QUANTITATIVE APPROACHES TO THE ANALYSIS OF STABLE ISOTOPE FOOD WEB DATA

STEPHANIE N. SCHMIDT,[1,3] JULIAN D. OLDEN,[2] CHRISTOPHER T. SOLOMON,[1] AND M. JAKE VANDER ZANDEN[1]

[1]*Center for Limnology, University of Wisconsin, 680 North Park Street, Madison, Wisconsin 53706 USA*
[2]*School of Aquatic and Fishery Sciences, Box 355020, University of Washington, Seattle, Washington 98195 USA*

Abstract. Ecologists use stable isotopes (δ^{13}C, δ^{15}N) to better understand food webs and explore trophic interactions in ecosystems. Traditionally, δ^{13}C vs. δ^{15}N bi-plots have been used to describe food web structure for a single time period or ecosystem. Comparisons of food webs across time and space are increasing, but development of statistical approaches for testing hypotheses regarding food web change has lagged behind. Here we present statistical methodologies for quantitatively comparing stable isotope food web data. We demonstrate the utility of circular statistics and hypothesis tests for quantifying directional food web differences using two case studies: an arthropod salt marsh community across a habitat gradient and a freshwater fish community from Lake Tahoe, USA, over a 120-year time period. We calculated magnitude and mean angle of change (θ) for each species in food web space using mean δ^{13}C and δ^{15}N of each species as the *x, y* coordinates. In the coastal salt marsh, arthropod consumers exhibited a significant shift toward dependence on *Spartina*, progressing from a habitat invaded by *Phragmites* to a restored *Spartina* habitat. In Lake Tahoe, we found that all species from the freshwater fish community shifted in the same direction in food web space toward more pelagic-based production with the introduction of nonnative *Mysis relicta* and onset of cultural eutrophication. Using circular statistics to quantitatively analyze stable isotope food web data, we were able to gain significant insight into patterns and changes in food web structure that were not evident from qualitative comparisons. As more ecologists incorporate a food web perspective into ecosystem analysis, these statistical tools can provide a basis for quantifying directional food web differences from standard isotope data.

Key words: circular statistics; food web change; food webs; stable isotopes; trophic niche.

INTRODUCTION

Food webs depict predator–prey and energy flow relationships in ecosystems (Paine 1980, Polis and Winemiller 1996), and an understanding of food web structure is of growing importance in ecosystem management (Kitchell 1992, Winemiller and Layman 2005, Vander Zanden et al. 2006). In recent years, stable isotope techniques have emerged as tools for elucidating trophic structure and inferring pathways of energy/mass flow in food webs (reviewed in Peterson and Fry 1987, Fry 2006). Ratios of stable isotopes (^{13}C/^{12}C and ^{15}N/^{14}N, expressed as "δ" notation relative to a known standard) vary predictably from resource to consumer tissues, whereby consumer δ^{13}C is generally an indicator of energy or carbon source and δ^{15}N is reflective of consumer trophic position (Post 2002). Stable isotope values of consumer tissues integrate diet variation over an extended period, offering a powerful tool for characterizing trophic pathways.

While stable isotopes provide a rich source of data, almost without exception food web studies have involved relatively simple qualitative analyses. These studies typically present descriptive statistics such as species mean and variance for each isotope and display these values as δ^{13}C (*x*-axis)–δ^{15}N (*y*-axis) bi-plots. Although these diagrams help visualize the trophic niche of individuals and species in what we refer to as "isotopic niche space," in recent years the sophistication of ecological questions has progressed beyond simple qualitative descriptions of food webs. Ecologists have examined patterns of trophic overlap and resource use (Jepsen and Winemiller 2002), food web impacts of species invasions (Vander Zanden et al. 1999), trophic overlap among populations across ecosystems (Koch et al. 1995, Campbell et al. 2003), and food web comparisons among impacted and reference sites in the context of restoration activities (Gratton and Denno 2006). Additionally, a growing number of retrospective studies have examined food web change over timescales ranging from tens to hundreds of years from isotopic analysis of archived specimens (e.g., Kiriluk et al. 1999, Perga and Gerdeaux 2003, Vander Zanden et al. 2003). These recent studies indicate the potential value of stable isotopes for studying ecological change and addressing

applied questions relevant to ecosystem conservation and management.

Despite increased interest in food web comparisons through time or space, statistical methodologies for hypothesis testing have lagged behind the growing field of stable isotope ecology. In fact, Schindler and Lubetkin (2004) called for a shift from qualitative treatment of stable isotope data to quantitative approaches for elucidating food web linkages. This paper addresses this need by using circular statistics for analyzing stable isotope food web data, in much the same way as these metrics are used to quantify the movement of individual organisms across the landscape (Batschelet 1981). We illustrate the manner in which this branch of statistics can quantify and test hypotheses regarding directional changes in trophic niches of species and communities across space and time. These methods complement traditional food web diagrams, providing a set of statistical tools for quantitative food web studies.

CASE STUDIES

To demonstrate our approaches for the statistical analysis of stable isotope data, we draw on recent examples that examined food web structure over both a spatial (Gratton and Denno 2006) and a temporal (Vander Zanden et al. 2003) gradient. Gratton and Denno (2006) used stable isotope data to describe the food web structure of an arthropod salt marsh community along a gradient of habitats impacted by an invasive plant. Vander Zanden et al. (2003) conducted a retrospective comparison using stable isotopes to characterize historical food web changes in fish communities of Lake Tahoe, California, USA, over the last century. In both studies, $\delta^{13}C$–$\delta^{15}N$ bi-plots were used to compare food webs.

