AACL BIOFLUX

Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Contribution to the biology of the endangered Mexican fish, *Zoogoneticus tequila*, and suggestions for its indoor management

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Abstract. The endangered Mexican fish, *Zoogoneticus tequila* Webb & Miller, 1998, represents a new challenge for scientists: its biology, quite unknown, could perhaps explain the reason for its extremely low consistency in natural environments. An experimental setting was prepared where four pairs of young *Z. tequila* from the only Italian colony were bred in two different (biotope and enriched) tanks. Breeding results showed that the animals have grown and presented a positive reproductive performance, in both the environmental conditions confirming that the choice of substrata, of the vegetable layout, of food administration and of breeding of fry, resulted optimal for the indoor breeding. Aggressive behavior was extremely low and, although inter-male interactions were unstable, no injuries were observed among the animals, apart from a few episode of cannibalism towards the young. The behavior of animals in stressful situation were also recorded, namely the encounter with a male displaying enhanced sexual characters or with a predator. The behavioral responses toward the male were mainly noticed in the enriched tank, where flicks of tail and display of fins were recorded compared to the biotope. On the other hand, the predator snake *Thamnophis sp.* elicited an innate flight response in both the experimental conditions and in both genders. All together those results seem to encourage the breeding of *Z. tequila* in captive condition, although more studies on the cognitive skills of the species are needed before its reintroduction in natural environment.
Key words: *Zoogoneticus tequila*, conservation biology, Goodeid Working Group.

Riassunto. La biologia di *Zoogoneticus tequila* Webb & Miller, 1998, un pesce originario del Messico centrale minacciato di estinzione, è praticamente sconosciuta e la mancanza di informazioni non permette una tutela adeguata della specie in natura. Dall'unica colonia allevata in Italia sono state prelevate quattro coppie di soggetti che hanno trovato alloggio in due tipologie distinte di vasche (ambiente arricchito e biotopo) allo scopo di studiarne gli aspetti riproduttivi, sociali e cognitivi. Nei due habitat allestiti la performance riproduttiva è risultata sostanzialmente simile e il comportamento aggressivo è stato sempre contenuto, ad eccezione di alcuni episodi di cannibalismo nei confronti dei giovani. La risposta comportamentale nei confronti di maschi zimbello ha mostrato qualche differenza nei soggetti dei due gruppi sperimentali mentre nei confronti dei predatori zimbello la risposta è stata univoca e immediata. I risultati delle registrazioni incoraggiano l'allevamento di *Z. Tequila* in cattività, anche se le capacità cognitive dei soggetti andrebbero attentamente valutate prima della loro reimmissione in natura.
Parole chiave: *Zoogoneticus tequila*, conservazione, Gruppo di lavoro Goodeidi.

Introduction. The success of the conservation and the possible reintroduction into the wild of endangered species is always related to the proper management of captive colonies; an incorrect management, indeed, could lead to the appearance of morphological, physiological, and behavioral changes of the original populations, thus causing a failure of conservation programs.

This aspect applies to *Zoogoneticus tequila* (also known as tequila splitfin) as well – a viviparous fish from the freshwater located in the highlands of central Mexico, belonging to the family Goodeidae (Dominguez-Dominguez et al 2005ab; De la Vega-Salazar 2006; Bayley et al 2007). Such a species, recently discovered and classified, has no longer tracked into its habitat and has been believed extinct (Webb & Miller 1998) until a small group of fewer than 500 individuals of various ages was seen in a small lake in 2001 (De La Vega-Salazar et al 2003ab). Since 2007, indeed, *Z. tequila* has been classified as "critically endangered" in the IUCN list of threatened species due to the currently decreasing of its population trend (IUCN 2010).

The reproductive peculiarity of Goodeid is their viviparity, in particular a matrotrophic livebearing fish (Meyer & Lydeard 1993). This reproductive choice could have been made by the family of Goodeidae, as well as the family of Poecilidae, Anablepidae and Jenynsiidae, because it allows to cope with confined and extreme places, subjected to sudden changes (Reznick & Miles 1989; Wourms & Lombardi 1992). Furthermore, the puppies, growing in the womb, would be less subjected to predation, would have larger size and greater autonomy at birth due to the presence of trophotaeniae.

