7.4 | DESCRIBING A SPECIES-RICH RIVER FOOD WEB USING STABLE ISOTOPES, STOMACH CONTENTS, AND FUNCTIONAL EXPERIMENTS

Craig A. Layman, Kirk O. Winemiller,
D. Albrey Arrington

Complexity and variability of ecosystems, especially in the tropics (e.g., rain forests, coral reefs, freshwater lotic systems), render obsolete simple solutions to describe how humans impact food webs. Yet this understanding is essential to help stem biodiversity loss and assess community- and ecosystem-level responses to human-induced habitat change (Chapin et al., 1998; McCann, 2000). Human impacts are especially difficult to assess in complex webs due to indirect (Wootton, 1993), diffuse (Yodzis, 2000), and emergent (Sih et al., 1998) effects. In this context, characterization of trophic dynamics (i.e., food web structure, energy flow) using multiple, complementary methodologies is most likely to lead to an understanding sufficient to derive useful predictions regarding effects of human-induced perturbations.

We describe research conducted in a species-rich, floodplain river in Venezuela, the Cinaruco, where we have employed three methodologies to describe different aspects of food web structure: (1) stable isotope

analyses, (2) stomach contents analyses, and (3) functional experiments. Because of the recent emphasis on stable isotopes in food web research (Post, 2002a; Vanderklift and Ponsard, 2003, and references therein), we devote much of this chapter to describing how we have used stable isotope ratios to characterize food web structure in the Cinaruco River. The importance of using empirically-derived stomach contents analysis has increasingly been acknowledged, and together stable isotope ratio and stomach contents analysis can provide detailed descriptions of energy flow through food webs. These approaches, however, do not reveal functional roles of organisms in the web (Paine, 1980). To illustrate, we compare results of experimental exclusion of large piscivores with results derived from stable isotope and stomach contents analysis of the same piscivores. Each of these methodologies may lead to different predictions regarding human impacts on food web structure, and we provide an example by examining how illegal commercial netters may affect the Cinaruco River food web through removal of large piscivore species.

METHODS

Study Site

The Cinaruco River is a moderate blackwater (sensu Goulding, 1980) floodplain river in southwestern Venezuela (6° 32´ N, 67° 24´ W). The river has a forested riparian zone, but open grassland dominates the drainage basin (ca. 10,000 km^2). Hydrology is strongly seasonal, with the river water level fluctuating more than five meters annually. In the wet season (May to October) the riparian forest is flooded, and dispersal of organisms is extensive (Lowe-McConnell, 1987). Rapidly falling water levels in the river from November to January increases fish densities, leading to intense biotic interactions (e.g., predation) during the dry season (February-April) (Winemiller and Jepsen, 1998; Layman and Winemiller, 2004). Maximum width of the main channel during the dry season is 50–200 meters. The river supports an extremely diverse fish assemblage (>280 species) with taxa representing a wide range of ecological attributes and life history strategies (Jepsen et al., 1997; Winemiller et al., 1997; Arrington and Winemiller, 2003; Winemiller and Jepsen, 2004).

Isotopic Analyses

Samples were collected 1999–2003 from a 25-kilometer stretch of the Cinaruco River. In this chapter, we discuss a sub-set of fish samples from an extensive isotopic ratio data base ($n > 1,900$ individual samples).

Collection, preservation, and preparation follow Arrington and Winemiller (2002). Briefly, fishes were collected using nets and hook and line, and represent a random sub-sample from the community. Immediately following capture, approximately 2 grams of dorsal muscle was removed and covered with 20 grams of non-iodized table salt. In the laboratory, salt-preserved samples were rinsed in distilled water, soaked in distilled water for 4 hours, and dried at 60°C for 48 hours. Once dry, samples were ground to a fine powder using a mortar and pestle and loaded into tin capsules. Samples were analyzed at the Stable Isotope Laboratory at the University of Georgia's Institute of Ecology for determination of percent carbon, percent nitrogen, and stable isotope ratios. Stable isotope values are reported using δ (delta) notation where:

$$\delta^{15}C \text{ or } \delta^{15}N = ([R_{sample} / R_{standard}] - 1) \times 1000 \qquad (1)$$