Coastal arthropod salt marsh communities, USA

We used stable isotope data from Gratton and Denno (2006) to characterize food web structure and infer feeding relationships among arthropod consumers in invaded and restored salt marsh habitats along a 5-km stretch of Alloway Creek (Salem County, New Jersey; 39°30.7′ N, 75°28.7′ W). The invasive macrophyte *Phragmites australis* has spread throughout brackish wetlands of the region that were once dominated by the native *Spartina alterniflora* Lois (Weinstein and Balletto 1999). Although this invasion has impacted arthropod assemblages, the restoration of *Spartina* can lead to the recovery of native assemblages (Gratton and Denno 2005). A large salt marsh restoration project led by the Public Service Enterprises Group (PSEG) involved the removal of the invasive *Phragmites* by herbicide applications (Weinstein and Balletto 1999), resulting in treated areas reverting back to *Spartina* domination within five years. Stable isotopes of salt marsh trophic guilds were examined within four different vegetated habitat types that spanned an invasion gradient. "*Phragmites*" habitats consisted of >90% *Phragmites*

cover. "Mixed" habitats represented a transitional stage of *Spartina* recovery (30–60% *Spartina*) after herbicide treatment to *Phragmites* stands. "Restored" habitats were areas of *Spartina* (>80%) previously dominated by *Phragmites*. "Reference" habitats consisted of areas that had no historical evidence of *Phragmites* invasion. Our analysis quantified directional food web changes in the trophic groups (herbivores, detritus/algal feeders, non-spider predators, spiders) and basal resources (dominant macrophytes, thatchy leaf litter, soil) as reported by Gratton and Denno (2006).

Freshwater fish communities of Lake Tahoe, USA

We use museum-archived (see Plate 1) and present-day stable isotope data from Vander Zanden et al. (2003) to describe the fish assemblages of Lake Tahoe at five time periods (1872–1894, 1904–1913, 1927–1942, 1959–1966, and 1998–2000) in response to introductions of a nonindigenous freshwater shrimp (*Mysis relicta*) and lake trout (*Salvelinus namaycush*). Both species are extremely abundant and are thought to have negative impacts on native biota of Lake Tahoe. Our study focuses on eight species for which at least two time periods of isotope data were available: *Gila bicolor* (tui chub), *Rhinichthys osculus robustus* (Lahontan speckled dace), *Richardsonius egregius* (Lahontan redside), *Cottus beldingii* (paiute sculpin), *Catostomus tahoensis* (Tahoe sucker), *Prosopium williamsoni* (mountain whitefish), *Salvelinus namaycush* (lake trout), and *Oncorhynchus clarkii henshawi* (Lahontan cutthroat trout).

METHODS

Stable isotope bi-plots are the traditional method for presenting stable isotope food web data. Quantitative analysis is restricted to error bars (±SE) on the bi-plots that provide a visual representation of feeding relationships and allow the comparison of trophic niches among species or populations. Overlapping error bars indicate species or groups that are not significantly different in trophic niche space. Testing hypotheses about community-wide differences using the $\delta^{13}C$–$\delta^{15}N$ bi-plots is not possible when dealing with multiple time periods or systems, because multiple bi-plots can create a complicated picture of species' means and overlapping error bars. In contrast, circular statistics allows for explicit hypotheses testing regarding community-wide directional changes or differences in food web structure through time and space.

Commonly used in behavioral ecology, circular statistics is an area of mathematics concerned with the analysis of angles ranging from 0 to 360°, or equivalently, from 0 to 2π radians (Batschelet 1981). Because the algebra of angles is somewhat different from traditional statistics, data from circular distributions generally may not be analyzed using traditional approaches for both theoretical and empirical reasons. For example, consider three compass directions, 10°, 30°, and 350°, for which we wish to calculate the arithmetic

mean. The mean calculation of $(25° + 10° + 355°)/3 = 390°/3 = 130°$ is obviously incorrect, for all the data are northerly in direction and the computed mean is southeasterly (Zar 1996). For this reason, circular statistics test for significance according to the von Mises distribution (also known as the circular normal distribution), unlike traditional statistics that are based on the Gaussian or normal distributions. Although statistical methods for describing and analyzing data from circular distributions are relatively new, the seminal work of Batschelet (1981) remains the most influential and comprehensive text, particularly for biologists.

Following the appropriate mathematical protocols for angular data (Batschelet 1981), we calculated the magnitude and direction of change in food web space for each species over consecutive time periods (fish communities) or across habitats (arthropod communities) using $\delta^{13}C$ and $\delta^{15}N$ means of each food web component as coordinates (x, y). Biogeochemical processes (i.e., anthropogenic nutrient additions) can cause $\delta^{13}C$ and $\delta^{15}N$ of primary producers to shift over time, which is then reflected up the food chain (Cabana and Rasmussen 1996). Vander Zanden et al. (2003) used zooplankton and zoobenthos end members to develop a baseline curve for $\delta^{15}N$ across all five time periods. Using this baseline curve, we corrected $\delta^{15}N$ values of all species ($\delta^{15}N[corrected] = \delta^{15}N[consumer] - \delta^{15}N[baseline]$) before conducting circular analyses for the Lake Tahoe case study. We assumed no change in $\delta^{13}C$ over time and used uncorrected values because Chandra et al. (2005) reported relatively constant $\delta^{13}C$ values of primary consumers in Lake Tahoe over the past 40 years, despite the gradual eutrophication of this water body. Some studies convert $\delta^{13}C$ and $\delta^{15}N$ to more ecologically meaningful metrics, such as percentage of benthivory and trophic position (Vander Zanden et al. 2003). The calculations for percentage of benthivory and trophic position incorporate baseline adjustments; however, these metrics are represented by different units of measurement in coordinate space. For example, a 1% shift in percentage of benthivory is not comparable to a change in one trophic position, whereas stable isotope values for carbon and nitrogen are expressed in the same units, a ratio of the heavy isotope to the light isotope, $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (expressed as "δ" notation relative to a known standard). In systems with a shifting isotopic baseline, appropriate baseline adjustments should be made to give baseline-corrected values of $\delta^{13}C$ and $\delta^{15}N$ before applying our statistical approach.

