

Diet overlap and food habits of slimy sculpin, deepwater sculpin, and round goby during winter and spring in offshore Lake Michigan

By

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Abstract

Ecological stability in offshore benthic food webs of the Laurentian Great Lakes has been recently altered by non-native species such as round goby *Neogobius melanostomus* and quagga mussels *Dreissena bugensis*, as well as steep declines in biomass of *Diporeia*, a native amphipod. Correspondingly, population dynamics, life histories and the diet composition of native sculpins may be affected. I examined food habits of slimy sculpin *Cottus cognatus*, deepwater sculpin *Myoxocephalus thompsonii*, and round goby collected from January to May in 2009 and 2010 in Lake Michigan offshore of Frankfort (FF), MI; Muskegon (MSK), MI; Two Rivers (TR), WI; and Sturgeon Bay (STB), WI in depths of 69-128m. Important prey (by dry weight proportion and % occurrence) for slimy sculpin were *Mysis* (0.34, 45%), *Diporeia* (0.16, 34%), and *Limnocalanus macrurus* (0.22, 68%). Prey important to deepwater sculpin were *Mysis* (0.74, 92%) and *Diporeia* (0.16, 54%). Round goby consumed mainly bivalves (0.68, 95%) and *Mysis* (0.15, 37%). Diet composition for all three species did not vary across days sampled in January through April, and little variance was explained by year or depth sampled. Variance in diet composition for each benthivore species was best explained by the site from which fishes were sampled. For example, *Diporeia* constituted high diet proportions in sculpins offshore of TR and STB, but was absent in sculpins offshore of FF and MSK. Significant diet overlap (Schoener's index ≥ 0.60) was identified between slimy and deepwater sculpin offshore of FF and STB but not offshore of TR. Significant diet overlap was not found between round goby and either sculpin species. Non-metric multi-dimensional scaling and cluster analyses revealed groups of fishes at each site with similar diets: 1) slimy sculpin only; 2) round goby only, and; 3) all deepwater sculpin, some slimy sculpin individuals, and very few round goby. Cluster analysis also distinguished groups for both sculpin species that reinforced the diet overlap results. Using genetic analyses on fish eggs taken from diet samples, bloater *Coregonus hoyi* and deepwater sculpin eggs were confirmed prey for slimy and deepwater sculpin in each month from February through May at all four sites sampled. Round goby consumed few deepwater sculpin eggs and no bloater eggs.

Introduction

Aquatic ecosystems have been dramatically altered from anthropogenic perturbations (Miller et al. 1989; Rahel 2002). Introduction of non-native species and extinction of native species (Christie 1974; Kaufman 1992), habitat destruction (Benke 1990; Scott and Helfman 2001), and overfishing (Smith 1968; Jackson et al. 2001) are some of the many stressors that have destabilized aquatic food webs. The rapidity of ecological change has clarified that ecosystem-based management is needed to maximize productivity, stabilize food webs, and ensure long-term sustainability in aquatic environments (Link 2002; Hilborn et al. 2003). Estimation of the diet composition of fishes, even for species without recreational or commercial value, is one of many data requirements needed to facilitate implementation of ecosystem-based fishery management (Francis et al. 2007; Zimmerman and Krueger 2009). For example, quantitative analysis of food habits has contributed to parameterization of ecosystem and bioenergetics models (Christensen 1995; Hanson et al. 1997), identification of fish recruitment bottlenecks via larval or egg predation (Tyus and Saunders 2000; Richardson et al. 2011), and the estimation of diet overlap and competition among fishes (Winemiller 1989; Garrison and Link 2000; Diana 2005).

Over the past century, the food webs of the Laurentian Great Lakes (hereafter Great Lakes) have been profoundly influenced by non-native species (Wells and McLain 1973; Mills et al. 1993), but a relatively new wave of non-native species have invaded multiple trophic levels since the mid-1980s (i.e., *Bythotrephes* spp., Gobiidae, *Dreissena* spp.) (Jude 2001; Vanderploeg et al. 2002; Fahnenstiel et al. 2010). Round goby (*Neogobius melanostomus*) was first discovered in 1990 in the St. Clair River (Jude et al. 1992), and now occurs in all of the Great Lakes. Round goby have caused recruitment failure of native mottled sculpin (Janssen and Jude 2001) and their diets overlap with small (<75 mm) native species, such as rainbow darter (*Etheostoma caeruleum*) and logperch (*Percina caprodes*) (French and Jude 2001). In lab settings, individual spoonhead sculpin (*Cottus ricei*) and logperch lost mass when paired with an individual round goby, likely due to the aggressive nature of round goby (Bergstrom and Mensinger

2009). Additionally, consumption of fish eggs by round goby has been observed in the field (Roseman et al. 2006) and may limit the recruitment of lake trout (*Salvelinus namayacush*, Chotkowski and Marsden 1999; Fitzsimmons et al. 2006) and smallmouth bass (*Micropterus dolomieu*, Steinhart et al. 2004). During winter, round goby move offshore into deepwater benthic habitats, likely in search of warmer temperatures (Miller 1986; Walsh et al. 2007). Here, biotic interactions may occur with native and ecologically important benthivores such as slimy sculpin (*Cottus cognatus*) and deepwater sculpin (*Myoxocephalus thompsonii*) (Van Oosten and Deason 1938; Fratt et al. 1997; Zimmerman and Krueger 2009). Because food habits for slimy sculpin, deepwater sculpin, and round goby are unknown for early winter and spring in the Great Lakes, managers and scientists do not know whether these fish are competing with one another for food or consuming eggs of native species

Ironically, diet overlap between round goby and native offshore sculpins could be alleviated by the proliferation of another invader, the quagga mussel (*Dreissena rostriformis bugensis*), which now exists at high densities in offshore lakes Michigan (Bunnell et al. 2009; Nalepa et al. 2010), Huron (Pothoven and Nalepa 2006), and Ontario (Mills et al. 1999). Slimy and deepwater sculpin lack similar pharyngeal teeth, jaws and digestive systems that allow larger round goby (those > 60 mm total length, TL hereafter) to be effective predators of bivalves (French 1993). Concomitant with the expansion of *Dreissena* spp. has been the precipitous decline of *Diporeia* spp. (hereafter *Diporeia*) (Nalepa et al. 2009), a native amphipod that historically was the most important prey by number, weight or volume for slimy and deepwater sculpin (Jacoby 1953; Wells 1980; Brandt 1986b; Wojcik et al. 1986; Selgeby 1988).

The extent to which these perturbations have affected the diets or population dynamics of slimy and deepwater sculpin in the Great Lakes remains largely unknown. In Lake Michigan for example, the biomass of deepwater sculpin and bloater (*Coregonus hoyi*, another native benthic fish) have reached near-record low levels based on data spanning a 37-year time series (Bunnell et al. 2011). Mechanisms underlying this decline are unclear, but may be related to skewed sex ratios for bloater (Bunnell et al.

2006), or the possibility that deepwater sculpin have recently begun migrating to depths beyond where monitoring has occurred (Madenjian et al. 2010). Conversely, slimy sculpin biomass has increased to near-record high levels over the same period, perhaps owing to lower vulnerability to predation from juvenile lake trout following changes in stocking policies (Madenjian et al. 1998; Madenjian et al. 2010). Round goby biomass, while interannually variable, also appears to have increased (Bunnell et al. 2011). Whether slimy sculpin, deepwater sculpin, and round goby experience competition for food, or potentially consume eggs and influence population dynamics of one another, or of bloater, is unknown as their winter and early spring diet composition are largely undescribed, except for sculpins in March (Wells 1980) and round goby in April (Walsh et al. 2007).

Diet composition of slimy and deepwater sculpin in seasons other than winter and early spring demonstrates significant diet overlap between these species (Brandt 1986a; Davis et al. 2007). Besides *Diporeia*, *Mysis* spp. (hereafter *Mysis*) is the primary prey for offshore sculpins, however, chironomids, copepods and cladocerans have also been commonly observed in the diet (Wells 1980; Davis et al. 2007). Conversely, nearshore diets of round goby >60 mm TL typically are dominated by *Dreissena* spp. (Ray and Corkum 1997; Jude 2001), although *Mysis*, and ostracods also occurred at sometimes high frequency of occurrences in their diets both inshore and offshore in the Great Lakes (Bunnell et al. 2005; Schaeffer et al. 2005; Walsh et al. 2007; Cooper et al. 2011).

Diet composition of slimy and deepwater sculpin has been found to vary little across seasons or interannually (Jacoby 1953; Wells 1980; Wojcik et al. 1986), but may vary in important ways across sites or depth strata. Hondorp et al. (2005) found that slimy sculpin consumption of *Diporeia* varied among sites in Lake Huron, but not among season or depths sampled. O'Brien et al. (2009) found that while the frequency of occurrence of *Diporeia* in deepwater sculpin diets in Lake Huron did not vary among years or sites, the mean number consumed by deepwater sculpin declined across years and variably between

sites. Owens and Weber (1995) found that as depth increased from 55-95 m, frequency of occurrence of *Mysis* increased and *Diporeia* declined in the diets of slimy sculpin from Lake Ontario.

