11 Assessment of Diets and Feeding Patterns

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11.1 INTRODUCTION

Quantitative assessment of food habits is an important aspect of fisheries management. Successful management of sport fishes often hinges on our ability to manage prey resources (Noble 1981; DeVries and Stein 1990). As a result, knowledge of prey resources can help guide management efforts aimed at increasing fish production. Accurate description of fish diets and feeding habits also provides the basis for understanding trophic interactions in aquatic food webs (Garvey et al. 1998a; Vander Zanden et al. 2000). Diet composition analysis or other techniques, such as stable isotope analysis, can be used to evaluate effects of ontogeny, habitat, or the establishment of exotic species.

Diets of fishes represent an integration of many ecological components that include behavior, condition, habitat use, energy intake and inter- and intraspecific interactions. As a result, food habit studies can be incorporated in a variety of different research objectives. In the simplest case, a food habits study might be conducted to determine the most frequently consumed prey or determine whether a particular food category is present in the stomach of fishes. In other instances, we may be interested in more complex questions, such as (1) determining the relative importance of different food types to fish nutrition, (2) quantifying the consumption rate of individual prey types (Chapter 12), or (3) understanding foraging trade-offs associated with predator avoidance (Chapter 16). Each of these questions requires information on fish diets but necessitates different approaches in how we collect and analyze data. In this chapter, we outline quantitative techniques used to describe food habits and feeding patterns of fishes.

11.2 QUANTIFYING DIET COMPOSITION

11.2.1 Sampling and Identifying Stomach Contents

Most studies of fish diets rely on examination of stomach contents to quantify prey abundance. This information characterizes foraging choices made over a relatively short time scale (e.g., usually <24 h). Hence, time of day, sampling location, prey

availability, and even the type of collecting gear used need to be considered before initiating a diet study or analyzing diet data. Investigators using historical diet samples or processed data must be aware of the sampling protocols, laboratory procedures, and preservation techniques used. Failure to understand how diet data were collected may preclude accurate interpretation of foraging patterns.

Stomach contents can be collected from live fish by means of the lavage technique (Seaburg 1957), whereby food items are flushed from the stomachs by use of pressurized water. Similarly, emetics can be used to induce regurgitation in live fish (Jernejcic 1969; see Bowen 1996 for review). Regardless of the method, investigators should ensure that the removal technique effectively samples all items in the gut. Otherwise, data will be skewed toward items that are more easily displaced from the stomach. Alternatively, fish can be sacrificed and stomach contents removed for analysis. If fish are to be sacrificed, they should be preserved immediately either by freezing or by fixing in formalin (Bowen 1996). Stomach contents will continue to digest, rendering rapid preservation of the fish or removed contents necessary to prevent loss of resolution. Various taxa digest at different rates (Sutela and Huusko 2000; Kim and DeVries 2001). As such, recently consumed taxa may be present in the foregut, but only resistant items remain in the hindgut. Investigators must consider the relative digestibility of prey when deciding on the section of the alimentary tract to sample. To avoid bias when both easily digested prey and resistant prey are present, only the immediate foregut (i.e., stomach) should be sampled (Sutela and Huusko 2000).

Prey items in fish stomachs are often not intact. Otoliths or other relatively indigestible hard parts, such as scales, pharyngeal teeth, cleithra, or backbones, have diagnostic, species-specific characteristics useful for identifying prey (Garman 1982; Holland-Bartels et al. 1990). Alternatively, partially digested prey may be identified using biochemical signatures, such as allozyme electrophoresis (Hartman and Garton 1992), immunoassays (Feller 1992; Schultz and Clarke 1995), or promising new techniques based on fatty acid analysis (Raclot et al. 1998).

Hard structures are often used to determine lengths or weights of prey items by regressing the dimension of an indigestible hard part (e.g., head capsule of an insect) against whole-body length or mass (least-squares regression models; Trippel and Beamish 1987; Scharf et al. 1997). Combining back-calculated estimates in this fashion may compound error in estimates of total prey weight (or volume). Thus, it is imperative that biometric relationships and measurements of hard parts used to reconstruct diet items are precise and not biased.

The proper taxonomic resolution for identifying stomach contents largely depends on the research question. Coarse taxonomic resolution is appropriate when quantifying ontogenetic changes in diet composition. Presence of fish in the diet may prove adequate for determining the size or time at which fish switch to piscivory. In other instances, finer taxonomic resolution may be needed, such as determining seasonal or spatial differences in diet composition or comparing percent composition of native versus exotic species.

Often, it is pragmatic to reduce the number of variables involved in the analysis by pooling diet items into categories based on taxonomy or habitat. Three types

of data pooling can be considered for prey items in fish stomachs: (1) necessary, (2) intuitive, and (3) statistical (Crow 1979). Necessary pooling occurs when unidentified prey are present in stomachs. If three categories of fish prey and one category of unidentified fish prey arise, then we should consider either pooling fish prey or dropping the unidentified category. An analysis with both identified and unidentified fish may be misleading because we do not know what proportion of unidentified fish were components of items we could successfully identify (Crow 1979). Intuitive pooling is based on taxonomic or ecological similarities among prey. Three species of calanoid copepods might be pooled into a single category (e.g., copepods) given similar morphological and behavioral characteristics. Similarly, we could pool species by habitat so that categories represent benthic, pelagic, or littoral prey. Finally, statistical pooling uses quantitative statistical procedures as a basis for pooling prey categories. Here, the investigator hypothesizes that two or more prey categories act as a single resource (Crow 1979). This hypothesis is tested using a 2×2 contingency table to identify whether prey are either positively or negatively associated (Box 11.1). Positive association implies that prey are acting as a single resource and may be pooled (Crow 1979).

11.2.2 Designing Appropriate Sampling Designs

11.2.2.1 Conducting Field Studies

Feeding patterns of fishes may be quantified in the field or with carefully designed experiments. In either case, the sampling design should be well considered before data are collected. As with other field studies, appropriate sampling designs for diet analysis include (1) simple random sampling, (2) stratified random sampling, (3) systematic sampling, and (4) multistage sampling (see Chapter 3). The choice of a particular sampling design depends on a variety of factors that include the research question, logistics, accessibility, and costs.

Prior to collecting diet data, attention should be given to factors that influence the quantity and quality of stomach contents. One important consideration in diet studies is that foraging behavior of fishes often varies with time of day (Shepard and Mills 1996). Hence, sampling plans should incorporate a diel component to determine how stomach contents change through time. Failure to standardize measurement times among sites or lakes may lead to erroneous conclusions about foraging patterns (see section 11.3.4).

Moreover, sampling approaches, such as electrofishing or gillnetting, may cause loss of stomach contents through regurgitation (Bowen 1996). Similarly, high-speed tow nets can eviscerate larval fish resulting in a loss of information (K. Arend, Ohio State University, personal communication). The use of active or passive gear types can also affect inferences about stomach fullness. Fish collected with passive gears can have more food in their stomachs than do fishes collected with active gears because passive gears often collect actively feeding fish (Hayward et al. 1989). Careful consideration should be given to sampling time and gear type to help reduce variability among samples.

Box 11.1 Pooling Prey Items as a Single Resource

Prey items in fish stomachs can sometimes be pooled prior to analysis. To determine whether two (or more) prey items act as a single resource, we can use chi-square contingency table analysis. In the example below, we are interested in whether prey *i* and prey *j* can be pooled prior to analysis.

We construct a 2 × 2 contingency table by totaling the number of fish that contain both prey types, either prey *i* or prey *j*, or neither prey type in the diet. In this example, the diets of 70 fish have been examined for prey *i* and prey *j*.

	Prey *j* present	Prey *j* absent	Total
Prey *i* present	18	9	27
Prey *i* absent	18	25	43
Total	36	34	70

Resulting output was obtained using the PROC FREQ procedure in SAS (SAS Institute1999). Here, the likelihood ratio chi-square value (*G*-statistic), 4.145, is larger than the critical value of a chi-square distribution (i.e., 1 df, $P = 0.041$), implying that the prey are either positively or negatively associated. Prey can be pooled only if they are positively associated. To determine association (*A*), we calculate the cross-product ratio of the contingency table as

$$A = (\text{cell } 11)(\text{cell } 22)/(\text{cell } 12)(\text{cell } 21)$$
$$= (18)(25)/(18)(9) = 2.8.$$

If *A* is greater than 1, then prey types are positively associated, implying that they are acting as a single resource and can be pooled (Crow 1979). If *A* were less than 1, then prey types would be negatively associated and should not be pooled.

Before initiating a field study, it is desirable to know how many samples are needed to describe the diet. Cumulative prey curves are useful for determining when a sufficient number of stomachs have been sampled. In this approach, the cumulative number of prey types is plotted against the cumulative number of pooled stomachs (Cortés 1997). The point at which the curve becomes asymptotic provides a minimum number of stomachs needed to characterize prey composition.

It is not uncommon to find empty stomachs. However, investigators must be cautious about how increasing sampling effort to find fish containing food affects their estimates. To our knowledge, the impact of this practice remains unexplored. Presumably, greater sample sizes arising when empty guts are frequent would affect variance estimates relative to other samples. Often, investigators restrict their analyses to the subset of individuals containing diet items (i.e., dropping individuals with empty guts) to explore diet preference (see section 11.3.7). This practice also must be approached cautiously. Diet characteristics of fish populations for which empty stomachs were frequent may be quite different than those for which empty stomachs were rare.

When sampling diets in the field, large numbers of fish are typically encountered, requiring subsampling across sizes. Stratifying samples as a function of body size is important because size often affects both the quantity and composition of items within diets (Schael et al. 1991; Bremigan and Stein 1994). The number of subsamples taken can be stratified by the relative proportion of individuals within each size-class or as some set number of individuals per size-class. Subsamples taken randomly in proportion to the actual number within each size-class reasonably reflect size-based patterns within the whole sample (Kimura 1977). However, this sampling design may poorly represent the diets of the largest individuals within the population, which are infrequently encountered. To remedy this, many sampling designs incorporate the set number per size-class approach. In a similar example with length-at-age data, Bettoli and Miranda (2001) demonstrated how simply pooling data from such a stratified sampling distribution poorly reflects the distribution within the overall sample. As such, extrapolating diet patterns within each size-class to that of the whole sample requires weighting the stratified diet data by the relative proportion of individuals within each size-class.

11.2.2.2 Conducting Experimental Studies

Food can be a limiting resource to fish populations. As a result, we are often interested in how competition for prey affects foraging success. Field data on stomach contents are inadequate to address competition questions. Rather, competition studies are generally performed under controlled experimental settings. Several approaches can be used to determine whether one species affects the foraging behavior of another. In all cases, it is important that densities of species be manipulated within the range of those in the environment to determine how variation in abundance affects competition. Three approaches are generally recognized in competition experiments: (1) substitutive, (2) density-gradient, and (3) response surface experiments (Goldberg and Scheiner 2001). The substitutive experimental approach involves varying treatment levels by substituting individuals of one species with an equal number of the other (Figure 11.1A). Total density is kept constant across all treatments. This approach tests for only the relative strengths of intra- and interspecific competition. The absolute magnitude of interspecific competitive effects is not isolated. A density-gradient approach involves holding the density of one focal species constant while varying that of another (Figure 11.1B). A problem with this approach is that foraging responses of the focal species are potentially confounded by an increase in frequency of the competitor and an overall increase in density (see Welker et al. 1994). A response surface experiment, which includes all density combinations of both competitors, avoids potential confounding effects but requires a large number of treatment combinations (Figure 11.1C). Clearly, designing an experiment to determine how competition affects foraging requires foresight about potential responses. The design of any experiment in which diet is a response variable requires careful consideration of the hypotheses being tested.

