Advances in the reintroduction of *Zoogoneticus tequila* in the springs of Teuchitlán, Jalisco, Mexico

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Have you ever seen tropical palm trees or banana trees in a temperate pine-oak forest? That is one example of the success of non-native species introduced by man in native habitats. Man not only changes environmental characteristics to build houses, streets and cities, he substitutes the local flora and fauna in a direct or indirect way, sometimes to obtain food or services according to the new environment he has created, but sometimes just because it is pretty and he can afford it. In this process of modification, man pushes the native species towards extinction. According to the International Union for Conservation of Nature (IUCN), 39% of extinctions have resulted from the introduction of species, 36% have resulted from habitat destruction, and 23% have resulted from hunting and planned extermination. The most worrisome situation is when one species is extinct worldwide, as this means that this species will never be recovered and we have lost it forever. In some luckier cases, species are extinct in the places where they used to live, but are found in captivity (e.g. zoos, scientific and private collections, research institutes). Under these captive conditions some species have thrived, giving the opportunity to think about the potential for reintroduction of species into their original habitat. One good example is the case of *Zoogonetiucs tequila* in the Teuchitlán River in México.

The reintroduction of an endangered species may be considered as the last opportunity to recover a species in its natural habitat and to reintegrate it into that native ecosystem. Native species reintroduction is feasible if the native environment maintains its ecological “native” properties, or if ecological restoration can be done to reach as close as possible to an “optimum” condition in order to allow the species to live and reproduce. The success of this reintroduction effort will be positive if the reintroduced individuals survive, are able to avoid predators, resist parasites, and produce successful offspring. However, this is not a “just go and put back the species and pray for success” task. We first need to answer some questions such as: 1) what are the environmental “optimal” conditions for the individuals to live, thrive, and leave successful ofspring? 2) Are conditions of the habitat where the species will be reintroduced suitable to maintain the new population for the long term? and 3) Are the local people conscious of the importance of maintaining the ecological services of the area where the new species is to be restablished? These “simple” questions are not easily answered, but in order to have responses, plenty of data need to be collected, before, during, and after the reintroduction process; data on habitat and water quality, amount and availability of food sources, potential parasites, non-native species, potential competition and predation, and so on. Analyzing these data can give important information about the success or failure of the reintroduction, and this information can be used to guide other reintroduction initiatives or to improve those already in progress. It also is very important to conduct an educational and conciousness-raising program involving the local and regional people, in order to communicate a positive attitude about the aquatic environment and to promote endangerd species conservation.Because of these efforts, the local people may become more protective of the species and its environment.

All of these issues are especially complicated in central Mexico for three reasons. First, the geological history of central Mexico is complex, resulting in a wide variety of aquatic ecosystems with different characteristics and seasonal dynamics, including lakes, rivers, springs, and wetlands. In some cases, the rainy season promotes temporary connections among them. The mix of geological complexity and environmental diversity among aquatic ecosystems makes a perfect combination for cooking biodiversification centers for fishes with many endemics (species limited to a specific small area and found nowhere else). Second, beginning in the 1930’s and 1940’s, a program to populate freshwater ecosystems with carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) took place in Mexico, the goal of which was to supply a potential source of protein to local people. Since then, these species have been intentionally and constantly introduced in many aquatic ecosystems. At the same time, the release of small fish kept ornamental purposes has resulted in the spreading of many other species throughout Mexico (see Swift et al. 1993; Miller 2005). Consequently, species such as non-native poeciliids are now very common in many waterbodies in Mexico. Third, most of the Mexican human population (73%) inhabits the central part of the country, and 67% of industrial activities take place in this same region (see Domínguez-Domínguez et al 2008), casuing major impacts on aquatic environments via water consumption, water pollution, and habitat destruction.

Under this scenario, the restoration and resettlement of the native fish fauna is quite complicated. To be successful in the challenge of getting the ecological services and biodiversity back, we must consolidate efforts from different teams and work together in a multidisciplinary way, considering local-people needs, knowledge, and interests (local-people team); ecological properties of the environment and the biology of the species present (biology-study team); options to transfer the technology to simplify techniques for monitoring the fauna, flora, and environment in a long term (monitoring team); the interest of national and international organizations to conserve these unique fish species (sponsor-funding team); and proposed options to manage and conserve the environment, and, at the same time to obtain stable and long-term benefits for the local people (social team). Of course, more teams can be included according to the goal being pursued!

One good example of re-establishing a local fish fauna in Mexico is now taking place in the locality of Teuchitlán, Jalisco. This town possesses characteristics that make it particularly attractive as a model for ecological restoration and re-population of the native fish fauna, (e.g., *Zoogoneticus tequila*): 1) an important archeological zone with major tourism potential represented by a large prehispanic settlement known as "Huachimontones"; 2) the use of the Teuchitlán springs and river as a water supply for the people of Teuchitlán; 3) some native fish species that are stillpresent; 4) commercial and ornamental non-native fish species that are quite abundant; 5) river flow that has been modified by a dam used mainly for crop irrigation; 6) local extinction of three endemic fish species which took place around 1990 (*Zoogoneticus tequila*, *Skiffia francesae* and *Notropis amecae*); and espcially 7) local people interested in recovering the local aquatic native biodiversity and promoting conservation, educational programs, and national and international tourism and 8) interest by international conservation organizations to restore the lost ichthyofauna of this unique place.

With the help from researchers from the Universidad Michoacana de San Nicolás Hidalgo (UMSNH) and sponsored by Chester Zoo Garden, Mohammed Bin Zayed Species Conservation Found, Haus des Meeres - Aqua Terra Zoo, Poecilia Scandinavia, Poecilia Netherlands, Missouri Aquarium Society, Deutsche Gesellschaft für Lebendgebärende Zahnkarpfen, British Livebearer Association, Goodeid Working Group, American Livebearers Association, Mexican Commission for the Knowledge and Use of Biodiversity, and Association Beauval Nature Pour la

Conservation et la Recherche, a multidisciplinary project began in January 2015 in order to restore and re-populate the Teuchitlán River with native fish species, particularly the goodeid *Zoogoneticus tequila* and the minnow *Notropis amecae.*

In this article we will give a sumary of the work that the team from the UNSNH did in 2015 and early 2016 on the reintroduction project. During that period the work was focused on fish community monitoring and basic species biology (non-native and native and semi-captive and in-situ), to find out which fish species were more competitive and produced more offspring, whether each species carried parasites, and their distribution in relation to river characteristics. The goal was to find the best place for reintroducing the native species based on water quality, and food resources such as zooplankton, phytoplankton, and benthic invertebrates, and the physio-chemical variables that are being monitored. Pooling together data on water and environmental quality indices offers a holistic view to allow selection of the best place for the reintroduction program to begin.

