Patterns of integration of invasive round goby (*Neogobius melanostomus*) into a nearshore freshwater food web

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Abstract

Understanding trophic interactions of non-native species is a key step in elucidating their ecological role in recently invaded systems. The benthic fish species round goby (*Neogobius melanostomus*) has successfully established in aquatic systems across the world, with abundances increasing dramatically over relatively short time periods. Though this (at times) voracious benthivore can become an increasingly important forage fish for piscivores, relatively little is known about how prey and production pathways that support round gobies vary in space and time. In 2010, we collected round gobies from ten nearshore sites, over three seasons, in Lake Michigan, U.S.A. Due to recent changes in Lake Michigan, the dynamic nearshore region may be crucial for stability of the whole-lake food web. We assessed the role of round gobies in the nearshore Lake Michigan food web using stomach contents, fatty acid profiles, and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Patterns in all of these measures were highly influenced by site, suggesting that local conditions, such as substrate composition or proximity to riverine inputs, were important in structuring round goby trophic interactions. By contrast, season of sampling and depth of collection had relatively weak associations with observed patterns. Few broad, regional patterns were evident, including a relatively high reliance on benthic production pathways on the western side of Lake Michigan. The observed variety in feeding patterns of round gobies, including potential for exploitation of different production pathways, may contribute to long-term persistence of this aquatic invader in new habitats.

Keywords: stable isotope; fatty acid; diet; benthic; Lake Michigan; spatial

1. Introduction

While food webs are often depicted as static in space and time, inter-specific predator-prey interactions (i.e., individual food web linkages) can vary ontogenetically (e.g., Werner et al. 1983; Warren and Lawton 1987), spatially, and seasonally (e.g., Warren 1989; Winemiller 1990). In large aquatic systems, dominant primary production and consumption pathways (i.e., the bottom of the food web) may vary spatially and temporally and thereby differentially affect composition and condition of lower trophic levels (e.g., Brooks and Edgington 1994). Physical processes may mediate magnitude and characteristics of primary production (e.g., through nutrient delivery, re-suspension, and distribution). Consistent physical features (e.g., sediment characteristics, proximity to tributaries) and prevailing conditions (e.g., water currents and gyres) may continuously structure food webs (e.g., Larson et al. 2012), while episodic hydrological events, such as river discharges and upwellings, may vary temporally in duration and intensity (e.g., Beletsky and Schwab 2001; Schwab and Beletsky 2003) and affect local areas or broad regions (Lee and Hawley 1998; Eadie et al. 2002). Ultimately, such structuring processes may not only lead to spatio-temporal differences in primary producers and consumers, but may also be reflected in the space- and time-specific diets and dietary signatures of secondary and tertiary consumers as energy is passed up the food chain.

Introductions of aquatic invasive species create additional complexity in understanding food web structure. These introductions are often linked to community compositional changes and may contribute to altered food webs and trophic regimes (e.g., Kuhns and Berg 1999; Vander Zanden et al. 1999; Janssen and Jude 2001; Vanderploeg et al. 2002; Hecky et al. 2004). As population numbers increase and species transition from “invasive” to “established”, these new species may interrupt or eliminate previously important trophic pathways (e.g., Vander

Zanden et al. 1999; Baxter et al. 2004). At the same time, the new species may facilitate energy transfer via new pathways, which may or may not lead to similar overall food web function as that of the pre-invasion state (e.g., Almqvist et al. 2010; Rogers et al. 2014). Effects of factors including substrate, vegetation, tributaries, light penetration, and temperature on the species will still vary both spatially and temporally, and thus may differentially shape the development of trophic connections between the new species and the pre-invasion food web.

In light of recent species invasions, nearshore food web interactions in the Laurentian Great Lakes have likely changed (Rennie et al. 2009). Several recent invaders, including dreissenid mussels (zebra mussel, *Dreissena polymorpha*; quagga mussel, *D. rostriformis bugensis*) and round gobies (*Neogobius melanostomus*), have contributed to shunting of energy from pelagic zones to the benthic nearshore via biomass accumulation (e.g., Vanderploeg et al. 2002; Hecky et al. 2004; Turschak et al. 2014). As a consequence, the relative importance of nearshore areas for lake-wide consumer production may have increased. An extreme example of this has occurred in Lake Michigan, where filtering action of dreissenids has caused the lake at large to transition toward a more oligotrophic state (Barbiero et al. 2012), especially in offshore waters. Combined with the broad range of physical and chemical characteristics displayed in the nearshore region, (e.g., Creque et al. 2010, Yurista et al. 2015), Lake Michigan may provide an ideal location to examine how the trophic role of an invasive but increasingly important species, the round goby, varies across seasons and habitats.

Round gobies are benthic fish from the Ponto-Caspian region which have successfully invaded habitats across the globe (reviewed in Kornis et al. 2012). While they were first introduced to the Laurentian Great Lakes during the late 1980s (Jude et al. 1992; Charlebois et al. 1997; Charlebois et al. 2001), as their range and abundance increased, round gobies preyed upon, competed with, and subsequently displaced multiple native species including several sculpin, darter, and invertebrate species (e.g., Jude et al. 1995; Dubs and Corkum 1996; French and Jude 2001; Janssen and Jude 2001; Vanderploeg et al. 2002; Lauer et al. 2004; Cooper et al. 2009). Over time, they also began to serve as important prey for several native and commercially important piscivores such as lake trout (*Salvelinus namaycush*), yellow perch (*Perca flavescens*) and smallmouth bass (*Micropterus dolomieu*) (e.g., Steinhart et al. 2004; Truemper and Lauer 2005; Dietrich et al. 2006; Jacobs et al. 2010; Madenjian et al. 2011).

Where small-bodied fishes invade, they may provide novel links in the food web as secondary consumers and prey for piscivores (as in Almqvist et al. 2010). Round goby consumption has been linked to population increases in a variety of predators across the globe (e.g., Jakubas 2004, Steinhart et al. 2004, King et al. 2006). As benthivores, round gobies may serve an important link to benthic production pathways, which has been demonstrated to be at least as important to lake-wide food webs as pelagic production pathways in many lake systems (Vander Zanden and Vadeboncoeur 2002). Conversely, there may be energetic or health tradeoffs associated with increased round goby consumption, given that round gobies may be less energetically dense than other prey (e.g., Van Guilder and Seefelt 2013) and are associated with increased contaminant transfer and botulism contamination (reviewed in Kornis et al. 2012). While it is clear that round gobies are becoming increasingly important members of Great Lakes food webs, to date, few broad-scale studies of round goby feeding patterns have been conducted.

A suite of approaches have emerged to examine aquatic food web interactions. Traditional diet (gut content) analysis, where researchers identify organisms found within digestive tracts, allows for describing recent prey consumption at fine taxonomic resolution but prey-specific digestion and retention rates likely introduce bias. In addition, diet analyses reflect recent feeding patterns but may not accurately reflect the relative integration of energy from particular prey into an organism over an extended time period. By contrast, the relative tissue composition of different fatty acids and stable isotope ratios, such as $\delta^{13}C$ and $\delta^{15}N$, allow researchers to quantify prey incorporated by consumers independent of digestive rate and over longer time scales (i.e., 4-12 weeks for fatty acids, Kirsch et al. 1998; 3-4 months for $\delta^{13}C$ and $\delta^{15}N$, Tieszen et al. 1983), albeit at low taxonomic resolution. In addition, certain fatty acid and stable isotopes signatures suggest reliance on pelagic versus benthic production pathways (e.g, Happel et al 2015a).

Signatures of fatty acids and stable isotope ratios in fish tissue complement traditional diet analysis, ultimately providing a comprehensive approach to examine feeding interactions (e.g., Pasquaud et al. 2008; Davis et al. 2012). In this study, we combined three trophic indicator methods (i.e., diet analysis, fatty acids, and carbon and nitrogen stable isotope ratios) to investigate how round goby feeding patterns in nearshore areas of Lake Michigan vary with location, season, and ontogeny. Specifically, we examined how spatial and temporal, (i.e., season, size of round goby), attributes might affect trophic roles of round gobies in the food web. Examining the prey items consumed by round gobies plus the relative composition of fatty acids and the $\delta^{13}C$ and $\delta^{15}N$ signatures in round goby tissues from Lake Michigan may suggest whether round gobies provide links to a single production pathway (i.e., benthic) or may allow predators of round goby to exploit multiple production pathways (e.g., pelagic or benthic primary producers).

2. Methods

2.1 Field Collections

We sampled 10 sites in nearshore Lake Michigan in May, July, August, and September 2010, selected to vary spatially and in terms of habitat characteristics (Table 1; Fig. 1). Sites in different parts of the lake were sampled during the same 2-week period within a season (May = Spring, July and August = Summer, September = Fall), but not all sites were sampled in all seasons (Table 1). We collected round gobies using 2-hour bottom-set, micromesh gillnets (6-mm, 8-mm, 10-mm, and 12-mm bar mesh sizes, 10 m of each mesh size per gillnet). For most season-site combinations, we deployed three 2-hour sets at three discrete depths (3 m, 7–9 m, and 14–16 m) starting at approximately 7 am EST and ending at approximately 1 pm EST (i.e., total effort: 9 x 2-hour net sets per site per season). Past studies indicated that round goby diets varied with individual size, with a shift from non-dreissenid diets to primarily dreissenid diets occurring somewhere between 60 and 100 mm (e.g., Ray and Corkum 1997; Campbell et al. 2009; Andraso et al. 2011). In an attempt to capture distinct signatures from these different stages, we targeted three *a priori* selected size classes during each sampling event (i.e., < 60 mm, 60–100 mm, and > 100 mm; target n = 20 per size class). In some areas where small numbers of round gobies were collected, we supplemented our catches by conducting beach seines (15 m sweep) and/or procuring fish from other organizations that were sampling in the region (i.e., Department of Natural Resources personnel in all states, Ball State University from sites in southern Lake Michigan; Table 1).

In the field, up to 20 fish total, collected across all times and depths sampled, were blotted dry and frozen on dry ice prior to subsequent storage in a -80°C freezer for fatty acid analyses plus diet content and/or stable isotope analyses. Up to 40 additional fish collected were stored in water on ice and frozen after transfer to a -20°C freezer (within 6 hours of collection). These fish were used for diet content analyses and/or stable isotope analyses. We collected potential benthic macroinvertebrate prey using a petite PONAR at soft substrate sites (opening = 0.023 m^2, mesh = 500μm) and used SCUBA divers to collect benthic scrapings with a pneumatic airlift system (Mackey 1972; Pearson et al. 1973) at hard substrate sites (Table 1). PONAR samples were collected in triplicate at all soft bottom sites and depths during all sampling events. Airlift samples were collected at FP during all seasons, while SB was sampled in summer and fall, and HP and CA were sampled in summer only (see Table 1 for definition of sites). The hard-bottom AR site was not sampled for benthos.

2.2 Laboratory Analyses

We measured total length (standard length when caudal fins were missing; ± 1 mm) and weight (± 0.001g) of individual round gobies stored at either -80°C or -20°C. We converted standard length measurements to total length estimates using a regression equation developed on a subset of fish (M. Henebry, unpublished data). Since round gobies do not have a clearly defined stomach, we removed whole digestive tracts and stored them in 95% ethanol until further processing. After removing whole digestive tracts of fish, we homogenized the remaining tissue using a Waring commercial blender. Homogenate from up to three individual fish per size class were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures. Homogenate from up to 20 individual fish per size class were analyzed for fatty acid signatures. Only fish stored at -80°C were used for fatty acid analyses. Non-gut fish tissue and resulting homogenate were kept on ice throughout the process to minimize the effect of thawing on results.

For up to 50 fish per site, season, and depth of sampling, we identified and counted all identifiable items in the digestive tracts to the lowest practical taxonomic level. We photographed up to 20 individuals from each taxonomic group using a dissecting microscope (Olympus SZ61, Unitron Z850 or Leica MS5) plus camera (Micrometrics 318CU), and used

ImageJ image analysis software to measure lengths of each photographed item (Rasband 2009). We converted these measurements to weights using length-weight regressions (Burns 1969; Eckblad 1971; Dumont et al. 1975; Nalepa and Quigley 1980; Makarewicz and Jones 1990; Sprung 1993; Benke et al. 1999; Sabo et al. 2002; Baumgartner and Rauthhaupt 2003; Conroy et al. 2005; C. Riseng and L. Eaton, unpublished data, University of Michigan School of Natural Resources and Environment, Ann Arbor, MI, U.S.A.) and used mean weight (mg) to estimate biomass of each taxonomic group in a given fish. In the case of fish eggs, nematodes, and ostracods, rather than convert lengths to weights with an equation, we used a single estimate for mean weight of the organism based on expert opinion (nematodes 0.9 μg, Nalepa and Quigley 1980; ostracods 21.2 μg, S. Pothoven, personal communication, NOAA Lake Michigan Station, Muskegon, MI, U.S.A.) or calculations based on multiple studies (e.g., fish eggs, 1.29 mg, C. Foley, unpublished data). We similarly estimated biomass of benthic macroinvertebrates available in the environment. All benthos samples were sorted by eye. For PONAR samples, we used the same methods and equations described above to estimate weight from length. For airlift samples, biomass estimates were quantified directly by lyophilizing the sample until dry then weighing the resulting tissue.