All three benthivore species have been shown to consume fish eggs and deemed able to limit recruitment of other fishes through this mechanism. For example, through egg predation slimy and deepwater sculpin have been implicated in limiting recruitment of bloater (Luecke et al. 1990), slimy sculpin have been hypothesized to limit sockeye salmon recruitment (Foote and Brown 1998), and round goby likely have impacts on recruitment of smallmouth bass (Steinhart et al. 2004) and lake trout (Fitzsimmons et al. 2006). Thus far, no examination of egg predation rates by these benthivore species has been completed during winter and early spring in offshore habitats of the Great Lakes. However, recruitment of three year old bloater was inversely correlated to the biomass of adult slimy sculpin that was present at the time when those bloater recruits were incubating as eggs in winter seasons across a 37-year time series (D. Bunnell, USGS unpublished data). Given that slimy sculpin, deepwater sculpin, and round goby in Lake Michigan spatially and temporally overlap with incubating eggs of deepwater sculpin and bloater, predation on these eggs could explain the recent record low biomass trends for both species. Fish eggs and larvae in diets can be difficult to identify due to putatively rapid digestion rates or ambiguous morphometric features (Ahlstrom and Moser 1976; Hunter and Kimbrell 1980). As a result, scientists use genetic techniques to identify these prey to species (Rosel and Kocher 2002; Carreon-Martinez and Heath 2010; Carreon-Martinez et al. 2011), but to my knowledge this research has not yet been applied to offshore benthivores in the Great Lakes.

In this study I sought to describe the winter and spring diets of slimy sculpin, deepwater sculpin and round goby in offshore (69-128 m) waters of Lake Michigan. I evaluated whether variation in diet proportions of prey taxa for each benthivore species could be explained by year, day of year (DOY), site, or sampling depth. I also evaluated whether interspecific diet overlap occurred and hypothesized that diet overlap would be significant between sculpin species but insignificant between round goby and

either sculpin species. Finally, I estimated the proportion and species of fish eggs that comprised benthivore diets using genetic techniques. I hypothesized that deepwater sculpin and bloater eggs would be consumed by all three benthivore species and that slimy sculpin would consume higher proportions of bloater eggs given the inverse relationship between bloater and slimy sculpin.

Methods

Slimy sculpin, deepwater sculpin and round goby were sampled from January through May in 2009 and 2010 using bottom trawls for 5-10 minutes at depths from 69-128 m taken offshore of four sites in Lake Michigan. Offshore of Frankfort, MI (FF; 44 30.66 N, 086 20.30 W) and Sturgeon Bay, WI (STB; 44 42.03 N, 087 07.52 W), a 13-m Yankee trawl was used to sample fish. Offshore of Two Rivers, WI (TR; 44 17.96 N, 087 21.44 W), a 31-m otter trawl was used to sample fishes. Lastly, offshore of Muskegon, MI (MSK; 43 11.99 N, 086 34.19 W), an 8-m skate trawl was used to collect fishes. Exact locations of offshore collections varied slightly across different depth strata sampled (Figure 1). Upon sorting of fishes collected in each individual trawl, all fish or 30-60 randomly subsampled specimens per species were immediately frozen. Because each site was not sampled monthly, the sampling design was unbalanced (Table 1).

Preserved fish were thawed, individually identified to sex, weighed (to 0.1 g) and measured (TL, in mm). Whole stomachs (esophagus to pyloric valve) of sculpins and entire digestive tracts of round gobies (esophagus to anus, since no similar valve exists) were excised and preserved in tris-ethylenediaminetetraacetic acid (ETDA) enriched 95% ethanol to preserve genetic material. Under a dissecting microscope, stomach contents from individuals were placed into a Ward counting wheel using fine forceps and water. The stomach lining of sculpins was scraped into a separate watch glass to account for embedded prey.

At 6 to 25X magnification, prey were classified to species, genus or family and, when possible, identified to life stage (Table 2). I recorded the length of the first ten individuals encountered for each prey type in each individual diet sample. For unmeasured prey, I averaged prey- and, if possible, life stage-specific lengths from intact prey of the same type measured across all sample dates, locations, and predator species in this data set. After identifying and measuring all prey, dry weight values for each prey item at ingestion were calculated using prey- and, life stage-specific length to dry weight

regressions or dry weight values published in literature for the same or similar species (Table 2). Because more than 90 prey types including different life stages, species, genera and families were encountered in benthivore diets, I grouped prey into one of 12 categories (Table 2). An additional category combined rare prey into miscellaneous taxa. For each fish, diet proportions were then calculated from dry weights of the 13 respective taxa groups. Because many invertebrates can be digested rapidly, I counted only specific body parts (such as copepod rami, cephalic segments of *Diporeia*, *Mysis* eyes, and head capsules of chironomids) to limit double counting (Table 2).

Two prey categories were bloater eggs and deepwater sculpin eggs. Initially, I assumed that fish eggs would belong to bloater, slimy or deepwater sculpin because these are the only species for which spawning and embryonic development occurs during winter and spring at the depths I sampled (Rottiers 1965; Emery and Brown 1978; Rice et al. 1987; Geffen and Nash 1992). Given that bloater, slimy sculpin and deepwater sculpin eggs all have similar morphology, coloring and diameter, visually identifying eggs to species was not feasible. Thus, I used genetic analysis on whole, unpunctured fish eggs found in diets. Up to 10 eggs from the stomach of each fish sampled for diet analysis were placed in bullet tubes and immediately preserved in EDTA enriched 95% ethanol for further analysis.

To cross-compare DNA from eggs in diets to known DNA for each species, standards were developed from fin clips of bloater, slimy sculpin and deepwater sculpin collected from Lake Michigan. DNA barcoding from approximately 700bp of the mitochondrial cytochrome C oxidase I gene (COI) was used (Hebert et al. 2003). DNA was extracted from samples using protocols from the DNeasy kit (Qiagen©, Valencia CA) and then 3 mAU proteinase K was added to ensure complete digestion of eggs. The composition of extracted DNA was examined on 2% agarose gels and quantified using fluorometry. DNA amplification via polymerase chain reaction (PCR) was performed using primers VR1_t1 and VF2_t1, which are recommended for high throughput sequencing (Ivanova et al. 2007). The PCR had a total volume of 30 μ L consisting of: the recommended buffer from Promega, 0.5 μ M of each dNTP, 0.2 μ M of

each primer, 2.5 μ M MgCl₂ and 1 U Taq DNA polymerase. The thermocycler profile consisted of an initial denaturing step at 95 °C for 3 min, followed by 15 cycles of 94 °C for 1 min, 52 °C for 45 s, and 72 °C for 10 s, followed by 20 cycles of 94 °C for 30 s, 52 °C for 30 s and 72°C for 10 s, and followed by a final extension at 72°C for 5 min. Unincorporated nucleotides and primers were removed from the amplicon using the Qiaquick PCR purification kit (Qiagen®, Valencia CA) and gene sequences were determined from both strands on an Applied Biosystems Model 3730 XL sequencer. Sequences were aligned by eye and then queried against sequences from GenBank using the BLAST search algorithm (Altschul et al. 1990). Four matching sequences were selected from GenBank (Accession numbers: bloater = EU523964.1; deepwater sculpin = EU524918.1; slimy sculpin = EU524520.1; <http://www.ncbi.nlm.nih.gov/sites/query>) and were used to find a restriction enzyme (RE) that would cut the COI region in all species by using RestrictionMapper (version 3.0, <http://www.restrictionmapper.org/>). Analysis of COI sequences indicated that RE HaeIII would cut the COI fragment in all four species. Using restriction fragment length polymorphisms enabled faster processing of large numbers of eggs than sequencing would have allowed. Restriction enzyme digests were performed in 15 μ l, including 90 U of the RE and manufacturer's recommended buffer (New England Biolabs). DNA fragments were separated on 3.5% agarose gels, stained with ethidium bromide and then photographed under UV light. Banding patterns of standards were compared to the patterns from unknown samples to identify each unknown egg to species.

I determined species identities of all remaining unidentified fish eggs based on DNA analysis of identified eggs. In cases where all eggs from the subset of eggs submitted for DNA analysis from an individual benthivore diet were identified to one species only, remaining eggs were assigned the same species. When multiple species of eggs were confirmed in the diet of an individual benthivore, the ratio of species from the tested eggs was assigned to any remaining eggs in the diet. Mean predator-specific ratios of egg species composition were determined for each sampling net and used to assign the egg

species composition for other benthivores from that net containing all unidentifiable eggs. For individuals that contained all unidentifiable eggs and were in a net with no fish having identified eggs, I used mean species composition of eggs from the same benthivore species determined from trawls at the same site first by the nearest sampling depth, and then at the nearest sampling date.