Limnological characterization and water quality

The watershed of Teuchitlán River is located in the southern portion of the volcano of Tequila, Jalisco. It is comprised of springs, a first-order river, and an artificial reservoir. It presents a longitudinal progression with changing

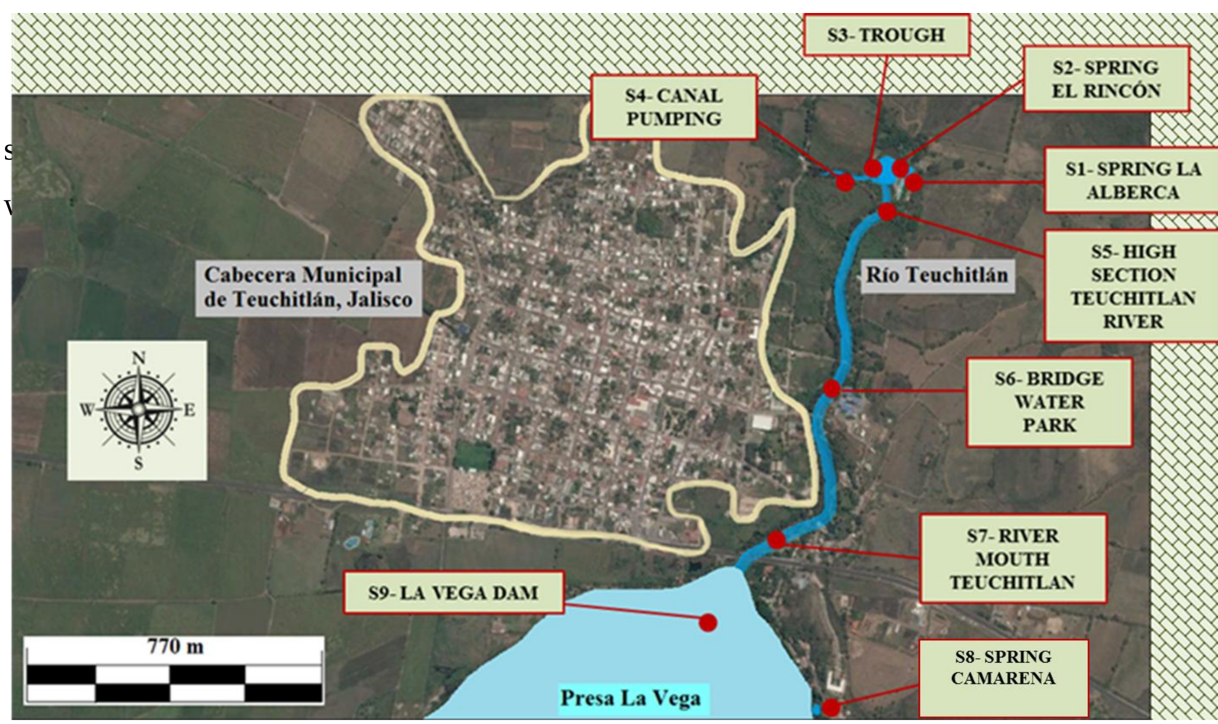

Figure 1.- Sampled sites aloing the Teuchitlan river.

A total of four environmental varibales, 34 physicochemical and five microbiological parameters were obtained for water following the Mexican Norms standard procedures established for each parameter. The water bodies show basic and moderately mineralized water with high solids loading and ionized compounds. 90% of the sites have a transparency of 100%, with a gradual increase in turbidity downstream, suggesting the accumulation of colloidal and particulate matter in the water. Regarding the nutrient loads, 40% of the sites are classified as mesotrophic to oligotrphic, mainly in the springs, while the remaining sites show a mesotrophic condition, prone to eutrophication. Regarding the water quality index established by the National Water Commission (SEMARNAT, 2007), the sites with better water quality are the springs (S1 and S2 in Figure 2). The sites with high variation in water quality correspond to the "pump channel" (S4) and "presa de la Vega" (S9). The sites with signs of water pollution, are from S3) to spring "Camarena" (S8) except upper river S5 (Figure 2). The physicochemical parameters that affect the water quality index in the study period are nine, of which the total alkalinity and total phosphorus have the highest ranges, indicating the presence of pollution, followed by coliform bacteria load, dissolved oxygen and turbidity.

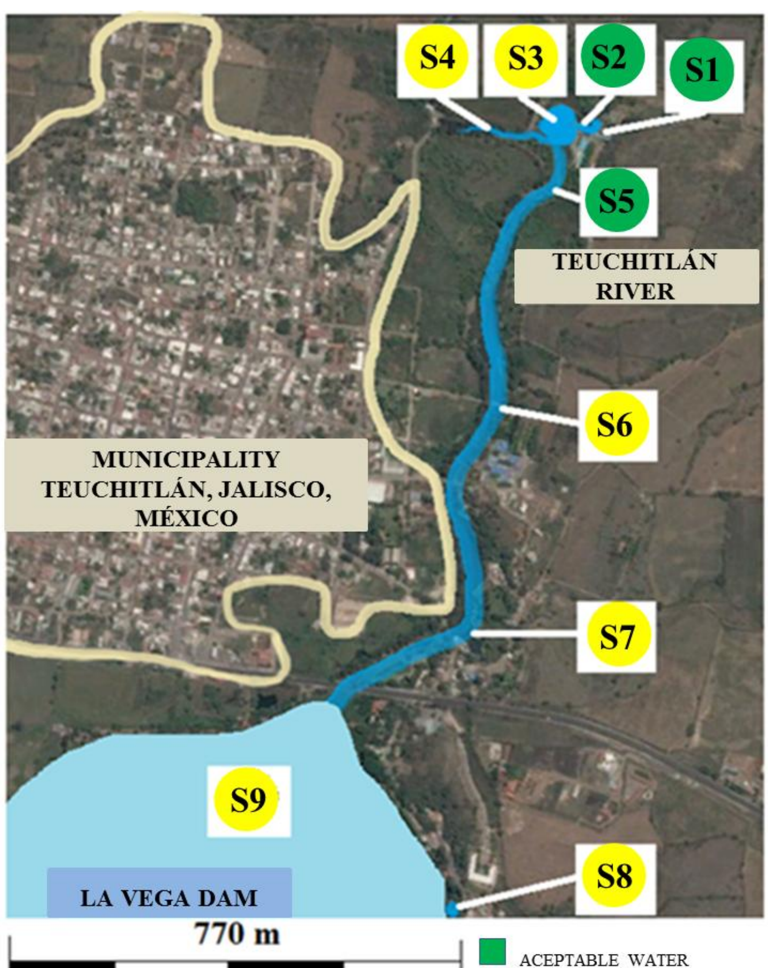

Figure 2.- Results of water quality index of the nine sample sites. In green is acceptable water quality and in yellow sites with signs of water pollution. **S1**. Spring La Alberca, **S2**. Spring El Rincon, **S3**. Trough, **S4**.Canal Pumping, **S5**.High Section Teuchitlán River, **S6**.Teuchitlan bridge water park, **S7**. River mouth Teuchitlan, **S8**. Spring "Camarena", **S9**. De La Vega Dam "Lic. Santiago Camarena".

