Annual report January 2022 to December 2022

Project: Reintroduction of the extinct species *Skiffia francesae* to their natural habitat, the springs of the Teuchitlán River

Introduction:

Skiffia francesae is Extinct in the wild (Mexican Official Red List; DOF, 2010, IUCN red list 2020, Domínguez-Domínguez et al., 2010, Lyons et al 2019). Accordingly, our project has the main objective to bring back to the wild the extinct *Skiffia francesae* to the Teuchitlán area. The primary beneficiary is the native people from Teuchitlán area since we are planning to continue restoring the habitat and recover the rich fish fauna that flourished in the area before human-induced extinction. At the same time, we will continue with the environmental education program that includes the local capacity building to secure the approval of the project by the local people. Meetings were made with "The Guardians of the River", an enthusiastic group in the conservation and protection of species and their environment. We worked together in planning activities for 2022. The Goodeids are a biologically and ecologically unique group that is considered a biological treasure of cultural significance to local communities, so this reintroduction is the final interest of biologists, governments, zoos, researchers, and conservationists around the globe. Establishing viable populations of Extinct in The Wild species is for the benefit of all.

Objectives:

1.- Carry out an experiment to obtain an effective deworming treatment for *S. francesae*.

2.- Set up mesocosm in the river, and conduct experiments of feeding habits, water quality treatments, competition, population growth, parasites, and condition factor experiment to figure out the potential adaptation of the *Skiffia* to Teuchitlán area and prevent possible problems for a new reintroduction event in the springs.

3.- Reintroduce organisms of *S. francesae* into the spring areas, with the participation of the local community of Teuchitlán.

4.-Monitor water quality at the release site. Study the biology of the reintroduce specie in the area and the ecology and habitat quality of the area were *Skiffia francesae* was reintroduce. Continue with the control and eradication of no native species in the river and spring area.

5.-During the field trips we are going to do an intensely monitoring thought the river, to identify if the species are prospering in other areas of the river.

6.-Continuing the environmental education program in coordination with the local community

Resume of the objectives:

Objetive	**Estimated time to spend on completing objective (in weeks and months)**	**Next steps**
Deworming treatment for *S. francesae*	six months	Reintroduce the individuals to the river
Experiments in mesocosm in the river	six months	evaluate feeding, reproduction, and other biological variables of the fish species
Reintroduce organisms of *S. francesae* into the spring areas	one year	Monitoring the survival of *S. francesae* in the springs
Study the biology of the reintroduce specie in the area	one year	Monitor water quality at the release site. Study the biology of the reintroduce specie in the area
Intensely monitoring of all the species reintroduced	five years	Monitoring all the species reintroduce in Teuchitlán river

Environmental education	five years	Continuing the environmental education project in coordination with the local community

Results

1.- Deworming treatment of *Skiffia francesae*

To characterize the parasitism in the population of *S. francesae* of the Botanical Garden, 60 fish (30 males and 30 females) were collected from the rustic pond (Figure 1). We sample the fish with minnow traps. The fish were euthanized with an overdose of benzocaine. Organisms were dissected, the intestine and liver were analyzed with the help of stereoscopic and optical microscopes to search for parasites. The crushing or "squash" technique was performed to facilitate the identification of the parasites. Then the number of parasites was counted. A total of 14 organisms presented parasites in the intestine, gonads, gills and muscle, with a prevalence of 26 parasites in total. We found the nematode *Spiroxys* sp. (larva and cyst) (Figure 2), it is an indirect endoparasite.

To carry out the anthelmintic treatment, organisms were collected from the rustic pond located in the UMSNH Botanical Garden. A total of 450 males and 450 females were collected and transported in plastic containers with water from the site to the Aquatic laboratory. The collected organisms were placed in 60-liter fish tanks with 50 organisms each, where they were kept for a period of 15 days at a temperature of 22 ± 0.5 °C, with a photoperiod of natural light and dissolved oxygen of 7 ± 0.5 mg/L. Dry and live food was provided twice a day.

For the antiparasitic treatment, the organisms were weighed (0.0001 g precision Ohaus Adventurer analytical balance) and measured (Fisher Scientific digital vernier) individually. The organisms used had a standard length greater than 20 mm. The system was controlled with a photoperiod of 12 hours of Light and 12 hours of darkness, maintaining a 22 ± 0.5 °C, pH of 7 ± 0.5 and dissolved oxygen of 7 ± 0.5 mg/L. The water used for acclimatization and anthelmintic treatments comes from tap water, previously filtered with a polypropylene sediment filter, granular and block activated carbon, ultrafiltration membrane, and ultraviolet light.