We calculated the directional change for each food web component, which consisted of two properties: direction (or angle of change, θ) and length (or magnitude of change). Directional change was measured as the difference between two points in trophic niche ($\delta^{13}C - \delta^{15}N$) space, where points in this calculation refer to the mean of a species at consecutive time periods or across habitats. For the Lake Tahoe example, we also calculated the mean directional difference between the

position of *Mysis relicta* in food web space and all species in 1959–1966 prior to the date of invasion. For the entire community the mean vector was calculated as the mean of all angles of change. The mean vector also has two properties: direction (the mean angle, μ) and length (referred to as r). Note, in this case, that length (r) of the mean vector does not correspond to magnitude or distance. Length provides a unit measure of concentration (i.e., dispersion) of turning angles. The parameter r has no units and may vary from 0, at which there is no concentration of data and the directions are more uniformly distributed, to 1.0, at which all data are concentrated at the same direction. Angular standard deviation was also calculated and provided a measure of dispersion or variance in angle directions. Taken together, these descriptive statistics for circular data enable ecologists to assess the trophic niche positions of species or groups of species through time and across ecosystems. By taking the average isotopic signature across all individuals of a species or trophic group we make the assumption, as do all food web studies, that the mean is representative of the entire population of individuals at a site. Formulas and example calculations for the mean vector and angular standard deviation are presented in Batschelet (1981:7–36) and Zar (1996: 598–603).

At the community level (i.e., the collection of fish species in Lake Tahoe at each time period or the collection of arthropod trophic groups in each habitat), we evaluated the angles and magnitudes of change using circular graphs and statistical methods for testing hypotheses on a circular scale. We constructed arrow diagrams to explore how much and in what direction species' trophic niche position changed over time in Lake Tahoe and across the invasion gradient in a coastal salt marsh. Each arrow represents the directional movement (θ) of a single food web component while the length of the arrow corresponds to the magnitude of change for that component. These arrow diagrams provide a visualization tool for expressing directions and magnitudes of change in position of species or trophic groups in food web space and therefore are particularly useful for understanding patterns in stable isotope data.

Rayleigh's test for circular uniformity was used to assess whether the distribution of mean angles of direction departed nonrandomly from uniformity. The null hypothesis for this test states that the parent population is uniformly distributed, or in other words, movements occur in all directions with equal frequency or have no mean direction. In the context of this analysis, the Rayleigh's test examines whether mean angular change (μ) in trophic niche food web space among food web components is nonrandom over time. Watson-William's two-sample test of homogeneity was used to compare two time periods or habitats to determine whether the mean angles differed significantly. This test can be performed in a pairwise fashion, so
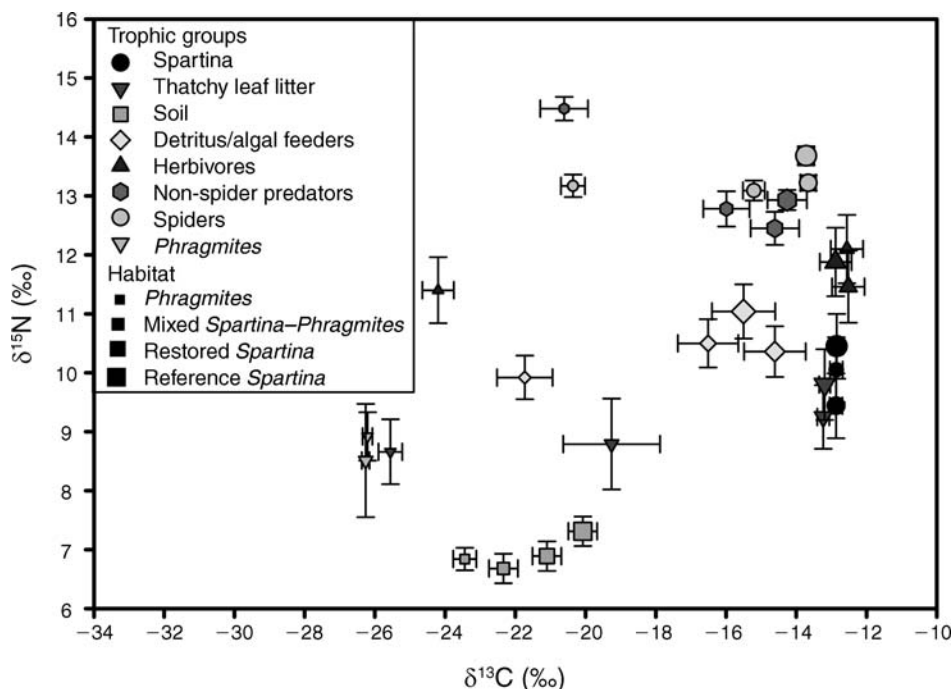
Fig. 1. Stable isotope food web diagram for all habitat types of an arthropod salt marsh community, New Jersey, USA (data are from Gratton and Denno [2006]). Each symbol represents the mean $\delta^{13}C$ and $\delta^{15}N$ for a given trophic group. The size of the symbol corresponds to the habitat type. Error bars show ±SE from the mean.

