Original Research

Historical analysis of an imperiled fish species: environmental variables modeling, biotic interactions, extirpation, and current restricted-range

Rodrigo Moncayo-Estrada1,*, José De La Cruz-Agüero1, Eugenia López-López2, Pablo Del Monte-Luna1, María Magdalena Díaz-Argüero2, Arturo Chacón-Torres3, Arely Ramírez-García4, Omar Domínguez-Domínguez5, Juan Pablo Ramírez-Herrejón6

1 Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional s/n Col. Playa Palo de Santa Rita, 23096 La Paz, B.C.S., México
2 Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prol. de Carpio y Plan de Ayala s/n, Col. Sto. Tomás, 11340 México, D.F., México
3 Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo, Avenida San Juanito Itzicuaro s/n, Nueva Esperanza, 58330 Morelia, Michoacán, México
5 Laboratorio de Biología Acuática, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Francisco J. Múgica S/N, 58030 Morelia, Michoacán, México
6 Conacyt-Universidad Autónoma de Querétaro, Facultad de Ciencias Naturales, Campus UAQ-Aeropuerto, Carretera a Chichimequillas s/n, Ejido Bolaños, 76140 Santiago de Querétaro, Querétaro, México
*Correspondence: rmoncayo@ipn.mx (Rodrigo Moncayo-Estrada)
Academic Editor: Graham Pawelec
Submitted: 23 December 2021 Revised: 30 January 2022 Accepted: 12 February 2022 Published: 20 May 2022

Abstract

Background: Analyses of spatial and temporal patterns and interactions are important for determining the abiotic factors limiting populations and the impact from other species and different anthropogenic stressors that promote the extirpation of species. The fish *Hubbsina turneri* de Buen (1940) was studied as a model species in a historical context at varying locations. Originally distributed only in the Lerma-Chapala basin, the main lake complex in Mexico, this species has not been collected from Lake Cuitzeo (LC) and now is restricted to Lake Zacapu (LZ). At present, the Highland splitfin is classified as critically endangered.

Methods: Historical information of LC and historical and current information from LZ were explored by applying cluster analysis and generalized additive mixed model (GAMM) to describe the biotic interactions among fish species and the relationship between density and environmental variables, respectively. The two lakes’ contrasting abiotic/biotic characteristics provided elements to describe some species distribution limits in chemical ion gradients. Extirpation calendar dates were estimated using an optimal linear estimation method. Finally, a bibliographic review was conducted on the causes that promoted the extirpation and restriction of *H. turneri* and the prognosis for its reestablishment and conservation.

Results: Clusters showed the fishes relationship according to their distribution along the lakes. GAMM indicated that high *H. turneri* density is related to low hardness/fecal coliforms, medium depth/suspended solids, and high oxygen concentration. Estimated extirpation dates were between the years 2013 and 2018. The extirpation was linked to an abrupt drop in the LC volume, water quality degradation, increased biotic interactions within macrophytes habitats with native and introduced species, and fisheries bycatches. The current restricted range of *H. turneri* resulted from the draining of the larger lake, forcing the remaining populations to small spring-fed remnants. Recent samplings in LZ resulted in a low number of individuals.

Conclusions: The integration of ecological interactions derived from statistical models, extirpation dates from nonparametric tests, and the exhaustive analysis of historical information can be applied to define the current situation of imperiled, ecologically relevant species, in different aquatic ecosystems. We are confident that this general framework is important for determining (1) the requirements and limitations of populations regarding abiotic variables, (2) biotic interactions (trophic and spatial) with native and introduced species, and (3) different anthropogenic stressors within and around the ecosystem. This knowledge will also allow understanding those aspects that contribute to the extirpation of populations and could help the restoration of the habitat and the reintroduction of extirpated species.

Keywords: endangered species; sub-tropical lakes; GAMM; OLEM; degradation; urban development
1. Introduction

There are three main ideas regarding important aspects while studying endangered species conservation [1], the first being environmental characteristics that determine habitat use, habitat requirements, and distribution [2]. Some local and restricted-range species are important indicators of environmental quality that can be used to prevent biodiversity loss, establish conservation priorities, and promote restoration practices [3]. Although different studies have reviewed the bio-ecological characteristics of fishes (e.g., [4,5]), an important constraint in Latin American research is the poor or incomplete historical scientific knowledge, particularly on imperiled species [6]. Previous studies have mainly included statistical models of species responses to environmental factors. Different studies have analyzed the patterns of multiple interacting factors affecting autoecological profiles with more flexible statistical models to create a comprehensive framework that relates these factors to species abundance [7–9].

The second topic is the determination of the extent to which different anthropogenic stressors can change the structure and function of freshwater ecosystems at different scales and promote the decrease of native populations and, in some extreme cases, local extinctions. Increasing demand for water by humans, significant depletion of water body volumes, pollution, habitat degradation, and the presence of introduced or invasive species, are closely correlated to population declines of endangered species [10–12]. For example, in Central Mexico, 25% of the sites with fish records had conditions that were no longer capable of sustaining life at the end of the 1980s [13]. In 2000, this figure increased to 40%, in 2012 to 53%, and in 2020 to 64% of the sites were classified as polluted or strongly polluted [14,15]. As a consequence, 40% of the fish species in the country exhibited some degree of risk, and three of them are extinct [16]. Information from historical sources and past field research can potentially reveal evidence about the drivers, rates, and magnitudes of declines [17]. Understanding the course of events related to an extinction incident is critical to prevent such incidents from occurring in the future [18].