Unfortunately, in recent years, together with human population growth and economic development, the conditions of *Z. tequila*'s native biotope, the Rio Teuchitlan, have significantly changed due to the exploitation of watercourses for agricultural, zootechnical and recreational purposes (in fact, this is a global trend, Szabo et al 2010). For example, a few years ago, a water park, with pools, slides and a picnic area, arose upstream of the river; this transformation significantly altered the original structure of the habitat, making the survival of original fish species extremely difficult, because of environmental pollution and noise generated. The worsening of the biotope is also caused by the uncontrolled placing of extraneous species, such as *Oreochromis spp., Ictalurus nebulosus, Poecilia spp., Xiphophorus spp., Cyprinus carpio, Carassius auratus* (John Lyons personal communication). Therefore, in order to encourage the creation of colonies of *Z. tequila* in captivity and to study projects of reintroduction into the nature, on May 1st, 2009 the "Goodeid Working Group", an international working group, which combines hobbyists, universities, public aquaria and zoos, was created in Stoholm, Denmark. The main aims of this group are: preserving the populations in captivity, allowing the exchange of subjects among group members, implementing a continuous monitoring of the colonies, and finally cooperate internationally to create plans to safeguard wild populations.

At the same time the Goodeid Working Group try recommended to the Mexican authorities to find solutions already on the field; the group hopes for a better management of the Rio Teuchitlan sources, for both allochtonous fauna control and the creation of an area for reintroduction projects. That alternative may also provide revenues to local micro-economy, today only focused on recreational fun, by involving people who live near these habitats.

The right way to be undertaken is a more correct breeding in order to re-create colonies whose aspects are as similar as possible to those of the original population, to this day almost completely lost, and to combine the captive subjects with the wild type. This can be achieved only after a correct management of the habitat by the removal (at least in part) of allochtonous fish and the closure of well-defined areas of springs, in which re-entering the specimens of *Z. tequila*.

The breeding of captive populations with the latest survivors in nature has been proposed for several species, including Sweden's *Vipera berus* (Madsen et al 1999). This choice could be a good compromise between the risk of keeping a few subjects into the nature, in any case genetically changed from the native population, and the risk of reintroducing subjects with characteristics too different from the original, that carry genes that may be negative for future generations.

This study aimed at assessing *Z. tequila* management in an indoor environment by evaluating two different habitat conditions and their effects on reproduction, survival of the young, and their ability to cope with various natural cues.

Material and Methods. Even if *Z. tequila* is included in IUCN red list as a "critically enangered" species, it is not included in CITES, so it can be legally exchanged worldwide. The original stock of *Z. tequila* was obtained in year 2008, at an auction sale at the meeting of the Association France Vivipare (Amboise, France) and delivered to one of the authors. This original stock was composed by five males and eight females that gave birth to the new generations of *Z. tequila*. In the present study four pairs of fishes were used: two young females and two young males of an average length of two cm. The assessment of biological, reproductive and behavioral attitudes of the species was carried out by rearing the pairs of *Z. tequila* in the following environments: i) a controlled, natural environment (as compatible as possible to the original habitat), and ii) a so-called "enriched" environment (not-biotope). This strategy was chosen in order to verify the best and bearable management condition of the species in captivity, and to increase its fitness, an indispensable prerequisite for a possible re-entry in the wild. Ethological and managerial information obtained are not only essential for maintaining and increasing the number of colonies in captivity, they can become a way to consider and prevent the problems that captive subjects might encounter after their reintroduction into the original habitat.

1. Tanks. The formal installation of the two farming systems began one month before the start of this study, postponing the placing of the fish to the stabilization of the different habitats; the observation period and recording of data lasted 18 months. The two types of environments (Table 1) included: a first tank with biotope set-up, having the characteristics of the original habitat (the Teuchitlan Rio sources); a second tank (not-biotope), while reproducing an environment consistent with the needs of the species, contained plant species easier to find on the market as well as easier to manage. This second environment was considered "enriched" by the presence of more places for the fish to hide as well to provide conditions for better access to resources, mating, stimulation of territoriality, fleeing during the fight or from predators and the like.