where R is $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$. Working standards were bovine ($n = 49$, $\delta^{13}C = -22.11\%$, SD = 0.06%, 48.8% C, $\delta^{15}N = 7.47$, SD = 0.07%, 10.0% N) and poplar ($n = 81$, $\delta^{13}C = -27.34\%$, SD = 0.10%, 48.1% C, $\delta^{15}N = -2.47$, SD = 0.16°/%, 2.7% N). We did not perform lipid extraction prior to stable isotopic analysis of samples because the range of lipid content among samples was relatively small (Arrington, *unpublished data*), and there was typically no correlation between the relative amount of lipid in a sample and the isotopic signature of either carbon (e.g., *Semaprochilodus kneri*, $n = 91$, $R^2 = 0.015$; $P = 0.25$) or nitrogen (e.g., *S. kneri*, $n = 89$, $R^2 = 0.012$; $P = 0.31$).

A common use of stable isotope ratios is to estimate the trophic position of secondary consumers using $\delta^{15}N$, and a crucial step in this calculation is determination of baseline $\delta^{15}N$. It is now widely acknowledged that primary consumers provide the best baseline because they integrate temporal and spatial variation in isotopic signatures of basal resources (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Post, 2002a, b). When consumers acquire nitrogen from more than one food web module (sensu Holt, 1997, e.g., the littoral and pelagic food web in a lake), the following model is typically used to assess trophic position of a secondary consumer (S):

$$\text{Trophic position} = \lambda + (\delta^{15}N_S - [\delta^{15}N_{B1} \times \alpha + \delta^{15}N_{B2} \times (1 - \alpha)])/\Delta \qquad (2)$$

where λ is the trophic position of the organism(s) used to estimate baseline values (in this study λ = 2, the trophic position of primary consumer

taxa), S is the consumer in question, B1 and B2 are the two baseline taxa, α the proportion of nitrogen in the consumer derived from the food web module which B1 represents, and Δ is the enrichment in $\delta^{15}N$ per trophic level. If it is assumed that nitrogen and carbon move through a food web in similar fashion, α can be estimated as:

$$\alpha = (\delta^{13}C_S - \delta^{13}C_{B2})/(\delta^{13}C_{B1} - \delta^{13}C_{B2}) \tag{3}$$

where the consumer taxa of interest is designated by S and baseline taxa are designated by B.

A species used to estimate baseline should: (1) temporally integrate isotopic changes at a scale near that of the secondary consumer of interest, and (2) capture spatial variability of resources supporting the secondary consumer (Post, 2002b). More generally, baseline taxa should integrate (temporally and spatially) the main sources of basal production that support consumers, thereby representing the specific energy sources for the overall food web. In tropical lotic waters, fish often occupy foraging niches that in temperate aquatic systems are dominated by invertebrates (e.g., processors of particulate organic matter) (Winemiller, 1990; Flecker, 1992; Winemiller and Jepsen, 1998), and thus detritivorous and herbivorous fishes may serve as ideal baseline organisms. Tissue turnover rates in invertebrates are much higher than that of fish (Fry and Arnold, 1982; Peters, 1983; Hesslein et al., 1993), and thus using fish to characterize the isotopic baseline in order to estimate trophic position of secondary consumers (i.e., typically other fish species) provides a better match of temporal integration of resources. Fish also are more vagile than most invertebrates, providing a spatial integration of basal production sources. This integration is especially important in floodplain rivers that have a high-level of habitat heterogeneity (Arrington, 2002; Hoeinghaus et al., 2003a) and numerous basal resources (Hamilton and Lewis, 1992; Forsberg et al., 1993; Lewis et al., 2001; Bunn et al., 2003).

Based on these considerations we identified two species, which integrate the two dominant source pools of primary production in the Cinaruco River: (1) *Semaprochilodus kneri* (Characiformes: Prochilodontidae) for autochthonous algal/detrital resources, and (2) *Metynnis hypsauchen* (Characiformes: Characidae) for allocthonous C_3 (and to a lesser extent C_4) plant material (see also Hamilton and Lewis, 1992; Lewis et al., 2001). *S. kneri*, similar to other prochilodontids, feed on a mixture of algae and detritus (Bowen, 1983; Bowen

et al., 1984; Goulding et al., 1988), integrating *in situ* benthic algae and periphyton production, as well as detrital matter derived from macroalgae, periphyton, macrophytes, and phytoplankton. The $\delta^{15}N$ of *S. kneri* is representative of $\delta^{15}N$ of other algivore/detritivore taxa in the river (e.g., the con-generic *S. laticeps*, loracariids, and curimatids, *n* of taxa = 15, *unpublished data*; see also Jepsen, 1999). Allochthonous production may also be important in tropical floodplain rivers, especially since numerous taxa consume parts of terrestrial plants (Gottsberger, 1978; Goulding, 1980; Hamilton and Lewis, 1987; Goulding et al., 1988). For example, in the blackwater Río Negro, 79 fish species were found to consume forest fruit and seeds (Goulding et al., 1988). *M. hypsauchen* feeds primarily on terrestrial plant material, including leaves, fruits, and seeds (Layman, *unpublished data*), and its isotope signature reflects a combination of C_3 and C_4 plants, weighted heavily toward to the former source (Jepsen, 1999).