The third topic is the opportunity for the reestablishment, recovery, and conservation of wild populations of imperiled fish species. In different countries, the restoration and conservation of aquatic systems have been intensively discussed over the last three decades and ecological studies have been encouraged for reestablishing fish populations [19–21]; however, progress has been limited, primarily due to the high cost of restoration and the low economic importance of the target species [22]. Therefore, greater emphasis has been placed on the ecosystem services provided by water bodies and the biological and ecological characteristics of the species as study models in different research fields (i.e., viviparity, diversification, adaptation, toxicity bioindicators) [23,24].

Different studies have used multivariate methods to explore fish assemblages, particularly hierarchical clustering [25]. Cluster analysis helps group species according to their redundant pattern’s similarity [26], which can be approximated to the ecological relationships [27]. Generally, information is aggregated temporally (e.g., year, season, month), spatially (e.g., location, depth), or even combining different types of capture gear [28,29]. Habitat models have been used to analyze species correlated and clustered responses to environmental variables, applying nonparametric techniques such as Generalized additive mixed models (GAMMs), which incorporate random effects [30]. GAMMs have been implemented in different aquatic ecosystems to account for environmental fluctuating variables’ temporal and spatial impact on endangered fish distribution within protected areas [9]. The optimal linear estimation method is regularly used from the sighting or collections records to determine the potential dates of extinction of an organism’s population and colonies in a conservative way [31]. The importance of establishing a potential time interval is that it allows the historical-critical revision of the possible events, both natural and anthropogenic, that promoted changes in the ecosystem and the loss of the species. This knowledge also provides those key elements that must be addressed for the restoration of the species [32].

The goodeid Hubbsina turneri was selected as a model species for analysis because, according to the data as far collected, it was endemic to Lake Cuítzoo (LC) and Lake Zacapu (LZ), Mexico, which are ecosystems with contrasting habitat characteristics [33,34]. This species currently has a restricted-range and is found only in LZ, which is the primary reason for the endangered status under the Mexican Official Norm (NOM-059-SEMARNAT-2010) and is listed as critically endangered by the IUCN [35]. The biological characteristics of this species have been studied at different time periods [33]; as a result, there is sufficient ecological information to analyze the species-habitat, species-species relationships, and estimate potential extinction dates. Our goals were to (1) determine and contrast the species interactions and water quality variables that have influenced the populations of this species in both lakes by using nonparametric statistical analyses; (2) estimate possible extinction dates of the species in LC, using linear estimation methods, aiming at establishing and identifying potential threats that may have led to this extinction; and (3) review and discuss the historical process that led to the restriction of H. turneri to LZ and the constraints to its reintroduction to LC.

2. Materials and Methods

2.1 Study Sites

LC and LZ are located in Central Mexico, within the Lerma-Chapala basin (Fig. 1). According to a morphostructural geological analysis, both lakes were jointed at the Pliocene-Pleistocene boundary by wide channels re-
Fig. 1. Location of Lake Cuitzeo and Lake Zacapu within the Lerma-Chapala Basin, indicating the sampling sites. Names describe the affluents for Lake Zacapu (La Angostura and La Zarcita) and for Lake Cuitzeo (Grande de Morelia River), as well as the effluent from Lake Zacapu (Angulo River). The star in Lake Zacapu represents some samplings in La Angostura springs. The dotted line in Lake Cuitzeo is the man-made effluent.

lated to morphotectonic structures and then separated during the Pleistocene-Holocene period [36]. LC, the second largest natural lake in the country, is a shallow tropical system (375 km²) located between 20°04'34" and 19°53'15"N and 101°46'45" and 101°47'25" W, with one main tributary (Grande de Morelia River) and a manmade effluent (Yuriria Channel) [37]. In an east-west direction, this lake is divided into three zones according to the horizontal physical, chemical, and biological patchiness; it has frequent volume fluctuations and the western zone dries up in years of severe droughts [37]. Several impacts affect this lake, including watershed degradation by deforestation and soil erosion, water withdrawal from its main tributary, water pollution from the state capital city, industry, and agricultural activities. As for the most common threats within the ecosystems are the introduction of non-native species, increasing fishers and nets, organizational capacity loss, and overfishing [37].

LC is a small remnant (0.335 km²) of a larger wetland (261 km²) located between 19°49'26" and 19°49'40" N and 101°46'45" and 101°47'25" W. The main tributaries to the lake are two groups of springs (La Angostura and La Zarcita), and the natural effluent is the Angulo River [33]. This small spring-fed lake is threatened by the proximity to the urban area, the agricultural use of the southern shoreline, the water subtraction from the springs that feed the lake, and the introduction of exotic species [38].