Field-derived patterns of foraging preference are by nature correlative. Only experiments definitively show how changes in food quality or quantity affect dietary

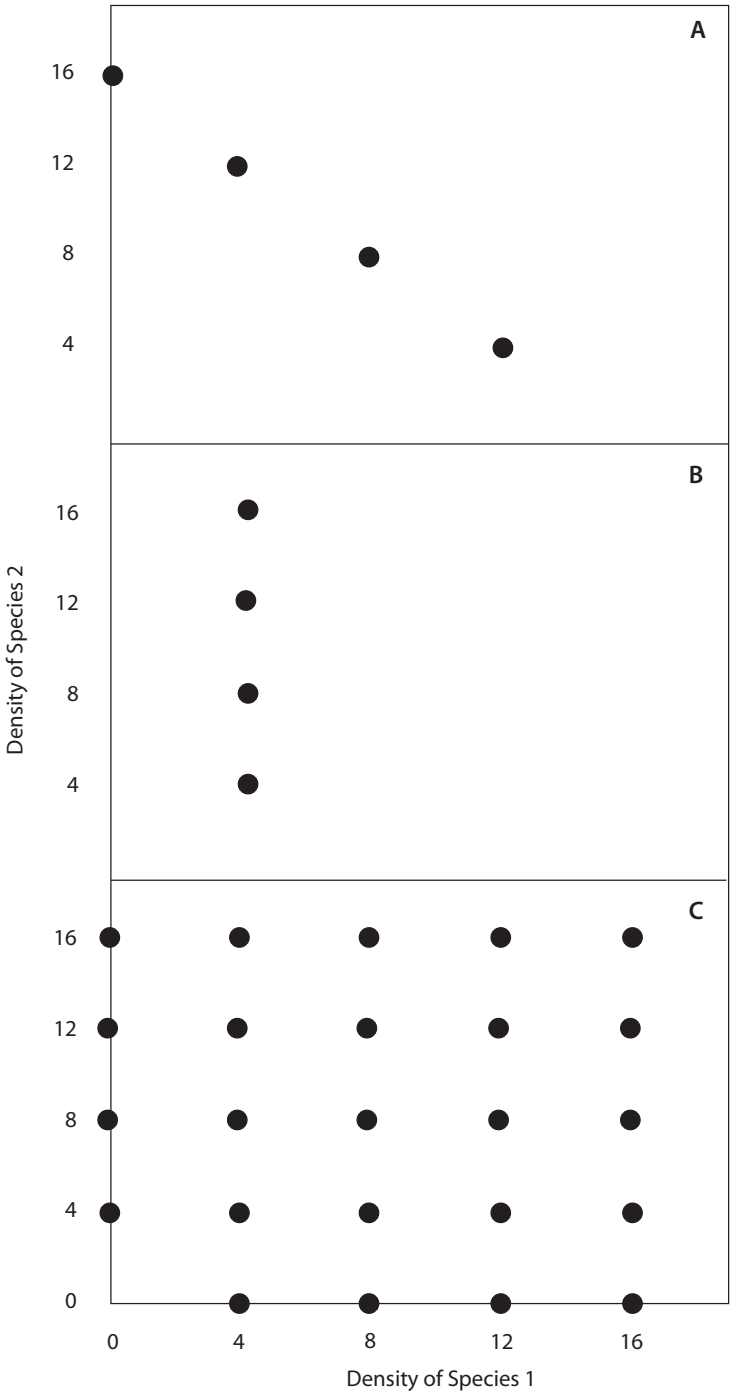

Figure 11.1 Three potential designs for competition experiments in which the impact of species 1 on the diet composition of species 2 is assessed. These experiments are **(A)** substitutive, **(B)** density gradient, and **(C)** response surface. Each point represents a single experimental treatment (adapted from Goldberg and Scheiner 2001).

choice. Size-dependent selection by larval fish for zooplankton prey in reservoirs (Bremigan and Stein 1997), selection of spot by piscivorous southern flounder in marine estuaries (Wright et al. 1993), and foraging preference for snails by pumpkinseed sunfish in natural lakes (Mittelbach et al. 1999) are examples of carefully designed experiments that provided insight into field dietary patterns. The outcome of foraging experiments such as these can be affected by many conditions. Using the same prey or predators across experimental trials may influence learning, which may cause foraging patterns to change through time (Reiriz et al. 1998). Using naïve consumers and prey in each replicate will remedy this confounding problem. Interactions among prey items within experimental units may cause different patterns of vulnerability relative to prey being exposed to consumers independently (Huang and Sih 1991). Similarly, changes in prey frequency as individuals are removed may influence their relative conspicuousness, thereby affecting selection patterns through time (Werner and Hall 1974). Hence, investigators may replenish prey throughout an experiment to keep densities as constant as feasible. Hunger levels of the consumer and prey and the size and realism of the experimental arena also may alter responses. In summary, foraging experiments can be insightful but also greatly misleading. Their design and interpretation must be carefully considered.

11.3 ANALYZING FOOD HABITS DATA

11.3.1 Selecting a Diet Index

A variety of measures have been used to quantify diet composition of fishes (Bowen 1996). Selecting an appropriate diet measure is strongly dependent on the research question; no single index is likely to provide a useful measure of prey importance under all conditions (Bowen 1996). For questions regarding the seasonal use of a prey resource, simple indices, such as frequency of occurrence, are usually adequate. Alternatively, we may want to quantify the energetic contribution of different prey types—a process that requires data on the abundance, weight, and caloric content of prey.

Traditional indices used for stomach content analysis include percent composition by number (N_i), percent composition by weight (W_i), and frequency of prey occurrence (O_i) (Bowen 1996; Table 11.1). It is important to recognize that each index emphasizes different information about the diet of fishes (Hyslop 1980; Cortés 1997). When evaluating percent composition by number, small prey can represent a dominant component of the diet. In contrast, percent composition by weight tends to emphasize the relative contribution of larger prey. Frequency of occurrence can provide information on how often a particular prey item was eaten but provides no indication of the relative importance of prey to the overall diet.

When calculated from the entire sample, N_i and W_i represent single measures with no corresponding variance estimate. If interest lies in evaluating the potential impact of predators on prey populations, then calculating N_i and W_i for the entire sample is appropriate. However, if diet data are to be used for statistical

Table 11.1 Equations for calculating diet indices (adapted from Pope et al. 2001). Symbols in equations are food or prey item (subscript *i*); fish (subscript *j*); number of fish (*J*); number of fish with food in their stomachs (*P*); number of fish containing prey *i* (J_i); number in food category *i* (N_i); number of food types (*Q*); weight of prey type *i* (W_i); weight of fish *j* (F_j); volume (mL) of food category *i* (V_i);. caloric density ($J \cdot g^{-1}$ wet weight) of food type *i* (X_i); and stomach capacity (mL) of fish *j* (C_j).

Diet index	Index symbol	Computational equation
Frequency of occurrence	O_i	$= \frac{J_i}{P}$
Proportion by number	N_i	$= \frac{N_i}{\sum_{i=1}^{Q} N_i}$
Proportion by weight	W_i	$= \frac{W_i}{\sum_{i=1}^{Q} W_i}$
Mean proportion by number	MN_i	$= \frac{1}{P}\sum_{j=1}^{P}\left(\frac{N_{ij}}{\sum_{i=1}^{Q} N_{ij}}\right)$
Mean proportion by weight	MW_i	$= \frac{1}{P}\sum_{j=1}^{P}\left(\frac{W_{ij}}{\sum_{i=1}^{Q} W_{ij}}\right)$
Mean proportion body weight	MBW_i	$= \frac{1}{P}\sum_{j=1}^{P}\left(\frac{W_{ij}}{F_j}\right)$
Mean stomach fullness	MSF_i	$= \frac{1}{P}\sum_{j=1}^{P}\left(\frac{V_{ij}}{C_j}\right)$
Prey importance index	PII_i	$= \frac{1}{P}\sum_{j=1}^{P}\left(\frac{W_{ij}X_i}{\sum_{i=1}^{Q} W_{ij}X_i}\right)$
Index of relative importance	IRI_i	$(\%N_i + \%W_i)(\%O_i)$
Relative importance index	RI_i	$\frac{100\mathrm{AI}_i}{\sum \mathrm{AI}_i}$, where $\mathrm{AI}_i = O_i + N_i + W_i$

comparisons then N_i and W_i should be calculated for individual fish and then averaged for each prey type (see MN_i and MW_i in Table 11.1). In this way, we treat individual fish as the sampling unit and assume that they represent a random sample (Table 11.2). Diet items in the stomachs of individual fish are not independent and generally should be measured to provide proportional data for individual fish (Hurlbert 1984; Krebs 1989).

When one is evaluating diet composition, prey weights are often more useful than are prey counts because weights are measured in comparable units. Consider

Table 11.2 Summary of prey weights for 10 bluegills. All weights are given in grams, and values in parentheses represent prey proportions for each fish. Mean proportion by weight (MW_i) and frequency of occurrence (O_i) are given in the last two rows. Note that dipteran larvae had the highest frequency of occurrence but contributed the least to the overall diet by weight—illustrating some of the problems associated with interpreting different diet measures.

		Prey weight and proportion				Total
Bluegill	Fish weight	Amphipods	Larval fish	Dipteran larvae	Mayfly nymphs	prey weight
A	150	0.3 (0.52)	0.24 (0.42)	0.02 (0.03)	0.016 (0.03)	0.576
B	91	0.09 (0.78)	0 (0)	0.018 (0.16)	0.008 (0.07)	0.116
C	99	0.11 (0.66)	0 (0)	0.024 (0.14)	0.032 (0.19)	0.166
D	123	0.03 (0.25)	0 (0)	0.052 (0.43)	0.04 (0.33)	0.122
E	210	0 (0)	0.12 (0.83)	0.001 (0.01)	0.024 (0.17)	0.145
F	102	0.22 (0.92)	0 (0)	0.003 (0.01)	0.016 (0.07)	0.239
G	124	0 (0)	0 (0)	0.006 (0.10)	0.056 (0.90)	0.062
H	199	0.015 (0.09)	0.12 (0.71)	0.003 (0.02)	0.032 (0.19)	0.170
I	101	0.45 (0.91)	0 (0)	0.015 (0.03)	0.032 (0.06)	0.497
J	111	0.26 (0.39)	0.36 (0.53)	0.054 (0.08)	0 (0)	0.674
MW_i		45%	25%	10%	20%	
O_i		80%	40%	100%	90%	

the difficulty in determining the relative importance of 1,500 zooplankton versus 1 fish. When measured as dry weight, we can directly compare 0.06 g of zooplankton to 0.2 g of fish in the diet. For this reason, prey weights are more appropriate when interest lies in comparing the energetic importance of different prey types (Bowen 1996). To correct for effects of fish size, it is often useful to express prey weight as a percentage of predator body mass.

Other indices used for diet analysis include mean stomach fullness and the prey importance index (Table 11.1). Early methods for measuring stomach fullness in fishes included subjective techniques such as the points method whereby food items were awarded points proportional to their estimated contribution to stomach volume (Swynnerton and Worthington 1940; Hynes 1950). Although easy to apply, these techniques have been criticized for their subjectivity (Windell and Bowen 1978). A more objective approach is to calculate the ratio of observed prey volume to estimated stomach capacity (Kimball and Helm 1971; Knight and Margraf 1982). Here, total volume of prey in each stomach is estimated either directly by water displacement or indirectly by means of geometric measurements. Maximum total prey volume is then regressed against fish size to estimate maximum stomach volume as

$$V = aL^b, \tag{11.1}$$

where V = maximum stomach capacity, a = regression coefficient, L = total length, and b = instantaneous rate of change (Knight and Margraf 1982). The ratio of observed prey volume (v) to maximum stomach volume (V) provides an index of

stomach fullness that accounts for fish length. The mean stomach fullness index (MSF_i) has several desirable advantages including it (1) eliminates subjectivity associated with the points method, (2) is relatively quick and easy to apply, (3) can be obtained from preserved or live fish, and (4) can be analyzed by a variety of statistical procedures (Knight and Margraf 1982). Furthermore, the MSF_i correlates well with prey caloric contribution, providing a robust index for evaluating the energetic contribution of different prey types (Pope et al. 2001).

The prey importance index (PII_i) combines information on the abundance, weight, and caloric content of prey (Table 11.1). Given sufficient information on prey assimilation efficiencies, the caloric densities of prey can be adjusted to account for energy actually metabolized by fishes (Probst et al. 1984). In most cases, this type of information is not readily available, so that total energy of prey is used. The usefulness of a caloric-based index such as the PII_i is that it provides a quantitative measure of the nutritional benefit of individual prey rather than relative importance based on numbers, weight, or occurrence in the diet (i.e., N_i, W_i, or O_i).

Diet measures each provide unique information about relative prey importance. In an attempt to consolidate the desirable properties of individual diet measures (e.g., N_i, W_i, and O_i), compound indices were developed that combine two or more diet measures into a single index (Table 11.1). The belief is that compound indices capture more information than do single, component measures. Several authors, however, argue that compound indices, such as the index of relative importance (IRI_i) and the relative importance index (RI_i), provide little or no additional information than that provided by single indices (MacDonald and Green 1983; Hansson 1998). Proponents of compound indices, on the other hand, have argued that (1) compound indices provide a more balanced view of fish diets because they capture all of the unique properties affecting individual measures (e.g., N_i, W_i, or O_i), and (2) there is a need for a standardized method for reporting relative prey importance (Cortés 1997). This argument has been criticized on the basis that the addition or multiplication of percentages has no biological meaning because both quantities are dimensionless ratios (Bowen 1996).

The usefulness of compound indices is constrained by several limitations. Comparisons with single measures indicate that compound indices can be a redundant source of information (MacDonald and Green 1983). A more significant problem, however, is that compound indices can be affected by the taxonomic resolution of prey items (Hansson 1998). At different taxonomic resolutions, the importance of different prey types can change, rendering the IRI_i a poor choice for a standard index in diet analyses (Hansson 1998).

The search for an index that best describes relative prey importance has led to much controversy over which diet index is best (Hyslop 1980; Cortés 1997; Hansson 1998). No doubt much of this confusion stems from the fact that relative prey importance is context specific and can be defined in a variety of ways. If we intend to evaluate energy flow, prey composition by weight (or volume) would be a better choice than composition by number. On the other hand, prey numbers could be used to assess prey preference if corresponding information on in situ prey

abundance was available (Hansson 1998). A general framework for selecting diet measures is given in Figure 11.2.

11.3.2 Presenting Data with Graphical Techniques

Diet measures such as N_i, W_i, and O_i are usually presented in tabulated format, making it difficult to interpret two or more indices simultaneously. Graphical techniques attempt to overcome this problem by combining two or more diet measures in two-dimensional space (i.e., bivariate plots). By examining relationships between different diet measures, graphical techniques can be used to interpret (1) predator feeding strategies, (2) relative prey importance, and (3) diet variability.

