





Creating a Participatory Action Plan for conservation of *Ambystoma granulosum* (Ajolote)

FINAL REPORT



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Introduction

The impact of human activities on freshwater ecosystems have led to the extinction of 122 species since 1980, currently a third (32%) of amphibian species worldwide are threatened and at least 43% of the species are experiencing events of population decline. In America 46% of salamander species are threatened or extinct (de Sá, RO 2005).

México ha experimentado en los últimos años un alto porcentaje de deforestación y cambio de uso de suelo, lo cual ha llevado a un impacto negativo en las poblaciones de los anfibios (Parra-Olea et al, 1999). En este grupo encontramos al *Ambystoma mexicanum* endémico de la Ciudad de México; *A. andersoni y A. dumerilli* endémicos de Michoacán; *A. granulosum y A. lermaense* endémicos del estado de México. Estas especies de salamandras se encuentran clasificadas actualmente por la IUCN como en peligro crítico de extinción y por la NOM-059-SEMARNAT-2010 como especie en peligro de extinción (P) *A. mexicanum* y el resto sujetas a protección especial (Pr).

Mexico has experienced in recent years a high rate of deforestation and land use change, which has led to a negative impact on amphibian populations (Parra-Olea et al, 1999). In this group we find the *Ambystoma mexicanum* endemic in Mexico City; *A. andersoni* and *A. dumerilli* endemic to Michoacan; *A. granulosum* and *A. lermaense* endemic to State of Mexico. These salamander species are currently classified by the IUCN as critically endangered. Mexico normativity (NOM-059-SEMARNAT-2010) classify the *A. mexicanum* as endangered species (P) and the rest is subject to special protection (Pr).

Species loss leads to the alteration of the structure and functions of ecosystems, mainly the loss of environmental services as climate regulation, humidity of the environment, carbon sequestration, water catchment and filtration, dust storms or flood mitigation, etc. These are intangible benefits that humans obtain from natural resources resulting in human health and quality of life.

Therefore decisions and actions taken on economy, transportation, social justice and governance topics, should always consider the citizen participation, as it is in last term, the user and beneficiary of a healthy ecosystem.

In turn, the generation of knowledge by the scientific and technical field is essential to assess the state of ecosystems and propose measures that contribute to a better management, under the framework of sustainable development, meaning the conservation of ecosystems alongside the social and economic development.

For the foregoing reasons, it is outstanding the initiative of Alstom Foundation on supporting projects with both components, the social and the environmental with the goal to achieve a sustainable development.

Participation of citizenship, Academy, NGO and local Authorities, is also a characteristic of the projects founded by Alstom.

Study area

Brockman and Victoria dams are located at El Oro, Mexico province, 94 km northwest from Toluca City, capitol of Mexico State, 2,748 m above sea level. Nearby towns are at west, Tlalpujahua, Michoacán; at south San José del Rincón, Estado de Mexico (Figure 1). These dams are located in Lerma-Santiago hydrographic region within the Lerma-Toluca river basin and contribute to the recharge of the nowadays overexploited lxtlahuaca-Atlacomulco aquifer (SMA, Estado de México 2007).

Dams are part of the Park "Santuario del Agua y Forestal Presas Brockman y Victoria" which was decreed on October 13th, 2004 by Estado de Mexico government with an area of 1,554.98 hectares.

Weather type is classified as C(w2) temperate, subhumid with an annual average temperature between 12 and 18 °C. The coldest month is between -3 and 18 °C and the warmest month is less than 22 °C. Rainfall during driest month is less than 40 mm reaching to above 200 mm in July. This region presents rain in summer and winter with only 5 to 10.2% of the overall annual rain (INEGI, 2012).

Soil surrounding the dams is classified as humic acrisol. It has a medium textural class, characterized for its acidity and it develops mainly on alteration products of acid rocks with elevated levels of highly altered clays. The zone is characterized by a rugged landscape, with altitudes ranging from 2,500 to 3,200 m.

On the southern edge of the Brockman dam the mountain "Cerro Cedral" is located, where forests of Oak, Oyamel (*Abies religiosa*) and Juniper-oak are distributed. The rest of the landscape between both dams has temporal agricultural production and small settlements of which "Presa Brockman and Campo Azul" are the main locations of major influence to the dams due to its proximity and the population settled.

Runoff from Cerro Cedral and a non-permanent stream from Cerro Llorón (southeast from the dam) are the main contributors to Brockman dam.

Brockman and Victoria dams have an extent of 30 hectares, providing a flow of 6 to 12 liters per second. This implies a daily volume of extraction between 518,000 to over 1 million liters per day.

Water provided by both dams represents almost 90% of the supply for El Oro, and it is released as drinking water quality by the water treatment plant located next to Victoria Dam, providing between 50 to 60 liters per second (SMA, Estado de México 2007).

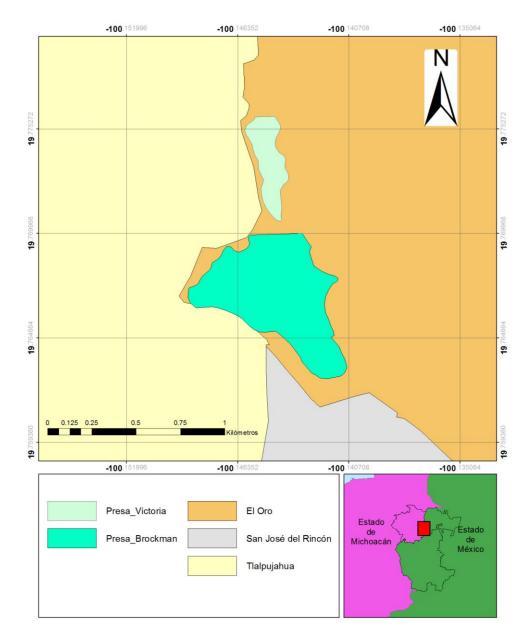


Figure 1. Study area in which the location of the Brockman and Victoria dams shown. Geopolitical influence of three adjacent municipalities observed in two states (states).

In 2009, the municipality of El Oro had 34,218 people, of which 3,469 speak an indigenous language. There are 6,545 households. According to calculations of the National Population Council (CONAPO), the municipality has medium degree of marginalization. This responds to factors such as inequality in income distribution and unemployment. The municipality is predominantly rural, 82.81% of people are living in communities of less than 2,500 inhabitants.

Regarding the municipality El Oro, there are four main economic activities. In this municipality an area of 8023 hectares is used for the production of basic grains such as corn and oats. It is grown in rainfed and irrigated modalities. Apple, pear, plum, walnut and wild cherry is also grown in approximately 66. Vegetables (tomato) and flowers are

produced in greenhouses. As for the livestock of El Oro, sheep is produced. In recent years it has developed the processing and marketing of rabbit meat. El municipality of El Oro is mainly forest, it currently has an area of 6024 Pine Forest, 1036 Native oak and pine forest-cedar is present. By its natural and historical riches, tourism is a major activity in the town. ¹

In the municipality of Tlalpujahua, the total population in 2010 was 27,587 people and there were a total of 6,591 households, of which women headed 1,683. 14,374 inhabitants in the municipality have a public health care, and 3,456 are beneficiaries of social program "Oportunidades". In Tlalpujahua an area of 3,246 hectares is cultivated. The municipality has an important forestry activity; the volume of lumber production (cubic meters roll) in 2011 was 16,454. For its scenic and cultural beauty, the town has an important tourism sector².

Section I. Environmental Analysis

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Environmental variables

Objective

Evaluate the environmental characteristics of the region and the possibility of *A. granulosum* presence.

Methods

Environmental variables

In order to understand dynamics of abiotic and some biotic parameters in one year, measurements were taken during the rainy (June to September 2013) and dry season (April 2014). In total, we conducted four visits: June (24-29), September (9-11) and April (1-3 and 27-30). Components analyzed were water and sediment.

Location of sampling points was the same as established in the first field trip in June 2013 with the aim of identifying the general characteristics of water and the internal variations at Brockman and Victoria. However, from rainy to dry season (September 2013 to April 2014), three additional sampling points were measured in the flooding area of Brockman Dam (BP11, BP12 and BP13) see Figure 2.

¹Official website of the municipality El Oro: http://www.eloromexico.gob.mx/poblacion.html. Accessed 26 September 2014

²INEGI official website http://www3.inegi.org.mx/sistemas/mexicocifras/default.aspx?e=16. Accessed 26 September 2014; and Official website of the municipality Tlalpujahua: http://www.tlalpujahua.com/. Accessed 26 September 2014.

Physical-chemical parameters measured during all field trips were: temperature (°C), dissolved oxygen (mg L⁻¹), pH, conductivity (K25 µS cm⁻¹), total dissolved solids (mg L⁻¹) and water clarity. The first five parameters were recorded with a multiparameter probe (Hanna® 9828) at surface (≈1m deep) and a Secchi disc for transparency measurement.

We also placed two sensors at each dam (ONSET HOBO®) which recorded every 30 minutes water temperature, from June to November (2013) when the first data were extracted. Data were recorded continuously 24 h from June – November 2013. In June 2013 temperature loggers were placed in both dams at two different depths, surface (1.5 m from the water surface) and deep (at 1 m from the bottom). Total depth for Brockman at the place where the loggers were located, was 2.8 m and for Victoria, it was 3.3 m.

However sensors were not found during the first field trip in April (2014) for the second data set extraction. Therefore in the second field trip in April 2014, a second set of sensors were placed and again data couldn't been obtained because water level increased and covered the sensors being impossible to find them. Therefore data for water temperature refers to punctual measurements.

Water analysis comprised nutrients, heavy metals and detergents. Samples were collected, preserved and stored in an ice cooler at 4°C and delivered to the laboratory the day after the collection.

Nutrients (nitrates (NO₃-), orthophosphates (PO₄-3) y ammonia (NH₄+) were measured in order to know eutrophication degree of water. According to the mineral activity of the region in previous years, heavy metals (arsenic (As), cadmium (Cd), copper (Cu), hexavalent chromium (Cr+6), lead (Pb) and mercury (Hg) were also measured to discard any pollutant source.

For arsenic, cadmium, copper, lead and mercury tests, analytical method used was based on Mexican regulation NMX-AA-051-2001 SCFI; for hexavalent chromium was based on NMX-AA-044-2001 SCFI and for detergents (SAAM) the analytical method is described in NMX-AA-039-2001 SCFI.

Detergents were measured only in April, as a result of the community concerns for the activity of "laundry" at one of the tributaries in Brockman dam, as a result of the participatory workshops.

In order to get *in situ* results for nutrients and heavy metals measurement (lead, cadmium, hexavalent chromium, copper, nitrates and orthophosphates), a LaMotte colorimeter Smart 3 was used. This equipment will also be used during participative monitoring workshops to be delivered mid-October as part of the project closing sessions.

As for pathogenic pollution, we used *E. coli* and total coliform bacteria as indicators. At sampling sites, a 3M Petrifilm [™] plate was inoculated and incubated with 1 ml of water from 10 points in total, for Brockman and Victoria. The bacteriological variables were measured previous and after April holyday season in order to assess the impact of visitors,

since during participatory workshops these situation was presented as risk for water quality.

In the case of sediment, samples were collected with an Ekman type dredge. Laboratory analyses were applied to determine the following parameters: fungi, fecal coliforms, Salmonella spp and helminth eggs. Also copper (Cu), hexavalent chromium (Cr⁺⁶), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb).

Analysis were performed according to Mexican normativity NOM-004-SEMARNAT-2002 for fungi, fecal coliform, salmonella spp., helminthes eggs, arsenic, cadmium, copper, lead and mercury. In the case of hexavalent chromium, analytical method was based on NMX-AA-044-SCFI-2001 regulation.

Laboratory analysis were all done by *Investigación y Desarrollo de Estudios de Calidad del Agua (IDECA) S.A. de C.V.* with certifications: EMA: AG-010-154112 (from 2012-08-24); CNA-GCA-836; PADLAIDF/047/AARI2012.

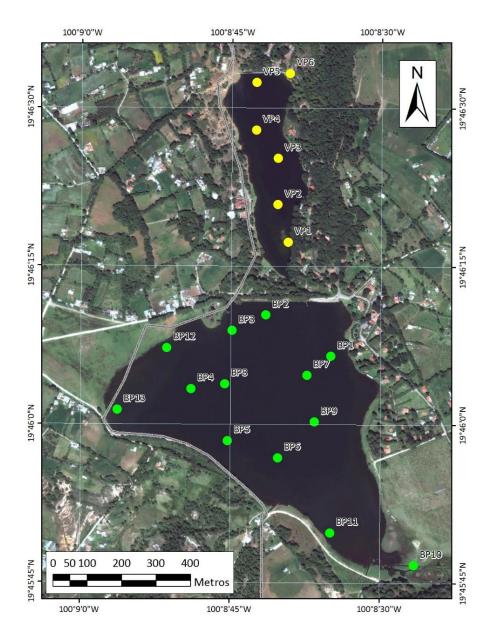


Figure 2. Distribution of sampling points of abiotic variables in both dams. Water bodies are observed very close to its maximum capacity.

Data analysis for physico-chemical variables temperature, pH, dissolved oxygen, conductivity and total dissolved solids, was done with spatial interpolations for the annual average of each variable.

Specifically, the interpolation applied was the Inverse Distance Weighted (IDW). This method is recommended when there are few sampling points or irregular spatial distribution points. The method is based on the assumption that nearby points have more influence than farthest points when estimating in points with unknown values (Kravchenko 2003). The model has two assumptions: a) the power which define the influence of the nearest points on the prediction of the new cell or pixel and b) the search radius can be

fixed or variable. The pixel size was 2 x 2 m and we used the QGIS program V 2.4., to run the data.

Catching and logging of organisms (amphibians, phytoplankton, zooplankton and macro-invertebrates).

In order to look for the ajolotes, we used minnow traps placed in Brockman (20 traps) and Victoria (6 traps) (Figure 3), during dry and rainy season. In the rainy season in Brockman dam, the traps remained 72 hrs. and 48 h at Victoria dam. During dry season time lap was reduced because we observed local people were taking out the traps. At Brockman dam traps remained submerged 48 hrs. and 24 h at Victoria dam. An indirect catching were fishes.

Phytoplankton and zooplankton, are the base of the aquatic food chain. In order to identify the base line for isotope analysis and the species of phyto and zooplankton, we did a dragging along 500 m in the Brockman dam and 300 m in Victoria dam for each one of the seasons, to pelagic level with a depth of 1m. Equipment used were trawl nets with a mesh size of 250 and 63 microns respectively and an entrance mouth diameter of 30 cm.

Phytoplankton samples were identified and counted at the Limnology laboratory of UNAM (UIICSE; FES Iztacala). The method used was Utermöhl (1958), which is based on the deposition of an aliquot of a known volume of a water sample using an inverted microscope. The cells, previously fixed fall by gravity and settle to the bottom of sedimentation plate.

In the case of zooplankton, the sample was also previously fixed with an iodine solution. For Victoria samples, direct counting was done with 1 ml of water on a gridded plate, repeating the procedure five times. For Brockman dam samples, as they presented a high density, 1 ml of water sample was placed as well on the gridded plate and three grids were counted. Result was extrapolated to the total of grids in order to do a homogenous counting in relation with Victoria. The procedure was also performed five times.

Finally, we carried out the collection of other organisms as macro-invertebrates, to characterize the food web of both dams. Samples for isotopes analysis were frozen and taken to the laboratory of the Biology Institute in order to do the corresponding processing. For clean tissues of fish and ajolotes, samples were dried at a desiccation stove at 50°C for 24-48 hours. For macro-invertebrates without a carapace the procedure was the same, but for insects or mollusks with carapace, it was first removed and the tissue was equally dried.

Once tissues are completely desiccated, they get pulverized and enveloped in special foil paper. For plants dry weight required is 5 mg and for animal tissues it is required 3 mg. Therefore collecting effort might be huge to get enough organisms for the dry weights needs.

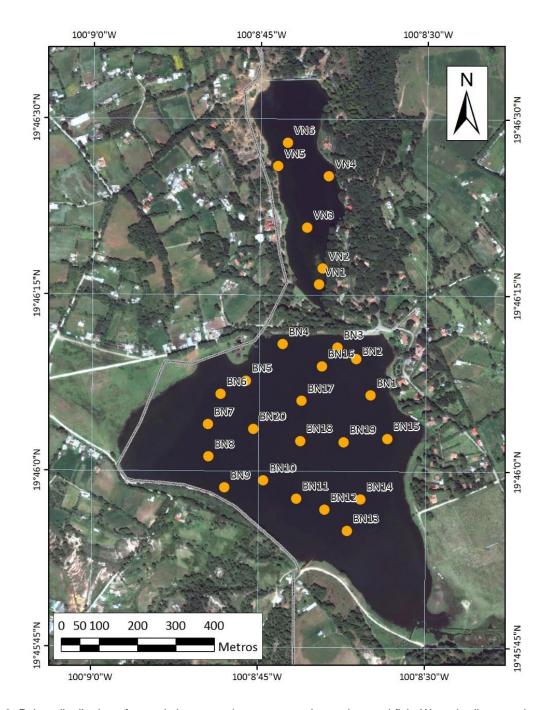


Figure 3. Points distribution of traps (minnow trap) to capture salamanders and fish. Water bodies are observed very close to its maximum capacity.

Results Temperature

Data from loggers were extracted twice in September and November 2013. Registers indicates that until November, Brockman temperature ranged between 15°C and 20 °C and Victoria's ranged between 16°C and 21 °C, variations in temperature along the study

period showed the same pattern at both dams and a slightly higher temperature was observed at Victoria reservoir (Figure 4).

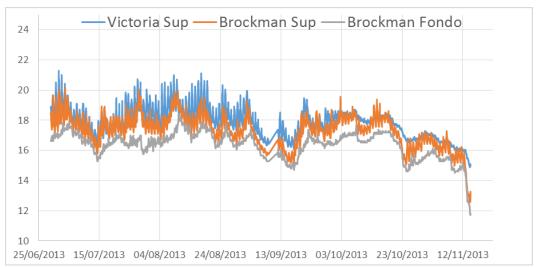


Figure 4. Water Temperature record during the rainy season in Brockman an Victoria dam. Victoria sup= surface temperature at Victoria; Brockman Sup= Surfae temperature at Brockman and Brockman Fondo= bottom temperature at Brockman.

In November, the air temperature dropped to 0 °C due to a cold front, which was reflected in the water temperature recorded: 11 °C Brockman-bottom, 13 °C for Brockman-surface and 15 °C for Victoria dam-surface (Figure 4). These were the last measurements obtained with data recorders.

In Figure 5 temperature variation between sampling points is shown similar for September and April. The PB10 point corresponds to the stream at the southern part of the dam which has a slightly higher temperature than the rest of the points in both seasons of the year. The PB11 point registered the highest temperature in both seasons, probably because of the low depth at this sampling point.

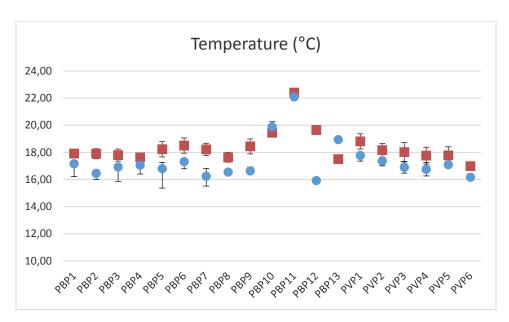


Figure 5. Average water temperature for each of the seasons and sampling point. Red boxes= rainy season (2013), blue circles= dry season (2014)

At Brockman dam cold temperatures occurred from the center toward the northern area where the spillway is located. This area has a greater depth (Figure 6). Warmer temperatures were registered in the south and east area of the dam which are part of the floodplains, where water is withdrawn in the dry season. Victoria dam presents a less temperature range than Brockman because it has a greater depth, its shape is elongated and has a gentle slope on its shores lines.

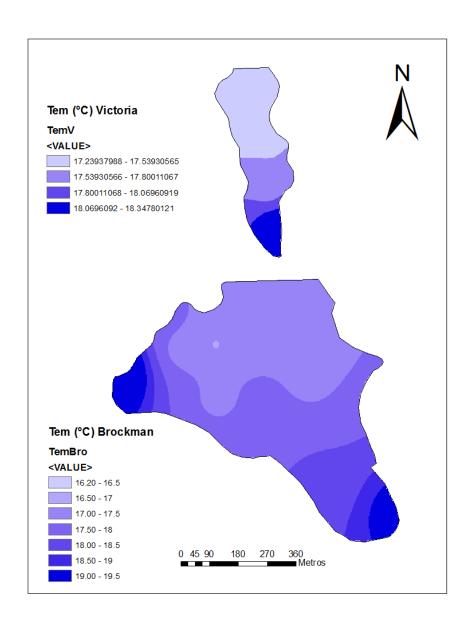


Figure 6. Water temperature interpolation for the annual average sampling.

pН

pH is one of the most important parameters for freshwater, since it is a measure of the acidity or alkalinity of solutions. It is related to the balance of the hydrogen ions [H+] and ions hydroxyl [OH-] in water. It also has a close relationship with the dissolution of carbonates and bicarbonates insolubilization. Also, photosynthesis of primary producers in the water (phytoplankton, algae and macrophytes) can significantly reduce the concentration of CO₂, so the pH is the result of the carbon balance and the process of living aquatic microorganisms.

According to the values registered along the monitoring, both water bodies tend to basicity (Figure 7). pH show higher values in the rainy season, perhaps caused by the input of allochthonous material. The only sampling point with lower pH values (7-8.2) is BP10 which is the entrance from the stream at the southern part of the dam.

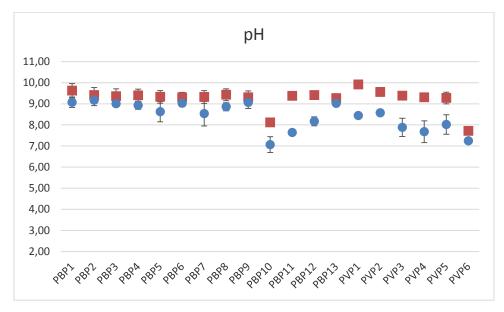


Figure 7. Average pH of water for each of the seasons and sampling point. Red boxes= rainy season (2013), blue circles= dry (2014)

Variation in pH values is greater in Brockman than in Victoria. In Figure 8 we can observe correspondance values between north of Brockman and south of Victoria dams which could be the result of the spillway from Brockman to Victoria. Also the presence of aquatic macrophytes in abundance at south of Victoria dam could be influencing higher pH values.

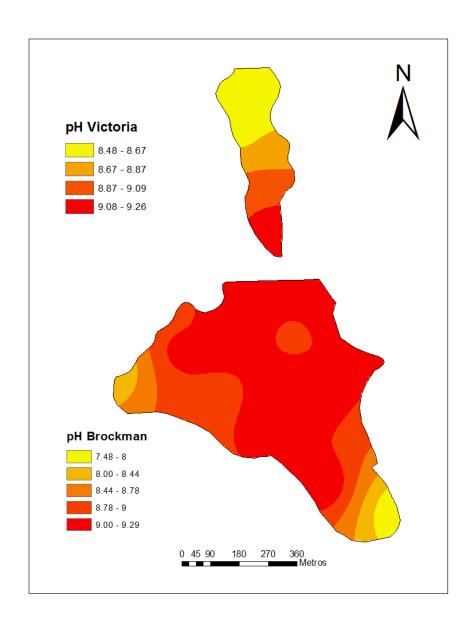


Figure 8. Interpolation of the pH of the water to the annual average sampling.

Dissolved Oxygen

Dissolved oxygen is vital for most organisms inhabiting aquatic systems, it is consumed by living organisms as well as by the bacteria acting in the decomposition process.

Concentration of dissolved oxygen vary according to several factors, such as depth, water temperature or presence of aquatic macrophytes. Nevertheless in spite of these constraints, oxygen values at Brockman and Victoria dam showed to be above 3 mg L⁻¹ (Figure 9) which is the minimum required for aquatic organisms to live. The only exception is point VP6, which has almost anoxic values. This measurement was taken in the spillway

from the water treatment plant and it might be due to the own chemical processes of the plant. This value was not included to avoid outliers and also considering it is actually an external sampling point.

The next low value of oxygen for Victoria dam is VP4 which is located in front of the spillway and maintain a high density of aquatic macrophytes, so this value might be a combination of both factors.