The treatment consisted to the addition of the anti-parasitic Albendazole in a concentration of 102 mg/kg in the dry food (Vitalis Flake food for Tropical Fish) (Figure 3). The medicine was crushed with a porcelain mortar until obtaining a uniformity in the powder obtained. The solution was prepared with the required amount of drug in each of the treatments where it was administered orally, these solutions were used to impregnate the flakes with the appropriate dose. The experiment lasted 15 consecutive days; the organisms were fed once a day with 3% of their body weight with the diet designed with the treatments. At the end of the experiment, a sample of 60 fish, 30 males and 30 females, was taken to be sacrificed and review the efficacy of the treatment. The fish were euthanized with an overdose of benzocaine, and we used the same technique that was used in the characterization of the parasitism ("squash" technique).

The results showed that both sexes presented an average of 0.06 parasites.

2.- Experiment in mesocosms

The organisms that were dewormed (section 1) were marked with elastomers sensitive to ultraviolet light, dividing males and females with different colors of elastomers (yellow females and orange males) (Figure 4). The mark was made on the right side of the organism, in the upper part of the back, where it was easily visible with a UV lamp. After marked were kept in observation for two weeks looking for changes in behavior, swimming, or appetite. Once this period ended, the fish were transported in sealed plastic containers with water from their tanks and constant aeration to the reintroduction site in Teuchitlán, Jalisco.

Twenty mesocosms were set in the first part of the Teuchitlán River (Figure 5). Five treatments with four replicates were established, each treatment being combined *S. francesae* and the exotic *Pseudoxiphophorus bimaculatus* in different proportions (Treatment 1: 148 organisms of *S. francesae*, Treatment 2: 180 organisms of *P. bimaculatus*, Treatment 3: 74 organisms of *S. francasae* and 74 organisms of *P. bimaculatus*, Treatment 4: 48 organisms of *S. francesae* and 104 organisms of *P. bimaculatus*, Treatment 5: 104 organisms of *S. francesae* and 46 organisms of *P. bimaculatus*).

A monitoring was carried out every three days for four months, the environmental variables of the water were evaluated, using a multiparametric probe. Two months later the organisms of the mesocosms were counts.

The results showed that *S. francesae* can survive in the environmental variables of the river (T1 76% survival) and can support the presence of the exotic species, however, it showed mortality in its presence (T3: 31% survival, T4: 36% survival, T5: 24% survival) and external damage to their fins, regardless of the sexual proportions found. Organisms that died are all marked (100%), these organisms will be check for stomach contents, reproduction, and parasites. In the treatment with only *S. francesae* some females were pregnant, at the end of the experiment we counted 400 fries, without mark of elastomer, indicating new fish born in the mesocosm. However, the presence of *P. bimaculatus* is an important factor for the development of *S. francesae*, limiting its number to very few organisms, but its presence is supported.

3.- Reintroduce organisms of *S. francesae* to the spring areas

We reintroduce specimens maintained in two conditions at least one month before reintroduction, the differences were as follows:

Method one: it consisted of 400 organisms (200 females and 200 males) dewormed following the protocols of section 1 (Deworming treatment of *S. francesae*) and marked with a blue elastomer (following the same technique and position of the elastomer that was used in the mesocosms experiment, section 2). The fish were transported from the aquatic biology laboratory (in Morelia Michoacán) to the Teuchitlán river in Jalisco (4 hours and a half - 346 km approximately) the same day of the reintroduction. The fish were transported in plastic containers with water from their fish tanks, anti-stress solution and constant aeration

Method two: it consisted of reintroducing 400 organisms (200 females and 200 males) marked with pink elastomer, these organisms were also from aquatic biology laboratory fish tanks, but these organisms were transported to the Teuchitlán river and placed at the mesocosms that were previously installed in the first part of the river two weeks before reintroduction. They were monitored daily in search of

organisms that had died, which were removed (a mortality of 6 individuals was found).

On November 4, the reintroduction of both populations (method 1 and method 2) was carried out in the sociocultural event described in the environmental education part (section 6). The specimens were liberated in two springs in the upper part of the Teuchitlán River (Figures 6) distributed in similar numbers of organisms.