With regard to the vulnerability of water quality, it is noteworthy that one of the sites with greater variation in this parameter is "La Vega" Reservoir (Dam) (S9), which is considered as a vulnerable system, followed by S7, both attributed to frequent changes in their physicochemical composition. Sites with less vulnerability correspond to the springs "El Rincon" (S1), spring La Alberca (S2), the meander and the upper section of the Teuchitlán river (S5) were the river begins (Fig. 2).

Phytoplankton community

Planktonic microflora is essential for the optimal development of insect larvae, copepods, cladocerans and other primary consumers, and also as a food source for the endemic fish of Teuchitlán. For the phytoplankton community, 47 taxa were identified belonging to four Phyla, 5 classes, 15 orders, 24 families, 33 genera and 46 species. Ochrophyta had the highest species richness, followed by Chlorophyta, Cyanobacteria and Charophyta. Algal abundance was led by Ochrophyta followed by Chlorophyta and Cyanobacteria. In general, the phytoplankton was dominated by diatoms at all sites, followed by green algae, cyanobacteria and charophytes (Fig. 3).

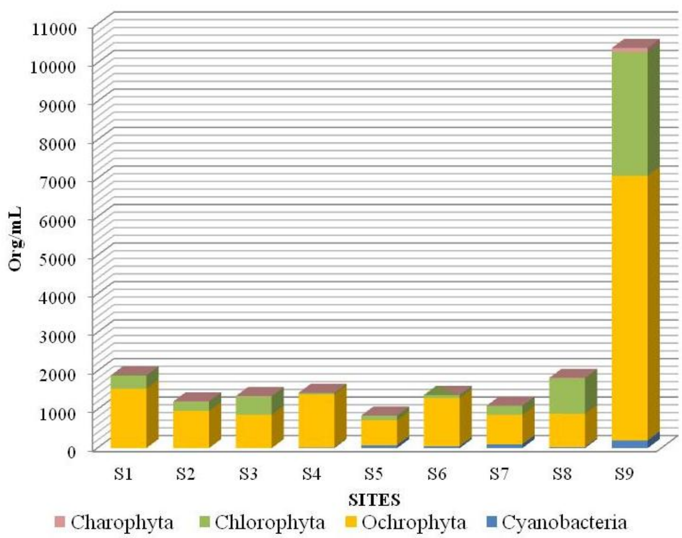

Figure 3. Variation in the abundance of phytoplankton by site. Site legend corresponds to those in Figure 1.

The most abundant species during the study were: *Scenedesmus dispar, Scenedesmus opoliensis, Pediastrum simplex, Cyclotella menenghiniana, Achnanthidium minutissimum, Gomphonema olivaceum, Surirella elegans, Nitzchia amphibia, Thalassiosira weisflogii, Cyclotella meneghiniana, Tetraedron minimum, Chrooccocus dispersus* and *Monoraphidium contortum* (Fig. 4).

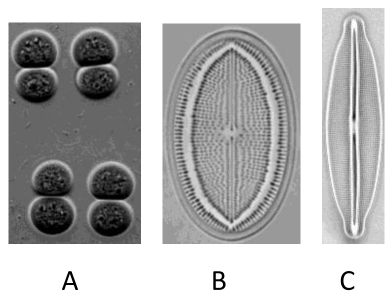

Figure 4. Genera of phytoplankton identify in the area. A *Chroococcus,* B. *Cocconeis* and C. *Frustulia.*

The evaluation of trophic status in the study area based on algal association indicates a mesotrophic to eutrophic condition in these aquatic systems (Nygaard index; Table 1), suggesting that the watershed is in the process of eutrophication. Sites with greater availability of autotrophic planktonic organisms are S4, S8 and S9, while S1 and S2 havea small number of organisms. The mesotrophic condition prevails in the springs of the upper Teuchitlán River, while from the S4 downward the algal associations suggest the development of eutrophic conditions culminating in a eutrophic category with a tendency to hypertrophy at the S9 (dam "La Vega") (Table 1).

Table 1. Trophic status based on the index Nygaard

SITE	TROPHIC STATE
S1 Spring "pool"	Mesotrophic
S2 Spring "El Rincón"	Mesotrophic
S3 Trough	Mesotrophic
S4 Pumping channel	Eutrophic
S5 Teuchitlan upper river	Eutrophic
S6 Teuchitlan middle river	Eutrophic
S7 Teuchitlan mouth river	Eutrophic
S8 Spring "Camarena"	Eutrophic

S9 Dam “La Vega”	Eutrophic to Hypertrophic

Zooplankton community

Twenty-eight taxa of zooplankton were identified, belonging to 7 Phylum, 1 Superclass, 8 classes, five subclasses, 5 superorder, 10 orders, 3 subfamilies, 10 families, 16 genera and 28 species. Zooplankton is represented in most places by Copepoda, Rotifera, Cladocera, Ostracoda and protozoa groups (Fig 5).

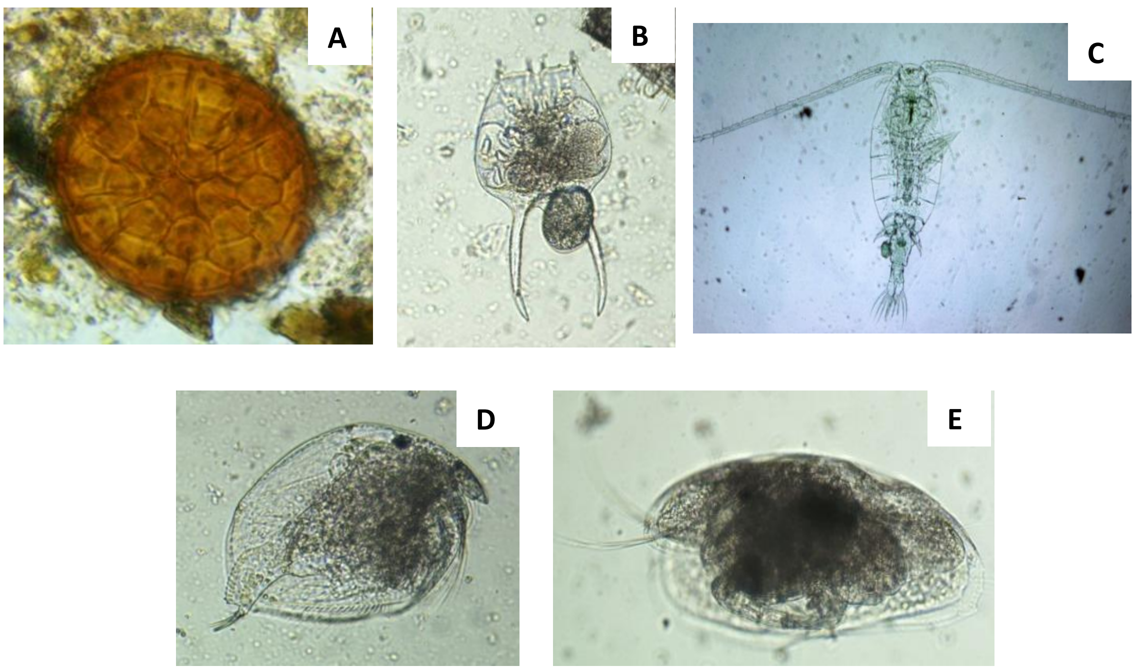

Figure 5. Zooplnkton community. A) protozoa, B) Rotifer, C) Copepod, D) Cladoceran, E) Ostracod.