We quantified fatty acid signatures following procedures described in detail in Czesny et al. (2011), Happel et al. (2015 a and b) and Henebry (2014). Briefly, lipids were extracted from fish tissue homogenate (Folch et al. 1957), fatty acids were transmethylated (Metcalfe and Schmitz 1961), and fatty acid signatures were analyzed using a gas chromatography - mass spectrometer (GC/MS Agilent Technologies Inc., Wilmington, DE). Stable isotope sample procedures are similarly described in detail in Happel et al. (2015 a and b), Henebry (2014) and Turschak et al. (2014). Briefly, samples were lyophilized with a Labconco freeze dry system for

1–3 days. Dry tissue (3–4 mg per sample) was weighed into tin capsules, compressed into cubes, and kept in a desiccator until mass spectrometer analysis. Stable isotopes were analyzed using a Finnigan MAT delta S stable isotope ratio mass spectrometer, with Carlo Erba NA 1500 NCS elemental analyzer front end and ConFlo II interface. Standards (N_2: air, CO_2: Pee Dee Belemnite carbonate) were included with samples to determine sample isotope ratios in per mil (‰), where $\delta^{13}C$ or $\delta^{15}N = (R_{Sample}/R_{Standard} - 1)*1000$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. We calculated the mean molar C:N ratio of all round gobies analyzed as 4.4 (range 3.5-5.7). As such, we chose to lipid-correct our $\delta^{13}C$ signatures using the equation developed specifically for round gobies by Turschak et al. (2014): $\delta^{13}C_{Normalized} = \delta^{13}C_{untreated} +[(C{:}N-3.54)*0.342+0.3132]$. This also helped address potential concerns with using whole fish homogenate rather than muscle tissue only (similar to Turschak et al. 2014).

2.3 Statistical Analyses

Given the multivariate nature of both the diet and fatty acid data, we analyzed these datasets similarly (but differently from the stable isotope data). We first summarized diet data by taxon as proportion of total count (hereafter, count) and proportion of total dry biomass (hereafter, biomass) found in a given fish's digestive tract. We pooled taxa that were found in less than 5% of all fish into an "Other" category. Taxa that were abundant enough for their own category were: Acari, Amphipoda, Chironomidae (larvae and pupae separate), Chydoridae, Copepoda, Dreissenidae (*D. r. bugensis*), Eggs, Gastropoda (primarily Planorbidae but also Physidae), Isopoda, Ostracoda, and Sphaeriidae. Taxa included in the "Other" category were *Bosmina*, *Bythotrephes*, *Daphnia*, Decapoda, Nematoda, Oligochaeta, Trichoptera, and dreissenid veligers. We detected 28 fatty acids in fish tissue (though not all were included in analyses; see Section 3.2), and expressed fatty acid data as proportion of total fatty acids detected (similar to Happel et al. 2015a).

To explore overall patterns in fatty acid and diet data, we performed non-metric multidimensional scaling (NMDS) analyses on all fish (n=560 for diets, 339 for fatty acids) using the metaMDS function in the R vegan package (R Core Team 2013; Bray-Curtis distance, n=1000 permutations maximum). We performed multiple NMDS models with k = 2 to 7 dimensions and selected the final model by determining where the scree occurred in a plot of stress versus k. We examined centroids along each axis in order to identify which factors (site, season, size category, depth sampled) were shaping patterns of diet contents or fatty acids in round gobies.

Round goby catches were inconsistent across space and time (Table 2), and large numbers of round gobies from each target size class were not collected upon each sampling occasion. To facilitate understanding of spatial and seasonal patterns, and to account for uneven sample sizes, we conducted a second suite of NMDS analyses on a subset of fish, namely those that fell into the intermediate size category (60–100 mm) collected during summer and fall from five sites (n=183 for diets, 136 for fatty acids). Two sites were in western Lake Michigan (FP, HP), one was in the southwestern region (CA) and two were in eastern Lake Michigan (SA and AR), and all were identified *a priori* as being predominantly hard-bottomed sites. This subset created a more balanced dataset across the factors of interest. We again examined a plot of stress versus k to select the best model, and we explicitly tested the importance of site and season of collection on this subset of fish using the ADONIS functions in the R vegan package (R Core Team 2013). ADONIS is a non-parametric test that identifies significant differences between two or more groupings by first creating a distance matrix then permuting the ranks of distances.

ADONIS also incorporates centroids in the data and squared deviations between centroids in determination of significance. The main outputs are an R-squared value and a p-value, based on pseudo F-tests. We performed the suite of analyses (NMDS on full dataset, NMDS and ADONIS on reduced dataset) on diet biomass, diet count, and fatty acid proportion data.

In order to visualize whole-lake patterns in stable isotope signatures, we plotted $\delta^{13}C$ and $\delta^{15}N$ for all fish (n=225). To explicitly test the effect of site, season, and depth of collection, we performed two separate Analyses of Variance ($\delta^{13}C$ or $\delta^{15}N$ as a response variable) on the more balanced subset of fish (5 sites, 2 seasons, 60-100 mm, n = 61) with the aov and TukeyHSD functions in the main R package (R Core Team 2013). The aov function returns Type I error, therefore we tested multiple models, placing factors in different order, and using the drop1 function to determine whether a factor could be removed in order to create a more parsimonious model. The drop1 function removes terms one by one and calculates an Akaike Information Criterion (AIC) for each model. The model with the lowest AIC is the model that should be retained. In our analyses, if two models had AIC values within 2 points of each other, we retained the model with more factors so as to be sure to include all possible interpretations.

3. Results

3.1 Collection patterns and digestive tract analyses

Round gobies were caught at all sites sampled, though abundances were much higher at sites identified *a priori* as hard-bottomed sites (Table 2). Round gobies were among the more dominant species at these hard-bottomed sites, comprising > 49% of the overall catch at three sites (SB, FP, SA; Table 2). Benthic prey available to round gobies were highly variable with site and season (Fig. 2). Some trends in benthic macroinvertebrate samples were evident, particularly that dreissenids were dominant components of hard-bottomed sites, and chironomid larvae and "other" macroinvertebrates became relatively larger components of the samples in summer and fall (Fig. 2). Diet content data (i.e., fish from all sites, seasons, and fish sizes available) indicated that round goby diets were similarly quite variable (Fig. 2). Highly localized patterns were evident (e.g., relatively high consumption of dreissenids at SA; variable diets at CA; Fig. 2). Round gobies tended to consume more chironomids, chydorids, and ostracods when they were smaller than 100 mm. Dreissenids became a more substantial part of the diet for the largest round gobies (i.e., those > 100 mm). In addition, dreissenids constituted a relatively greater component of diets for all sizes of round goby later in the year (Fig. 2). Consumption of fish eggs appeared to increase after May though relative proportion of fish eggs as prey was lower than for chironomids, chydorids, and dreissenids (Fig. 2).

The best NMDS models for the entire diet content biomass and count datasets, respectively, had four dimensions (stress = 0.09 for biomass and 0.08 for counts). Results were very similar for both analyses (C. Foley, unpublished data); thus we only present biomass data herein. The associations between biomass of prey found in round goby diets and fish size, sampling season, and location were complicated (Fig. 3 and supplementary material). As noted in section 3.1, dreissenids were positively associated with larger fish and the fall (supplementary material). However, they were also positively associated with sites along the eastern shore of the lake (AR, SA, Fig. 3c) and fish caught at the deepest sampling point (14-16 m) regardless of site (supplementary material). Chironomid larvae and other taxa were positively associated with sites along the western shore (SB, HP, Fig. 3c) while chironomid pupae, isopods, and other taxa were positively associated with spring (supplementary material). There was some association between smaller-bodied items (ostracods, copepods, Acari and sphaeriids) and the Muskegon and

Whitefish Bay sites (MK, WB, Fig. 3d), though larger chironomid pupae also trended along this axis. Amphipods and isopods, which are preferred prey of round gobies in lab experiments (e.g., Diggins et al. 2002, Polačik et al. 2009), may not have been associated with any factor that we measured (Fig. 3b, Axis 4). The axis scores for this and all other NMDS analyses are included as supplementary material.

Using the more balanced subset of fish (i.e., 60-100 mm, caught at one of five sites in summer and/or fall), the best NMDS model for biomass of diet contents also had four dimensions (stress = 0.08, Fig. 4). These results emphasized the importance of site as a factor influencing round goby diets, with a more obvious gradient from east to south to west (Fig. 4c). This gradient was associated negatively with dreissenids (Fig. 4a) especially in fall (supplementary material), and positively with chironomid larvae, gastropods, chydorids, eggs and other taxa (Fig. 4a) especially in summer (supplementary material). Though the best model suggested 4 axes, axes 3 and 4 do not appear to reflect factors measured in our study. The taxa associated with axes 3 (Acari, chironomid pupae, amphipods and isopods) and 4 (amphipods and Acari) were found in relatively few fish (17-53, depending on taxon), and these were collected from all sites in all seasons. Based on ADONIS analyses of the subset of fish, site ($F_{4,173}$=14, R^2 = 0.22, p<0.001) and season ($F_{1,173}$=10.6, R^2 = 0.04, p<0.001) were each significant factors affecting biomass of diet items, and there was a significant interaction term between the two ($F_{4,173}$=3.6, R^2 = 0.06, p<0.001). Site ($F_{4,173}$=26, R^2 = 0.31, p<0.001) and season ($F_{1,173}$=11.8, R^2 = 0.04, p<0.001) were similarly significant factors affecting count diet data and also had a significant interaction term ($F_{4,173}$=9.7, R^2 = 0.12, p<0.001). In both cases the R^2 values for season and the interaction terms were low, thus we question their ultimate influence.

3.2 Fatty acid analyses

The most dominant fatty acids in round goby tissues were 16:0 and 20:5n-3, though 18:1n-9, 18:1n-7, and 22:6n-3 were also relatively common (Table 3). 12:0 was found in so few fish (n=3) that we excluded it from NMDS analyses. For fatty acid profiles, the best NMDS solution including all fish had four dimensions (stress = 0.06, Fig. 5). Happel et al. (2015a*)* describe a suite of fatty acids that have been associated with benthic (16:1n-7, 18:3n-3, and 20:5n-3) or pelagic (22:6n-3, 20:4n-6) resources in various systems. All of these trend along the first NMDS axis, suggesting a clear association with pelagic versus benthic resources (Fig. 5a, c). The Saugatuck (SA) site had the most pelagic signature while Highland Park (HP) and Dead River (DR) had the most benthic signatures (Fig. 5a, c). There was slight differentiation by season along the second NMDS axis, where fish collected in spring were separate from those collected in summer and fall (supplementary material). Along this axis, 22:1n-9, 22:6n-3, and 22:5n-6 were most associated with spring samples while 16:1n-7, 18:1n-7, 18:2n-6, 18:3n-3, and 18:3n-6 were most associated with summer and fall samples (supplementary material). At least one fatty acid seemed to be associated with larger gobies (18:4n-3; supplementary material).

The NMDS for the more balanced subset of fish (stress = 0.05, Fig. 6) further underlined the importance of site, season, and depth of sampling (Fig. 6 and supplementary material). Site was the most important factor identified through the ADONIS ($F_{4, 128} = 33.3$, $R^2=0.44$, $p<0.001$). Season ($F_{1, 128} = 32.9$, $R^2=0.11$, $p<0.001$) and the interaction term ($F_{2, 128} = 4.5$, $R^2=0.03$, $p<0.001$) were also significant, though the interaction term had a very low R^2 value. The relative concentration of fatty acids varied spatially (Table 3); for example, round gobies from SA contained the highest relative proportion of docosahexaenoic acid (pelagic signature) and round gobies from the four sites near Milwaukee and Chicago (FP, WB, HP, and DR) had some of the highest relative proportions of palmitic and eicosapentaenoic acids 20:5n-3 (benthic signature).

3.3 Stable isotope analyses

Lipid-corrected stable isotope ratios similarly demonstrated differences between site, season, depth of net set, and size of round goby (Fig. 7 and supplementary material). In general, the carbon gradient had greatest differentiation among groupings regardless of factor of interest (Fig. 7). Fish that were caught at deeper depths and/or were larger tended to have a more depleted $\delta^{13}C$ signature (supplementary material), as did fish caught at Saugatuck, Muskegon, Michigan City, and Arcadia (SA, MK, MC, AR, Fig. 7a). Though sites and seasons were distributed along the nitrogen gradient, the distance between these does not appear to span more than one trophic level (~ 3‰, Fig. 7a). No other factor appeared to vary in nitrogen values (supplementary material).

