 1992. Examination of the carbon base in sourthern Ontario streams using stable isotopes. Journal of the North American Benthological Society 11(1):1-10.

Rudstam, L.G., K. Danielsson, S. Hansson and S. Johannsson. 1989. Diet vertical migration and feeding patterns of *Mysis mixta* (Crustacea, Mysidacea) in the Baltic Sea. Marine Biology 101:43-52.

Rudstam, L.G., A.L. Hetherington and A.M. Mohammadian. 1998. Effect of temperature on feeding and survival of *Mysis relicta*. Canadian Journal of Fisheries and Aquatic Sciences 25(2):363-371.

Selgeby, J.H. 1988. Comparative biology of the sculpins of Lake Superior. Journal of Great Lakes Research 14(1)L:44-51.

Sell, D.W. 1982. Size-frequency estimates of secondary production by *Mysis relicta* in Lakes Michigan and Huron. Hydrobiologia 93:69-78.

Smock, L.A. 1980. Relationships between body size and biomass of aquatic insects. Freshwater Biology 10:375-383.

Stapleton, H.M., C. Masterson, P. Ostrom, J. Skubinna and J.E. Baker. *In prep.* Accumulation of atmospheric and sedimentary PCBs and toxaphene in a Lake Michigan food web.

Takahashi, K., T. Yoshioka, E. Wada and M. Sakamoto. 1990. Temporal variations in carbon isotope ratio of phytoplankton in a eutrophic lake. Journal of Plankton Research 12(4):799-808.

Tatrai, I., K. Matyas, J. Korponai, G. Paulovits, P. Pomogyi and M. Presing. 1999. Stable isotope analysis of food webs in wetland areas of Lake Balaton, Hungary. Archiv fuer Hydrobiologie 146(1):117-128.

Tieszen, L.L., T.W. Boutton, K.G. Tesdahl and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for delta carbon-13 analysis of diet. Oecologia 57(1-2):32-37.

Toda, H. and E. Wada. 1990. Use of $^{15}N/^{14}N$ ratios to evaluate the food source of the mysid, *Neomysis intermedia* Czerniawsky, in a eutrophic lake in Japan. Hydrobiologia 194:85-90.

Wells, L. 1980. Food of alewives, yellow perch, spottail shiners, trout-perch, and slimy and fourhorn sculpins in southeastern Lake Michigan. Technical Papers of the U.S. Fish and Wildlife Service #98 12pp.

Wojcik, J.A., M.S. Evans and D.J. Jude. 1986. Food of deepwater sculpin, *Myoxocephalus thompsoni*, from southeasrtern Lake Michigan. Journal of Great Lakes Research 12(3):225-231.

Wong, W.W., L.L. Clarke, G.A. Johnson, M. Llaurador and P.D. Klein. 1992. Comparison of two elemental-analyzer gas-isotope-ratio mass spectrometer systems in the simultaneous measurement of $^{13}C/^{12}C$ ratios of carbon content in organic samples. Analytical Chemistry 64:354-358.

Yoshioka, T., E. Wada and H. Hayashi. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. Ecology 75(3):835-846.

Zohary, T., J. Erez, M. Gophen, I. Berman-Frank and M. Stiller. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnology and Oceanography 39(5):1030-1043.