Regarding its distribution, zooplankton was common in all the sites of the stream, principally Copepod and Cladocera. “La Vega” dam (S9 in Fig. 1) had the most zooplankton diversity.

The most abundant zooplankton group was calanoid copepods, followed by cyclopoid copepods and rotifers.. Cyclopoid copepods and rotifers had the greatest species richness. The calanoid group was not present at S6 and S7, while rotifers and cladocerans were absent at S1 and S2, ostracods at S1, S2 and S3, and cyclopoids only at S2. Protozoa was the only group present at all sample sites. The sites with most diversity and abundance of zooplankton were S9 (Presa La Vega), followed by S8 (spring Camarena). Sites S1 and S2 had the lowest diversity and abundance (see Figure 1). The most common species across all sites was *Mastigodiaptomus patzcuarensis.* The presence of this species is related to a warm, oxygenated, slightly basic column of water that was moderately soft and productive, with moderate concentrations of nutrients derived from organic matter mineralization.

Aquatic vegetation

Sampling sites had high differences in submerged, emergent and rooted floating aquatic vegetation. For S1 (Spring La Alberca) and S2 (Spring El Rincon) aquatic vegetation was absent (Fig. 6), and shade and fish refuges were provided by the canopy and roots of riparian trees.

Figure 6. Srping el Rincon location (S2).

In other locations some species of macrophyta were present, mainly the genus *Pistia*. Another important component of aquatic flora is the genus *Typha*, which is present in most of the Teuchitlán River; its presence is in scattered-discountinuous patches (Fig. 7).

Figure 7. A) *Pistia* at site S5 and B) *Thypa* at site S6.

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At the lowest part of the river (S7), two species of aquatic vegetation were found, *Eichornia* spp and *Eigeria* spp, the first of which provides substantial biomass to the system, while the second has been found near the untreated wastewater discharge into the river (Fig. 8).

Figure 8. A) *Eichornia* spp at the lower part of the river Teuchitlan (S7) and B) *Eigeria* spp in the wastewater discharge.

Fish community

The fish community is composed of ten species belonging to four families; four native, five exotic, and one translocated (Table 2). *Chapalichthys encaustus* is translocated because its native distribution is restricted to the nearby but separate Chapala Lake basin and surrounding areas. This is one of the few documented cases of a translocated fish species in the family Goodeidae, and therefore, little is known about the effects this translocation may have on fish the native fish community.

Table 2. Fish species collected during surveys in the Teuchitlán River.

Family	Species	Mexican name	English name	Origin
Cichlidae	*Oreochromis* spp.	Tilapia/Mojarra	Tilapia	Exotic
Goodeidae	*Ameca splendens*	Mexclapique mariposa	Butterfly Goodeid	Native
	Chapalichthys encaustus	Pintito de Ocotlán	Barred Splitfin	Translocated
	Goodea atripinnis	Tiro oscuro	Blackfin Goodea	Native
	Zoogoneticus purhepechus	Picote Purépecha	La Luz Splitfin	Native
Ictaluridae	*Ictalurus dugesii*	Bagre del Lerma	Lerma catfish	Native
Poeciilidae	*Heterandria bimaculata*	Repotete	Twospot livebearer	Exotic

Poecilia sp	Molly	Shortfin molly	Exotic
Xiphophorus helleri	Cola de espada	Green swordtail	Exotic
Xiphophorus maculatus	Platy	Southern platyfish	Exotic

The most diverse fish sites are S2 and S3. The relative abundance of exotic species was greater than that of the natives; across all sites, and the overall percentage of exotics was greater than 80% (Fig. 9). Native fish decreased in number and presence in the direction of the river flow, wth the opposite pattern for the exotic fishes. The most abundant species overall was *Heterandria bimaculata*, representing ~60% of the total fish abundance. This poecilid have been listed in other water bodies as a "risk to native fauna" because of its aggressive behavior and its potential competion for food resources. The next two most abundant species were *Xiphophorus helleri* (16%) and *Poecilia* sp. (14%), both non-native. The combined relative abundance of all native species (*Ameca splendens, Goodea atripinnis*, *Ictalurus dugesii* and *Zoogoneticus purhepechus*) was less than 10%. Although the genus *Oreochromis* has low abundance in number of individuals, some organisms of this genus can reach a size exceeding 30 cm in length and up to 1 kg, so it represented a substantial proportion of total fish biomass. Abundance fluctuations of the community apparently were determined mainly by temperature variation.