A graphical technique that relates prey abundance (N_i or W_i) to frequency of occurrence (O_i) was developed by Costello (1990) and later modified by Amundsen et al. (1996). In the Amundsen method, prey-specific abundance is plotted against frequency of occurrence, where prey-specific abundance is defined as the proportion a prey item constitutes of all prey items in only predators that contain prey i (Amundsen et al. 1996). The equation used to calculate prey-specific abundance (P_i) is

$$P_i = (\Sigma S_i / \Sigma S_{ti})100, \tag{11.2}$$

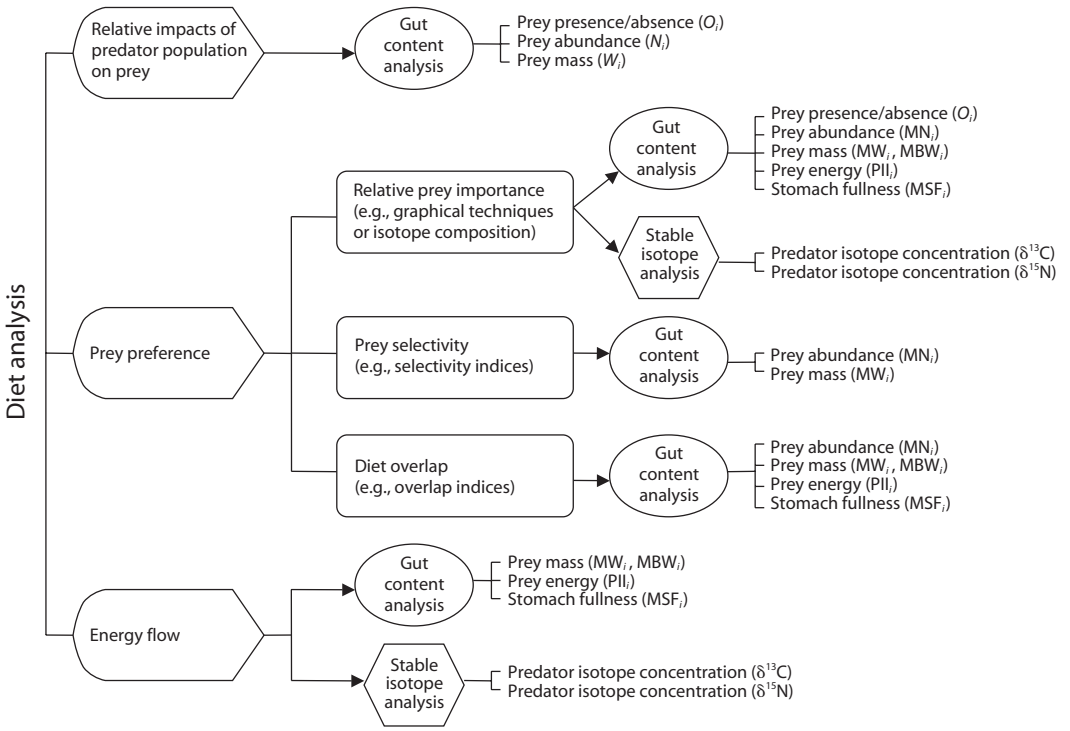

Figure 11.2 Diet measures commonly used to address questions about predator impacts, prey preference, or energy flow. See Table 11.1 for calculation of different diet measures.

where P_i equals prey-specific abundance (numbers, mass, or volume) of prey i, S_i equals the abundance of prey i in stomachs, and S_{ti} equals the total abundance of prey in predators that contain prey i. As an example, consider three fish, respectively with 1, 2, and 3 g of prey i and 8, 7, and 5 g of total stomach contents. We calculate P_i as follows:

$$P_i = \frac{1+2+3}{8+7+5}(100) = \frac{6}{20}(100) = 0.3(100) = 30\%.$$

When plotted against frequency of occurrence, prey-specific abundance can be used to evaluate three important aspects of the fish diet: (1) feeding strategy (specialized versus general), (2) prey importance (dominant versus rare), and (3) niche width (Figure 11.3). In practice, four interpretations can be made by relating prey-specific abundance to frequency of occurrence that could otherwise not be determined from single diet measures (Box 11.2).

Because prey-specific abundance and frequency of occurrence are calculated for the entire sample of fish, graphical techniques that use these indices represent analysis at the population level. To assess feeding patterns at the individual level, graphical methods have been developed that incorporate the use of prey diversity and number of prey in individual stomachs (Bridcut and Giller 1995). In this approach, individual prey diversity for each fish is calculated using a diversity index and then plotted against the total number of prey in the stomach. A generalist feeding strategy is characterized by high prey diversity and low abundance of each prey type, whereas a specialist strategy is represented by low prey diversity and high utilization of a few prey types. Methods for defining high prey diversity, however, have not been developed. As a result, this technique involves subjective interpretation but can be useful for examining patterns of diet specialization across time or space (Bridcut and Giller 1995).

11.3.3 Exploring Variation in Prey Size

Often investigators are interested in the relationship between prey size and predator size, particularly as it relates to gape limitation in fishes. Hence, the maximum linear dimensions of each diet item are plotted against predator length (Juanes 1994). Resulting distributions are often wedge-shaped because small fish are generally limited to small prey, whereas large fish can incorporate a variety of prey sizes in their diet (see example in Box 11.3). In many cases, identifying maximum and minimum prey sizes, rather than the average size, is desirable. Although the maximum and minimum edges of these bivariate scatter plots can be described using least squares regression (LSR), the choice of what edge data to include in the analysis is arbitrary. In addition, LSR is sensitive to the effects of outliers in the chosen edge distribution. A promising method involves the use of a quantile regression technique called least absolute values regression (Scharf et al. 1998; Cade and Noon 2003), in which the sum of the absolute values of the residuals are minimized (rather than the sum of squares of residuals as in conventional LSR).

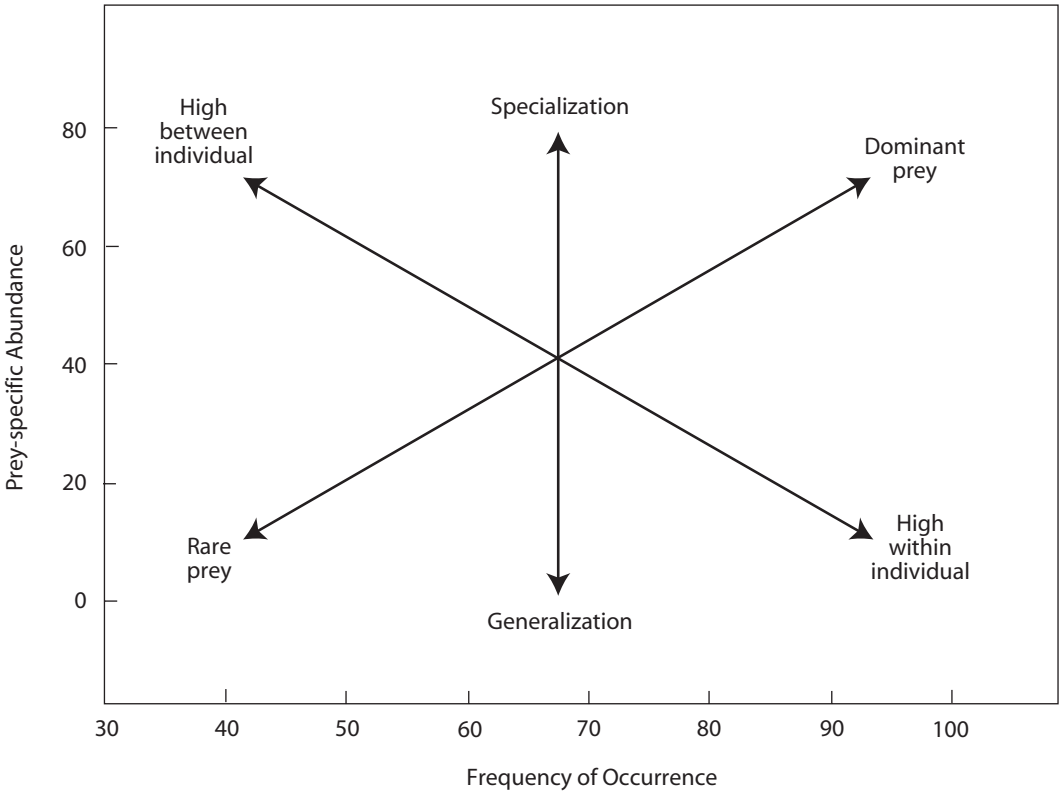

Figure 11.3 Graphical model that depicts feeding strategy (specialized or generalized), relative prey importance (dominant or rare), and niche variation (individual versus population patterns) based on the distribution of individual prey types. Prey-specific abundance is calculated from only those predators that contain prey *i* and is plotted against frequency of occurrence for each prey (O_i). Prey points located in the upper left of the plot indicate prey that are consumed by few individuals displaying specialization; points located in the lower right reveal prey items that have been eaten occasionally by most individuals (Amundsen et al. 1996). Figure adapted from Amundsen et al. (1996) as first described by Costello (1990).

Estimates are obtained through minimization of

$$\sum_i |y_i - \beta_0 - \beta_1 X_i| h_i, \tag{11.3}$$

where h_i is a multiplier equal to a chosen quantile value (e.g., 0.5 for the median) if the residual within the absolute value symbols is positive or one minus the quantile value if the residual is negative (Scharf et al. 1998, Cade and Noon 2003). This technique was quite robust in identifying upper and lower bound slopes in scatter diagrams (Scharf et al. 1998; Cade and Noon 2003) and is very useful for characterizing prey size–predator length relationships (Box 11.3).

Box 11.2 Presenting Diet Measures Graphically

By combining different diet measures in two-dimensional space, graphical techniques can relay important information about feeding behavior of fishes. Using Figure 11.3, we can interpret feeding strategies of each predator population in the graphs presented below.

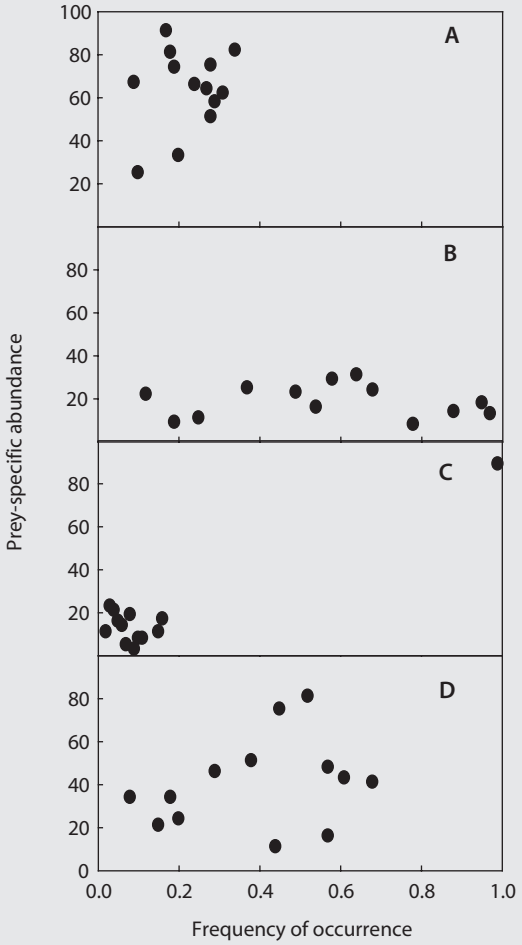

Figure Graphs showing abundance of 13 prey types for four predator populations—A, B, C, and D. Each point represents a different prey type and is expressed as prey-specific abundance plotted against frequency of occurrence (adapted from Admundsen et al. 1996).

We see that fish from population A specialize on individual prey types. As a result, these fish show a high degree of between-individual variation in diet breadth. In population B, predators have a more generalized diet and higher within-individual variation in diet breadth. In population C, the predator population is specializing on a single prey type while occasionally consuming other prey. Finally, population D represents a mixed feeding strategy in which some individuals have a specialized diet and other fish have a more generalized feeding strategy. Graphical techniques, such as the one illustrated here, provide insight about fish feeding patterns that might not be inferred from single diet indices.

Box 11.3 Determining the Minimum and Maximum Sizes of Prey

The maximum size of prey in fish diets often increases with body size. However, the minimum size of prey may change relatively little. In addition to determining the mean or median size of prey consumed by use of bivariate plots, investigators may want to characterize the maximum and minimum sizes consumed (i.e, the edges of the scattergrams). Least absolute values regression (LAV), also called least absolute deviations regression, can be used to evaluate these types of diet data (Scharf et al. 1998). An extension of LAV, quantile regression, fits any specified quantile as a linear regression model. The LAV is the 50th percentile (median) in quantile regression. Such an analysis is available in the Blossom Statistical Software Package (Cade and Richards 2000; available at http://www.mesc.usgs.gov). This program generates test statistics by permutations of the original data through re-randomization.

For example, we want to characterize the upper and lower bounds of lengths of fish prey in age-0 largemouth bass diets from Tappan Lake, Ohio, during 1994 through 1996 (data from Garvey et al. 1998b).