Stomach Contents Analyses

From 1999–2003, piscivores were collected from the Cinaruco River by gill netting and hook and line. Stomachs were analyzed using one of two methods. *Cichla* spp. were examined by pressing down the posterior region of the tongue and pushing gently on the fish's stomach while holding the fish in a head-down position (Layman and Winemiller, 2004). Other piscivore species were euthanized, and stomachs removed for examination. All prey items were identified to the lowest taxonomic level possible, measured (SL ± 1.0 mm), and quantified volumetrically.

Experimental Manipulations

A large-bodied fish exclusion experiment conducted in the dry season (January–March) of 2001 evaluated, at a relatively large-scale (approximately 500 m²), whether prey altered their spatial distribution in the absence of large-bodied piscivores. Exclosures were made of poultry wire (mesh 2.5 cm) which allowed most prey taxa to move freely in and out of experimental areas, while preventing entry of large-bodied piscivores. Control areas were of an equivalent size, but gaps in the wire allowed passage of all fishes. After two weeks, fish assemblages in experimental areas were sampled, and abundance of fishes were used to assess potential community-level responses to piscivore exclusion. Complete methods can be found in Layman and Winemiller (2004).

DISCUSSION AND CONCLUSIONS

Isotopic Analyses

What do $\delta^{13}C$ and $\delta^{15}N$ tell us about a secondary consumer, *Cichla temensis*, one of the most common piscivores in the Cinaruco River (Jepsen et al., 1997; Winemiller et al., 1997)? Since the trophic fractionation of $\delta^{13}C$ is assumed to be close to zero (Rounick and Winterbourn, 1986; Peterson and Fry, 1987; Post, 2002b), $\delta^{13}C$ suggests that *C. temensis* assimilates a large proportion of its energy from the autochthonous food web module (Figure 1), perhaps via *S. kneri* (Winemiller and Jepsen, 1998; Winemiller and Jepsen, 2004). The variability in $\delta^{13}C$ values observed among individual *C. temensis* indicates that a diversity of prey types support individuals of this top predator species, and emphasizes the importance of large sample sizes to characterize this variability. Significant overlap in $\delta^{13}C$ of basal resources in tropical floodplain rivers (Hamilton and Lewis, 1992; Lewis et al., 2001) complicates determination of resources supporting upper trophic levels. Other sources of information, such as additional isotopes (Wainwright et al., 2000; Hsieh et al., 2002; Currin et al., 2003; Litvin and Weinstein, 2003) or stomach
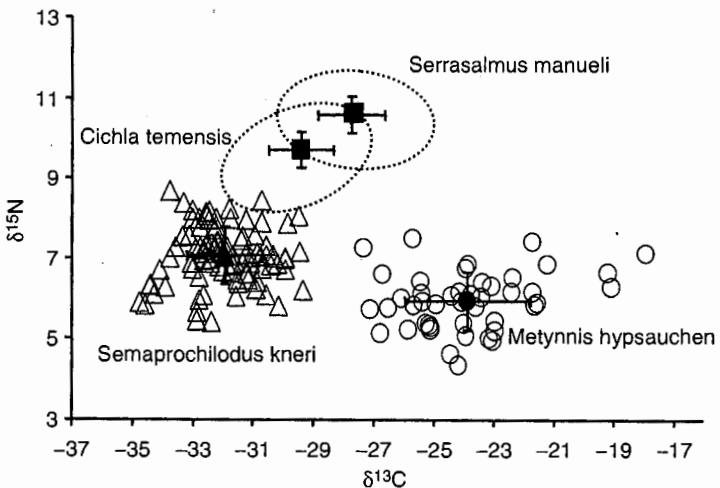

FIGURE 1 | Biplot of carbon and nitrogen stable isotope signatures of individual *Metynnis hypsauchen* (circles; $n = 45$), and *Semaprochilodus kneri* (triangles; $n = 95$). The two ellipses represents ninety-five percent confidence intervals of *Cichla temensis* ($n = 184$) and *Serrasalmus manueli* ($n = 148$) stable isotope signatures. Open symbols are values for individual specimens, and filled symbols means of all individuals of the species (± 1 SD).

contents, are needed to further identify the importance of specific resources to secondary consumers.

