

Article

Diet and Trophic Structure of the Fish Community in a Small Sub-Tropical Lake in Central Mexico

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Abstract: Analyses of trophic structure and feeding habits of the fish community can provide information on the complex biotic and abiotic interactions in lake ecosystems. Based on stomach content and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analyses, we conducted a comprehensive study of the diet of the fish community and its trophic structure in subtropical Lake Zacapu in central Mexico. Overall, there was good agreement between the results based on the diet and the isotope analysis. Fish diets consisted mainly of aquatic macroinvertebrates, which were abundant in the lake. Most species were secondary consumers and trophic generalists across the four sites and two seasons. The food web structure did not differ significantly between the sites or seasons. Our results suggest a low trophic position of native species having a wide spatial trophic niche and niche width. Trophic diet overlap was greater between native species (especially between species from the same family) than between non-native species. Our study provides new information on the trophic interactions in a subtropical lake, rich in endemic species and an important resource for human communities.

Keywords: fish; goodeidae; stable isotopes; $\delta^{13}\text{C}$; $\delta^{15}\text{N}$; diet analysis; SIBER analysis



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1. Introduction

Small lakes are diverse and often productive [1], and they provide water sources for human use, as well as a natural habitat for aquatic organisms, including endemic species [2]. They are often influenced by human activities that can affect the structure and function of their food webs [3]. Understanding complex interactions between lake communities and their effects on energy flow and community structure is essential for the effective management of lake systems [4].

Fish are important drivers of food webs in lakes; they occupy a great variety of trophic niches and circulate matter and energy from basal resources to the highest levels of the web [5]. Fish and ecosystem trophic structure studies are essential for defining conservation strategies for aquatic ecosystems and for integrated assessment of their resources [6]. Evaluation of the feeding habits of fishes in different parts of their life cycle is useful

to determine the status of the species within the food web and, thus, contributes to the understanding of the community structure [7].

Analyzing the diets of different fishes in a community can provide information about inter- or intraspecific interactions and the mechanisms that determine the distribution of resources [8]. For example, competition occurs when species share the same prey [9] and the resources available are limited [10]. Competitive interactions are usually much stronger within than between species of a community [11]. When competition is for the same resource, it can affect habitat selection patterns and niche overlap [12]. It has been reported that competition is stronger between native species than between non-native species [13,14]. Coexistence is the degree to which phylogenetic relatedness between species reflects their ecological similarity, and it can, thus, be used to understand community assembly [15], and when the relationship is closer, for example, the same family or genus, the niche overlap increases [16].

Elucidating the trophic position of different fish species in a community may help to understand the complexity and functioning of a food web [17]. Changes in the trophic position of species are often associated with abiotic and biotic factors, such as hydrological stability, ecosystem area, diet composition, the availability of prey, and the body size of organisms [18,19]. Identifying the trophic position also helps to reveal the functional role of a species within the trophic web and its specific contribution to energy flow pathways [17].

Stomach content analysis constitutes a classical method to elucidate the diet of aquatic organisms [20]. However, data obtained by this method only provides a snapshot of the diet of the consumer at the time of sampling [21]. Stable isotope analysis techniques have been used to integrate energetic information over longer periods [22]. In stable isotope studies, carbon signatures identify energy sources, while nitrogen signatures are related to the trophic position of a consumer within the food web [23,24]. The joint use of stomach and stable isotope analyses allows a more comprehensive examination of the food web relationships and modeling of the trophic structure in an ecosystem [25] and further improves the understanding of the trophic position of species in the web and of how species (for example native versus non-native species) interact [26]. The combination of the two methods has been successfully used for elucidating food webs in freshwater and marine systems [27–30]. However, there are few such studies from lakes [31,32].

Lake Zacapu is shallow (average ~2.7 m) and is maintained by the contribution of numerous tributaries and springs [33]. The hydraulic renewal is fast (approx. five days) due to the continuous flow of water from springs, leading to good water quality and a high buffering capacity for pollutants. The lake has little human disturbance [33,34] and has many native and endemic fish species [35,36].

Here, we describe the diet of fishes in Lake Zacapu temporally (two seasons) and spatially (4 sampling sites) and document their trophic position in the food web. Our hypotheses were that (1) the trophic position of fishes evaluated from stable isotope data will mimic the diet data, (2) homogeneous environmental characteristics, different substrates, and the hydrodynamics of the lake result in high availability and diversity of food resources within the lake for fish and low contribution from external sources, and (3) trophic diet overlap will be greater for native species than for non-native species.

2. Materials and Methods

2.1. Study Area

Lake Zacapu is a small sub-tropical lake (ca. 20 ha) located in central-western Mexico at 1980 m.a.s.l. and is part of the Lerma-Chapala River basin (Figure 1).