that the selected samples are compared with each other, a pair at a time, or an overall test can be calculated for all samples. The null hypothesis for a Watson-William's test states that the mean angle of change for the community is the same for each consecutive time period or location. With this case study, the Watson-William's test compares between time steps or habitat types and evaluates whether movements in food web space, at the community level, are significantly different from one another. For the Lake Tahoe example, we also used this test to examine the directional change in food web structure after the invasion of *Mysis relicta*. All circular graphs, descriptive statistics, and statistical tests were performed using Oriana 2.0 (Rockware, Inc., Golden, Colorado, USA). However, a number of other Windows-based programs (e.g., Vector Rose), modules for commonly used software packages (e.g., Circ toolbox for MatLab), and libraries for different programming languages (e.g., CircStats Package for the R-package) are also available for conducting circular analyses.

RESULTS

Coastal arthropod salt marsh communities

Isotope data from all four habitat types are presented in a single $\delta^{13}C$–$\delta^{15}N$ bi-plot (Fig. 1; see Gratton and Denno 2006). Progressing from the most impacted site (*Phragmites*) to the least impacted (reference *Spartina*) there is a clear trend of increased reliance on *Spartina*-based food sources by arthropod consumers, as indicated by enriched $\delta^{15}N$ values. This pattern is most evident

when comparing the habitat dominated by *Phragmites* to the transitional mixed *Spartina*–*Phragmites* habitat. Any subsequent changes in carbon resources are relatively small. Additionally, it appears that arthropod consumers in the reference *Spartina* have elevated $\delta^{15}N$ values compared to the restored *Spartina* habitat. However, soil $\delta^{13}C$ and $\delta^{15}N$ values also increase in a fairly stepwise fashion over this spatial gradient (Fig. 1).

Angle (θ) and magnitude of change for each food web component along this spatial gradient quantify our qualitative observations based on the C–N bi-plot (Fig. 2). For all three spatial intervals, there is a significant directionality in movement in food web space for all trophic groups (Rayleigh's test, $P < 0.01$ all cases; Table 1). That is, progressing from *Phragmites* to a mixed *Spartina*–*Phragmites* habitat results in a significant increase in reliance on *Spartina*-based carbon sources (Fig. 2A). This trend is also significant when comparing the mixed habitat to the restored *Spartina* habitat; however, as indicated by a lower magnitude of change among all components, the subsequent trend is relatively small. Further corroborating this trend, the mean direction of change in C–N food web space among all arthropod consumers was not significantly different along a gradient of *Phragmites* to mixed habitats and then mixed to restored *Spartina* habitats (Watson-William's test, $P = 0.68$; Table 1). However, the mean direction of change for the community in food web space was significantly different between the restored
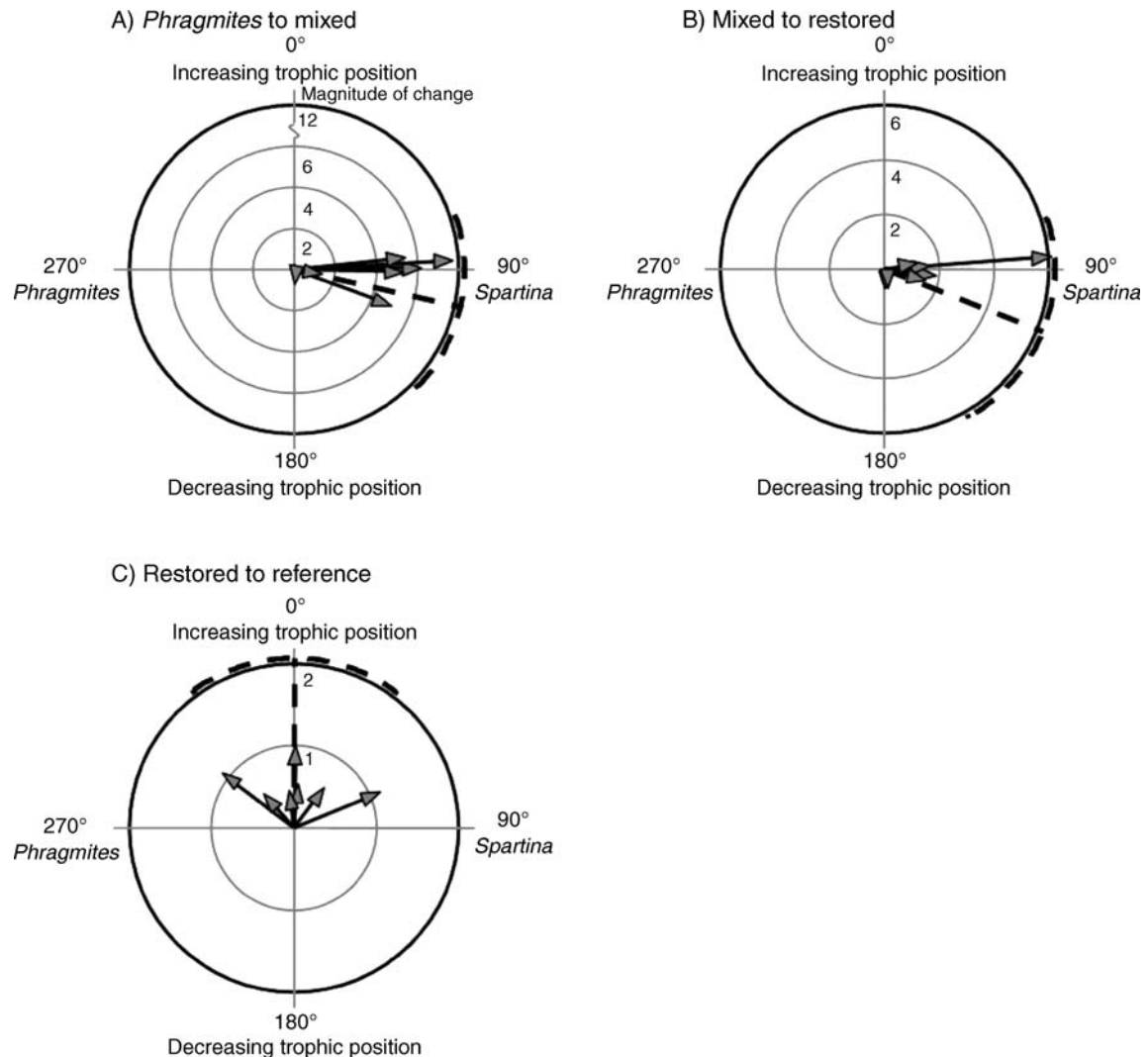
FIG. 2. Arrow diagrams for angle of change (θ) for all trophic groups along a gradient of habitats impacted by invasive *Phragmites*. Each diagram represents a progression from one habitat to another: (A) directional food web differences between *Phragmites* habitat and the mixed *Phragmites–Spartina* habitat; (B) differences between the mixed habitat and restored *Spartina* habitat; (C) differences between the restored and reference *Spartina* habitat. Each arrow represents a single trophic group and the direction in trophic niche space the group moved. The length of the arrow represents the magnitude of change for that particular trophic group. Concentric circles correspond to magnitude (i.e., distance) of change. The straight dashed line is the mean vector of change (μ) among all species; the curved dashed line on the rim indicates the 95% confidence interval around the mean vector of change. Note that axes for magnitude are different among the three arrow diagrams and that panel (A) has a broken axis.