Ratios of stable isotopes indicate that large-bodied piscivores feed relatively low in the food web. The piranha *Serrasalmus manueli* occupied the highest estimated mean trophic position in the web, 3.6 (using a trophic level fractionation, Δ, of 2.54, see Vanderklift and Ponsard, 2003), with individuals varying from 2.9–4.4 (trophic position 1 = primary producers). Trophic position of individual *C. temensis* ranged from 2.8 to 3.7 (mean 3.2), suggesting *C. temensis* predominantly feed on organisms at trophic position 2 (herbivore or detritivores). A predator's foraging strategy is optimal when it exploits the thermodynamically richest resource possible (MacArthur and Pianka, 1966; Hastings and Conrad, 1979). By selectively feeding on herbivorous (e.g., *M. hypsauchen, Myleus* spp.) and algivorous/detritivorous (e.g., *S. kneri*, hemiodontids) taxa, piscivores in the Cinaruco River exploit short, productive food chains. Lewis et al. (2001) describe a similarly "compressed" food web for the Orinoco River, which may explain why secondary fish production is so high in these floodplain rivers.

Estimation of trophic position is especially sensitive to two factors. First, fractionation may vary with taxonomy (DeNiro and Epstein, 1981; Macko et al., 1982; Minagawa and Wada, 1984; Hobson and Clark, 1992; Vanderklift and Ponsard, 2003), form of nitrogen excretion (Vanderklift and Ponsard, 2003), quality of food (DeNiro and Epstein, 1981; Hobson, 1993; Hobson et al., 1993; Webb et al., 1998; Adams and Sterner, 2000), and turnover time of tissues (Schmidt et al., 2003). Thus, a given trophic transfer may vary substantially from the mean fractionation value employed (Post, 2002b). Reliance on a single assumed value of Δ is the weakest component of stable isotope use to estimate trophic position (Vander Zanden and Rasmussen, 2001; Phillips and Koch, 2002), and this is especially true in species-rich systems where community members vary greatly in taxonomy, body size, diet, excretion, etc. Second, it is possible that the excessive enrichment of *S. kneri* $\delta^{15}N$ values, relative to sources of primary production, may have resulted in an underestimation of trophic position of secondary consumers. For example, $\delta^{15}N$ of filamentous algae was found to be 1.3 ± 0.9 and $\delta^{15}N$ of *S. kneri* 7.1 ± 0.7 (see Figure 1). Such measures represent a greater enrichment in *S. kneri* than would be expected even when using the largest estimated mean values of Δ (Post, 2002b). Enriched primary consumer $\delta^{15}N$ values (relative to expected values based on temporally and spatially integrated $\delta^{15}N$ of primary producers) also have been found in temperate lakes (Post, 2002b) and a river in the Andean piedmont of Venezuela (Peter McIntyre, personal communication). Hamilton and Lewis (1992) showed that

algae can have a significantly different isotopic signature from bulk algal/detrital samples, suggesting that the heterogeneous nature of detritus (and the microbial community associated with it) may result in *S. kneri* assimilating organic matter enriched in $\delta^{15}N$ relative to isolated algae samples. *All* of the resources integrated by consumers must be carefully considered when determining baseline $\delta^{15}N$.

Stomach Contents Analyses

Stable isotope ratios are most informative when combined with stomach contents analysis. This is especially important in species-rich systems where predators may consume diverse prey items, and it is impossible to identify particular predator–prey interactions from stable isotopes ratios alone. Table 1 illustrates the diversity of prey taxa consumed by the seven most common large-bodied piscivores in the Cinaruco River. Dietary breadth was high, yet these are likely conserva-

Table 1. Stomach contents analysis of the seven most common large piscivore species of the Cinaruco River. Prey taxa were identified to the species or genus level. The last column gives the proportion of identified taxa (i.e., excluding contents of unknown identity) of diet contents (by volume) that were *Moenkhausia* af. *lepidura*, the prey fish species that responded most significantly in large-bodied piscivore exclusion experiments.