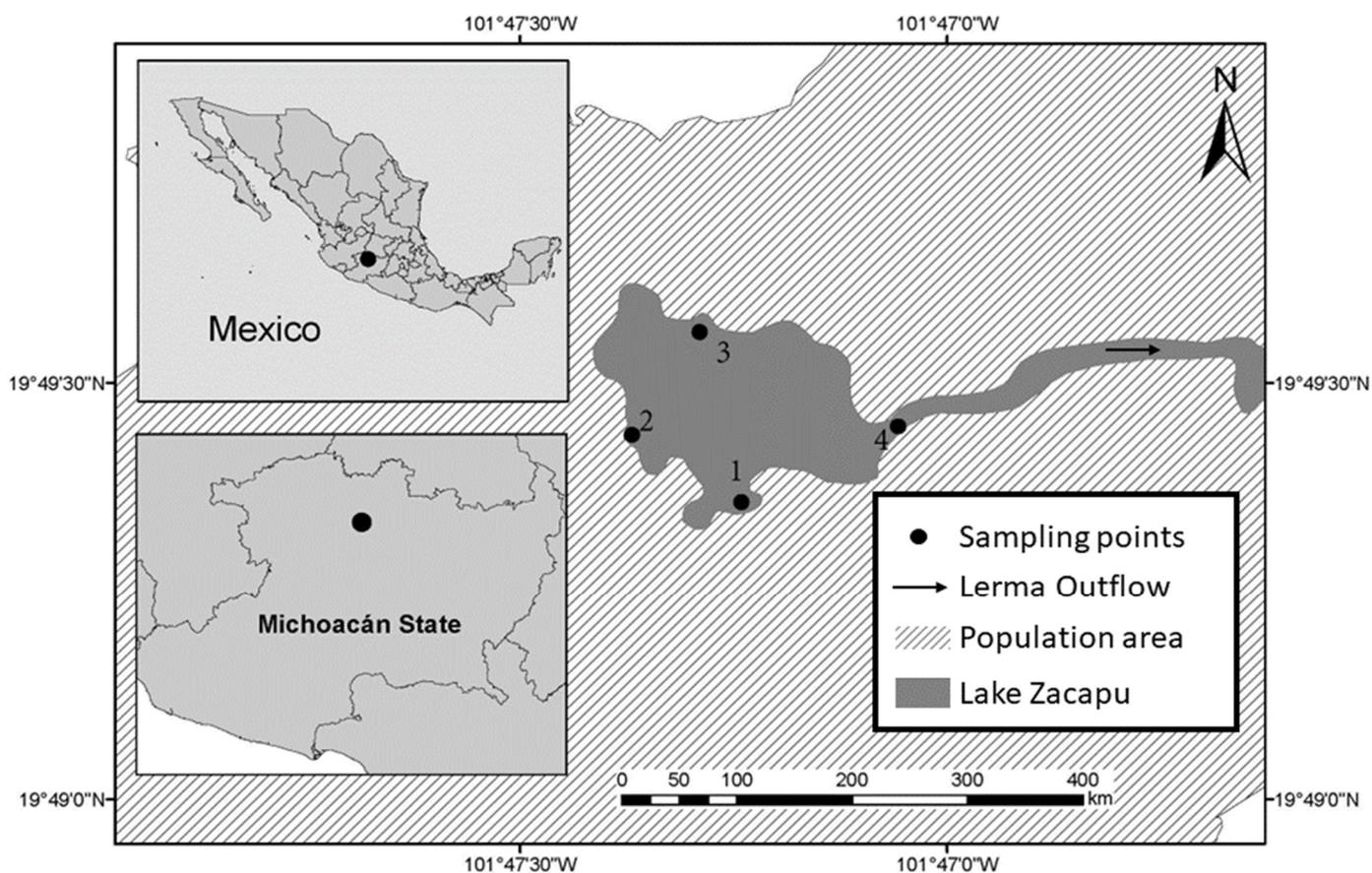


Figure 1. The geographical location of the study area and sampling sites (S1–S4) are located in Lake Zacapu, Michoacán, México.

The air temperature varies from 14 to 18 °C during the season, and the total annual precipitation is 800–1200 mm [37]. In 2003, the lake was declared a Natural Protected Area; its management plan was published in 2005 [38]. Lake Zacapu is a monomictic subtropical ecosystem with low turbidity [30–32]. This shallow lake (average depth~2.7 m) is fed by 12 springs, and it has a high hydraulic renewal rate (approx. 5 days) and a high buffering capacity [33,34]. The lake is also considered homogeneous, with good water quality because of little human disturbance and the short renewal time, and spatial variations in physical and chemical variables are low [35,36,39–41]. Four sampling sites were chosen to represent the different shorelines (Figure 1), including two major spring-influenced areas (sites 1 and 3; [35,36]).

2.2. Environmental Variables

Environmental variables were measured at each site during the samplings. We used a multiparameter probe (YSI EXO2; YSI Inc., Yellow Springs, OH, USA) and measured conductivity ($\mu\text{S}/\text{cm}$), temperature ($^{\circ}\text{C}$), dissolved oxygen (O_2 mg/L), reduction-oxidation potential (mv), total dissolved solids (TDS mg/L⁻¹), ammonia (NH_3 , mg/L), nitrate (NO_3 , mL/L), ammonium (NH_4 , mg/L), and pH. We compared two seasons (wet and dry). Other studies from the lake have reported that there are no significant differences between the environmental variables between different points of the lake or between different months of the year [35,36,41], and most environmental variables have remained in the same range for decades (1995 to 2019) [36]. Whether the feeding strategy of the fish species change between the two seasons of the year is, however, not known.

2.3. Fish Collection

We conducted fish sampling over an annual cycle from May 2019 to May 2020 for diet analysis every three months and for stable isotopes in two seasons (dry and wet). Multiple fishing methods were used to sample the fish. We used a seine net (25 m length, 1.8 m height, and 5 mm mesh size) to sample fish in areas with a maximum depth of 1.5 m. We also used minnow traps (stainless steel, square mesh 0.5 m, cylindrical, 42 cm long and 19 cm in diameter, with two 2.5 cm holes with inverted cone inlets) set for one hour per site. Shallow (<1 m) areas near shores within site were sampled with electrofishing for half an hour (DC-backpack electrofisher model ABP-3, ETS electrofishing systems, LLC, average power~200 W, peak voltage~250 V, peak current~10 A). In addition, specimens were also obtained from local fishermen. We identified fish species using published keys [42]. The fish were euthanized with an overdose of benzocaine. Fishes for stable isotope analysis were preserved in ice water, and fish for stomach content analysis were preserved in 4% formaldehyde and transported to the Aquatic Biology Laboratory from the Universidad Michoacana de San Nicolás de Hidalgo. All field sampling techniques and fish handling protocols were reviewed and approved by the Mexican Ministry of Environmental and Natural Resources (SEMARNAT-SGPA/DGVS/00012/19), Ministry of the Environment, Climate Change and Natural Resources (SEMACCDET-OS. 0084/2019), and the Secretariat of Agriculture and Rural Development (SAGARPA: PPF/DGOPA-014/20).

2.4. Invertebrate Collection

In addition to fish, we gathered samples of other food web components. Thus, we sampled benthic aquatic macroinvertebrates using a 500 μm D-shape net. The collected specimens were separated in situ from the abiotic material and subsequently transported to the laboratory. Macroinvertebrates were processed per individual when the size of the organism was sufficient to obtain at least 2 mg (dry weight); if individuals were not large enough (i.e., *Hyalella*), we pooled all available individuals from each site per season. Plankton was collected by filtering 300 L of water covering the entire water column using two nets of different mesh sizes (64 μm and 200 μm). These bulk samples contained a mix of phytoplankton (larger sizes) and zooplankton. All the samples were frozen at $-20\text{ }^{\circ}\text{C}$ until laboratory processing.

2.5. Laboratory Analysis

Standard length and weight were measured for each individual fish. Each fish's stomach was dissected, and the stomach was preserved in 5% formaldehyde. Later the content was analyzed manually using a dissecting microscope at 10x magnification; prey items were identified to the lowest possible taxonomic level using invertebrate keys (for insects: [43] and non-insects: [44]). For the species *Cyprinus carpio* and *Algansea tincella*, we could not evaluate the stomach content due to the low capture of organisms, and they were, therefore, only considered in the stable isotopic analysis.

From the fish used for stomach analysis, muscle tissue samples (2 cm^3) from the lateral back region of three specimens of all fish species were taken, labeled, transferred to plastic bags, frozen at $-20\text{ }^{\circ}\text{C}$, and stored until processing.

Fish muscle tissue, plankton samples, and macroinvertebrate individuals were dried and pulverized, and~1 mg was weighed into tin capsules and analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ stable isotopes. Stable isotope analysis was performed at the University of New Mexico Department of Earth and Planetary Sciences. Two percent of all samples were processed in duplicate (mean standard error 0.5‰ for C and 1.5‰ for N). The stable isotope analyses were conducted based on 112 fish, 4 phytoplankton samples, 4 zooplankton samples, and 29 invertebrate samples from the wet season and 118 fish, 4 phytoplankton samples, 4 zooplankton samples, and 26 invertebrate samples from the dry season.

2.6. Data Analysis

2.6.1. Environmental Variables

We tested for differences in environmental variables among seasons (dry and wet) and sites (four sites) using Kruskal Wallis non-parametric analyses of variance (Dunn test, *posteriori*). The analysis was performed only for the samples obtained to describe isotopes because the abiotic characteristics related to the diet analysis have already been described and analyzed elsewhere [36].