4.-Study the biology of the reintroduce species in the area

This objective will begin to be evaluated once the *Skiffia* population is established, since the reintroduction was during this month of November, and it is still too early to carry out a follow-up of its biological aspects of the species in the reintroduced area.

5.- Intensely monitoring of all fish species in the river

Four samplings were carried out to determinate biomass, abundance, sex, and species data from headwater along the river and some spring close to La Vega Dam (Figure 7). Environmental data were taken with a Hanna-type probe in each site. According to results, the highest abundance of the species is in the springs close to La Vega Dam sites (Figure 7).

Ten species were found along the river, five native and five exotics. The data indicates that the most abundant and dominant species along the river are the exotic species *Xiphophorus hellerii* and *P. bimaculatus*.

In the headwaters, in the spring sites, the native species have a greater abundance than exotic species. Through the river the abundance of the native species decreases and the exotic increase. The characteristics of the spring water are more suitable for native species.

A total of 69 individuals of *Z. tequila* were capture (38 females and 31 males) in the spring of the upper part (original release site), the species showed a normal condition (bases on the weight and the length of the organisms). In the springs close to La Vega dam also were found organisms of *Z. tequila*, a total of 159 were found (89

females and 70 males) (Figure 8). It is notorious that in the springs areas close to La Vega Dam the population is increasing.

6.-Environmental education activities

Environmental education activities have continued under the direction of the Guardians of the River. A week of orientation and information for tourism was held, where the group of guardians of the river prepared and delivered an informative diptych to create awareness in people that comes to visit the river (Figure 9). A total of 300 persons were attended.

In April, a puppet theater was held about the conservation of river species, attended by 100 children (Figure 10).

In June, children of guardians of the river participated in a camp at the Guadalajara Zoo, where they interacted with 200 children from other communities. The objective of the camp was to make children aware of water care (Figure 11).

Playful workshops on ecological awareness were held in July, with the participation of 80 children, held in the Teuchitlán public square. Talks, traditional games, and board games were held, where the children had fun and learned about the fish of the Teuchitlán River and the conservation of the river (Figure 12).

Infographics with themes of caring for the environment were made, where they were disseminated through the social networks of the project link: https://www.facebook.com/profile.php?id=100069923225348 (Figure 13).

Every Tuesday from April to July, interpretative trails called "the Zoogy trail" of the Teuchitlán River were carried out, where 120 children participated, and they were explained about the importance of the river and its species (Figure 14).

In the month of August, a recognition of endemic and exotic fish in the El Rincón springs was done, which was called "a day as a biologist" where 80 children participated (Figure 15).

During the development of the activities indicated above, meetings were held with the new municipal authorities to present the project and establish collaboration agreements.

In September the Guardians of the river and our team started working on the organization of the “Skiffia forever” event that took place at the beginning of November.

On November 4, as part of the Night of the Dead celebration, the event called "Skiffia forever" was held in Teuchitlán, Jalisco. It was carried out in two moments, the first one was carried out in the morning at the Guachimontones Interpretive Center and in which local, ecclesiastical, and academic authorities and Guardians of the River participated. The objective was to publicize the participation of local actors such as the church and Guardians of the River in conservation actions for the river and its endemic species. There was also the participation of international organizations such as Chester Zoo and Shoal, among others (Figure 16). This event was attended by educational authorities, students and the people from Teuchitlán, with an attendance of more than 100 people.

The second moment of this great event was held at night at the El Rincón springs. It consisted of cultural artistic activities, regional and pre-Hispanic dances, a theater play represented by biology students about the reintroduction and conservation of endemic species of the Teuchitlán river. There was the participation of children belonging to Guardians of the river with an acrostic forming the word *S. francesae*, at the end of the event there was a “night of the dead” procession led by a local dance and followed by educational and local authorities and the public to observe the release of the first specimens of *S. francesae* to their home (Figure 17 and 18). In total, around 400 people attended, making possible the inclusion of the local people in the actions that are carried out for the conservation of endemic species of the Teuchitlán River and achieve a greater participation of society in them.

Preliminary conclusions

Through the experiments in mesocosms, we conclude that *S. francesae* organisms tolerates the present environmental conditions and is capable of survive with the dominant exotic species of the river (*P. bimaculatus*).

The first reintroduction of *S. francesae* to the Teuchitlán springs area was made, a total of 800 organisms were reintroduced on November 4 through a socio-cultural

event. To the next day to a following week, a monitoring to observe the Skiffia organisms in the springs were made, with the use of Snorkel, we only found organisms with the elastomer color pink (see methodology two, section two), these individuals have been found with the caudal fin quite rotten and mistreated, so in the following months we will continue with monitoring of the reintroduced species to have more robust conclusions.