Askoll Environment® acquaria (80 x 35 x 49 cm), with a capacity of 112 L, were equipped with two 20W lamps (sun light and phyto: Askoll Aquaglo®, Askoll Sunglo®), an electronic thermostat Askoll Termoaktive® 150 W, an automatic feeder used exclusively for the administration of food during the weekends (1 administration/day). A PVC reflector was placed under the cover, to enhance the light radiation and to avoid neon-light dispersion. Each aquarium was combined with a system, custom built, consisting of an external filter with a pump for re-circle (maximum flow 680 L / H), (21 x 34.5 x 16 cm) a filtration-sterilization (UVC) and a diffusion of CO_2 (Askoll System®) system. A brass pressure reducer, 2.4 Bar (working pressure) was installed on the CO_2 cylinder. It was opted for a distribution of 4 bubbles per minute, resulting in a concentration of about 7 mg / L CO_2, KH 6, GH 8, pH 7.3 (Sera test®).

Table 1

Experimental tanks' layout

Tanks' layout	*Biotopic tank*	*Non biotopic tank*
Litres	112	112
T°	25°	25°
Substrate	Akadama soil	Akadama soil
CO_2 system	yes	yes
UV sterilizer	yes	yes
External filtering system	yes	yes
Water conditioner	Sera Aquatan	Sera Aquatan
Light system	2 neons 8h/day	2 neons 8h/day
Vegetation	Microphytes (mosses and algae)	Macrophytes
Fishes	2 M, 2 F	2 M, 2 F
Food administration	1/day	1/day
Ethological observation	30 min/day	30 min/day

The environment in the tanks has been designed on the basis of scant information obtained from rare bibliographic data (Webb & Miller 1998) and suggestions by Professor John Lyons, Curator of the Museum of Zoology at the University of Wisconsin, an expert on origin sources, who reported the presence of a bottom with pebbles, sand and debris.

For this reason, we chose the Akadama or Kanuma clay that does not make the water turbid, and allows a correct plant rooting too. Its geological origin makes this substrate "mineral-rich" (Kg Kg^{-1}) Al_2O_3 0.334, Fe_2O_3 0.157, CaO 0.0022, MgO 0.0144, K_2O 0.0096, SiO_2 0.47, TiO_2 0.0129 (Asaoka & Aono 2006) and very similar to that one it has been found in central Mexico areas, as the Jalisco region. In order to obtain a substrate 5 cm thick, 5x4 L (Aquaground bios®) granules of small size (1.5-2 mm) were used.

Because of the scarce information on the native vegetation in the original environment, also the choice of the vegetation arrangement in the biotope tank has been developed after an intensive exchange of information with Professor John Lyons. He recommended the use of microphytes because there are no spontaneous macrophytes in the sources: as a result, the choice fell on algae and mosses of fresh water, in particular, on a green ornamental alga, *Aegagropila linnaei*, known as *Cladophora aegagropila* (Hanyuda et al 2002), and *Taxiphyllum alternans* (Cardot). The aquatic moss *Taxiphyllum* is a plant typical of springs, is a widespread microphyte, with many species in all continents, 26 of which have been, to date, grown in aquarium (Benl 1958; Cook et al 1974; Takaki et al 1982; Gradstein et al 2003; Tan et al 2004).

For the not-biotope tank, we used easily cultivable and very widespread in trade plants: *Shinnersia rivularis* or *Trichocoronis rivularis, Cryptocoryne weendtii*, brown and green varieties, species common in slow-moving watercourses (Dötsch 1984).

Regarding the chemical and physical properties of water in the Rio Teuchitlan sources, they are referred as having a neutral and slightly alkaline pH, medium hardness (John Lyons, personal communication); these physical and chemical properties can be easily re-created also using watercourses from the common Italian domestic distribution network – in fact, the groundwater that often runs on calcareous substrates, has a neutral and basic pH and medium hardness. The water used, springing from the aqueduct of Ruzzo (Teramo, Italy), showed, at the time of collection, the following physical and chemical characteristics: pH 7.3 KH 8, GH 6. These are very similar to those ones of original biotopes; in spite of this, it was also used a reverse osmosis system in the laboratory (Ruwal Aquapro®) and specific salt for the recovery of water (Sera, Mineral salts®).