2.6.2. Diet Analysis

To determine whether the number of analyzed stomachs accurately described the diet of the species, a prey accumulation curve followed the criteria of [45]. We calculated the percent of a prey-specific index of relative importance (%PSIRI) to reveal the dominance of prey using the following equation [46]:

$$\%PSIRI = \%FO_i \times (\%PN_i + \%PW_i) / 2$$

%PSIRI includes the percent frequency of occurrence (%FO_i = a number of stomachs with prey *i* divided by the total number of stomachs). Additionally, it includes the prey-specific abundance (%PN_i, %PW_i): %PA_i = $\sum_{j=1}^n \%A_{ij} / n_i$, the average percent abundance (%N_i, %W_i): %A_i = $\sum_{j=1}^n \%A_{ij} / n$, where %A_{ij} is the abundance (counts or weights) of prey *i* in stomach sample *j*, *n_i* is the number of stomachs containing prey *i*, and *n* is the total number of stomachs. The trophic level of each taxon allowed us to gain information on the position that organisms occupied in the food web and was estimated using the TrophLab software [47]. The results were expressed as a trophic level between 1 and 5, where 0–1 corresponds to decomposers, 1–2 to primary producers, 2–3 to primary consumers, 3–4 to secondary consumers, and >4 to tertiary consumers. The omnivory level was estimated by the omnivory index (OI) proposed by [48]. The index is dimensionless and describes the distribution of interactions at each trophic level, and the values are in the range between 0 and 1. When OI is close to 0, the consumer is considered a specialist. If the value of OI is close to 1, the consumer feeds on several trophic levels. Diet overlaps between different fish taxa were assessed via Horn's index [49] using the following equation:

$$Ro = \sum (P_{ij} + P_{ik}) \log (P_{ij} + P_{ik}) - \sum p_{ij} \log P_{ij} - \sum p_{ik} \log P_{ik} / 2 \log 2$$

where Ro = is Horn's niche overlap index between the species; P_{ij} is the proportion of resource *i* that is used by species *j*; P_{ik} is the proportion of the resource *i* that is used by the species *k*; and *N* is the total number of resources. The values in this index vary from 0 to 1. Values between 0 and 0.29 indicate low superposition, values between 0.30 and 0.65 indicate medium superposition and values > 0.66 indicate high overlap [49]. Fish species diets according to the PSIRI index were compared using cluster analysis following the Bray–Curtis ordination method and Euclidian distance as an index of dissimilitude. To complement the cluster analysis and to visualize the main prey and fish species, we used a principal component analysis (PCA). To test for significant differences in the diet composition between the groups that emerged from the cluster analysis, a non-parametric Permutational Multivariate Analysis of Variance (PERMANOVA; Bray Curtis index) was performed.

2.6.3. Stable Isotopes

Differences in carbon and nitrogen isotopic signatures between species from different sites were analyzed with ANOVA with a posthoc Tukey test to identify significant differences; for differences between seasons, a student's test was applied. A bivariate plot of the mean (±1 SE) isotopic values (δ¹⁵N on the y-axis; δ¹³C on the x-axis) was made for all fishes (per species), including grouped benthic invertebrates, zooplankton, and phytoplankton.

The niche breadth and isotopic overlap degree of the species were estimated by computerizing the standard Bayesian ellipses area in R (SIBER) in the bidimensional isotopic space of δ¹⁵N and δ¹³C values [50]. These metrics reflect assemblage trophic niche

space as the area occupied within a $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ biplot as a range in $\delta^{13}\text{C}$ (CR), range in $\delta^{15}\text{N}$ (NNR), mean distance to the centroid (CD), and total area (TA). CR is a measure of basal $\delta^{13}\text{C}$ variation, NNR provides information on the food chain length, CD indexes trophic diversity within the assemblage, and TA represents the total trophic niche space occupied by the assemblage. The extent of trophic redundancy within the assemblage was measured by two additional metrics: mean nearest neighbor distance (MNND) and standard deviation of the nearest neighbor distance (SDNND). Theoretically, smaller MNND and SDNND indicate greater trophic redundancy (groups with similar trophic niches; [51,52]). Corrected Bayesian standard ellipse area (SEAc) was used to quantify the trophic niche space of the fish community, and the overlap niche was obtained between seasons and sites [48]. To determine the trophic position of the fish species, we used a Bayesian model using the package tRophicPosition in R for the calculation of consumer trophic position using C and N stable isotopes [53].

3. Results

3.1. Environmental Variables

Kruskal-Wallis test showed no significant differences between the dry and wet seasons for temperature ($\chi^2 = 3.6, p = 0.056$), dissolved oxygen ($\chi^2 = 0.2, p = 0.599$), TDS ($\chi^2 = 0.8, p = 0.369$), conductivity ($\chi^2 = 1.8, p = 0.170$), NO_3 ($\chi^2 = 2.6, p = 0.102$), and NH_4 ($\chi^2 = 2.2, p = 0.134$) (Table 1).

Table 1. Physical and chemical water characteristics of Lake Zacapu, Michoacán, Mexico. Temp = water temperature ($^{\circ}\text{C}$), DO = dissolved oxygen (O_2 mg/L), pH = potential for hydrogen, TDS = total dissolved solids (mg/L), Cond = conductivity ($\mu\text{S}/\text{cm}$), NH_3 = ammonia (mg/L), NO_3 = nitrate (mg/L), NH_4 = ammonium (mg/L). Average and standard deviation ($\bar{X} \pm \text{SD}$). Kruskal Wallis differences among seasons and sites. * Significant differences.

Parameter	Dry Season	Wet Season
Temp	18.5 \pm 1.5	19.9 \pm 0.7
Season		$\chi^2 = 3.6486, p = 0.056$
Sites		$\chi^2 = 1.723, p = 0.631$
DO	13.7 \pm 2.7	14.3 \pm 0.2
Season		$\chi^2 = 0.2757, p = 0.599$
Sites		$\chi^2 = 9.3971, p = 0.064$
pH	7.7 \pm 0.4	7.3 \pm 0.2
Season		$\chi^2 = 6.419, p = 0.011$ *
Sites		$\chi^2 = 0.74108, p = 0.86$
TDS	107.9 \pm 2.1	109.2 \pm 2.4
Season		$\chi^2 = 0.80397, p = 0.37$
Sites		$\chi^2 = 1.0738, p = 0.78$
Cond	136.7 \pm 2.6	138.8 \pm 1.8
Season		$\chi^2 = 1.875, p = 0.17$
Sites		$\chi^2 = 1.0429, p = 0.79$
NH_3	0.07 \pm 0.02	0.11 \pm 0.03
Season		$\chi^2 = 6.1799, p = 0.012$ *
Sites		$\chi^2 = 0.18107, p = 0.98$
NO_3	7.4 \pm 1.0	6.7 \pm 0.6
Season		$\chi^2 = 2.6694, p = 0.10$
Sites		$\chi^2 = 0.13889, p = 0.98$
NH_4	0.06 \pm 0.02	0.09 \pm 0.05
Season		$\chi^2 = 2.2443, p = 0.13$
Sites		$\chi^2 = 0.0171, p = 0.99$

Significant differences were, however, found for pH ($\chi^2 = 6.4, p = 0.0113$) and NH_3 ($\chi^2 = 6.1, p = 0.012$). Dunn test shows that pH in the dry season was higher than in the wet

season ($\alpha = 0.05$, $p < 0.01$), and vice versa for NH_3 ($\alpha = 0.05$, $p < 0.01$). We did not find significant differences among the sites for any of the other variables (Table 1).

3.2. Diet Analysis

Eleven species were recorded in the lake, and these belonged to four families, Goodeidae being the most common (68% of all the species) (Table 2).

Table 2. Diet and trophic classification of fish species sampled in Lake Zacapu. The values for each item type and fish species represent the Variation in the Prey-Specific Index of Relative Importance (PSIRI). Abbreviations of minor taxa identified: Fish/R = fish and fish remains; AcIn/R = Aquatic insect remains; Chi = Chironomidae; Hya = *Hyalella*; Cam = *Cambarellus*; Ost = Ostracods; Bos = *Bosmina*, Cop = copepods; Gas = gastropods, Biv = Bivalvia, Ker = *Keratella*, Frag = Fragilaria; Dia = diatoms; Veg/R = vegetal remains; Pot = *Potamogeton*; Plu = Plumatelidae. Origin of the species: * = native, ** = endemic, + = nonnative.