To explain variation in food habits of slimy sculpin, deepwater sculpin and round goby I developed two series of general linear models (GLMs). Because my sampling design was unbalanced, Model 1 examined the effect of DOY on diet composition and used only diets from offshore of TR given that sampling here from January through April was the most temporally thorough of sampling at any site (Table 1). Model 2 GLMs determined if variation in diet composition could be explained by spatial effects resulting from the site sampled. Whether Model 2 GLMs used data from all time periods or just April (when all sites were sampled) was dependent upon results from Model 1 GLMs. In all Model 2 GLMs, I excluded slimy sculpin data from MSK during May and June because of low sample sizes ($N = 3$ fish), but included deepwater sculpin sampled from all months and all sites in analysis. Both models included year as a categorical variable and depth as a continuous variable. For the sampling unit I used mean diet proportions (by dry weight) from benthivore species caught within the same nets rather than individual fish diets to avoid pseudoreplication. In each model, the number of individual predator diets sampled from each net was used as a weighting factor. For each GLM series, I developed individual models for each predator and corresponding prey categories constituting $\geq 90\%$ of their overall diets by dry weight. This resulted in five taxa categories for slimy sculpin, four for deepwater sculpin and three for round goby in the GLM series. For each predator I made a Bonferroni adjustment to alpha by dividing 0.05 by the number of prey categories used (i.e., $w/4$ prey categories, $\alpha = 0.05/4 = 0.0125$). To determine if prey proportions used in GLMs were influenced by predator size I regressed TL of each individual fish caught within a site against its corresponding diet proportion for each important prey taxon. No visual patterns

emerged and all r^2 values were low (< 0.02), thus effects of TL on the diet proportions of benthivores were ignored.

To assess whether food habits of slimy sculpin, deepwater sculpin and round goby were similar at a given site I calculated diet overlap. I used mean proportions by dry weight of all thirteen prey categories (Table 2) calculated from all individual predator species collected at a given site (i.e., pooling individuals across all depths and dates) to calculate Schoener's (1970) index of diet overlap, which equaled:

$$D_{x,y} = 1 - 0.5 \left(\sum_{i=1}^n |P_{ix} - P_{iy}| \right),$$

where $D_{x,y}$ = Schoener's overlap index between species x and y ; P_{ix} = proportion of food category i used by species x ; and P_{iy} = proportion resource i of total resources used by species y . Values ≥ 0.6 indicated significant overlap and possible competition, if resources were limiting (Martin 1984).

Lastly, I performed multivariate nonmetric multidimensional scaling (NMS; Kruskal 1964; Mather 1976) to produce a visual representation of how diets varied between predators within each site. NMS is iterative, seeks to minimize the "stress" of a k -dimensional configuration, is well suited for non-normal data, and avoids assumptions of linear relationships among variables (McCune and Grace 2002). As in GLM analyses, I calculated the mean diet proportion by dry weight for each of the 12 prey categories for each benthivore species captured within a given net. I excluded the miscellaneous category because its composition varied between predator species. Because I was unable to weight sampling units by the number of fishes in a net (as done in GLMs), I excluded diet data from nets where ≤ 2 individuals were caught. To run each NMS procedure I used PC-ORD (McCune and Mefford 1999) and selected the Sorenson (Bray-Curtis) distance measure to calculate the dissimilarity matrix, and the "slow and thorough estimation method" (McCune and Grace 2002). PC-ORD determined the optimal number of dimensions for each ordination. To avoid local minima, I checked that ordination stability and standard

deviation of stress was ≤ 0.00010 over the last 15 iterations (McCune and Mefford 1999; McCune and Grace 2002). To understand which prey species were associated with each ordination axis, I correlated the ranks of axis scores with the ranks of up to each of eight prey types that were required to account for > 90% of each predators diet proportion at each port (these were the same prey types I used in GLMs) using Kendall's tau. To avoid spurious correlations, and avoid potentially including all prey types required in analyses on each axis, I used a Bonferroni adjustment by dividing alpha by the number of prey categories (e.g., $p = 0.05/8 = 0.0063$), so only prey types with significant correlations were visual displayed alongside each axis.

To identify where diets varied between species, I used PC-ORD to perform cluster analyses within each ordination. For consistency, I used a Sorenson (Bray-Curtis) distance matrix and a flexible beta linkage (also known as flexible clustering) where $\beta = -0.25$ (McCune and Grace 2002). Clusters were identified using Wishart's objective function (which measures information loss as agglomeration of clustering proceeds, Wishart 1969). Minimal number of clusters and maximum amount of original information was retained by visually determining a point on each dendrogram where this balance was achieved (McCune and Mefford 1999). As a result, each cluster was assumed to include diets with similar composition. When clusters were comprised of only one benthivore species, it suggested minimal interspecific diet overlap. Conversely, when clusters contained multiple benthivore species it suggested interspecific diet overlap (McCune and Mefford 1999; McCune and Grace 2002).

Results

A total of 2,266 fishes with prey in stomachs were used in analyses (slimy sculpin N=1016, deepwater sculpin N=699, round goby N=552). Ranges, means and standard deviations of TL and wet weight of fishes were broad for each species overall and within sites (Table 3). Pooling across all sites, slimy sculpin consumed mainly *Mysis*, *Limnocalanus macrurus* (hereafter *Limnocalanus*) *Diporeia*, and chironomids (Figure 2). Deepwater sculpin consumed mostly *Mysis* and *Diporeia*, and round goby consumed mostly bivalves, *Mysis*, and ostracods. Of the bivalves found in round goby, 72% were quagga mussels, 24% unidentified dreissenids, 2% zebra mussels, and 2% other bivalves. *Diporeia* was never identified in diets from any species offshore of FF and MSK (Table 4).

Of 615 eggs used for DNA analysis, 373 were identified as deepwater sculpin, 87 as bloater, 1 as a slimy sculpin and 154 were unidentified due to insufficient DNA (Table 5). Deepwater sculpin eggs were identified in diets of all three benthivore species at all four sites from January through May. Of the 2,586 eggs assigned as deepwater sculpin, 55% were cannibalized by deepwater sculpin, 31% were consumed by slimy sculpin and 14% were consumed by round goby. The ranges of TL of predators that ate deepwater sculpin eggs differed: slimy sculpin = 42-102 mm, deepwater sculpins = 79-162 mm, and round goby = 78-144 mm. Slimy sculpin offshore of FF and deepwater sculpin offshore of TR, STB and MSK contained relatively high diet proportions by dry weight of deepwater sculpin eggs (0.03–0.06) and high frequency of occurrence (11-25% of predators contained eggs, Table 4) across many depths, and dates sampled (Table 5).

Bloater eggs were identified in diets of slimy and deepwater sculpins at all four site in February through May (Table 5). Of the 338 eggs assigned as bloater, 65% were consumed by slimy sculpin and 35% by deepwater sculpins. The size range of TL of benthivore species that consumed bloater eggs differed: slimy sculpins = 40-102 mm and deepwater sculpin = 92-163 mm. Slimy sculpin diets offshore of FF and

deepwater sculpin diets offshore of MSK contained relatively high proportions of bloater eggs by dry weight (0.04-0.07) at frequencies of occurrence ranging from 21-23% (Table 4).

The total number of fishes (2263) and trawls (121) included in the GLM analyses (Table 6) revealed the importance of site in explaining variation in benthivore diet proportions and the relative unimportance of year, DOY and depth (Table 7). For example, in Model 1 GLMs (which used only data at TR), mean diet proportions of 5 taxa consumed by slimy sculpin, 4 consumed by deepwater sculpin, and 3 consumed by round goby never varied as a function of DOY on which nets were sampled. Additionally, year sampled was also never significant, and depth was only significant for deepwater sculpin consuming *Diporeia* (Table 7).

Because DOY did not explain variation in diet proportions of benthivore species in Model 1 GLMs, Model 2 GLMs only used diets grouped by all nets site. Mean diet proportions of important prey species differed significantly between sites. For example, in six of twelve Model 2 GLMs, mean diet proportions of *Mysis* varied between sampling sites for all three benthivore species, of *Diporeia* for both sculpin species, and of bivalves for round goby. Diet proportions also differed between years for *Mysis* and bivalves consumed by round goby. Depth was never an important explanatory variable for any Model 2 GLMs (Table 7).

As hypothesized, diet overlap was never significant between round goby and either sculpin species using Schoener's index (Table 8). The lack of diet overlap between round goby and sculpin species was also reflected in NMS and cluster analyses. For each site, I identified three clusters, retaining 45-50 % of the original information for each ordination. Unlike the diets of sculpins, the diet composition of round goby plotted in different ordinations clustered only with other round goby. The clusters comprised of round goby diets were always associated with high proportions of quagga mussels and ostracods (Figure 3, 4), and sometimes with *Senecella* (Figure 3B). Additionally, the large distance

between the focal point for clusters of round goby diets and the focal points for clusters containing sculpin diets supported low overlap (Table 8, Figure 3, 4).

Schoener's index also revealed significant diet overlap between sculpin species offshore of FF and STB, but not offshore of TR (Table 8). NMS and cluster analyses offered a visualization of how diets overlapped. At FF, diets of deepwater sculpins, and most slimy sculpin were in the same cluster and these diets were highly associated with *Mysis* (Figure 3A). Besides *Mysis*, important prey taxa to slimy sculpin in this cluster included *Senecella*, *Limnocalanus*, and chironomids. A second cluster that contained the remaining slimy sculpin was associated with high diet proportions of *Senecella*, *Limnocalanus*, and chironomids and lower proportions of *Mysis* (Figure 3A). Similar to FF, one diet cluster at STB contained 100% of the deepwater sculpin and the majority of slimy sculpins (Figure 3B). This mixed-species cluster was positively correlated with *Diporeia*. A second cluster at STB contained the remaining slimy sculpin, which were associated with high proportions of *Senecella* and *Mysis* and low proportions of *Diporeia*.