In order to allow the maturation of the chemical-biological system, we chose a product based on bacteria, also used in aquaculture (Prodibio Biodigest®), single-dose darkened vial with a bacterial concentration of 20 billion bacteria /vial. The bacterial inoculum was previously experienced, with excellent results, in more stressful breeding conditions compared to those of the project underway (data not shown). The composition of the bacterial inocula includes *Nitrosomonas europea*, *Nitrobacter winogradskyi* (nitrifying), *Paracoccus denitrificano*, *Pseudomonas stuzerii* (heterotrophic). Then, a periodic replenishment of bacteria (2 vials per week for each aquarium) has been made, at the same time of the cleaning of the sponges that are part of the filtering system, and at the end of the 4^{th} monthly water change.

2. Fish. Each aquarium contained two young pairs of *Z. tequila*, born in captivity in the only Italian amateur fish breeding where the subjects chosen for such a study were born and bred in a fry breeding before being sexed and randomly caught. The eight subjects formed the initial project's colony, carried on over eighteen months. Throughout the experimental period food was administered once a day at not fixed times, keeping the light off in the laboratory and avoiding entering the animals' visual field, in order to prevent taming. In addition, the introduction of food was made in different parts of the tank, using the side and rear openings of the lamp-holders bridge. Only at weekends we used a system of automatic administration of food (Askoll Robofood®), loaded with dry food (SHG®) and freeze-dried invertebrates (Sera®). In order to avoid overfeeding, we administered the food ensuring that only small amounts remained on the bottom, in order to stimulate the foraging behavior, and making the search for food on tanks bottom occupy most of the animals' time. Works on Goodeid bred in captivity have shown that an excessive administration of food decreases the ability of foraging in their environment, and the chances of success in a potential reintroduction (Kelley et al 2006).

In order to meet the needs of this omnivorous species, without any nutritional problems, and using at the same time natural food, we used a high-quality granulated fish food, with a slow precipitation (SHG Microgranules®), with flake food (SHG Spirulina®, SHGs *Artemia salina* flakes®) and lyophilized food (Sera FD Tubifex®, Sera Daphnia® Sera Artemia shrimps®). Furthermore, "living food" has been used: *Artemia salina, Daphnia magna, Chironomus sp.* and black mosquito larvae, balanced with spirulina and enriched with a multivitamin complex (vitamins A, B1, B2, B6, B12, D, E) and coenzymes (Azoo Fishvitamin®). Their use enable to stimulate subjects in the tank to search, and to evaluate their behavior towards several types of food that are identical or very similar to those that may be commonly found in their origin biotopes.

In addition, small breeding of living invertebrate animals have been set up, usable as food for fish, that is cultures of terrestrial worms, *Enchytraeus buchholzi* and *Artemia salina*.

3. Observation. Various biological and ethological aspects, useful to assess the fish have been monitored: 1) courtship; 2) reproductive data; 3) social interactions between subjects (observations done three times a day of 10 minutes each per tank); 4) response to dummy exposure: a) conspecific adult male (a bound-in-paperboards specimen) or a strip of yellow sticker; b) dummy of predators (*Esox sp.* and *Thamnophis sp.*); 5) evaluation of foraging.

4. Statistical analysis. Data reported in this paper are represented as the median ± 5°-95° percentile. The data were checked for normal distribution by Shapiro-Wilks W test, and were compared by Mann Whitney U-Test or by Kruskal-Wallis followed, when necessary, by Dunn's *post-hoc* test (GraphPad Prism 5). The differences were considered significant and highly significant at f $p<0.05$ and $p<0.01$, respectively.

Results and Discussion

1. Courtship. The observation of the fish that established the early colony of *Z. tequila*'s in both tanks has led to draw up a list of typical behaviors of this species.

Mating is preceded by a ritual of courtship, characterized by an active search of a female; often, if the male meets a rival during this stage, he shortly chases him and then returns to his own home range, whereas rarely a fight occurs. In this experience, however, we have never recorded physical injuries or, worse, the death of the subject moved away. The female is followed by the male that stands alongside keeping very close and showing his coat (especially the terminal yellow stripes of the caudal fins) with quick movements of his body. In most cases, the truly mating does not follow these rituals of courtship; finally, before the actual copulation, the male places himself at a 45° angle respect to the female. There are no recorded cases of courtship towards any pregnant female; conversely, after delivery, the first attempts occur from 1-2 days later. Rarely some females, especially those of larger size, have chased away her suitors, without causing them any physical harm.