and reference *Spartina* habitats (Watson-William's, $P <$ 0.001; Table 1).

Fish communities of Lake Tahoe, USA

Isotope data from all time periods for Lake Tahoe are presented in a single $\delta^{13}C–\delta^{15}N$ bi-plot (Fig. 3; see Vander Zanden et al. 2003). It is difficult to identify patterns of food web change from this condensed bi-plot, although some species have clearly shifted in trophic niche space over time and some species may have exhibited similar directional changes towards reliance on pelagic production. In fact, species exhibited varying

angles and magnitudes of change in Lake Tahoe over the last century (Table 2).

Arrow histograms represent angle and magnitude of change for each species between consecutive time periods for Lake Tahoe (Fig. 4). In Lake Tahoe, there is no pattern of directional change among species for the first three time steps (Fig. 4A–C); however, in the final time step, all species are shifting in a similar direction in food web space (Fig. 4D). In support of these patterns, the Rayleigh's test for the first three time steps indicated no significant pattern of consistent change among species ($P > 0.30$ in all cases), whereas in the final time step (1959–1966 to 1998–2000) all species within the

TABLE 1. Directional statistics quantifying change in isotope niche space for arthropod trophic groups along a gradient of salt marsh habitats in New Jersey, USA, impacted by invasive *Phragmites*.

Habitat gradient	No. observations	Mean vector Direction (μ)	Mean vector Length (r)	Circular SD	Rayleigh's test Z	Rayleigh's test P	Watson-William's test $F_{1,12}$	Watson-William's test P
Phragmites to mixed	7	102.7	0.85	32.8	5.047	**0.003**	0.18	0.68
Mixed to restored *Spartina*	7	111.6	0.77	41.7	4.123	**0.01**	21.80	**<0.001**
Restored *Spartina* to reference	7	0.3	0.79	39.1	4.391	**0.007**		

Notes: Rayleigh's test assesses whether the distribution of mean angles of direction (i.e., angular variance) depart from uniformity. Watson-William's two-sample test of homogeneity tests for differences between mean angles of direction for different habitat transitions. *P* values in boldface type are significant at the $\alpha = 0.05$ level.

community showed similar directionality in their movement in food web space ($P < 0.001$, Table 3).

The community-wide direction of change in C–N food web space has not been constant over the last century (Watson-William's test, global $F_{4,20} = 6.257$, $P < 0.01$). The mean direction of community food web change in time step A (1872–1894 to 1904–1913) was significantly different from that in time step B (1904–1913 to 1927–1942; 251.1 ± 67.4 vs. 49.3 ± 75.7, respectively; [mean ± circular SD] $P < 0.05$); similarly, the mean direction in time step C (1927–1942 to 1959–1966) was significantly different than that in time step D (1959–1966 to 1998–2000; 74.9 ± 65.6 vs. 235.7 ± 13.8, respectively; $P < 0.01$; Table 3). The Watson-William's test also allows for comparison of the community at a given time step to any angle in food web space. For example, *Mysis relicta*, a large, nonnative pelagic crustacean, was introduced