Nevertheless oxygen is not a limiting factor for ajolotes or any other aquatic organism, as it is easily observed in Figure 10.

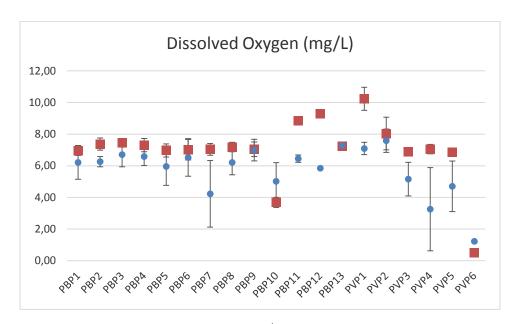


Figure 9. Dissolved oxygen average concentration data (mg L^{-1}) for each of the seasons and sampling point. Red boxes= rainy (2013), blue circles= dry (2014)

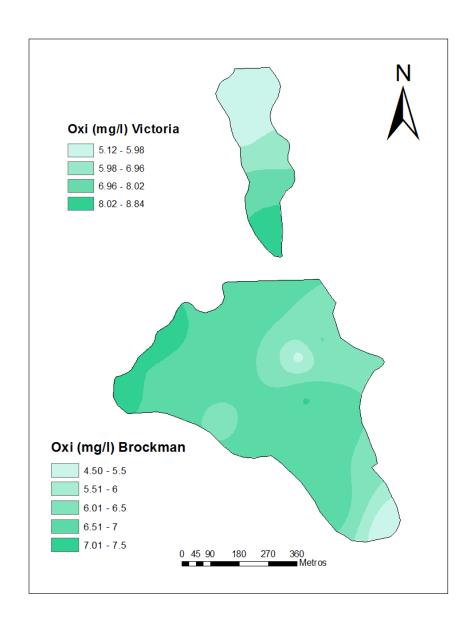


Figure 10. Interpolation of dissolved oxygen in the water for the annual average sampling

Conductivity and total solids dissolved

Given that conductivity is a measurement of salts dissolved in water and dissolved solids are in more or less degree also salts, both measurements are correlated. These parameters are influenced by dissolution of the rocks in the substrate, pH, water temperature and the nature of the type of salts present, among others. For example, in a body of water undisturbed by human activity total dissolved solids must be below 1,000 mg L⁻¹, which is the case for both dams.

Brockman has a range between 70 - 138 S/cm, with more variation in the rainy season (Figure 11 and Figure 12). In Brockman the BP10 point was opposite with respect to the rest of values registered, probably as a result of entrance of dissolved solids from the runoff area and / or due to water evaporation, since the site is a shallow area.

Based on interpolation of conductivity in both dams (Figure 13 and 14), Victoria shows to be similar in conductivity/dissolved solids to the deepest area of Brockman which has stable conditions. Therefore these parameters are neither a limiting factor for aquatic organisms *in situ*.

The change of land use in the catchment area of dams can generate an increase in total dissolved solids as it can increase the supply of soil materials to the water body. Therefore the measurement of these parameters are very useful indicators for land planning as a governmental policy.

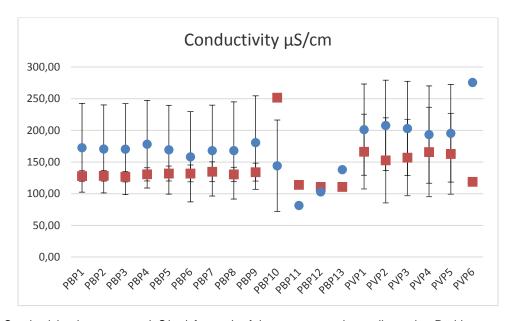


Figure 11. Conductivity data average (μ S/cm) for each of the seasons and sampling point. Red boxes= rainy (2013), blue circles= dry (2014).

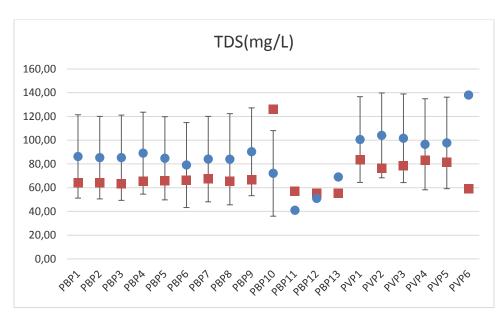


Figure 12. Total dissolved solids data (mg L^{-1}) average for each of the seasons and sampling point. Red boxes= rainy (2013), blue circles= dry (2014).

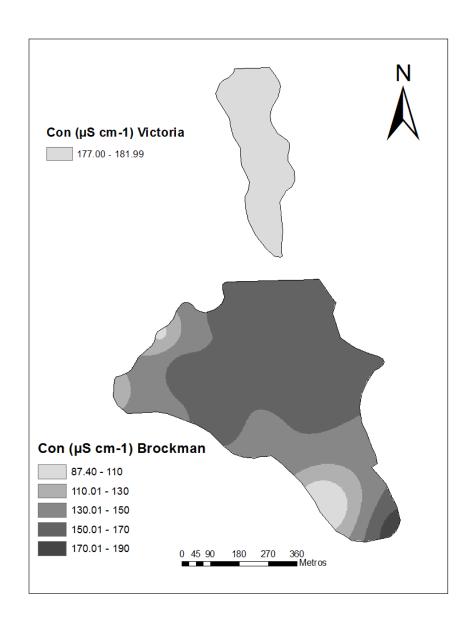


Figure 13. Interpolation water conductivity (μ S/cm) to the average annual sampling.

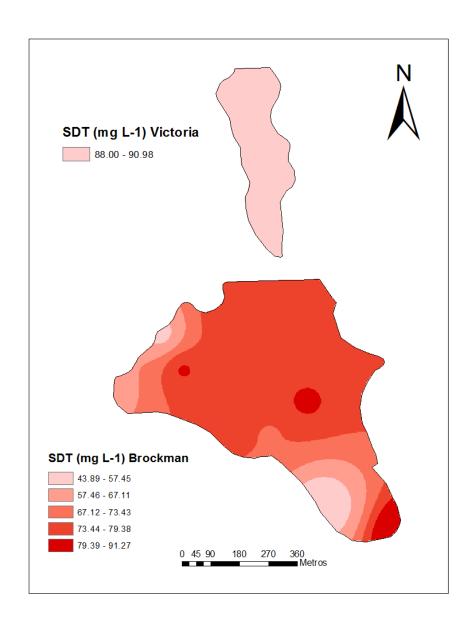


Figure 14. Interpolation of total dissolved solids (mg L-1) of water for the average annual sampling.

Depth and transparency

Transparency of water is restraint to dissolved and suspended solids. Usually the difference of total depth minus the depth where the sunligth penetrates into the water show how stable or "healthy" the water body is. In eutrophized water bodies, algae prevent light penetration and aquatic vegetation can't grow which in turn diminishes food and reproduction areas for aquatic organisms and also diminish oxygen concentration in water.

For Brockman and Victoria, monitoring of limnological parameters started during rainy season of 2013, when dams contained the water of the previous rainy season. Maximum level in both dams was reached in October 2013 and August 2014.

Variation of water level in Brockman was higher than 3 m but water depth is less than in Victoria dam, probably because Victoria's morphology resembles an inverse cone while Brockman has a less pronounced slope from the edge to the center of the dam.

According to transparency data, both dams are considered mesotrophic (Table 1), this means that nutrients, algae and humic substances are balanced but a higher input of nutrients from human activities might easily change this condition and gradually turn it into an eutrophized state.

The presence of *E. densa*, the aquatic macrophyte is the result of water transparency and has beneficial impact in water quality because it retains suspended solids (Sand-Jensen y Pederson 1999) and provides food and reproduction niches for aquatic organisms. However high density of the plant could consume oxygen from water affecting other organism's survival.

To determine the adequate density of the aquatic plant is necessary specialized studies but as we have seen in Brockman, suddenly in the edges appear dead *E. densa* which might mean that it is self-regulating its density population.

Again, nutrients and soil material input, due to external human activities, can change this condition and reverse the environmental quality of the dams.

In the case of Victoria dam transparency is even higher because Brockman receives the input of suspended solids which sediment in there and since water from Brockman to Victoria enter through the spillway or by opening the floodgates of Brockman, when this happens, many suspended solids have settled and Victoria receives clean water. Also around Victoria's edge *E. densa* constitutes a barrier for the soil eroded from the surroundings increasing water transparency.

One of the results of workshops with local inhabitants was that there are two groups who have different opinions about the presence of *E. densa*, one thinks is damaging water quality and other thinks is beneficial. Our point is that it is working in favor of the water quality but is important to control density and it can be done as long as land use with damaging human activities as fertilizers use, deforestation or urbanization is avoided around the dams.

Table 1. Depth, transparency, and atmospheric and water conditions at the time of making the data. Z=dept, Transp=transaparency and Con atm/water=

| | | Rainy sea | ason 2013 | | Dry sea | son 2014 |
|-------|-------|-----------|---------------------------|-------|---------|-------------------------------------|
| Point | Z (m) | Transp | Con atm/water | Z (m) | Transp | Con atm/water |
| PBP1 | 3.00 | 0.80 | Sunny wind/ | 3.65 | 1.7 | |
| PBP2 | 2.80 | 0.90 | Sunny / | 2.80 | 1.8 | |
| PBP3 | 1.70 | 1 | | 1.70 | 1.60 | Sunny/ |
| PBP4 | 1.00 | 0.90 | | 2.25 | 1.50 | |
| PBP5 | 1.50 | 0.90 | /Egerea densa presence | 2.10 | 1.70 | /Egerea densa presence |
| PBP6 | 1.50 | 1 | | 2.50 | 1.85 | |
| PBP7 | 1.60 | 1 | | 3.60 | 2.20 | Sunny/ |
| PBP8 | 2.20 | 1.10 | | 2.75 | 1.90 | Cloudy/ |
| PBP9 | 2.70 | 1.05 | /Egerea densa presence | 3.00 | 1.60 | |
| PBP10 | 0.40 | | Cloudy/ | 0.30 | | Cloudy/ |
| PBP11 | 0.60 | | Cloudy/ | _ | | |
| PBP12 | 0.60 | | Cloudy/ | | • | e of season the point recorded data |
| PBP13 | 0.60 | | Cloudy/ | | | |
| PVP1 | 1.00 | 1.40 | / Egerea densa presence | 3.8 | 0.35 | Cloudy /Egerea densa presence |
| PVP2 | 2.90 | 1.30 | /Egerea densa presence | 5.1 | 3.15 | Cloudy/ Egerea densa presence |
| PVP3 | 3.30 | 1.35 | Cloudy/ | 5.85 | 3.5 | Cloudy/ |
| PVP4 | 2.30 | 1.20 | Cloudy/ | 8 | 3.1 | Cloudy/ |
| PVP5 | 1.90 | 1.30 | Sunny/ | 6.2 | 3.3 | Cloudy/ |

Nutrients

Nitrogen is an element basic for living organisms, since it form part of the growing of tissues or constituent structures.

In water, nitrogen is present in three varieties, total nitrogen N, nitrates NO₃ and ammonium NH₄+. For total nitrogen the normal concentration is 10 mg L⁻¹

Ammonium is important for primary producers because they use it as a nitrogen source for the synthesis of amino acids. In well-oxygenated water bodies, its concentration is low and when it reaches high values (> 0.5 mg L⁻¹) there may be a massive fish kills (Roldán & Ramirez, 2008).

Phosphate (orthophosphate) is a limiting nutrient for primary producers (Roldán & Ramirez, 2008). In nature, the primary source of phosphorus is derived from igneous rocks, although anthropogenic activities enrich phosphorus water bodies causing eutrophication of aquatic ecosystems.

For the rainy season, in Brockman and Victoria, almost all values were below the method detection limit (<0.02, 0.25 and 0.01 mg L-1). Only nitrates in dry season showed a slight increase but ammonium and orthophosphate maintained low values as in rainy season.

Although apparently there is not a high input of nutrients (Table 2, Item PB10), another factor that might be reducing the concentration of nutrients in the water is the presence of *E. densa* since according to researches (Feijoo et al 2002) this plant absorb ammonium from water.

While some classifications to categorize the trophic status of lakes are given in concentrations of nitrogen and total phosphorus, and other variables (chlorophyll *a*; water transparency), for this study only nutrient concentrations and transparency data were considered to determine that the dams have mesotrophic conditions (Nürnberg 1996).

Table 2. Nutrients variables measured in the aquatic system in two seasons. PB= Brockman dam and PV= Victoria dam. Orthopho= Orthophosphate. Values are in mg L⁻¹.

| | F | Rain season 2 | 013 | Dry season 2014 | | |
|-------|----------|---------------|----------|-----------------|----------|----------|
| Point | Nitrates | Ammonium | Orthopho | Nitrates | Ammonium | Orthopho |
| PBP2 | 0.04 | <0.25 | <0.01 | 0.03 | <0.25 | <0.01 |
| PBP4 | <0.02 | <0.25 | <0.01 | <0.02 | <0.25 | <0.01 |
| PBP6 | <0.02 | <0.25 | <0.01 | 0.02 | <0.25 | <0.01 |
| PBP7 | <0.02 | <0.25 | <0.01 | 0.09 | <0.25 | <0.01 |
| PBP9 | <0.02 | <0.25 | <0.01 | 0.06 | <0.25 | <0.01 |
| BP10 | <0.02 | <0.25 | <0.01 | 0.02 | <0.25 | <0.01 |
| PVP1 | <0.02 | <0.25 | <0.01 | 0.02 | <0.25 | 0.01 |
| PVP3 | <0.02 | <0.25 | <0.01 | 0.02 | <0.25 | <0.01 |
| PVP5 | 0.11 | <0.25 | <0.01 | 0.02 | <0.25 | <0.01 |
| PVP6 | <0.02 | <0.25 | <0.01 | 0.13 | <0.25 | <0.01 |

Heavy metals in water (arsenic, cadmium, copper, chromium, lead and mercury)

Arsenic (As) is a metalloid considered not essential for living organisms. Cadmium (Cd), hexavalent chromium (Cr +6), lead (Pb) and cupper (Cu) are non-essential trace elements and can become toxic to aquatic life if its concentration in water is high. Mercury (Hg) is also a non-essential trace element, and effects on aquatic organisms and humans can be fatal, affecting the ability of fertilization and the early stages of development (eg, benthic organisms).

In both dams heavy metals values were below the detection limit of method, meaning concentrations are not detectable (Table 3) and of course, below the maximum limits permissible defined in Mexican Official Standard NOM-001-SEMARNAP -1996

Table 3. Values of heavy metals in water on rainy (2013) and dry (2014) season. PB= Brockman dam and PV= Victoria dam. Values in mg L⁻¹.

| | Rain season (2013) | | | | | Dry season (2014) | | | | | | |
|-------|--------------------|---------|--------|------------------------|-------|-------------------|---------|---------|--------|------------------------|-------|---------|
| Point | Arsenic | Cadmium | Copper | Hexavalent Chromium | Lead | Mercury | Arsenic | Cadmium | Copper | Hexavalent Chromium | Lead | Mercury |
| PBP2 | 0.0005 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 | 0.0003 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0002 |
| PBP4 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0005 |
| PBP6 | 0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0004 |
| PBP7 | 0.0003 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 |
| PBP9 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 |
| BP10 | 0.0003 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 | 0.0003 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0005 |
| PVP1 | 0.0003 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0002 | 0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 |
| PVP3 | 0.0003 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0003 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0003 |
| PVP5 | 0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0002 | <0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | <0.0002 |
| PVP6 | 0.0002 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0002 | 0.0011 | <0.005 | <0.006 | <0.007 | <0.02 | 0.0003 |

Some water variables comparing colorimetric test against laboratory analysis.

In order to evaluate the accuracy of colorimetric tests to be used in participative monitoring against lab analysis, we measured *in situ* some parameters and some of the sampling points (Table 4).

Table 4. Comparison of variable concentration. H Chro= Hexavalent Chromium, Col= colorimeter, Orthopho= Orthophosphate and AL = laboratory analysis.

| | Le | ead | Cac | lmium | Н | Chro | Co | pper | Nitr | ates | Orto | pho |
|-------|------|-------|------|--------|------|--------|------|--------|------|------|------|-------|
| Sitio | Col | AL | Col | AL | Col | AL | Col | AL | Col | AL | Col | AL |
| PBP2 | 0.50 | <0.02 | 0.09 | <0.005 | 0.00 | <0.007 | 0 | <0.006 | 0.00 | 0.03 | 0.00 | <0.01 |
| PBP 6 | 0.77 | <0.02 | 0.01 | <0.005 | 0.00 | <0.007 | 0.02 | <0.006 | 0.05 | 0.02 | 0.00 | <0.01 |
| PVP1 | 0.82 | <0.02 | 0.01 | <0.005 | 0.00 | <0.007 | 0 | <0.006 | 0.02 | 0.02 | 0.00 | <0.01 |
| PVP6 | 1.00 | <0.02 | 0.03 | <0.005 | 0.00 | <0.007 | 0 | <0.006 | 0.03 | 0.13 | 0.00 | <0.01 |

Advantages of using colorimetric tests is that results are obtained immediately *in situ*, it is not required a highly specialized equipment and any person can perform the test with only a basic training. Nevertheless colorimetric test do not replace a lab analysis for accurate results.

For stakeholders, colorimetric test can be essential in emergency situations like a massive fish death, a sudden water color change or an algae bloom.

According to results obtained, high variations were observed for lead and cadmium. Considering that in lab analysis the result for these parameters were almost not detectable, accuracy of test is important and if colorimetric test is used, and high values are obtained, a lab analysis should be performed.

For nutrients analysis however, results of both methods were very similar, so colorimetric test could be used on a routine basis, provided there is a responsible for equipment and the budget for reagents.

Bacteria in water

Coliform bacteria are a common indicator for sanitary quality of foods and water. Coliform can be found in the aquatic environment, soil and crops and are present in large amount in the feces of warm-blooded animals. Among coliform bacteria there are pathogens and non-pathogens types but all of them are an indicator of fecal pollution.

To reinforce the test, an *Escherichia coli* analysis is also performed. *E. coli* is also a coliform bacteria and according with the number of colonies formed in the culture media, we can find out the extent of pollution.

Concentration of bacteria in laboratories is measured in samples of 100 ml of water. For our study we used the 3M brand Petrifilm plates, which needs only 1

ml of water sample to inoculate the culture media. Number of counting presented in Table 55 were extrapolated to 100 ml of water for final results.

Samples were taken in September and twice in April 2014, before and after the Easter season when tourists arrive to the dam.

Total coliforms were recorded at points BP4 and BP10 in September and at points BP2, BP4, BP6, BP7 and BP10 in April (Table 55).

During dry season *E. coli* was present in BP4 and BP10 sampling points. The first point lies on the west end zone of the dam, where a drainage, football fields and houses are observed. The second point is located in the stream flowing from the settlement nearest to Brockman.

At the moment of the sampling in April, the stream had standing water so this condition may have allowed an increase in bacteria. Furthermore, in the catchment area of the stream livestock and horses grazes freely, so they are a direct pollution source.

In general results show that most of total coliforms and *E. coli* are present during dry season in higher concentration than those allowed for drinking water by Mexican Official Standard NOM-127-SSA1-1994³, which is 2 UFC/100 ml.

Regarding the issue about the impact of tourism in water quality which also was a question done during workshops, we found that it is not a determinant variable for fecal pollution but the constant presence of cattle, dogs and stream/drainage from the nearest settlements is determinant.

Table 5. Results of *Escherichia coli* and coliform for the rainy and dry season for the sampling period. PB = Brockman dam, PV = Victoria Dam and CFU= colony forming units.

| | Escherichi | a coli (CFU | /100ml) | Total Coliform (CFU /100ml) | | |
|--------|--|----------------------------|---------|--|----------------------------|-----|
| Points | Rainy season 2013 (September) | Dry season 2014 (April) | | Rainy season 2013 (September) | Dry season 2014 (April) | |
| PBP2 | 0 | 0 | 0 | 0 | 500 | 100 |
| PBP4 | 100 | 0 | 0 | 500 | 500 | 0 |
| PBP6 | 0 | 0 | 0 | 0 | 200 | 100 |
| PBP7 | 0 | 0 | 0 | 0 | 100 | 0 |
| PBP9 | 0 | 0 | 0 | 0 | 0 | 0 |
| PBP10 | 100 | 600 | 200 | 400 | 800 | 500 |
| PVP1 | 0 | 0 | 0 | 400 | 100 | 200 |

³ Rule name in Spanish: "Salud ambiental, agua para uso y consumo humano - límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilización"

| PVP3 | 0 | 0 | 0 | 0 | 100 | 200 |
|------|---|----|---|---|-----|-----|
| PVP5 | 0 | 0 | 0 | 0 | 700 | 200 |
| PVP6 | 0 | NA | 0 | 0 | NA | 0 |

Detergents in water

A detergent is a product that is used for cleaning and are characterized by lowering the surface tension of the liquid in which it dissolves, to reduce the adhesion of different particles ("dirt") to a given surface.

Different chemical compounds added to detergents makes difficult the lab analysis but the phosphate content which is not biodegradable and cause foaming, is toxic to aquatic life and promote the growth of algae and macrophytes in excess.

In general, concentrations of anionic detergents, which are more common in domestic wastewater, are between 1 and 20 mg L⁻¹, while on the surface of natural water bodies, the concentrations do not exceed of 0, 5 mg / I except in the vicinity of the drainages.

Results for both dams in April (Table 6), show that values are below 0, 5 mg / I, so it is considered that currently there is no impact affecting water quality of dams. However, it is a warning that this activity should not increase.

Table 6. Detergent concentration in Brockman and Victoria dam on April 2014. PB=Presa Brockman y PV= Presa Victoria. Values are in mg L⁻¹.

| Point | Detergent (SAAM ⁴) |
|-------|--------------------------------|
| PBP2 | <0.007 |
| PBP4 | <0.007 |
| PBP6 | <0.007 |
| PBP7 | <0.007 |
| PBP9 | <0.007 |
| PBP10 | 0.3229 |
| PVP1 | <0.007 |
| PVP3 | <0.007 |
| PVP5 | <0.007 |
| PVP6 | <0.007 |

⁴ SAAM in Spanish, sustancias activas al azul de metileno.

Heavy metals in sediments

Sediment can accumulate chemical elements over time, so it is often used as a historical register of the study area. Natural concentrations of heavy metal are related to volcano activities or soil erosion.

Since Brockman region has had as a main economic activity for many years the mineral extraction, our aim was to know whether sediment contained heavy metals potentially toxic for aquatic life and humans since the water is used for drinking.

In Mexico there is not a normativity for heavy metals in sediment, so we used the Canadian Environmental Quality Guidelines (Canadian Council of the Minister of Environment, 1999).

Table 7. Heavy metal concentration in sediment in rain season June (2013). PB= Brockman dam and PV= Victoria dam. Values are in mg/kg.

| Point | Arsenic | Cadmium | Copper | Chrome | Hexavalent Chromium | Mercury | Lead |
|-------|---------|---------|--------|--------|------------------------|---------|-------|
| PBP2 | 0.386 | 1.168 | 16.1 | 12.07 | 0.12 | <0.001 | 28.84 |
| PBP4 | 0.176 | 0.95 | 11.9 | 12.68 | 0.398 | 0.351 | 26.44 |
| PBP6 | 0.153 | 1.197 | 16.1 | 11.5 | <0.09 | <0.001 | 19.80 |
| PBP7 | 0.361 | 1.189 | 15.2 | 13.58 | 0.4 | 0.011 | 19.02 |
| PBP9 | 0.16 | 1.243 | 17.2 | 11.71 | 0.279 | 0.266 | 22.7 |
| PVP1 | 0.110 | 1.345 | 17.8 | 12.76 | 0.314 | 0.394 | 20.1 |
| PVP3 | 0.314 | 0.987 | 16.8 | 12.41 | 0.314 | <0.001 | 19.3 |
| PVP5 | 0.043 | 1.044 | 16.01 | 14.15 | 0.52 | 0.368 | 19.1 |

Table 8. Heavy metal concentration in sediment in dry season (April 2014). PB= Brockman dam and PV= Victoria dam. Values are in mg/kg⁵.

| Point | Arsenic | Cadmium | Copper | Chrome | Hexavalent Chromium | Mercury | Lead |
|-------|---------|---------|--------|--------|------------------------|---------|-------|
| PBP2 | 0.015 | 1.35 | 9.54 | 12.09 | <0.09 | <0.001 | 22.33 |
| PBP4 | 0.015 | 0.8 | 5.44 | 11.49 | <0.09 | <0.001 | 28.07 |
| PBP6 | 0.015 | 1.39 | 10 | 9.26 | <0.09 | <0.001 | 23.91 |
| PBP7 | 0.007 | 1.55 | 20.44 | 8.26 | <0.09 | <0.001 | 25.68 |
| BP10 | 0.007 | 1.04 | 4.5 | 9.69 | <0.09 | <0.09 | 13.8 |
| PVP3 | 0.007 | 1.53 | 14.45 | 8.27 | <0.09 | <0.001 | 24.06 |
| PVP5 | 0.007 | 1.11 | 11.79 | 8.8 | <0.09 | <0.001 | 23.74 |

A second more suitable for natural water sediments parameter are Guides Canadian Sediment Quality for the Protection of Aquatic Life (Canadian Environmental Quality Guidelines Canadian Council of Ministers of the Environment, 1999). Table 9 shows the limits established by these sediment quality guidelines (GCS) for heavy metals in freshwater sediments and the concentration of a probable effect level (NEP) on aquatic life.