2. Reproductive data*.* Birth recording was performed daily for a total duration of 65 weeks (18 months). It was not possible to record the deliveries of fry, since they always occurred during the night. For each tank, fry found alive and dead and females that gave birth were recorded daily. The reproductive results, with reference to born fry, inter-birth periods and months during which births occurred, are shown in the following tables (Tables 2 and 3).

Table 2

Reproductive outcomes in biotope and non-biotope (enriched) tanks

	Biotope tank		*Non-biotope tank*	
	Female A	Female B	Female C	Female D
N. of births/n. of fry	7/43	7/38	8/77	5/28
Mean borns/birth	6.14	5.42	9.62	5.6
N. of alive (%)	29 (67.4%)	27 (71.05%)	53 (68.83%)	17 (60.71%)
Inter-birth (min-max days)	36-119	24-259	35-88	31-178

Table 3

Births distribution during the experiment

		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
		1st year											2nd year						
Biotope tank	Female A		Δ▲ ▲▲	▲▲				ΔΔ ΔΔ ΔΔ Δ▲ ▲▲			Δ ΔΔ ΔΔ ▲▲				ΔΔ ΔΔ ΔΔ	Δ ΔΔ ΔΔ ▲▲			Δ ΔΔ ΔΔ ▲▲
	Female B		▲▲	Δ Δ▲		Δ ΔΔ ΔΔ ΔΔ	ΔΔ ΔΔ ▲▲									ΔΔ ΔΔ ΔΔ ▲▲	Δ ΔΔ ΔΔ ▲▲	Δ ΔΔ ▲▲	
Non-biotope tank	Female C				ΔΔ Δ▲ ▲▲	ΔΔ ΔΔ Δ▲ ▲▲	ΔΔ ΔΔ ΔΔ ΔΔ ▲▲			Δ ΔΔ ΔΔ Δ▲ ▲▲ ▲▲		ΔΔ ΔΔ ΔΔ Δ▲ ▲▲			ΔΔ ΔΔ ΔΔ ΔΔ ▲▲	Δ ΔΔ ΔΔ ΔΔ Δ▲ ▲▲	Δ ΔΔ ΔΔ ΔΔ Δ▲ ▲▲		
	Female D						Δ Δ▲							ΔΔ ΔΔ Δ▲ ▲▲	Δ ΔΔ ▲▲	Δ ΔΔ Δ▲ ▲▲	Δ ΔΔ ▲▲		

Live (Δ) and dead (▲) subjects of *Z. tequila*, recovered during the observation period of 18 months, separated by type of tank (biotope or not) and by pregnant females (A, B, C, D). The incidence of births was higher in the period from May to August, during the first year of observation, and from March to June in the second year, during which all females bred.

3. Predatory behavior. The first case of cannibalism was documented in the non-biotope tank: an adult female attacked a three-day-old fry that, along with others, was standing on the surface. Behavior dynamic was similar to that one normally observed during feeding from the top: the female was placed sideways and still, with the front of her body facing upward, quickly sprang towards her prey (fry), which then was chased and eaten. Subsequently three other cases of assault were observed: two in the enriched tank (not-biotope), and one in the biotope tank, for a total of four cases, equally distributed.

At the beginning of June in the first year, against a total of three living members in the biotope tank and eight in the not-biotope one, only two young had survived (the birth of female B followed the establishment of the dead), only in the not-biotope tank, with a death rate of 100% in the biotope tank and 25% in the not-biotope one.

With the exception of four subjects for that it was possible to record predation, the causes of death of those died outside the periods of observation are not known. But, following these events, from the subsequent birth all the new fry have been previously isolated in floating birth rooms, and then they have been moved to an appropriate fry breeding, until their reaching of sub-adult size (2 cm in length). This management has allowed us to bring up to adult size 64 subjects out of a total of 126 fry (fry alive on day 1, biotope tank and not-biotope tank), representing 50.79% of survival.

Reproductive results obtained have allowed the creation of new colonies both in Italy and outside that will ensure a greater spread of this species. Five new colonies of *Z .tequila* have been created, respectively in Lanciano, Pescara, Rieti, Verona (Italy) and one in Switzerland, in addition to having achieved, to this day, a substantial increase of the starting colony (data not shown).

4. Aggressiveness between genera. Aggressive phenomena between genera are summarized in Table 4. The interactions were described using the symbol "/", which separates the offender (on the left), by the subject who has suffered the aggression (on the right). For example, in the interaction m/f we consider a male who has started the conduct of threat, while the second (f) is the one who suffered the aggression. No significant differences were recorded in the two different habitats.