The local community of Teuchitlán has responded favorably to the various activities organized by the Guardians of the River, where many children and adults have been involved in them. Work will continue with more activities around the conservation of nature, native species, and the Teuchitlán River.

Figures

Figure 1.- Botanical Garden, UMSNH

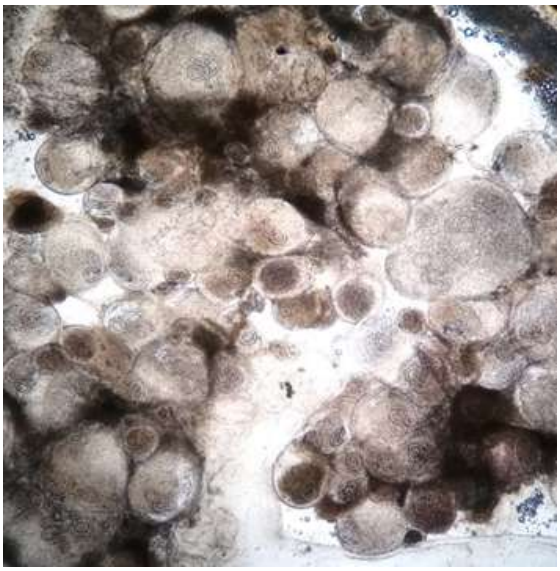

Figure 2.- Parasites found in the organisms of *S. francesae*

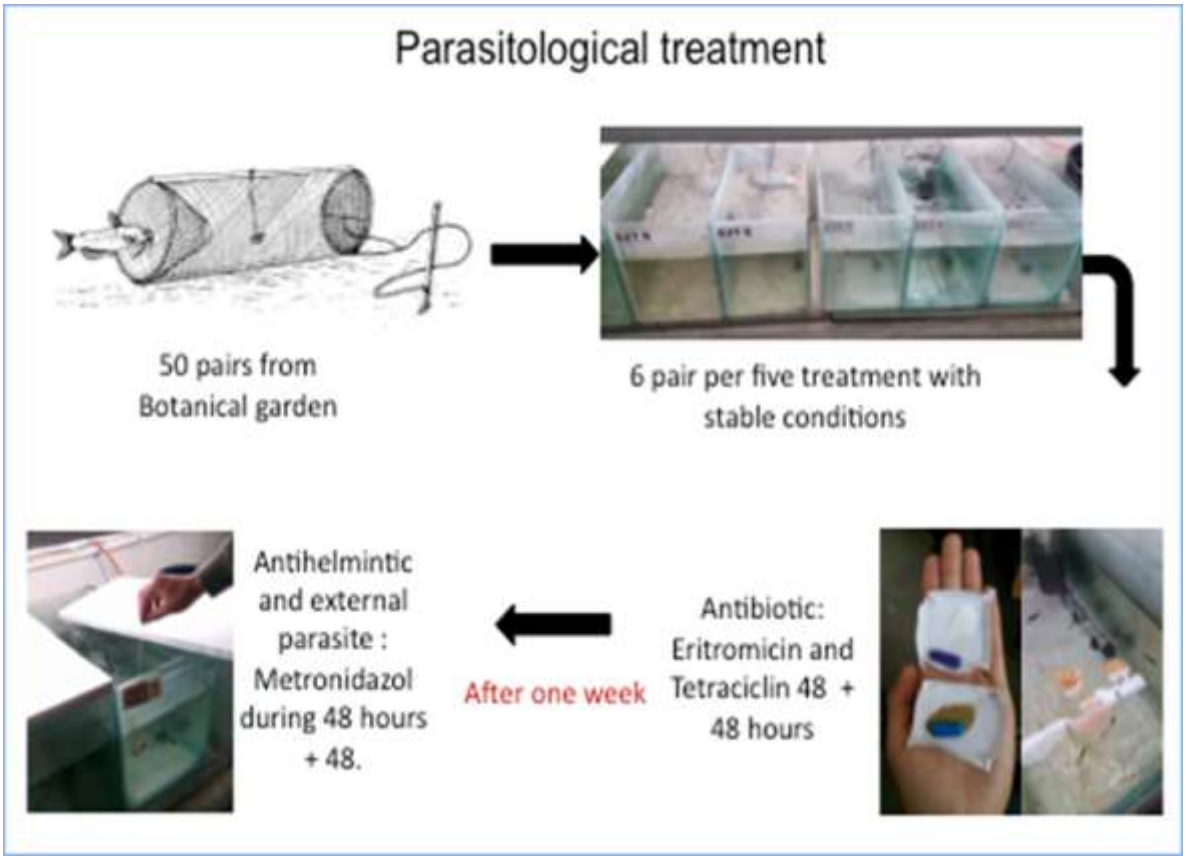

Figure 3.- Antiparasitic treatment

Figure 4.- Organisms of *S. francesae* marked with elastomer

Figure 5.- Mesocosms set in the first part of the river Teuchitlán.

Figure 6.- Reintroduction of the *S. francesae* to the springs areas

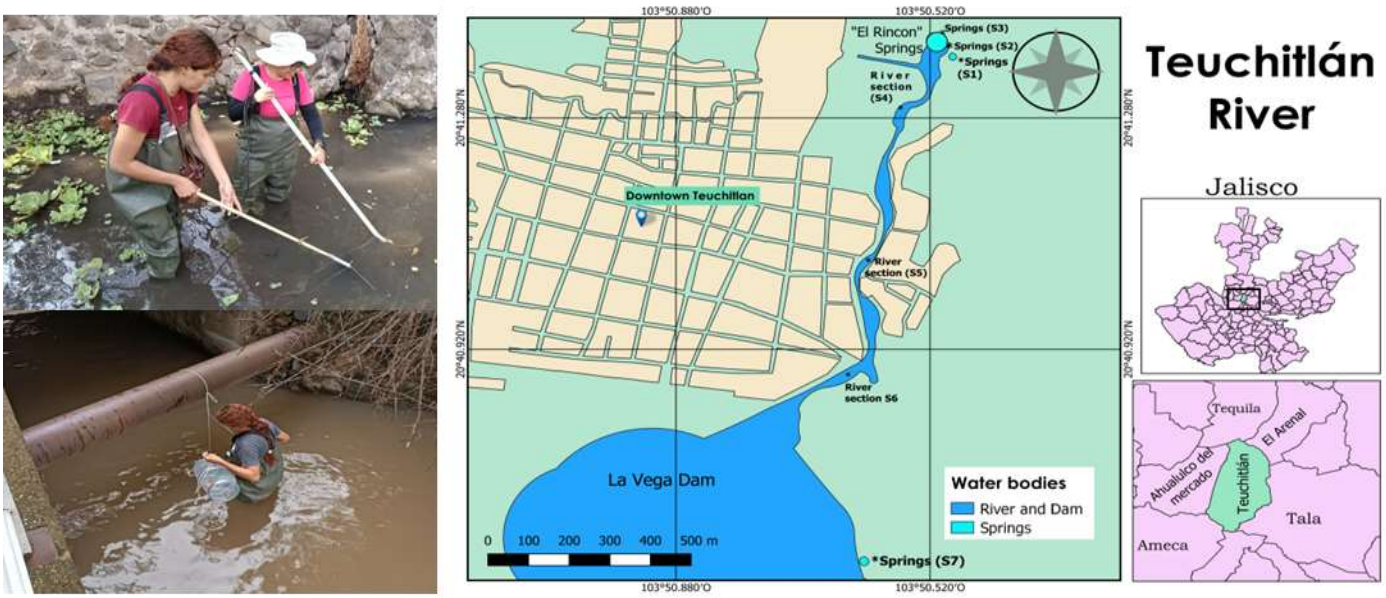

Figure 7.- Monitoring of fish species in the springs and river (left) and location of the sampling sites (right).

Figure 8.- Monitoring of *Z. tequila* in the springs close to La Vega Dam

Figure 9.- Informative diptych to create awareness in tourism that comes to visit the river

Figure 10.- Puppet theater in the square of Teuchitlán

Figure 11.- Camp at the Guadalajara Zoo

Figure 12.- Workshops on ecological awareness

Figure 13.- Infographics with themes of caring for the environment

Figure 14.- “The Zoogy trail"

Figure 15.- "A Day as a biologist"

Figure 16.- Event of the “Skiffia Forever” in the Guachimontones Interpretive Center

Figure 17.- Cultural artistic activities in the event “Skiffia Forever”

Figure 18.- Reintroduction of the organisms of *Skiffia francesae* to the springs areas.