Comparing results obtained (Table 7 and 8) during dry and rainy season with the limits of CEQG, even the highest values recorded are below the limits in sediments and therefore the likely effects on aquatic life.

Table 9. Interim sediment quality guidelines (ISQGs) and probable effect levels (PELs) to Heavy metals.

| Variables | Chemical symbol | Unit | Freshwater (ISQGs) | Freshwater (PELs) |
|-----------|-----------------|-------|-----------------------|----------------------|
| | | | Value | Value |
| Arsenic | As | mg/kg | 59 | 170 |
| Cadmium | Cd | mg/kg | 6 | 35 |
| Chromium | Cr | mg/kg | 373 | 900 |
| Copper | Cu | mg/kg | 357 | 1970 |
| Lead | Pb | mg/kg | 350 | 913 |
| Mercury | Hg | mg/kg | 1.7 | 4.86 |

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⁵ Due to the requirement of paragraph PVP1; presence of a large amount of macrophyte *Egeria densa* (Elodea) could not be collected in the sediment sample.

Potential pathogenic organisms in sediments

Depending on the texture of sediment, water flow strength and accumulation speed, bacteria get attached to the sediment to a minor or major extent (WHO, 1996).

For sediment we analyzed Salmonella sp, helminthes eggs and fecal coliforms. According with Mexican regulation, NOM-004-SEMARNAT-2002, concentrations of Salmonella sp. and Helminthes eggs in both seasons (Table 1010Table 1111) are below or equal to the minimum limit.

For fecal coliforms the same regulation establish that if there is direct contact with humans, concentrations should be under 1000 NMP/g.

This limit was exceeded at sampling point BP4 in rainy and dry seasons, probably as a result of the runnoff from the grazing area. For rainy season also the point VP1 at Victoria overpassed the limit and during dry season another register was for the point BP10, the stream from the settlement.

Table 10. Presence of microorganisms in the sediment in June 2013 (rain season). PB= Brockman dam, PV= Victoria dam and MPN= most probable number.

| Point | Fungus (MPN/g) | Salmonella (MPN/g) | Coliforms (MPN/g) | Helminths eggs (HH/g) |
|-------|-------------------|-----------------------|----------------------|-----------------------------|
| PBP2 | 40 | <3 | 23 | 2 |
| PBP4 | 1760 | <3 | 1500 | 0 |
| PBP6 | 390 | <3 | 930 | 0 |
| PBP7 | 150 | <3 | 40 | 0 |
| PBP9 | 1240 | <3 | 40 | 0 |
| PVP1 | 310 | <3 | >2400 | 0 |
| PVP3 | 260 | <3 | 70 | 0 |
| PVP5 | 110 | <3 | 40 | 0 |

Table 11. Presence of microorganisms in the sediment in April 2014 (dry season) PB= Brockman dam, PV= Victoria dam and MPN= most probable number.

| Point | Fungus (MPN/g) | Salmonella (MPN/g) | Coliforms (MPN/g) | E. Coli (MPN/g) | Helminths eggs (HH/g) |
|-------|-------------------|-----------------------|----------------------|--------------------|-----------------------------|
| PBP2 | 2000 | <3 | 230 | 40 | 1 |
| PBP4 | 3800 | <3 | 2100 | 2100 | 0 |
| PBP6 | 2400 | <3 | 230 | 40 | 1 |
| PBP7 | 4000 | <3 | 230 | 40 | 0 |
| BP10 | 4700 | <3 | 1100 | 1100 | 0 |
| PVP3 | 2000 | <3 | 230 | 90 | 0 |
| PVP5 | 1000 | <3 | 230 | 90 | 0 |

Abundance of Ambystoma granulosum

Sampling for *Ambystoma granulosum* was done in June (2013) and April (2014) using minnow traps adapted to fit the salamanders, with a sampling effort of 2,112 hours in total (Table 12).

Thirty three ajolotes were collected at Brockman dam, 23 in June during rainy season and 10 in April during dry season. At Victoria dam we didn't catch any *A. granulosum.*

During rainy season 2013, the area where *A. granulosum* were catched, comprised five points of Brockman: points BN3 y BN17 with 3 individuals each one; point BN14 with 4 individuals; at point BN7 11 individuals were collected and at points BN1 and BN5, 1 individual were trapped at each point.

Also during macro-invertebrate collects, ajolotes were watched twice near point BN4 and southern area of the dam as well as at a temporary affluent 180 m from BN7 point.

In our experience, the area without *A. granulosum* observations, comprised from the center of the dam toward the south side.

At the west side of the dam 86.9% of 23 individuals, were recorded of which, 18 were catched at BN7 point (Figure 15) and at the opposite site of the dam only 1 ajolote was catched at a depth <3m (BN1).

Sites with high density of macrophyte *E. densa* and with catching of ajolotes, were BN1 (one individual), BN3 (one individual) and BN14 (4 individuals).

Macrophytes are a potential shelter to *A. granulosum* and other organisms, however presence of *Ambystoma* at every patch of aquatic vegetation is not a

rule. It might depend on the individual preferences or needs of food of each individual.

Characteristics of the area (BN5-BN8) are: depth, .90-2m in both seasons; temperature 16.5 - 17.5 °C; pH 8.7-9.3; dissolved oxygen 6.5-7.0 mg L⁻¹; low conductivity ,150-190 K₂₅ μ S cm⁻¹ and total dissolved solids,150-190 mg L⁻¹.

Table 12. Number of hours of effort to show per season, number of individuals registered salamanders and depth recorded on the site. Green sites had E. densa. H/E = hours of effort and Z= depth.

| | Rainy | / season 201 | 3 | D | ry season 20 | 014 | Tot | tal annual |
|-------|-------|--------------|-------|-----|--------------|----------|------|------------|
| Traps | H/E | Organisms | Z (m) | H/E | Organisr | ms Z (m) | H/E | Organisms |
| BN1 | 72 | 1 | 3.06 | 48 | 0 | 3.25 | 120 | 1 |
| BN2 | 72 | 0 | 3.00 | 48 | 0 | 3.90 | 120 | 0 |
| BN3 | 72 | 3 | 3.07 | 48 | 0 | 3.90 | 120 | 3 |
| BN4 | 72 | 0 | 2.10 | 48 | 0 | 2.70 | 120 | 0 |
| BN5 | 72 | 1 | 1.70 | 48 | 0 | 1.90 | 120 | 1 |
| BN6 | 72 | 0 | 0.70 | 48 | 1 | 1.40 | 120 | 1 |
| BN7 | 72 | 11 | 0.90 | 48 | 7 | 1.60 | 120 | 18 |
| BN8 | 72 | 0 | 1.20 | 48 | 0 | 2.00 | 120 | 0 |
| BN9 | 72 | 0 | 1.60 | 48 | 0 | 2.70 | 120 | 0 |
| BN10 | lo | oss | 1.80 | 48 | 0 | | 2.40 | 0 |
| BN11 | 72 | 0 | 1.70 | 48 | 0 | 2.30 | 120 | 0 |
| BN12 | 72 | 0 | 2.00 | 48 | 0 | 1.90 | 120 | 0 |
| BN13 | 72 | 0 | 0.95 | 48 | 1 | 1.90 | 120 | 1 |
| BN14 | 72 | 4 | 1.40 | 48 | 0 | 2.40 | 120 | 4 |
| BN15 | 72 | 0 | ND | 48 | 1 | 2.25 | 120 | 1 |
| BN16 | 48 | 0 | 3.00 | 48 | 0 | 3.40 | 96 | 0 |
| BN17 | 48 | 3 | 2.70 | 48 | 0 | 3.35 | 96 | 3 |
| BN18 | 48 | 0 | 3.00 | | loss | 4.00 | 4.00 | 0 |
| BN19 | 48 | 0 | 3.00 | 48 | 0 | 3.45 | 96 | 0 |
| BN20 | 48 | 0 | 2.30 | | loss | 3.00 | 3.00 | 0 |
| | 1248 | 23 | | 864 | 10 | | 2112 | 33 |

West side of Brockman also has a slight slope and ajolotes might move easily when water level increase or decrease. These fluctuations mark a sharp change in the spatial conditions and feeding area for *A. granulosum*, as other organisms with less moving capacity like diptera larvae (Chironomidae sp), a main food for *A. granulosum* get exposured to dry conditions.

At these area and especially toward the dam curtain, we could observe abundance of crayfish and *Physella* and *Helisoma* sp. snails, which are a main food source for ajolotes.

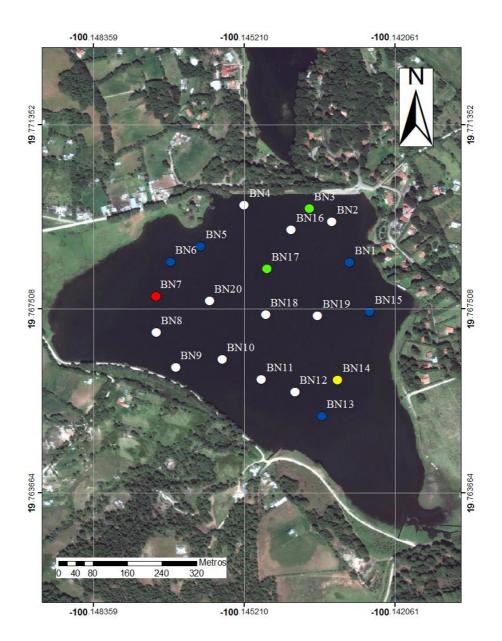


Figure 15. Points distribution of traps (minnow trap) to capture salamanders. = 18 individuals red circle, yellow circle = 4 individuals, green circle= 3 individuals, blue circle= 1 individual and white circle= 0 individuals.

In order to study the biology and reproductive behavior of A. granulosum under controlled conditions, 16 individuals in total were taken to UNAM Laboratorio de Restauración Ecológica, twelve individuals from the rainy season (June 2013) and four from the dry season (April 2014).

In the case of Victoria dam, the sampling effort was 408 h, without any catchment registered. Even though water quality and presence of macro-invertebrates which could be a potential food for Ajolotes are very similar to Brockman, apparently the morphological characteristics of Victoria with a pronounced slope which fall to reach 6.2 m depth and a small flooding area, are determinant for the absence of *A. granulosum*.

Phytoplankton Phytoplankton is a set of mostly photoautotrophic organisms adapted to open water suspension depending of passive movement by wind and

currents commonly present on the surface of water in the photic zone (Roldan 2008). It is made up of many groups of organisms within several classes in the taxonomy, so that phytoplankton contains a great diversity of species.

Los valores que se presentan en la Table 13 son los concentrados de una muestra de 500 ml derivado de los arrastres con la red de fitoplancton, la cual se filtraron 84,823 litros de la presa Victoria y 141,370 litros para la presa Brockman. Se registraron siete clases de fitoplancton los cuales son: Cyanophyceae, Dinophyceae, Fragilariophyceae, Bacillriophyceae, Chlorophiceae, Coscinodiscophyceae y Zygnemophyceae.

The values presented in Table 13 are concentrated in a 500 ml sample derived from the net hauls phytoplankton. At Victoria dam 84.823 liters were filtered in and 141.370 liters at Brockman dam. Seven kinds of phytoplankton were recorded: Cyanophyceae, Dinophyceae, Fragilariophyceae, Bacillriophyceae, Chlorophiceae, Coscinodiscophyceae and Zygnemophyceae.

There were identified 14 species for Brockman Dam and 26 for Victory dam. In several cases we were unable to identify the organism to species, such as diatoms or simply is estimated that there are several species of the same genus as *Cycloptella spp.*

Table 13. Cell numbers of species per milliliter in Brockman and Victoria for each of the seasons prey.

| Especie (cells per ml) | Brockman rainy (2014) | Brockman dry (2014) | Victoria rainy (2013) | Victoria dry (2014) |
|------------------------------|--------------------------|------------------------|--------------------------|------------------------|
| Selenastrum sp. | 302847 | | 52728 | |
| Asterionella Formosa | 484015 | | 550264 | 836307 |
| Eutetramorus sp. 1 | | 1368222 | 79768 | 189279 |
| clorofita 1 | | | | 3862 |
| Coelastrum afin. pulchrum | | | | 61805 |
| Diatomea. 4 | | | 1352 | |
| Cyclotella sp.1 | | | 181168 | |
| Cyclotella sp. 2 | | | 225784 | |
| Cyclotella sp.3 | | | 44616 | |
| Fragilaria sp.diatomea 5 | | | 4056 | |
| diatomea 6 | | | 2704 | |
| diatomea 7 | | | 2704 | |

| Fragilaria crotonensis | | | 4056 | |
|---|---------|----------|--------|--------|
| Peridinium sp. | | | | 7725 |
| Ceratium hirundinella | | | | 1931 |
| filamento cianobacteria no id. | | | | 36697 |
| filamento clorofita sp. 1 | | | | 30902 |
| Microcystis (free cells) | 3101485 | | | |
| Microcystis flosaquae | | 321775 | 114920 | 778365 |
| Microcystis sp. 1 | 754415 | | | |
| Microcystis muscicola | | 849055 | | |
| microcystis small colony (sp. 2) | 127087 | | | |
| microcystis sp. 2 | 1176239 | 475903 | | 722353 |
| Microcystis aeruginosa | | | 197392 | |
| Dolichospermum sp. | 354223 | 14917956 | | 197005 |
| Pseudanabaena muscicola (Not adhered) | | 205503 | | |
| Pediastrum tetras | | | 16224 | |
| Desmodesmus sp. | | | 20280 | |
| Desmodesmus quadricauda | | | 8112 | |
| Staurastrum sp. 1 | | 37855 | 4056 | |
| Staurastrum sp. 2 | | 5407 | | 5794 |
| Eutetramorus sp. 1 | | | | 164171 |
| Pseusdoanabaena. muscicola (adherida a colonias Microcystis) | | 3274541 | | |

Zooplankton

El zooplancton constituye una parte esencial en la alimentación de peces y macroinvertebrados acuáticos. Ellos pueden llegar a modificar la estructura del fitoplancton por medio del consumo diferencial de alagunas especies de acuerdo a sus características y facilidad de captura. El zooplancton es un término aplicado a un grupo de animales que se alimentan por ingestión de materia orgánica ya elaborada y que tiene como hábitat principal la columna de agua. El zooplancton está constituido principalmente por protozoos, rotíferos y microcrustaceos (cladóceros y copépodos).

Zooplankton is an essential part in the feeding of fish and aquatic macro-invertebrates. They may modify the structure of phytoplankton by the differential consumption of some species according to their features and ease of capture. Zooplankton is a term applied to a group of animals that organic matter and and whose main habitat is the water column. Zooplankton consists mainly of protozoa, rotifers and microcrustaceans (cladocerans and copepods).

Based on the results obtained a greater number of copepods were observed in the rainy season (.

Table 14), with an estimated 1.75 individuals per liter for Brockman. For the Victoria dam in the rainy season 0.12 individuals / L were recorded and in this case the number of individuals in the dry season increased. Copepods in the water bodies are distributed at shoreline, pelagic and benthic level.

Cladocerans belong to the subclass Branchiopoda. They are one of the main components of food webs. Most species eat algae and supplement their diet with debris and bacteria. Their way of feeding is by filtering, which allows to concentrate dispersed particles in the water column and. They can also be feed by copepods, cladocerans other predators, small fish and larvae or juveniles of larger fish (Havel 2009).

For Brockman the largest number of cladocerans was recorded in the rainy season (.

Table 14) with an estimated of 1.89 individuals / L., decreasing the number during dry season. At Victoria dam a greater number of individuals of cladocerans was present in the rainy season and diminished by two-thirds in the dry season.

Table 14. Summary records observed in samples for zooplankton prey in both seasons. Cop = copepods, Cla= cladocerans Cla=, Indi= approximate number of individuals per sample and Prom ind / L= average number of individuals per liter.

| | | Presa B | Presa Victoria | | | | | |
|---------------|---------|---------|----------------|---------|--------|---------|--------|--------|
| | Llu | vias | Se | cas | Llu | vias | Secas | |
| Campo | Сор | Cla | Сор | Cla | Сор | Cla | Сор | Cla |
| 1 | 396 | 495 | 160 | 176 | 22 | 8 | 120 | 48 |
| 2 | 495 | 594 | 264 | 280 | 17 | 15 | 64 | 88 |
| 3 | 396 | 462 | 288 | 536 | 21 | 12 | 56 | 64 |
| 4 | 660 | 594 | 288 | 488 | 19 | 15 | 128 | 80 |
| 5 | 528 | 528 | 312 | 472 | 24 | 17 | 64 | 64 |
| Indi ≈ | 247,500 | 267,300 | 131,200 | 195,200 | 10,300 | 138,020 | 43,200 | 34,400 |
| Prom ind/l | 1.75 | 1.89 | 0.93 | 1.38 | 0.12 | 1.63 | 0.51 | 0.41 |

Macrophyte (Egeria densa)

This is an introduced species from South America and is considered an aquatic weed invading soon the sites where it is introduced. It is a freshwater perennial submerged plant (macrophyte), which is found in both lotic (flowing water) and lentic (standing water).

Commonly, it is rooted within 1 to 2 m below the surface, but at Victoria and Brockman have been recorded above 3 and 5 m respectively (Figure 16). Its reproduction is asexual meaning that stems develop as independent individuals (Lot & Olvera 2013).

According to bibliography, it has a great ability to adapt and modify ecosystems. Grows at a relatively fast rate, adapts to different light regimes, and is flexible in its ability to absorb nutrients from the water; even by diffusion from water column (Camargo et al, 2006).

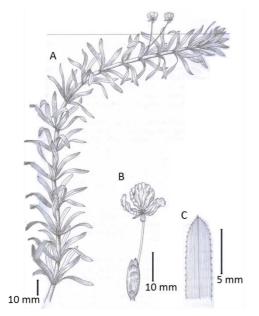




Figure 16. Left side: A, upper portion of plant; B, flower with stamen; C, leaf detail, modified from Lot & Olvera 2013 Right: flower above the water surface at the Victoria Dam

Introduction of *E. densa* in reservoirs improve features such as water clarity and nutrient reduction. However, its rapid growth generates a large mono-specific coverage which induce the reduction of diversity through competition and exclusion space.

At reservoirs such as Brockman y Victoria *E. densa* promotes water transparency by retaining dissolved solids, allowing a higher light penetration (Figure 17).

However in high densities contribute to decrease nutrients which in turn reduces phytoplankton population and develops a chain impact on the rest of the food chain.

Also in long term, the retention of suspended solids increase sedimentation of the reservoirs and reduce the depth which for artificial reservoirs such as Brockman and Victoria is a negative impact.

Specifically in the case of Brockman and Victoria, apparently the population of *E. densa* experience a self-control process in terms that seasonal changes in water level expose to the plant to dry conditions, producing death of the plant. However a more specialized study is required to completely understand the ecological process of this macrophyte at both dams.

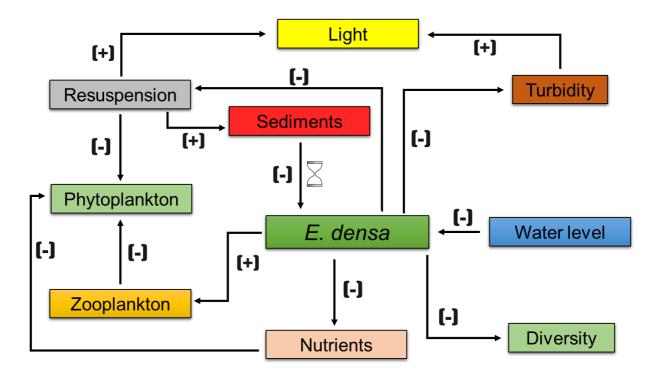


Figure 17. Modifying some ecosystem properties generated from the presence of *E. densa*. Modified sensu Jones et al. 1994.

Conclusions

Based on results obtained for all studied parameters, the water quality of both reservoirs enables good conditions for aquatic organisms.

Neither eutrophication processes nor heavy metals concentrations are limiting factors for the species. Also regarding to the pathogenic risk for human health produced by fecal coliforms, we can grade it as low.

However we observed that certain human activities around the dams are potential risk to degrade current conditions. Regulation policies measures need to be taken among authorities and local inhabitants in terms of:

- Illegal logging
- Land use change to create agriculture areas around the dams
- Detergent input from settlements around the dams
- Urbanization

Seasonal flooding is an important process for *Ambystoma granulosum* and *Egeria densa*, so an immediate need is to do eco-hydrological research to determine the minimal water level needed to preserve ecological process which sustain biodiversity at both reservoirs.

Food chain at Brockman and Victoria Reservoirs, a first approach.

State of the art

Dietary patterns or more colloquially, who eats who in a terrestrial or aquatic ecosystem, is the best way to understand the flow of energy and give us a clear vision of how conserved or how degraded is the system under study.

There are two levels of approach to the study of feeding relationships in an ecosystem. The first level refers to the food chain, in which case you can get an elaborate scheme of interactions between species, which shows the organisms who feed on whom specifically.

The second approach relates to the trophic level of the studied organisms, i.e. organisms of different species are grouped by the type of feeding. This form of classification is called functional groups (Table 15). In the first classification, there are autotrophs, which are those organisms that capture sunlight or chemical sources (sulfates, ammonium and nitrite) to make food. This group forms the basis of the food chain and has played a crucial role in the evolution of life on Earth. Heterotrophs are all organisms who need to feed on others to survive. This group is divided into primary consumers, secondary consumers and decomposers (saprotrophic).

Each group affects differently the structure and functioning of ecosystems, while the primary consumers transfer energy and materials from autotrophs to the other links in the food chain, detritivores recycle elements and matter available to primary producers so they can continue their function. In turn, these large trophic groups can be segregated into many other functional attributes considered more specific. The services provided by ecosystems that are critical to the development of human societies depend on the activity of these functional groups (Martinez-Ramos, 2008)

Table 15. Types of Functional Groups. (From Martínez-Ramos M., 2008, in Capital Natural de México).

| TIPO DE ALIMENTACIÓN | Fuente de energía | Variantes metabólicas | HÁB | TATI |
|----------------------|-----------------------------|--|------------|-----------|
| Autótrofos | Quimiosintéticos | Sulfatos Amonio Nitritos | | |
| Autotrolos | Fotosintéticos | C3 C4 CAM | | |
| Heterótrofos | Consumidores primarios | Herbívoros Bacterias FN Hongos micorrizógenos Fitopatógenos Fitoparásitos Polinívoros Nectarívoros Granívoros Frugívoros Folívoros | Terrestres | Acuáricos |
| | Consumidores secundarios | Micoheterótrofos Depredadores Zoopatógenos Zooparásitos Parasitoides | | |
| | Degradadores | Saprófagos Saprófitos | | |

A preserved ecosystem will present several links in the food chain and will often observe multiple interrelationships between organisms, whereas in a degraded system, there are gaps between one link and another and energy flows can occur in reverse, i.e., the energy source of primary producers can come from an external element to the aquatic system, rather than originated in the same body of water by the action of decomposers that recycle organic matter.