At TR, the optimal ordination included three dimensions (Figure 4). The TR ordination differed from that of FF and STB in that the primary mixed species cluster included all diets of deepwater sculpins and a few (i.e., 30%) slimy sculpin. The other cluster with sculpins contained diets of all slimy sculpin except for one sample of deepwater sculpin. Closer visual examination of this cluster revealed that Axis 2 separated these two clusters. The cluster dominated by slimy sculpin was associated with high proportion of *Limnocalanus*, chironomids, and *Senecella* and lower proportions of *Mysis* and deepwater sculpin eggs. Conversely, the primary mixed species cluster was correlated with higher proportions of *Mysis* and deepwater sculpin eggs. Overall, the greater segregation of diets of slimy and deepwater sculpins in clusters from the TR ordination was consistent with the lack of significant diet overlap between sculpins at this site.

Discussion

My study revealed that *Mysis* and *Diporeia* were the most important prey for slimy and deepwater sculpin as has been previously documented (Wells 1980; Selgeby 1988; O'Brien et al. 2009). Slimy sculpin also preyed upon notable numbers of calanoid copepods (e.g., *Limnocalanus* and *Senecella*), chironomids, and fish eggs. Deepwater sculpin also consumed these prey, but in lower proportions and frequencies, except for deepwater sculpin eggs at some sites. Where *Diporeia* were absent from deepwater sculpin diets, *Mysis* comprised nearly 100% of diet composition, whereas slimy sculpin diet composition was more diverse whether *Diporeia* were present or absent. For round gobies, quagga mussels were the primary prey, but *Mysis* and ostracods contributed important proportions at some sites. Diet composition for each benthivore species varied little across years, DOY, or depths sampled in this study. Conversely, the site sampled explained high levels of variation in the diet composition for each benthivore species. Genetic analyses used on fish eggs found in benthivore diets revealed that slimy and deepwater sculpin preyed upon deepwater sculpin eggs as has been hypothesized in other studies (Wells 1980; O'Brien et al. 2009). Deepwater sculpin eggs were also consumed in low numbers by round goby. Bloater eggs were identified in diets of slimy and deepwater sculpin, but not in diets of round goby. Diet overlap was significant between slimy and deepwater sculpin but was minimal between round goby and either sculpin species.

Although fishes were sampled offshore of TR from multiple days within each month from January through April over a two year period, DOY did not explain variation in diet composition of any benthivore species. Previous research has also failed to identify significant temporal variation in food habits of sculpins. For example, Wojcik et al. (1986) sampled deepwater sculpin in Lake Michigan monthly from April to August at a depth of 100 m but found no monthly or seasonal effects on diet composition. With the exception of higher proportions of fish eggs in slimy sculpin diets in October, Kraft and Kitchell (1986) found that the diet composition of both slimy and deepwater sculpin did not

vary between fish collected in October or June. Wells (1980) found that the diet composition of slimy and deepwater sculpin collected monthly from March through November in 31-110 m depths did not vary across time, with the exception of higher proportions of fish eggs in deepwater sculpin in November. Lack of temporal variation can be attributed to relatively homogenous conditions experienced by benthivore species during all seasons in the Great Lakes at depths >70 m, including relatively constant temperatures, high pressure and low light levels (Wells 1968; Patterson et al. 1993; Janssen et al. 2007). Thus, when sampling Great Lakes benthivores from offshore habitats, accounting for temporal variation over days or months may be less important than accounting for other variables, such as location (see below), that could better explain variation diet composition.

Although DOY on which fishes were sampled did not influence diet composition, year sampled explained variation in the proportions of *Mysis* and dreissenids consumed by round goby in this study. Because only two years were sampled, determining if the year effect was random variation or an emerging pattern with ecological implications is difficult. Schaeffer et al. (2005) hypothesized that increased frequency of occurrence of quagga mussels and decreased frequency of occurrence of *Diporeia* in the diet of round goby sampled between 2001 and 2002 from depths of 27-46 m resulted from declines in *Diporeia* biomass and increases in quagga mussel biomass over those years in Lake Huron. My results are limited because of the duration of my study, but similarly suggested that round goby consumed more dreissenids, and fewer *Mysis* between 2009 and 2010 in offshore Lake Michigan. While there were likely increases in quagga mussels from 2009 to 2010 in Lake Michigan (Bunnell et al. 2009; Nalepa et al. 2009), data on mysids is unavailable. Future work will be required to determine if annual variation in round goby diet is a common response to annual ecological changes in prey species compositions, or biomass levels.

While I did not detect significant annual variation in sculpin diets, my results can be used to indicate a reduction in importance of *Diporeia* as a prey compared to previous research. For example, in

2003, French et al. (2010) found 100% frequency of occurrence of *Diporeia* in slimy sculpin sampled from > 55 m depths offshore of both FF and STB. By 2009-2010, *Diporeia* was never found in diets of sculpins offshore of FF, and occurred in only 46% of diets of slimy sculpin offshore of STB. Further, in this study sculpin never contained *Diporeia* offshore of MSK, but at the same site in 2000-2001 proportions of this prey averaged 0.66 (by dry weight) for slimy sculpin, and 0.45 for deepwater sculpin (Hondorp et al. 2005). Comparing my results to studies completed prior to the invasions of *Dreissena* spp. and the concomitant decline of *Diporeia* (e.g., Wells 1980; Kraft and Kitchell 1986; Wojcik et al. 1986), the proportions of *Diporeia* in diets from my study were markedly lower. There were sites in this study where *Diporeia* remained a component of the diets of sculpins (i.e., TR, STB), and these locations generally agreed with abundance estimates (Nalepa et al. 2009) that revealed higher densities of *Diporeia* on the western side of the lake.

Sculpin species appeared to differentially respond to either the decline of *Diporeia* or other recent food web changes. Whereas deepwater sculpin replaced *Diporeia* mainly with *Mysis*, slimy sculpin diversified their diet with other prey. For example, chironomids represented as much as 15% of the diet in slimy sculpin at some sites, and this could be explained by the results of Owens et al. (2003) and Hondorp et al. (2011) that demonstrated positive selection by slimy sculpin for chironomids after the decline of *Diporeia*. Further, slimy sculpin may also be taking advantage of prey that appear to be increasing in abundance. For example, both pre- (Wells 1980; Brandt; 1986b; Kraft and Kitchell 1986), and post-*Diporeia* decline (Owens and Dittman 2003; Hondorp et al. 2011) descriptions of sculpin diets lacked any mention of copepods as an important component. Slimy sculpin in my study contained high mean diet proportions and frequencies of occurrences of the copepod *Limnocalanus* at Two Rivers (0.48, 90%) and lower mean levels at Frankfort and Sturgeon Bay (0.09, 56-58%). Correspondingly, recent studies have reported *Limnocalanus* densities as increasing in recent years. Barbiero et al. (2009) reported densities three times higher from 2004-2006 than for the mean 19 years prior, and

Vanderploeg et al. (2011) estimated a +78% percent change in biomass of *Limnocalanus* post-expansion of quagga mussels into offshore habitats (2006-2008). Overall, my results corroborate those of Owens and Dittman (2003) and Hondorp et al. (2011) that slimy sculpin have diversified diets in response to food web changes, whereas deepwater sculpin diets appear to have become even more simplified with greater reliance on *Mysis* as *Diporeia* declined.

Given that diets of benthivores varied across sites, I evaluated whether diet overlap occurred within each site. Previous studies identified diet overlap between slimy and deepwater sculpin (Brandt et al. 1986a; Davis et al. 2007), and as hypothesized, significant diet overlap was found in this study. Significant diet overlap between sculpins offshore of FF and STB largely resulted because *Mysis* and *Diporeia* predominated in the diets of both species: their sum proportions by dry mass ranged from 0.82- 0.97 for deepwater sculpin and 0.57-0.58 for slimy sculpin. Conversely, at Two Rivers, diet overlap was not significant between sculpins because diet of slimy sculpin was dominated by calanoid copepods and chironomids (sum = 0.54) while diet of deepwater sculpin remained dominated by *Diporeia* and *Mysis* (0.81). Thus, diet overlap between sculpins may be reduced when slimy sculpin diversify their food habits (Owens and Dittman 2003; O'Brien et al. 2009; Hondorp et al. 2011).

When diet overlap between sculpin species did occur, however, it is not clear whether interspecific competition was occurring. Specifically, competition is dependent on several factors, including spatial overlap and whether common prey items were limiting. In this study, the weighted mean depth of capture for slimy sculpin (93m) was less than that of deepwater sculpin (122m) offshore of Sturgeon Bay and Frankfort, suggesting low spatial overlap. Madenjian and Bunnell (2008) observed similar depth segregation between sculpins in Lake Michigan in the fall, and hypothesized that it facilitated coexistence by mediating competition for food despite diet overlap (Madenjian et al. 2005). Additional explanations for depth-related segregation include preferences for different prey types and sizes (Owens and Weber 1995; Hondorp et al. 2011), different temperature preferences (Brandt et al.

1980), differential predation by lake trout or burbot *Lota lota* (Owens and Bergstedt 1994; Madenjian 2005), and interspecific predation or egg consumption (Brandt 1986a; Kraft and Kitchell 1986).

Whatever factors ultimately drove spatial segregation between sculpins, diet overlap may well increase in response in response to further *Diporeia* decline and increased reliance on *Mysis*.