For the more balanced subset of sites and sampling seasons, the best statistical model for lipid-corrected $\delta^{13}C$ indicated that site was significant ($F_{4,55} = 31.7$, $p<0.001$, Fig. 7b), while season was not ($F_{1,51} = 1.3$, $p=0.26$, supplementary material). The best statistical model for $\delta^{15}N$ ratios also indicated that site was significant ($F_{4,51} = 22.1$, $p<0.001$, Fig. 7b) while season was not ($F_{1,51} = 2.7$, $p=0.11$, supplementary material), though there was a significant interaction term between site and season ($F_{4,51} = 5.4$, $p<0.01$). Tukey's HSD tests applied *post hoc* indicated two significant groupings of sites along the nitrogen gradient (SA+CA and AR+FP+HP, Fig. 7b) and three along the carbon gradient (SA, AR+CA and FP+HP, Fig. 7b). Specifically, the Saugatuck (SA) and Calumet (CA) sites were enriched in $\delta^{15}N$. The Saugatuck (SA) site was the most depleted in $\delta^{13}C$ while the Fox Point (FP) and Highland Park (HP) sites were most enriched in $\delta^{13}C$ (Fig. 7b).

4. Discussion

Invasive round gobies are among the more dominant small-bodied fishes found on hard-bottomed sites in nearshore Lake Michigan. Our results suggest that the specific prey categories and production pathways which support round gobies vary across locations. While some patterns are highly localized, trophic relationships of round gobies also seem to follow regional trends described for other, indigenous species in Lake Michigan (Happel et al. 2015 a and b*)*. Specifically, round gobies generally seem to rely on a greater relative proportion of pelagic production in the eastern nearshore zone and benthic production in western Lake Michigan. Given that round gobies have become important prey in the Laurentian Great Lakes (e.g., Steinhart et al. 2004; Truemper and Lauer 2005; Dietrich et al. 2006; Jacobs et al. 2010; Madenjian et al. 2011; Jakubas 2004, King et al. 2006), spatial variation in round goby trophic interactions may affect which production pathways ultimately support top predators in different regions. In addition, the flexibility of round gobies' trophic niche may suggest that this invasive fish will be able to persist as a key component of a seemingly continuously evolving Lake Michigan food web.

As previously noted in sections 3.1, 3.2 and 3.3, in the southern basin of Lake Michigan the relative importance of pelagic pathways in supporting round gobies appears to decrease from east to west. Similarly, regardless of analysis method, the largest round gobies were associated with more pelagic signals than their smaller counterparts. According to diet analyses, the largest round gobies consumed the greatest proportion of dreissenids, thus we suggest that the pelagic signature is likely influenced by comparatively high dreissenid consumption. Dreissenids are a benthic prey item that relies on pelagic pathways of production and likely reflects a more pelagic signature than other benthic prey (e.g., Foley et al. 2014; Turschak et al. 2014). The influence of dreissenids in round goby diets is well-documented (e.g., Pennuto et al. 2012) and it has been suggested that round gobies present an important link between dreissenids and the greater food webs of some freshwater lakes (Charlebois et al. 2001; Kornis et al. 2012). Our findings suggest that this may indeed be true, particularly if piscivores consume larger round gobies or feed on round gobies in regions where dreissenids comprise large portions of round goby diets. However, given their slow retention time in round goby guts, dreissenids may be overestimated via traditional diet analyses (Coulter et al. 2011; Brush et al. 2012; but see Hamilton 1992). They are also not necessary to round goby success, as round gobies have established and proliferated in dreissenid-poor areas (e.g., Polačik et al. 2009; Cooper et al. 2012). And while our results indicate that larger round gobies do derive more energy from pelagic pathways than their smaller counterparts, they by no means indicate that larger round gobies rely solely on dreissenids.

We suggest that the observed regional patterns of round goby trophic interactions are related not only to dreissenid and other prey consumption patterns, but also to differences in relative riverine inputs, dominant substrates, and/or water circulation patterns. In addition to demonstrating reliance on pelagic pathways of production, depletion of $\delta^{13}C$ can demonstrate importance of riverine inputs (Dufour et al. 2005; Larson et al. 2012). The Saugatuck (SA), Muskegon (MK), Michigan City (MC), and Arcadia (AR) sites in our study are relatively geographically distant from each other, yet collectively demonstrated the greatest depletion of $\delta^{13}C$ of all sites examined. Each of these sites is located relatively close to a river or drowned rivermouth lake (Kalamazoo River, Muskegon Lake, Trail Creek, and Arcadia Lake, respectively). Regionally, riverine inputs into the main basin of Lake Michigan are much greater in the east as compared to the west, where the largest river (Fox River) flows into Green Bay. Thus, tributary inputs may subsidize production to a greater extent along eastern Lake Michigan, both at a local and regional scale.

Similarly, the regional differences in round goby prey consumption may not simply reflect very local substrate conditions, but rather broader substrate conditions. Though our study sites were selected *a priori* to include a combination of hard- and soft-bottomed sites and these were spread somewhat evenly around the lake, most sites included a combination of substrates or were relatively close to other substrate types. For example, the Saugatuck site can essentially be characterized as a rocky island in a sea of sand, while the Whitefish Bay site is in a sand gap between two rocky areas. Western Lake Michigan substrates are predominantly hard (Janssen et al. 2005). Non-dreissenid benthic macroinvertebrate densities can be high on hard substrates in Lake Michigan (e.g., Janssen et al. 2005), and such hard substrates may also support growth of various benthic algae. Given active and passive movement of organisms and organic material and the high prevalence of along shore currents in nearshore Lake Michigan (Höök et al. 2006), it is likely that relatively high benthic production in the nearshore zone influences trophic connections beyond very local scales. Our study would have been improved by inclusion of more sites in the northern basin of the lake, especially the hard substrates of the northeast (Janssen et al. 2005).

Production pathways supporting round gobies may also be shaped by regional prevalence of up- or down-welling events. Lake Michigan's southern basin is characterized by mean cyclonic, near-surface, circulation patterns (Beletsky et al. 1999). Frequently, circulation patterns coupled with prevailing winds favor formation of upwelling events in western Lake Michigan and down-welling events in eastern Lake Michigan (Höök et al. 2004; Plattner et al. 2006). These have the potential to force cold, bottom waters or less dense, surface water close to shore, respectively, and may redistribute nutrients and organisms (Schelske et al. 1971; Yaguchi 1977; Bell and Eadie 1983). Specifically, prevailing down-wellings may bring offshore, near-surface materials (including primary producers) into the nearshore zone of eastern Lake Michigan, while upwelling events may concentrate benthic material in the nearshore zone of western Lake Michigan. Primary consumers in these two regions may differentially rely on these two (pelagic versus benthic) production pathways and such effects may in particular be reflected by biochemical trophic indicators.

Though the regional east versus west trends generally held true, two sites indicated very localized round goby signatures. Fish from the Saugatuck (SA) site tended to demonstrate the strongest reliance on pelagic pathways of production. It was also the only site where dreissenids made up a large component of round goby diets through all seasons and size classes, and it had some of the greatest proportions of dreissenids in the benthic invertebrate samples (almost all samples were entirely dreissenids). Despite the strong reliance on pelagic pathways of production, round gobies caught at this site did contain a variety of items in digestive tracts. Round gobies do not prefer dreissenids but rather consume them when preferred prey such as chironomids or amphipods are not as available (Diggins et al. 2002; Polačik et al. 2009; Coulter et al. 2011), and invasion success of round gobies has been linked to ability to consume energy-rich, non-mollusc prey items (Polačik et al. 2009). It is possible that round gobies are acquiring diet items from the nearby Kalamazoo River and/or that taxa such as amphipods, ostracods and Acari are benefiting from using dreissenids as habitat in this region (e.g., Kuhns and Berg 1999; Janssen et al. 2005), thus increasing the probability that they will be eaten along with the dreissenids. Whatever the mechanism, this site had the highest relative proportion of round gobies present, suggesting perhaps that round gobies are able to exploit a habitat that is not favorable for other species.

The Calumet (CA) site near the Illinois-Indiana state border trended with eastern sites for the shorter-term analyses (diet contents and fatty acids), but with the Arcadia (AR) site for longer-term resource gradient ($\delta^{13}C$). This suggests that something more than the broad, regional characteristics described above is shaping round goby feeding at CA. One possibility is a relationship between round gobies and local primary production. The CA and AR sites are close in proximity to regions of large mats of the benthic algae *Cladophora* (MTRI 2014), which demonstrates a relatively high $\delta^{13}C$ signal in Lake Michigan (Turschak et al. 2014). Though we only sampled the benthos at CA once, we noted that the substrate was gravel covered by a thick layer of *Cladophora* (H. Bootsma, unpublished data). Round goby density has been positively associated with *Cladophora* biomass in Lake Ontario (Pennuto et al. 2012), potentially because *Cladophora* provides habitat for benthic macroinvertebrates (Higgins et al. 2008). Though relative abundances of round gobies at the CA and AR sites are lower than at other hard-bottomed sites, our findings suggest that round gobies might also be able to effectively feed in *Cladophora*-dominated regions. While this was untested in our study, it is possible that it is another example of flexibility in feeding patterns of round gobies, which would add to their value as a trophic conduit.

Depth of collection of round gobies may affect feeding patterns. We expected that round gobies would move relatively freely among our sampling locations at a given site, as they have been shown to move up to 50 m in a given day (Lynch and Mensinger 2012). Though at some sites the distance between the shallowest and deepest net sets was up to 3 km, we might still have expected fairly homogenous trophic signatures for depth, particularly for analyses that reflect integration of prey over the longer term. However, even the long-term measure of trophic position (stable isotopes) indicates that round gobies caught in the deepest waters had a consistently different, more pelagic signature than those closer to shore. Pennuto et al. (2012) found a significant increase in round goby densities from the 5-m to the 10-m depth. They attribute this to seasonal migrations offshore and did not examine any aspect of round goby diets. Round gobies do commonly overwinter in offshore Lake Michigan, thus broad seasonal movements are possible and individuals may not inhabit the same nearshore region from one year to the next (Kornis et al. 2012). However, round gobies tend to have a relatively small home range for most of the year (Ray and Corkum 2001; Lynch and Mensinger 2012), and our results may reflect round goby behavior (i.e., territoriality). It is also possible that the effect of depth was confounded by a fish-size effect. Though there were few overall correlations between size of round goby and depth of capture, there do appear to be some positive trends within the subset of fish analyzed such that deeper depths yielded larger fish (Supplementary material).

As in other studies (e.g. Brush et al. 2012, Happel et al. 2015a), we found that using complementary techniques to examine trophic patterns of round gobies greatly enriched our understanding of results. All three analytical methods used to examine integration of diet items into round gobies of nearshore Lake Michigan underlined the importance of site in structuring trophic patterns. Season was statistically significant when examining digestive tract content and fatty acid data, which makes sense given that diet contents, fatty acids, and stable isotope signatures reflect the past 1-2 days, 3-12 weeks, and 3-4 months, respectively. However, the effect of site is much stronger, and evident despite biases associated with each method (e.g., diet contents are affected by different rates of digestion, fatty acids and stable isotopes may provide limited taxonomic resolution among very diverse prey). The consistency across methods may reflect round goby behaviors, namely that they have a relatively small home range throughout the sampling period (May through September) and that they are highly territorial (e.g., Corkum et al. 1998, Ray and Corkum 2001). Further, the observed round goby trophic interactions were responsive to a variety of conditions and habitats. Brandner et al. (2013) suggested that round gobies along an invasion front displayed differential trophic characteristics than established round goby populations. We observed similar flexibility in feeding regimes, including ability to rely on different production pathways in different regions, and suggest that this ability may lead to long-term persistence of round gobies in a particular system.

High intra-specific spatial variation in trophic connections has previously been documented in Lake Michigan, other Laurentian Great Lakes and other large freshwater systems (e.g., Winemiller 1990; Roswell et al. 2013; Happel et al. 2015a and b), though many studies considered overall community structure rather than focusing on a single species (e.g., Vander Zanden et al. 1997). At both individual- and population-levels, the consistency of such spatial variation in trophic connections is often unknown because trophic connections are not described across multiple sampling occasions or because trophic indicators do not reflect multiple time scales. By sampling across multiple seasons and by using three methods which capture trophic connections over different temporal scales (diet content analysis is short term; fatty acid analysis is intermediate term; stable isotope analysis is long term), we demonstrate that the spatial trophic patterns of round gobies are consistent over time. Moreover, we somewhat surprisingly highlight a scenario where a single species appears to play a multifaceted role in a food web (i.e., round gobies appear to effectively assimilate energy over varied habitats and from multiple production pathways). Given the high abundance of round gobies and their increasing importance as a prey item, it is possible that they will serve as a linchpin in this system in the coming decades. It is unclear whether this will lead to instability of the system (e.g., McCann 2000), thus we suggest that future research explicitly consider the potential impacts, both positive and negative, that round gobies may provide to top predators. To maximize understanding of trophic connections, we suggest that in addition to sampling over multiple time periods and spatial locations, food web studies should consider analysis via multiple trophic indicators.

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Table 1. Descriptions of sampling sites in Lake Michigan. See Fig. 1 for map. Site abbreviations from this table correspond with site abbreviations used in other tables and figures.