Fish Species	Fish/R	AcIn/R	Chi	Hya	Cam	Ost	Bos	Cop	Detritus
Goodeidae									
* <i>Allophorus robustus</i>	7.1	1.2		1.9	13.0			0.3	0
** <i>Allotoca zacapuensis</i>		13.8	4.8	22.9		0.6		0.8	0
* <i>Goodea atripinnis</i>	4.5		1.5			6.9	7.5	2.5	9.7
* <i>Hubbsina turneri</i>		3.9	2.7	31.2		2.3	4.5	3.9	0
* <i>Skiffia lermæ</i>	0.7	4.6		0.9		0.4	6.0	1.6	5.1
* <i>Xenotoca variata</i>	8.1		8.2	27.4		9.3		4.9	0.3
* <i>Zoogoneticus quitzeoensis</i>	7.6	2.2	3.9	13.7			0.4	10.9	3.6
Atherinopsidae									
* <i>Chirostoma humboldtianum</i>	1.5	0.5	0.5	27.1	0.4	2.2	31.0	5.2	0
Poeciliidae									
* <i>Poeciliopsis infans</i>	3.1	0.2	0.2	0.6		0.9	24.6		6.1
Cyprinidae									
+ <i>Ctenopharyngodon idella</i>									21.3
** <i>Notropis grandis</i>				20.3		13.3	9.6	1.9	0
Fish Species	Gast	Biv	Ker	Frag	Dia	Ve/R	Pot	Plu	
Goodeidae									
* <i>Allophorus robustus</i>	5.7	1.2							
** <i>Allotoca zacapuensis</i>						0.4			
* <i>Goodea atripinnis</i>	1.0	0.4	3.0	4.4	13.9				
* <i>Hubbsina turneri</i>		3.6				0.3			0.9
* <i>Skiffia lermæ</i>			0.2	14.8	1.1	0.6			
* <i>Xenotoca variata</i>		0.6	0.1	0.1	0.1	0.1			0.2
* <i>Zoogoneticus quitzeoensis</i>					0.4				
Atherinopsidae									
* <i>Chirostoma humboldtianum</i>	0.9	0.9	2.6				0.2		
Poeciliidae									
* <i>Poeciliopsis infans</i>					1.8				
Cyprinidae									
+ <i>Ctenopharyngodon idella</i>								48.2	13.2
** <i>Notropis grandis</i>	6.8			0.6	0.1				

Apart from two (*C. idella* and *C. carpio*), all fish species present in the lake are native to Mexico. From a total of 3403 stomachs, 3229 (94%) were analyzed and used for establishing feeding groups. The remaining stomachs were empty or had indeterminate dietary content.

The food items recorded were classified as follows: Chordata (including whole fish, scales, fins, and fish remains); Arthropoda (including insect remains and the family Chironomidae); Crustacea (including Malacostraca [genera *Hyalella* and *Cambarellus*], Ostracoda, Branchiopoda [genus *Bosmina*], and Maxillopoda [copepods]); Mollusca (including Gastropoda and Bivalvia); Rotifera (comprising Monogononta, genus *Keratella*); Diatomista (including Diatomea [genus

Fragilaria, *Synedra*, *Cymbela*]; Magnoliophyta (including vegetal remains and Liliopsida within the genus *Potamogeton*), and Bryozoa (genus *Plumatella*) (Table 2).

The PSIRI index revealed that *A. robustus* mainly fed on *Cambarellus* (13.0), followed by fish remains (7.1). *Hyalella* was the item occurring in the highest proportion for many species, *Z. quitzeoensis* (13.7), *N. grandis* (20.3), *A. zacapuensis* (22.9), *H. turneri* (31.2), *X. variata* (27.4), and *C. humboldtianum* (27.1). The species *C. idella* was the only one consuming *Potamogeton* (21.3). *Goodea atripinnis* mainly fed on detritus (9.7), *S. lermæ* mainly on aquatic insect remains (4.6; 28.6, respectively), and *P. infans* on *Bosmina* (24.6) (Table 2).

C. idella had the most specialized niche breath, and *G. atripinnis* was the most generalist species (Table 3).

Table 3. Trophic level (average ± SD) and omnivory index for the fish species caught in Lake Zacapu.

Fish Species	Trophic Level	Omnivory Index
<i>A. robustus</i>	3.5 ± 0.52	0.20
<i>A. zacapuensis</i>	3.3 ± 0.45	0.10
<i>G. atripinnis</i>	2.8 ± 0.37	0.29
<i>H. turneri</i>	3.1 ± 0.40	0.12
<i>S. lermæ</i>	2.8 ± 0.35	0.22
<i>X. variata</i>	2.8 ± 0.37	0.10
<i>Z. quitzeoensis</i>	3.0 ± 0.39	0.12
<i>C. humboldtianum</i>	3.3 ± 0.44	0.25
<i>P. infans</i>	3.1 ± 0.46	0.05
<i>C. idella</i>	2.6 ± 0.14	0.01
<i>N. grandis</i>	2.9 ± 0.38	0.15

All species occupied two positions in the trophic web: primary and secondary consumers (Table 3). The omnivory index indicated that most species were generalists (Table 3). *G. atripinnis*, *S. lermæ*, and *A. robustus* had high omnivory index values, indicating that these species fed on more trophic levels than the other species. Horn’s niche overlap index suggests high food overlap between the species *A. zacapuensis* vs. *H. turneri*, *X. variata*, *Z. quitzeoensis*; *S. lermæ* vs *C. idella*, *G. atripinnis*; *X. variata* vs *Z. quitzeoensis*, *C. humboldtianum*, *N. grandis*; *C. humboldtinum* vs. *N. grandis*; and *G. atripinnis* vs. *S. lermæ* (Table 4).

Table 4. Values of Horn’s niche overlap index for fish species in Lake Zacapu. *Ar* = *Allophorus robustus*, *Az* = *Allotoca zacapuensis*, *Ht* = *Hubbsina turneri*, *Sl* = *Skiffia lermæ*, *Xv* = *Xenotoca variata*, *Zq* = *Zoogoneticus quitzeoensis*, *Ch* = *Chirostoma humboldtianum*, *Pi* = *Poeciliopsis infans*, *Ci* = *Ctenopharingodon idella*, *Ng* = *Notropis grandis*, *Ga* = *Goodea atripinnis*. White = low superposition, light gray = medium superposition, black color = high overlap between the species.

	<i>Ar</i>	<i>Az</i>	<i>Ht</i>	<i>Sl</i>	<i>Xv</i>	<i>Zq</i>	<i>Ch</i>	<i>Pi</i>	<i>Ci</i>	<i>Ng</i>	<i>Ga</i>
<i>Ar</i>	1	0.33	0.35	0.16	0.40	0.38	0.30	0.19	0.00	0.29	0.15
<i>Az</i>		1	0.87	0.37	0.83	0.72	0.54	0.13	0.00	0.52	0.20
<i>Ht</i>			1	0.47	0.88	0.79	0.79	0.33	0.00	0.72	0.38
<i>Sl</i>				1	0.45	0.52	0.49	0.51	0.66	0.50	0.76
<i>Xv</i>					1	0.93	0.67	0.24	0.03	0.72	0.36
<i>Zq</i>						1	0.63	0.33	0.07	0.66	0.45
<i>Ch</i>							1	0.68	0.01	0.80	0.50
<i>Pi</i>								1	0.10	0.45	0.57
<i>Ci</i>									1	0.04	0.38
<i>Ng</i>										1	0.52
<i>Ga</i>											1

The combination of the PSIRI values for each dietary item (Table 2) used in a cluster analysis revealed a classification into three trophic groups: Carnivore (one species, mostly feeding on insects and mollusks), omnivore-carnivore (six species feeding mostly

on copepods, ostracods, *Hyaella*, and fish remains), and omnivore-herbivore (four omnivorous species feeding mostly on rotifers, cladocerans, aquatic plants, diatoms, and detritus) (Figures 2 and 3).