As hypothesized, diet overlap between round goby and both sculpin species was not significant in all comparisons ($D = 0.08 - 0.39$) because of the dominance of quagga mussels in round goby diets. This finding on the dominance of dreissenids in round goby diet is similar to other studies in the nearshore Great Lakes (Ray and Corkum 1997; French and Jude 2001). Likewise, the 95 % of round goby in this study contained quagga mussels in their stomachs, similar to that observed in round goby sampled at 55 and 95 m during April in Lake Ontario (94 %, Walsh et al. 2007). Other main prey consumed by round goby included *Mysis* offshore of FF (62 %, 0.33), and ostracods offshore of STB (71%, 0.14). Consumption of each of these native invertebrates by round goby contributed to a lack of diet overlap between gobies and sculpins, since ostracods were always rare in sculpin diets (Table 4). Similarly high frequencies of occurrence of *Mysis* (58 %) and ostracods (70%) were also found in diets of offshore round goby sampled during April in Lake Ontario (51%, Walsh et al. 2007), and during fall in Lake Huron (91%, Schaeffer et al. 2005). So long as quagga mussels are abundantly available to round goby in offshore habitats of the Great Lakes, the diet overlap of round goby and sculpins should remain low.

All three benthivore species in this study consumed eggs of deepwater sculpin. Most eggs were cannibalized (55%), whereas slimy sculpin and round goby consumed smaller percentages of all those identified (31 %, 14 %, respectively). For some individuals, a surprisingly high number of deepwater sculpin eggs were found. For example, 10 slimy sculpin each contained more than 22 eggs in their stomachs, and 3 contained more than 90 eggs. Also, 17 deepwater sculpin each contained more than 20 deepwater sculpin eggs, and 8 contained more than 70 eggs. Finally, 3 round goby each contained more

than 61 of this egg species in their stomachs. Wells (1980) similarly reported up to 102 fish eggs in the diet of an individual deepwater sculpin during November, but other studies (e.g., Owens and Dittman 2003; O'Brien et al. 2009) did not provide details, or provided comparatively low values for egg consumption by individual deepwater sculpin (Wojcik et al. 1986, maximum = 15) and slimy sculpin (Wells 1980, maximum = 32). The proportion of deepwater sculpin eggs cannibalized did not vary by depth, day of the year, site, or year. Further modeling of predation and cannibalism could help determine whether consumption of deepwater sculpin eggs has negatively affected the recruitment of deepwater sculpin

Bloater eggs were only consumed by deepwater and slimy sculpin in this study. This prey was identified at all four sites sampled, during February through May. The maximum mean proportion of bloater eggs (i.e., 0.07) occurred for slimy sculpin offshore of Frankfort (i.e., 0.07) and nearly equaled the highest site level proportion (0.06) of deepwater sculpin eggs in sculpin diets from any site. The similarity was somewhat surprising because relative to deepwater sculpin eggs, bloater eggs were fewer in number when observed in individual sculpin diets. In fact, the maximum number of bloater eggs observed in an individual slimy sculpin was 14, and was 10 for an individual deepwater sculpin. Likewise, the median number of bloater eggs consumed by individual sculpins that contained this prey was 1. As hypothesized, of bloater eggs in diets, slimy sculpin consumed a greater percentage (66%) than did deepwater sculpin (34%). Interestingly, none of the bloater eggs identified to species in February to March diets were eyed or well developed, but during April to May, more than 50% of the eggs identified were well-developed, eyed, and appeared nearly ready to hatch. These observations suggest that spawning occurred from February to March and hatching from April to May, which corresponded with previous observations (Rice et al. 1987). Although this study confirmed slimy sculpin consumption of bloater eggs, further modeling will be required to determine whether it is sufficient to have an impact on bloater recruitment.

As in all studies, some caveats should be considered when interpreting findings herein. For example, not all sites were sampled in all months. My approach to this unbalanced design was to analyze temporal effects at the site with the best temporal coverage. A more balanced design would have examined DOY effects on benthivore diet composition at all sites in January through March. Another bias that could have influenced diet composition results was that prey do not digest at equal rates. For example, bivalve shells (Prejs et al. 1990; French 1993) and chitinous chironomid heads (Hershey and McDonald 1985) may resist digestion, which would result in an apparently higher abundance in the diet compared to items with faster rates of digestion. Another concern in this study was the underestimation of fish eggs in diets given their potential to be quickly digested (Daan et al. 1985). However, egg casings are relatively resistant to digestion (Ohtsuka 1960; Bailey and Houde 1989) and I have found that the parts I used to count *Mysis* (*eyes*) and casings of bloater eggs could remain identifiable up to 5 days after ingestion by slimy and deepwater sculpins kept at temperatures 2° to 4° C (Londer unpublished data). Thus, the long gut residence times of these prey pieces may have reduced any bias to overestimate bivalves or chironomid content of the diet. Lastly, this study was a snapshot in time and space, and extrapolating these results into southern Lake Michigan or into other Great Lakes should be approached cautiously.

These descriptions of diet composition from deepwater sculpin, slimy sculpin and round goby provide valuable data to inform ecosystem-based management by filling data gaps for ecosystem models and by providing data on egg consumption to support future recruitment models (GLFC 2001; Zimmerman and Krueger 2009). Results clearly indicated that sculpins and round goby varied their diets by site, likely as a consequence of site-specific differences in prey composition. Another important finding was that round goby appeared to have minimal impacts on offshore sculpins in Lake Michigan as they relied primarily upon quagga mussels and generally avoided *Diporeia* and fish eggs. However, if the population of round goby continues to proliferate, their relatively low consumption of *Mysis* (0.15 mean

diet proportion across all ports) could impact upon *Mysis* production and availability. Lastly, although the frequency of bloater and deepwater sculpin eggs) in sculpin diets seemed comparable to frequencies at earlier times, egg consumption may be more important today than in the past because bloater and deepwater sculpin biomass (and therefore egg production) are currently near record low levels, while slimy sculpin biomass is very high compared to historic levels. As a result, even small proportions of eggs consumed by sculpins could have a disproportionate impact upon recruitment of these important native species.

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Table 1: The number of trawl hauls done to collect benthivores at each site, month, and year from depths of 69 – 128 m in Lake Michigan.

Slimy sculpin								
Month	<u>Two Rivers</u>		<u>Frankfort</u>		<u>Sturgeon Bay</u>		<u>Muskegon</u>	
	2009	2010	2009	2020	2009	2010	2009	2010
January	3	2	-	-	-	-	-	-
February	2	2	-	-	-	-	-	-
March	6	2	-	-	-	-	-	-
April	3	2	6	4	3	4	-	-
May	-	-	-	3	-	3	1	-
June	-	-	-	-	-	-	1	-
Deepwater sculpin								
January	3	2	-	-	-	-	-	-
February	1	2	-	-	-	-	-	-
March	6	2	-	-	-	-	-	1
April	3	-	5	3	2	3	-	1
May	-	-	-	2	-	2	1	1
June	-	-	-	-	-	-	1	-
Round goby								
January	3	2	-	-	-	-	-	-
February	1	2	-	-	-	-	-	-
March	5	2	-	-	-	-	-	-
April	2	1	7	4	3	2	-	-
May	-	-	-	1	-	1	-	-
June	-	-	-	-	-	-	-	-

Table 2: Summary information for the 12 prey categories used in diet analyses. Measurement identifies body parts measured for regression analyses to estimate dry weight.

Prey category	Life stages	Taxonomic resolution	Identified from	Measurement	Sources
Bivalves	veliger to shelled	<i>Dreissena bugensis</i>	visual, right septa,	shell, septa	Hillbricht-Ilkowska and Stanczykowska 1969; Prejs et al. 1990; Nalepa et. al 2010
	veliger to shelled	Other bivalves	visual, right septa	shell, septa	
Bloater eggs	egg, embryo	<i>Coregonus hoyi</i>	whole, DNA	radius, color	Wells 1980
Deepwater sculpin eggs	egg, embryo	<i>M. thompsonii</i>	whole, DNA	radius, color	Geffen and Nash 1982
Chironomidae	larvae to adult	<i>Heterotrissocladius</i>	head, mouth parts	total length	Nalepa and Quigley 1980
	larvae to adult	<i>Micropsectra</i>	head, mouth parts	total length	Nalepa and Quigley 1980
	larvae to adult	<i>Paracladopelma</i>	head, mouth parts	total length	Nalepa and Quigley 1980
<i>Cladocera</i>	egg, adult	<i>Daphnidae</i>	intact foot	head to foot	Rosen 1981
	egg, adult	<i>Bythotrephes</i>	whole intact spines	spine length	Makarewicz and Jones 1990
	egg, adult	<i>Bosminidae</i>	complete rostrum	total length	Bottrell et al. 1976; Andrew and Herzig 1984
<i>Diporeia</i> spp.	adult	<i>Diporeia</i> spp.	whole heads	head to telson	Pothoven et al. 2001
<i>Mysis</i> spp.	adult	<i>Mysis</i> spp.	eyeballs	antennal scale	Grossnickle and Beeton 1979; Shea and Makarewicz 1989
Fish remains	unknown, adults	Cottidae, nine spine	visual, cleithra	length, weight	Londer unpublished data
	unknown, adults	smelt, unidentified,	visual, cleithra	length, weight	Londer unpublished data
<i>Ostracoda</i> spp.	unknown, no eggs	<i>Ostracoda</i> spp.	shells	shell length	Nalepa and Quigley 1980
Other copepods	nauplii to adults	<i>Leptodiaptomidae</i>	rami, morphology	head – urosome	Pace and Orcutt 1981
	nauplii to adults	<i>Cyclopoida</i> spp.	rami, morphology	head – urosome	Hudson et al. 2003
	nauplii to adults	<i>Harpactacoida</i> spp.	rami, morphology	head – urosome	Pace and Orcutt 1981
<i>Limnocalanus macrurus</i>	nauplii to adults	<i>Limnocalanus macrurus</i>	rami, morphology	head – urosome	Pace and Orcutt 1981; Doubek and Lehman 2011
<i>Senecella calanoides</i>	nauplii to adults	<i>Senecella calanoides</i>	rami, morphology	head – urosome	Lesko et al. 2003; Pace and Orcutt 1981

Table 3: Summary statistics for benthivore species from which diets were estimated.