Site name	Abbreviation	State	Substrate	Seasons sampled
Sturgeon Bay	SB	WI	Rock	Summer**, Fall
Fox Point	FP*	WI	Rock	Spring**, Summer, Fall
Whitefish Bay	WB*	WI	Sand	Spring, Summer, Fall
Highland Park	HP	IL	Rock	Spring, Summer, Fall
Dead River	DR*	IL	Sand	Spring, Summer, Fall
Calumet	CA	IN	Gravel	Spring, Summer, Fall
Michigan City	MC*	IN	Sand	Spring, Summer**, Fall
Saugatuck	SA*	MI	Sand/Rock	Spring, Summer, Fall
Muskegon	MK*	MI	Sand	Summer, Fall
Arcadia	AR	MI	Sand/Boulder	Summer, Fall

*Benthos collected during all seasons.

**Some fish specimens collected via additional sampling.

Table 2. Round goby total catch distribution including the proportion of round gobies in the overall catch (small mesh gill net data only). Values listed under each season are for total catch across depths, with shallow (2-3 m), intermediate (7-9 m), and deep (14-16 m) depths in parentheses. Bold values were included in the subset analyses. See Table 1 for definition of station locations.

Site	Size category	Spring	Summer	Fall	Prop. round goby in overall catch
SB	< 60 mm	-	7 (0, 7, 0)	1 (0, 1, 0)	0.49
	60-100 mm	-	17 (0, 17, 0)	9 (0, 9, 0)	
	> 100 mm	-	5 (0, 5, 0)	0 (0, 0, 0)	
FP	< 60 mm	25 (13, 3, 9)	8 (4, 4, 0)	7 (1, 6, 0)	0.55
	60-100 mm	76 (39, 19, 18)	**34 (17, 17, 0)**	**11 (4, 7, 0)**	
	> 100 mm	5 (5, 0, 0)	6 (0, 6, 0)	0 (0, 0, 0)	
WB	< 60 mm	3 (0, 0, 3)	0 (0, 0, 0)	0 (0, 0, 0)	0.036
	60-100 mm	3 (1, 0, 2)	3 (2, 1, 0)	0 (0, 0, 0)	
	> 100 mm	1 (0, 0, 1)	3 (0, 0, 3)	0 (0, 0, 0)	
HP	< 60 mm	2 (0, 0, 2)	12 (8, 3, 1)	4 (0, 2, 2)	0.37
	60-100 mm	89 (14, 36, 39)	**56 (14, 20, 22)**	**45 (0, 20, 25)**	
	> 100 mm	26 (3, 11, 12)	6 (0, 5, 1)	2 (0, 0, 2)	
DR	< 60 mm	0 (0, 0, 0)	0 (0, 0, 0)	0 (0, 0, 0)	0.048
	60-100 mm	9 (5, 3, 1)	1 (0, 0, 1)	16 (0, 3, 13)	
	> 100 mm	1 (0, 0, 1)	0 (0, 0, 0)	1 (0, 0, 1)	
CA	< 60 mm	1 (0, 1, 0)	3 (0, 3, 0)	7 (0, 5, 2)	0.15
	60-100 mm	7 (0, 7, 0)	**22 (0, 17, 5)**	**50 (0, 24, 26)**	
	> 100 mm	0 (0, 0, 0)	0 (0, 0, 0)	4 (0, 1, 3)	
MC	< 60 mm	0 (0, 0, 0)	0 (0, 0, 0)	0 (0, 0, 0)	0.0061
	60-100 mm	0 (0, 0, 0)	0 (0, 0, 0)	1 (0, 0, 1)	
	> 100 mm	0 (0, 0, 0)	12 (0, 0, 12)	1 (0, 0, 1)	
SA	< 60 mm	23 (0, 23, 0)	34 (19, 10, 5)	17 (0, 0, 17)	0.68
	60-100 mm	29 (0, 29, 0)	**54 (26, 14, 14)**	**57 (1, 11, 45)**	
	> 100 mm	7 (0, 7, 0)	5 (0, 3, 2)	7 (0, 0, 7)	
MK	< 60 mm	-	0 (0, 0, 0)	0 (0, 0, 0)	0.0072
	60-100 mm	-	6 (0, 0, 6)	0 (0, 0, 0)	
	> 100 mm	-	0 (0, 0, 0)	0 (0, 0, 0)	
AR	< 60 mm	-	2 (2, 0, 0)	2 (0, 0, 2)	0.12
	60-100 mm	-	**5 (0, 0, 5)**	**32 (0, 32, 0)**	
	> 100 mm	-	1 (0, 0, 1)	3 (0, 3, 0)	

Table 3. Mean fatty acid values for round gobies by sampling site (2010 only, all sizes and seasons combined) in Lake Michigan. Abbreviations for site names are found in Table 1.

Fatty acid	FP	WB	HP	DR	CA	MC	SA	AR	SB
12:0	0.013	0.004	0.003	0.003	0.024	0	0	0	0.032
14:0	1.64	1.49	1.86	2.1	2.18	2.49	1.72	2.11	1.56
15:0	0.35	0.42	0.41	0.56	0.62	0.61	0.61	0.67	0.59
16:0	15.0	15.1	16.5	15.9	14.5	13.3	14.5	14.5	15.4
16:1n-9	0.40	0.43	0.41	0.34	0.61	0.43	0.48	0.43	0.68
16:1n-7	10.7	11.5	14.0	11.6	9.39	11.0	9.9	9.5	8.73
17:0	0.39	0.4	0.40	0.49	0.73	0.71	0.63	0.63	0.83
17:1	0.32	0.39	0.39	0.66	0.63	0.12	0.36	0.39	0.63
18:0	5.66	4.68	5.43	5.09	5.38	4.6	5.61	4.86	5.69
18:1n-9	8.16	8.09	9.35	10.1	9.75	10.6	8.89	9.85	10.7
18:1n-7	7.33	7.50	8.64	8.88	7.07	7.32	5.91	5.70	6.08
18:2n-6	4.05	3.19	4.79	5.53	4.28	3.74	2.79	3.92	6.08
18:3n-6	0.50	0.34	0.46	0.42	0.55	0.44	0.31	0.33	0.48
18:3n-3	2.12	1.30	2.26	2.64	2.85	2.44	1.93	2.6	3.67
18:4n-3	1.03	0.66	0.86	1.43	1.24	1.95	1.74	1.32	1.03
20:1	0.98	1.13	0.66	1.37	1.98	2.54	2.32	2.18	1.42
20:2n-6	0.20	0.25	0.13	0.21	0.30	0.31	0.34	0.27	0.36
20:3n-6	0.16	0.15	0.089	0.099	0.34	0.27	0.22	0.25	0.28
20:4n-6	5.43	6.92	4.39	4.12	7.04	5.39	6.70	7.52	6.59
20:3n-3	0.13	0.13	0.094	0.12	0.26	0.15	0.15	0.13	0.25
20:4n-3	0.31	0.26	0.23	0.35	0.45	0.43	0.41	0.35	0.39
20:5n-3	18.8	18.0	16.6	16.4	14.2	15.2	11.9	13.6	13.3
22:1n-11	0.12	0.086	0.086	0.067	0.13	0.15	0.22	0.17	0.16
22:1n-9	0.13	0.16	0.10	0.11	0.15	0.27	0.40	0.35	0.16
22:4n-6	0.61	0.96	0.38	0.48	1.01	0.82	0.99	1.40	0.83
22:5n-6	1.40	2.38	1.08	1.65	2.69	2.86	3.59	3.73	1.78
22:5n-3	7.16	6.99	4.89	4.52	4.59	4.96	5.63	5.43	5.0
22:6n-3	6.83	7.1	5.5	4.74	6.99	6.89	11.8	7.81	7.34
n	49	3	70	4	58	2	70	27	20
Min TL (mm)	50	61	49	62	50	97	47	48	60
Max TL (mm)	167	155	158	141	106	101	137	116	94

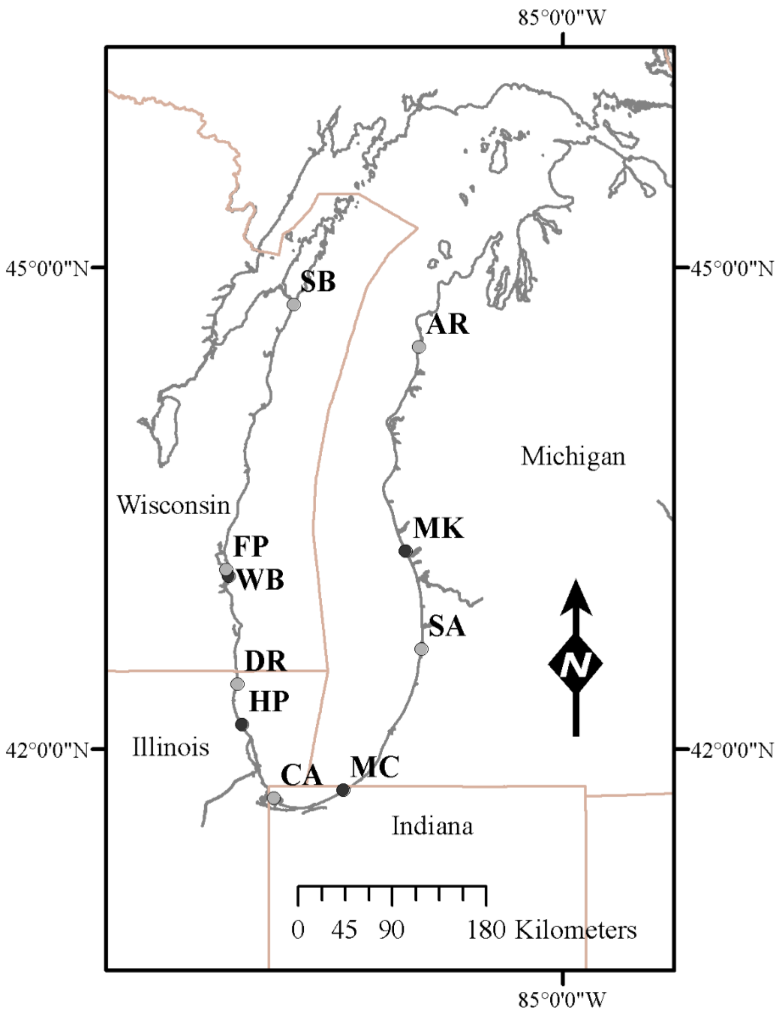

Fig. 1. Sites in Lake Michigan where round gobies and benthos were collected in 2010. Sites with substrate defined *a priori* as "hard" or "soft" are depicted with light and dark circles, respectively. Full descriptions of sites, including seasons of sampling, are listed in Table 1.

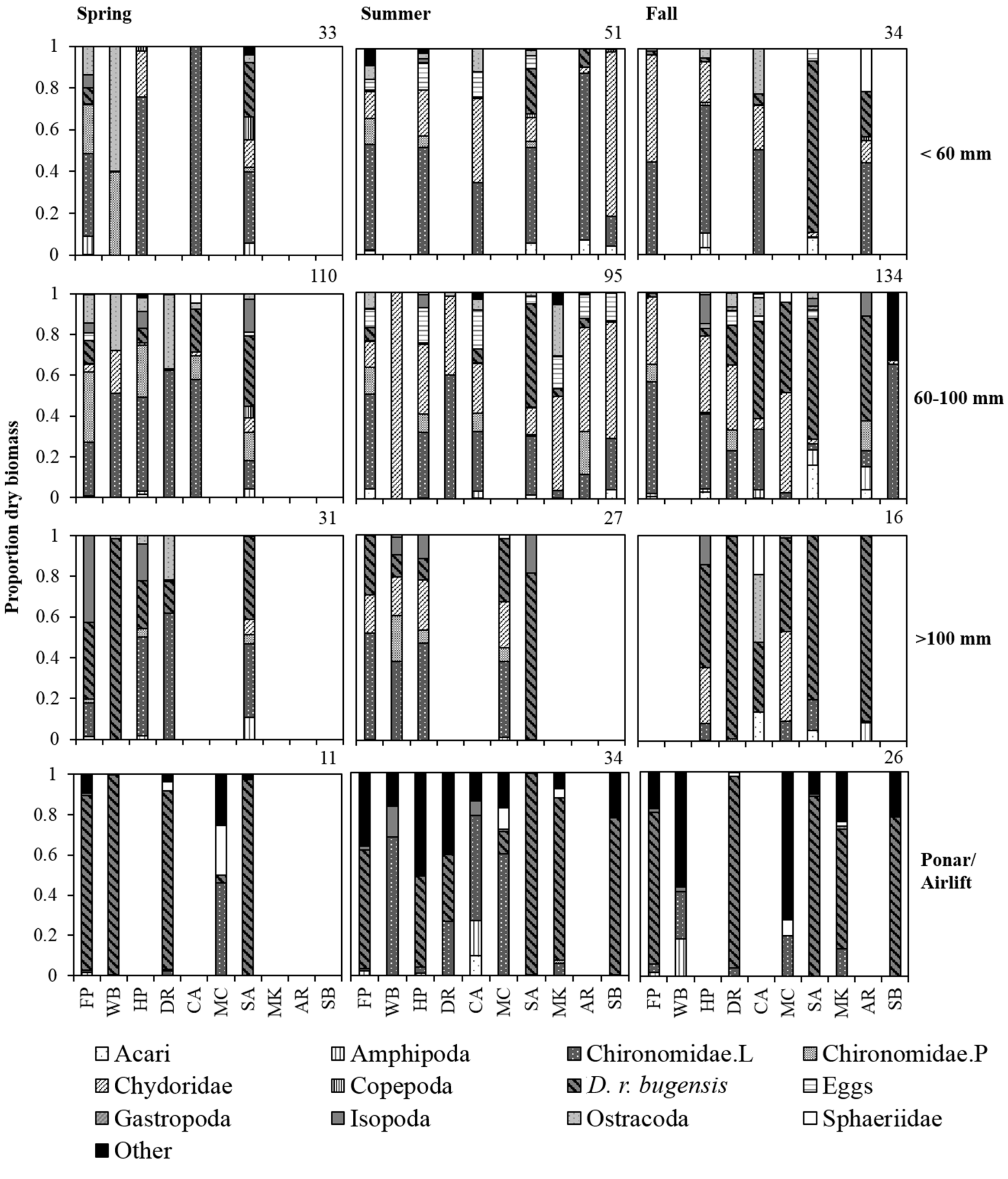

Fig. 2. Composition of benthic macroinvertebrates found in round goby diets (mean proportion of dry biomass; top three rows) and the environment (mean proportion of dry biomass; bottom row) at Lake Michigan sites (see Table 1) sampled in 2010. Taxa correspond to the same categories included in NMDS and ADONIS analyses. Data are presented by site (along the x-axis of each plot), and grouped by size category (row) and season (column). Sample sizes are included at the top right of each plot, with n as the number of individual round goby diets (top three rows) or number of benthic invertebrate samples examined (bottom row; petite PONAR or

scraping with airlift sampler), respectively. Total length information was missing for 29 fish, so these were not assigned to a size category nor depicted here. L = larvae, P = pupae, D. = *Dreissena.*