into Lake Tahoe between 1963 and 1965 (Linn and Frantz 1965). By the final time period (1998–2000), *Mysis relicta* was well established in the pelagic zone of Lake Tahoe (Vander Zanden et al. 2003). We found no significant difference between the directional change in the Lake Tahoe community between 1959–1966 to 1998–2000 and the directionality between the 1959–1966 community and position of *Mysis relicta* in food web space (235.7 ± 13.8 vs. 238.2 ± 9.6, respectively; $P = 0.74$; Table 3). Furthermore, comparing individual angles of movement in trophic niche space from 1959–1966 to 1998–2000 to the location of *Mysis relicta* revealed that Lahontan speckled dace, paiute sculpin, Lahontan redside shiner, and Tahoe sucker showed strong movement in the direction of *Mysis relicta*, whereas Tui chub and lake trout showed a weaker response (Table 2).
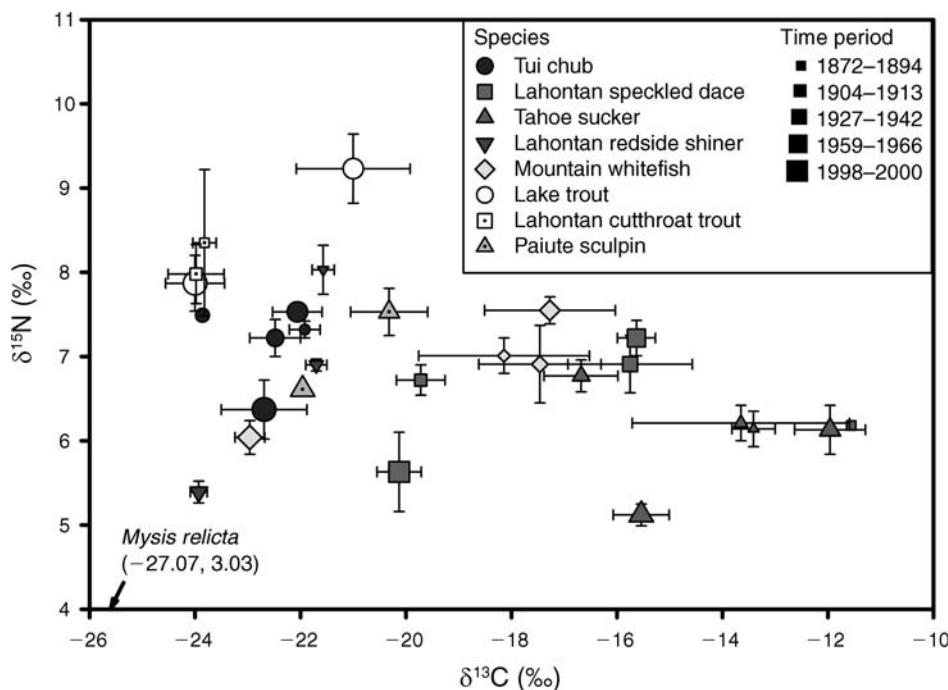

FIG. 3. Stable isotope food web diagram for fish communities across all time periods of Lake Tahoe (data are from Vander Zanden et al. [2003]). Each symbol represents the mean $\delta^{13}C$ and $\delta^{15}N$ (baseline-corrected) for a given species. The size of the symbol corresponds to the time period. Error bars show ±SE from the mean. The position of *Mysis relicta* in food web space is noted.

TABLE 2. Directional change (magnitude [Mag.] and angle [θ]) for each species across all time steps in the Lake Tahoe, USA, fish community, in which angle is expressed in degrees.

Species	1872–1894 to 1904–1913		1904–1913 to 1927–1942		1927–1942 to 1959–1966		1959–1966 to 1998–2000		1959–1966 to *Mysis*
	Mag.	θ	Mag.	θ	Mag.	θ	Mag.	θ	θ
Tui chub† (*Gila bicolor*)	1.94	270.0	1.39	95.8	0.55	50.2	1.37	207.3	242.1
Lahontan speckled dace (*Rhinichthys osculus robustus*)	8.16	268.4	4.01	81.9	0.34	20.5	4.93	245.9	232.7
Lahontan redside shiner (*Richarsonius egregius*)					1.15	186.5	2.82	232.4	225.2
Tahoe sucker (*Catostomus tahoensis*)	0.24	281.5	3.04	275.2	4.72	92.4	3.82	249.4	245.3
Paiute sculpin (*Cottus beldingii*)							1.96	236.8	230.8
Mountain whitefish (*Prosopium williamsoni*)	0.68	93.0	0.68	16.1					
Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*)	0.42	202.6							
Lake trout (*Salvelinus namaycush*)							3.42	241.3	253.3

Note: The far right column is the angular difference between all species' trophic niche position in 1959–1966 to the position of *Mysis relicta* in C–N food web space.

† Pelagic.

DISCUSSION

The pattern of resource shifting among arthropod consumers toward dependence on *Spartina* is evident from examination of the condensed bi-plot, but is further corroborated and quantified by using circular statistics (Figs. 1 and 2, Table 1). Gratton and Denno (2006) stated that interactions among arthropods within the restored *Spartina* sites are indistinguishable from those in the reference *Spartina* sites. Use of circular statistics, however, reveals a slight difference between the two sites, whereby trophic groups from reference *Spartina* sites are elevated in $\delta^{15}N$ (Fig. 2C, Table 1). In this particular case, inclusion of basal sources in the statistical analysis helps determine whether resources shifts reflect a food web difference. The elevation of $\delta^{15}N$ is likely due to biogeochemical differences among habitats, since both consumers and basal resources have similar directional shifting in food web space (Figs. 1 and 2C). Regardless, our statistical approach detected a significant pattern among habitats that was previously overlooked by comparing bi-plots.