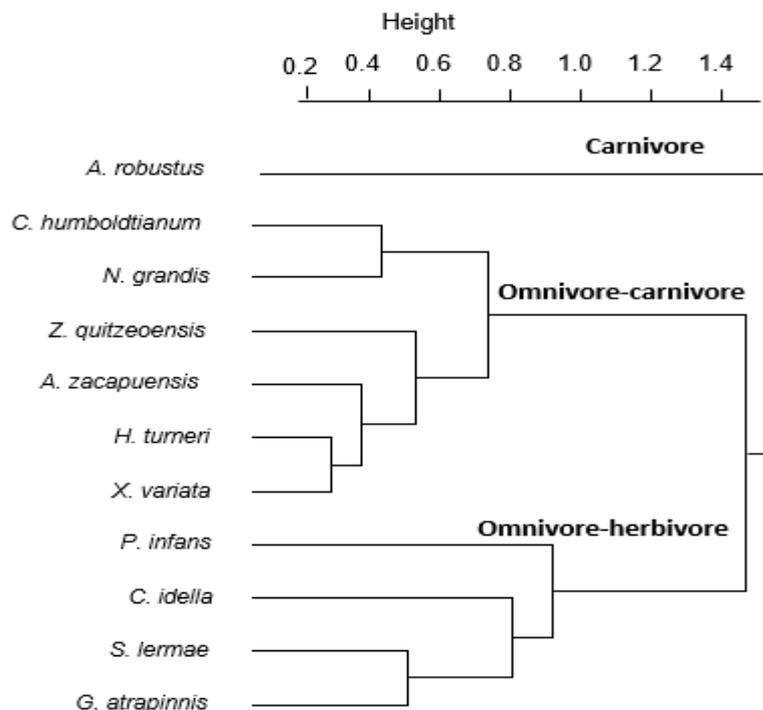


Figure 2. Cluster analysis of the trophic classification of fish species from Lake Zacapu.

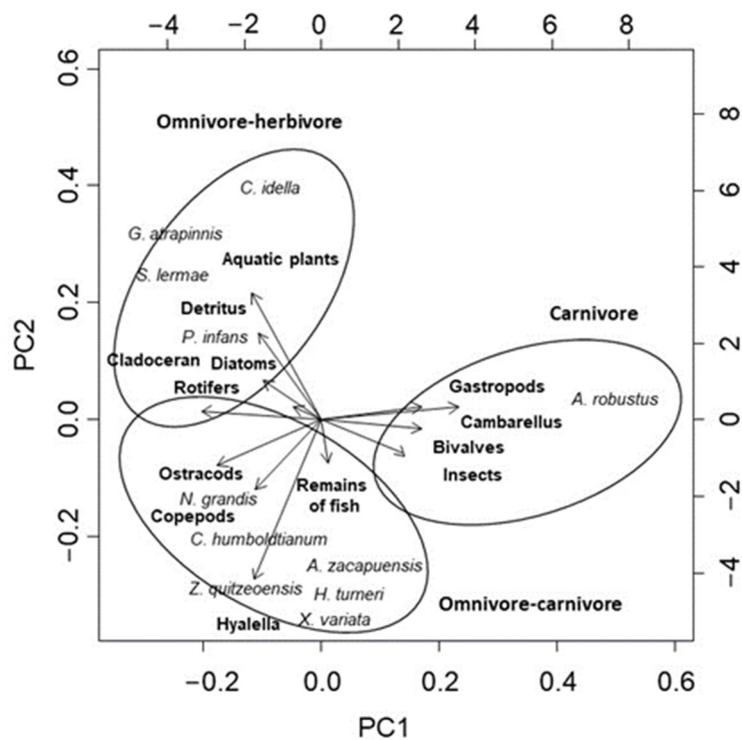


Figure 3. Principal component analysis showing the position of the trophic groups of fish species in Lake Zacapu.

The principal component analysis (PCA) separated out the same three trophic groups (Figure 3), with the first two axes explaining 66% of the variation (PC1 = 47% and

PC2 = 19%). The PERMANOVA test gave strong statistical support to this classification, with significant differences in PSIRI index values for the dietary items between the groups ($F = 6.35, p < 0.001$).

3.3. Stable Isotopes

We found significant differences between seasons in $\delta^{13}\text{C}$ for *A. tincella* ($t = 0.506, p = 0.016$) and *C. carpio* ($t = 0.947, p = 0.002$); the post hoc test showed the most enriched values in the wet season. Significant differences between seasons in $\delta^{15}\text{N}$ were found for *H. turneri* ($t = 0.320, p = 0.021$) and *P. infans* ($t = 0.238, p = 0.005$), and the post hoc test showed the most enriched in the dry season. Significant differences among sites in $\delta^{13}\text{C}$ ($F = 8.047, p = 0.001$) and $\delta^{15}\text{N}$ ($F = 4.149, p = 0.021$) were only found for *G. atripinnis*; the Tukey test showed that site 3 had the highest values of $\delta^{13}\text{C}$ and the lowest values of $\delta^{15}\text{N}$ ($p < 0.05$). *P. infans* showed significant differences among sites in $\delta^{15}\text{N}$ ($F = 3.4811, p = 0.033$), and *Z. quitzeoensis* exhibited differences in $\delta^{13}\text{C}$ ($F = 3.436, p = 0.037$); the Tukey test showed that site 1 had the lowest values for both species. *A. robustus* occupied the highest fish trophic position in the food web, based on relative $\delta^{15}\text{N}$ values and food components (Figure 4), while *G. atripinnis* and *C. idella* had the lowest position. *A. tincella* had the most enriched $\delta^{13}\text{C}$ values of all the food web components. Except for *C. humboldtianum* and *A. tincella*, the fish species had average $\delta^{13}\text{C}$ values, ranging between -28 and -18% .