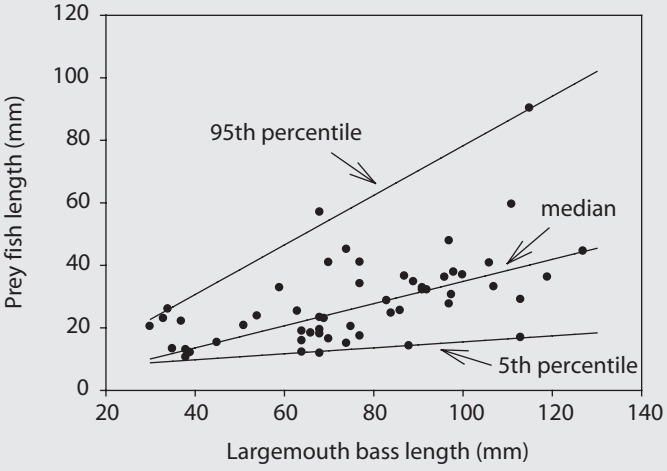

Figure Prey length versus age-0 largemouth bass length (from Garvey et al. 1998b).

Using quantile regression, we first determine the median regression model that minimizes the least absolute differences between the observed values and the residuals. We then determine the quantile regression models that fit the 5th and 95th percentiles of the data. The form of each linear regression model is $y = \beta_0 + \beta_1 x$. The test statistic generated for the LAV regression (i.e., quantile = 0.5) is equivalent to that of a typical least-squares regression comparing the proportional reduction in deviations when passing from a reduced to a full model. Because quantile regression involves weighted absolute deviations (see equation [11.3]), we cannot assume identical error distributions across the independent variables. As such, Cade and Richards (2000) recommend using a rank-sums test for quantile regression (i.e., quantile $\neq$ 0.5), in which the statistic is based on the sign of the residual from the reduced parameter null model.

(Box continues)

Box 11.3 *(continued)*

Table Model values for the full LAV (median), 95th percentile, and 5th percentile regressions. The absolute values of the residuals for the 95th and 5th quantile regression are weighted, and the *P*-value is based on a rank-sums test.

Quantile	β_0	β_1	Sum of absolute values of residuals	*P*
0.5	–0.5748	0.3541	433.07	0.0002
0.95	–1.0507	0.7930	112.92	0.077
0.05	5.9543	0.0957	41.73	0.055

The full LAV regression model (quantile = 0.50) was significantly different than the reduced model. The quantile regression models describing the 95th and 5th percentiles had slopes greater than 0, and only 7.7% and 5.5% of the corresponding test statistics generated by the permutation procedure had more significant values. We conclude that this technique effectively characterizes the median as well as the upper and lower bounds of prey sizes consumed by age-0 largemouth bass in Tappan Lake.

11.3.4 Evaluating Nonindependence of Diet Data

The compositional nature of diet measures (i.e., proportions) has important implications for data analysis. Interpretations about the relatedness of prey items or sites can be very different when using compositions relative to unstandardized (e.g., raw) data (Jackson 1997). In practice, arcsine transformations are often applied to compositional data prior to analysis in attempts to normalize the data. Such transformations should not be applied arbitrarily; rather, data should be examined (for normality) to verify that transformations are needed. Traditional statistical techniques (e.g., *t*-test and analysis of variance [ANOVA]) can be applied when assumptions of normality are met or large sample sizes are obtained. In cases in which compositional data are not normally distributed, nonparametric rank procedures can be useful for detecting differences in individual prey proportions.

Fisheries scientists must be aware of the nonindependence trait of diet data. A well-considered experimental design will avoid the pitfall of pseudoreplication (Hurlbert 1984). Diets from individual fish often contain multiple items that cannot be treated independently. In addition, fish diets are usually sampled either repeatedly through time or at the same location. A variety of statistical techniques can be used that account for autocorrelation within diet data. When designing a study, fisheries scientists should determine if the assumptions of these tests are met.

Temporal and spatial variation in diet data may be analyzed using conventional parametric statistical techniques such as ANOVA if stomachs of individual fish are collected from independent experimental units. For example, temporal dietary patterns in an experiment may be analyzed using ANOVA if they derive from

independent mesocosms or aquaria sampled only once during an experiment. In this case, each stomach would represent a one date–replicate combination. Obviously, meeting this assumption may require a large number of replicates in an experiment because variation among individuals within treatments will likely be high.

In field studies, stomach contents are often collected from groups of fish at the same location during multiple sampling trips. Diet data also may be collected from the same live fish multiple times during an experiment. In these cases, diet data are not independent. For fish captured at the same location, time, or both, stomach samples will likely be more similar than those collected at other times and locations. These potentially confounding problems of spatial or temporal autocorrelation may be addressed statistically using several techniques including repeated-measures ANOVA.

Repeated-measures designs use the same subject (e.g., site or fish) for each of the treatments in a study (Neter et al. 1990). The subject is considered a block, and the treatment(s) are applied to each subject in random order. In a randomized-complete-block repeated-measures ANOVA, each subject receives all of the treatment combinations. If the subject is being followed through time, then time is the repeated measure within each subject (i.e., the within-subject effect; Box 11.4). It is assumed that the variance within each subject (i.e., individual) will be less than that among subjects (Neter et al. 1990). The randomized-complete-block approach is often difficult to employ because it may be impossible to apply all treatments to all subjects. A split-plot ANOVA is a special case of the repeated-measures design that allows subjects to be included in only some of the treatments (see Maceina et al. 1994). To illustrate, let us explore temporal variation in fish diets both within days and among weeks. If we consider each fish to be a fixed subject, then biomass consumed by each may be quantified during morning for half of the fish, while the remaining half is sampled during afternoon. All diets are quantified on a weekly basis. In this case, variation in biomass consumed must be partitioned due to (1) individual fish, (2) time of day, (3) week, and (4) interactions among fish, time of day, and week. Fish with similar characteristics are blocked in pairs, and each is randomly assigned a morning or afternoon sampling time (Table 11.3). Samples are then taken for several weeks. We perform an ANOVA exploring the effects of block, time of day, and their interaction, called the main-plot effects. We also determine the subplot effects of week and the week × time of day interaction. The allure of this approach is that individual fish can be followed through time, and not all fish need to be handled twice each sampling date.

Randomized-complete-block repeated-measures ANOVAs involve strict assumptions about the sphericity of the variance–covariance matrix of the within-subject factor (e.g., time). For the matrix to be spherical, the variance of the difference between any two levels of the within-subject factors must be constant. This property is tested by determining the sphericity of the variance–covariance matrix, such as with a Mauchly's test of sphericity (SAS Institute 1999). If assumptions of sphericity are not met, then the likelihood of rejecting the null hypothesis of no within-subject effect (e.g., time) is inflated, and an adjusted test must be used (Box 11.4).

Box 11.4 Analyzing Diet Data with Repeated-Measures Analysis of Variance (ANOVA)

Diet data often arise from multiple samples within the same system or from multiple observations of the same individual. A repeated-measures ANOVA approach is useful for teasing apart variation as a function of independent effects (e.g., between subject) and nonindependent effects (e.g., within subject).

In this hypothetical example, we conducted a 5-week experiment to determine the effect of gizzard shad on the mean percent by weight (MW_i) of zooplankton in diets of bluegill. Four bluegills were sampled from each replicate once weekly. The resulting data were MW_i for each replicate and date. Data were arcsine(x) transformed prior to analysis.

Table Zooplankton (MW_i) in diets of bluegill ($n = 4$ per replicate per sampling period) in treatments with and without gizzard shad. Data in the table are untransformed.

Treatment (gizzard shad) and replicate	Week				
	1	2	3	4	5
Absent					
1	0.6	0.57	0.59	0.68	0.67
2	0.36	0.37	0.4	0.41	0.49
3	0.43	0.39	0.48	0.49	0.48
4	0.55	0.54	0.6	0.58	0.52
Present					
1	0.72	0.45	0.4	0.32	0.29
2	0.65	0.4	0.38	0.27	0.1
3	0.53	0.46	0.38	0.29	0.23
4	0.45	0.4	0.29	0.23	0.25

Program

```
data one;
input treat $ rep week1-week5;
cards;
[input data];
proc glm;
class treat;
model week1-week5=treat;
repeated time/ printe;
run;
```

Other techniques are available for analyzing autocorrelation in temporal or spatial diet data. Long-term observations of diets may be analyzed using time-series techniques, which are particularly useful in unreplicated systems such as lakes or reservoirs. Autoregressive integrated moving average (ARIMA) models and related techniques may be used to identify nonrandom patterns through time (Rasmussen et al. 1993), assuming that observations are available in discrete, evenly spaced intervals. These models can be extended to compare the treatment response

Interpretation
The test for the gizzard shad effect (between subjects) was significant at $P = 0.057$.

Table The effect of gizzard shad on the MW_i of zooplankton in diets of bluegill.

Effect	*df*	Mean square	*F*-value	*P*
Treatment	1	0.222	5.51	0.0572
Error	6	0.040		

When testing for a time effect (within subjects), a Mauchly's test for sphericity was rejected ($P = 0.0021$), indicating that the variance–covariance matrix was not circular. This is typical for data that are sampled repeatedly through time. A test such as the Greenhouse–Geisser epsilon (G–G) must be used to adjust the error rate. These tests are automatically computed by the SAS procedure.

Table Test for time effect and time*treatment effect of gizzard shad on zooplankton in bluegill diet.

Effect	*df*	Mean square	*F*-value	G–G adjusted *P*
Time	4	0.0350	10.06	0.0040
Time*treatment	4	0.0746	21.42	0.0002
Error	24	0.0034		

We conclude from this analysis that MW_i changed in both treatments through time. The time*treatment effect indicates that the treatments changed in different ways through time, probably because zooplankton increased in diets in the absence of gizzard shad but declined in treatments with gizzard shad.

of a single, unreplicated experimental system to that of a reference system. A limitation of these moving average techniques is that they usually require large sample sizes (>50 dates; Rasmussen et al. 1993).

Autocorrelated spatial patterns in diet data can be analyzed in a variety of ways. The Mantel test is a randomization test that determines whether differences between two $n \times n$ distance matrices are random (Fortin and Gurevitch 1993). Spatial variation among individuals (distance matrix 1) can be compared to the

Table 11.3 Split-plot repeated-measures ANOVA design for biomass consumed by individual fish ($n = 6$) blocked into pairs and then randomly selected to be sampled in the morning or evening (effect A). Consumption of each fish was quantified once a week (effect B).

Block and fish	Time of day (effect A)	Week (effect B)		
		1	2	3
Block 1				
1	AM	$A_{AM}B_1$	$A_{AM}B_2$	$A_{AM}B_3$
2	PM	$A_{PM}B_1$	$A_{PM}B_2$	$A_{PM}B_3$
Block 2				
3	PM	$A_{PM}B_1$	$A_{PM}B_2$	$A_{PM}B_3$
4	AM	$A_{AM}B_1$	$A_{AM}B_2$	$A_{AM}B_3$
Block 3				
5	AM	$A_{AM}B_1$	$A_{AM}B_2$	$A_{AM}B_3$
6	PM	$A_{PM}B_1$	$A_{PM}B_2$	$A_{PM}B_3$

relative proportion of a specified diet item in the stomachs (distance matrix 2). The Mantel test will determine if nonrandom spatial patterns in diet composition exist. Alternatively, two or more bivariate plots of spatial distributions of prey occurrence in diets of individual fish may be compared using a multiway, two-dimensional Kolmogorov–Smirnov test (2DKS; Garvey et al. 1998c). The 2DKS test can also be used to determine if spatial distributions within single plots differ significantly from randomly generated ones. An example of using 2DKS to assess spatial variation in predator diets is given in Box 11.5.

Diel changes in diet have important implications for choosing sampling times or understanding gastric evacuation patterns for fishes (see section 11.2.2.1). Using analysis of covariance (ANCOVA), the content of fish stomachs (e.g., weight) can be regressed against fish weight (the covariate) using conventional least-squares regression during each sampling time. If these relationships can be transformed such that they are linear, the slopes of each line can be compared (Box 11.6). If slopes are the same (i.e., parallel), then the intercepts among the regression lines can be compared. Significant among-intercept differences indicate a diel pattern in the abundance of prey in stomach contents.

11.3.5 Comparing Diet Composition

11.3.5.1 Employing Multivariate Analysis of Variance with Randomization

The multivariate nature of fish diets often requires approaches other than univariate statistics when interest lies in the simultaneous evaluation of all prey categories. By example, consider the decisions we make when buying fishing gear. Before making a purchase, we (e.g., anglers) often consider at least four factors: (1) price, (2) quality, (3) brand name, and (4) style. One angler may rate their decision on (1) style, (2) quality, (3) brand name, and (4) price, whereas another angler may consider the purchase based on (1) price, (2) quality, (3) style, and (4) brand name. Here, we are interested in asking whether anglers use the same

Box 11.5 Assessing Spatial Patterns in Diet with the Two-Dimensional Kolmogorov–Smirnov Test

Several statistical methods are available to relate diet patterns to the distribution of habitat in aquatic systems. Mantel and partial-Mantel tests are powerful techniques that test whether spatial patterns are random or due to some treatment (or time). These tests are not specifically discussed here. More information can be obtained in Fortin and Gurevitch (1993) and Chapter 18. If spatial data can be arranged into bivariate spatial coordinates, a two-dimensional Kolmogorov–Smirnov (2DKS) test can be used to (1) identify whether a single distribution has arisen by random effects or (2) compare two bivariate distributions (see Garvey et al. 1998c for a review). This nonparametric test finds the maximum difference, D_{bks} (where bks represents bivariate Kolmogorov–Smirnov), in integrated probabilities for four quadrants around each point in a plane. If the maximum D_{bks} between two distributions exceeds that expected randomly, we conclude that they differ. The significance of the test statistic D_{bks} is determined by rerandomizing the original data 5,000 times and then comparing this randomly generated distribution to the observed value.