When there is a lack of organisms which meet key functions within the ecosystem as in the case of detritivores or saprotrophic, disappearance of important links in the food chain of the site can result in loose of functionality of ecosystem. This condition makes ecosystem more vulnerable and therefore its degradation can occur at a greater speed.

In aquatic systems, compared with terrestrial, studying food chains acquire a higher level of complexity due to the difficulty of observing and directly sample the organisms living in the water body.

One of the most innovative methods that have been developed since the decade of 80-90, is the use of stable isotopes.

Isotopes are atoms having the same number of electrons and protons but differ in the number of neutrons. They are "stable" atoms from an energy point of view because the number of protons and neutrons are very similar and do not degrade as quickly as radioactive isotopes. In Figure 9 a graphical representation of carbon isotopes, ₁₂C and ₁₃C used in the study of food chains is observed.

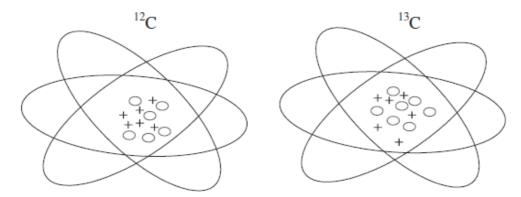


Figure 18. Stables Isotopes of Carbon. The only difference is the number of protons, marked in circles. Carbon is one of the main constituents of matter.

In the study of food webs and trophic levels, the stable isotope technique has been widely applied (Peterson and Fry, 1987; Vander Zanden et al. 1999 b; Post et al. 2000), enabling a continuous measurement trophic position of each organism, i.e., identifying whether the organism in question is consistently a primary or secondary consumer. It also assesses the complex interactions that integrates the assimilation of energy or mass flow through all the various trophic pathways leading to an organism (trophic omnivory). For example for ajolote, a carnivore, isotope analysis ideally reflects its prey and also who is the prey of their prey, as some may feed on other fish or macro-invertebrates, but may also feed on aquatic or terrestrial vegetation and these differences are reflected in stable isotopes studies.

The main elements used in stable isotope analysis for ecological research are carbon, nitrogen and sulfur. Stable isotopes of nitrogen (15N and 14N) and carbon (13C and 12C) reflect the trophic position and food source respectively, in a trophic structure (Gannes et al. 1997; Minagawa and Wada, 1984; De Niro and Epstein, 1978).

The ratio of stable isotopes of nitrogen (d15N) can be used to estimate trophic position because the d15N of a consumer is typically enriched by 3-4 % relative to its diet (Minagawa and Wada, 1984).

In contrast, the carbon isotope ratio (d13C) changes little (0.5 - 1 ‰) as carbon moves through food webs (Peterson and Fry, 1987). Consequently, it can be used to evaluate the farthest carbon source of an organism when the isotopic signature of sources is different.

Method

To carry out the study of isotope analysis in Brockman and Victoria Reservoirs, the collection of representative organisms of large functional groups such as macro-invertebrates, mollusks, crustaceans, fish and amphibians, as well as phytoplankton, zooplankton, sediment and aquatic vegetation was planned.

One of the advantages of the application of isotope technique is that it only requires between 1 and 5 mg of plant or animal tissue, respectively, for the analysis, however when the organisms are very small, it requires a great effort collection in order to accomplish the dry weight needed. In the case of vertebrates, it is not necessary to sacrifice them. Specifically for the Ajolotes, we used anesthesia to collect the isotope sample.

Sampling was done during four field trips. We did the field trips during the rainy season (26 June -1 ° July and September 8 to 12, 2013) and dry season (March 31-April 4 and April 27 to 30, 2014). The specific equipment and material for each of the types of organisms collected for processing and isotope is described in ANNEX 1.

In order to capture organisms at the central and coast areas of reservoirs we had the valuable support of the Department of Public Safety, Civil Protection and Firefighters from El Oro. Specifically we would like to recognize the support of Juan Miguel Mondragon, Arnulfo Legorreta, Isaac Martinez, William Daniel Leon, Armando Sánchez and José Antonio, who accompanied us in every sampling. We also would like to thank to Mr. Juan José López Ummarino, who kindly offered a glass fiber canoe which was quite helpful during the short incursions to Brockman.

Points and collection areas of organisms for isotope analysis are shown in Figure

Isotope analysis was applied to 80 samples for rainy season and 87 samples for dry season, in the Stable Isotope Facility of California Davis, where samples can be efficiently analyzed by continuous flow Isotope Ratio Mass Spectrometer (IRMS).

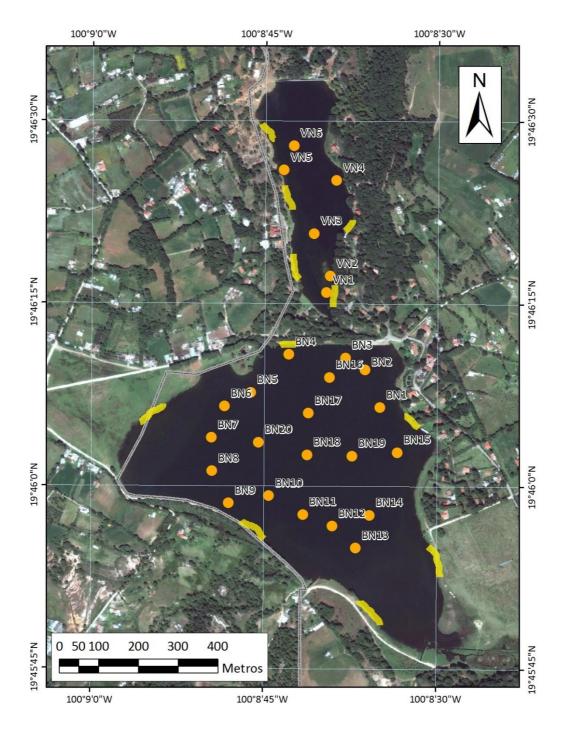


Figure 19. The mustard colored dots indicate the location of the traps to catch salamanders; yellow lines indicate the sampling area of other organisms for isotope analysis.

Results

In Tables 16_a and 17_b it is shown the result of the samples analyzed for rainy and dry season. The number of samples refers to the number of vials sent for analysis of each taxa, but this amount does not reflect the number of organisms collected and dried required to fill the vials. The results used to do the graphic are the notation d13C and d15N for carbon and nitrogen isotopes respectively.

Table 16a. Isotopic signatures of the organisms analyzed from Brockman and Victoria dam, El Oro, Estado de México during rainy season.

| | | Samples number | Carbon Isotopic Mean (d13C) | Standard Deviatio n d13C | Carbon Mean (µg) | Nitrogen Isotopic Mean (d15N) | Standard Deviation (d15N) | Nitrogen Mean (μg) |
|--------------------------|------------------|-------------------|--------------------------------------|--------------------------------|------------------------|--|---------------------------------|--------------------------|
| | Acaros | 2 | -22.30 | 0.37 | 528.33 | 12.05 | 0.09 | 113.19 |
| | Acocil | 6 | -20.69 | 0.53 | 398.90 | 9.35 | 0.51 | 110.77 |
| | Ajolote | 12 | -19.39 | 1.88 | 512.98 | 9.64 | 0.30 | 130.37 |
| | Caracol Ellisoma | 2 | -21.39 | 0.67 | 472.53 | 6.99 | 0.75 | 116.19 |
| | Caracol Physella | 1 | -21.19 | 0.00 | 376.77 | 8.13 | 0.00 | 96.06 |
| nan | Carpa | 6 | -22.48 | 1.04 | 528.24 | 9.18 | 1.88 | 151.02 |
| Organisms/ Brockman | Coleoptero | 2 | -22.87 | 0.27 | 432.70 | 6.32 | 0.36 | 98.21 |
| sms/ E | Corixidae | 2 | -22.01 | 0.81 | 455.34 | 7.51 | 0.83 | 106.50 |
| Jrgani | Efemeroptera | 1 | -28.87 | 0.00 | 781.19 | 7.41 | 0.00 | 133.50 |
| O | Hyalella | 2 | -19.55 | 5.85 | 154.18 | 5.26 | 0.38 | 29.54 |
| | Mexcalpique | 2 | -23.24 | 4.01 | 471.13 | 11.18 | 0.24 | 133.28 |
| | Planaria | 4 | -22.16 | 3.38 | 528.45 | 9.03 | 1.07 | 125.27 |
| | Zigoptera | 2 | -22.36 | 0.63 | 2894.59 | 9.64 | 0.34 | 309.36 |
| | Zooplancton | 3 | -24.26 | 0.76 | 514.25 | 9.29 | 0.27 | 109.68 |
| | Elodea | 4 | -15.88 | 3.34 | 832.56 | 5.48 | 2.33 | 86.98 |
| Others/ Brockman | MOP | 1 | -24.25 | 0.00 | 2087.15 | 3.77 | 0.00 | 411.32 |
| Pro G | Sedimento | 3 | -22.64 | 0.17 | 2894.59 | 3.78 | 0.24 | 309.36 |
| | Acocil | 3 | -20.27 | 3.76 | 456.05 | 11.56 | 1.02 | 134.79 |
| ıria | Carpa | 2 | -20.39 | 2.50 | 434.26 | 11.38 | 2.78 | 127.46 |
| Organisms/Presa Victoria | Corixidae | 2 | -22.14 | 1.93 | 515.30 | 8.56 | 0.64 | 120.62 |
| Presa | Efemeroptera | 2 | -19.14 | 0.15 | 466.60 | 11.10 | 0.10 | 86.34 |
| isms/ | Hyalella | 3 | -17.20 | 0.89 | 345.57 | 9.05 | 2.11 | 64.08 |
| Orgar | Zigoptera | 2 | -18.30 | 4.20 | 473.33 | 11.36 | 0.85 | 99.40 |
| | Zooplancton | 3 | -24.77 | 1.40 | 553.97 | 6.30 | 5.18 | 112.12 |
| | Elodea | 7 | -18.88 | 0.20 | 771.73 | 6.34 | 0.51 | 67.59 |
| Others/ Victoria | MOP | 2 | -24.03 | 2.53 | 2401.39 | 5.15 | 2.39 | 477.67 |
| ζŻ | Sedimento | 2 | -24.15 | 0.01 | 2492.50 | 3.07 | 0.14 | 282.56 |

Table 17b. Isotopic signatures of the organisms analyzed from Brockman and Victoria dam, El Oro, Estado de México during dry season.

| Dry Seas | son | Samples number | Carbon Isotopic Mean (d13C) | Standard Deviation d13C | Carbon Mean (µg) | Nitrogen Isotopic Mean (d15N) | Standard Deviation (d15N) | Nitrogen Mean (µg) |
|--------------------------|---------------------|-------------------|--------------------------------|-------------------------------|---------------------|-------------------------------------|---------------------------------|-----------------------|
| | Acocil | 4 | -18,90 | 1,42 | 1773,70 | 8,51 | 0,29 | 526,40 |
| | Ajolote | 4 | -18,38 | 0,73 | 1936,62 | 9,06 | 0,68 | 563,27 |
| | Caracol Helisoma | 1 | -21,37 | | 394,33 | 4,50 | | 93,71 |
| | Caracol Physella | 1 | -23,11 | | 405,06 | 5,73 | | 94,84 |
| rman | Carpa | 5 | -22,76 | 2,73 | 2330,83 | 9,47 | 0,80 | 638,68 |
| , Brock | Coleoptero | 1 | -22,18 | | 504,10 | 6,98 | | 112,43 |
| Organisms/ Brockman | Corixidae | 1 | -19,68 | 1,06 | 1588,25 | 6,54 | 0,75 | 360,56 |
| Orga | Hyalella | 5 | -18,70 | 0,76 | 1710,23 | 4,71 | 0,60 | 327,31 |
| | Mexcalpique | 1 | -19,37 | | 514,94 | 10,46 | | 139,67 |
| | Planaria | 1 | -19,74 | | 527,76 | 8,72 | | 118,67 |
| | Zooplancton | 3 | -21,66 | 0,32 | 1377,27 | 7,78 | 0,81 | 232,40 |
| | Zygoptera | 2 | -19,92 | 0,91 | 882,61 | 7,32 | 0,31 | 207,84 |
| an | Alga | 4 | -18,77 | 0,39 | 3887,44 | 5,53 | 0,14 | 278,48 |
| Others/ Brockman | Elodea | 4 | -20,69 | 0,41 | 4264,39 | 5,73 | 0,14 | 399,22 |
| ners/ B | МОР | 2 | | | 7008,99 | 3,63 | 0,61 | 1443,67 |
| ‡ O | Sedimento | 12 | -23,03 | 1,68 | 17859,52 | 3,85 | 0,90 | 2169,40 |
| | Acocil | 4 | -19,71 | 0,94 | 1927,44 | 10,99 | 0,21 | 583,72 |
| | Anisoptera | 2 | -25,03 | 0,34 | 1214,95 | 8,73 | 0,15 | 262,88 |
| oria | Carpa | 2 | -21,22 | 1,68 | 1097,00 | 10,83 | 0,53 | 320,24 |
| Organisms/Presa Victoria | Corixidae | 3 | -21,62 | 0,75 | 1388,09 | 7,87 | 1,09 | 328,80 |
| is/Pres | Efemeroptera | 2 | -18,53 | 0,43 | 950,11 | 6,01 | 0,20 | 159,65 |
| yanism | Hyalella | 1 | -18,64 | | 363,16 | 5,41 | | 68,77 |
|)iO | Planaria | 1 | -22,85 | | 488,36 | 9,03 | | 109,02 |
| | Zooplancton | 5 | -24,05 | 0,43 | 2645,49 | 10,65 | 0,43 | 588,25 |
| | Zygoptera | 5 | -23,26 | 0,65 | 1985,39 | 8,93 | 0,24 | 351,90 |
| storia | Elodea | 4 | -25,66 | 0,48 | 4218,85 | 2,98 | 0,12 | 303,41 |
| Others/ Victoria | МОР | 2 | -15,87 | 0,50 | 10142,72 | 6,30 | 0,61 | 2853,03 |
| Othe | Sedimento | 4 | | | 4365,35 | 6,16 | 0,14 | 593,59 |

In a first approach to the isotope analysis, Carbon isotopic values (X) against Nitrogen (Y) with the standard deviation for each sample are plotted in order to visualize the differences in carbon sources and trophic levels. Given that we collected ajolotes only at Brockman dam and the purpose of isotope analysis is

to find out the preferred preys of Ajolote, we applied data analysis only to Brockman reservoir results.

To examine how different sources contribute to nutrition of *A. granulosum* we proceeded to analyze the stable isotope data collected in the Brockman for prey organisms. Data were analyzed with the improved MixSir 1.0.4 software (Seemens and Moore, 2008) version. Mixture models (mixing models) offer a unique solution for samples with two isotopes (carbon and nitrogen) and describe in percentage how much potential prey contribute to the isotopic signature of consumers.

Each one of ajolotes collected was considered as a consumer and the means and standard deviation of the potential prey were used. Groups considered as prey were crayfish (*Cambarellus lermensis*); Helisoma snail; Physella snail; Coleoptera; Corixidae; Ephemeroptera; Hyalella; Planaria; Zygoptera and Zooplankton.

To run the model we used mean and standard deviation of isotopic fractionation as reported in the literature (McCutchan *et al.*, 2003; Vanderklift and Ponsard, 2003). The results are presented according to Seemens and Moore (2008) proposal.

Table 18. Ajolote diet, with % of contribution from each prey

| | Crayfish | Helisoma snail | Physella snail | Coleoptera | Corixidae | Hephemeroptera | Hyallela | Planaria | Zygoptera | Zooplancton |
|--------------|----------|----------------|----------------|------------|-----------|----------------|----------|----------|-----------|-------------|
| Rainy season | 11,1 | 6,8 | 8,6 | 4,7 | 5,5 | 1,8 | 33,3 | 6,6 | 6,4 | 4 |
| Dry season | 14,7 | 4,1 | 2,3 | 2,8 | 8,6 | | 37,7 | 5,7 | 6,6 | 3 |
| Average | 18,45 | 8,85 | 9,75 | 6,1 | 9,8 | 1,8 | 52,15 | 9,45 | 9,7 | 5,5 |

Statistical significance: posterior draws > 1000; no duplicates;

radio < .01

According to results obtained from the isotope mixing model, which assumes all preys have same abundances the main food for Ajolotes at Brockman reservoir are crayfish and Hyalella. This result can be confirmed with the presence of Hyalella sp and Crayfish in the stomach content of Ajolotes (Table 19),

Both are crustaceans, Hyalella is an amphipod usually found on soft bottom or at the leaves of aquatic plants, which are a natural shelter. It consumes mainly detritus, as well as the crayfish which is a major crustacean.

The usual distribution of preys is in littoral areas of water body, therefore we assume that Ajolote also look for the edges looking for its food and this approaching makes them also vulnerable to predators like birds and dogs, as well as an easy catchment for fishers.

Table 19. Stomach content analysis for Ajolote

| BROO | CKMAN | CONTENIDO | VIC. | TORIA |
|------------------|------------------|------------------------|--------------|--------------|
| | | | | |
| LLUVIAS | SECAS | ESTOMACAL | LLUVIAS | SECAS |
| AJOLOTE | AJOLOTE | | | |
| Ácaros | Acocil | Corixidos/notonectidos | Acocil | Anisóptera |
| Acocil | Caracol Helisoma | Diptera | Carpa | Acocil |
| Caracol Ellisoma | Caracol Physella | Lombriz | Corixidae | Carpa |
| Caracol Physella | Carpa | M.N.I | Efemeróptera | Corixidae |
| Сагра | Coleóptero | Materia vegetal | Hyalella | Efemeróptera |
| Coleóptero | Corixidae | Odonato | Zygóptera | Hyalella |
| Corixidae | Hyalella | Quironomidos | Zooplancton | Planaria |
| Efemeróptera | Mexcalpique | Sanguijuela | | Zygóptera |
| Hyalella | Planaria | Zooplacton | | Zooplancton |
| Mexcalpique | Zygóptera | | | |
| Planaria | Zooplancton | | | |
| Zygóptera | | | | |
| Zooplancton | | | | |

Section II Social participation

González Hernández Citlalli A.

Introduction

The methods applied in this project are part of the social science research aimed at facilitating the understanding of socio-ecological phenomena. These are qualitative methods based on a participatory approach; and the recognition of the diverse perspectives of social groups and local actors. The set of tools are based on the subjectivity of the local perception; therefore, it is important to guide the dynamics with a clear objective. It is also suggested to validate the information received with complementing workshops and with the use of different social research methods. In this case we proposed six different workshops that took place from June 2013 to April 2014.

In this section, we provide information regarding the participatory workshops. We briefly describe the objectives, the method, the development, the results and conclusions for each one of them.

Finally, we present the analysis of information obtained in a final table in which we identify the main socio-ecological problems, the strategies suggested by local actors as well as the actions. The strategies can be organized in three groups: 1) Collective action and social organization; 2) Promoting new research; 3) Project development and management.

To conclude the section we present the main general results and conclusions of the social participation section.

Workshop 1 "Our map: Brockman and Victoria dams"

Objective

Identify local knowledge regarding the ajolote (*A. granulosum*): identification of the specie and its spatial location. Also, obtaining information on the main economic activities of the area, land use, roads and local perceptions of environmental problems in the dams.

Method

This participatory workshop aims at generating community maps of the study area (Brockman and Victoria dams) from a participatory mapping method.

The map seeks to capture the perception of communities regarding the use of space and resources, as well as obtaining relevant spatial information for the project being developed. Community maps can provide information on issues such as agricultural areas, urban areas, fishing areas, boundaries and environmental problems in a geographic area. Questions are proposed by the facilitator in order to obtain information on: socio-economic issues of the surrounding area, environmental issues, recognition and location of ajolote in the area.

Material

Flipchart, markers.

- Maps locating key sites of the area (roads, water bodies, etc.). This maps could guide and/or initiate the discussion.
- Printed photos of different species of ajolote to motivate the discussion and to identify the local species and their characteristics.
- PowerPoint presentation with images and key questions.

Development

Supported by the Municipality and Guadalupe Avilés (neighbor and local leader), a group of people from the municipality of El Oro (State of Mexico) and Tlalpujahua (Michoacán), where the two dams are located, got together.

Presentation of the team (REDES AC and the Ecological Restoration Laboratory of the Institute of Biology IB-UNAM). The team was introduced as well as the objectives of the project. A brief summary of previous work was presented, including how the team has worked with ajolote (A.mexicanum) in the Xochimilco wetland. Alstom Foundation representative provide a short presentation as well.

The goals of the workshop and the importance of local participation were presented. Participants were able to recognize how important is their knowledge regarding the most relevant socioeconomic and environmental issues, as well as on the ajolote. The next step was to present questions to the participants to begin the map making (see Annex 2). Working groups were formed.

To begin the mapping, the facilitators - members of REDES AC- assisted the working groups, locating landmarks and facilitating the exchange of ideas in the group based on key questions. The key generating questions were divided into two parts, first there was a half-hour to discuss general socio-economic and environmental issues in the dams. Later more specific questions were presented regarding the ajolote as main specie of interest.

After completing their community maps, each team presented their map to the whole group. Open exchange of ideas among participants took place. This allowed the enrichment of ideas between teams; also, some concerns and proposals were raised in order to continue the work in the area.

Results

Seven maps were generated where the following aspects were located:

- Economic activities: agriculture, tourism (Eco-touristic Park), fishing.
- Land use and vegetation: forest areas, agricultural areas
- Roads
- Environmental problems: pollution (garbage), use of agricultural pesticides, water scarcity, presence of water hyacinth, loss of species or endangered species (trout, crayfish, sea bass, ajolote, frog), contamination by septic tanks, logging.
- Identification of main flora and fauna in the study site.

Location of areas were ajolote has been normally seen.

The participatory workshop also identified perceptions and concerns of both towns of El Oro and Tlalpujahua. Importantly, the qualitative nature of this participatory dynamic, *ie*, the information presented at this point of the project reflects the vision of the workshop participants, so it is highly subjective. They would have more participatory workshops to validate this information with different groups and applying other methods of social research and contrast it with the results gained from environmental sampling performed.

- Need to improve the schemes of collaboration between the municipal governments of El Oro (State of Mexico) and Tlalpujahua (Michoacán) in order to conserve the environment of the dams.
- · Lack of desilting of the dams.
- Need for cleaning canals
- Rationalization of water from the treatment plant
- Improve forms of social participation: schools, the youth, women's groups, fishermen, settlers. For example, encourage participation in clean-up campaigns.
- It was proposed to ban illegal logging and that the two municipalities have to be involved in regular monitoring.
- Improve the collaboration between the two municipalities
- Improve environmental care and signage.
- Regulate that houses do not pour water into the dams, particularly in Victoria.
- Find alternatives regarding the problem of unemployment and generate economic opportunities from tourism.
- Potential of ecotourism alternatives using the ajolote as a symbol of the dams.

Conclusions: Identify interests of the Community

The participants expressed their interest in continuing workshops on the theme of conservation of the ajolote and the environment. Possibilities of working on the theme of ecotourism and about the economic viability of projects related to the conservation of the ajolote were raised. Participants were motivated by the conservation of the ajolote in Xochimilco, and showed interest to learn from this case. Another proposal was to conduct workshops in the Eco-turistic Park, closer to the communities, as well as in Tlalpujahua.

Participants also expressed their intention to carry out similar practices than the ones that are being performed by REDES AC to protect the ajolote in Xochimilco (eg. creating shelters). The facilitators explain that the case of Xochimilco is different, so it is necessary to generate specific alternatives considering the local conditions in the dams.

Workshop 2. The "ajolote" as part of the ecosystem and its relationships with human activities (November 2013)

Objective:

- To identify local community perceptions regarding the specie of Ambystoma granulosum and its relationships with other elements of the socio—ecological landscape.
- To encourage a systemic view of the landscape, considering the biophysical elements as well as the society–nature relationships considering the main economic activities and their potential impacts.

Theoretical background and methods

The workshop aimed at generating a diagram of relationships between elements of a landscape. The cognitive basis of this relationship map is the recognition of socio-ecosystems from its systemic complexity.