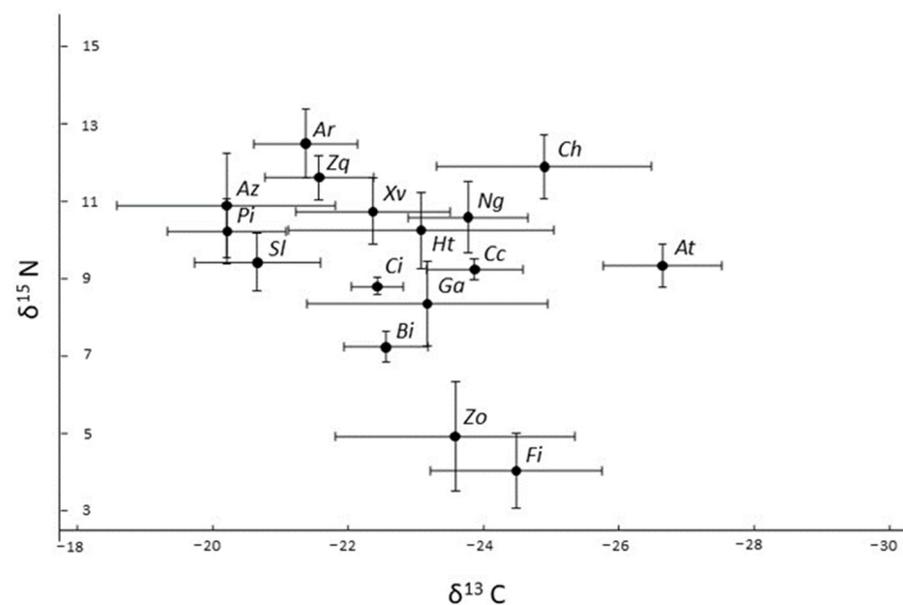


Figure 4. Lake Zacapu food web plot based on analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope values, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($\pm 1\text{SE}$). Acronyms: Ar = *Allophorus robustus*, Az = *Allotoca zacapuensis*, Ht = *Hubbsina turneri*, Sl = *Skiffia lermæ*, Xv = *Xenotoca variata*, Zq = *Zoogoneticus quitzeoensis*, Ch = *Chirostoma humboldtianum*, Pi = *Poeciliopsis infans*, Ci = *Ctenopharingodon idella*, Cc = *Cyprinus carpio*, Ng = *Notropis grandis*, At = *Algansea tincella*, Ga = *Goodea atripinnis*, Bi = benthic invertebrates, Zo = zooplankton, Fi = phytoplankton.

No clear seasonal pattern was found in the community-wide trophic structure (Figure 5, Table 5). However, the degree of trophic diversity, measured as CD, was greatest in the dry season. Site 3 had the lowest CD value, while sites 1 and 4 had the same or a higher value (2.77) (Table 5).

Table 5. Metrics of the niche breadth and isotopic overlap between the species per site and season. Abbreviations: CR = range in $\delta^{13}\text{C}$, NNR = range in $\delta^{15}\text{N}$, TA = total area, SEA = Bayesian standard ellipse area, SEAc = Bayesian standard ellipse area corrected.

	NNR	CR	TA	SEA	SEAc
Wet Season	11.3	10.7	82.4		
Fish			49.5	9.1	9.2
Phyto			1.7	2.3	3.5
BMI			21.5	4.8	4.9
Zoo			4.3	5.3	7.9
Dry Season	10.8	12.7	88.3		
Fish			49.5	9.1	9.2
Phyto			1.7	2.3	3.5
BMI			21.5	4.8	4.9
Zoo			4.3	5.3	7.9
Site 1	10.7	8.9	64.0		
Fish			38.2	9.7	9.8
BMI			15.1	5.7	6.1
Phyto-Zoo			3.0	3.6	5.4
Site 2	9.1	12.6	74.8		
Fish			39.2	9.6	9.7
BMI			10.7	4.8	5.2
Phyto-Zoo			8.6	10.5	15.7
Site 3	10.6	9.8	63.6		
Fish			34.2	7.5	7.5
BMI			10.6	5.2	5.8
Phyto-Zoo			5.1	6.3	9.5
Site 4	10.3	9	68.19		
Fish			32.7	9.0	9.2
BMI			22.8	9.6	10.5
Phyto-Zoo			1.8	2.2	3.3

The metric MNND, which declines in webs with overlap, indicated a divergent trophic niche or low overlap in both seasons and at all sites. The metric SDNND, which measures the evenness of species isotopic distributions, was highest in the dry season and at site 2 (Figure 5, Table 5).

The $\delta^{13}\text{C}$ isotope mean values of the baseline (macroinvertebrates) were -21.9 ± 1.4 for site 1, -22.8 ± 1.1 for site 2, -22.8 ± 1.3 site 3, and -22.8 ± 1.9 for site 4; for $\delta^{15}\text{N}$, the mean values were 7.1 ± 1.5 for site 1; 7.3 ± 1.4 for site 2; 7.6 ± 1.4 for site 3, and 7.8 ± 1.9 for site 4 (Figure 6). The $\delta^{13}\text{C}$ values for the consumers (fish) were 23.0 ± 2.2 for site 1, -22.8 ± 2.0 for site 2, -21.9 ± 1.8 for site 3, and -22.7 ± 2.1 for site 4, while the $\delta^{15}\text{N}$ values were 10.6 ± 1.4 for site 1, 10.5 ± 1.6 for site 2, 10.6 ± 1.3 for site 3, and 10.8 ± 1.4 for site 4 (Figure 6).

There were no significant differences between sites for either $\delta^{13}\text{C}$ ($F = 1.6057$, $p = 0.188$) or $\delta^{15}\text{N}$ ($F = 0.6551$, $p = 0.580$). In the wet season, the values of the consumers were 10.6 ± 1.4 for $\delta^{15}\text{N}$ and -22.5 ± 2.0 for $\delta^{13}\text{C}$. For the dry season, the $\delta^{13}\text{C}$ value was -22.7 ± 2.1 and the $\delta^{15}\text{N}$ value 10.7 ± 1.4 ; the baseline (macroinvertebrates) in the dry season was -22.5 ± 1.8 for $\delta^{13}\text{C}$ and for 7.5 ± 1.6 $\delta^{15}\text{N}$; in the wet season $\delta^{13}\text{C}$ was $0-22.5 \pm 1.0$ and $\delta^{15}\text{N}$ 7.3 ± 1.6 . The baseline of Zoo in the wet season was -24.6 ± 2.1 for $\delta^{13}\text{C}$ and 4.4 ± 1.1 for $\delta^{15}\text{N}$, and in the dry season it was -22.9 ± 5.2 for $\delta^{13}\text{C}$ and 6.1 ± 1.1 for $\delta^{15}\text{N}$ (Figure 6). There were no significant differences between the dry and wet seasons for either $\delta^{13}\text{C}$ ($F = 0.5600$, $p = 0.455$) or $\delta^{15}\text{N}$ ($F = 1.4406$, $p = 0.231$).