In the following hypothetical example, we want to know how vegetation in a large lake affects piscivory in age-0 smallmouth bass. We partition the bottom of a shallow lake into 80 habitat quadrants (20 × 4) and determine whether each contains vegetation. Within each quadrant, we sample smallmouth bass diets by means of gastric lavage and note whether piscivory is present or absent.

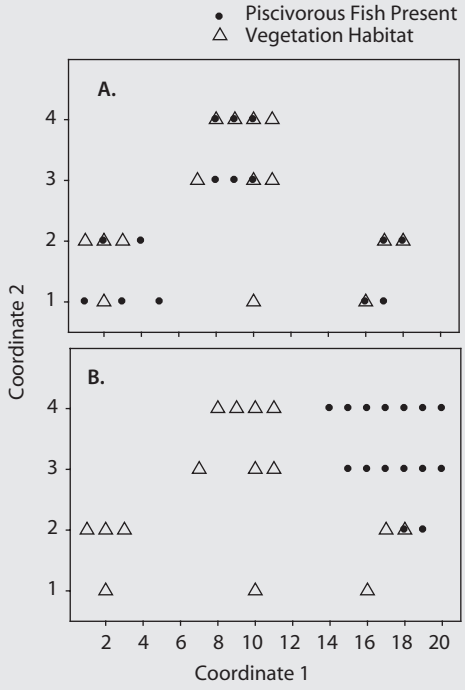

Figure Two hypothetical scenarios of smallmouth bass in a shallow lake. The bottom of the lake is partitioned into 80 habitat quadrants (20 × 4), and it is determined whether each contains vegetation. Within each quadrant, smallmouth bass diets are sampled to determine whether piscivory is present or absent.

(Box continues)

Box 11.5 *(continued)*

In the scenario depicted in the upper panel **(A),** piscivorous smallmouth bass (closed circles) appeared to be closely associated with quadrants containing vegetation (open triangle). In confirmation, a 2DKS test comparing the bivariate distributions of vegetation and piscivorous smallmouth bass revealed no difference ($D_{bks} = 0.143$, P = 0.999). In the scenario depicted in the lower panel **(B),** the distribution of vegetation is identical to that in A. However, the spatial distribution of piscivorous smallmouth bass appears to be associated with some other factor. The 2DKS test detected a difference between the spatial distributions of vegetation and piscivorous fish ($D_{bks} = 0.714$, P = 0.002).

Of course, the 2DKS test is useful for determining only presence and absence in this example. We also must assume strong site-fidelity of fish within habitats and that fish are homogeneously distributed among vegetated and nonvegetated sites. Cells with missing data are acceptable. It is important to note that Mantel tests incorporate quantities within each cell, allowing us to compare other responses such as the frequency of occurrence of fish in diets.

decision factors before purchasing fishing gear. To address this issue, we treat the purchase as a multivariate response by evaluating these decisions simultaneously. In the same way, we can treat the diet of fish as a multivariate response defined by the abundance of different prey items in the stomach.

There are a variety of approaches for analyzing multivariate diet data. For the convenience of modeling and performing statistical tests, techniques such as multivariate analysis of variance (MANOVA) require the assumption of multivariate normality (Khattree and Naik 1999). Other multivariate methods, such as cluster analysis and ordination techniques, are largely distribution free in nature and are useful for generating biologically meaningful patterns from multivariate data.

When diets are expressed as prey weight or volume, MANOVA can be useful for testing differences in diet composition. The assumptions of MANOVA require that prey proportions have a multivariate normal distribution and a similar variance–covariance structure among samples. Prior to performing MANOVA, tests for multivariate normality should be applied to data to evaluate this assumption (Khattree and Naik 1999). When diet composition data do not meet the assumption of multivariate normality (as is often the case), a nonparametric-based randomization procedure can be applied to test for differences in diet composition between samples (Crow 1979; Somerton 1991). In this approach, MANOVA is combined with a randomization procedure. Randomization procedures are not new to ecological analysis but have received little attention in the analysis of fish diets (but see Somerton 1991). Randomization procedures are relatively straightforward and proceed as follows.

1. Combine diet proportion data from time or area samples.
2. Randomly sort data into n new samples equal in size to the original data.
3. Calculate a test statistic based on the new samples.
4. Repeat steps 2 and 3 a large number of times (e.g., 5,000).

Box 11.6 Determining Diel Patterns in Diet Data with Analyis of Covariance (ANCOVA)

We often want to determine if diel patterns in diet data occur. This has important implications for designing sampling protocols and interpreting diet data. One way to determine whether diel variation in feeding occurs is by sampling fish during different times of the day.

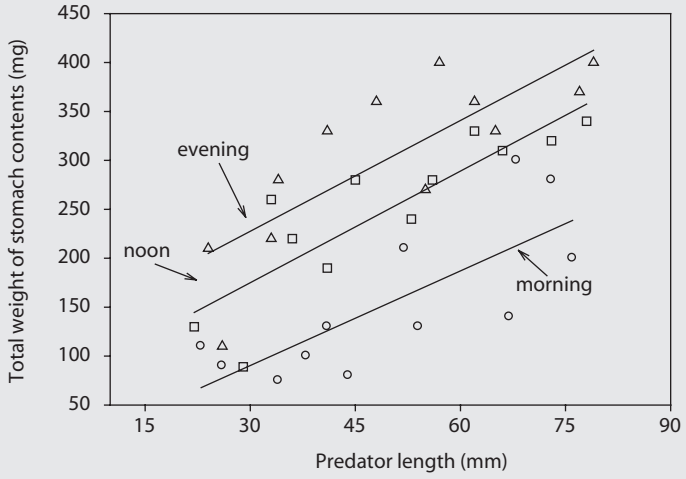

Figure The total weight (mg) of food found in the diets of different size fish are given for three time periods—morning, noon, and evening.

Program

An ANCOVA was used to test the null hypothesis that the three regression lines are equal. For an ANCOVA to be valid, the slopes of the regression lines must be parallel ($slope_{dawn} = 0.0322$; $slope_{noon} = 0.0380$; and $slope_{dusk} = 0.0377$). If this assumption holds, then we can use the general linear model (GLM) procedure in SAS in which length is the continuous covariate and time is the categorical variable. The assumption of parallel slopes is rejected if a length*time interaction is detected.

```
data one;
input time $ length diet;
cards;
[input data];
proc glm;
class time;
model diet = time length;
run;
```

Interpretation

Table Effect of time of day and length of fish on weight of items in fish stomachs.

Effect	*df*	Mean square	*F*-value	*P*
Time	2	6.770	28.65	0.0001
Length	1	14.431	61.06	0.0001
Error	32	0.236		

No length × time interaction occurred in the initial model. We then assumed that slopes were parallel and dropped the interaction from the model. The ANCOVA revealed that total weight of items increased in guts with increasing body size. In addition, the amount of food varied with time of day, suggesting that sampling time be carefully considered when developing a protocol.

From these data, a probability distribution of the randomized test statistic is generated. If the observed test statistic is within the upper (or lower) 5% tail of the randomized distribution, then the result is significant at the 5% level. Similarly, if our observed value falls within the 1% tail, then we can conclude that the difference is significant at the 1% level and so on. The choice of a test statistic depends on the research question being addressed and the characteristics of the test statistic. In a two-sample case, we could perform a randomization procedure on the Hotelling's T^2-statistic and test for a difference between sample means. Similarly, the *F*-statistic could be used to test for a treatment effect among three or more factors (e.g., lakes, seasons, and sites). An example of a nonparametric MANOVA that tests for diet differences is given in Box 11.7.

11.3.5.2 Examining Prey Numbers with Log-Linear Contingency Tables

When diet data are expressed as prey numbers, a multiway contingency table analysis can be used to assess diet variation (Cortés 1997). In this approach, data are arranged in an $R \times C$ contingency table, where R is the number of prey categories and C is the number of predator categories. Each cell in the table contains the total number of the ith prey category found in the stomachs of the jth predator category. One limitation of contingency table analysis is that large samples sizes are needed so that less than 20% of the cells have an expected frequency less than five. One way to remedy this situation is to pool prey species, so that we reduce the total number of categories and increase the sample size for the remaining categories (see section 11.2.1; Crow 1979).

Contingency table analysis begins by testing for significant interactions. In this way, we are testing a hierarchy of models starting with the most complex. In a three-way contingency table, we would start by examining the three-way interaction. If this term were not significant, we would delete it from the model and then proceed to test all the two-way interactions. The advantage of this approach is that by proceeding with posthoc tests, we can readily identify the rows (prey types) and columns (predators) that contribute the most to diet variation (Cortés 1997). An example of a three-way contingency table is given in Box 11.8.

11.3.5.3 Applying Ordination Techniques

Ordination techniques, such as principal components analysis (PCA), are widely used in ecological data analysis. Because diet data are often measured as proportions, analytical techniques are affected by the constant-sum constraint (i.e., as the abundance of one taxa increases, one or more taxa must decrease; Jackson 1997). To deal with compositional data, two alternative ordination methods have been proposed. The first approach is a log-ratio analysis performed on the logarithms of the percentages; this approach is most appropriate when compositional data do not contain zeros (Aitchison 1983). Although not new to the ecological literature, log-linear PCA techniques have only recently been applied to fish diet data (De Crespin De Billy et al. 2000). Termed %PCA, this technique is based on a PCA performed on a proportion table in which each column is defined by a prey

Box 11.7 Comparing Diet Data from Different Locations or Times with Multivariate Analysis of Variance (MANOVA)

Because of the multivariate nature of diet data, we are often interested in determining whether diet composition differs among fishes sampled from different locations or at different times. When diet data are measured as prey mass (or volume), MANOVA can be useful for testing an overall location (or time) effect.

Table Hypothetical diet data for three bluegill populations. Data are presented as mean percent composition by weight (MW_i) for four different prey items.

	Prey type			
Bluegill	Chironomids	Amphipods	Odonates	Copepods
		Lake A		
1	0.12	0.35	0.44	0.09
2	0.09	0.22	0.63	0.06
3	0.12	0.35	0.5	0.03
4	0.26	0.38	0.22	0.14
5	0.27	0.29	0.27	0.17
		Lake B		
6	0.49	0.01	0.38	0.12
7	0.36	0.04	0.59	0.01
8	0.34	0.05	0.57	0.04
9	0.42	0.03	0.24	0.31
10	0.57	0.11	0.21	0.11
		Lake C		
11	0.08	0.34	0.49	0.09
12	0.06	0.27	0.59	0.08
13	0.02	0.33	0.58	0.07
14	0.11	0.57	0.28	0.04
15	0.01	0.57	0.31	0.11

Program

Here, we are interested in testing for an overall lake effect in diet composition and perform a MANOVA analysis. The MANOVA procedure was used in the following SAS program to generate output.

```
data test;
input lake $ fish chiro amph odon zoo;
cards;
[input data];
proc glm;
class lake;
model chiro amph odon zoo=lake;
manova h=lake / printe printh;
title 'Manova of diet data';
run;
```

(Box continues)

Box 11.7 *(continued)*

Interpretation

Results from the MANOVA test for the hypothesis of no overall lake effect are presented below. Several statistics are produced from MANOVA analysis and provide similar results. For randomization procedures, we will consider the Wilk's lambda test statistic. From the output shown below, we would conclude that there is an overall lake effect on bluegill diets.

Table Test statistics comparing MANOVA results of diet composition for bluegills among three lakes.

Statistic	Value	*F*-value	*P*
Wilk's lambda	0.079	8.52	0.0001
Pillais trace	0.92	3.16	0.021
Hotelling–Lawley trace	11.58	17.38	0.0001
Roy's greatest root	11.58	42.46	0.0001

To determine which prey types vary among bluegill populations, individual ANOVAs are performed on each prey type. Because these tests are a posteri, an appropriate alpha level can be obtained using the Bonferroni inequality by which adjusted alpha levels are equal to the overall alpha divided by *n*. In this case, we would consider individual ANOVAs to be significant at 0.0125. In the table below, we see that chironomids and amphipods vary significantly among lakes.

Table Analysis (ANOVA) of which prey types vary among the three bluegill populations.

Prey item	Source of variation	*df*	Sum of squares	*F*-value	*P*
Chironomid	Lake	2	0.379	31.38	0.0001
Amphipod	Lake	2	0.363	20.98	0.0001
Odonate	Lake	2	0.007	0.13	0.87
Copepod	Lake	2	0.004	0.34	0.71

type and each row represents an individual fish. An advantage of this technique is that individual fish and their prey are analyzed simultaneously and can be displayed on the same graph (De Crespin De Billy et al. 2000). A second approach offered as a solution to the problem of compositional data is correspondence analysis (CA; Jackson 1997). This approach is particularly well suited to handle compositional data and provides advantages over other methods (e.g., PCA; Digby and Kempton 1987; Jackson 1997). Furthermore, unlike log-ratio analysis, CA is not constrained by the presence of zeros in the data, providing a robust approach for analyzing compositional data.