While there was temporal variation in species' trophic niches for the Lake Tahoe fish community, this approach indicated no significant directional food web change until the most recent time step. This trend of increased pelagic reliance between the two final time periods was noted by Vander Zanden et al. (2003), suggesting a shift in trophic basis for fish production from benthic to pelagic over the last century. The use of circular statistics allowed us to advance the findings of Vander Zanden et al. (2003) by quantifying this trend of shifting production reliance and testing whether it differed from random expectations. Additionally, Vander Zanden et al. (2003) focus their analysis on species-specific shifts in trophic niche space over time; the nonnative lake trout shifted to a trophic niche that is similar to the historical trophic niche of the extirpated Lahontan cutthroat trout. Using circular statistics, we were able to assess community-wide differences in trophic structure over time; our analysis revealed no significant difference between the community shift during the final time step and the angular difference from all species in 1959–1966 to *Mysis relicta* in food web space. After *Mysis relicta* became established in Lake Tahoe, most fish species shifted in the direction of *Mysis relicta* in food web space, becoming more dependent on pelagic-based production (Fig. 4, Table 3). Importantly, the food web shift towards pelagic production is not due to *Mysis relicta* alone; Lake Tahoe has become increasingly eutrophic over this same time period (Chandra et al. 2005), which could also shift whole-lake productivity to the pelagic zone (Vadeboncoeur et al. 2003). This analysis extends the qualitative analysis of Vander Zanden et al. (2003) by quantifying the dynamic nature of the Lake Tahoe community in response to environmental change over the past century.

In this study, we illustrate the utility of circular statistics to quantify complex patterns and test explicit hypotheses regarding changes in food web structure using two complementary case studies. Both Gratton and Denno (2006) and Vander Zanden et al. (2003) provide qualitative descriptions based on the constructed bi-plots for each community over space or time, inferring patterns of change within and among food web components. Our statistical approach allows qualitative statements such as "it appears that" to be replaced with test statistics that examine explicit hypotheses regarding differences in food web structure over time and space.

Recent interest has focused on using stable isotopes to characterize changes in community-wide feeding relationships in the context of ecological restoration (Vander Zanden et al. 2006). The statistical tools described here provide a quantitative approach of exploring questions and testing trajectories of community "recovery" and "perturbation" over time and space. However, we acknowledge several limitations of this
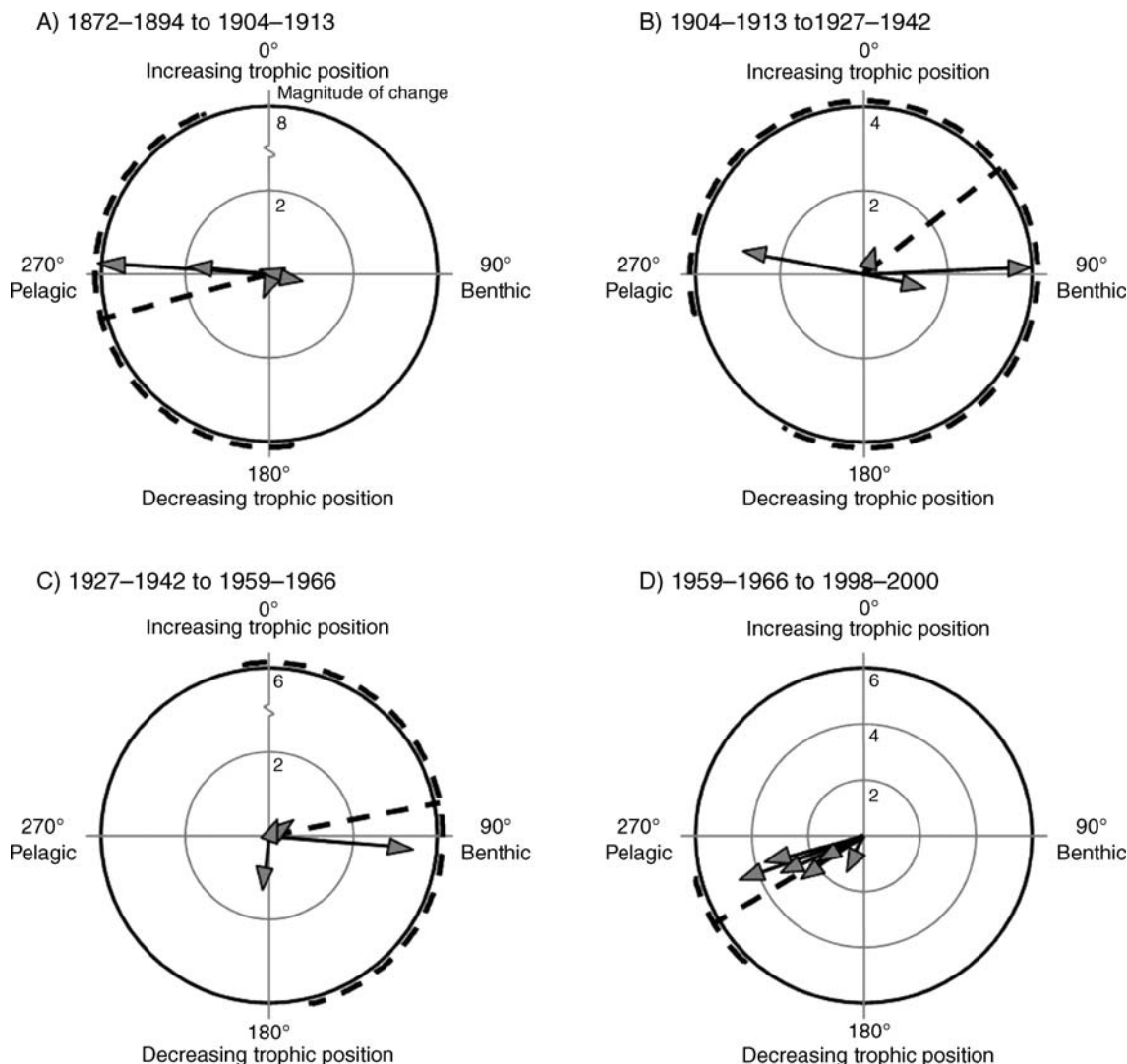
Fig. 4. Arrow diagrams for angle of change (θ) for all fish species in Lake Tahoe at each time step. Each diagram shows directional food web changes between consecutive time periods. Each arrow represents a single species and the direction in trophic niche space the species moved. The length of the arrow represents the magnitude of change for that particular species. Concentric circles correspond to magnitude (i.e., distance) of change. The straight dashed line is the mean vector of change (μ) among all species; the curved dashed line on the rim indicates the 95% confidence interval around the mean vector of change. Note that axes for magnitude are different among the four arrow diagrams and that panels (A) and (C) have broken axes.