Piscivore Species (number of individuals examined)	Number of Unique Prey Taxa	% Stomach Contents *Moenkhausia* af. *lepidura*
Boulengerella cuvieri (*n* = 257)	17	2.7%
Boulengerella lucius (*n* = 272)	11	3.5%
Cichla intermedia (*n* = 97)	10	0
Cichla orinocensis (*n* = 703)	29	0
Cichla temensis (*n* = 1,159)	40	<1%
Hydrolycus armatus (*n* = 406)	24	0
Serrasalmus manueli (*n* = 668)	31	0

tive estimates of the diversity of food resources because: (1) the number of unique prey items was significantly related to number of stomachs examined for each piscivore species ($R^2 = 0.92$, $p < 0.0001$; n of piscivore stomachs examined >5,000), and (2) the high frequency of empty stomachs for many top predators (Arrington and Winemiller, 2002). *C. temensis* were found to consume 40 unique prey taxa characterized by substantial variation in body size, morphology, trophic position, and life history strategy. *S. kneri* made up 41% (by volume) of the total diet of *C. temensis*, and reliance on *S. kneri* as a food source is consistent with $\delta^{15}N$ values suggesting *C. temensis* feeds relatively low in the food web. No other prey item accounted for more than 7% of diet contents. Other important components of *C. temensis* diet included hemiodontids (algivore/detritivores), juvenile *Cichla* spp. (piscivorous), *Brycon* spp. (omnivorous), other cichlid taxa (insectivorous/piscivorous), and various small characiform species (insectivorous/omnivorous).

Although integration across time and space is desirable when selecting taxa to serve as baseline $\delta^{15}N$ indicators, specific temporal or spatial feeding patterns may not be apparent using stable isotope ratios without associated stomach contents data. For example, in November (when the river water level typically begins to drop) *S. kneri* comprised 59.8% of the identifiable prey items found in the stomachs of *C. temensis*, with a guild of small characid species (e.g., *Bryconops caudomaculatus*, *Moenkhausia* spp., *Hemigrammus* spp.) comprising only 5.8% of their diet. At the end of the dry season (April), no *S. kneri* were identified in *C. temensis* stomachs, and 26.7% of *C. temensis* stomach contents were characids. Likewise, piscivores undergo ontogenic diet shifts (Werner and Gilliam, 1984; Winemiller, 1989c; Jepsen et al., 1997; Post, 2003), necessitating stable isotope analyses across size-groups or age classes. Opportunistic feeding likely contributes to variability in $\delta^{13}C$ and $\delta^{15}N$ of Cinaruco piscivores (e.g., see *C. temensis* in Figure 1). Prey species are not homogeneously distributed among habitats (Arrington, 2002; Hoeinghaus et al., 2003a; Layman and Winemiller, 2004; Layman et al., 2005), and piscivores may feed on different taxa depending on foraging location. Moreover, some individual piscivores may move long distances and others remain restricted to one area (Hoeinghaus et al., 2003b), which also affects prey species consumed.

Energy Flow and Functional Food Webs

Food webs developed using stable isotopes or stomach contents can depict the flow of energy in an ecosystem, but do not allow for determination of "functional" roles of organisms (Paine, 1980; Polis, 1991; Polis

and Strong, 1996). Field experiments are often used to examine the effect that one organism has on any defined attribute, such as a community (e.g., abundance of other species) or ecosystem (e.g., primary production) level parameter. In the Cinaruco River, both stomach contents and isotopic analyses suggested relatively low trophic position of the piscivores *Cichla temensis* and *Serrasalmus manueli*, but these methodologies do not reveal what functional effect these species have in the food web.

In a large-scale experiment (approximately 500 square meters), we evaluated the effect of large-bodied piscivore exclusion on spatial distribution of prey. After two weeks, abundance of fishes in a specific size range (40–110 millimeters) was significantly higher in exclusion areas, suggesting a size-based behavioral response by prey (Layman and Winemiller, 2004). The most commonly collected taxa in this size range, *Moenkhausia* af. *lepidura*, was significantly more abundant in exclusion than control areas (Kruskal-Wallis, $P = 0.002$). This prey species, however, comprises a relatively small proportion of diets of excluded piscivorous fishes (see Table 1; based on complete stomach contents data set across all seasons and sampling sites). Although this experiment examined only one component of a community-level functional response (effect of predators on prey), it demonstrates how experimental manipulations can provide insights not ascertainable from either stable isotopes or stomach contents analyses.