	<u>Wet Weight (g)</u>			<u>Total Length</u>		
	Range			Range		
	Mean ± Standard Deviation			Mean ± Standard Deviation		
	slimy sculpin	deepwater sculpin	round goby	slimy sculpin	deepwater sculpin	round goby
Overall	0.6 - 20.6 6.0 ± 3.1	0.6 - 90.2 17.8 ± 12.4	3.0 - 91.6 20.3 ± 13.1	36 - 112 74.1 ± 12.1	38 - 192 113 ± 13.1	59 - 178 105 ± 23.1
Frankfort	0.6 - 20.6 5.4 ± 3.3	0.6 - 39.7 6.5 ± 7.6	3.4 - 76.2 19.9 ± 12.9	38 - 112 71.8 ± 13.3	38 - 146 77.7 ± 31.1	59 - 178 104.4 ± 20.9
Sturgeon Bay	0.6 - 14.4 6.6 ± 3.2	1.2 - 51.4 21.1 ± 9.8	3.0 - 40.0 11.4 ± 6.9	38 - 102 75.3 ± 12.9	55 - 165 124.1 ± 23.7	60 - 135 87.5 ± 14.8
Two Rivers	0.6 - 16.0 6.2 ± 2.8	5.8 - 90.2 26.2 ± 12.3	3.4 - 91.6 26.3 ± 15.4	36 - 102 75.1 ± 10.6	60 - 192 131.5 ± 20.3	63 - 168 116.1 ± 23.8
Muskegon	n/a n/a	0.8 - 18.7 10.1 ± 3.2	n/a n/a	n/a n/a	48 - 125 100.6 ± 13.7	n/a n/a

Table 4: Diet proportions by dry weight and frequency of occurrence (in parentheses) for each site and benthivore species. Summations for proportions may not equal one because of rounding and because the miscellaneous category was excluded. SS = slimy sculpin, DWS = deepwater sculpin and RG = round goby. All three benthivore species are listed at each site, except at Muskegon where only diets of deepwater sculpin are listed.

Species	Frankfort			Muskegon	Two Rivers			Sturgeon Bay		
	SS	DWS	RG	<u>n</u> DWS	SS	DWS	RG	SS	DWS	RG
Bivalves	<0.01 (1)	<0.01 (1)	0.58 (96)	<0.01 (1)	<0.01 (2)	<0.01 (1)	0.83 (95)	<0.01 (1)	<0.01 (2)	0.62 (95)
Bloater eggs	0.07 (23)	<0.01 (1)	0.00 (0)	0.04 (21)	0.02 (5)	0.02 (12)	0.00 (0)	0.01 (2)	0.01 (10)	0.00 (0)
<i>Chironomidae</i>	0.05 (53)	<0.01 (21)	<0.01 (44)	<0.01 (8)	0.10 (79)	<0.01 (7)	0.01 (17)	0.15 (83)	0.01 (41)	0.01 (59)
Deepwater sculpin eggs	0.03 (11)	0.01 (4)	0.01 (1)	0.05 (21)	0.01 (2)	0.06 (13)	0.02 (3)	0.03 (8)	0.05 (25)	<0.01 (1)
<i>Diporeia</i> spp.	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.11 (19)	0.20 (32)	0.01 (3)	0.33 (46)	0.42 (87)	0.03 (3)
<i>Limnocalanus macurus</i>	0.09 (58)	<0.01 (20)	<0.01 (10)	0.01 (17)	0.48 (90)	0.03 (20)	0.02 (26)	0.09 (56)	<0.01 (16)	<0.01 (8)
<i>Mysis relicta</i>	0.58 (72)	0.97 (100)	0.33 (62)	0.88 (95)	0.19 (26)	0.61 (82)	0.03 (5)	0.24 (37)	0.50 (90)	0.10 (15)
Ostracoda	<0.01 (8)	<0.01 (11)	0.02 (59)	<0.01 (1)	0.01 (10)	<0.01 (1)	0.04 (44)	<0.01 (3)	<0.01 (1)	0.14 (71)
<i>Senecella calanoides</i>	0.13 (79)	0.02 (80)	0.05 (72)	0.01 (17)	0.05 (43)	0.01 (23)	0.01 (9)	0.11 (56)	0.01 (53)	0.03 (35)
Other copepods	0.03 (38)	<0.01 (4)	<0.01 (11)	<0.01 (2)	0.01 (34)	<0.01 (2)	<0.01 (14)	0.02 (35)	<0.01 (4)	<0.01 (21)

Table 5: Summary of months and locations where eggs from the diets of 3 benthivore species were identified to species. sites: TR = Two Rivers, WI; FF = Frankfort, MI; MSK = Muskegon, MI; and STB = Sturgeon Bay, WI. SS = slimy sculpin, DWS = deepwater sculpin, RG = round goby. * N = 2 fishes, ** N = 6 fishes.

Month	Port	Predator	Bloater eggs				Deepwater sculpin eggs			
			# eggs identified by genetics	Total # of eggs after assignments	Depths (m) from which eggs were identified or assigned	Percent occurrence of eggs in all diets from these depths	# eggs identified by genetics	Total # of eggs after assignments	Depths (m) from which eggs were identified or assigned	Percent occurrence of eggs in all diets from these depths
Jan	TR	SS	0	0	-	-	15	31	98, 106	08
		DWS	0	0	-	-	15	169	82, 98, 106	14
		RG	0	0	-	-	1	75	98	09
Feb	TR	SS	11	15	82	37	7	35	85	09
		DWS	3	5	85	22	74	347	85, 98, 106	19
Mar	TR	SS	1	11	84	25	0	0	-	-
		DWS	1	22	104	20	2	43	99	50
Apr	MSK	DWS	4	8	107	14	31	175	107	21
		TR	SS	0	1	87	03	1	5	91
	TR	DWS	4	7	87	30	3	1	87	11
		MSK	DWS	1	5	107	17	12	51	107
	FF	SS	31	52	91, 110, 128	23	81	176	91, 128	23
		DWS	0	3	110	07	14	2	128	03
		RG	0	0	-	-	9	142	82, 128	02
May	STB	SS	1	8	110	0	24	151	110, 128	83**
		DWS	9	36	110, 128	15	42	340	110, 128	20
	MSK	SS	7	17	100	100*	13	90	100	100*
		DWS	7	12	100, 107	17	7	23	100	58
	STB	SS	0	0	-	-	5	37	110	23
		DWS	0	0	-	-	9	48	110, 128	19
		FF	SS	5	24	128	53	6	28	128
		DWS	0	0	-	0	0	1	-	07

Table 6: Descriptive statistics of the number and sizes of predator species caught in a given net at a given site and used in general linear modeling. SS = slimy sculpin, DWS = deepwater sculpin, and RG = round goby.

Port	Predator	N nets	N fish	Range of N fish in each net	Mean N of fish in each net	Standard deviation of N fish in each net
Frankfort	SS	13	325	15-30	25	6
	DWS	10	142	1-31	14	13
	RG	12	305	1-50	23	18
Sturgeon Bay	SS	10	220	2-34	22	12
	DWS	7	189	1-39	27	12
	RG	6	91	1-37	15	15
Two Rivers	SS	22	466	1-33	21	9
	DWS	19	238	1-30	13	11
	RG	18	156	1-33	9	10
Muskegon	DWS	4	130	28-30	29	1

Table 7: *F* and *P* values from General Linear Models that sought to explain variation in diet proportions by dry weight for each benthivore species for a given prey taxa category. The sampling unit in these analyses was the mean diet proportions for a given prey and predator species caught within the same net. Model 1 used only data from benthivore species caught at Two Rivers. Model 2 used data from benthivores caught at all sites. Significant *P*-values that were less than the Bonferroni-adjusted α are in bold.