2.2 Specimens Analyzed and Environmental Variables

To relate species densities to environmental variables, a data set of *H. turneri* in LC was used, including 448 individuals collected from five sites in four months during 1979 (February, April, August, and November). This is the last report of the species in the area after comprehensive ichthyological surveys [13]. The environmental data available for this lake consisted of annual average values at each site (Table 1). The data were collected at the surface during the day simultaneously with the fishes’ catches. In LZ, 2,135 individuals were caught and analyzed in four months of 1995 (January, May, July, and October). For this lake, water quality information was also recorded simultaneously with fish capture at the surface at four sites during the day and nighttime (Table 1). Also for LZ, environmental data and fish samples were collected during 2019 and 2020 at the same sites and months during the day (274 individuals). The sites in both lakes were selected in such a way to represent the different habitat characteristics (water inflow from springs and rivers and the effluents, marshy areas and zones with distinct submerged macrophytes species, and nearshore influence from agricultural activities and urban development). Fishes were collected in 1979 and 1995 using a 50 m long trawl net with a 4 m × 1.8 m rectangular mouth and 5 mm × 5 mm mesh wings with a cod end; the sample area at each site was approximately 165 m². The fish density was adjusted for the net area, and the results were expressed in the number of fishes per square meter. In 2019 and 2020 we used a smaller long trawl net of 25 m length with a 4 m × 1.8 m rectangular mouth and 5 mm × 5 mm mesh wings with a cod end. Due to the small number of individuals captured, we also used five cylindrical minnow traps set for one hour per site (stainless steel, 42 cm long and 19 cm in diameter, stretch mesh 0.5 cm, with two 2.5 cm holes with inverted cone inlets) and electrofishing (Power ~200 W, peak voltage ~250 V, peak current ~10
Table 1. Average values of environmental variables of Lake Cuitzeo (1979) and Lake Zacapu (1995). Bold numbers represent 2019–2020 values.

<table>
<thead>
<tr>
<th>Water parameters</th>
<th>Lake Cuitzeo</th>
<th>Lake Zacapu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Site 1</td>
<td>Site 2</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>20.78</td>
<td>21</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg L⁻¹)</td>
<td>4.55</td>
<td>6.6</td>
</tr>
<tr>
<td>pH</td>
<td>9.4</td>
<td>8.34</td>
</tr>
<tr>
<td>Conductivity (mS cm⁻¹)</td>
<td>1.11</td>
<td>0.6</td>
</tr>
<tr>
<td>Salinity (g L⁻¹)</td>
<td>0.7</td>
<td>0.38</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO₃ L⁻¹)</td>
<td>151</td>
<td>58</td>
</tr>
<tr>
<td>Transparency (m)</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Suspended Solids (mg L⁻¹)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Turbidity (FTU)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate (mg L⁻¹)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ammonia (mg L⁻¹)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Phosphorus (mg L⁻¹)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorophyll a (mg m⁻³)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total Bacteria (MPN)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coliform Bacteria (MPN)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

2.3 Data Analysis

The population density and frequency of *H. turneri* were analyzed and compared with the information of other fish species, aiming at describing the status of the species and identifying potential interactions, mainly with the introduced organisms in LC. We used Spearman’s correlation coefficients to measure the strength of the spatial association between species. In addition, we implemented a cluster analysis, with the sites as the grouping units, to recognize the species distribution affinities in multidimensional space. We applied the Bray-Curtis dissimilarity measure and Ward’s minimum variance method to link similar points [39]. The correlation coefficients and significant values were calculated with the ‘Hmisc’ package (v. 4.5-0) [40], and clusters were obtained with the ‘vegan’ package (v. 2.5-7) [41]. Both methods were performed in the R statistical language (v. 4.1.2, Vienna, Austria) [42].

The relationships between environmental aspects and *H. turneri* abundance were explored with a generalized additive mixed model (GAMM) in 1995 in LZ. Environmental variables were analyzed to search for outliers and screened for collinearity via Cleveland dotplots and by applying Pearson’s correlation coefficients (>0.75), respectively [43]. Bacteria’s counts were log-transformed, and nitrates square root transformed to avoid large values. Correlations were found between temperature and DO (r² = –0.76, p < 0.001) and temperature and conductivity (r² = –0.76, p < 0.001), as well as between nitrite and hardness (r² = 0.89, p < 0.001). We removed different covariates according to collinearity (e.g., temperature and nitrite), low average spatial variation within optimal values for *H. turneri* (e.g., pH, conductivity), and to avoid skewness because several parameters represent similar water properties (e.g., turbidity, total bacteria).

A GAMM was applied, assuming a Poisson distribution because species counts include a low proportion of zeros (9.4%). Five covariates were used as smoothers (spline functions), assuming no strong relationships between covariates and response variables. We used random effects to model the correlation in space and time [43] because spatial and temporal autocorrelation was expected, since distance among sites is small (average 375.5 m) and samples were obtained every three months. We included a random intercept of Month (impose correlations between observations in the same sampling event) and Site (nested in Month) as the random intercept to allow for correlations between ob-
servations made in the same month (small scale spatial and temporal correlation). The final GAMM construction for \( H. \text{turneri} \) count data followed the equation:

\[
H. \text{turneri}_{ijk} \sim \text{Poisson}(\mu_{ijk})
\]

\[
\log(\text{H. turneri}_{ijk}) = \alpha + \text{Total phosphorus}_{ijk} + f\text{Dissolved oxygen}_{ijk} + f\text{Depth}_{ijk} + f\text{NH}_{ijk} + f\text{Suspended Solids}_{ijk} + f\log(\text{Total coliforms})_{ijk} + \text{Month}_i + \text{Site}_j
\]

The model establishes that the counts of \( H. \text{turneri} \) at month \( i \), site \( j \), and observation \( k \) follow a Poisson distribution with mean \( \mu_{ijk} \). The log link function is implemented with the expectation that the values of \( H. \text{turneri} \) and \( \mu_{ijk} \) are a function of the covariates and avoid non-negative fitted values. We assessed the overdispersion of the model by dividing the sum of squared Pearson residuals by the difference of the number of sampling units minus the degree of freedom from each component in the model [43]. The GAMM was fitted using the gammas package (v. 0.2-6) within the R language (v. 4.1.2, Vienna, Austria) [42].