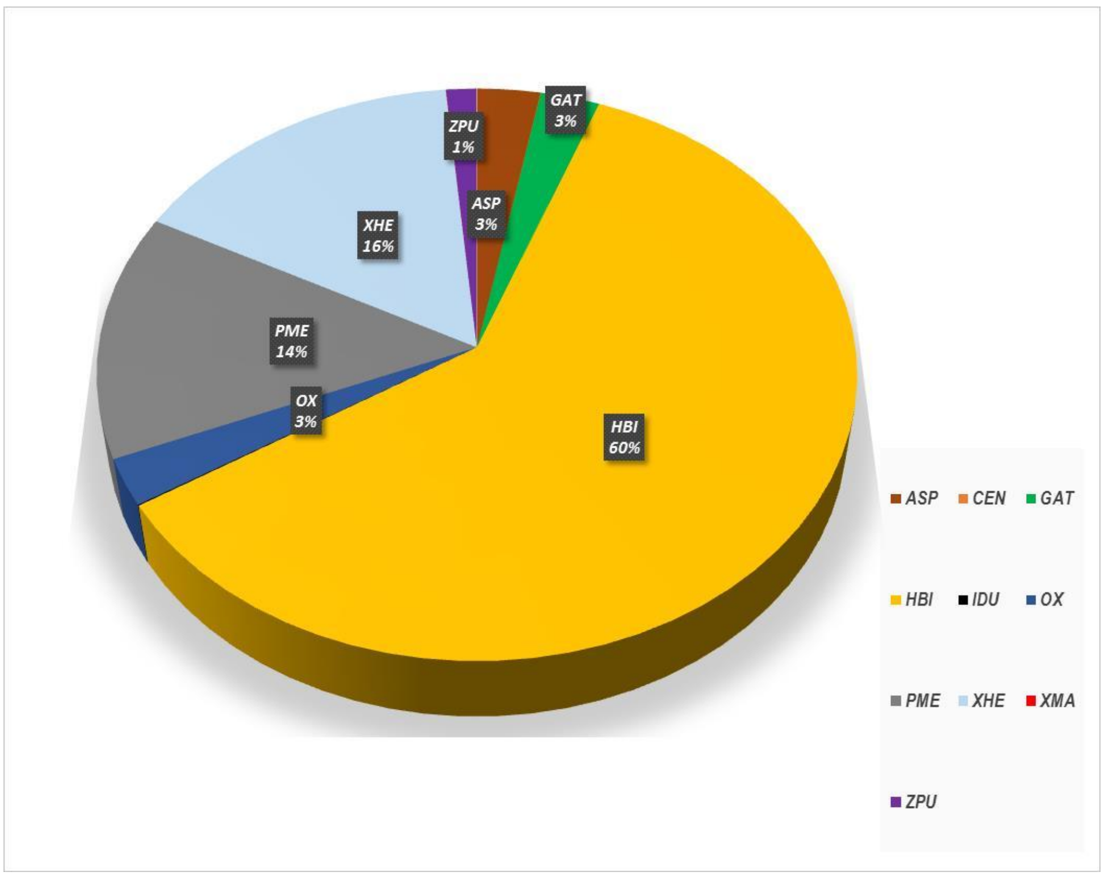

Figure 9. General abundance of fish species in Teuchitlán River. **ASP**= *Ameca splendens*, **CEN**= *Chapalichthys encaustus*, **GAT**= *Goodea atripinnis*, **HBI**= *Heterandria*

bimaculata, **IDU=** *Ictalurus dugesii*, **OX** = *Oreochromis* spp., **PME**= *Poecilia* sp, **XHE**= *Xiphophorus helleri*, **XMA**= *Xiphophorus maculatus* y **ZPU**= *Zoogoneticus purhepechus*.

Native species had their greatest abundance at sites S1, S2 and S3. *Ameca splendens* had its maximum abundance at S2 and S7 with a few individuals at the rest of the sites. *Zoogoneticus purhepechus* was most abundant at S1 and S2, declining in abundance downstream (Fig. 10). The abundance of *Z. purhepechus* is generally low, and the maximum number of organisms obtained for this species was in March. Although native species had lower density, their biomass by site was greater than that of the non-native species.

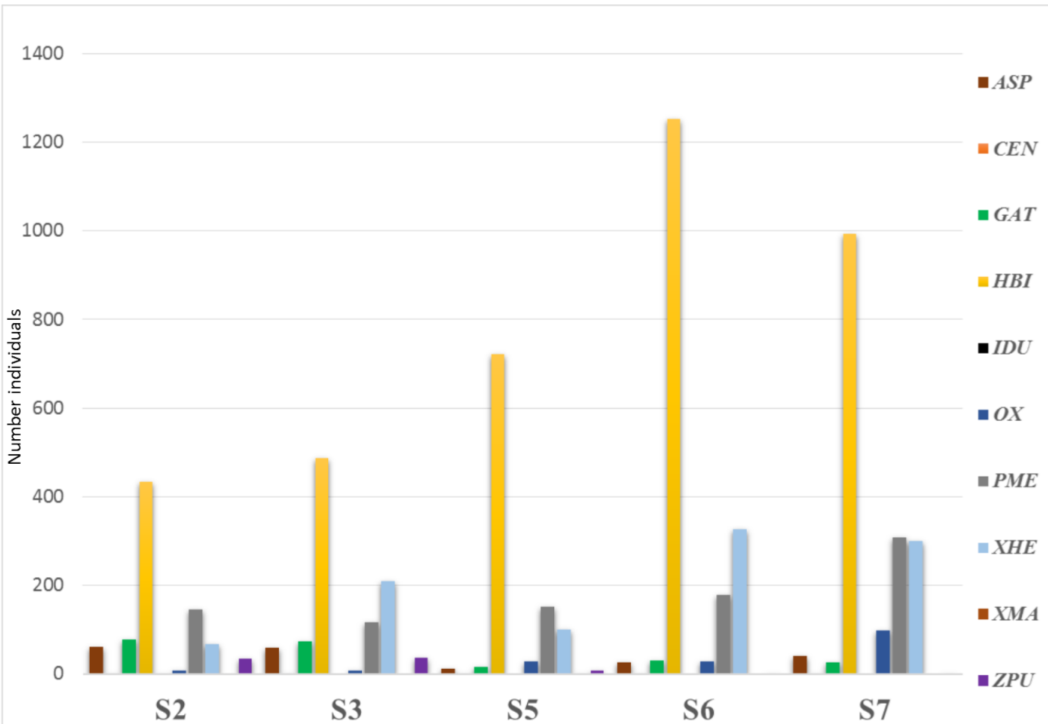

Figure 10. Abundance of fish species by site. **ASP**= *Ameca splendens*, **CEN**= *Chapalichthys encaustus*, **GAT**= *Goodea atripinnis*, **HBI**= *Heterandria bimaculata*, **IDU=** *Ictalurus dugesii*, **OX** = *Oreochromis* spp., **PME**= *Poecilia* sp, **XHE**= *Xiphophorus helleri*, **XMA**= *Xiphophorus maculatus* y **ZPU**= *Zoogoneticus purhepechus*.

Trrophic analyses

The trophic web of the fish community was composed of 20 diet elements. Only omnivorous species consummed organic matter and plant debris. Two trophic guilds were present: omnivorous and carnivorous-insectivorous. The fish community is formed mostly by primary consumers with atrophic level of 2. Of the seven species analyzed, only two were secondary consumers (*Z. purhepechus* and *H. bimaculata*) (Table 3).