Although the mathematical derivation of these techniques is beyond the scope of this chapter, a user-friendly program for running these analyses is CANOCO

Once we obtain our observed test statistic (e.g., Wilk's lambda = 0.079), we then perform a randomization test (Crystal Ball 7.0, Decisioneering, Inc., Denver, Colorado; http://crystalball.com/crystal_ball/) to evaluate the significance of our statistic. After performing 4,999 simulations, we obtain the following probability distribution of the test statistic based on our data.

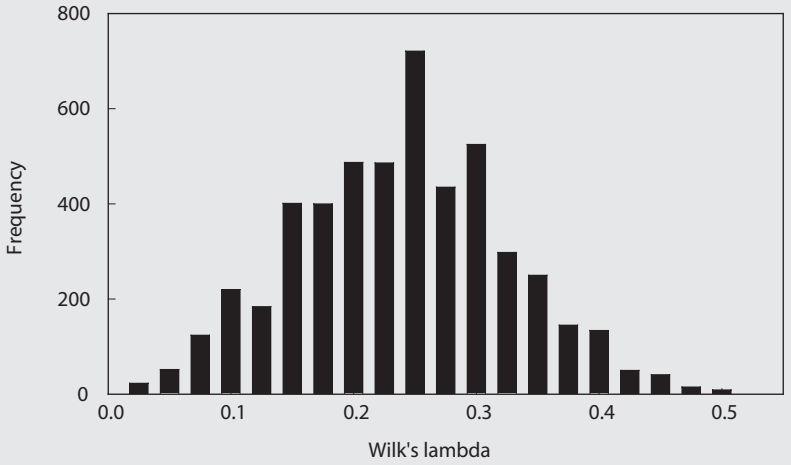

Figure Randomized frequency distribution for Wilk's lambda based on 4,999 simulations.

From the randomized frequency distribution, we see that our observed value (0.079) easily falls in the lower 5% of the observations. In fact, only 75 observations were less than our observed Wilk's value (0.079). We can estimate a *P*-value as 75/5,000 or 0.015. Hence, it is unlikely that we would obtain a value of 0.079 if the null model is true, and we would conclude that diets are significantly different among lakes. Similarly, randomization procedures could be performed on the individual ANOVAs (e.g., *F*-value) to confirm that chironomids and amphipods account for these differences.

for Windows (ter Braak and Smilauer 1998). To demonstrate the usefulness of log-ratio analysis (%PCA), we used diet data provided in Table 11.2 to assess individual variation in bluegill food habits (Box 11.9).

11.3.6 Estimating Diet Overlap

Niche overlap indices are often used to measure the magnitude of resource overlap among different species. Although these indices are sometimes used to infer competition, we should recognize that high resource overlap between two species may not indicate competitive bottlenecks. Rather, it may be indicative of high resource abundance, such as seasonal peaks in prey availability.

Box 11.8 Testing Prey Counts with Multiway Contingency Table Analysis

When diet data are measured as prey counts, multiway contingency table analysis can be used to test for treatment effects.

Table The following data represent numbers of prey for two different life stages of fish collected from two different environments. A three-way contingency table is used to test for differences among the three different levels: (1) life stage, (2) habitat, and (3) prey type.

	Prey type			
Habitat and life stage	Amphipods	Chironomids	Mayflies	Ostracods
Littoral				
Adult	29	69	9	10
Juvenile	19	43	4	6
Pelagic				
Adult	6	21	6	4
Juvenile	1	8	5	4

Program
The following SAS program was used to generate output.

```
data test;
input prey $ stage $ habitat $ number;
cards;
[input data];
proc catmod;
weight number;
model prey*stage*habitat=_response_ / pred=freq;
loglin prey|stage|habitat;
run;
```

The summary statistics below show that the three-way interaction, prey*stage*habitat, is not significant. If this term were significant, there would be no reason to examine two-way interactions or main effects.

Table Summary statistics for multiway contingency table analysis.

Source	*df*	χ^2	*P*
Prey	3	71.27	0.0001
Stage	1	8.99	0.002
Habitat	1	26.75	0.0001
Prey*stage	3	1.70	0.637
Prey*habitat	3	12.88	0.0049
Stage*habitat	1	0.18	0.674
Prey*stage*habitat	3	3.09	0.377

Deleting the prey*stage*habitat term from the model, we obtain a significant interaction for prey*habitat ($P = 0.0095$). To determine which prey items are responsible for the significant prey*habitat interaction, we can delete individual prey categories and revaluate the interaction term. Below, we see that by deleting individual prey types, we are unable to obtain a nonsignificant interaction term for amphipods, chironomids, or ostracods. However, when we delete two groups of prey from the analysis we find that amphipods and chironomids are responsible for the significant interaction observed in the prey*habitat term.

Table Analysis to determine which prey items are responsible for the significant prey*habitat interaction.

Prey type deleted	*P*-value for interaction term
Single prey	
Amphipods	0.024
Chironomids	0.007
Mayflies	0.115
Ostracods	0.006
Combined prey	
Amphipods & chironomids	0.377
Amphipods & ostracods	0.010
Chironomids & ostracods	0.002

By tabulating the observed and expected frequencies (in parentheses) for amphipods and chironomids, we can make inferences about how these prey types differ across fish life stages and habitats. Here we see higher than expected numbers of chironomids in adult diets from both habitats. For both prey types, adult fish also showed higher than expected values compared to juvenile fish.

Table Comparison of prey type across life stage and habitats. Given are observed and expected (in parentheses) frequencies of prey type in diet; note that observed and expected frequencies are not equal because other prey types are not shown.

Prey type and life stage	Littoral habitat	Pelagic habitat
Amphipod		
Adult	29 (22)	1 (5)
Juvenile	19 (22)	6 (5)
Chironomid		
Adult	69 (57)	8 (12)
Juvenile	43 (57)	21 (12)

Box 11.9 Exploring Diet Data with Principal Component Analysis (PCA)

Traditional multivariate techniques, such as PCA, can be constrained by the compositional nature of diet data in so much as the row sums must equal one. Log-ratio analysis, such as %PCA (see text), is performed on the logarithm of proportions and can be useful for exploring individual variation in diet data. For values equal to zero, very small numbers (e.g., 0.00001) are entered prior to analysis as recommended by Aitchison (1983). A %PCA analysis was performed on the diet composition data given in Table 11.2. The first two components accounted for 94% (%PC1 = 60%; %PC2 = 34%) of the total variation in diet data.

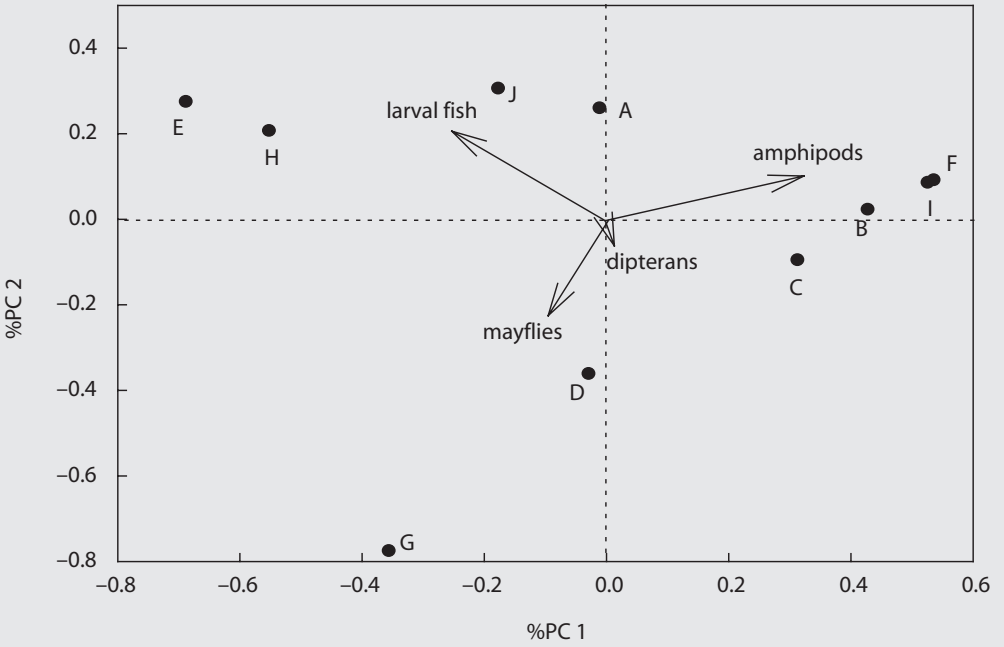

Figure The graph shows each prey type linked to an arrow for which the length of the arrow is proportional to the relative abundance of the prey. The %PCA results for individual bluegills (A–J) are then superimposed on the prey distribution to show individual variation in diet composition. Amphipods, larval fish, and mayflies accounted for much of the variation in individual diets, whereas dipterans accounted for little variation and were distributed near the population centroid (origin).

The patterns represented in the figure above can be compared to physical or biological characteristics to help identify factors affecting diet variability. For example, we might be interested in whether fish size accounts for variation in stomach contents. Correlation analysis reveals a significant relationship between the first axis scores (%PC1) and fish size ($r = -0.84$; $P = 0.002$). Smaller fish (i.e., B, C, I, and F) consumed more amphipods, whereas larger fish (i.e., H and E) were more likely to contain larval fish in their diet. Similarly, this approach could be used to assess a variety of factors such as habitat characteristics, limnological parameters, or fish community attributes.

A variety of indices have been proposed to quantify diet overlap, and there is controversy as to which index is best (Krebs 1989). In cases where prey numbers are available, Morista's index has been recommended as the most robust index (Smith and Zaret 1982). Morista's index is calculated using the equation

$$M = \frac{2\Sigma_i^n p_{ij} p_{ik}}{\Sigma_i^n p_{ij}[(n_{ij}-1)/(N_j-1)] + \Sigma_i^n p_{ik}[(n_{ik}-1)/(N_k-1)]}; \quad (11.4)$$

M = Morista's index of niche overlap between species j and k;
p_{ij} = proportion resource i is of the total resources used by species j;
p_{ik} = proportion resource i is of the total resources used by species k;
n_{ij} = number of individuals of species j that use resource category i;
n_{ik} = number of individuals of species k that use resource category i; and
N_j, N_k = total number of individuals of each species in sample.

If data are not expressed as prey numbers (e.g., biomass or volume), then Horn's index is recommended (Krebs 1989) and is calculated as

$$H = \frac{\Sigma(p_{ij}+p_{ik})\log(p_{ij}+p_{ik}) - \Sigma p_{ij}\log p_{ij} - \Sigma p_{ik}\log p_{ik}}{2\log 2}, \quad (11.5)$$

where H = Horn's index of overlap between species j and k. In equation (11.5), any base of logarithms may be used.

Confidence limits or tests of significance can be calculated for diet overlap values. One way to estimate confidence limits on diet overlap values is to use bootstrapping techniques. Bootstrap techniques are relatively simple and proceed as follows.

1. Using the original data with n observations, randomly select n diet overlap values with replacement. Because we are sampling with replacement, some values may be selected one or more times or not at all. Repeat this step at least 100 times (preferably 1,000).
2. Calculate a mean diet overlap value for each bootstrap sample.
3. Estimate the mean and standard error from the replicate bootstrap values.

Because bootstrap procedures estimate the sample mean, rather than the population mean, they contain a bias that can be corrected using the equation,

$$\text{Bootstrap mean}_{\text{adj}} = 2\bar{x}_s - \bar{x}_B, \quad (11.6)$$

where $\bar{x}_s$ = observed mean of original sample and $\bar{x}_B$ = bootstrap estimate of mean (Krebs 1989).

11.3.7 Estimating Prey Preference

When given a variety of prey types, most fishes select some food categories over others. To measure this selectivity, a variety of indices have been developed that incorporate measures of prey use and prey availability (see review in Bowen 1996). While prey use can be easily determined from gut content analysis, accurate description of prey availability can be problematic. What we quantify as prey availability may be quite different than what fish perceive under natural conditions. Furthermore, because different prey can occupy different habitats, a single sampling technique may not adequately quantify the relative abundance of different prey items in the environment. This is important because we cannot use volumetric estimates of zooplankton abundance (e.g., number/L) and real densities of benthic invertebrates (e.g., number/m^2) as simultaneous measures of prey availability. Only in cases where prey are collected with the same gear type, such as open-water zooplankton, can we begin to compare use versus availability.

Like diet and overlap indices, there is much controversy over which preference index is best (Wallace and Ramsey 1983). Comparisons of different indices have revealed that the Manly–Chesson (Chesson 1983) and the linear (Strauss 1979) indices are good choices for quantifying prey preference (Smith and Zaret 1982; Wallace 1981; Krebs 1989). The Manly–Chesson index is frequently used to quantify prey preference and can be calculated for two scenarios (Krebs 1989).