The optimal linear estimation method proposed by Solow and Roberts [45] was applied to estimate a calendar date interval when \( H. \text{turneri} \) might be considered extirpated or locally extinct from LC with 95% statistical confidence. Collection records from several scientific collections were used to implement the model, including records from the University of Michigan Museum of Zoology, National Museum of Natural History-Smithsonian Institution, Tulane University, and the Colección Nacional de Peces Dulceacuícolas Mexicanos de la Escuela Nacional de Ciencias Biológicas, IPN (Table 2, Ref. [13]). This approach is based on the shape parameter of the Weibull distribution for the interval between successive dates. The model is described as:

\[
v = \frac{1}{k-1} \sum_{i=1}^{k-2} \log \frac{T_i - T_k}{T_i - T_{i+1}}
\]

where \( T_1 > T_2 > \ldots > T_k \) are the \( k \) most recent sighting times of a species, ordered from most recent to least recent. The confidence interval is given by:

\[
T_1 + \frac{T_1 - T_k}{S_L - 1} T_1 + \frac{T_1 - T_k}{S_U - 1}
\]

where the lower \( (S_L) \) and upper \( (S_U) \) limits are calculated as:

\[
S_L = \left(-\log \frac{1 - \frac{\alpha}{k}}{k}\right)^{-v}
\]

\[
S_U = \left(-\log \frac{\alpha}{k}\right)^{-v}
\]

were confidence intervals were set at \( \alpha = 0.05 \).

3. Results

3.1 Structural Characteristics of Hubbsina Turneri in Lake Cuitzeo and Lake Zacapu

\( H. \text{turneri} \) had the sixth-highest abundance and cohabited with 12 species of fishes in LC, and the dominant species were the native atherinopsid (\( C. \text{humboldtianum} \)) and goodeids (\( G. \text{atripinnis} \) and \( S. \text{lermae} \)), and atherinopsid (\( C. \text{humboldtianum} \)). \( H. \text{turneri} \) had the fifth-highest abundance. As in LC, \( H. \text{turneri} \) exhibited smaller abundance values in the main affluent (214 at site 2), but with higher values in the more stagnant site and the effluent (876 and 748 individuals at sites 1 and 4, respectively). The occurrence of \( H. \text{turneri} \) was also related to the presence of submerged macrophytes (\( P. \text{filiformis} \) and \( P. \text{pectinatus} \)). Temporally, 35.7% of the organisms (763 individuals) were captured in October and only 17% (362 individuals) were captured in January. During 2019 and 2020, from the trawl net information, the dominant fish changed and \( C. \text{humboldtianum} \) and the goodeid \( X. \text{variata} \) had the higher values. \( H. \text{turneri} \) only occurred in 4% of the samplings and occupied the eighth position of the nine species collected. The few places where the species was captured were the same where it was dominant in 1995. With the electrofishing and the minnow traps, we captured more individuals of \( H. \text{turneri} \) (218), and at more sites, than with the trawl net (56).

In LC, \( H. \text{turneri} \) was highly correlated with \( A. \text{robustus} \) \( (r^2 = 1, p < 0.001) \) and \( P. \text{infans} \) \( (r^2 = 0.97, p = 0.005) \), and the species was more related to \( C. \text{auratus} \) \( (r^2 = 0.8, p = 0.1) \) than to other introduced species (\( O. \text{niloticus} \): \( r^2 = 0.7, p = 0.19 \) and \( C. \text{carpio} \): \( r^2 = 0.37, p = 0.54 \)). Three species groups were identified in the multidimensional space analysis: group 1 included the dominant fish, group 2 included the two introduced cyprinids and one native species, and group 3 included the intermediate dominant species including \( H. \text{turneri} \), two other goodeids, and the introduced cichlid but the poecilid (Fig. 2). In 1995 \( H. \text{turneri} \) in LZ was more related to the goodeids
Zoogoneticus quitzeoensis ($r^2 = 0.74, p < 0.001$), A. robustus ($r^2 = 1, p < 0.001$) and X. variata ($r^2 = 1, p < 0.001$) than other species. About the introduced species H. turneri was more related to Pienophringodon idella, which was captured at site 2 ($r^2 = 0.45, p = 0.01$). Two groups were differentiated from the cluster analysis: (1) fishes with high densities including H. turneri and (2) species with low abundance values (Fig. 2). In 2019 and 2020, we found no relation of H. turneri with the other species because of the low individual number. Two species groups were identified in the multidimensional space analysis: (1) The intermediate and dominant species and (2) the species with lower abundance, where H. turneri is now located along with the predator A. robustus (Fig. 2).

### 3.2 Linking Hubbsina Turneri to Environmental Variables and Extirpation Analysis

Both lakes showed contrasting environmental characteristics. On the one hand, LC was shallow and saline with higher temperatures and higher values of different chemical parameters (conductivity, hardness, salinity, alkalinity, and pH) than LZ. On the other, LZ reached deeper areas, with very high dissolved oxygen (DO) values and low turbidity, conductivity, and hardness (Table 1). The correlation analysis revealed a close relationship between H. turneri with depth ($r^2 = -0.9, p = 0.04$) and DO ($r^2 = 0.9, p = 0.04$) in LC. In LZ, H. turneri was negatively correlated with total coliform bacteria ($r^2 = -0.55, p = 0.03$) and DO ($r^2 = -0.49, p = 0.06$).