Prey Type	Predator	Day of Year	Model 1		Site	Model 2	
			Depth	Year		Depth	Year
Bivalves	Round goby	$F_{1,14}=0.66$ $p=0.4316$	$F_{1,14}=0.03$ $p=0.8753$	$F_{1,14}=2.61$ $p=0.1283$	$F_{2,31}=4.34$ $p=0.0219$	$F_{1,31}=2.74$ $p=0.1080$	$F_{1,31}=17.33$ $p=0.0002$
<i>Chironomidae</i>	Slimy sculpin	$F_{1,18}=6.61$ $p=0.0192$	$F_{1,18}=0.04$ $p=0.8406$	$F_{1,18}=1.80$ $p=0.1963$	$F_{2,40}=3.34$ $P=0.0403$	$F_{1,40}=4.62$ $P=0.0878$	$F_{1,40}=3.52$ $p=0.0677$
DWS eggs	Deepwater sculpin	$F_{1,15}=0.41$ $p=0.5314$	$F_{1,15}=1.26$ $p=0.2800$	$F_{1,15}=3.70$ $p=0.0738$	$F_{3,34}=2.23$ $P=0.1021$	$F_{1,34}=0.64$ $p=0.4297$	$F_{1,34}=2.54$ $p=0.1203$
<i>Diporeia</i> spp.	Slimy sculpin	$F_{1,18}=2.10$ $p=0.1646$	$F_{1,18}=1.05$ $p=0.3200$	$F_{1,18}=0.35$ $p=0.5610$	$F_{2,40}=9.91$ $p=0.0003$	$F_{1,40}=4.55$ $p=0.0392$	$F_{1,40}=0.09$ $p=0.7604$
	Deepwater sculpin	$F_{1,15}=5.56$ $p=0.0324$	$F_{1,15}=11.69$ $p=0.0038$	$F_{1,15}=0.00$ $p=0.9606$	$F_{3,34}=12.55$ $p<0.0001$	$F_{1,34}=5.59$ $p=0.0239$	$F_{1,34}=1.42$ $p=0.2424$
<i>Limnocalanus macrurus</i>	Slimy sculpin	$F_{1,18}=1.70$ $p=0.2092$	$F_{1,18}=1.76$ $p=0.2016$	$F_{1,18}=2.11$ $p=0.1637$	$F_{2,40}=15.86$ $p<0.0001$	$F_{1,40}=3.62$ $p=0.0642$	$F_{1,40}=5.27$ $p=0.0269$
	Deepwater sculpin	$F_{1,15}=1.19$ $p=0.2934$	$F_{1,15}=1.75$ $p=0.2055$	$F_{1,15}=3.52$ $p=0.0803$	$F_{3,34}=0.32$ $P=0.5743$	$F_{1,34}=0.64$ $p=0.4297$	$F_{1,34}=1.39$ $p=0.2473$
<i>Mysis</i> spp.	Slimy sculpin	$F_{1,18}=2.14$ $p=0.1608$	$F_{1,18}=8.04$ $p=0.0109$	$F_{1,18}=2.25$ $p=0.1507$	$F_{2,40}=19.83$ $p<0.0001$	$F_{1,40}=0.87$ $p=0.3570$	$F_{1,40}=0.88$ $p=0.3539$
	Deepwater sculpin	$F_{1,15}=4.92$ $p=0.0424$	$F_{1,15}=6.40$ $p=0.0231$	$F_{1,15}=1.87$ $p=0.1911$	$F_{3,34}=11.88$ $p<0.0001$	$F_{1,34}=3.70$ $p=0.0629$	$F_{1,34}=0.06$ $p=0.8084$
	Round goby	$F_{1,14}=0.38$ $p=0.5489$	$F_{1,14}=2.91$ $p=0.1102$	$F_{1,14}=0.53$ $p=0.4786$	$F_{2,31}=14.81$ $p=0.0001$	$F_{1,31}=3.65$ $p=0.0650$	$F_{1,31}=9.52$ $p=0.0052$
<i>Ostracoda</i>	Round goby	$F_{1,14}=0.87$ $p=0.3654$	$F_{1,14}=2.07$ $p=0.1719$	$F_{1,14}=0.16$ $p=0.6932$	$F_{2,31}=4.50$ $p=0.0192$	$F_{1,31}=4.19$ $p=0.0492$	$F_{1,31}=3.46$ $p=0.0726$
<i>Senecella calanoides</i>	Slimy sculpin	$F_{1,18}=1.88$ $p=0.1871$	$F_{1,18}=1.38$ $p=0.2548$	$F_{1,18}=7.11$ $p=0.0157$	$F_{2,40}=4.68$ $p=0.0149$	$F_{1,40}=1.80$ $p=0.1879$	$F_{1,40}=0.67$ $p=0.4174$

Table 8: Schoener's index of diet overlap estimated for each species pair at each site. Values ≥ 0.60 indicate significant diet overlap and are indicated in bold.

Port		Slimy sculpin	Deepwater sculpin
Frankfort	Slimy sculpin	-	-
	Deepwater sculpin	0.6170	-
	Round goby	0.3581	0.3939
Two Rivers	Slimy sculpin	-	-
	Deepwater sculpin	0.3893	-
	Round goby	0.1078	0.0829
Sturgeon Bay	Slimy sculpin	-	-
	Deepwater sculpin	0.6233	-
	Round goby	0.1384	0.1297

Figure 1: Sites where benthivores were sampled for diet analyses. The dark solid circles represent the nearest city for nearby offshore sampling locations.

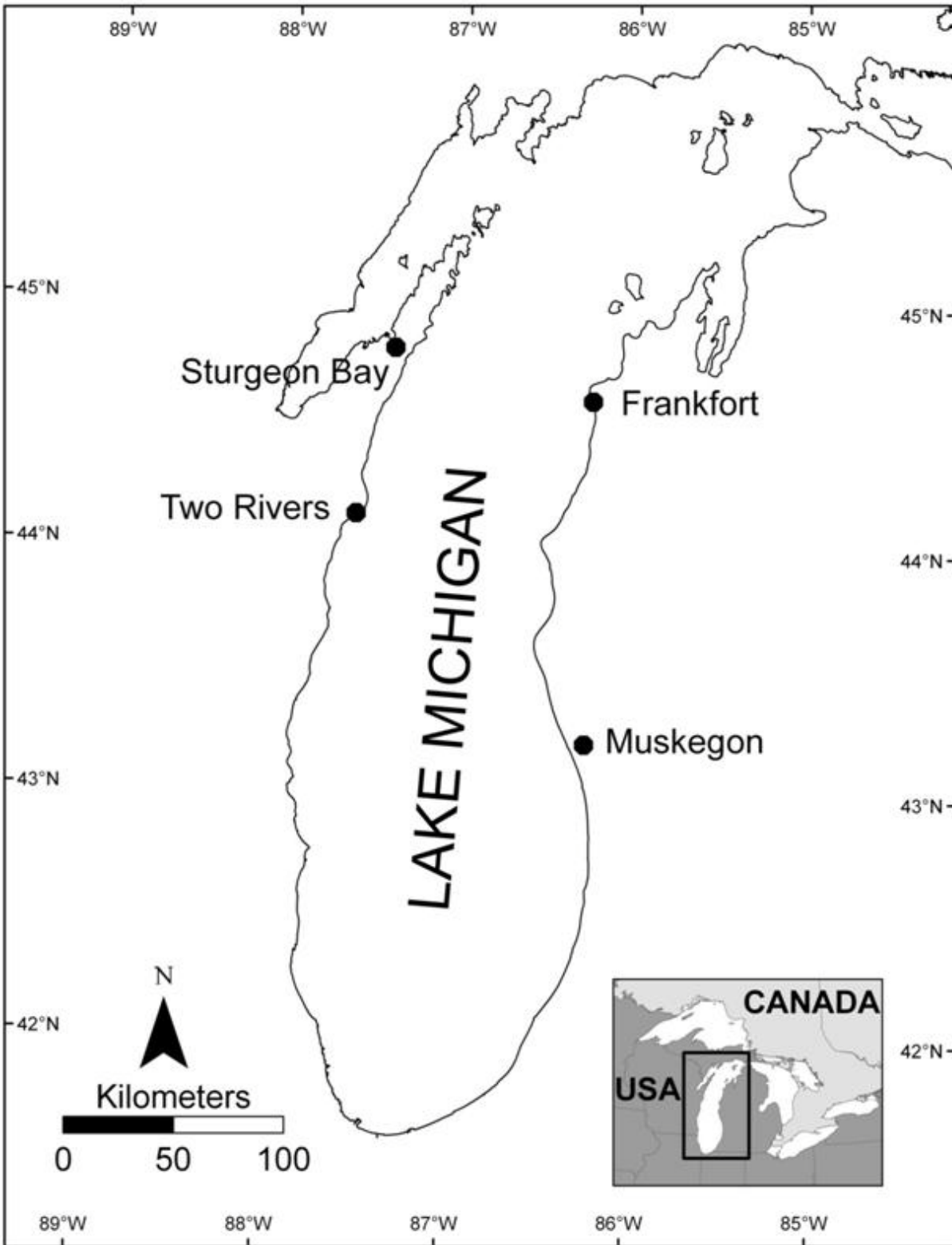


Figure 2: Pooling across all individuals, mean diet proportions (A) and frequency of occurrence (B) of prey items in slimy sculpin, deepwater sculpin, and round goby sampled in Lake Michigan in January-June 2009 – 2010. May and June slimy sculpin samples at Muskegon were excluded because of low sample size (N = 3 fish).