Constant prey abundance. This form of the Manly–Chesson index is used when the number of prey eaten is very small relative to that prey item's total population or when prey are replaced, as in laboratory studies. The equation for the Manly–Chesson index under constant prey abundance is

$$\alpha_i = \frac{r_i}{n_i} \frac{1}{\Sigma (r_j / n_j)}; \tag{11.7}$$

α_i = Manly's alpha for prey type i;
r_i, r_j = proportion of prey type i or j in the diet;
n_i, n_j = proportion of prey type i or j in the environment; and
m = total number of prey types.

Values of α_i are normalized so that $\sum_{i=1}^{m} \alpha_i = 1.0$.

Prey preference is indicated when α_i values are greater than $1/m$. Conversely, α_i values less than $1/m$ imply that prey species i is avoided in the diet because it is used in lower proportion than its availability in the environment.

Variable prey abundance. This form of the Manly–Chesson index is used when the number of prey eaten is large relative to that prey item's total population in the environment or when, in experimental studies, prey are not replaced after being eaten. The Manly–Chesson index for variable prey populations is calculated using the equation

$$\alpha_i = \frac{\log P_i}{\sum_{j=1}^{m} P_j}; \tag{11.8}$$

α_i = Manly's alpha for variable prey populations;
P_i, P_j = proportion of prey i or j remaining at the end of the experiment (e_i/n_i);
e_i = number of prey type i remaining at the end of experiment;
n_i = number of prey type i at the beginning of the experiment; and
m = total number of prey types.

In equation (11.8), any base of logarithms can be used.

It is recommended when using the Manly–Chesson index for variable prey populations that the number of prey eaten and the number of prey remaining are greater than 10 (Manly 1974; Chesson 1983; Krebs 1989). In practice, indices such as the Manly–Chesson can be used to test for differences in prey selectivity providing important information about preferred (or vulnerable) prey types (Box 11.10).

Box 11.10 Assessing Prey Preference

Differences in prey selectivity provide important insight about foraging patterns of fishes. In many cases, these type of data are collected under controlled, experimental settings in which changes in the absolute abundance of prey can be accurately determined.

Catalano et al. (2001) examined the effects of tag color on vulnerability to predation. Age-0 bluegills were marked with either brightly colored fluorescent tags or cryptic tags and then exposed to largemouth bass predators in a series of tank experiments. Manly's alpha was calculated using the equation for variable prey populations (equation [11.8]).

Table Vulnerability of age-0 bluegills with differently colored tags to predation by largemouth bass (data from Catalano et al. 2001).

Trial and tag color	Initial number of prey	Final number of prey	Proportion remaining	Manly's alpha
1				
Bright	120	22	0.183	0.618
Cryptic	60	21	0.350	0.382
2				
Bright	96	26	0.271	0.529
Cryptic	48	15	0.313	0.471
3				
Bright	150	64	0.427	0.528
Cryptic	75	35	0.467	0.472

Here, we are interested in whether marking pattern (bright versus cryptic tags) influences prey selectivity. A Student's *t*-test reveals a significant difference in selectivity between brightly colored and cryptic tags ($t = 2.76, P = 0.05$); fish marked with brightly colored tags had a higher selectivity ($\bar{x} = 0.56$) than did fish marked with cryptic tags ($\bar{x} = 0.44$).

11.4 TRACKING ENERGY FLOW WITH STABLE ISOTOPE ANALYSIS

Stomach content analysis provides a high degree of taxonomic precision but is limited in many ways because it provides only a snapshot in time of consumer diets. Stable isotope analysis is an alternative approach to traditional food habit studies that provides time-integrated information useful for tracking energy flow in aquatic food webs (Fry and Sherr 1984; Peterson and Fry 1987). Stable isotope analysis has several advantages as a method for quantifying feeding patterns: (1) it reflects materials actually assimilated by fish, enhancing our ability to detect subtle but important feeding interactions that might go undetected by traditional gut content analysis; (2) it allows more efficient use of sampled fish because there is no loss of information when stomachs are empty; and 3) it can be used to evaluate within-population variation in fish feeding habits.

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are the most commonly used isotopes in aquatic food web studies. In general, $\delta^{13}C$ signatures of consumers are similar to those of their prey and can be used to identify carbon sources at the base of the food chain. Conversely, $\delta^{15}N$ signatures exhibit a step-wise increase from prey to predator. A 3–4‰ enrichment of the heavy nitrogen isotope represents a typical trophic level increment (e.g., zooplankton to fish). Hence, $\delta^{15}N$ signatures can be used to identify important feeding relationships and energy pathways.

Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (ppt; ‰) deviation from a standard material (Peterson and Fry 1987). The formula for calculating $\delta^{13}C$ or $\delta^{15}N$ is

$$\delta^{13}C \text{ (or } \delta^{15}N) = ([R_{sample}/R_{standard}] - 1) \times 1{,}000, \tag{11.9}$$

where R equals the ratio of $^{13}C/^{12}C$ (or $^{15}N/^{14}N$). Standard materials are represented by Pee Dee belemnite limestone for $\delta^{13}C$ or atmospheric nitrogen for $\delta^{15}N$, where both standards have a ppt value set to 0. A positive (or less negative for carbon) isotopic value indicates the sample is "isotopically" enriched and contains more of the heavy stable isotope (^{13}C or ^{15}N; Vander Zanden et al. 2000).

Samples for stable isotope analysis are usually collected from white dorsal muscle tissue (1–2 g wet weight) of individual fish and frozen until analysis. Although samples are usually collected from sacrificed fish, biopsy punches (6–8 mm) are useful for obtaining nonlethal samples in the field where fish can be quickly treated with an antibiotic ointment and released. For invertebrates or larval fish, whole samples are obtained in the field and then frozen. Prior to freezing invertebrates and larval fish, it is recommended that they be placed in filtered water for up to 12 h to allow gut evacuation. Prey items in the guts of small invertebrates and larval fish can affect $\delta^{15}N$ signatures (Yoshioka et al. 1994). Because 0.1 g dry weight is usually required to analyze stable isotopes, samples should consist of about 1–2 g wet weight. Samples are then dried at 70°C to a constant weight, ground into a fine powder, and packed into 4 × 6-mm tin capsules for isotopic analyses. Isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ is performed using a mass spectrometer.

11.4.1 Applying Stable Isotope Data

A promising new technique for assessing energy flow in aquatic ecosystems involves the calculation of fish trophic position, a continuous variable that quantifies the average energy pathway to a consumer (Vander Zanden and Rasmussen 1999). Trophic position is useful for assessing feeding patterns because it incorporates the relative contribution of different trophic levels to fish diets. Both dietary data and stable isotope ratios can be used to calculate trophic position of fish. Because trophic position incorporates omnivorous feeding behavior, it provides an advantage over food chain studies that fail to consider omnivorous trophic interactions and food web studies that fail to weight food links according to their energetic importance (Polis 1991; Gaedke et al. 1996; Vander Zanden and Rasmussen 1999).

To demonstrate how trophic position can be estimated from dietary data, consider a lake trout population that has a diet consisting of 20% herbivorous zooplankton (trophic level = 2) and 80% planktivorous fish (trophic level = 3). These data, usually obtained from numerous fish within a size-class, can be used to calculate trophic position (TP_{diet}) as

$$TP_{diet} = \Sigma(V_i T_i) + 1, \tag{11.10}$$

where V_i is the percent volumetric contribution of the ith prey item (e.g., 0.2 or 0.8) and T_i is the trophic position of the ith prey item (e.g., 2 or 3; Vander Zanden et al. 1997). Hence, the size-specific trophic position for this lake trout population is 3.8.

Alternatively, trophic position can be estimated for individual fish from stable isotope ratios as

$$TP_{fish} = [(\delta^{15}N_{fish} - \delta^{15}N_{baseline})/3.4] + 2, \tag{11.11}$$

where $\delta^{15}N_{fish}$ is the isotope signature of the fish, $\delta^{15}N_{baseline}$ is the "corrected" isotope signature of the fish, 3.4 is the assumed per mil increase in $\delta^{15}N$ per trophic level, and 2 represents the number of trophic levels involved (Vander Zanden and Rasmussen 1999).

Because $\delta^{15}N$ values vary greatly among organisms at the base of the food chain, the $\delta^{15}N$ value of a consumer cannot be regarded as an absolute measure of trophic position. Hence, it is necessary to correct the $\delta^{15}N$ signatures of fish to account for $\delta^{15}N$ variation among primary consumers (e.g., zooplankton, chironomids, and amphipods; Angradi 1994; Vander Zanden and Rasmussen 1999). To accomplish this, bivariate plots of $\delta^{15}N - \delta^{13}C$ are used to describe the relationship between nitrogen and carbon signatures for primary consumers. This relationship can then be used to calculate baseline conditions ($\delta^{15}N_{baseline}$) that are used to correct $\delta^{15}N$ values of secondary consumers (see Vander Zanden and Rasmussen 1999).

Isotopically derived measures of fish trophic position can be used to assess diet variability within a population. Bivariate plots that depict trophic position–body

size relationships are first constructed to assess variation in energy flow among different-sized fish. Because trophic position normally increases with fish size, variance estimates may be higher for populations with steep trophic position–body size slopes. To remedy this, variance estimates can be estimated as the mean absolute residual value from trophic position–body size relationships (Box 11.11). This variation is independent of body size and can be used to assess factors affecting fish trophic position (Vander Zanden et al. 2000).

Variation in trophic position reflects the magnitude of two diet components: (1) diet breadth–the overall range of prey consumed, and 2) diet consistency–the

Box 11.11 Determining Trophic Position of Fishes with Stable Isotope Analysis

Stable isotope data is often used to estimate the trophic position of fishes (Vander Zanden et al. 2000). Variation in trophic position can then be used to evaluate factors affecting fish foraging patterns across space or time.

In this example, isotope data were used to calculate the following trophic position estimates (TP) for different size walleyes. The relationship between TP and walleye size was then used to develop the equation

$$TP_{predicted} = 2.797 + 0.001445(L),$$

where predicted trophic position ($TP_{predicted}$) is estimated as a function of walleye length (L = total length in mm).

Table Trophic position (TP) versus walleye size. From this relationship, predicted TP and residuals are calculated.

Walleye size (mm)	TP	$TP_{predicted}$	|Residual|
152	3.5	3.0166	0.4834
254	3.6	3.1640	0.4360
305	3.4	3.2377	0.1623
355	3.5	3.3100	0.1900
381	3.61	3.3475	0.2625
457	3.42	3.4574	0.0374
508	3.45	3.5311	0.0811
584	3.61	3.6409	0.0309
609	3.59	3.6770	0.0870
660	3.7	3.7507	0.0507
Mean residual			0.1821

Residual values were calculated from the trophic position–body size relationship as the difference between TP – $TP_{predicted}$. Absolute residual values are then averaged to obtain a measure of trophic position variation that is independent of fish body size (i.e., 0.1821). Variation in trophic position can then be compared across time or space or correlated with biotic or abiotic variables to assess factors affecting diet variation of walleyes.

degree to which an individual fish repeatedly consumes the same prey type. High levels of variation indicate high diet breadth and high diet consistency, whereas low variation can represent either (a) high diet breadth and low consistency (all individuals consume similar proportions of a wide range of prey), or (b) low diet breadth (e.g., all individuals specialize on a few prey types; Vander Zanden et al. 2000). Variables, such as lake area, prey diversity, number of competitor species, food chain length, and lake productivity, are just a few parameters that can be compared with trophic position variation in an attempt to understand factors affecting feeding patterns. Similarly, variation in trophic position can be compared across seasons as a method for evaluating temporal changes within a population.

11.5 CONCLUSIONS

Food habit assessments are an integral part of many research and management plans. While specific goals of food habit studies vary, the usefulness of diet data relies on the accurate quantification of diet composition. Factors such as time of year and sample location can profoundly affect prey availability and diet composition of fishes. At smaller scales, time of day, habitat characteristics, and collecting gear can influence diet composition. The degree to which these factors affect interpretation of fish diets largely depends on the research question. As a result, it is important in diet studies to have well-defined research objectives that account for factors affecting diet composition.

Because diets can be quantified in many different ways, it is unlikely that a single diet index will be useful in all circumstances. Rather, we should rely on the unique properties of individual measures to select a method that is most appropriate for our study. As previously discussed, single indices based on prey number, weight (or volume), or occurrence each have their place in studies of diet composition. Similarly, graphical techniques that incorporate two or more single indices can provide important insight into feeding strategies, niche breadth, and relative prey importance. For questions concerning energy flow in aquatic ecosystems, techniques such as stable isotope analysis can provide powerful tools for quantifying important energy pathways to fishes.

Diet data have several important characteristics that affect analysis and interpretation: data are usually (1) multivariate in nature, (2) proportional, (3) variable at the individual level, and (4) autocorrelated across space and time. By appreciating and understanding these characteristics, we can design appropriate studies and select sound analytical techniques for assessing food habits and feeding patterns of fishes.

11.6 REFERENCES

Aitchison, J. 1983. Principal component analysis of compositional data. Biometrika 70:57–65.

Amundsen, P. A., H. M. Gabler, and F. J. Staldvik. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data—modification of the Costello method. Journal of Fish Biology 48:607–614.

Angradi, T. R. 1994. Trophic linkages in the lower Colorado River: multiple stable isotope evidence. Journal of the North American Benthological Society 13:479–495.