When we checked the overdispersion value in the GAMM, the result was sufficiently close to 1 (1.14), meaning that the model had low overdispersion and an adjusted R-squared of 0.54. The GAMM outputs, and the H. turneri distribution according to the general water quality parameters in both lakes, indicated the characteristics of the sites where it would be more likely to find higher densities of the species (Table 3). Shallow to medium depth (up to 3–4 m); high oxygen concentrations, where LZ had oversaturation and the species was found in medium values (from 13 mg L$^{-1}$); meanwhile in LC only in the higher values (>6.5 mg L$^{-1}$); suspended solids (between 8.5 to 9 mg L$^{-1}$); soft to moderate hardness water (35–40 in LZ, and 100–140 mg CaCO$\_3$ L$^{-1}$ in LC); a pH from neutral to basic (7.0–8.7); and the species was found in medium values (from 13 mg L$^{-1}$).

### Table 2. Different historical records of *Hubbsina turneri* at four localities in the Lake Cuitzeo basin from distinct scientific literature and ichthyological collections. Records highlighted in bold represent years without captures of the species. N = number of individuals.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Year</th>
<th>N</th>
<th>Catalog Number</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coatzio Reservoir</td>
<td>1940</td>
<td>1</td>
<td>UMMZ-143299</td>
<td>F de Buen</td>
</tr>
<tr>
<td>Coatzio Reservoir</td>
<td>1945</td>
<td>11</td>
<td>ENCB-IPN-1863</td>
<td>V Villaseñor</td>
</tr>
<tr>
<td>Coatzio Reservoir</td>
<td>1985</td>
<td>-</td>
<td>ENCB-IPN-8169</td>
<td>E Díaz &amp; J Barragán</td>
</tr>
<tr>
<td>Coatzio Reservoir</td>
<td>1993</td>
<td>-</td>
<td>ENCB-IPN-6331</td>
<td>E Soto</td>
</tr>
<tr>
<td>Irrigation canal near Alvaro Obregón</td>
<td>1963</td>
<td>1</td>
<td>UMMZ-30829</td>
<td>CD Barbour &amp; S Contreras-B.</td>
</tr>
<tr>
<td>Irrigation canal near Alvaro Obregón</td>
<td>1969</td>
<td>33</td>
<td>UMMZ-192403</td>
<td>CD Barbour &amp; RJ Douglass</td>
</tr>
<tr>
<td>Irrigation canal near Alvaro Obregón</td>
<td>1993</td>
<td>-</td>
<td>-</td>
<td>Soto-Galera et al. [13], 1998</td>
</tr>
<tr>
<td>La Mintzita spring</td>
<td>1963</td>
<td>2</td>
<td>ENCB-IPN-1865</td>
<td>M. Rosas &amp; R. Galicia</td>
</tr>
<tr>
<td>La Mintzita spring</td>
<td>1968</td>
<td>1</td>
<td>UMMZ-189036</td>
<td>RR Miller &amp; HL Huddle</td>
</tr>
<tr>
<td>La Mintzita spring</td>
<td>1997</td>
<td>-</td>
<td>UMMZ-245055</td>
<td>Webb &amp; J Lyons</td>
</tr>
<tr>
<td>La Mintzita spring</td>
<td>2004</td>
<td>-</td>
<td>TU-202145</td>
<td>Bart, Lyons &amp; Clements</td>
</tr>
<tr>
<td>Lake Cuitzeo</td>
<td>1957</td>
<td>3</td>
<td>ENCB-IPN-5584</td>
<td>I. Mendoza</td>
</tr>
<tr>
<td>Lake Cuitzeo</td>
<td>1968</td>
<td>9</td>
<td>UMMZ-189040</td>
<td>RR Miller &amp; HL Huddle</td>
</tr>
<tr>
<td>Lake Cuitzeo</td>
<td>1991</td>
<td>-</td>
<td>IBUNAM-10204</td>
<td></td>
</tr>
<tr>
<td>Lake Cuitzeo</td>
<td>2000</td>
<td>-</td>
<td>ENCB-IPN-7980</td>
<td>G Rangel &amp; E Díaz</td>
</tr>
<tr>
<td>Lake Cuitzeo</td>
<td>2013</td>
<td>-</td>
<td>-</td>
<td>Personal communication</td>
</tr>
</tbody>
</table>

### Table 3. GAMM models summary implemented in the analysis of Hubbsina turneri in samples from Lake Zacapu. Estimated degrees of freedom (or linear coefficient in the case of parametric terms) and statistical significance (***p ≤ 0.001, **p ≤ 0.01).

<table>
<thead>
<tr>
<th>Variable</th>
<th>edf/lc</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phosphorus$_{ijk}$</td>
<td>3.6</td>
<td>0.0014**</td>
</tr>
<tr>
<td>fDissolved oxygen$_{ijk}$</td>
<td>4.9</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>fDepth$_{ijk}$</td>
<td>5.9</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>fNH$<em>3$</em>{ijk}</td>
<td>1.0</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>fSuspended solids$_{ijk}$</td>
<td>7.9</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>flogTotal coliforms$_{ijk}$</td>
<td>3.3</td>
<td>&lt;0.001***</td>
</tr>
</tbody>
</table>

The optimal linear estimation analysis indicated that the time interval within which H. turneri may be regarded as extirpated from LC is between the years 2013 and 2018; in other words, the species may have already been extir-
Fig. 2. Hierarchical cluster analysis of the fishes’ density in the different lakes analyzed. (A) Lake Cuitzeo. (B) Lake Zacapu in 1995. (C) Lake Zacapu in 2019–2020. The assignment of species to significant clusters is shown with blue rectangles.

Fig. 3. GAMM model for H. turneri abundance and the predictor variables in Lake Zacapu.