Food Web Perturbations and Predictive Models

A central goal of food web research is to produce predictive models, especially those that can be used to assess human-induced impacts on food web structure (see Winemiller and Layman, Chapter 1.2). One of the most acute environmental problems in Venezuela is overexploitation of fisheries (Rodríguez, 2000). In the Cinaruco River, illegal commercial netting is a relatively new development, but is becoming increasingly intense (Hoeinghaus et al., 2003b). Populations of many large-bodied fish taxa, including *C. temensis* and *S. kneri*, are in decline, which will likely lead to shifts in food web structure. Employing both energy flow (stable isotope and stomach contents analyses) and functional/experimental approaches allow the most comprehensive assessment of effects induced by this netting activity.

For example, stable isotope and stomach contents analyses both suggest that netting may serve to *increase* food chain lengths in the system. A decline in populations of *S. kneri*, one of the most important prey of large-bodied piscivores such as *C. temensis*, may cause piscivores to consume

larger quantities of omnivorous and invertivorous taxa, thereby increasing mean food chain length. Further, removal of *C. temensis*, the primary predator of *S. kneri*, results in a shift in the community to piscivore taxa that feed at higher trophic levels (as is reflected by their $\delta^{15}N$ values (Jepsen and Winemiller, 2002). Consequently, we hypothesize that average food chain length (sensu Post, 2002a) will increase with increased commercial harvest (Figure 2). This hypothesis differs from the conventional belief that "fishing down food webs" (sequentially removing top predators from food webs) results in decreased average food chain length (Pauly et al. 1998). Such an approach to analyzing human impacts based on realized measures of trophic position (typically with stable isotopes) are becoming increasingly common (France et al., 1998; Pauly et al., 1998; Jennings et al., 2001; Jennings et al., 2002).

Field experiments suggest additional effects of piscivore removal by commercial netters. Piscivore exclusion on sand bank habitats results in increased abundance of prey fishes in the size range most commonly consumed by piscivorous fishes, likely a behavioral response to piscivore exclusion (Layman and Winemiller, 2004). Subsequent work has shown this pattern to hold at the landscape scale, as distinct size-based differences in fish assemblage structure between netted and un-netted lagoons that correspond closely to those at the experimental scale (Layman et al., unpublished manuscript). Two small characins, *Moenkhausia* sp. af. *lepidura* and *Bryconops caudomaculatus*, displayed

(A) Natural Feeding Pathway:

3   2   1

Cichla spp.   *Semaprochilodus kneri*   Algae
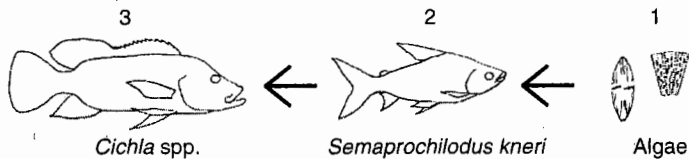
(B) Feeding Pathway Following Illegal Commercial Netting:

4   3   2   1

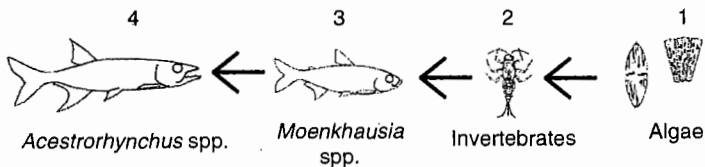*Acestrorhynchus* spp.   *Moenkhausia* spp.   Invertebrates   Algae

FIGURE 2 | Hypotheses of primary paths of energy flow in the Cinaruco River: (A) natural feeding pathway; (B) feeding pathway following removal of *Cichla* spp. and *Semaprochilodus kneri* by illegal commercial net fishermen. Numbers represent estimated trophic position.

the most significant responses to piscivore exclusion, and also had the greatest difference in abundance between netted and un-netted lagoons. These are among the species we expect will increase in abundance if commercial netting activity continues to increase, a prediction that could not be derived from energy flow methodologies alone.

The questions of interest in any food web study will determine the methodologies that must be employed in that particular instance. But in most food webs, particularly species-rich systems, even seemingly simple questions may be impossible to answer adequately without multiple research techniques. When moving from abstraction to prediction, research paths may lead in different directions, but going down each may be necessary to provide the information necessary to assess human impacts on food webs.

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