Table 3. Trophic classification of fish species

Species	Trophic guild	No. prey	Main Prey	Trophic Level
Oreochromis spp.	Omnivore	7	Detritus	2
A. splendens	Omnivore	3	Detritus	2
C. Encaustus	Insufficient data (ISD)	ISD	ISD	ISD
G. Atripinnis	Omnivore	4	Detritus	2
Z. purhepechus	Carnivore-insect.	12	Aquatic chironomidae larvae	3.26.±43
Heterandria bimaculata	Carnivore-insect.	25	Aquatic true-bugs and ants	2.88±0.35
***Poecilia* sp.**	Omnivore	7	Detritus	2.01±0.04
Xiphophorus Helleri	Omnivore	9	Detritus	2.01±0.03

Food resources that were used by all the species were plant debris and organic matter. *Poecilia* sp and *X. helleri* consumed small amounts of aquatic insects. Only *H. bimaculata* and *Z. purhepechus* had a trophic level greater than 2.5. *Heterandria bimaculata* used a large number of different resources, but it also used significant amounts of organic matter (30%) and for this reason, its trophic level is less than that of *Z. purhepechus*, which has the highest trophic level in the system. *Zoogoneticus purhepechus*is carnivorous-insectivorous and a specialist consumer of the insect family Chironomidae (Relative Importance Index-RII<70). Its consumption of organic matter is also the lowest of all the studied species. Due to its consumption of macroinvertebrates and zooplankton, *Z. purhepechus* is a potential competitor with *H. bimaculata*.However, *H. bimaculata* has a greater diet breadth, which may make it more adaptable to to changes in prey availability.

Zoogoneticus tequila in semi-captive conditions ate mostly insect larvae and adults (>90% of the Relative Importance Index-RII), copepods, cladocerans, algae, mollusks, diatoms, ostracods and plant debris. The proportion of each item by month is show in Figure 11.

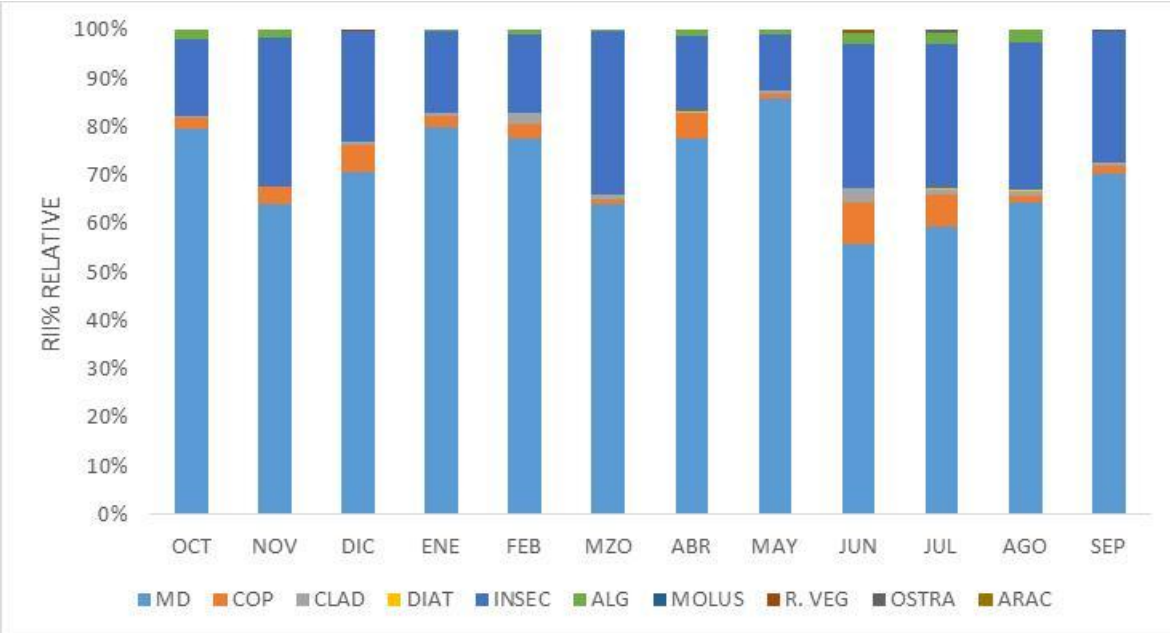

Figure 11. Prey importance in the stomach contents of *Zoogoneticus tequila* expressed as % according to RII. **MD=** Digested material (insect remains), **COP=** Copepods **CLAD=** Cladocerans **DIAT=**diatoms, **INSEC=** rest of insects, **ALG=** Algae**, MOLUS =** Mollusc, **R.VEG. =** plant remains, **OSTRA=** Ostracoda **ARAC=** Arachnidae.

Its diet niche breadth, determined by the Levin index, had a maximum value of 0.24 and a minimum of 0.11, indicating that the diet of this species is not very broad and does not change in different seasons.It is also a diurnal food consumer, feeding mainly during daylight. Its trophic level places it as a secondary consumer.

Reproductive aspects

Preliminary data on the reproductive aspects of 7 species in the natural environment of Teuchitlán indicate that the alien species *Poecilia* sp. had the highest fecundity (Table 4). Five native and non-native species had similar but lower fertility values, , and the fertility of non-native *Oreochromis* spp was not evaluated because the captured individuals were not sexually mature. *Heterandria bimaculata* was sexually mature at the smallest length size, 21mm standard length (SL), and the native *Goodea atripinnis* at the largest size (48.35 mm SL). The native *Ameca splendens* had the second largest size at 38.36 mm SL, while the other 4 species reached maturity below 30 mm SL (figure 34).

Under semi-captive conditions, *Zoogoneticus tequila* had a sex ratio of 2:1 (F / M), , a fertility varying between 1 to 4 fry per female, and a size at first maturity of females of 23.63mm and of males of 20.48. There were two reproductive periods, winter (January) and during the rainy season, August.

Table 4. Reproductive aspects of viviparous and oviparous species from the Teuchitlán River, Jalisco. ND= Not Determined.

Specie	Reproductive Strategy.	Size at first maturity (mm)	Sexual Proportion (Female:Male)	Absolute Fertility (mean number of embryos per female)
G. atripinnis	**viviparous**	48.35	ND	5
H. bimaculata	**viviparous**	21	1.18:1	5
A. splendens	**viviparous**	38.36	0.25:4	5
***Poecilia* sp.**	**viviparous**	27.78	0.68:1	14
X. hellerii	**viviparous**	31.18	1.38:1	7
Z. purhepechus	**viviparous**	27.34	0.75:1	7
***Oreochromis* spp.**	**oviparous**	27	0.66:1	ND

Index of Biological Integrity

In terms of their tolerance, habitat, and feeding guilds, 70% of species in the Teuchitlán River fish community were classified as tolerant, 20% as moderate and only 10% as sensitive. Ten percentused benthic habitats and the remainder primarily used the water column. Three trophic guilds were found; 60% were omnivores, 30% were carnivores, and only 10% were herbivores (Table 5).

Table 5. Classification of fish species according to the criteria of Lyons *et al.* (1985).