4. Discussion

By accounting for the structural characteristics of the fish fauna in Lake Cuitzeo (LC) and Lake Zacapu (LZ), we found that Hubbsina turneri was placed in a similar position in both communities decades ago. This result is important because both places have similar species composition values compared with other aquatic ecosystems in the region (e.g., Simpson’s similarity index = 0.8) [27]. Additionally, this denotes that the species may have been in the optimal ecological and environmental conditions during the collection period in LC. However, nowadays the species had a reduced population in a restricted area. The association of H. turneri with other fishes reflected the different biological interactions: (1) the relation with Zoogoneticus quitzeoensis results from the consumption of similar prey (e.g., Horn’s trophic niche overlap index = 0.87 in LZ) [33]; (2) a prey-predator interaction with Allophorus robustus; (3) and the association with the introduced Oreochromis niloticus may be more related to the habitat structure.

The analysis was restricted by the information available in both lakes, as only water quality was recorded and some variables were absent in LC, such as nutrient levels...
and bacteria counts. However, the main parameters of the limnological characteristics of each lake were recorded (LC as a hyposalinic lake and LZ as an oligohaline lake). The combined contrasting characteristics between lakes allowed for the identification of some potential optimal ranges of variables. Consequently, the ionic composition of the water, depth, and dissolved oxygen were the most important limiting factors for the distribution of *H. turneri*. The typical habitat of the species, which must be considered for conservation purposes, was related to shallow sites with transparent littoral areas, abundant aquatic vegetation, low-alkalinity, medium-hard water, basic pH, and medium to high-oxygen levels. These aspects are associated with the absence of *Hubbsina turneri* in the more saline and alkaline west zone of LC.

4.1 Probable Disappearance of *Hubbsina Turneri* in Lake Cuitzeo Basin: Impacts and Biological Characteristics

The survival rate of a species is a historical indicator of the differential anthropogenic impacts on communities among aquatic systems. In goodeids, this value is 0.57 for LC and 0.94 for LZ [46]. A decline in the number of fish species in LC was described between 1970 and 1980 [13]. Of the 15 native species originally documented, only 4 occurred in the 1990s [13]. The fishery showed an important production in the lake at the beginning of the 1990s, followed by a drop in the second half of the decade related to a severe drought, which was then followed by a continuous decline in the 2000s (3250 t in 1994, 730 t in 1999, and 300 t in 2006). It is unclear what aspects contributed specifically to the disappearance of the species in the lake. Although the probable main causes are noted, we assume that the severity of the effects depends on the magnitude, frequency, duration, and combined impacts of the causes [47], which created circumstances that did not allow the population to recover, some of them are:

First, habitat reduction negatively affects population size and viability [48,49]. There were significant surface fluctuations in LC due to hydrometeorological variation. The last report of the species in the lake coincided with a dry period (1980–2000) [50]. Two-thirds of the lake remained dry in 1988, and the maximum depth was one meter [51]. Volume drop also relates to water use because the state capital city is located within the lake basin. The population almost doubled between the 1970 to 1980 decades [52], and the water demand increased likewise (18 × 10^3 m^3 in 1970 and 33 × 10^3 m^3 in 1980) [53]. The substantial exploitation and management of water resources (e.g., dams, water diversion structures, and over-extraction of aquifers) had caused severe ecological damage and had imposed real threats to imperiled species in basins everywhere [54–56]. Lake volume fluctuation was associated with mineralization changes, which in LC was reflected in alkalinity (lake average in mg CaCO₃ L⁻¹: 856 in 1980, 1407 in 1985, and 403 in 2002) and conductivity (lake average mS cm⁻¹: 3 in 1980, 9 in 1985, and 1 in 2002) [57]. Ion concentration could exceed the organisms’ physiological adaptability, leading to the cessation of growth and increased mortality [58], an aspect that could squeeze *H. turneri* into marginal conditions. This pattern has been described temporally with environmental variables in other ecosystems [58].

Second, eutrophication is one of the most threatening effects in lakes because a drastic increase in nutrient concentration affects water quality and promotes changes in biodiversity and biogeochemical processes [11,13]. The distribution area of *H. turneri* in LC (eastern zone) presented the highest Chlorophyll-a and nitrate concentrations associated with inputs from the Grande de Morelia River [59]. This river crosses the capital state city, where the treatment plant wasn’t constructed until 2007 [59]. In addition, the river flows and is channelled in the Morelia-Queréndaro irrigation district, which has a surface area of 200 km² [60]. In the 1970s, a significant change related to the green revolution occurred in Mexican agriculture, when mechanization and agrochemicals use significantly increased. In this region, between 1970 and 1980, the cultivar structure switched from cereal grains for human consumption to forage species [61]. From the 1980s records, a consistently increasing trend in phosphates has been reported (lake average in mg L⁻¹: 0.28 in 1980, 0.23 in 1985, and 72.4 in 2002) [57]. In addition, gully erosion increased from 9 km² in 1975 to 23 km² in 2000 [44].