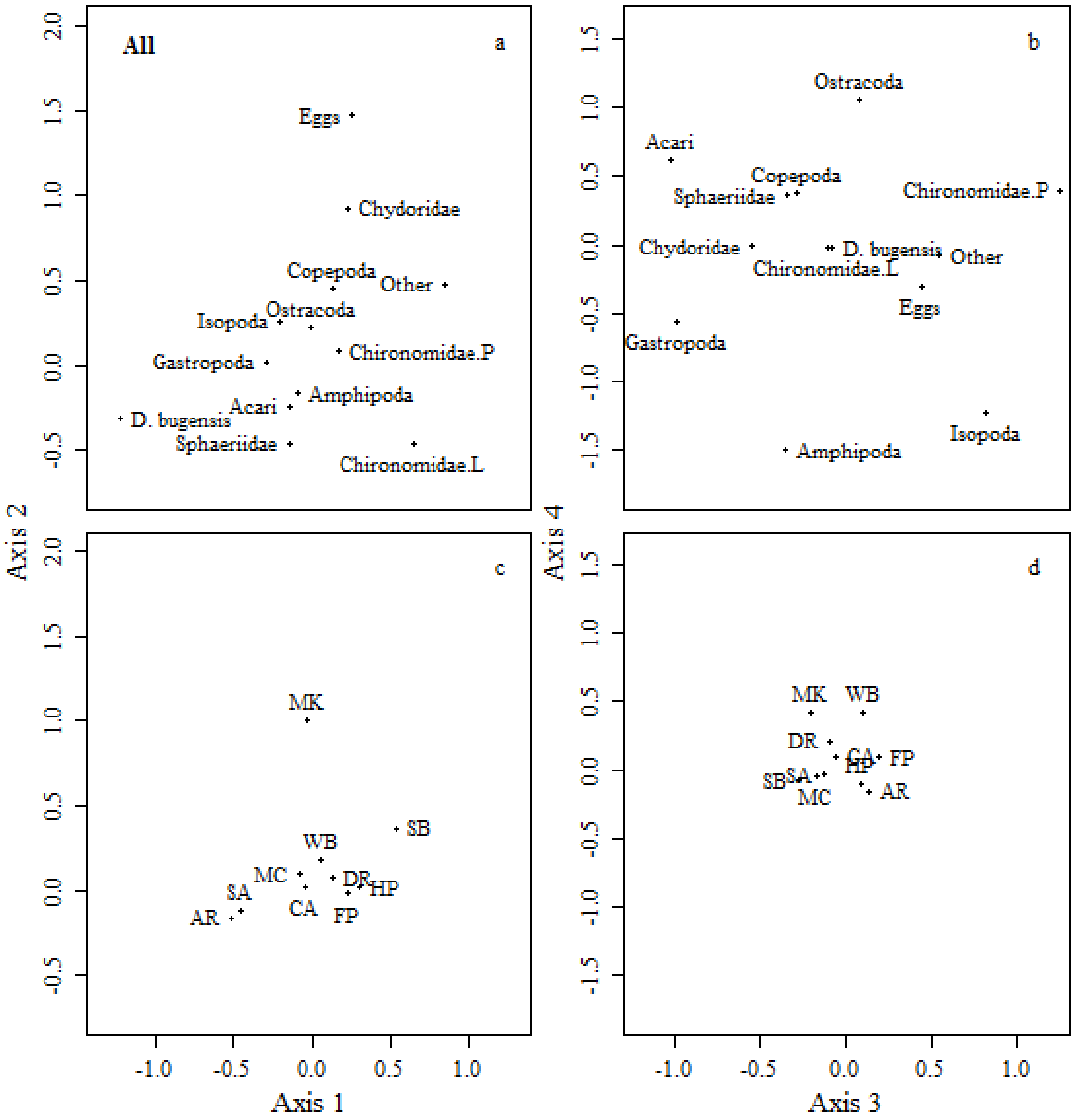

Fig. 3. Non-metric multidimensional scaling analysis of diet contents (proportion dry biomass) including all sites (see Table 1) and seasons sampled during 2010 in Lake Michigan. Dots represent centroids for taxa (a, b) and site (c, d). The full list of taxa found in round goby diets is included in the section 2.3. Centroids for season, depth of collection, and size category of fish, as well as axis scores for all taxa, are included as supplementary material.

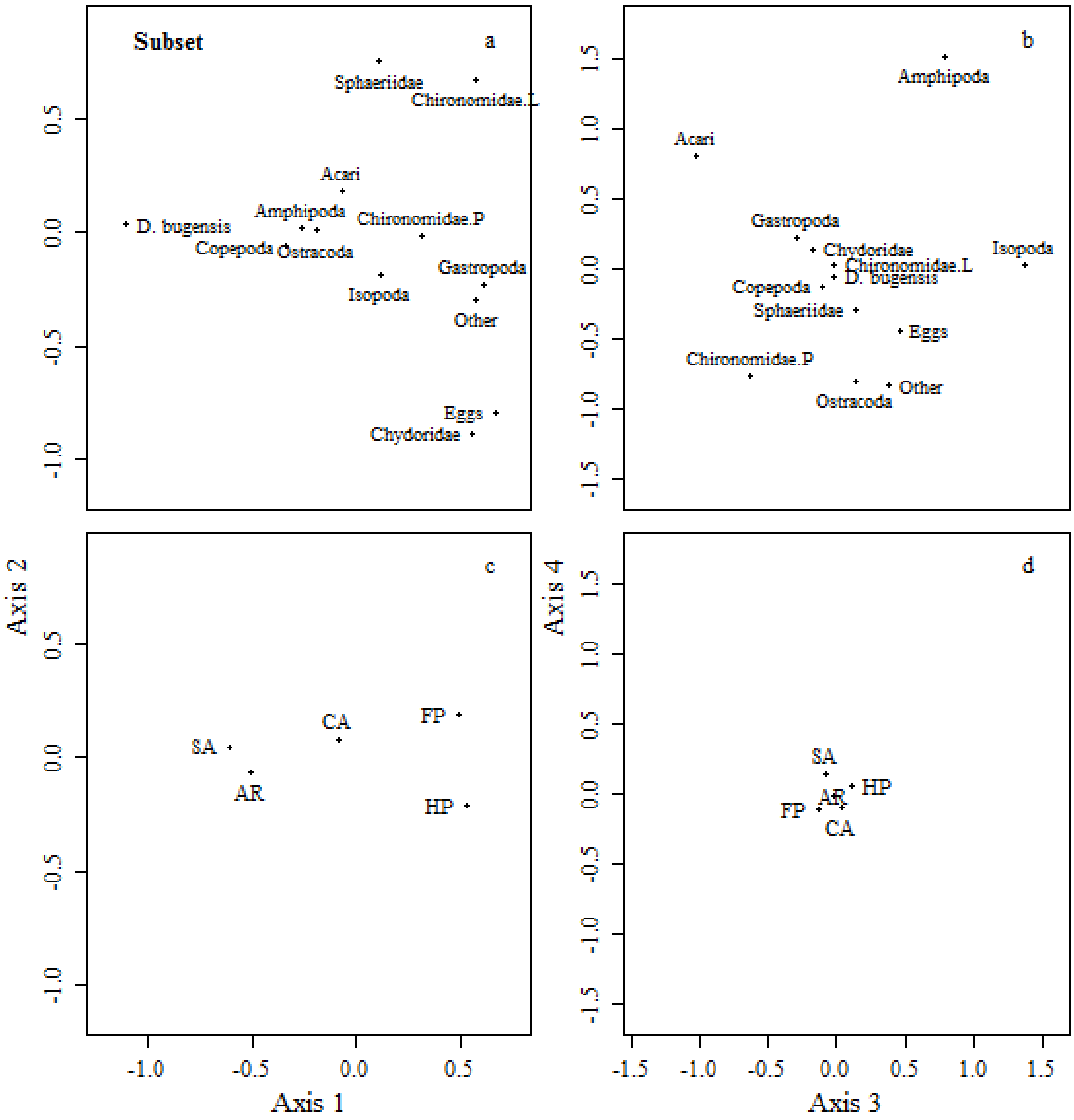

Fig 4. Non-metric multidimensional scaling analysis of diet contents (proportion dry biomass) of intermediate-sized (60-100 mm) round gobies collected in 2010 from five Lake Michigan sites (see Table 1) in summer and fall only. Dots represent centroids for taxa (a, b) and site (c, d). The full list of taxa found in round goby diets is included in the section 2.3. Centroids for season and depth of collection, as well as axis scores for all taxa, are included as supplementary material.

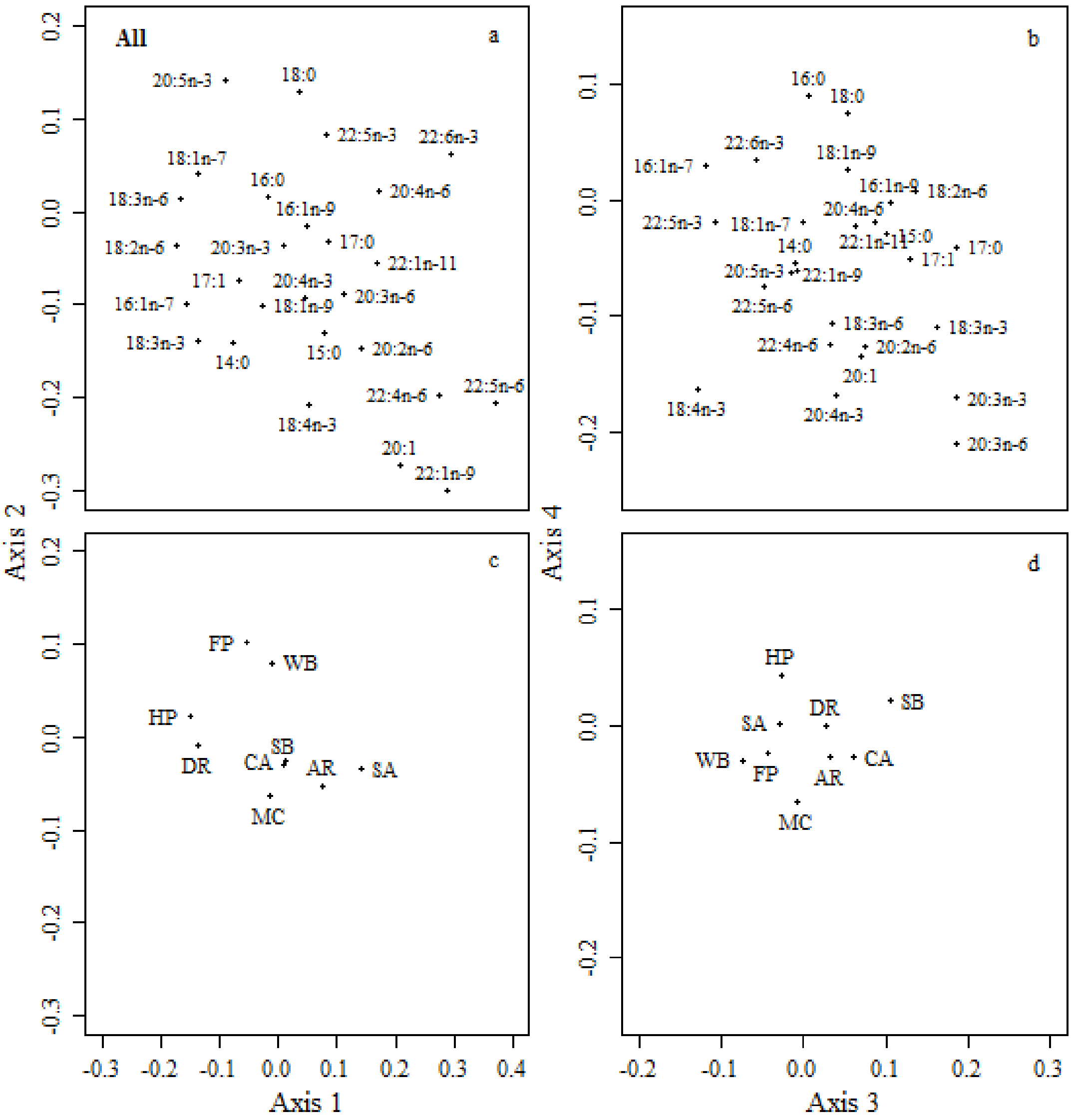

Fig 5. Non-metric multidimensional scaling analysis of fatty acids (proportion of total fatty acid detected) including all sites (see Table 1) and seasons sampled during 2010 in Lake Michigan. Dots represent centroids for taxa (a, b) and site (c, d). The full list of fatty acids found in round goby tissue is included in Table 3. Centroids for season, depth of collection, and size category of fish, as well as axis scores for all taxa, are included as supplementary material.