From the complexity approach, social-ecological systems can be understood as integrated systems of human and natural beings (Cumming 2011). Biophysical and social elements are interrelated and interdependent and so what affects or benefits a system element has a positive or negative impact on the rest of the set. The relations in the system can be then analyzed as processes of positive and negative feedback. Human groups are included in the system and human actions have an implication in all biophysical elements. Similarly it is assumed that human societies depend on the basis of nature. In this regard, the state of the ecosystem impacts directly on human activities and the use of natural resources.

A diagram of positive and negative feedback in the socio-ecological landscape allows us to visualize how these processes occur. Especially if we are looking for facilitating dialogue around how communities perceive relationships between elements of the nature and the impact of their actions on the ecosystem. In this case it is proposed that both positive and negative feedbacks should be identified and discussed, as well as the possible reasons of their perception. This dialog allows us to identify the environmental problems in an integrative way. It also encourages reflection on possible actions to tackle the problems. The relationship map is an instrument for collective discussion, exchange of knowledge and awareness, considering a systemic and holistic understanding of the socio-ecosystems.

Material Flipchart

Markers (at least two different colors)

Printed images of ajolote and other elements of flora and fauna, water and soil; also from human activities such as agriculture, fishing, tourism.

Development

1. Participants were organized in small groups of 5 or 6 persons to promote dialogue and allow everyone to express their opinion. The groups should

- incorporate diversity of actors, considering the age, gender, economic activity, etc.
- 2. The facilitator suggested placing the "ajolote" in the center of a flipchart. They were given a package of images of flora and fauna from the dams, both native and introduced species, and two different colored markers for positive and negative relationships. The facilitator encouraged the groups to draw or write other elements that they would consider important. Participants were suggested to place the species in relation to the ajolote wondering: Does this species are related to the ajolote? How do they relate? Your relationship is positive and / or negative? Why do you think that is? Does these species are related to other species or landscape elements (eg. Water)? Why and how these relationships are given?
- 3. Participants placed the images and identified relationships between different elements. The center of the diagram was the ajolote, this element triggered reflection and participants pointed out the relationships between the various elements of biodiversity and water. The groups were also encouraged to identify environmental issues, for example in relation to the effects of introduced species regarding populations of native species.
- 4. The next part of the activity was to identify both positive and negative relationships between biophysical elements of human activities. In order to achieve this goal, each team received a set of images of human activities and also was asked to place the images on the relationship map. Participants discussed how their activities and actions are linked to biodiversity and water. The groups talked about the relationships and if these could be improved by identifying the problem and through collective discussions. While the dialogue took part, the facilitator emphasized that there are both positive and negative interactions. It was also highlighted how important is for communities to participate in the conservation of the ecosystem from which they are part.
- 5. Finally, each team presented their relationship diagram in plenary and a feedback exercise and collective reflection took place. (in this case two diagrams were presented the next day for lack of time)

Results

Three teams of between 5 and 6 people were formed. The teams made their diagrams of positive and negative relationships (Annex 3). As in the previous workshop the community had already dealt with the socio-ecological problems in the area, this diagram gave continuity to that discussion. The following subjects were discussed:

- Threats related to introduced species
- Maintenance of the canals
- Water quality
- Importance of research regarding water quality and soil
- Use of chemicals in agriculture,

- Illegal logging
- Garbage and waste management
- Clandestine discharges
- More sustainable and profitable activities for the area (eg, ecotourism and sustainable agriculture)

Conclusions

All of the teams raised the above-mentioned general topics. The role of community organizations was highlighted to find proper solutions. This was discussed in Workshop that took part the next day. Participants expressed significant knowledge about their ecosystem. They also recognize the various impacts of human activities as part of the socio-ecosystem. Although many assumed to know little of the ajolote, they could identify the relationships of this amphibian with other species as part of the food chain. The importance of appropriate water quality was also identified for the species to maintain good health. They talked about how other species of fish (introduced species) can compete aggressively with the ajolote, threatening its population.

Workshop 3: Collective proposal of activities regarding the socio-ecological problems in the Brockman and Victoria damns (November 2013)

Objective

To identify activities prioritized regarding the socio-ecological problems using collective discussion and dialogue.

Theoretical background and method

Whereas previous discussions were about the socio-ecological problems and conservation of *Ambystoma granulosum*, this activity was proposed to trigger community dialogue in order to identify priority areas for conservation and sustainable activities. The 1987 Brundtland report defined sustainable development as the development that "meets the needs of the present without compromising the ability of future generations to meet their needs" (Cumming 2011). To Holling (2001) sustainability has "the aim of promoting adaptability and creating opportunities "(Holling, 2001 in Cumming, 2011:15). Sustainability has been defined by Norberg and Cumming as "equitable, ethical and efficient use of natural resources" (in Cumming, 2011:15).

During previous workshops, the communities had been discussing the interconnection and interdependence between biophysical elements and human activities. Participants talked about the importance of appropriate actions for the use and management of natural resources without compromising ecosystem functions. They proposed actions to improve environmental quality in the dams. The groups considered the importance of community organization and actions needed for the conservation and sustainable management. They also defined axis for the sustainable management of dams and conservation of *Ambystoma granulosum* for the governmental agencies.

In this dynamic it was proposed to imagine that the community was standing on one side of a river and across the river there was the objective they wanted to achieve (in this case conservation of ajolote and sustainable management of Brockman and Victoria dams). To reach their goal they have to cross the river, but there was no bridge. They had to build a bridge with blocks, each block was an action meant to reach the target. Thus, each group discussed what actions would be their blocks to build the bridge.

Material

Flipchart and markers

Development

- 1. Two teams of 5 or 6 people were made. Each team brainstormed to identify actions regarded as necessary to promote conservation of ajolote and sustainable management in the damns. The facilitator gave a flipchart and markers to each team and also suggested the following questions: What actions are necessary to reach your goal? How do you begin the journey?
- 2. A member of each team wrote the actions in a flipchart

- 3. Participants were encouraged to identify the most important actions required
- 4. Finally, each team presented their bridge blocks in plenary. Feedback was received.

Results

Teams discussed the following subjects:

Team 1:

- The first step is the "organization" and union among the people from two municipalities: El Oro and Tlalpujahua.
- An individual-based training scheme should take place first. Then the knowledge obtained can be transferred to the community.
- Knowledge and skills obtained during training should be applied.
- There must be a proper management of resources. In order to achieve that:
- Schools can be platforms to start projects.
- The invitation to participate in activities and projects should be done in sites were a lot of people assist. For instance, where they already have meetings of other programs, such as the "Opportunities Program", the Church, the school.
- The need of productive projects with a long-term approach.
- The transmission of knowledge and skills is considered essential to maintain long-term projects.

Team 2:

- The first step is the unity and organization among communities.
- There should be awareness on environmental issues in the dams.
- Communities can start with some short-term solutions:
- Collect trash
- Rational use of water
- Use of organic fertilizers
- Ask for water quality and soils research
- Find alternative ways for cropping and having gardens considering the weather.
- Environmental information in schools
- Search methods for pests control
- Find the sources of water lily growth and find mechanisms to control it
- Implement ecological bathrooms for places where there is tourism.
- Coordination between national, regional and municipal authorities
- Create security and cleaning groups for the forests, rivers and dams.
- Provide information regarding the ajolote.

- Installation and training regarding bio-digesters so households could avoid discharging gray waters into rivers and dams.
- The use of ajolote as a symbol of identity and conservation and attract more tourism without disturbing the environment.
- Investigate the chemicals used on the production of Christmas trees
- Request students from different schools to create teams for garbage collection in forests and Brockman and Victoria dams.

The list of participants can be found in Annex 4.

Conclusions

It should be noted that both of the groups had very similar proposals, which in plenary was important as a statement of the communities common perception. This allowed them to identify themselves as a group and strengthened their collective action. The workshop also allowed validating information that had been generated around the socio-ecological problems of dams in previous workshops. The ideas of community organization emerged as a way to achieve the goal of conservation and sustainable development. It was relevant to identify mechanisms to promote community participation and community organization. The technical team of REDES A.C., proposed an upcoming workshop and activities to enhance leadership and reinforce the importance of community organization.

Strategic planning workshops: The environmental awareness campaign for tourists in Brockman and Victoria dams– Easter season 2014 (1, 2 and 3 April 2014)

Introduction

From the previous workshops (held in November 2013) the importance of community organization was identified to achieve the objectives of *Ambystoma granulosum* conservation and sustainable management of dams. In order to support the processes of community participation and organization it was proposed to conduct two workshops. On the one hand, they were aimed at discussing the importance of the organization. On the other hand a participatory approach for strategic planning took place so they could organize themselves to approach a practical experience. This allowed the strengthening of ties as a working group and realizing concrete results from the organization and strategic planning.

The local group had decided to conduct an environmental awareness campaign for tourists visiting Victoria Brockman and dams during the Easter season. As a group they recognized the importance of a campaign that would provide relevant information to tourists in order to reduce impacts normally generated during the Easter season. It was decided to support the group enhancing organizational strengthening through an exercise of collective strategic planning of the environmental awareness campaign.

A third extra workshop was held during the fieldwork, this one was a different approach to environmental concerns through emotions and artistic activities.

Session 1

Objectives

Reflect on the learning process of local actors since the implementation of the project took part.

Identifying actions required for the environmental awareness campaign for Easter season (2014).

Method

Participants thought over their learning process discussing the following question: What has been the most significant learning that you have achieved since the project took place? During the first part of the workshop, each participant wrote their thoughts on a card, and then this was shared within the group. Collective enrichment and feedback was generated. This led to a reflection on whether they identify the importance of community organizing for environmental conservation and sustainable management in dams.

Subsequently, participants presented their progress in the Easter awareness campaign and were invited to identify the main activities they were planning to develop.

Finally to begin the strategic planning it was proposed to draw a map of the dams. They were able to identify key elements and sites for organizing the campaign: entry sites and main exits, main travel routes, major concentration areas, places where garbage accumulates.

Development

Each participant wrote on a card their ideas.

Some reflections taken from the oral presentation of participants were:

- "I have been able to do more things that I knew I could do and I have also meet people with similar ideals than mine"
- "I have learned to take care of nature (animals) and to share experiences with other people"
- "I was unaware of the specie of ajolote and the importance of its protection"
- "With REDES A.C. and GRUPEDSAC I have learned about the importance of working together as a group"
- "I have learned to finish what I start and I gained interest in nature"
- "I have learned to live with the best green technologies and to take care of nature"
- "I now care for the environment and implement green technologies (ecological toilet, cistern and solar heater)"
- "I now have the experience of sharing ideals with others and realize a way of life caring for the environment"
- "I learned the beginning of organization"

After this activity participants presented their general ideas regarding the upcoming awareness campaign. Some important comments were:

- To talk with people to prevent littering, burning the trash or using powerboats in the water bodies.
- Importance of placing containers with legends to separate trash. It was said that a member of the group is lobbying with local government to get some containers. It was still not defined the number of containers or their location.
- It was considered the need to place some welcoming signs for tourists with the message: "You are reaching a water sanctuary".
- The members of the group would use a badge or pin that would identify them.
- They would place an information spot to attend any question from tourists. They would provide first aid assistance, emergency phone numbers. They would have a book of comments.
- The group should be prepared with water, food and protection from the sun. They had to define equipment required, work shifts and invite more people to participate in the campaign.

 The two areas of the dams (El Oro and Tlalpujahua) were seen as common territory.

Finally, they draw a map of the Brockman and Victoria dam, wherein key elements and sites were identified for organizing their campaign.

Session 2

Objective

Perform collective strategic planning for the Easter season. Identify the main objective of the awareness campaign and strategic areas of action.

To identify previous activities required as well as activities during the Easter season days. Define responsible and/or coordinators for each activity. Define a schedule and materials required.

Method

Three activities were proposed for this workshop. The first was to define the goal of the awareness campaign collectively. We use the following question: What do you want to achieve with the campaign?

Subsequently we proposed a collective reflection called "The tourists that visit us today and tourists that we would like for the future". A brainstorm activity took part regarding the characteristics that current tourists have and how they would like the tourist to be in the future. Participants thought about conservation issues and sustainable management of dams.

Finally, participants elaborated a matrix of strategic priorities for the campaign. Strategic Axes for action were identified. They collectively established the main tasks in each axis. Both previous work as well as activities during the Easter season days were defined. Responsible or coordinators were chosen for each activity.

Development

1. Campaign objective

The workshop started defining the objective of the campaign, which was designated collectively as "Environmentalist Caravan".

Participants made contributions to establish the "main purpose" of the campaign.

Some ideas expressed were:

- Create awareness to protect the environment
- Clean the dams to benefit communities
- To sensitize people regarding the preservation of the environment, water and what inhabits it.
- Educate people to protect the environment
- Boosting awareness so in the future there would be no need for an awareness campaign
- Conservation of a common territory
- 2. "The tourists that visit us today and tourists that we would like for the future"

This activity was based on the collective reflection over the main characteristics of tourists that usually visit the dams in Eastern. We use the question: Who is the Tourist visiting the dams? Subsequently we suggested the question: Who we would like the tourist to be? Participants described features that an ideal tourist visiting the dams should have.

3. Outlining a collective strategic plan for the "Environmentalist Caravan".

The next activity was to define the strategic priorities and the specific activities and tasks to be performed to achieve the goal.

Three areas of strategic action were identified:

- 1) Provide information to tourists (information spot)
- 2) Place trash container in strategic locations and promote separation (RRR)
- 3) Place signs and messages

Participants elaborated a matrix of strategic axis. Tasks and responsibilities were defined, considering both previous work and activities taking place during Eastern days. Since not all participants of the caravan came to the workshop, the group decided to make the schedule in the following days when all participants were present.

Results

1. The objective of the Environmentalist Caravan was defined as follows:

"Objective: To raise awareness and sensitize the tourism and the surrounding communities for the care and preservation of the environment, water and biodiversity living in it"

2.

Who are the tourists?

- Young destroyers in the area (they get into the dams)
- Alcohol drinkers
- They leave garbage, bottles
- Consume drugs
- Violent

- Make campfires
- Bring animals, horses and motorcycles, including motor boats
- Families that come to picnic. They leave trash; get into the dam to swim
- Families who come to enjoy the place and respect the space
- Users of rest / country houses
- Tourist can cause traffic congestion
- Who would we want the / tourists coming to the dams?
- Responsible clean (collect their waste)
- Respectful of others
- They care for the environment: do not break or throw bottles and trash into the water, do not swim in the water, do not drink alcoholic beverages, do not burn tires
- They are educated
- 3. Finally the following scheme was obtained -strategic planning matrix.

See Annex 5 for more information regarding personal experiences of some participants.

Strategic planning matrix

| | Provide information to tourists | Locate trash containers | Locate signals and messages for tourism | Information spot |
|------------------------------------|---|--|---|---|
| Previous activities | Develop a script for the brigade Study the script Rehearse the script Revise strategic location to establish meeting points Create a format to register tourists data Develop brigade badge | Request trash containers Pick up containers If needed, build more containers Define the number of containers Put labels for separating and organizing trash Get garbage bags Set a garbage gathering place Define where containers are placed Conduct a visit to recognize the locations Defining transportation | Define the dimensions of the signs and messages Define the legends Choose pictures for the signs and take them if we don't have them Define location Search for materials to place the signs and messages Design the material | Define location Define responsible and timetables Define the information to be given Define the first aid kit and put it together Having information for security and safety emergencies (emergency numbers) Having the book reviews Getting a table, chairs and a tarp |
| Activities during the caravan days | Comply with the principles, agreements and attitudes. Talking with tourists Take information to register tourists Wear badges at all times Provide information Provide information of regulation in the territory | Transport trash containers Locate trash containers Change the trash bags during the day Pick up trash Transport garbage to the gathering place | Locate signs and messages Pick up signs and messages and take them to the site | Bring the table, chairs and tarp to the site Provide information Pick up table and materials |

| Denound | ce irregularities |
|-----------|-------------------|
| Finishing | the day |

| General activities | |
|---------------------|--|
| Previous activities | Determine whether the group will be organized or they will ask family to provide food and water for participants |
| | Buy ice to preserve food |
| | Buy water |
| | Buy candy and / or fruit |
| | Prepare distinctive |
| | Prepare camera and / or video to document the activities |
| | Prepare raincoat |
| Activities | Having water and food |
| during the | Carrying a cooler, water, candy and / or fruit to the gathering place |
| caravan days | Wear badges or distinctive |
| | Having the team for documenting activities: take pictures and / or video |
| | Bring a raincoat |

Session 3 Sculpture workshop

Facilitator: Félix Carranza

Objective

Expressing emotions about the socio-ecological problems of the Brockman and Victoria dams through a more ludic and artistic tool: modeling clay.

Method

More playful and artistic tools enable an emotional approach to environmental awareness. Humans can connect to the sensitivity from the emotional and sensory stimuli using art as a tool. The playful strategies offer the possibility to reach a deeper level of reflection, which refers to different types of emotions about a situation or problem. In this case the modeling activity was based on the emotions around the socio-ecological problems that are happening in Brockman and Victoria dams. In this session, we invited an artist, Felix Carranza who has an expertise in this kind of approach.

Development

The strategy was primarily introspective. Participants modeled a sculpture depicting emotions. Each participant built a sculpture from their feelings regarding the environmental problems in the territory. Then, they shared and interpreted their work in a group chat. They broke this first sculpture to make another sculpture that proposes a solution to the problem. Finally, participants shared with colleagues the emotions and significance of the sculptures in clay to generate reflections collectively.

Results

Participants made their sculptures and reflected as a group about their emotions. They were able to share and "connect" with their feelings regarding the socio-ecological problems of dams. They thought over the history of the place and how they were personally related to processes in the territory (participants in his childhood and youth for example). They talked about their perceptions of change in the landscape of the dams and the local importance of those processes. In the end they emphasized the importance of acting as individuals and as a group to protect the ecosystem and seek alternative management and sustainable use.

Conclusions

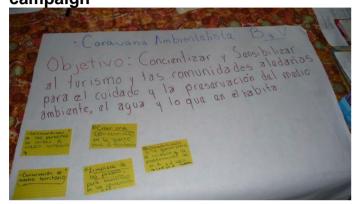
During the three days of workshops we collectively thought about learning processes of local actors since the implementation of the project. Strategic planning for the environmental awareness caravan (Easter 2014) was performed. This target was achieved collectively. The axes of main actions were identified. Tasks and activities for each axis considering both previous and Easter days were also defined. The strategic planning was performed as an exercise in organizational skills as a group. Participants were invited to construct meanings for environmental leadership and community involvement. This exercise also allowed raising awareness on organization and planning skills importance. Visible results and possibilities for collective analysis and reflection were found.

Finally workshops in April 2014 concluded with a playful and artistic approach. We made a call for more environmental awareness based on the emotions of participants. Participants made sculptures that represented their feelings around socio-ecological problems of dams. They also made a sculpture representing actions to face the identified problem. The group proposed that REDES AC should focus on strengthening the formation of a group of environmental leaders.





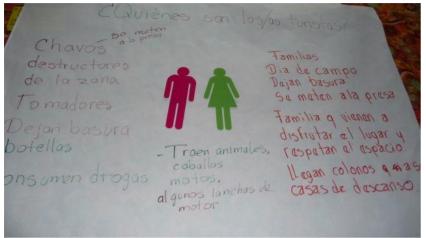
Day 2. Definition of an objective for the awareness campaign



Day 2. Defining tourists features



2. Features from the tourists







Advidades Generales - Massaches Alphier Many Invitor a Familiares a anigos a participar con alimentos y aqua. 13/0 definir si el grupo va a organizar Ma para proparar los alimentos y llevar agoa. Can grupo o cada govern) - Hiclera pava conservar alimentos. Goodalupo - Comprar garrafon Juanta y Aurelia - Comprar carametos y/o frutas c/ quien - Tener listos los distintivos. (uniforme/gafete) - com Preparar cámar de Poto y lo video para documentar los actividades. Nancy - Preparar impermeables / ? - Tener comida y agua un el lugar - Hevar la historia, garratón, carameto gobit on a lugar de acopio Llevar los distintivos / gafete. - Tener el eguipo para la documentación tomar las fotos y/o video Dang - Llevon impermedale / 2

Day 3. Modeling clay workshop









Analysis of the socio-ecological problems of the Brockman and Victoria Dams using participatory workshops (June 2013 - April 2014)

From participatory workshops with communities around Brockman and Victoria Dams, (June 2013– April 2014), we were able to obtain valuable information regarding socio-ecological problems and *Ambystoma granulosum* conservation. The workshops allowed us to validate the information from different participatory methodologies (participatory mapping, interaction diagrams, identifying community actions, strategic planning workshops, modeling clay). Parts of participatory workshops are recorded in voice and/or video. Activities have been also documented throughout a photographic archive.

REDES AC has synthesized the information in the following tables. We were able to analyze the socio-ecological problems, strategies and priority actions identified (see Table 1). Diagram analysis of the socio-ecological problems and strategies for the conservation and sustainable management was also performed (see Figure 20).

From this analysis three main groups of strategies were identified:

- 1. Collective action and social organization. This group was considered the first building block to tackle socio-ecological issues. These strategies are related to the need of improving social participation in the environmental issues, the access, use and management of the natural resources from the dams. Local participants proposed to assume civil society responsibility and generate collective actions towards environmental issues (the specific actions proposed are explained in the summary table.). The participation can be boosted from environmental education. Social participation and responsibility is considered by local communities as the corner stone to preserve the dams and the ajolote. Some of the specific actions expressed were: to organize security and cleaning crews focused on forests, rivers and dams and to generate campaigns for tourists to collect their garbage. Local actors were able to organize an environmental awareness campaign in the last Easter season that turned out to be a success with plenty learning experience. Participants are considering to become an environmentalist group.
- 2. Promoting new research. Local actors considered the need to ask for more research on different environmental fields, such as water and soil quality, biodiversity (mainly regarding the knowledge of ajolote), environmental treats such as invasive species, agrochemical impacts on the environment, among others. Besides generating the research, local actors consider that these information should be provided to communities and so the results should be useful for them. In conclusion, the research must respond to social concerns and also be useful to implement actions regarding the environmental issues.

3. Project development and management. A third group of strategies is related to generating and managing development productive projects with a sustainable view. For local actors, there is a need of economic alternatives that are also environmentally friendly. This is related to collective action strategies. The local communities need to be organized in order to get productive projects, implement them and manage them properly. In order to accomplish their objectives, local communities (beginning with local leaders) need to be educated and get the necessary set of skills. They realize activities mainly related to ecotourism and sustainable agriculture.

Conclusions

From the workshops and activities of this project, we could:

- Identify how local actors perceive the socio-ecological problems related to the ajolote (*A. granoulosum*). We also identified which social and environmental issues they consider the more important and the strategies and actions necessary to face that problems.
- Obtain information regarding the local knowledge on the specie of ajolote (A. granulosum). Local actors expressed their knowledge on the specie and some stories related to them, but also how a lot of people had very little information on this important amphibian. Some people did not have any knowledge and so they appreciated the information provided by the project and currently recognize its importance.
- The activities allowed people to acknowledge the importance of social organization and collective action to protect the ajolote, and also their habitat in the dams. Local actors from El Oro and Tlalpujahua related to de socio-ecological problems and some of them decided to organize themselves as one group. They recognize one territory, and the need to be together from both of the municipalities. They called this territory Brockmand-Victoria.
- Participants expressed that they need jobs and economic assets to improve their livelihoods, but the improvement in their quality of life was also depending on the protection of the dams. So, they suggested to generate only sustainable projects and activities around the area: mainly eco-tourism and sustainable agriculture.
- Potential environmental leaders were identified. They began using their knowledge and skills to generate a strategic plan to develop and awareness campaign for tourists. Leaders were able to organize the group, from the planning to the implementation of the plan. The campaign that they designed had plenty of success. They decided to learn from the experience and improve next year campaign. Local authorities recognize their campaign. Participants decided to continue generating local actions and negotiating with authorities for improving collaboration.
- A group of young graphic design students from the Faculty of Arts and Design-UNAM under coordination of Mto. AV. Claudia Mena González, developed an image for the new environmentalist group. The group

received a logo and an image for the group, the design of some products like T-shirts, bags, stuffed animals, and others to generate environmental awareness (See Annex 6). This products were thought as part of a new eco-tourist approach in the area, where the ajolote is seen as the center of the campaign. The students also proposed new signs, different products to sell, and fun presentations for environmental information. Local actors also received products to enhance environmental awareness from local communities, especially for children.