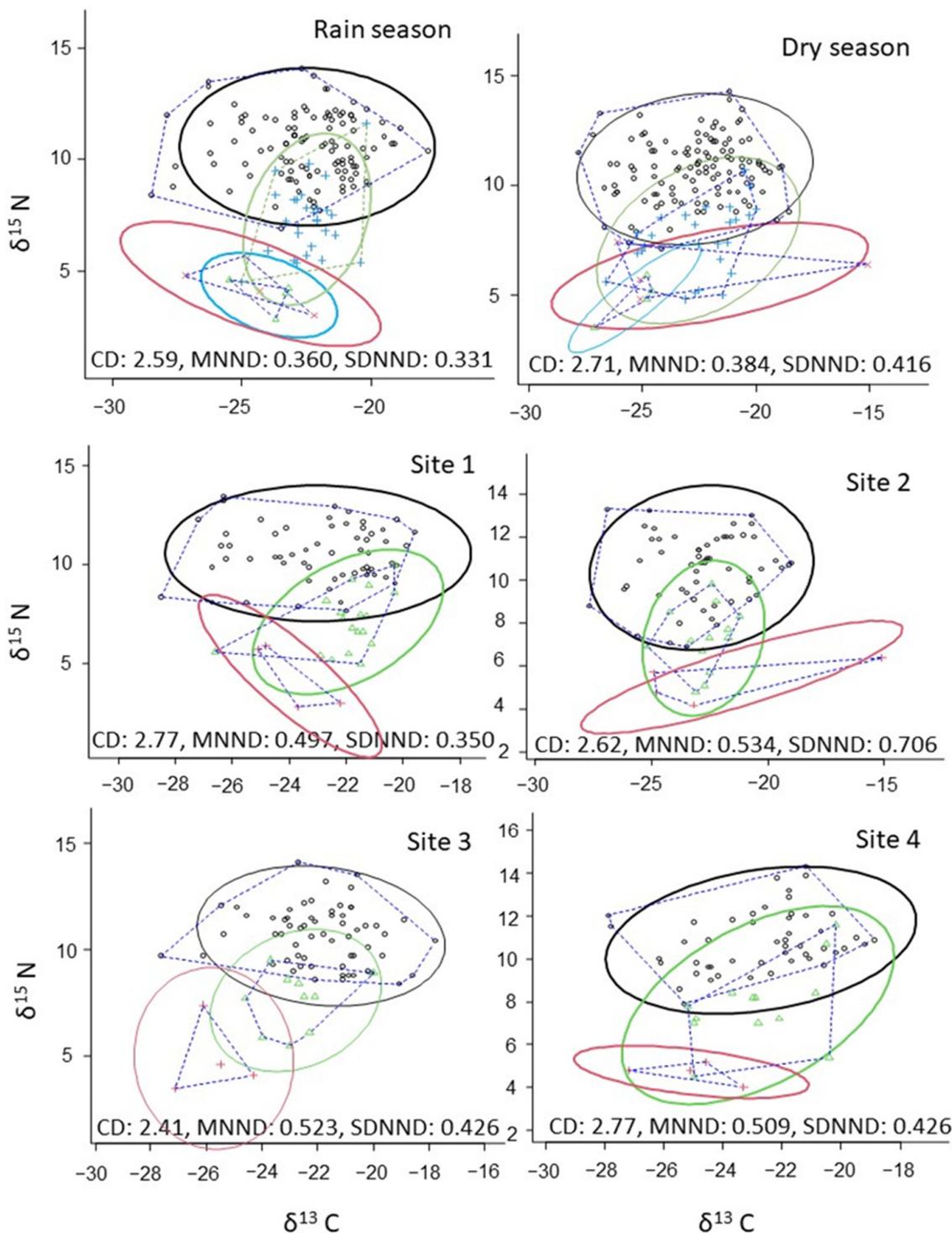


Figure 5. Stable isotope ellipses encircling one standard deviation of the bivariate data for fish species (black line), macroinvertebrates (green line), phytoplankton (blue line), and zooplankton (red line) collected in Lake Zacapu. Layman metrics for each season and site are shown in the plots; phytoplankton and zooplankton communities per site are shown together (red line). CD mean distance to centroid; MNND mean nearest neighbor distance; SDNND standard deviation of nearest neighbor distance.

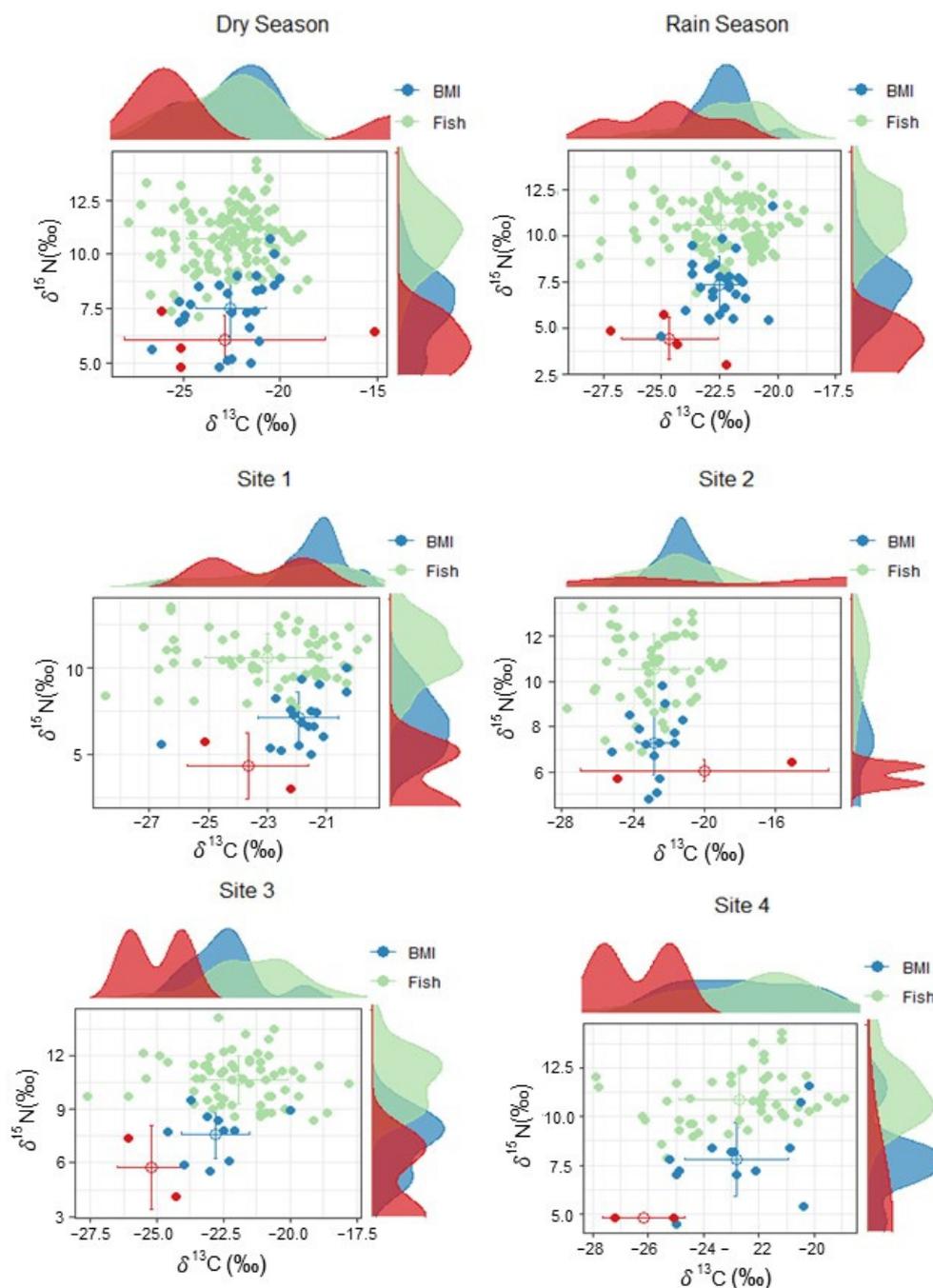


Figure 6. Bayesian model for the calculation of consumer (fish species: green color) trophic position using stable isotopes of C and N with BMI (macroinvertebrates: blue color) and Zooplankton (red color) as baseline for the different sites and seasons in Lake Zacapu.

4. Discussion

This study focused on elucidating the diet and trophic structure of the fish species present in Lake Zacapu. We demonstrated that (1) analysis of stomach content and analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ complement each other, (2) there was a great availability and diversity of food resources for the fish species in the lake, (3) the native species had a low trophic position, as well as a wide spatial trophic niche and niche width at all sites and both seasons studied, indicating that they were mainly generalists, (4) the trophic structure of a group of related species (the family Goodeidae) revealed similar feeding habits, (5) Trophic diet overlap was greater between native species than between non-native species.

The stomach content data and isotopic signatures provided overall similar results for the trophic positions for the different species (Figure 4), confirming our first hypothesis. However, it is well documented that the stomach content does not fully capture the trophic position. Diet data typically only provide a snapshot of the consumption (hours to days), while the stable isotopes integrate over a longer time (weeks to months) [20]. Moreover, some fish specimens had empty stomachs. In our study, *C. carpio* and *A. tincella* could not be considered for the stomach content analyses, for example, as only a few fish were captured and most had empty stomachs, in part because some were captured by the fishermen who use gill nets set over-night, leading to defecation. Else the stomach content provides valuable information on the taxonomic composition of the diets (Table 2), which could not be obtained by the stable isotope analysis. Combining both techniques (stomach content and stable isotopes), therefore, improves our interpretation of the diet of the species and the food web structure in the lake.