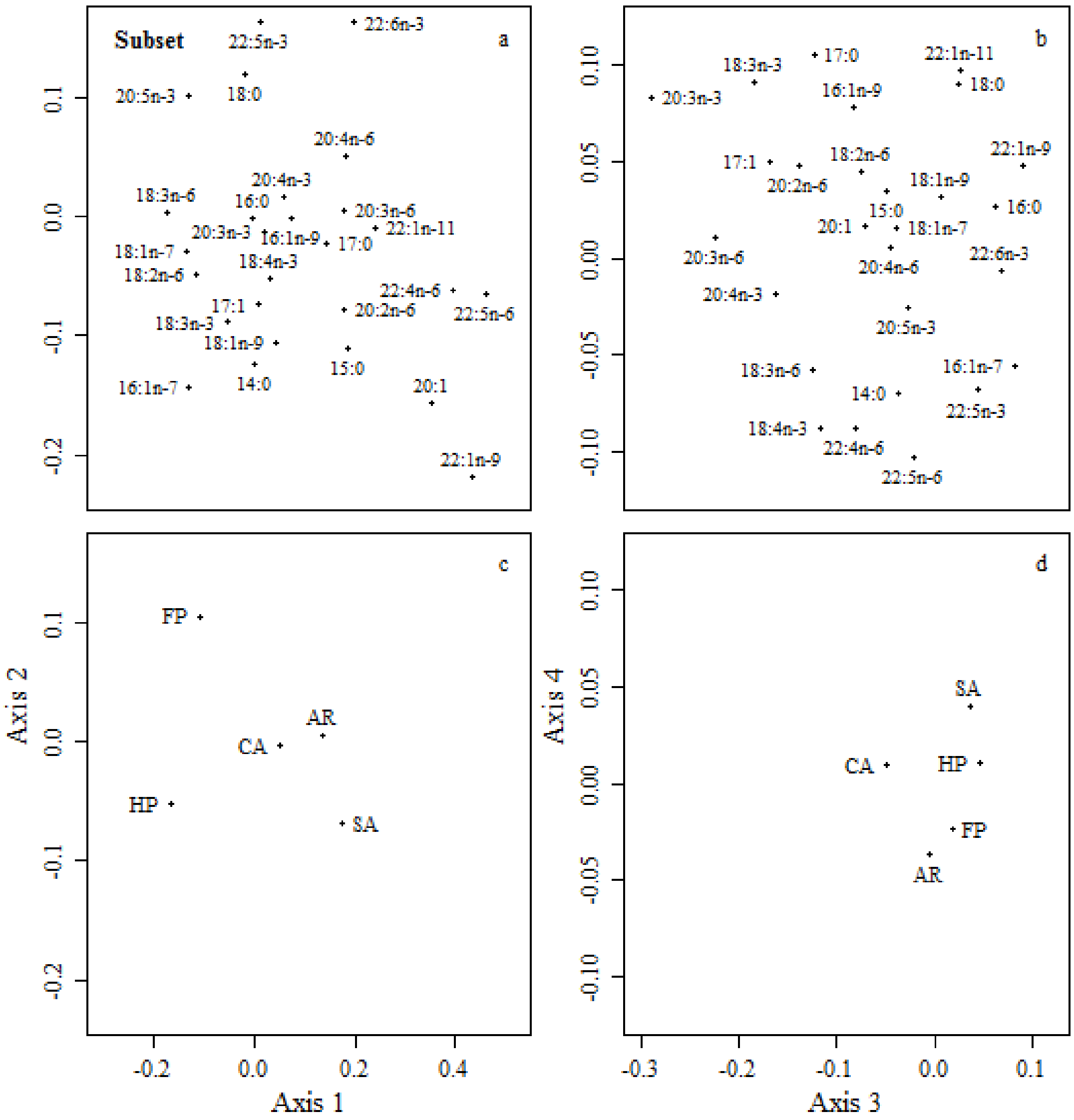

Fig 6. Non-metric multidimensional scaling analysis of fatty acids (proportion of total fatty acid detected) of intermediate-sized (60-100 mm) round gobies collected in 2010 from five Lake Michigan sites (see Table 1) in summer and fall only. Dots represent centroids for taxa (a, b) and site (c, d). The full list of fatty acids found in round goby tissue is included in Table 3. Centroids for season, and depth of collection of fish, as well as axis scores for all taxa, are included as supplementary material.

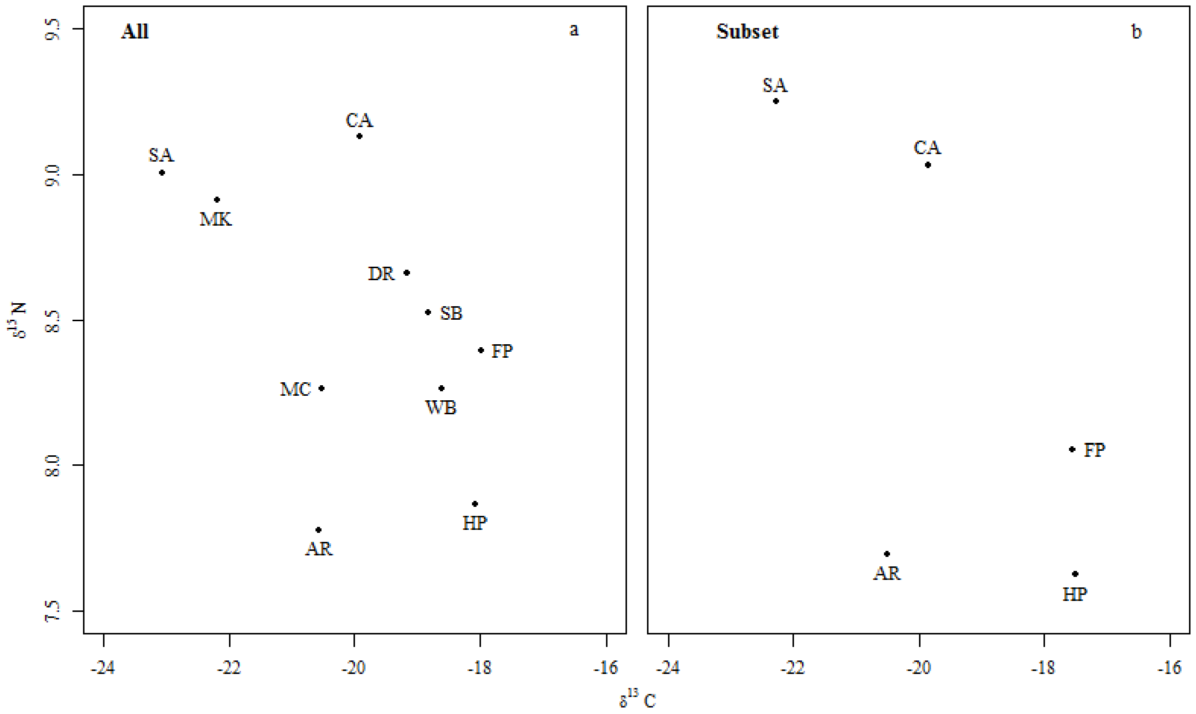

Fig. 7. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope signatures for Lake Michigan (a) round gobies of all size classes, collected from all sites (see Table 1) and seasons sampled in 2010 and (b) 60-100 mm round gobies collected from five sites in summer and fall 2010 only. Dots represent centroids for sites. Centroids for season, depth of collection, and size category of fish, as well as axis scores for all taxa, are included as supplementary material.

7. Supplementary material

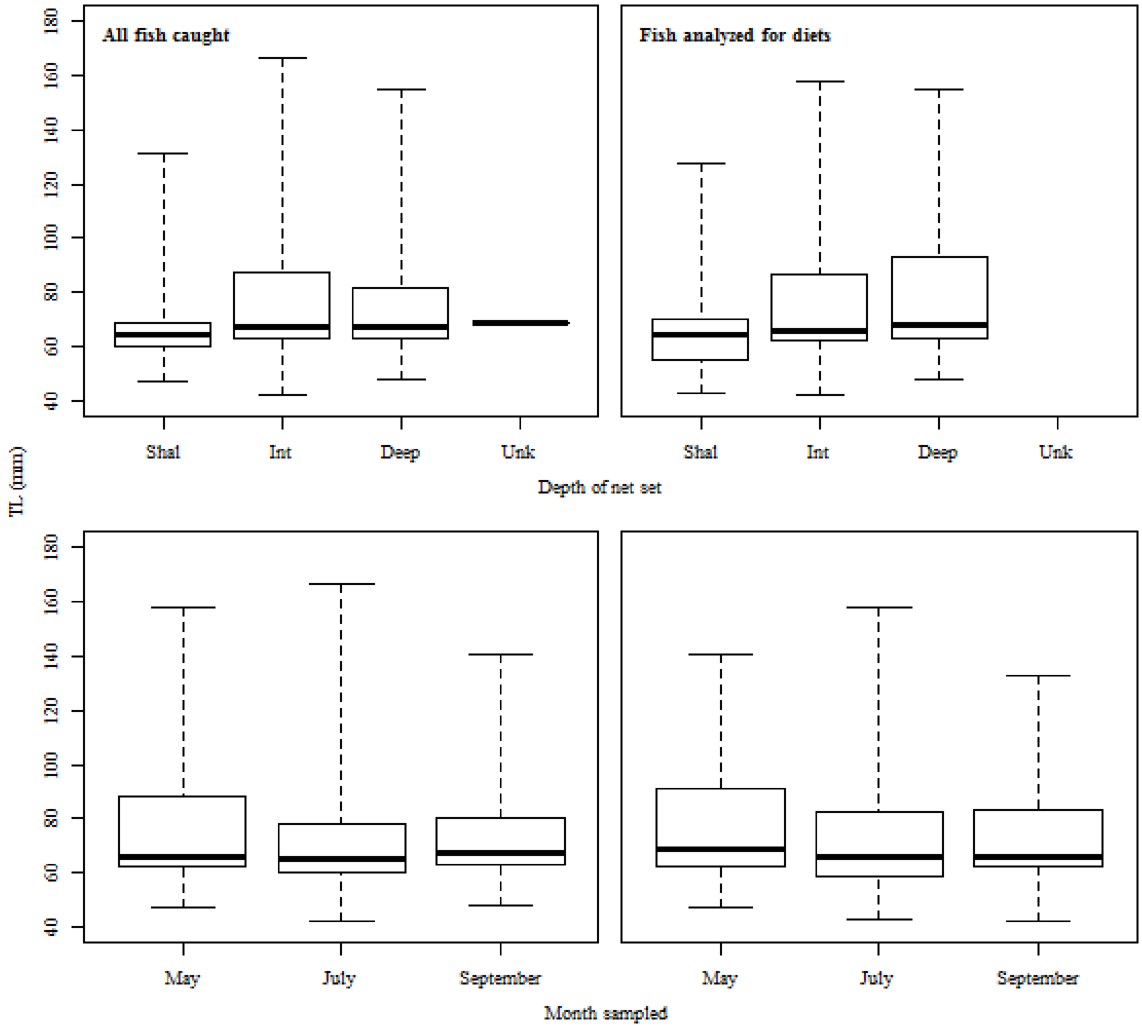

Fig. S1. Boxplot distribution of size of round gobies caught in Lake Michigan in 2010 versus depth of capture (top panels) and month sampled (bottom panels). Boxes depict the lower and upper quartiles, respectively, as well as the median (bold line). Whiskers depict minimum and maximum values of data. The top right panel indicates that depth of collection may be confounded by size of fish analyzed in our non-metric multidimensional scaling analyses.