Species	Origin	Water column position	Feeding	Tolerance	Reproduction
A. splendens	N	Column	Omnivore	Sensible	Viviparous
C. encaustus	N	Column	Omnivore	Tolerance	Viviparous

G. atripinnis	N	Column	Omnivore	Tolerance	Viviparous
H. bimaculata	E	Column	Carnivore	Tolerance	Viviparous
Ictalurus dugesii	N	Benthic	Carnivore	Moderate	Viviparous
***Oreochromis* spp**	E	Column	Omnivore	Tolerance	Oviparous
***Poecilia* sp**	E	Column	Herbivore	Tolerance	Viviparous
X. helleri	E	Column	Omnivore	Tolerance	Viviparous
X. maculatus	E	Column	Omnivore	Tolerance	Viviparous
Z. purhepechus	N	Column	Carnivore	Moderate	Viviparous

The Index of Biological Integrity (IBI) scored fair to poor in the Teuchitlán River system. The highest rated sites were located in the upper part and the lowest-rated sites ones were close to the river mouth (Fig. 12). This pattern mirrored the longitudinal trends in environmental degradation in the Teuchitlan River.

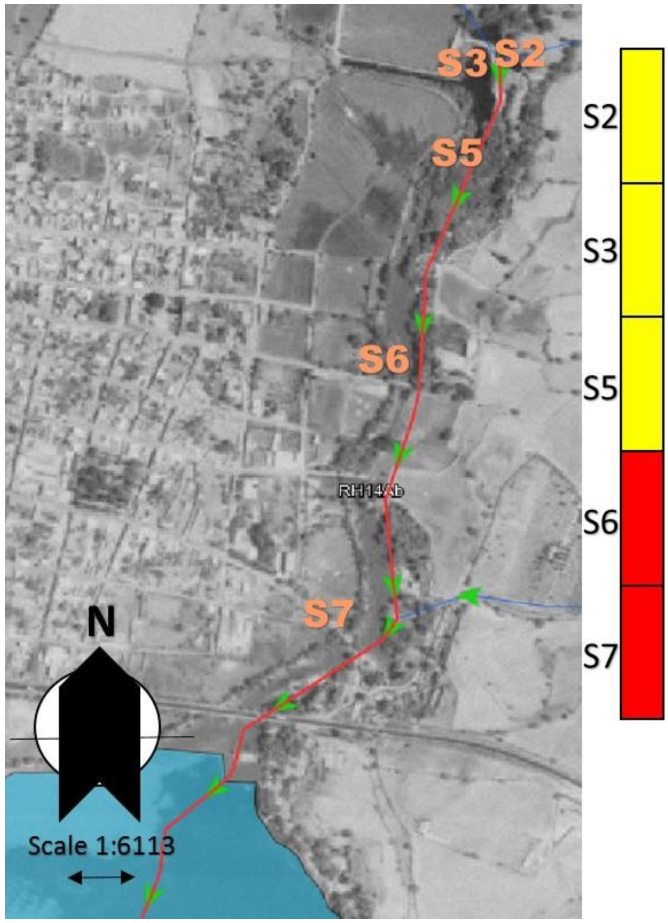

Figure 12. General trend of Index of Biotic Integrity (IBI) ratings. The green arrows on the map indicate the direction of flow of the river. Sites are indicated on the far right. Color represent; Yellow: fair IBI; Red: poor IBI.

Parasitology

From seven species of fish, five genera of helminth parasites (*Contracaecum* sp Larva, *Eustrongylides* sp Larva, *Diplostomun* sp, *Rabdochona* sp Larva, *Bothriocephalus acheilognathi* Adult) have been identified with an overall prevalence of 25%, an abundance of 0.8 helminths per analyzed host and an average intensity of 3 helminths per infected host. Throughout the year infection tended to remain at low levels, indicating an absence of parasitological problems in resident fish populations (Table 6).

Among the parasites found, the nematode larvae of *Contracaecum*, *Eustongylides* and *Rabdochona*, as well as the trematode metacercaria of *Diplostomum,* were parasites with complex life cycles. The larval phases occur in the aquatic system, with aquatic vertebrates as intermediate hosts (mainly fish but also amphibians), and they must use terrestrial vertebrates as their final host to reach adulthood. The cestode *B. acheilognathi* has its complete life cycle in the water, using microcrustaceans as intermediate hosts. This cestode is an exotic parasite from Asia. It has been reported to have negative effects on native fish (??) faunas.

Table 6. Parasites fauna in fish species analyzed.

Host	**Parasite**	**Taxonomic group**
G. atripinnis	*Contracaecum* sp L2-L3*	Nematoda
	Eustrongylides sp L3*	
	Diplostomum sp Mc**	Platyhelminthes
A. splendens	*Rhabdochona* sp	Nematoda
***Oreochromis* spp**	*Bothriocephalus acheilognathi*	Platyhelminthes
***Poecilia* sp**	*Contracaecum* sp L2-L3*	Nematoda
	Eustrongylides sp L3*	
X. hellerii		
Z. purhepechus	*Diplostomum* sp Mc**	Platyhelminthes
H. bimaculata	*Contracaecum* sp L2-L3*	Nematoda
	Diplostomum sp Mc**	Platyhelminthes

* L = Larvae, **Mc = Metacercariae

Two parasites were found in *Zoogoneticus tequila* living in semi-captive conditions, *Lernaea cyprinacea*, a crustacean ectoparasite, and the nematode *Spiroxys* sp Larva. From December through May the infection rate was zero, in June it rose to 20%, with an average intensity of 3 parasites per infected host and an abundance of 1.2 parasites per analyzed host, and in July it reached a maximum, with a prevalence of 70%, an average intensity of 9 parasites per infected host, and an abundance of 3.9 parasites per analyzed host. These values remained constant until October, and in November the values decreased to a prevalence of 40%, an average intensity of 2 parasites per infected host, and an abundance of 8 parasites per analyzed host (Fig. 13).

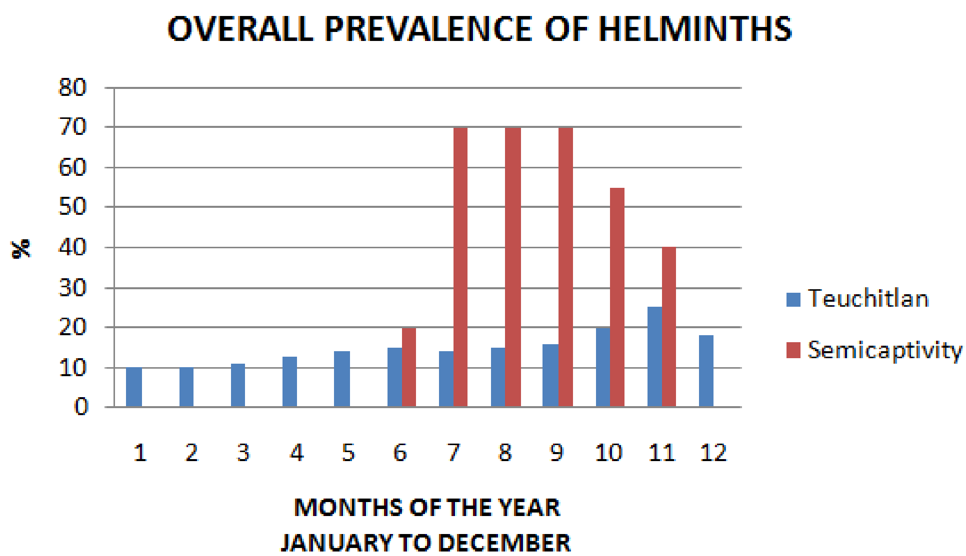

Figure 13.Prevalence of parasites in *Z. tequila* in the semi-captive area and in 7 fish species from Teuchitlan.