Third, biological interactions with native as well as exotic species at different niche aspects, which impose direct impacts like competitive exclusion, niche displacement, predation, and indirect impacts, such as habitat alteration and the spread of emerging diseases, can be particularly severe in imperiled populations [62,63]. Volume drop could reduce survival rates of vulnerable fish in lake zones due to limiting similarity processes like competition for food [64,65]. *Hubbsina turneri* and *Z. quitzeoensis* are both species that inhabit similar habitats and consume analogous prey (chironomids). However, *Z. quitzeoensis* was more abundant (1.03 org m⁻² and 0.53 org m⁻², respectively) and consumed other common prey (the insect Notonecta). Predators influence prey’s population structure and dynamics; *H. turneri* is prey for *A. robustus* and may have been more vulnerable to predation if macrophyte-covered habitats no longer concealed it. This assumption has been corroborated in other predator-prey interactions, such as the higher success of largemouth bass (*Micropterus salmoides salmoides*) predation on bluegill (*Lepomis macrochirus*) in low macrophyte stem densities [66]. In the small, conserved lake La Mintzita, *H. turneri* disappears, and *Z. quitzeoensis* and *A. robustus* persist with higher abundances [67]. The interaction for space and food could also occur with introduced species [68]. In general, introduced species are strong competitors, and some studies have described the competitive interaction between *O.
Fig. 4. The three localities where Hubbsina turneri was most recently collected. (A) Lake Zacapu. (B) Naranja de Tapia channel. (C) Jesús María spring and creek. The boy captured H. turneri with the bucket in 2006.

**niloticus** and native fishes that results in the displacement from the structured habitats occupied by the native fishes [69].

Fourth, fisheries could contribute to the disappearance of populations and species in some distribution areas. The continuous decline of several species, including those captured as bycatch, is an indirect effect of using non-selective fishing gear, which is an aspect that is discussed in different environments [70,71]. In LC, seine nets have been used to capture the more valuable and locally appreciated silverside *Chirostoma jordani*. Because of the mesh size of these nets, it is common to capture other juveniles and adults of native and introduced species.

### 4.2 Shrinkage of Hubbsina Turneri Habitat and Actual Status

Official records described a 150 km² lacustrine zone in the Zacapu wetland with depths up to eight meters and several springs that were mainly along the south shore [72]. However, in 1896, the federal government approved the desiccation of approximately 123 km², and the region turned into the main grain producer in the state [73]. This action fragmented the *H. turneri* populations, which are now restricted to LZ, and it was also present in the years 2006 and 2007 in the spring of the Naranja de Tapia town (12 km SE from LZ), and a small creek in the town of Jesús María (33.42 km NE from LZ) [33]. The water and habitat quality and biotic integrity evaluations in LZ fluctuate between regular and good, and the fish composition has remained the same [38]. However, the number of native species in the Naranja de Tapia spring has declined from five reported in 1995 to only one in 2006, and it presents poor biotic integrity; *H. turneri* and other species only persisted in a channel leading away from the system (Fig. 4) [38]. The situation of the species is also precarious in the Jesús María creek because it is located behind a livestock barn and the habitat is continually altered by cattle trampling (Fig. 4). Livestock grazing and trampling alter the complexity of the habitat in stream channels and the structure, pattern, and processes of riparian vegetation and, ultimately, the survival of other aquatic species like fishes [74].

A synthetic fiber factory was installed in 1948 on the shore of the LZ effluent [75]. This factory accelerated the urban growth, and the population of the area doubled between 1940 and 1950 (from 6 169 to 14 346 inhabitants). In 1995 the population grew 3.5 times more (48 307 inhabitants) and the last population census in 2020 reported 55 287 inhabitants. This growth exponentially increased the demand for basic services as well as the production of wastewater [75]. The city of Zacapu had a complete wastewater network and treatment plant with a treatment capacity of 120 L s⁻¹ installed until 2000 [15]. However, there were some reports of wastewater discharges in LZ from the city meat processing facility in 2010 [76]. Most environmental variables remain similar at the different decades but NO₃, which had an important increase in the recent samples (average ± sd in 1995: 1.1 ± 0.3 mg L⁻¹; in 2019–2020: 7.3 ± 0.8 mg L⁻¹). The natural level in the surface water of nitrates and other forms of nitrogen is typically less than 1 mg L⁻¹, but the excess of nitrates is related to eutrophication processes [77]. The habitat structure in lakes is important for supporting biota and it is related to the presence of springs, riparian vegetation and littoral cover complexity, high diversity of underwater plant species, substrate diversity, and anthropogenic disturbance [78]. Lake Zacapu had a spatial heterogeneity general gradient in an east-west direction from low to high values [79], and something that has been perceived between decades, but not quantified, is a greater modification in the south shore due to the establishment of agricultural activity and removal of submerged vegetation in the west zone. This modification in the habitat structure may be related to the reduction in the population of some species, influencing the change in the dominance from littoral to more limnetic fish. Another imminent threat found in the most recent sampling on the springs of La Angostura, the main affluent of LZ, was the presence of the species *Pseuodoxiphophorus bimaculatus* and *Xiphophorus hellerii*, because negative interactions with native fishes have been reported for both species in other aquatic ecosystems [80].
4.3 Hubbsina Turneri Reintroduction Opportunities and Conservation Measures

The current distribution of *H. turneri* is of special concern because fishes with restricted distributions are generally more prone to extinction than widely distributed species [81,82]. To devise conservation and restoration measures for this species, it is important to understand the status of the lakes the species has been inhabiting. Lake Cuitzeo (as part of an extensive region) and LZ (by itself) are recognized as priority hydrological regions by the National Commission for the Knowledge and Use of Biodiversity [83]. Specifically, LZ was declared as a natural protected area in 2003 [84] and is a Ramsar-listed wetland (No. 1465, designation date 05-06-2005) [85].

The central issue regarding the reintroduction of *H. turneri* in LC is that the system requires a major intervention, including habitat restoration at multiple spatial scales [86]. Several plans have been elaborated by the government [84,87] and research institutions [50,88], and they have already answered some critical questions, including those related to the disturbances associated with land use, the amount of natural land cover in the basin, the impact and treatment of residual solids, and the implementation of wetlands to reduce nutrient inputs [1,89]. Unfortunately, the misperception of the lake as a decaying, senescent, and hypereutrophic water body has overshadowed the understanding of the multiple ecosystem services it provides, which has reduced the support for restoration efforts [46].