TABLE 3. Directional statistics quantifying change in isotope niche space for fish communities in Lake Tahoe over time.

Time step	No. observations	Mean vector Direction (μ)	Length (r)	Circular SD	Raleigh's test Z	P	Watson-William's test F	df	P
1872–1894 to 1904–1913	5	251.1	0.50	67.4	1.25	0.30	6.39	1, 7	**0.04**
1904–1913 to 1927–1942	4	49.3	0.42	75.7	0.70	0.53	0.19	1, 6	0.68
1927–1942 to 1959–1966	4	74.9	0.52	65.6	1.08	0.37	18.06	1, 8	**0.003**
1959–1966 to 1998–2000	6	235.7	0.97	13.8	5.66	<0.001	0.12	1, 10	0.74
1959–1966 to *Mysis*	6	238.2	0.99	9.6	5.84	<0.001			

Notes: The bottom row compares location of all species in 1959–1966 to the position of *Mysis relicta* in C–N food web space. *P* values in boldface type are significant at the α = 0.05 level.

PLATE 1. Preserved fish specimens at the University of Michigan Museum of Zoology (UMMZ; Ann Arbor, MI). The museum collection contains over three million catalogued fish specimens from around the world. Specimens from the United States and Mexico are the most abundant in the collection. Many of the samples used in the Lake Tahoe case study came from the UMMZ. Photo credit: S. N. Schmidt.

approach. Establishing analytical tools that incorporate variance among individuals of species and test hypotheses regarding magnitude would be an improvement to this method. Additionally, our approach depends on unique positions in trophic niche space. Ecosystems having either multiple or basal resource pools with similar $\delta^{13}C$ values will have less unique niche separation. However, the dual-isotope approach weakens this source of bias by examining trophic niches in two-dimensional rather than one-dimensional space. Basal resources may also exhibit significant temporal variation in isotopic signatures, which are then reflected up the food chain (Cabana and Rasmussen 1996). As such, it is important to establish an isotopic baseline against which all food web components are corrected (Vander Zanden and Rasmussen 1999, Post 2002). Furthermore, individual species could be moving in different directions, but would be interpreted as random change at the community level. Grouping species according to ecological function, such as trophic groups (Gratton and Denno 2006), would help address this issue. Building on the framework outlined here, we encourage the exploration of other methods to strengthen the tools available to ecologists using stable isotopes.

While we were able to quantify directional food web change for the Lake Tahoe fish community and a coastal salt marsh arthropod community, there are other possible quantitative approaches for analyzing food web differences. For example, it is also important to understand how the overall shape of the food web changes over time and space. Layman et al. 2007 quantify food web change by calculating polygon dispersion metrics, borrowing from two-dimensional

geometric morphometrics in which shape variables can be calculated from x, y coordinates. This shape dispersion method differs from the directional method outlined here, but the statistical approaches are complementary, and both provide the tools necessary for answering important ecological questions regarding food web change. Additionally, it is possible to explore changes in C or N (separately) over time or space using traditional quantitative approaches such as a t test, Mann-Whitney or other paired univariate test, and univariate or multivariate regression analyses (e.g., Vander Zanden et al. 1999). These statistical approaches may be useful for examining a single species over time/space, but assessing community-wide changes across multiple time periods or food webs would not be as feasible. The circular statistics proposed here allow for such community-wide food web analysis. Furthermore, while the calculation of angles combines $\delta^{15}N$ and $\delta^{13}C$ differences, our approach is capable of adequately examining explicit trophic level ($\delta^{15}N$) or basal resource ($\delta^{13}C$) shifts already. For example, an angle of 0 or 180° represents a strict shift in $\delta^{15}N$; whereas, an angle of 90° or 270° represents a strict shift in $\delta^{13}C$.

In conclusion, our study demonstrates that circular statistics provide an addition to the ecologist's toolbox for testing hypotheses regarding food web change over time and space. The case studies highlighted here represent systems with varying degrees of food web complexity and patterns of food web structure, thereby demonstrating the versatility of our statistical approach. The development of quantitative methods in stable isotope ecology will lead to more rigorous inquiry and powerful interpretation of stable isotope data. As the

study of food webs continues to grow, we believe the statistical approaches presented here will provide a foundation for quantifying and testing hypotheses regarding food web differences across space and time. We urge ecologists to employ and further develop novel statistical approaches for assessing spatial and temporal patterns of food web structure.

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