Bettoli, P. W., and L. E. Miranda. 2001. Cautionary note about estimating mean length at age with subsampled data. North American Journal of Fisheries Management 21: 425–428.

Bowen, S. H. 1996. Quantitative description of the diet. Pages 513–522 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Bremigan, M. T., and R. A. Stein. 1994. Gape-dependent larval foraging and zooplankton size: implications for fish recruitment across systems. Canadian Journal of Fisheries and Aquatic Sciences 51:913–922.

Bremigan, M. T., and R. A. Stein. 1997. Experimental assessment of the influence of zooplankton size and density on gizzard shad recruitment. Transactions of the American Fisheries Society 126:622–637.

Bridcut, E. E., and P. S. Giller. 1995. Diet variability and foraging strategies in brown trout *(Salmo trutta)*: an analysis from subpopulations to individuals. Canadian Journal of Fisheries and Aquatic Sciences 52:2543–2552.

Cade, B. S., and B. R. Noon. 2003. A gentle introduction to quantile regression for ecologists. Frontiers in Ecology and the Environment 1:412–420.

Cade, B. S., and J. D. Richards. 2000. User manual for Blossom Statistical Software. Midcontinent Ecological Science Center, U.S. Geological Survey, Fort Collins, Colorado.

Catalano, M. J., S. R. Chipps, M. A. Bouchard, and D. H. Wahl. 2001. Evaluation of injectable fluorescent tags for centrarchid fishes: mark retention rate and effects on vulnerability to predation. North American Journal of Fisheries Management 21:911–917.

Chesson, J. 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology 64:1297–1304.

Cortés, E. 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Canadian Journal of Fisheries and Aquatic Sciences 54:726–738.

Costello, M. J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. Journal of Fish Biology 36:261–263.

Crow, M. E. 1979. Multivariate statistical analysis of stomach contents. Pages 87–96 *in* S. J. Lipovsky and C. A. Simenstad, editors. Fish food habits studies: proceedings of the second Pacific Northwest technical workshop. Washington Sea Grant Program, University of Washington, Seattle.

De Crespin De Billy, V., S. Doledec, and D. Chessel. 2000. Biplot presentation of diet composition data: an alternative for fish stomach contents analysis. Journal of Fish Biology 56:961–973.

DeVries, D. R., and R. A. Stein. 1990. Manipulating shad to enhance sport fisheries in North America: an assessment. North American Journal of Fisheries Management 10:209–223.

Digby, P. G. N., and R. A. Kempton. 1987. Multivariate analysis of ecological communities. Chapman and Hall, New York.

Feller, R. J. 1992. Potential applications of immunoassays in studies of flatfish recruitment. Proceedings of the First International Symposium on Flatfish Ecology 29:1–3.

Fortin, M., and J. Gurevitch. 1993. Mantel tests: spatial structure in field experiments. Pages 342–359 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Chapman and Hall, New York.

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

Gaedke, U., D. Straile, and C. Pahl-Wostl. 1996. Trophic structure and carbon flow dynamics in the pelagic community of a large lake. Pages 60–71 *in* G. A. Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics. Chapman and Hall, New York.

Garman, G. C. 1982. Identification of ingested prey fish based on scale characteristics. North American Journal of Fisheries Management 2:201–203.

Garvey, J. E., N. A. Dingledine, N. S. Donovan, and R. A. Stein. 1998a. Exploring spatial and temporal variation within reservoir food webs: predictions for fish assemblages. Ecological Applications 8:104–120.

Garvey, J. E., E. A. Marschall, and R. A. Wright. 1998c. From star charts to stoneflies: detecting relationships in continuous bivariate data. Ecology 79:442–447.

Garvey, J. E., R. A. Wright, R. A. Stein, K. H. Ferry, and S. M. Micucci. 1998b. Assessing the influence of size on overwinter survival of largemouth bass in Ohio on-stream impoundments. Ohio Division of Wildlife, Final Report, F-69-P, Columbus, Ohio.

Goldberg, D. E., and S. M. Scheiner. 2001. ANOVA and ANCOVA: field competition experiments. Pages 77-98 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Oxford University Press, New York.

Hansson, S. 1998. Methods of studying fish feeding: a comment. Canadian Journal of Fisheries and Aquatic Sciences 55:2706–2707.

Hartman, K. J., and D. W. Garton. 1992. Electrophoretic identification of partially digested prey of piscivorous fish. North American Journal of Fisheries Management 12:260–263.

Hayward, R. S., F. J. Margraf, C. T. Knight, and D. J. Glomski. 1989. Gear bias in field estimation of the amount of food consumed by fish. Canadian Journal of Fisheries and Aquatic Sciences 46:874–876.

Holland-Bartels, L. E., S. K. Littlejohn, and M. L. Huston. 1990. A guide to the larval fishes of the upper Mississippi River. U.S. Fish and Wildlife Service Publication, LaCrosse, Wisconsin.

Huang, C., and A. Sih. 1991. Experimental studies on direct and indirect interactions in a three-trophic level stream system. Oecologia 85:530–536.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211.

Hynes, H. B. N. 1950. The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in studies of the food of fishes. Journal of Animal Ecology 19:36–58.

Hyslop, E. J. 1980. Stomach contents analysis: a review of methods and their application. Journal of Fish Biology 17:411–429.

Jackson, D. A. 1997. Compositional data in community ecology: the paradigm or peril of proportions? Ecology 78:929–940.

Jernejcic, F. 1969. Use of emetics to collect stomach contents of walleye and largemouth bass. Transactions of the American Fisheries Society 98:698–702.

Juanes, F. 1994. What determines prey size selectivity in piscivorous fishes? Pages 79–100 *in* D. J. Stouder, K. L. Fresh, and R. J. Feller, editors. Theory and application in fish feeding ecology. University of South Carolina Press, Columbia, South Carolina.

Khattree, R., and D. N. Naik. 1999. Applied multivariate statistics with SAS software, 2nd edition. SAS Institute, Cary, North Carolina.

Kim, G. W., and D. R. DeVries. 2001. Adult fish predation on freshwater limnetic fish larvae: a mesocosm experiment. Transactions of the American Fisheries Society 130: 189–203.

Kimball, D. C., and W. T. Helm. 1971. A method of estimating fish stomach capacity. Transactions of the American Fisheries Society 100:572–575.

Kimura, D. K. 1977. Statistical assessment of the age–length key. Journal of the Fisheries Research Board of Canada 34:317–324.

Knight, R. L., and J. F. Margraf. 1982. Estimating stomach fullness in fishes. North American Journal of Fisheries Management 2:413–414.

Krebs, C. J. 1989. Ecological methodology. Harper Collins, New York.

MacDonald, J. S., and R. H. Green. 1983. Redundancy of variables used to describe importance of prey species in fish diets. Canadian Journal of Fisheries and Aquatic Sciences 40:635–637.

Maceina, M. J., P. W. Bettoli, and D. R. DeVries. 1994. Use of a split-plot analysis of variance design for repeated-measures fisheries data. Fisheries (Bethesda) 19(3):14–20.

Manly, B. F. J. 1974. Randomization and Monte Carlo methods in biology. Chapman and Hall, London.

Mittelbach, G. C., C. W. Osenberg, and P. C. Wainwright. 1999. Variation in feeding morphology between pumpkinseed populations: phenotypic plasticity or evolution? Evolutionary Ecology Research 1:111—128.

Neter, J., W. Wasserman, and M. H. Kutner. 1990. Applied linear statistical models: regression, analysis of variance, and experimental designs. Irwin Publishing, Boston.

Noble, R. L. 1981. Management of forage fishes in impoundments of the southern United States. Transactions of the American Fisheries Society 110:738–750.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Polis, G. A. 1991. Complex trophic interactions in deserts: an empirical critique of food web theory. American Naturalist 138:123–155.

Pope, K. L., M. L. Brown, W. G. Duffy, and P. H. Michaletz. 2001. A caloric-based evaluation of diet indices for largemouth bass. Environmental Biology of Fishes 61:329–339.

Probst, W. E., C. F. Rabeni, W. G. Covington, and R. E. Marteney. 1984. Resource use by stream-dwelling rock bass and smallmouth bass. Transactions of the American Fisheries Society 113:283–294.

Raclot, T., R. Groscolas, and Y. Cherel. 1998. Fatty acid evidence for the importance of myctophid fishes in the diet of king penguins, *Aptenodytes patagonicus*. Marine Biology 132:523–533.

Rasmussen, P. W., D. M. Heisey, E. V. Nordheim, and T. M. Frost. 1993. Time-series intervention analysis: unreplicated large-scale experiments. Pages 138–158 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Chapman and Hall, New York.

Reiriz, L., A. G. Nicieza, and F. Brana. 1998. Prey selection by experienced and naive juvenile Atlantic salmon. Journal of Fish Biology 53:100–114.

SAS Institute. 1999. SAS procedures guide, version 8. SAS Institute, Cary, North Carolina.

Schael, D. M., L. G. Rudstam, and J. R. Post. 1991. Gape limitation and prey selection in larval yellow perch *(Perca flavescens),* freshwater drum *(Aplodinotus grunniens),* and black crappie *(Pomoxis nigromaculatis).* Canadian Journal of Fisheries and Aquatic Sciences 38:1919–1925.

Scharf, F. S., J. A. Buckel, F. Juanes, and D. O. Conover. 1997. Estimating piscine prey size from partial remains: testing for shifts in foraging mode by juvenile bluefish. Environmental Biology of Fishes 49:377–388.

Scharf, F. S., F. Juanes, and M. Sutherland. 1998. Inferring ecological relationships from the edges of scatter diagrams: comparison of regression techniques. Ecology 79:448–460.

Schultz, D. R., and M. E. Clarke. 1995. An immunological study of predation on hatchery-reared, juvenile red drum *(Sciaenops ocellatus)*: preparation and assays of a "red drum-specific" protein for predator–prey experiments. Journal of Experimental Marine Biology and Ecology 189:1–2.

Seaburg, K. G. 1957. A stomach sampler for live fish. Progressive Fish-Culturist 19:137–139.

Shepard, W. C., and E. L. Mills. 1996. Diel feeding, daily food intake, and *Daphnia* consumption by age-0 gizzard shad in Oneida Lake, New York. Transactions of the American Fisheries Society 125:411–421.

Smith, E. P., and T. M. Zaret. 1982. Bias in estimating niche overlap. Ecology 63:1248–1253.

Somerton, D. A. 1991. Detecting differences in fish diets. U.S. National Marine Fisheries Service Fishery Bulletin 89:167–169.

Strauss, R. E. 1979. Reliability estimates for Ivlev's electivity index, the forage ratio, and a proposed linear index of food selection. Transactions of the American Fisheries Society 108:344–352.

Sutela, T., and A. Huusko. 2000. Varying resistance of zooplankton prey to digestion: implications for quantifying larval fish diets. Transactions of the American Fisheries Society 129:545–551.

Swynnerton, G. H., and E. B. Worthington. 1940. Notes on the food of fish in Haweswater. Journal of Animal Ecology 9:183–187.

ter Braak, C. J. F., and P. Smilauer. 1998. CANOCO for Windows, version 4.0. Center for Biometry, Wageningen, The Netherlands. (Distributed by Microcomputer Power, 113 Clover Lane, Department C8, Ithaca, New York, 14850-4930, USA. Available at http://www.microcomputerpower.com/.)

Trippel, E. A., and F. W. H. Beamish. 1987. Characterizing piscivory from ingested remains. Transactions of the American Fisheries Society 116:773–776.

Vander Zanden, M. J., G. Cabana, and J. B. Rassmussen. 1997. Comparing the trophic position of littoral fish estimated using stable nitrogen isotopes ($\delta^{15}N$) and dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54:1142–1158.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer $\delta^{15}N$ and $\delta^{13}C$ and the trophic position of aquatic consumers. Ecology 80:1395–1404.

Vander Zanden, M. J., B. J. Shuter, N. P. Lester, and J. B. Rasmussen. 2000. Within- and among-population variation in the trophic position of a pelagic predator, lake trout *(Salvelinus namaycush).* Canadian Journal of Fisheries and Aquatic Sciences 57:725–731.

Wallace, R. K. 1981. An assessment of diet-overlap indexes. Transactions of the American Fisheries Society 110:72–76.

Wallace, R. K., and J. S. Ramsey. 1983. Reliability in measuring diet overlap. Canadian Journal of Fisheries and Aquatic Sciences 40:347–351.

Welker, M. T., C. L. Pierce, and D. H. Wahl. 1994. Growth and survival of larval fishes: roles of competition and zooplankton abundance. Transactions of the American Fisheries Society 123:703–717.

Werner, E. E., and D. J. Hall. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish *(Lepomis macrochirus)*. Ecology 55:1042–1052.

Wright, R. A., L. B. Crowder, and T. H. Martin. 1993. The effects of predation on the survival and size-distribution of estuarine fishes—an experimental approach. Environmental Biology of Fishes 36:291–300.

Windell, J. T., and S. H. Bowen. 1978. Estimating food consumption rates of fish populations. Pages 227–254 *in* T. Bagnel, editor. Methods for assessment of fish production in fresh waters, 3rd edition. Blackwell Scientific Publications, Oxford, UK.

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