Lake Zacapu is important to protect because it provides many ecosystem services, including urban, industrial, recreational, and agricultural water use services to the town and wetlands of Zacapu. The lake has an artisanal fishery where ten local families harvest silverside (*Chirostoma humboldtianum*) and common carp (*Cyprinus carpio*) and extract clams (*Anodonta grandis grandis*) and crustaceans (*Cambarellus montezumae*) [33]. In addition, LZ is an important habitat for endemic fishes (*Notropis grandis* and *Allotoca zacapuensis*) and amphibian species (*Ambystoma andersoni*) [90]. This lake represents a groundwater-dependent ecosystem that is influenced by the chemical, ecological and hydrological characteristics of infiltration and water transport, and it is threatened by different land-use activities, pollution, and climate change [91]. In this context, higher elevation areas and adjacent recharge areas must be protected, particularly at the level of landscapes related to volcanic processes with pine-oak forests [92].

We suggest the implementation of three actions to preserve the LZ ecosystem: (1) protect spring zones and regulate the water extraction for the conservation of aquatic biodiversity, (2) restoration of the littoral areas, and (3) adapt decision-making initiatives of the lake use and urban development to long-term objectives to preserve the ecosystem structure and function [5,93]. Exotic species may represent a threat to native species, and non-native fish control measures must be implemented to reduce the already established populations (i.e., reducing restocking and increasing harvesting). New introductions must be avoided, and a well-implemented educational campaign is critical to prevent the release of organisms by aquarists into the lake [1]. *Hubbsina turneri* and goodeid fishes lack substantial economic fishery value, but the family has drawn attention from conservationists worldwide (i.e., Goodeid Working Group). Several populations are kept in captivity as a source of species data and as a gene bank by hobbyists, universities, public aquaria, and zoos. The data on this species include a detailed protocol on population treatment for restoration and reintroduction projects [94].

5. Conclusions

We identify a set of ecological, environmental, and conservation aspects that are particularly suited to the study of threatened species, such as the fish community structure, physical and chemical variables models, and local extinction. For instance, the contrasting characteristics of the lakes allowed for the identification of critical biological and habitat aspects for species conservation and restoration. However, the selection of predictor variables for modeling species habitats was limited to the information available in both lakes. Unfortunately, long-term monitoring is lacking, and complete ecological studies in the same ecosystem are scarce and infrequent. Consequently, it is important to promote long-term monitoring programs on species with a critical conservation status, and including the communities. The analysis also provided some clues to understanding why the species is now statistically extinct at LC. The lack of individuals was found to be related to habitat degradation as a consequence of the combination of several natural and anthropogenic effects. This study contributes information relevant to habitat restoration and species translocation; both aspects are important because different species of Goodeidae and other native fishes have been negatively and irreversibly impacted in several regions of Mexico and other countries where there is persistent increasing pressure on the hydrological resources [5,41]. An ecological analysis interpreted from a historical viewpoint of environmental damage and effects can help managers or landowners learn from past events to avoid those same practices from occurring in the future to prevent biodiversity loss. Additionally, this approach highlights the need for redefining the scale of the analyses, offers useful methods to evaluate the magnitude and geography of extinction debt of freshwater fishes, understand the synergistic effects of multiple stressors in freshwater ecosystems, and propose recommendations to avoid or reduce the impacts of harmful development projects [18].

### Abbreviations

LC, Lake Cuitzeo; LZ, Lake Zacapu; GAMM, Generalized Additive Mixed Model; OLEM, Optimal Linear
Estimation Method; TP, Total phosphorus; DO, Dissolved Oxygen; IUCN, International Union for Conservation of Nature; GWG, Goodeid Working Group.

Author Contributions
R.M.E., JDLCA, ELL, MMDA, ACT, ARG and ODD designed the research study. R.M.E., MMDA, ACT, and ARG performed the research. JDLCA, PDML, ODD, and JPRH provided help and advice on the sampling methodology and sample processing. R.M.E., MMDA, ACT, PDML, ARG, and JPRH analyzed the data. R.M.E., JDLCA, ELL wrote the manuscript. JDLCA, ELL, PDML, and ODD provided financial support. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate
Not applicable.

Acknowledgment
R.M.E. and M.M.D.A. were supported by Consejo Nacional de Ciencia y Tecnología (CONACyT) and Programa Institucional de Formación de Investigadores (PIFI). A.R.G. receive a CONACyT fellowship grant for PhD studies. R.M.E., J.D.L.C.A., E.L.L., P.D.M.L. are supported by the Comisión de Operación y Fomento de Actividades Académicas (COFAA) and Estímulo al Desempeño Académico (EDT). Thanks to the Unión de Pescadores de Zacapu Lake, the government of Zacapu, and Centro Regional de Investigación Pesquera, CRIAP Pátzcuaro and the members of the Laboratorio de Biología Acuática of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH).

Funding
This research was funded by CONACyT grant number CB A1S19598 and SIP-Instituto Politécnico Nacional grant number 20211495. In addition, funding was provided by Chester Zoo, The Rufford Foundation Small Grants, the Goodeid Working Group, the American Livebearing Association, and CIC-UMSNH.

Conflict of Interest
The authors declare no conflict of interest.

References


of Twente. 2006.


[66] Collingsworth PD, Kohler CC. Abundance and habitat use of juvenile sunfish among different macrophyte stands. Lake and Reservoir Management. 2010; 26: 35–42.