With respect to the environment where the reintroduction will take place, the annual sampling cycle has been completed, and results show that the parasites populations are not very high, with prevalence and abundance values always low. This indicates that the springs and bodies of water nearby provide a favorable environment for the reintroduction of *Z. tequila* in terms of parasites Conversely, the stock of *Z. tequila* kept in semi-captivity shows a typical annual cycle for some parasites species, in which parasite populations increase in the hot and humid seasons of the year and decrease in the dry and cool seasons. It is recommended to take fish for reintroduction in the dry months of the year to ensure that the parasites populations are low and to avoid problems when starting the reintroduction experiments. If necessary, fish used for reintroduction can be treated with anti-helminthics.

Social aspects

A program for environmental education and awareness is being conducted with the goal of recognizing the importance of *Z. tequila* and using it as symbol of the identity of Teuchitlán in environmental terms. This program has the potencial to diminish the introduction of non-native fish, protect the local aquifer and groundwater recharge areas, stimulate organic farming, and promote sewage treatment through conferences, environmental awareness workshops, and educational activities for different ages (e.g., playfulness theaters). These activities have been taking place in the town square and local elementary schools, explaining the importance and goals of the project.

Figure 14. Theater presentation in Teuchitlan main square

Together with the Centro Interpretativo Guachimontones, a research center for archeological research (Jalisco State Government), joint efforts have taken place in order to promote scientific and social results related to the importance of the aquatic ecosystems and the fauna inhabiting the area.

The project has three social aspects; i) engaging government and other official agencies, ii) environmental education program, and iii) involving the community in the monitoring program.

In terms of engaging government and other official agencies, we have already presented the project to the local government, and we have obtained support from the municipality governors, including involving government workers as monitors of the local fauna. We are also working with the local municipality in order to review the laws about land-use planning and to update them in light of the ecological data from the municipality. This effort will provide options and suggestions to better guide urban and agricultural development. Furthermore, the project will promote the declaration of the micro-basin of the Teuchitlán River as a Biological and Ecological Protected Area.

Through the environmental education work, we obtained data about the local level of knowledge of the aquatic fauna through polls and interviews with local people. Some local people recognized that *Z. tequila* was present and abundant ca. 30 years ago in the Teuchitlán River; this information came from people of ca. 40 years old. Furthermore, local people related the use of herbicides, a spill of sodium hydroxide in the Tala sugarmill (adjacent to the municipality of Teuchitlán), and collections by local people as causes of the decrease of *Z. tequila*. However, most of the local people, did not know of *Z. tequila*, nor of Picote tequila or any other local name for native fish.

In terms of community monitoring, intial efforts have resulted in the creation of local voluntary groups for monitoring environmental variables related to water quality, and biological variables related to biotic integrity. This monitoring will promote continuity in data collection for developing management and conservation strategies as well as advancingthe technology transfer process to the local people and society.Overall, 22 local volunteers are interested in participating as monitors, 12 of whom are highly interested in water quality monitoring and conserving sites for *Z. tequila* reintroduction. Up until now, workshops for monitoring environmental and biological variables are in the early stages, with the expectation that in the short term the local groups can work independently obtaining physicochemical and microbiological data and generating a database available from a website. This sampling will provide data in real time and allow for contingency actions to protect the aquatic environment if necesary

The future

Limnological, ecological, and biological surveys

In order to have a long-term perspective about the habitat quality for the reintroduction of *Z. tequila*, we will continue to take samples and collect data on limnology, habitat quality, biological and ecological aspects of fish (including *Z. tequila*), plankton, insects, and parasites. This information will give us a better understanding of the habitat for the reintroduction of *Z. tequila* and will help identify future management strategies and possible threats.

Mesocosm experiments

Using the data obtained from the previous surveys, experiments involvingmesocosms technicques will take place at sites where it is believed that reintroduction of *Z. tequila* has the highest chance of success. In this context, mesocosms are relatively small netted-off areas of the Teuchitlán environment where natural circulation of water and movement of microrganisms can take place but the density and compostion of fish and larger animals can be restricted and manipulated for experimental purposes. In these mesocosm experiments, the ecological and biological responses of *Z. tequila* will be measured and recorded in order to elucidate in the short term: the population growth rate in the "new environment"; local parasite sucesptibility related to reproduction responses; and competitive effects and responses to the non-native species. The mesoscosm will be designed to avoid accidental releases of *Z. tequila*.The data obtained from these experiments will help us understand the potential success of the reintroduction of a native species into its original environment. It will also help us develop and implement long-term monitoring techniques for the local people.

Reintroduction of Z. tequila strategy

We are planning to conduct genetic analyses in order to ensure the taxonomic integrity of *Z. tequila* planned for reintroduction We plan to survey the initial genetic diversity of the semi-captive source population and then compare it with the reintroduced population in futures surveys of Teuchitlán in order to better understand the role that genetic diversity plays in the success of reintroduction of fish species. We are also hoping to install a semi-natural pond near the spring at the Balneario el Rincon (S1, S2 and S3) in order to maintain a high population of *Z. tequila* for reintroduction. The pond will have a continous flow by gravity and excess water will godirectly to S3. We are still working on the design of the pond. One of the potential problems for reintroduction will be non-native species, so we are also designing a trap to keep the exotics out of the springs. The design of this trap needs to low maintenance and yet provide for long-term protection. Once the trap is in place, we will conduct an eradication campaign to remove all of the exotic species from the springs.

Training

The monitoring of water quality in the Teuchitlán River and La Vega Reservoir and data collection related to the determination of hydrolic vulnerability of reintroduction sites includes the training of three undergraduate students in limnology, one in the management of hydrographic basins, and one in phycology, all of them part of the Programa Institucional de Monitores Ambientales, in the Departamento de Educación Continua de la Universidad Michoacana. As well, the monitoring training includes local volunteers from Teuchitlan. Furthermore, five undergraduate theses students, two Master theses students, and one PhD theses student are working on this project. Thesestudents will have a high potential to develop excellent theses related to the conservation and management of aquatic ecosystems.

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We have covered most of anticipated costs for the planned work for 2016, but we are still loking for funds for the environmental education and social programs, the genetic analyses, the mesocosm experients, eradication of exotics from the springs, and parasite surveys. For 2017-2019 we have partial funding, but we are still looking for additional support.