The main result is that the group of local actors is motivated to continue working, they already implemented an action to promote environmental awareness. They also are planning to continue as a group and generate sustainable activities, such as eco-tourism. They plan to educate tourists and local communities to protect the ajolote and their habitat. They also are emerging as leaders within their communities and also negotiating with local authorities.

Continuity and enhancement

To implement strategies and plans that have been identified, it is necessary for local actors to establish a working agenda and strategic planning. From a series of workshops with different actors, agreements can be generated to continue the work. It is important to agree on responsibilities, timelines, and specific goals and targets. While conducting workshops it could also be possible to establish monitoring and evaluation schemes to achieve the goals and objectives properly. This is not an easy task, it requires commitment and continuity to protect dams, the ajolote and biodiversity of the area. Ecosystem protection may be accompanied by related production projects, with a vision of sustainability and feasibility to enhance the quality of life of local residents.

Table 20 Socio-ecological issues, strategies and actions

| Environmental problems | Strategies | Actions |
|--|--|---|
| Littering | Encourage garbage collection schemes (eg groups from local schools). Collective action. | Young people from different local schools can collect garbage in forests and the Brockman and Victoria dams. Organize security and cleaning crews focused on forests, rivers and dams. Campaigning for tourists to collect their garbage. |
| Illegal logging | Surveillance and control of illegal logging. Collective action. | Prohibit illegal logging Periodic monitoring of governments of the two municipalities. Create security crews |
| Water scarcity | Rational use of water. Collective action | |
| Use of chemicals for agricultural production | Obtaining information regarding chemicals for agriculture. Promoting new research avenues. | Research on the specific chemicals being used for producing Christmas trees. |
| | Improve agricultural practices. Encourage practices of sustainable agriculture. Project development | Use of bio–fertilizers Looking for biological methods for pest control |
| Invasive species (water hyacinth) | Finding information for better decision making Promoting new research avenues. | Looking for the origin of water hyacinth and finding mechanisms to control blooms. |
| Endangered species (ajolote, trout, crayfish, bass, frog) | Obtaining information about ajolote. Promoting new research avenues. | Getting more knowledge regarding ajolote to enable protection actions |
| Pollution from septic tanks. Dumping of sewage from homes. Disposal of soapy water and laundry bleach. | Wastewater management. Project development | Monitoring dumping of sewage from houses, mainly in Victoria dam. Installing bio–digesters for homes and training local communities to manage this technology. This would help to avoid gray water disposals into the dams. |
| Lack of environmental information. Local communities do not obtain the | Obtaining results from research on water quality and soils. Promoting new research avenues . | Demand research on water quality and soils. Obtaining the results. |

| research and studies on water quality and soils. | | |
|--|--|--|
| Social problems | Strategy | Actions |
| Lack of cooperation between municipalities of El Oro (Estado de Mexico) and Tlalpujahua (Michoacan) to conserve the environment of the dams. Lack of cooperation between different levels of government. | Promote coordination between authorities of the two municipalities and between different levels of government. Collective Action | |
| Lack of social groups participation (Schools, youngsters, groups | Encourage participation and social organization. Union among the residents of the two municipalities. Collective action. | Take advantage of spaces to encourage participation in cleaning campaigns, schools, opportunity meetings or churches. Schools as a starting point to enhance participation. |
| of women, fishermen, local inhabitants) | Environmental awareness, training and knowledge transfer. Collective action/Project Development | Suggest schemes for individual training in environmental issues and productive projects. To promote social participation with permanent callings. That trainees apply and transmit knowledge and learning |
| | Appropriate management of resources and projects. Project Development | Request productive projects with long-term vision. |
| Unemployment | Management of productive projects Project Development | Schemes to promote ecotourism as economic activity where you can use the ajolote as a symbol or icon of the dams' territory. The ajolote can become a symbol of identity. It is necessary to provide information about the specie. Implement composting, ecological toilets at tourist sites. Educate tourists about their impact on the environment, to collect their trash and respect the ecosystem. Improving preventive care and environmental signage. |

| | | Search for production alternatives adapted to the weather conditions. |
|---|-------------------|---|
| Managements and maintaining of the dams | Collective action | Rational management of the water treatment plant Cleaning the canals Local residents should be beneficiaries of the dams. Since |
| | | currently local communities are not beneficiaries. |

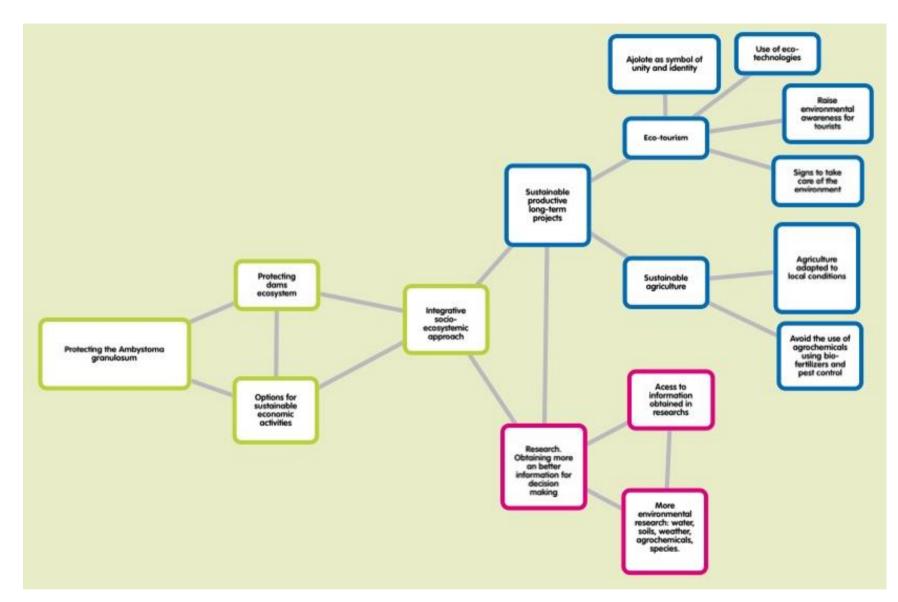


Figure 20. Analyzing socio-ecological issus ByV

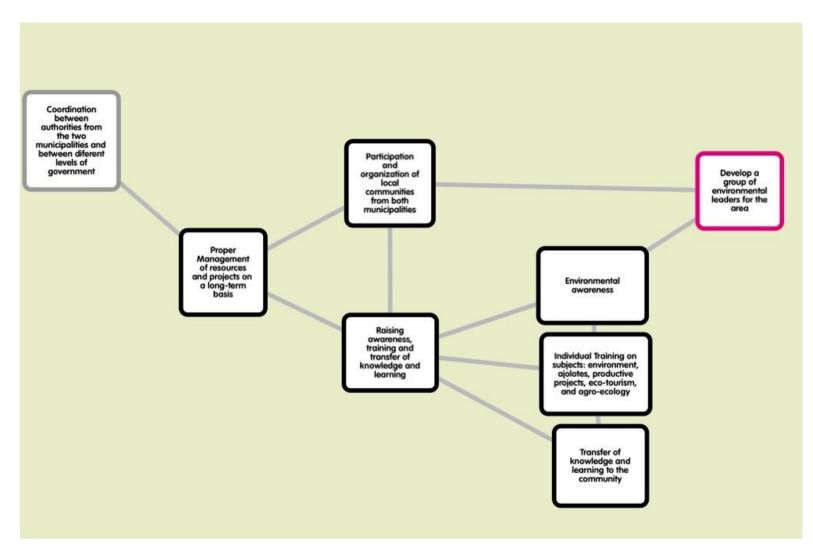


Figure 21. Analyzing socio-ecological issues 2 ByV.

Section III. Conservation *ex situ* of *Ambystoma granulosum* (Taylor, 1944) at Laboratorio de Restauración Ecológica IB-UNAM.

Mena González Horacio Rodríguez Flores Erika

Resume

In the season june 24, 2013 to august 22, 2014, four sampling were programmed to obtain ajolotes (Ambystoma granulosum) at the Brockman dam in "El Oro" State of Mexico. They were obtained and selected 14 ajolotes of less than a year old, during catches were not found adults. The organisms were identified with chips and transferred at Laboratorio de Restauración Ecológica IB-UNAM, where samples of tissue for stable isotopes, stomach content and tests of chytridiomycosis were taken. The adaptation to captivity was slow and 8 ajolotes were transformed into salamanders stimulated by stress like a change of environment, kind of food, number of organisms in the same area, surgery procedures such as an invasive marking and sampling procedures. Currently we have five organisms in larval phase and seven are salamanders, all they are maintained under a management protocol developed for this specie under laboratory conditions. The program provides adequate installations, cleaning and disinfection, quality of water and food, good temperature, health evaluation and habitat preference. Only one ajolote differentiated as a female and the relationship between male and female was 18:1, in this case are considered all the organisms that were captured in the four samplings seasons, the organisms with male characteristics were returned to the dam. The female was subjected to the same kind of stress and also turned into a salamander. Three more samplings were programed for obtaining females in larval stage or as salamanders and the result were negative, but we were found in a confined space four different life cycle phases about this specie, larval, juvenile, mature ajolote, and organism in metamorphosis. Currently do not consider it appropriate to force reproduction of the female through the use of hormonal products because the reproductive physiology of this specie is not clearly understood and it is not more than two years old. The life cycle of the species in the Brockman dam is not understood in terms of the organism's origin at the dam, female's location, maturation time, average age metamorphosis, first reproduction, salamander phase location, so it is suggested to address the following research efforts to learn more about the life history of the species in the Brockman dam.

Capture of ajolotes

Four efforts to capture ajolotes were planning in different dates, the objective to the last three efforts, were to obtain females in larval or salamander stage. In all the trapping were obtained animals less than one year old. Features such as a skin texture, less than 100 mm total length and little cloacal glands development indicate that these animals were

immature. Ten months later they matured and had glandular development, very common in males.

First sampling: from 24 to 26 June 2013 were captured 12 organisms of *A. granulosum* with 24 networks (Figure 22). Networks were put at different distances from the littoral zone of the dam and distributed with the intention to covering different depths in the water body. Subsequent to obtaining the 12 organisms, networks remained for 3 more days and we reviewed every 24 hours with the intention of capturing the greatest number of ajolotes and to find in them an evident sexual dimorphism, in order to generate information about the population status in the site. Also the physico-chemical parameters of the water were taken with a probe multiparameter (HANNA model 9828). The animals were transferred in ice buckets with dam water to minimize a possible physiological stress(Barnett*et al.* 2001) (Figure 23) to the sampling and analysis area. They were anesthetized with isoflurane (PISA Labs.) 3 ml/ I. of water and then, were evaluated through a physical examination, they were measured and identified with the implantation of microchips (AVID MUSSIC12 mm) (Figure 24); stomach content samples were also taken (Figure 25) to learn about your diet and for isotopes studies. Details of each organism were recorded on a sheet of initial collection.



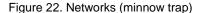




Figure 23. Transferring organisms



Figure 24. Surgery of chip implant for identification



Figure 25. Sampling stomach contents

Measures considered for the captured organisms: TL total length, snout vent length SVL, tail length Tl, gills length GL (Figure 26) identified sex. This information was recorded for to know de general state where the ajolotes were found.

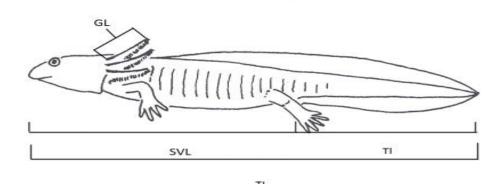


Figure 26. Morphometric measures (modified from Casas-Andreu y McCoy 1979)

For recovery from his intensive management the ajolotes were kept in dark containers and fed with live fishes. Later they were taken in plastic bags with water and air inside to cooler with ice to the Laboratorio de Restauración Ecológica IB-UNAM, were there continued with its recovery and began the captivity adaptation process.

Second sampling: the objective was to obtain some females. The effort was held from 1 to 4 April 2014 and was performed at the sites where the presence of organisms was evident in previous sampling. In these points we search for ajolotes without development of cloacal glands. Four organisms were selected and after three months three differed as a males and only one like a female. The majority of ajolotes in captivity were transformed into salamanders and we decided to plan a third capture.

Third sampling: was from 21 to 25 July 2014, night walks were conducted (Figure 27) and 16 pitfall traps (Figure 28) were used in the surroundings of the Brockman dam (T1-T16, Map 1). The potential sites selected, included protection from direct sunlight, zones with under water level, food, little disturbance and near bark of tress (Figure 29). During this sampling were observed four ajolote's males, two organisms from four months of age, one larval adult and the last one a male in metamorphosis process that presented eyelids development, gills and dorsal fin absorption (Figure 30) This is the first sighting of an organism in metamorphosis process in two years of follow-up at the Brockman dam.





Figure 27. Disposal of pitfall traps for terrestrial amphibian samples







Figure 29. Illustration of potential sites for salamanders



Figure 30. Organism in metamorphosis process (formation of eyelids and reabsorption of gills)

In the Pitfall traps we catch another kind of amphibians; three different frog species and two toads.

The fourth sampling: was held from 20 to 22 August 2014. The purpose was to obtaining aquatic or terrestrial females ajolotes, two networks were put in the Brockman dam to obtain the organisms in the aquatic phase, and eight pitfall traps were distributed in the surroundings of the dam (t17- t24, Map 1) for terrestrial phase. Two males were captured in the water, one in metamorphosis process and the other one in larval stage (Figure 31). After, we taking their morphometrics and evaluating their health status, and then both were returned to the capture point.





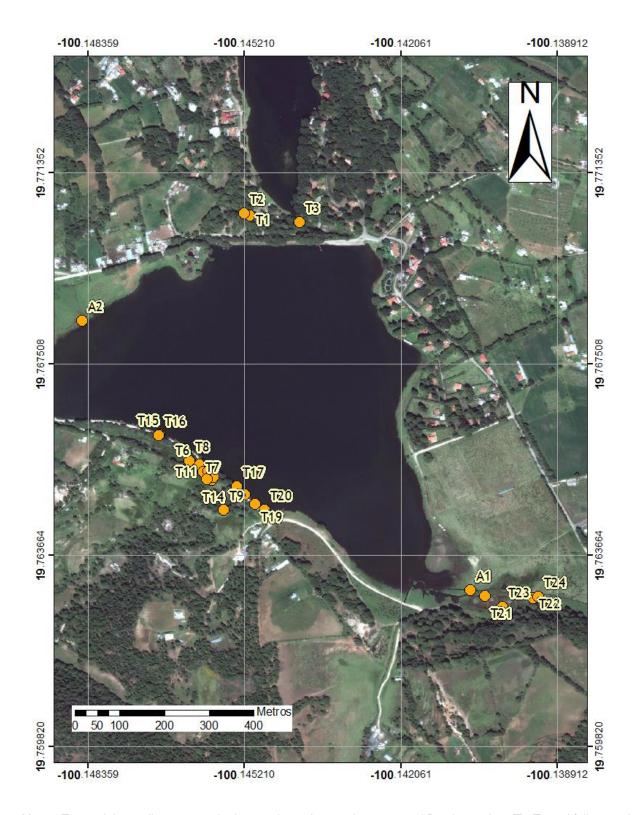
Figure 31. One larval male (with gills/down and left) and the other one in metamorphosis stage (up and right)

Overview of sampling efforts in water and land for obtaining organisms

In 10 days of sampling we employed 54 traps / 228 hours (Map 1) and 33 hours of night field trips 21).

Table 21. Summary of sampling effort taken from the project in Brockman dam

| Sample | Month | Days | Number of traps | Time – trap (hours) | Ncturnalsampling (hours) |
|--------|-------------|------|-----------------|------------------------|--------------------------|
| 1 | June 2013 | 3 | 26 (aquatic) | 72 | 0 |
| 2 | April 2014 | 3 | 2 (aquatic) | 72 (aquatic) | 0 |
| 3 | July 2014 | 2 | 16 (terrestial) | 36 (terrestial) | 18 |
| 4 | August 2014 | 2 | 2 (aquatic) | 48 (aquatic and | 15 |
| | | | 8 (terrestial) | terrestial) | |
| Total | | 10 | 54 | 228 | 33 |



Map 1. Terrestrial sampling area to obtain organisms *A. granulosum* around Brockman dam (T1-T24, pitfall traps; A1-A2 sighting in small bodies of waters)

Enabling space for adaptation and maintenance of Ambystoma granulosum

In the laboratory of Restauración Ecológica at IB-UNAM, was fitted out a dark space with 8 liters plastic containers to keep organisms in quarantine. In other space were three 40 L aquariums with shelters and filtration system. For the permanent ajolotes maintenance, was fitted a space with a tub terrarium of fiberglass 200 L of 1.60 m X 1.20 m, designed with two equal compartments and three levels of depth, two for the aquatic phase and one for the land phase. It contains fibreglass rock non-porous shelters, and filtration system (Figure 32, Figure 32. Container with 3 levels). Respect to the quality of water, temperature and lighting conditions, the ajolotes colony has god facilities like: clean water, climate controller, artificial light (timer) to provide conditions depending the year season.





Figure 32. Container with 3 levels

Figure 33. Space and facilities in the LREIB-UNAM

Adaptation and maintenance of organisms into captivity

The adaptation period and maintenance of any organism to a new space is slow, complex and demand greater knowledge about the biology of the species, so if this knowledge is insufficient adjustment period will be even more complicated. At this time the animals are more likely to live stressed and the likelihood of acquiring infectious diseases or metabolic rate increases. To adapt to the new space organisms went through a period of quarantine, preventive medicine programs, nutrition, health screenings were implemented, the most important morphometric measurements were also recorded, as well as morphological and behavioral changes.

Quarantine

Quarantine is a time period that is used to prevent and control disease, and the adaptation of organisms to their new space in the shortest time possible is encouraged. In this period it is important to keep a strict hygiene, keep animals in a sandbox (Shannon 2008) and reduce additional stress factors beyond capture as diet changes, transport and environmental changes (Fowler 1986).

The organisms caught were placed in a previously assigned space (Figure 34) within the ajolote's colony. In amphibians a minimum 60-day quarantine (Schad2007) is recommended, but there are diseases that can take longer to incubate. Such is the case of chytridiomycosis, a disease caused by the fungus Batrachochytriumdendrobatidis causing loss and decline of populations of large numbers of amphibians worldwide. This fungus can go through an incubation period and cause disease in a period of 9-76 days (Danszak et al. 1999). For this reason the quarantine period for *A. granulosum* was 90 days. During this period the salamanders were observed daily, the information was recorded individually and data on overall health status, presence of abnormalities in behavior, count and appearance of feces, amount of food consumed, weekly weight of organisms and morphometric measurements once a month were included (Figure 34, Figure 35, Figure 36,)



Figure 34. Quarantine space



Figure 35. Weight of organisms



Figure 36. Morphometric measurements

Preventive medicine

The preventive medicine program applied to *A. granulosum*, consists principally of four actions.

- 1. Administration scheduled of disposal of parasites and vitamin broad spectrum to reduce the presence of metabolic or infectious diseases.
- 2. Cleaning and disinfection. The organisms have permanent and temporary containers for its exclusive use. Also capture nets, filters, air pumps and shelters. All equipment is periodically cleaned and disinfected with powder detergents or disinfectants such as chlorhexidine, benzalkonium chloride or sodium hypochlorite, which are rotated in use to

keep parasites developing resistance. The program also includes the cleaning and disinfection of floors and walls and controlled via a timer photoperiod.

- 3. Comprehensive management program in nutrition that strengthens disease prevention through general disinfection and filter food container. The food is washed and disinfected before being administered and the debris removed promptly.
- 4. The control of water quality. It is a measure essential for the proper maintenance of the salamanders, for this reason it is evaluated through the measurement of physicochemical parameters such as pH, temperature, chlorine and the presence of ammonium (NH₄+). The Table 22 show the specific actions that the medicine program contain.

Table 22. Summary of medicine program

| Activity | Time | Products |
|---|---|--|
| Vitamins administration | Every 6 months | 2 drops (.01 ml) of Catosal ® B12 and Vigantol ® ADE (Bayer labs.) |
| Anthielmintic treatment | Every 6 months | Metronidazol 50 mg/ 1 lt of water as a bath for 24 hours every 2 days. |
| Cleaning and disinfection of filters | Cleaning weekly and disinfection every month | Benzalkonium chloride or chlorhexidine in water (1:10), sodium hypochlorite (1:100) |
| Cleaning and disinfection of substrate | Cleaning every 15 days disinfection every month | Benzalkonium chloride or chlorhexidine in water (1:10), sodium hypochlorite (1:100) |
| Cleaning and disinfection of containers | Cleaning weekly, disinfection every month | Benzalkonium chloride or chlorhexidine in water (1:10), sodium hypochlorite (1:100) |
| Cleaning and disinfection of floors and walls | Walls every 6 months and floors every week | Soap and sodium hypochlorite |
| Disinfection food (Tubifex tubifex) | Every week | Methylene blue, cooper sulphate, acriflavine, potassium permanganate. |
| Disinfection food (smallfish) | Every week | Methylene blue |
| Cleaninig and disinfection of food container | Cleaning every third day Disinfection every week | Benzalkonium chloride or chlorhexidine in water (1:10), sodium hypochlorite (1:100) |
| Waterqualityevaluation | Everyweek | Ammonia:HANNA checker HI 700 Chlorine: HANNA checker HI 701 Temperature, ph: HANNA Tester HI 98129 |

The program is also aimed at reducing the risk of transmission of animal diseases - human through the use of work clothing such as gowns, masks, washing hands frequently, the

restricted use of cell phones and food consumption. Additionally, a scheduled parasite disinfection of personnel working with the species is performed.

Quality of water

The water quality was evaluated from the measurement of physicochemical parameters such as temperature, ph, chlorine and ammonia, considered to be the most important for the well-being of the ajolotes. The Table 23 displays the ranges of physicochemical parameters obtained in this study. Follow-up organisms did not show signs of disease, for this reason these values can be considered as a reference to keep the species under conditions of captivity.

Table 23. Data comparision of the physochemical parameters in A. *granulosum*.

| | Ambystoma granulosum (LREIB-UNAM 2013- 2014) | Ambystoma granulosum (Shaffer 1989) | Order Caudata (Wright 2001b) |
|-------------|---|---|---|
| Temperature | 17.1- 17.6 °C | 16-18 °C | 16-20 °C |
| Ph | 8.2-9.1 | 6.5-7.3 | 6.5-8.5 |
| Ammonia | 0- 0.28 mg/l | | < 0.02 mg/l (optimal) 0.1 mg/l (maximum) |
| Chlorine | 0 mg/l | | 0 mg/l |

According to the table 3, *A. granulosum* is tolerant to neutrality and basicity environment; it tolerates levels near and greater than ph 7. Values of ph at the Brockman dam (ph 8-9) are very similar to those obtained in the laboratory (8.2-9.1) and the temperature is constant (17°C) and is within the ranges present in the water of the dam (17-19 ° C). Ammonium in the laboratory readings were slightly higher than mentioned in the literature by suggesting that this species can tolerate higher concentrations, very close to 0.3 mg/l, the chlorine must be always absent.

Feeding program in laboratory conditions

The diet of free-living organisms depends on the availability of food, time of year and stage of the life cycle in which they are; in salamanders even though the food is very similar in its different stages, often vary in the amount (Wright 2001).

The first observations of the type and content of the feces of salamanders captured in Brockman reservoir suggest that in the wild *A. granulosum* carries a diet of mollusks, crustaceans such as crayfish and small fish, these observations were made in organisms with size greater than 10 cm in length (snout-cloaca). The change in diet experienced by the organisms from free life to captivity is summarized in a long adaptation process. In this period all organisms present suggestive signs to metabolic disease and weight loss

(Table 24). The intensive management process like de capture stress, implantation of identification chips, the taking of tissue and gastric samples, surely favored the presence of immunosuppression in organisms further hampering its adaptation. The first week the organisms rejected the diet of small fish. At the beginning this diet was used in order to keep moving ajolotes, since the use of preys with little or no movement returns to animals sedentary and lowers their ability to hunt (Wright, 2001). In the second week the salamanders rejected the diet so the annelid *Tubifex tubifex* was added, this food had some degree of acceptance. Until the seventh week of observation, the organisms had shown a preference for quick *T. tubifex* and a gradual increase in the consumption of fish.