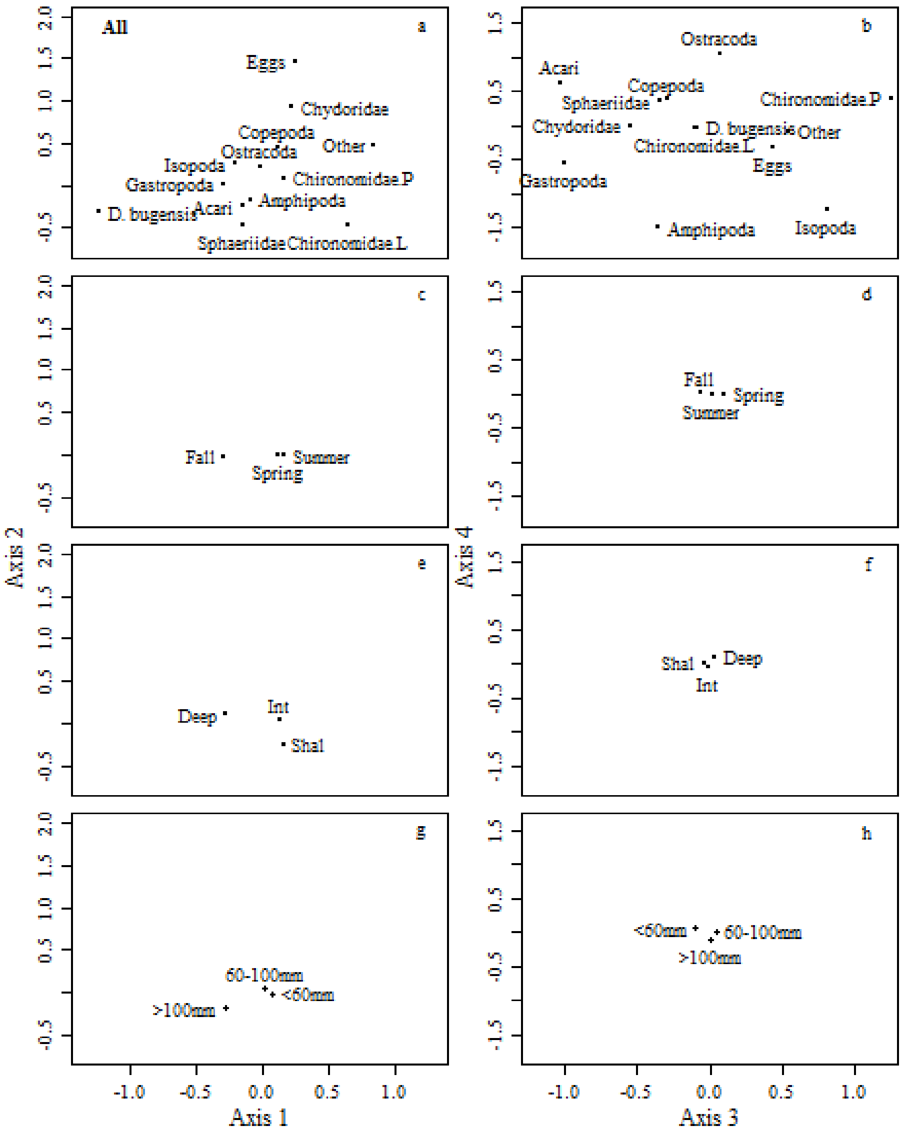

Fig. S2. Non-metric multidimensional scaling analysis of diet contents (proportion dry biomass) including all sites (see Table 1) and seasons sampled during 2010 in Lake Michigan. Dots represent centroids for taxa (a, b), season (c, d), depth of collection (e, f), and size category (g, h). The full list of taxa found in round goby diets is included in section 2.3.

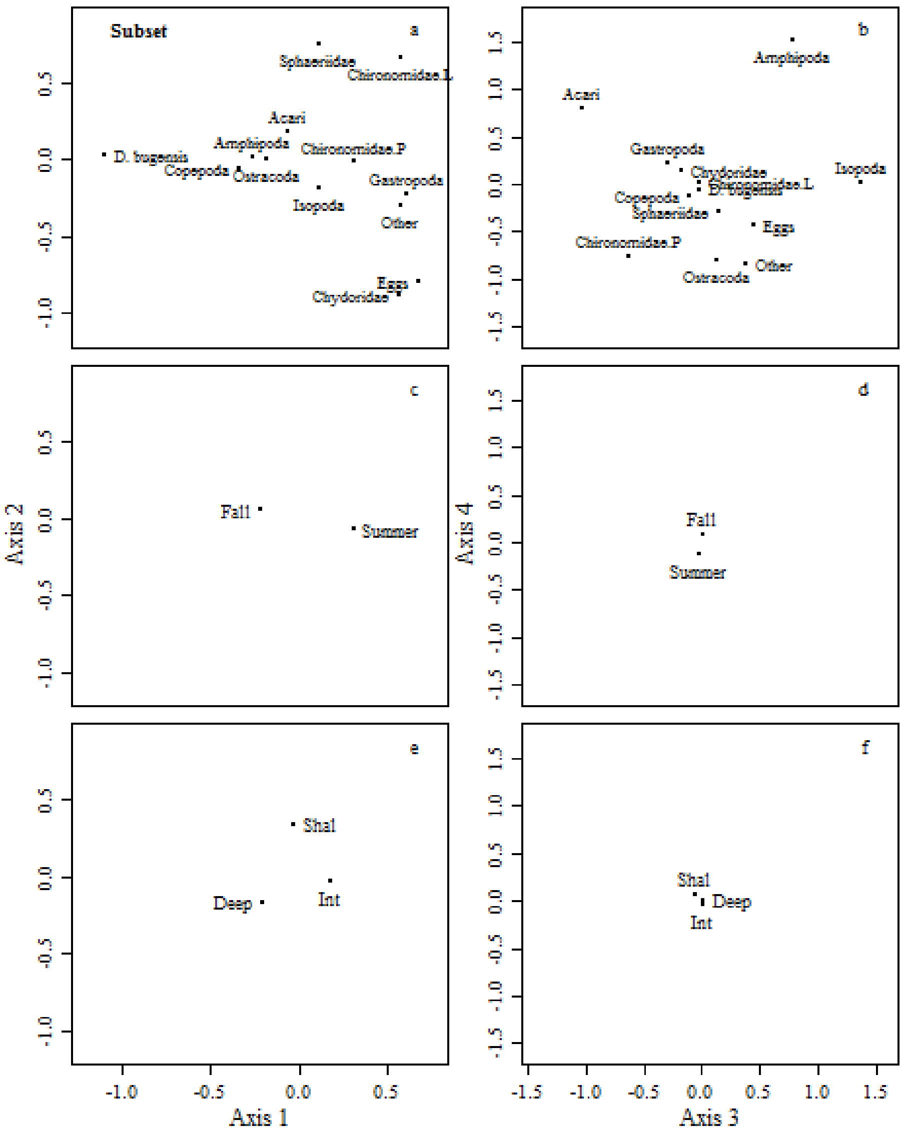

Fig. S3. Non-metric multidimensional scaling analysis of diet contents (proportion dry biomass) of intermediate-sized (60-100 mm) round gobies collected in 2010 from five Lake Michigan sites (see Table 1) in summer and fall only. Dots represent centroids for taxa (a, b), season (c, d), and depth of collection (e, f). The full list of taxa found in round goby diets is included in the section 2.3.

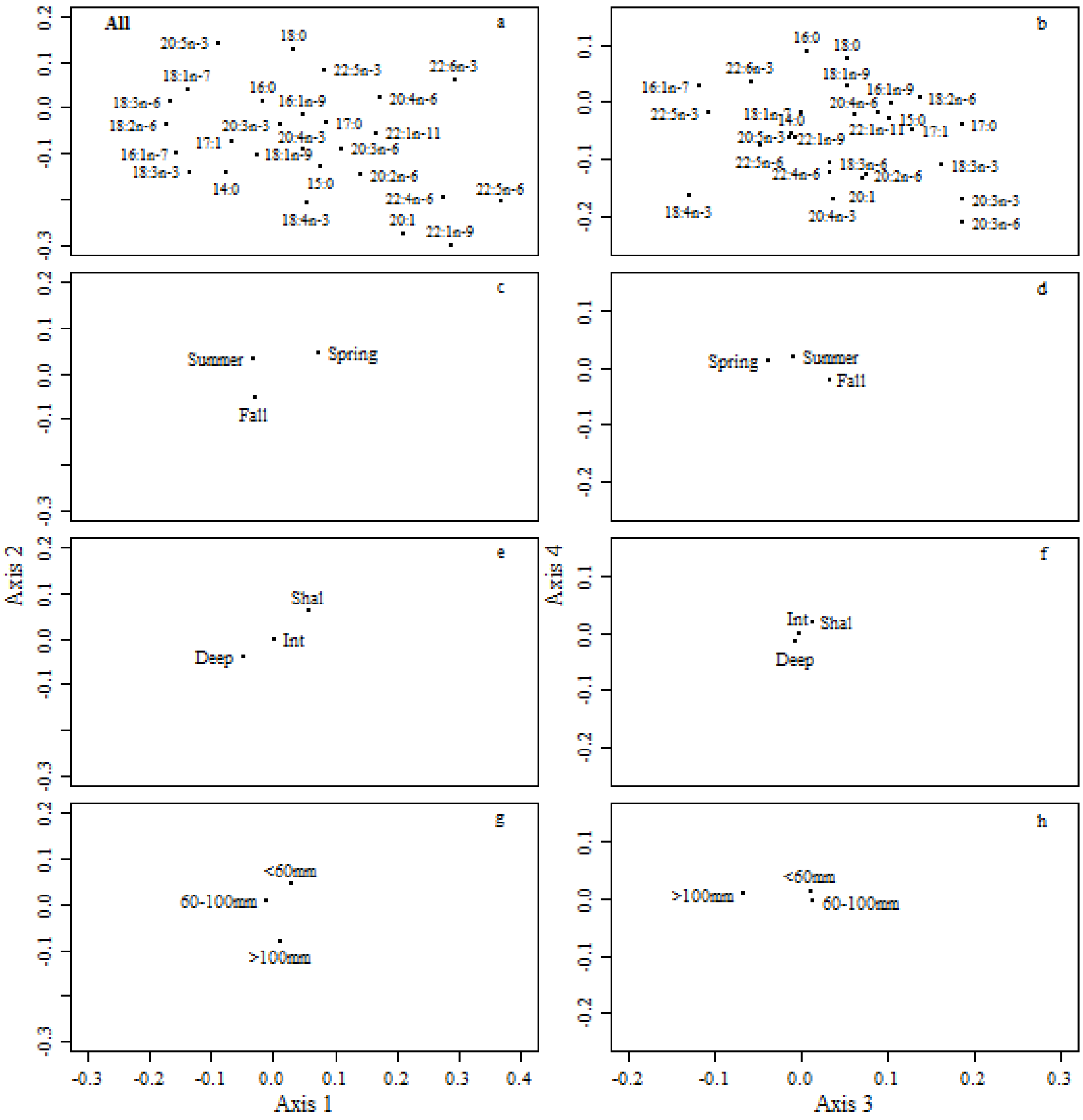

Fig. S4. Non-metric multidimensional scaling analysis of fatty acids (proportion of total fatty acid detected) including all sites (see Table 1) and seasons sampled during 2010 in Lake Michigan. Dots represent centroids for taxa (a, b), season (c, d), depth of collection (e, f) and size category (g, h).The full list of fatty acids found in round goby tissue is included in Table 3.