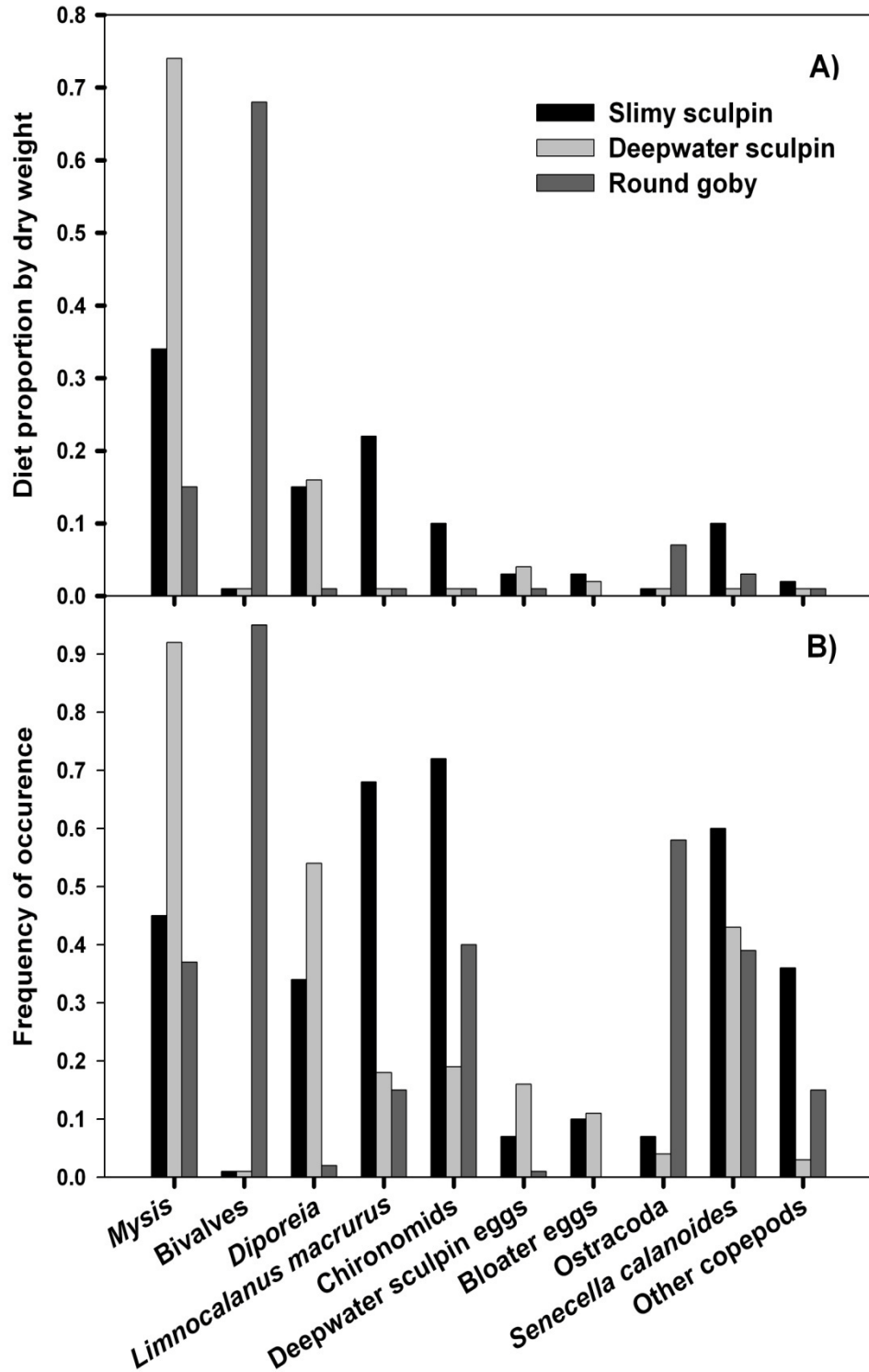


Figure 3: NMS results of mean diet proportions for benthivore species caught in nets offshore of Frankfort (A) and Sturgeon Bay (B). Each circled group was determined from cluster analyses, and contains species with similar diet composition. Prey species that were significantly correlated to an axis after alpha was adjusted by dividing it by the number of prey categories used ($p= 0.0063$) are listed alongside that axis. Arrows indicate low to high prey importance.

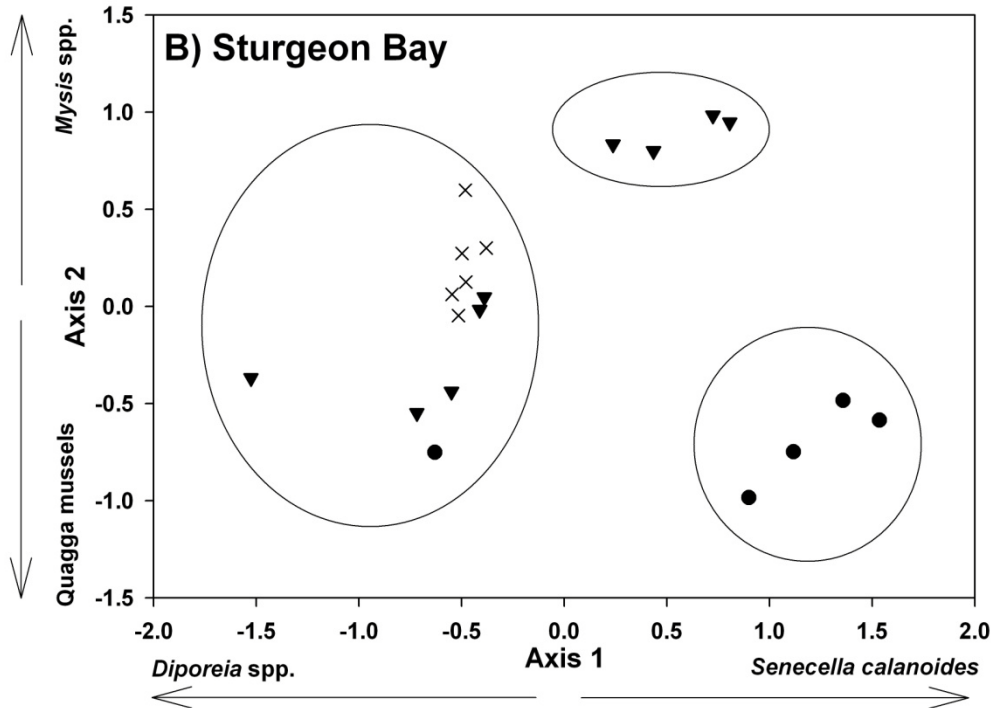
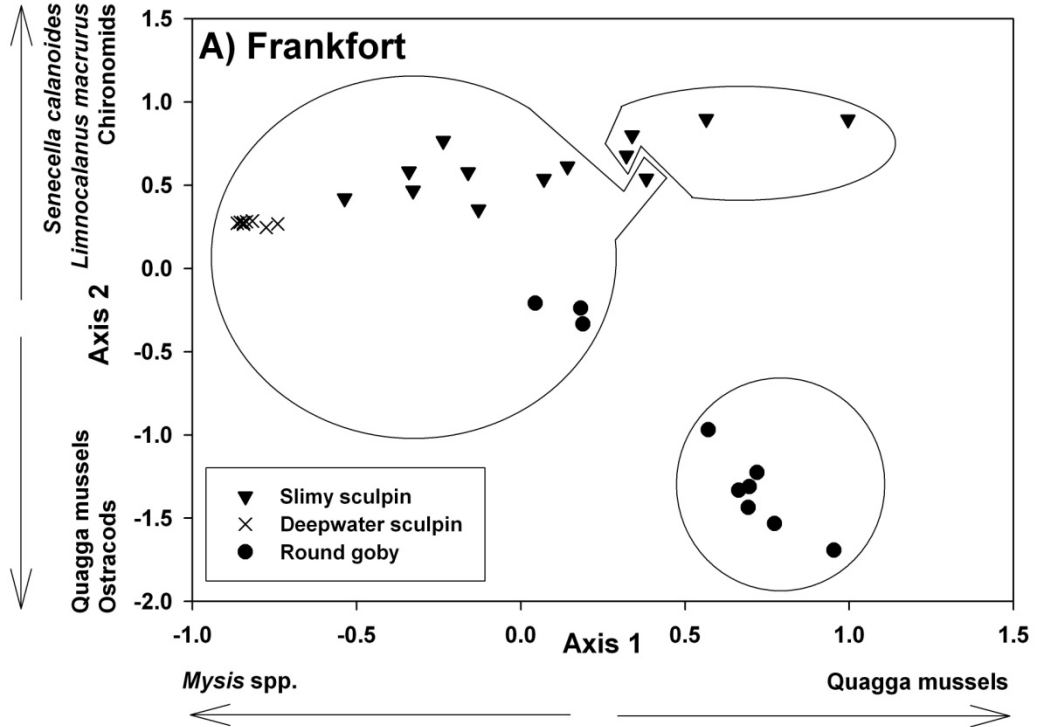


Figure 4: NMS results of mean diet proportions for benthivore species caught in nets offshore of Two Rivers. Each outlined group was determined from cluster analyses, and contains species with similar diet composition. Prey species that were significantly correlated to an axis after alpha was adjusted by dividing it by the number of prey categories used ($p = 0.0063$) are listed alongside that axis.