Currently in laboratory organisms fed *T. tubifex and Chirostoma sp* (charal) every other day. The leftover food is removed in order that the salamanders don't lose interest and keep waiting for the next administration. This form of feed accelerates the process of acceptance of the diet and to organisms that are not eating, something that can also be seen reflected in weight loss. So far, after 15 months of study organisms have stabilized weight, accept the two types of food, generate strategies to capture fishes and show great impetus to consume *T. tubifex*. Although the current supply of organisms is different from those in the wild (crayfish, insects, mollusks), nutritional content replaces the protein requirements of organisms, however is high in lipids (Albanila 2004). The oil excess can be decreased by regulating the amount of *T. tubifex*. Offering foods that contain or exceed the amount of nutrients may cause physiological disorders and predispose to metabolic diseases (Fowler 1986) or infectious one (Wright 2001a). Sometime during the adaptation period all organisms have presented signs suggestive of metabolic disease type and weight loss (Table 24).

Table 24.Metabolic disease observable in organisms during 15 study months

| Time | Organism | Signs Diagnosis | | Treatment |
|------------------|---------------|---------------------|-------------------|---------------------|
| 10-20 days after | All organisms | anorexy, tail hook, | Maladaptation | Vitamins ADE, B |
| collection day | | weight loss | Syndrome, | complex and |
| | | hyperactivity | infection, stress | antibiotics |
| 5 months after | 075057307 | Eyelid edema | Vitamina A | 1 drop vitamin A in |
| capture | (salamander) | | deficiency | each eye. |
| | | | | Improvement |
| | | | | after 24 h. |

Health Assessment

During maintenance in captivity several measures have taken different for early detection of diseases and risk reduction. In the second week of quarantine swabs from the mouths of all the salamanders were taken to determine the presence of the fungus Batrachochytrium dendrobatidis which causes the disease called chytridiomycosis (Danszak et al. 1999). The samples were transported to the laboratory of Mycology in the Facultad de Medicina Veterinaria y Zootecnia at UNAM where the fungus culture was performed. The result was negative.

From the first month signs of disease appeared in several organisms as aggravation of injuries caused by the capture netting, weight loss, decreased food intake, tail hook, lethargy, and lesions suggestive of the presence fungi such as Saprolegnia sp. and bacteria such as Aeromona hidrophila. With the appearance of these signs the appropriate medical treatment was initiated and decreased the causes of stress and excessive handling, inadequate exposure to sunlight, excessive water movement, improper temperature in the atmosphere and water replacement was intensified and isolation of stressed organisms. Welfare of organisms relates to their physiological responsiveness and adaptation to the environment in which they are (Fowler 1986). Stressors can be divided into somatic, psychological and behavioral. In the group of salamanders include somatic changes in tail position and tension in the muscles. Psychological factors in hyperactivity are manifested by the need to escape. Other stressors include poor nutrition, parasites, infectious agents, burns and surgery. During the adaptation process were identified and different techniques were used to reduce stress and avoid the risk of illness and death. The most critical period was the recovery of organisms after collection of tissue for the study of isotopes. This tissue sample is important to identify what is the possible food species consumed in the wild. Along with this procedure, organisms were labeled with an identification chip that was inserted into the muscle of the right dorsal region in the first third of the chest. This procedure allows the identification of the ajolote permanent lifetime. The second critical period was adapting to the new type of food that is now the foundation of its diet. After recovery of the organisms, there were behavioral changes such as hyperactivity. For example, two ajolotes jumped from their respective containers, so they were changed to 40 I tanks. This phenomenon is consistent with acclimatization and maladaptive syndrome (AMS) (Wright, 2001c) (Table 25).

Table 25. Causes of stress in A. granulosum from capture to adapt to captivity

| TIME | STRESS CAUSES | ACTIONS TO REDUCE STRESS | OBSERVATIONS |
|---------------------------|--|--|---|
| 24 hours after capture | Capture | After capture the ajolotes were put in non-abrasive containers and we prevent the direct light exposition. | Some ajolotes presented abrasive injuries caused by networks |
| 24-72 hours | Surgery (identification chip implant and we took tissue sampling from the tail and gastric sample to) | Use of anesthetic, intensive 12-hour recovery period, were subsequently monitoring the consumed food and feces production | The recommended dose of anesthesia was insufficient, required a larger amount. |
| 24-72 hours | Transport captured ajolotes to laboratory site | The ajolotes were put in air bags and covered with black plastic. | During the transfer, were kept at 19°C |
| Quarantine time (90 days) | Individual management | The ajolotes were put in dark containers to 19 ° C, a photoperiod was of 12 hours light for 12 hours of darkness, daily cleaning, abnormalities registry behavioural, feces and food consumed. | Two ajolotes showed bad adaptation syndrome, and the 12 collected ajolote's show signs of sickness like tail shaped hook, weight loss and anorexia. |
| Adaptation time | Direct manipulation, diet, different space, changing water conditions. | Use of shelters, simulation of vegetation, photoperiod control, assessment, monitoring weight once a week and length every month | 7 axolots had metamorphosis, and after from this event have stabilized. And 4 organisms adapted in larval stage. |
| Reproduction | Manipulation, lack of environmental enrichment (such as increase of vegetation) and lack of knowledge about development, and sexual dimorphism in this species | Implementation of a balanced diet, management of the temperature according to their habitat, adequate place for oviposition. Review of the literature on development and sexual dimorfism in other species of salamanders and implementation of invasive techniques to locate the stage of gonadal development | Deficient bibliographical information about sex and gonadal maturity of this species. |

Evaluation of size and weight

The height and weight assessment is an indirect way to know the health status of the animals, since the variation of these measures may indicate the presence of parasitic or infectious diseases, maladaptive syndrome; in salamanders may be indicative of the maturation process of the species itself, known as metamorphosis, in which they pass the larval to adult stage.

Twelve months after quarantine phase, the measurements obtained in ajolotes have been stable and their height and weight are greater than those recorded by Aguilar-Miguel (2005), and regardless of the stage of maturity they are passing (Table 26). This can be attributed to the quality and quantity of feed used, availability of living space, genetics, favorable environmental conditions (Lemos *et al.* 2005) and load capacity on the capture site, among others.

Table 26. Comparison of A. *granulosum* in two stages: average values of weight expressed in grams and length in millimeters (TL: total length; SCL: snout-vent length; Tl: tail length).

| | Larval stage | | | | Adu | lt stage (r | netamorpho | osis) |
|-------------------------------------|--------------|-----|-----|--------|---------|-------------|------------|--------|
| | TL | SVL | TI | Weight | TL | SVL | TI | Weight |
| Aguilar- Miguel 2005 | 160 | 90 | 70 | | 143-169 | 81-92 | 62-77 | |
| Brockman Action Plan Study | 236 | 124 | 110 | 69.97 | 230.9 | 127.6 | 104.6 | 63.76 |

Currently no data are available on female A. *granulosum* from Brockman reservoir; however males continue to have larger sizes as described by Taylor (1944) and Aguilar-Miguel (2009). These authors only describe the difference in size between males and females and do not consider the difference between larval and adult stage, apparently both descriptions refer to the larval stage (Table 27). It is important to mention that the described value of female's size in Brockman dam only refers to a one female.

Table 27.Comparison of A. *granulosum* between males and females: average values of weight in grams and length in millimeters (TL: total length; SVL: snout-vent length; Tl: tail length)

| | Females | | | | Males | | | |
|----------------------------------|---------|--------|------|--------|-------|-------|-------|--------|
| | TL | SVL | TI | Weight | TL | SVL | TI | Weight |
| Taylor, 1944 | 153.5 | 85 | 68.5 | | 164 | 90 | 74 | |
| Aguilar- Miguel, 2009 | 193.33 | 108.33 | | 60.07 | 207 | 112 | | 51 |
| Brockman Action Plan Study | 255 | 126 | 122 | 84.19 | 230 | 122.3 | 106.4 | 64.58 |

14 organisms became them tracking for 15 months. The minimum and maximum total length arose in terrestrial phase and body weight is greater in larval stage. In the process of metamorphosis organisms decrease their consumption of food, its size also undergo structural changes in your skin and skeleton (Table 28). At the time of his capture, the organisms showed slightly higher values in larval stage in comparison to those who are in the process of metamorphosis (Table 29).

Table 28. Capture data of morphometric measures; values of length expressed in millimeters.

| N° organisms | Stage larval/salamander | Total length maximum | Total length mínimum | Weight |
|--------------|----------------------------|----------------------|-------------------------|--------|
| 9 | salamander | 255 | 199.7 | 63.66 |
| 5 | larval | 250 | 209.6 | 69.97 |

Table 29. Data of morphometric measures during 15 months of study; average values of length expressed in millimeters.

| N° organisms | Stage | Total length | Total length | Weight |
|--------------|--------------------------|--------------|--------------|--------|
| | alevin/larval/salamander | maximum | mínimum | |
| 2 | salamander | 232 | 197 | 63 |
| 17 | larval | 265 | 185 | 64.30 |
| 2 | alevin | 136 | 135 | 18 |

Growth of organisms

Evaluation of body growth is essential in the management of a population. With this information in some species we can estimate the age of the animals (Lemos et al. 2005) and thus learn more about his life story. 8 (57%) of 14 organisms with their morphometrics tracking showed a growth (

Table *30*). In larval stage, 3 (60%) showed a growth and 5 (55%) salamanders growth to. Currently the larval and terrestrial phases continue to grow.

Table 30. Data of organisms that were observed with body growth; reference is snout-vent length because is not altered during metamorphosis; (values of snout-vent length (SVL) expressed in millimeters.

| Organisms | SVL (initial) | SVL (final) | Stage |
|-----------|---------------|-------------|------------|
| 77841636 | 130 | 135 | salamander |
| 75069321 | 126 | 134 | salamander |
| 75045317 | 108 | 116 | salamander |
| 77864298 | 125 | 128 | larval |
| 75076612 | 130 | 137 | larval |
| 75045874 | 120 | 126 | salamander |
| 75069338 | 102 | 115 | larval |
| 75093623 | 130 | 136 | salamander |

Morphological and behavioral changes in the species

After 15 months five organisms retain their larval characteristics (Figure 37) and seven became salamanders (Figure 38). Both groups exhibit behaviors consistent with would be an expression of animal welfare, water displacement is appropriate to the stage of development where they are, feed intake is constant on scheduled days, their physical development and weight are constant, manual restraint is allowed, defecation is solid and regular, use their shelters and have not been sick in a period of six months. Possibly these are organisms adapted to the stage in which they are located.



Figure 37. Organism in larval stage

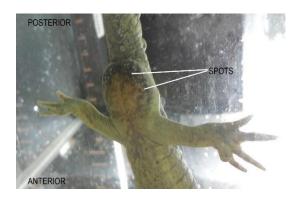


Figure 38. Organism after metamorphosis (salamander) in terrestial stage

When the organisms were captured in the wild, skin was smooth and now it has become thick and rough, with dark shades, its presents grains zones and change of color on the cloacal region and finger tips have been obscured (Figure 39). These changes are described and related to the maturity of organisms, since in this species adulthood presents granules across the back, a feature that distinguishes this species (Taylor, 1944).

Morphological changes in Ambystoma granulosum

Spots presence in the cloacal region (salamanders)



Color change in cloacal region tone light to dark (larvae and salamanders)

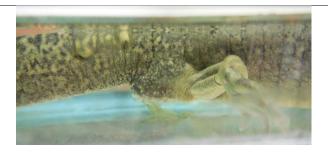
Larvae phase



Salamander phase



Increase in the width and height of the cloacal region and thickening of the skin (larvae and salamanders)



Presence of dark spots on the tip of the snout and the tips of the fingers (larvae)



Darkening of the fingertips (salamanders)



Increase of light and dark spots on the skin, mainly in the dorsal region of the body (salamanders)



Figure 39. Main morphological changes in Ambystoma granulosum during this study

Activity and artificial habitat preference of A. granulosum

The activity and time that the organism were in different levels of the container (Figure 40, Figure 41) allowed evaluating at different times of the day its activity and preference in the two phases (aquatic and terrestrial). Schedules of evaluation were from 8:00-19:00 hrs in a period of 10 months.

Container for larval stages (with gills)

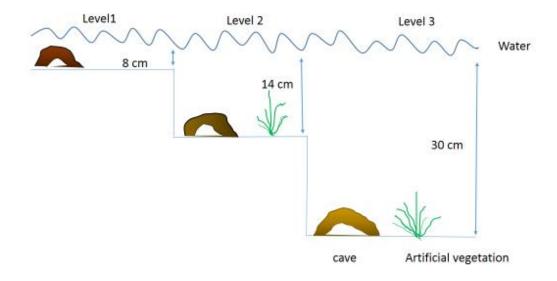


Figure 40. Showing the distribution of space used for larvae stage

Container for terrestrial stage (salamander)

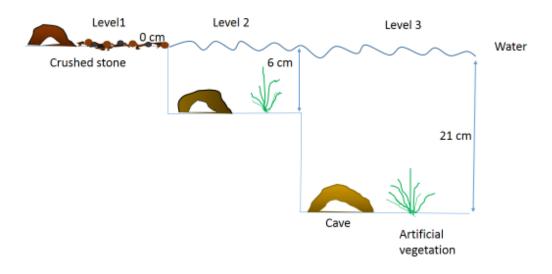


Figure 41. Showing the distribution of space used for metamorphic stage (salamanders)

Organisms in larval stage showed greater activity to the 15:00-18:00 hrs, without being related to foraging and preferred the level 2 and 3 in a time of 9:00 - 16:00 hrs. Your favorite refuge was simulated vegetation. From 9:00-12:00 organisms in terrestrial phase preferred to hide in the caves located at shallow levels (level 1 and 2); between 11:00-

14:00 and from 18:00-19:00 they showed greater activity, in both cases related to the pursuit of food and from 14:00-16:00 had preference for the level 3 and used the caves and simulated vegetation as a refuge. Both larval organisms such as salamanders were observed in the majority of cases they prefer to be in groups, provided that they are not looking for food.

Metamorphosis

Metamorphosis in captivity

The most significant morphological changes during metamorphosis are gill resorption, dorsal-caudal fin resorption and eyelid development (Figure 42). Other changes are the development of pigmentation in the skin, changes in the skeleton and musculature structure (Shi, 2000). For A. granulosum gills and dorsal fin reabsorption, eyelid presence, changes in the color and texture of the skin, and changes in the shape of the head (Figure 43) was observed. Of the 14 organisms caught 8 presented metamorphoses over a period of six months. These organisms were very unstable in the acceptance and consumption of food, presented constant hyperactivity, and had frequent changes of skin and gradual weight loss before metamorphosis. Several species of salamander use metamorphosis as a tool for adaptation to a different environment such as the captivity or the dry season in their places of origin (Kaplan 1980; Duellman y Trueb 1986) or in captivity conditions metamorphosis in A. granulosum was 22 days minimum and 56 days maximum with an average of 43 days (Table 31). Is possible that the early 22-day metamorphosis was caused by maladjustment to captivity and late metamorphosis that lasted 56 days has been part of the life cycle of the species itself. In A. talpoideum studies have been made about the time of metamorphosis and have been observed that the early or late in the process depends on several factors. If the organism is exposed to adverse conditions such as drought or salinity increased, the organism will conduct a rapid metamorphosis in order to colonize new environments to survive. Conversely if the organism lives in an adequate environment, metamorphosis takes longer to complete (Semlitsch & Wilbur, 1988).

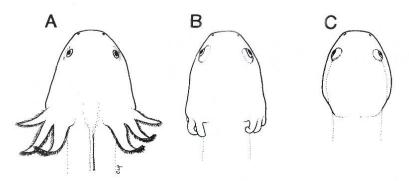


Figure 42.Dorsal view of the head in *Ambystoma tigrinum* showing the morphological changes during metamorphosis (loss of external gills, presence of eyelids, reduction and change in shape of the head); A. larval stage, B. premetamorphosis, C. salamander. (Lauder y Shaffer 1988)

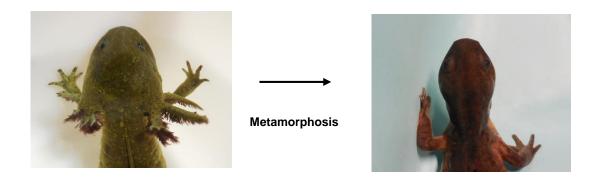


Figure 43.Morphological changes observed in *A. granulosum*: absorption of gills, presence of eyelids, change in shape of the head, change in the color and texture of the skin

Table 31. Days to metamorphosis in organisms of this study.

| Organisms | Period | Time of metamorphosis |
|-----------|----------------------|-----------------------|
| 075045874 | 5/aug/13- 10/sept/13 | 22 days |
| 075093623 | 26/jul/13-14/sep/13 | 50 days |
| 075057307 | 23/aug/13-18/oct/13 | 56 days |
| 077861117 | 10/sep/13-21/0ct/13 | 42 days |
| 075069321 | 17/sep/13-30/oct/13 | 43 days |
| 077841636 | 25/oct/13-4/dic/13 | 40 days |
| 075045317 | 10/dic/13-27/jan/14 | 48 days |
| 010635857 | 10/may/14-25/jun/14 | 46 days |

Metamorphosis in the wild

During the night trips in a space of 3 m, four ajolotes were found at different stages of development an alevin, a youth, an adult and one in the process of metamorphosis. Also, in a trap within the dam two organisms were located, an adult with characteristics of larva and the other one an adult in metamorphosis process, all the ajolotes mentioned above were males. A complex lifecycle for salamanders includes larvae and organisms adults in the same space of habitat. Here the species can benefit by plasticity in time to experience the metamorphosis (McMenamin & Hadly 2010). As in the Brockman dam water is constant and the possibility to the process of metamorphosis for each individual is not subject to a specific season but if for a certain age or the presence of a stressful event such as an increase in the intensity of currents, lack of food, or any contaminant. Permanent environments such as this metamorphosis may be postponed until the second or third year. Plasticity in the amphibian life history strategies is a common theme. Many species required forced metamorphosis from the aquatic to the terrestrial depending on the environmental conditions (McMenamin and Hadly 2010). Possibly this is not the case for *Ambystoma granulosum* in the Brockman dam.

Sexual gender identification

The sexual gender identification was carried out through the observation of the cloacal glands development, this characteristic is observed in sexually mature males and the females do not have this development (Wright & Whitaker 2001). During initial capture efforts were selected organisms without development of these glands, but after four months of watching these ajolotes which had been identified as females its began to present growth of cloacal glands. These changes indicated that the organisms obtained were not sexually mature despite having described for gonadal maturation sizes (organisms with long snout cloaca (LHC) greater than 100 mm) (Aguilar-Miguel et al. 2009). The development of cloacal glands is currently present in organisms in larval stage and those who already are salamanders

Gonadal development studies

In order to specify whether sex and sexual maturity in male organisms is expressed through the development of cloacal glands, made four histological studies in different organisms (Table 32); two ajolotes in larval stage without glandular development, one in larval stage with glandular development and the last one at salamander with glandular development phase. The method consisted in the extraction of the organisms gonads through a surgical procedure. Once obtained sample was fixed in solution of Bounin, went through a process of dehydration and was fixed in paraffin for its subsequent cut in rotation

microtome, once obtained the tissues fixed with their respective stains on a slide preparations were made for further observation in the optical microscope (Olympus Bx50). Staining techniques used were the technique of hematoxylin eosin (HE) and trichrome technique from Mallory to see secretions. These techniques were carried out with the support of the laboratory of tissue biology and reproductive of the Facultad de Ciencias at UNAM.

| Organism | Length total | Snout- vent length | Weight | Stage larval/salamander | Gonadal stage |
|----------|-----------------|--------------------------|--------|----------------------------|---|
| 1 | 248 | 128 | 69.46 | larval | swollen cloacal glands |
| 2 | 207 | 119 | 48.56 | salamander | swollen cloacal glands |
| 3 | 250 | 125 | 66.20 | larval | Littles wollen cloacal glands |
| 4 | 210 | 114 | 50.90 | Larval | flat cloacal glands (possibly a female) |

Table 32. Main characteristics of organisms A. *granulosum* used for the study of gonadal histology; lengths are represented in millimeters and weight in grams

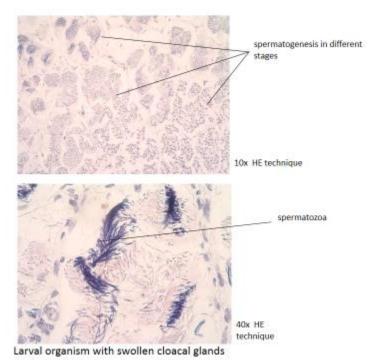
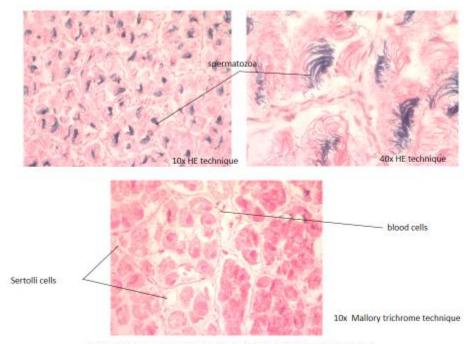
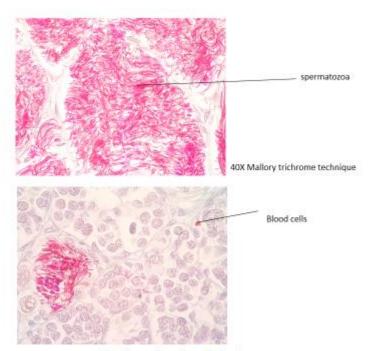


Figure 44.Organism 1 with swollen cloacal glands. Spermatogenesis at different stages, presence of mature sperm. Hematoxylin-eosin technique was used. Objectives of 40 x 10x.



Organism mature with swollen cloacal glands (salamander)

Figure 45 Organism 2 mature with swollen cloacal glands (stage salamander). Showing high sperm production. Mallory trichrome technique used; 10x and 40 x showing the lens used in the microscope.



Organism with little swollen cloacal glands

Figure 46. Organism 3 with Little swollen cloacal glands. Showing a little sperm production. Mallory trichrome technique used; objective 40x

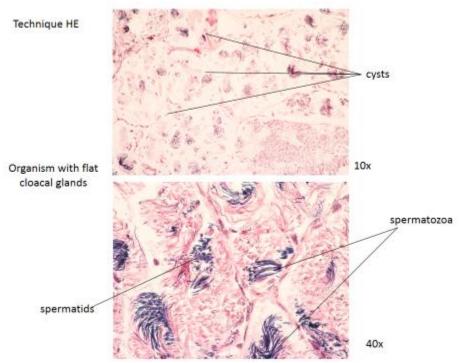


Figure 47 Organism 4 (up) with flat cloacal glands. Cysts are observed with hematoxylin eosin technique. The axolots present a quistic spermatogenesis with different stages. Hematoxylineosintechniquewasused; objectives 10x, 40x.

Figures 44, 45, 25 and 46 indicate that all organisms studied were males, sexually mature. Spermatogenesis in amphibians is the cystic type i.e. the ripening process takes place in each formed cysts (Figure 48).

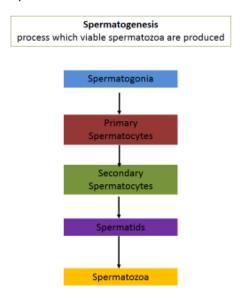


Figure 48. Diagram showing the different stages of spermatogenesis

In organisms with evident gonadal development(Figure 44, Figure 45) the degree of maturation is very similar to an organism has gills and the other is a salamander, this suggests the possibility that males of this species can be reproduced both in aquatic phase as land. Another way to externally identify the sexual maturity in males is the production of espermatoforos (sperm containers), they are structures (Duellman & Trueb 1986) conical and gelatinous consistency that sexually mature organisms produce. In the last 15 months the espermatoforos production was not observed, although in histological preparations, it can be seen that they are ready to reproduce.