As in other studies of Lake Zacapu [35,36,41], we found homogeneous environmental conditions, both spatially and temporally, reflecting continuous circulation with little zonation due to the shallowness of the system, high capacity for hydraulic renewal and small spatial dimensions [33]. Only ammonia and pH differed slightly between the two seasons (Table 1).

In accordance with our second hypothesis, the fish relied mainly on internal food sources, while terrestrial organisms were of minor importance. The aquatic macroinvertebrate data revealed 79 genera, and the analysis of the macroinvertebrate samples showed no differences among sites in community structure and organization [54]. The most abundant macroinvertebrate genera in the diet were *Hyalella* (40%), *Cambarellus* (4.5%), *Belostoma* (3.2%), and gastropods (*Physella*: 12.5%, *Pomatiopsis*: 6.3%, and *Valvata*: 8.0%), indicating ample food resources for the fish in the lake, reducing the competition between the species.

Macroinvertebrates played an important role as a food resource for most species (Table 2). For example, the endemic *N. grandis* is an omnivorous species with a tendency to carnivory (Figure 2), which fed mainly on *Hyalella* (Table 2), as also demonstrated in an earlier study of other species of the genus [55]. The relict species *H. turneri* also fed mainly on *Hyalella* and microcrustaceans (copepods and cladocerans), as also reported in other studies [39,56], and the micro-endemic species *A. zacapuensis* mainly ingested *Hyalella* and aquatic insects (Table 2). Three species are restricted to this small lake: *N. grandis* [57], *H. turneri* [58], and *A. zacapuensis* [59]. They had similar feeding habits (ingesting *Hyalella* and aquatic insects) but only occurred in low abundance in specific areas of the lake [36]. The three species occur in different habitats; *N. grandis* lives in the littoral zone of the lake [36], *A. zacapuensis* restricting to one of the springs (Site 3) [35] with dense vegetation [41], and *H. turneri* lives in the littoral areas with abundant aquatic vegetation [58].

Regarding our third hypothesis, we found higher trophic diet overlap between native species than for non-native. Species from the same family, such as *Z. quitzeensis*, *A. zacapuensis*, *H. turneri*, and *X. variata*, were grouped into the same guild due to the similarity of the resources that they consumed (Figures 2 and 3), likely reflecting the taxonomic proximity between the species [60], all of them native to this lake. In principle, closer species should be strongly competitive due to high diet overlap [61], as we found for species of the Goodeid family (Table 4). However, high overlap does not necessarily mean that the species are competing if resources are abundant enough [62], as was the case for macroinvertebrates in Lake Zacapu [54].

Three trophic guilds were identified in the lake: carnivore, omnivore-carnivore, and omnivore-herbivore, as also reported 27 years ago for this lake [39]. The fish species ranged from primary to secondary consumers, with *A. robustus* (Table 3) having the highest $\delta^{15}\text{N}$ value (Figure 4). This species is also considered a secondary consumer in other Mexican aquatic systems [63] and feeds principally on insects, and is considered being the most highly developed carnivore in the Goodeidae family [42].

In the non-native versus native species, we only found diet overlap between *C. idella* and *S. lermae* (Table 4); however, the native species *S. lermae* consumed various items,

including *Bosmina*, aquatic insects, copepods, and detritus as their main food items, while *C. idella* only consumed *Potamogeton*, Plumatelidae and detritus (Table 1). This indicates that the overlap mainly concerns detritus, as also seen in the PSIRI index values. The other non-native species, *C. carpio*, occupied a lower position, as revealed by the $\delta^{15}\text{N}$ analysis (Figure 3), classifying it as omnivorous, which corresponds with the reports for other aquatic systems in which these carps are non-native species, for example, Lake Pátzcuaro [63] and Lake Xochimilco [64], and elsewhere [65]. The abundance of *C. carpio* was relatively low in the lake, and their population was controlled by the fishermen [36]. The results of the isotope signatures of *C. carpio* (Figure 4) and the diet overlap of the *C. idella* (Table 4) indicated that trophic diet overlap was greater between native species (especially between species from the same family) than for non-native species, as also seen in another study [16].

Another species with a relatively low abundances in the lake is the native *A. tincella* [36]. This species showed the most enriched values of carbon and low values of $\delta^{15}\text{N}$, classifying it as an omnivore. The genus *Algansea* is considered omnivorous, feeding mainly on filamentous algae, phytoplankton, benthic invertebrates, and zooplankton [42,66], which concurs with the observed niche overlap with *A. tincella* in our isotopic results (Figure 4).

Lake size has been shown to be an important determinant of the trophic composition of shallow subtropical lakes [67,68]. In the present study, both the stable isotopes and stomach contents indicated that the mean trophic level (Table 3) and the trophic position (Figure 4) of the fish species were overall similar to those reported for other small shallow lakes [68], while the higher trophic position has been found in Mexican lakes with larger surface areas, such as Lake Chapala [69] and Lake Pátzcuaro [70], though may vary with fish community composition [71]. In Lake Zacapu, the dominant species were carnivorous, omnivorous-carnivorous and omnivorous-herbivorous, and the preferred food item of most species were macroinvertebrates (Table 1), emphasizing the phylogenetic relatedness of the species in the Goodeidae family.

Low $\delta^{15}\text{N}$ values correlated with great $\delta^{13}\text{C}$ variation [72], thus, a wider trophic niche area. The fish isotopic signatures from this lake differ from previous records of feeding habitats of the species in other, more eutrophic, aquatic Mexican systems [66,69,73]. We found the $\delta^{15}\text{N}$ values to be lower and the trophic niche areas wider, suggesting that the species are opportunistic and are able to switch food sources depending on their abundance [13]. The omnivory index (Table 3) provides similar evidence, indicating that these species fed on more than one trophic level.

The SIBER analysis revealed similar values in the isotopic niche space (SEAc) and a similar trophic diversity based on the NNR, CR, and CD values in the dry and wet seasons (Table 5, Figure 5), which is also supported by the similar values of MNND and SDNND (trophic redundancy) in the two seasons. This suggests that the fish in this lake exploited similar resources in the dry and wet seasons studied. We, however, found a tendency to lower CD and SEAc values at site 3 and lower MNND and SDNND values at site 1 (Table 5; Figure 5), indicating lower trophic diversity. These sites were spring fed and located where most of the water entered the lake, and here the fish had aquatic macroinvertebrates as their main source of energy. The stable isotope data, however, suggests that they also consumed food with enriched $\delta^{13}\text{C}$ values, such as zooplankton (Figure 6). We found little variation in isotope values and diet between seasons, likely reflecting the low variation in environmental variables, including temperature, as compared to the temperate regions [74].

5. Conclusions

Lake Zacapu is characterized by environmental conditions that favor trophic generalist fish. Our findings reveal that the local food web structure did not differ significantly between the four sampling sites (Figure 6), which we attribute to aquatic macroinvertebrates [54] and environmental homogeneity (Table 1). Our results suggest a low trophic position of native species having a wide spatial trophic niche and niche width, indicating

that they fed on more than one trophic level. We also found overall good agreement in the trophic position determined by gut content and isotope analysis. Trophic diet overlap was greater between native species (especially between species from the same family) than between non-native species. Our study provides new information on the trophic interactions in a subtropical Mexican lake, rich in endemic species and an important resource for the local human communities.

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