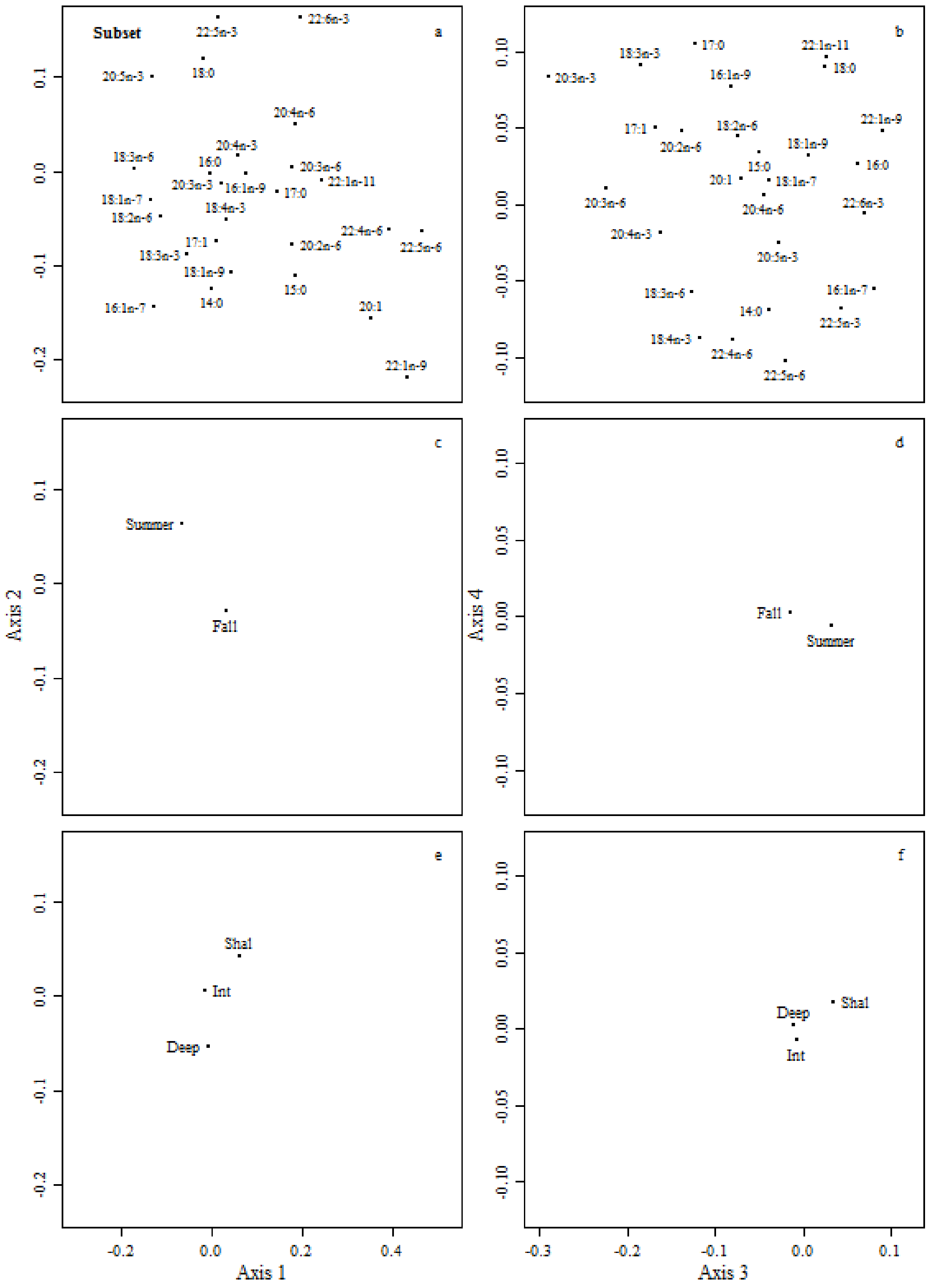

Fig. S5. Non-metric multidimensional scaling analysis of fatty acids (proportion of total fatty acid detected) of intermediate-sized (60-100 mm) round gobies collected in 2010 from five Lake

Michigan sites (see Table 1) in summer and fall only. Dots represent centroids for taxa (a, b), season (c, d), depth of collection (e, f) and size category (g, h).

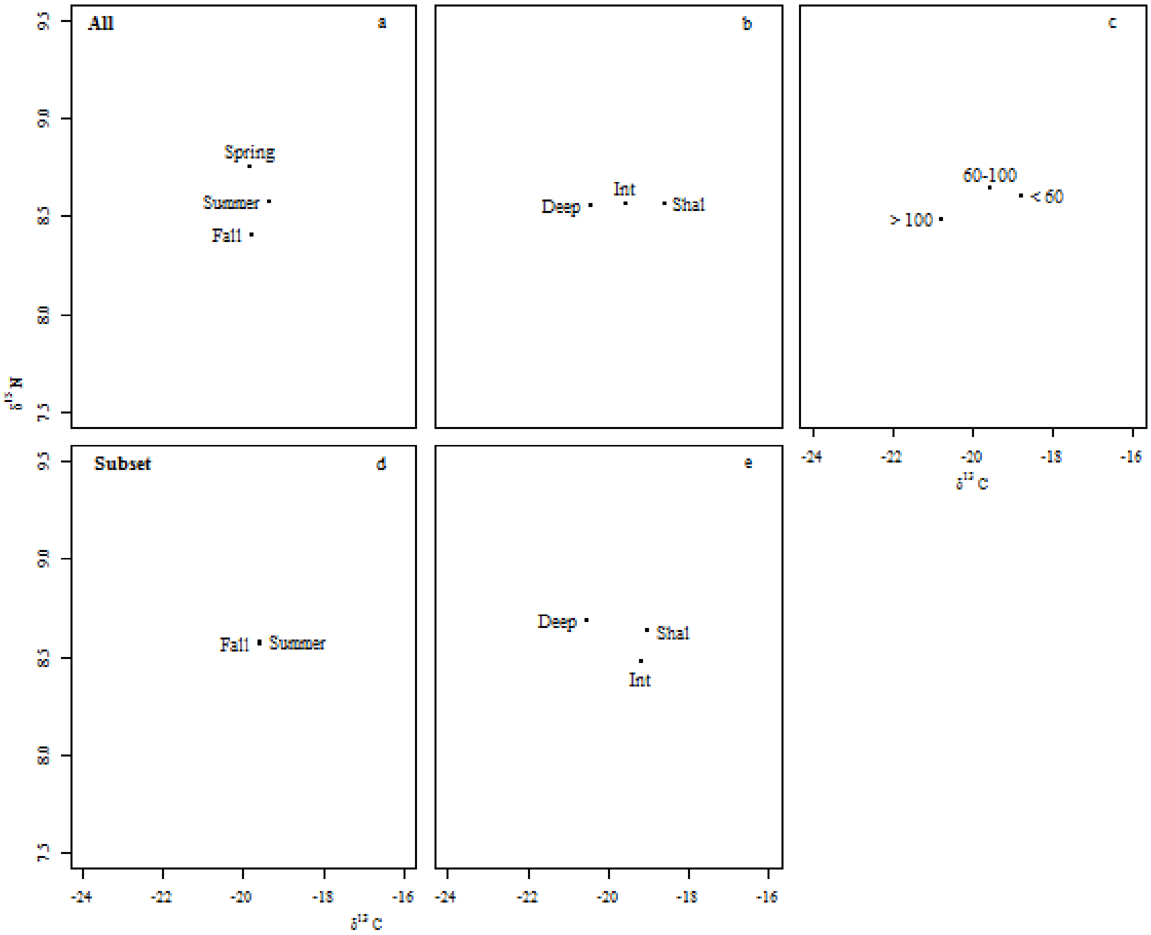

Fig. S6. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope signatures for Lake Michigan round gobies of all size classes, collected from all sites (see Table 1) and seasons sampled in 2010 (a-c) and 60-100 mm round gobies collected from five sites in summer and fall 2010 only (d, e). Dots represent centroids for season (a, d), depth of collection (b, e) and size category (c).

Table S1. Axis scores for biomass non-metric multidimensional scaling analysis of diet items eaten by round gobies during 2010. All sampling events and sites included.

Taxon	NMDS1	NMDS2	NMDS3	NMDS4
Acari	-0.14801	-0.24402	-1.02171	0.620864
Amphipoda	-0.09016	-0.16519	-0.35415	-1.49926
Chironomidae larva	0.642927	-0.46398	-0.10228	-0.02677
Chironomidae pupa	0.169441	0.087166	1.249856	0.395316
Chydoridae	0.228235	0.92621	-0.54535	-0.01093
Copepoda	0.126166	0.456832	-0.29048	0.38225
D. r. bugensis	-1.22148	-0.316	-0.08305	-0.02314
Eggs	0.249025	1.471541	0.437211	-0.30771
Gastropoda	-0.29405	0.01997	-0.99676	-0.55721
Isopoda	-0.20889	0.260913	0.810431	-1.22876
Ostracoda	-0.01412	0.22209	0.07587	1.061317
Sphaeriidae	-0.14539	-0.45787	-0.34299	0.356441
Other	0.846699	0.480011	0.542722	-0.08056

Table S2. Axis scores for biomass non-metric multidimensional scaling analysis of diet items eaten by 60-100 mm round gobies in 2010. Five sites in summer and fall only included.

Taxon	NMDS1	NMDS2	NMDS3	NMDS4
Acari	-0.06759	0.184464	-1.02661	0.806973
Amphipoda	-0.2671	0.021291	0.780305	1.511872
Chironomidae.larva	0.572619	0.66934	-0.01599	0.021477
Chironomidae.pupa	0.317502	-0.01129	-0.62754	-0.76578
Chydoridae	0.561174	-0.88499	-0.1714	0.138684
Copepoda	-0.33971	-0.06076	-0.10373	-0.12739
D. r. bugensis	-1.09948	0.033354	-0.02078	-0.05518
Eggs	0.674201	-0.79529	0.454432	-0.44236
Gastropoda	0.612199	-0.22505	-0.29173	0.218683
Isopoda	0.117913	-0.18416	1.373675	0.028656
Ostracoda	-0.18532	0.008698	0.134227	-0.80551
Sphaeriidae	0.111855	0.753404	0.138173	-0.29244
Other	0.578787	-0.29261	0.37977	-0.84053

Table S3. Axis scores for non-metric multidimensional scaling analysis of fatty acids detected in round gobies collected from Lake Michigan in 2010. All sampling events and sites included.

Fatty acid	NMDS1	NMDS2	NMDS3	NMDS4
14:0	-0.07753	-0.14129	-0.01094	-0.05475
15:0	0.077137	-0.12952	0.101805	-0.02955
16:0	-0.01618	0.016112	0.005721	0.089464
16:1n-9	0.048752	-0.01586	0.104928	-0.00162
16:1n-7	-0.15592	-0.09961	-0.11877	0.029179
17:0	0.086107	-0.0311	0.185568	-0.04021
17:1	-0.06828	-0.07363	0.12932	-0.0505
18:0	0.034194	0.128927	0.054259	0.074583
18:1n-9	-0.02768	-0.10159	0.052701	0.026746
18:1n-7	-0.13732	0.040053	0.000473	-0.01954
18:2n-6	-0.17318	-0.03727	0.137124	0.008157
18:3n-6	-0.16738	0.014204	0.034415	-0.10559
18:3n-3	-0.13493	-0.13959	0.162226	-0.10892
18:4n-3	0.05329	-0.20737	-0.12832	-0.16264
20:1	0.209588	-0.2736	0.07098	-0.13443
20:2n-6	0.142227	-0.14711	0.074436	-0.1262
20:3n-6	0.110891	-0.08932	0.187224	-0.20996
20:4n-6	0.172336	0.02206	0.063309	-0.02309
20:3n-3	0.009878	-0.03701	0.186293	-0.17029
20:4n-3	0.047036	-0.09275	0.038581	-0.16845
20:5n-3	-0.08914	0.141636	-0.00775	-0.06125
22:1n-11	0.16792	-0.05563	0.088054	-0.01833
22:1n-9	0.287694	-0.30038	-0.01417	-0.06314
22:4n-6	0.274565	-0.19746	0.032755	-0.12365
22:5n-6	0.369868	-0.20552	-0.04737	-0.0745
22:5n-3	0.082833	0.083459	-0.10683	-0.01944
22:6n-3	0.293294	0.061063	-0.05706	0.034424

Table S4. Axis scores for non-metric multidimensional scaling analysis of fatty acids detected in 60-100 mm round gobies in 2010. Five sites in summer and fall only included.

Fatty acid	NMDS1	NMDS2	NMDS3	NMDS4
14:0	-0.0009	-0.12403	-0.03809	-0.06932
15:0	0.18544	-0.1113	-0.04958	0.034537
16:0	-0.00348	-0.00195	0.062731	0.026472
16:1n-9	0.074104	-0.00164	-0.08228	0.077436
16:1n-7	-0.1298	-0.14389	0.081665	-0.05521
17:0	0.143615	-0.02202	-0.12357	0.105141
17:1	0.009674	-0.07395	-0.16775	0.050133
18:0	-0.01921	0.119245	0.025175	0.089617
18:1n-9	0.044286	-0.10631	0.006174	0.031874
18:1n-7	-0.13693	-0.02958	-0.03929	0.015798
18:2n-6	-0.11565	-0.04856	-0.07506	0.044402
18:3n-6	-0.17371	0.003628	-0.12552	-0.05738
18:3n-3	-0.05464	-0.08849	-0.18406	0.09076
18:4n-3	0.029938	-0.05142	-0.11707	-0.08777
20:1	0.35398	-0.156	-0.07084	0.017043
20:2n-6	0.178313	-0.07818	-0.13855	0.047488
20:3n-6	0.177888	0.005244	-0.22362	0.010818
20:4n-6	0.183319	0.050708	-0.04428	0.005813
20:3n-3	0.019461	-0.01299	-0.28932	0.083038
20:4n-3	0.057745	0.016126	-0.1619	-0.01841
20:5n-3	-0.13262	0.101085	-0.02681	-0.02541
22:1n-11	0.241371	-0.00909	0.025856	0.096792
22:1n-9	0.433063	-0.21868	0.09076	0.048198
22:4n-6	0.394016	-0.06128	-0.08003	-0.08803
22:5n-6	0.463575	-0.06435	-0.02065	-0.10284
22:5n-3	0.01235	0.164012	0.043465	-0.0682
22:6n-3	0.196428	0.162564	0.068723	-0.00602