Reproduction

In the present study the proportion obtained between males and females of the Brockman dam was 18:1. The female has had a long process of adaptation, and is currently in salamander phase. In the last two months it has adapted to the consumption of food but had to be fed with vitamin supplements to keep it alive. Although males seem to be in proper condition to reproduce, at the moment is not suitable to stimulate the female by its general health state and the lack of information for this species. It is important to consider that the metamorphosis in the female was stimulated by the bad adaptation syndrome, and not by natural situations. Currently it is known that the time and the reason why metamorphosis is presented affects directly organisms aspects such as the phenotype, survival, age at first reproduction, and reproductive success. Larvae which undergo metamorphosis at a young age can have a decline in fertility (McMenamin & Hadly 2010). Information about reproduction protocols and differentiation between males and females in this species is practically non-existent; the information that exists is confusing and unclear. Aguilar-Miguel (2009) refers to the use of hormonal products for stimulation of ovulation but 90% of eggs produced were infertile and the link between male and female, as well as the fertilization of eggs in a natural way is not clearly described and the success rate is less than 10%. Before suggesting the induced reproduction with hormonal products it would be important to know more about the life history of the species in the Brockman dam.

It has been identified that the age at first reproduction in 12 tropical species was between 6 and 15 months in males with an average of 10.7 months and females of 8 to 15 months with an average of 10.99 months. In contrast, age at first reproduction in 8 species in temperate regions was 2 to 4 years with an average of 3.4 years in males and 2.5 to 6 years with an average of 3.6 in females (Duellman & Trueb 1986). Today we know that the reproductive season for some amphibians at high altitudes is shorter and the number of encounters male female is restricted. The Brockman dam is located at 2870 meters of altitude. The frog *pretiosa* reproduces once every 1-3 years in North America but once a year at low altitudes (Licht 1975; Morrison and Marchero 2003). In low temperatures

anurans and urodeles tend to grow more and live well for long periods in larval stage. This is the case of *A. maculatum, tigrinum, opacum* (kaplan 1980) and could be to *A. granulosum.*

With regard to the proportion of male - female in the family Ambystomatidae that presented short breeding seasons, first males move to breeding sites. There have been cases where there are six or seven males per female when reproductive success is based on the presence of many reproductive potential males (Duellman & Trueb 1986) and also case studies where the survival of males is higher than females (Husting 1965), this phenomenon could be is presenting at the Brockman dam. Another possibility is the ecosystem alteration with the presence of endocrine disruptors(substances that alter hormone levels in animals causing change in the sex of organisms), these events are reported for fishes (Díaz et al., 2013), Currently not exist reports about this situation in amphibians but can be a cause of this sex ratio, another possibility is the dragging of males, ajolotes in different stages of development or eggs in rainy season and the Brockman dam functioning being as a receiver glass in.

Conclusions

Established guidelines for the maintenance of *Ambystoma granulosum* population for research in captivity are as follows:

- Ambystoma granulosum can be established under conditions of captivity with a high survival percentage.
- The process of maturation and sexual differentiation of organisms was conducted in the course of a year under laboratory conditions.
- Ambystoma granulosum is immunologically sensitive ininvasive processes even under anesthesia and analgesia protocols.
- Ambystoma granulosum in salamander phase was stronger than in larval stage to stressful events handling and exposure to different stimuli such as change of substrate, slight variation in the temperature and water levels.
- It is not determined the optimal reproduction time of the species in captivity.

- Ambystoma granulosum present a favorable and healthy behavior when is located with a high environmental enrichment (use of shelters and false vegetation).
- The favorable physico-chemical parameters are: temperature of 16 to 17 ° C, ph 8 to 9 and tolerates Ammonium concentrations near 0.3 mg/l.
- Sexual maturity in males is presented in aquatic and terrestrial phase

First approach to the Brockman dam and aspects to be considered

- In a non-intensive trapping the population of study of the Brockman dam had a sex ratio of 18 males per 1 female
- Not found Ambystoma granulosum in salamander phase in the dam surroundings
- Is required an intensive trapping to learn about fundamental aspects of the species life cycle. Stages of life expressed within the dam, the presence of the terrestrial phase within the zone, potential threats to the population, domestic animals as a predators of terrestrial stages and/or alteration of places of refuge

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References

Abarca, F. J. 2007. Técnicas para la evaluación y monitoreo del estado de los humedales y otros ecosistemas acuáticos, p. 113-144. In: O. Sánchez, M. Herzing, E. Peters, R. Márquez & L. Zambrano (eds.). Perspectivas sobre conservación de ecosistemas acuáticos en México. Instituto Nacional de Ecología (INE-Semarnat). 293 p.

Aguilar-Miguel, X. 2005. *Ambystoma granulosum*. Algunas especies de anfibios y reptiles contenidos en el Proyecto de Norma Oficial Mexicana PROY-NOM-059-ECOL-2000. Facultad de Ciencias, Centro de Investigación en Recursos Bióticos, Universidad Autónoma del Estado de México. *Bases de datos SNIBCONABIO. Proyecto W035*. México. D.F.

Aguilar-Miguel X., G. Legorreta y G. Casas-Andreu. 2009. Reproducción *ex situ* en *Ambystoma granulosum* y *Ambystoma lermaense* (Amphibia: Ambystomatidae). *ActaZoológica Mexicana* 25(3): 443-454 p.

Albanila, 2004. Tubifex. Boletin Electrónico Trimestral Killirium1 (2): 3-6

Barnett S.I., J.F. Cover y K.M. Wrigth. Amphibian husbandry and housing .In: Wright K.M. y B.R. Whitaker (eds). *Amphibian medicine and captive husbandry*. Krieger Publishing Company, Florida.35-61 p.

Casas-Andreu, G y C.J.McCoy, 1979. *Anfibios y reptiles de México: Claves ilustradas para su identificación.* Ed. Limusa 87 p.

Camargo, A., M. Pezzato, G. Henry-Silva & A. Assumpacao. 2006. Primary production of *Utricularia foliosa* L., *Egeria densa* Planchon and *Cabomba furcata* Schult & Schult.f from rivers of the coastal plain of the State of São Paulo, Brazil. Hydrobiologia 570: 35-39.

Cumming, G.S. 2011. Spatial Resilience in Social-Ecological Systems. Springer.

Danszak P., Berger L., Cunningham A., Hyatt A., Green E., Speare R., 1999 Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5 (6): 735-748.

Deniro, M.J. & S.E. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Geochimica et Cosmochimica Acta, 42:495-506

Díaz T., Gibson R., González F., Zarco A., Mazari H. 2013. Endocrine disruptors in the Xochimilco wetland, Mexico City. *Water air soil pollut* 224(1586): 2-11.

Duellman W.E. y L. Trueb. 1986. *Biology of amphibians*. McGraw Hill Book Company USA 670 pp.

Feijoo, C., M. García, F Momo & J Toja. 2002. Nutrient absorption by the submerged macrophyte Egeria densa Planch.: effect of ammonium and phosphorus availability in the water column on Growth and nutrient uptake. *Limnetica* 21: 03-104.

Fowler M.E., 1986.Stress.En: Fowler M.E. (ed). Zoo and wild animal medicine. Second edition. W.B. Saunders Company. 34-35 p.

Gannes, L.Z., D.M. O'Brien & C. Martínes Del Río. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78: 1271-1276

Havel, J.M. 2009. Cladocera. In: Encyclopedia of inland waters. G. Likens, 611-622 p.

Husting, 1965. Survival and breeding structure in a population of Ambystoma maculatum. Copeia 3: 352-362.

Kaplan, R.H.1980. Theimplications of ovum size variability for offspring fitnes and clutch size within several populations of salamanders (Ambystoma). *Evolution*34: 51-64

Kravchenco, A.N. 2003. Influence of spatial structure on accuracy of interpolation methods. *Soil Science Society of America Journal* 67: 1564-1571

Lauder, G.V y H.B. Shaffer, 1988. Ontogeny of functional design in tiger salamanders (*Ambystoma tigrinum*): are motor patterns conserved during major morphological transformations. *Journal of morphology* 197: 249-268

Lemos, E.J.A, R.M. RojasG & J.J.Zuñiga V. 2005. *Técnicas para el estudio de poblaciones de fauna silvestre*. Conabio. Primera edición. México 157 p.

Licht, L.E. 1975. Comparative life history features if the western spoted frog, *Rana pretiosa*, from low-and high elevation population. *Canadian Journal of Zoology*: 53, 1254-1258

Lot, A & M. Olvera. 2013. *Hydrocharitaceae*. In: Plantas acuáticas mexicanas una contribución a la Flora de México. A. Lot, R.M. Lemos & F. Chiang (eds.) Instituto de Biología. UNAM

Martínez-Ramos, M. 2008. Grupos Funcionales In: *Capital Natural de México, vol. I: Conocimiento actual de la Biodiversidad.* CONABIO, México, pp. 365-412.

McMenamin S.K. Hadly E.A. 2010.Developmental dynamics of *Ambystoma tigrinum*in a changing land scape. *BMC Ecology* 10: 1-10

Minagawa, M. & E. Wade. 1984. Stepwise enrichment of 15N along food chains: Further evidence and the relation between 15N and animal age. Geochim. Cosmochim. Acta, 48: 1135-1140.

Morrison Clare and Marchero-Jean. 2003. Geografic variation in life history characteristics of amphibians: *A review. Journal of Animal Ecology* 72: 270-279.

Nürnberg, G. 1996. Trophic state of clear and colored, soft- and hard-water lakes with special consideration of nutrients, anoxia, phytoplankton and fish. Lake and Reservoir Management 12: 432–447.

Peterson, B.J. & Fry B. 1987. Stable isotopes in ecosystem studies. Ann Rev. Ecol. Syst. 18:293-320.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83(3): 703-718

Semlitsch, R. y H. M. Wilbur, 1988. Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. *Copeia* 4: 978-983.

Schad, K., ed. 2007. *Amphibian Population Management Guidelines*. Amphibian Ark Amphibian Population Management Workshop, San Diego, CA, USA.www.amphibianark.org; 2008.31 p.

Secretaría del Medio Ambiente (SMA). Gobierno del estado de México. 2007. Programa de Conservación y Manejo del Parque Estatal Santuario del Agua y Forestal "Presas Brockman y Victoria".

Sand-Jensen, K & O Pederson. 1999. Velocity gradients and turbulence around macrophyte stands in streams. *Freshwater Biology* 42: 315-328.

Roldan Pérez, G. & Ramírez Restrepo, J.J. 2008. Fundamentos de limnología neotropical. Universidad de Antioquia, 440 p.

Secretaria del Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP). 1996. Norma oficial Mexicana que establece los límites máximos permisibles de contaminantes en las descargas de aguas residuales en aguas y bienes nacionales.

Secretaria del Medio Ambiente y Recursos Naturales (SEMARNA). 2002. Norma official Mexicana. Protección ambiental. Lodos y biósolidos. Especificaciones y

límites máximos permisibles de contaminantes para su aprovechamiento y disposición final.

Secretaría de Economía. 2001. Norma Mexicana. Análisis de aguas –determinación de sustancias activas al azul de metileno (SAAM) en aguas naturales, potables residuales y residuales tratadas- Método de prueba.

Shaffer, H.B., 1989. Natural history, ecology and evolution of the mexican axolotls. *Axolotl Newsletter* 18: 5-12

Shannon, T.F., 2008. Amphibian Quarantine Guidelines En: *Amphibian husbandry resources guide*. AZA's Amphibian Advisory Group. Ed 1.1. 63-72 p.

Shi, Yun-Bon. 2000. Amphibian metamorphosis. *From morphology to molecular biology*. John Wiley & Sons, Inc. Publication, USA 288 p.

Taylor, 1944.A new ambystomid salamander from the plateau-region of Mexico.The University of Kansas *Science Bulletin*.30 (5): 57-61.

Wrigth K.M. y B.R. Whitaker.2001a. Nutritional disorders (charper 7).In: Wright K.M. y B.R. Whitaker (eds). *Amphibian medicine and captive husbandry*. Krieger Publishing Company, Florida.73-83 p.

Wrigth K.M, 2001b. Waterquality (chapter 12). In: Wright K.M. y B.R. Whitaker (eds). *Amphibian medicine and captive husbandry*. Krieger Publishing Company, Florida 147-157 p.

Wrigth K.M. y B.R. Whitaker.2001c. Quarantine (capítulo 23). In: Wright K.M. y B.R. Whitaker (eds). *Amphibian medicine and captive husbandry*. Krieger Publishing Company, Florida 301-307 p.

ANEXOS Section I Environmental Analysis

ANNEXO 1. Collection methods for isotope samples: sediment, phytoplankton (POM), zooplankton, macroinvertebrates, salamanders and fish.

To collect sediment we used an Eckman dredge.





To collect phytoplankton and zooplankton samples two trawling net were used. Both has a mouth diameter of 30 cm and lenght of 120 cm. Mesh size for phyto is 63 μ and for zoo 250 μ . Trawling was done in a linear distance of 500 m, with low velocity and in diagonal transects. Hauls from Brockamn coast were also made:





Phytoplankton samples were processed same day with a vacuum pump. Once dried, Millipore filters containing phytoplankton, were protected with foil and frozen to preserve them until lab processing.





Macroinvertebrates collection was made with triangle nets, scraping the walls of water body and sweeping aquatic vegetation. Samples were processed same day to prepare them for further processing at lab. Macroinvertebrates were identified with systematic keys until Genus and preserved in formaldehyde at 4%.





Ajolotes and some small fish were collected with minnow traps adapted with a broad opening to protect ajolotes of hurting. It is noteworthy that the species of salamander of the Brockman Dams and Victoria, the *Ambystoma granulosum*, is protected by Mexican law (NOM-059-SEMARNAT) and is also considered endangered internationally (IUCN), so its capture for sale or exhibition purposes is prohibited.

Capturing the salamanders and other aquatic organisms was made for the purpose of scientific study and under the license of Dr. Luis Zambrano González, as a scientific collector (FAUT-0112).





Once made the collection of organisms, they were moved to the Laboratory of Ecological Restoration Institute of Biology, UNAM, where they were processed as follows:

The filter where the phytoplankton remained with particulate organic matter was put to dry in a drying oven at $50\,^\circ$ C for 48 hours and then it was encapsulated in metal containers 9 x 10mm. Zooplankton samples were cleaned of debris and particulate organic matter, dried in a muffle furnace at $50\,^\circ$ C for 24 hours and pulverized with a mortar.

For mollusks, crustaceans and some insects, the shell was stripped to remove the inorganic carbon isotopic value; since its biological basis is based on a precipitate carbonates (Post, 2002). Shells were also dried and pulverized.

In the case of fish, the same day of collect a muscle sample was extracted and frozen. Once at lab it was dried at 50 ° C and pulverized.

The salamanders captured, 23 in total, were transferred to the hotel and anesthetized 11 of them to obtain tissue samples for isotope analysis, for which isoflurane (laboratories PISA) was used at a dose of 3 ml per each liter of water. The salamanders were kept in 24 hours observation and subsequently rejoined the dam.

The remaining 12 salamanders were transferred to Colony of salamanders at Ecological Restoration Laboratory of the Institute of Biology, UNAM, for acclimatization and study. Once at laboratory, these were similarly anesthetized to extract tissue samples with a hollow punch, approximately 2 g of tissue per axolotl. The samples were dried in a muffle furnace at 50 ° C for 24 hours and subsequently pulverized.

Once the tissues have been turned to dust, were packaged in aluminum containers, which are sealed and placed in a special plastic tray for processing.

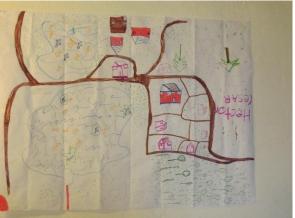
The powdered samples of all organisms were packed and sent to the Stable Isotope Laboratory at University of California Davis, for analysis. Specialized equipment for analysis including elements analyzer coupled with a mass spectrometer to determine the isotopic ratio in a continuous stream (IRMS) is used.



Tray to transport samples for isotope analysis

ANEXOS Section II Social Participation **ANNEXO 2.**







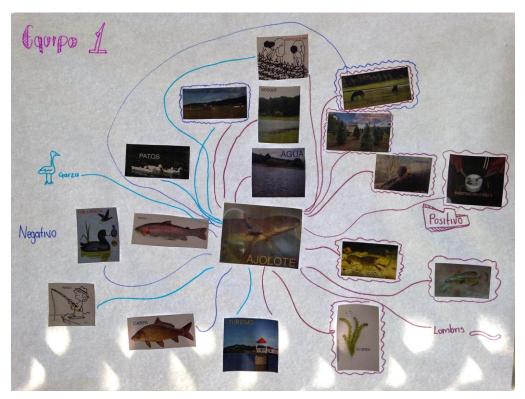




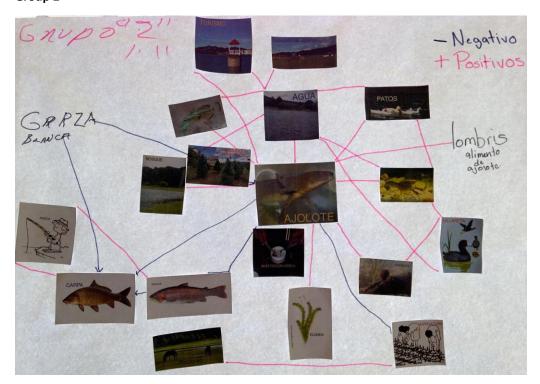


ANNEXO 3.- Diagrams of positive and negative feedbacks in the socio-ecological system

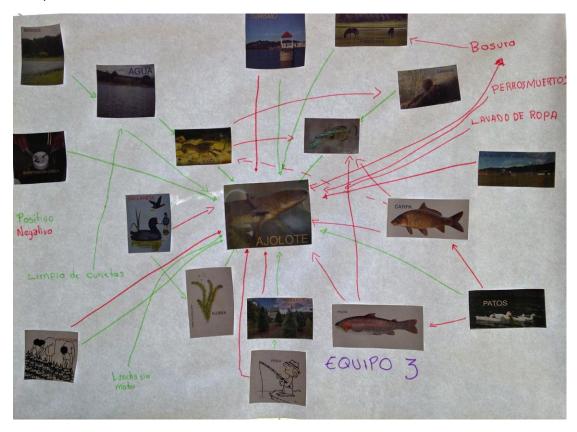
Group 1



Group 2

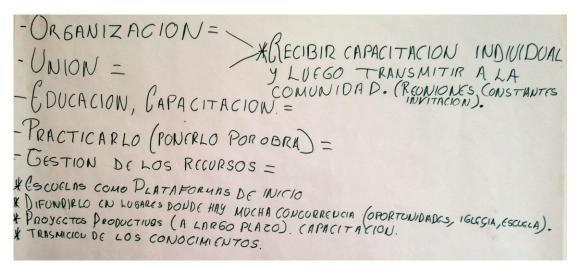


Group 3



ANNEX 4.- Actions to face socio-ecological problems

Team 1



Grupo de: 2

- @ Unidad y Organización
- © Concientización
- 6 Soluciones a Corto plazo
 - Colectar basurg
 - z Uso racional del agua
 - · Uso de abonos organicos
 - · Pedir estudios de suelo y agua
 - · Buscar alternativas de siembras y huertos debido alclima.
 - *Información ambiental en las escuelas
 - Buscar metodos para el control de plaga
 - = Buscar el origen del crecimiento del lirio y mecanismos para controlarlo.
 - =Implementar baños para los lugares donde exeiste el turismo
 - 2 Coordinación entre autoridades Federales, Estatales y Municipales. (Locales)
- G Formar brigadas de vigilancia enfocadas a los bosques, rios y
- @ Informar Sobre el Ajolote

Grupo (2) 6 Instalación de biodigestores en los hogares evitando salida de aquas grises hacia nos y presas 6 Crear dentro de nuestras comunidades que el ajolote sea nuestra identidad y asi atraer más turismo sin perfurbar el ambiente. @ Investigar el tipo de quimico que se usa en los árboles navioleño: 3 Solicitar a los Jovenes de los diferentes planteles a la recolección de basura en bosques y la presa brockman 9

ANNEX 5 -. Report of participants of the Environmentalist Caravan Easter 2014. Presented by the team Tlalpujahua-El Oro6.

JOEL:

On Thursday April 17, before the environmentalist caravan, we had a training facilitated by Citlalli Gonzalez and the REDES AC team. We discussed and prioritize the issues that we were going to develop on Friday and Saturday of Easter season. We agreed on the objective of raising awareness and sensitize the tourism and surrounding communities for the preservation of this place, water, flora and fauna.

On Friday 18th at 8am we began the day of the environmentalist caravan. We had participation of 85% of the people that originally was going to participate (25 persons).

First we installed the signs and messages in places previously designed with the following messages:

- 1. You're arriving to the sanctuary of water of Brockman and Victoria dams.
- 2. Welcome to the water sanctuary B and V.
- 3. Environmentalist caravan B y V.

Tlalpujahua-El Oro

Without frontiers

⁶ REDES AC team translated the document. Some parts were adapted considering the complexity of local expressions and vocabulary.

JOEL:

During the day we would work in two shifts, but seeing that with the hours passing by the more people arrived, thus we all decided to stay all day long since we could not leave unfinished the target.

Surveys were conducted to tourists which was well accepted and tourists expressed surprise as they had never seen before a campaign as the one we were doing, at least in the territory of Michoacan. On Saturday we installed five modules, for which I was assigned to oversight needs in each module. We had a dialogue with colleagues in which they expressed they felt more confortable since they had the experience from the first day.

We recorded circulation of approximately 400 vehicles (trucks, cars). There were about 100 merchants selling fruits, food, and toys. We visited the merchants and invited them to concentrate their garbage and to organize it into organic and inorganic. We also installed 4 garbage containers in each one the 5 modules.

Garbage was concentrated in my house and definitely Saturday was an exhausting day for all colleagues. Everyone was showing signs of joy in every so we came to a good conclusion. However, there were out of small incidents, as some people showed disagreement despite being people from the community, some told us that they were not scavengers and the council was to pick up litter. But I repeat, only few people showed their apathy, this is documented in the reports of the interviews. Finally, on Sunday we went to pick up the trash on the contour of the Brockman dam to give the image the place deserves. I also want to express that more participation of the biologists is required in fieldwork for obtaining better results.

ANETT:

For me it was a new experience, I accept that at first I was not entirely pleased or interested I did not even felt full confidence about what the results we would obtained from that campaign. The first day we arrived early to install all 5 modules set in strategic locations and near to tourists. We split to work in each module and we were given some formats in which we had to register the place of origin, number of people, comments and general suggestions of the tourists passing by the area.

When people started to arrive we addressed them to fulfill our goal. Some people were showing distrust on the questionnaire, which is somewhat understandable from my point of view. Others behaved very friendly and amazed by the work being carried out and even prompted us to continue, as there were people with despotic attitude and egocentric behavior that refused to give us the interview (just few people).

Each module was visible at a considerable distance since the tarps and trash containers made from white costal made the location visible. People from the community showed curiosity about what was taking place and we as neighbors and members of the caravan provide them the necessary information and apparently they liked the idea. Undoubtedly the most tiring day was Saturday since the largest influx of people was recorded that day. But finally got good results and I feel proud of the work of the whole team.

SOLEDAD:

As part of the environmentalist group I started to participate in the campaign of Easter in the Brockman Dam. I attended a large module to which people came without going and address them. I could not perform any interview because my writing ability is limited so I helped to monitor another module while the others went out to approach people. I would like to emphasize that the campaign was successful

because on Monday I observed around the dam and it was still clean, unlike previous years when there were immense garbage concentrations. I consider the importance to continue with the campaigns for the benefit of all the residents of my community.

DIANA:

The environmentalist caravan turned out to be a pleasant but very exhausting experience. I was assigned to be in a module and conduct some interviews, although many people could not be interviewed by many situations such as large quantity of people present, the confusion about whether they had been interviewed by another module and the unwillingness of some people to give us an interview. I believe that we successfully achieved our main objective, which was to preserve clean the surroundings of the Brockman, and Victoria dams. Only in part, but not entirely, it was possible to raise awareness in some people considering both locals and tourists, highlighting that the action to raise awareness is very complex. I am happy with the results.



Annex 6. Logo and image for the group.

A group of young graphic design students from the Faculty of Arts and Design-UNAM, developed an image for the new environmentalist group and some products to promote environmental awareness.

| Name of students | | | | |
|-----------------------------|--------------------------------|--|--|--|
| López Guzmán Neyra Patricia | Monzalvo Ramírez Abigaíl | | | |
| Marquez Pérez Alfredo | Tellez Domínguez Olivia Nohemí | | | |
| Mendoza Luciano Karen | | | | |