Table 4

Aggressive interactions among *Z. tequila* subjects

Week	*Interaction*	*Biotope tank*			*Non-biotope tank*		
		Me	5° p	95° p	Me	5° p	95° p
1	m/m	1	0	1.8	1	0	6.8
	m/f	0	0	0	0	0	0
	f/m	0	0	0	0	0	0
	f/f	0	0	0	0	0	0
2	m/m	0	0	1	0	0	1.8
	m/f	0	0	1.8	0	0	0
	f/m	0	0	0.8	2	0.2	3.8
	f/f	0	0	0.8	0	0	0.8
3	m/m	0	0	1.8	0	0	1.8
	m/f	2	1	3.6	0	0	1.8
	f/m	0	0	0	1	0	1.8
	f/f	0	0	0	0	0	0
4	m/m	4	2.2	6.4	0	0	4.8
	m/f	1	0.2	4.4	0	0	2.8
	f/m	1	0	2.6	0	0	3.4
	f/f	0	0	0	1	0	0.975
5	m/m	4	2.4	7.6	2	0.4	2
	m/f	1	1	4.6	0	0	2.6
	f/m	2	0.4	3	1	0	3
	f/f	0	0	5	0	0	0.8
8	m/m	4	0.4	9.4	5	0.5	11.3
	m/f	1	0.1	1	0	0	0
	f/m	2	1.1	3.8	3	1.2	6.6
	f/f	0	0	3.6	0	0	2.7
9	m/m	5	0	9.2	1	0	2.6
	m/f	1	0	1	0	0	0
	f/m	5	0.8	5.8	4	1.4	10.4
	f/f	1	0	1	2	0.2	4
10	m/m	5.5	3.15	10.4	1	0.15	3.55
	m/f	0	0	0	0	0	0
	f/m	0	0	1.7	10	8.15	11.85
	f/f	0	0	1.7	4	2.3	4.85
11	m/m	1	0	5.4	3	0.2	7.4
	m/f	2	1.2	3.8	7	0.8	9
	f/m	0	0	0	0	0	0
	f/f	1	0	2	2	1	4.8

m = male f = female; Me = Median, 95° = 95 percentile, 5° = 5 percentile. The observation was discontinued after the 11th week, because of the contemporaneous earthquake that hit the Abruzzo region in April 2009, which would have made the study of aggressive interactions difficult to interpret.

5. Reactions to the exposure to a conspecific dummy and on a releaser. Following the exposure (n = 3 presentations from right to left and vice versa) to an adult male *Z. tequila* dummy, characterized by a garish yellow stripe on the caudal fin (see Figure 1A), the following reactions have been reported: i) males housed in a not-biotope tank did show a behavior of placing the dummy side by side, darkening of the coat and small jerking movements both of the head (header) and the tail; ii) males housed in the biotope tank did not show any kind of behavior aimed at a territorial defense.

Female subjects housed in both the tanks have shown interest in the dummy, by carrying out an initial inspection, followed by a progress – i.e. a behavior similar to that found during the early stages of courtship, when the female exceeds the male to be followed by him. However, the female interest was not stimulated by the mere display of the yellow stripe (Figure 1A), exposed in the same way of the male dummy.

6. Reactions to the exposure to predatory dummies (*Esox sp.* and *Thamnophis sp.*). Following the exposure (n = 3 presentations from right to left and viceversa) to the *Esox sp.* dummy (Figure 1B), the males housed in both the tanks kept themselves aloof all the time of the exposure; on the contrary, the females showed an exploratory behavior. Both the subjects in the enriched tank and those in the biotope reacted by fleeing, following the movement of the tail or the body of the fake predator.

The use of a 3D model of *Thamnophis sp.* (Figure 1B) – i.e. a small snake that feeds on fish with a distribution area including Mexico and present in *Teuchitlan* (John Lyons personal communication) as well – allowed to observe the escape reaction.

The snake dummy was slowly put in the aquarium through a back opening: firstly it was kept on the surface (thus simulating the behavior of such a species in the wild) then it was immersed slowly. All fish showed avoidance, even if different according to each experimental group: the individuals in the enriched tank remained at about 5 cm from the object, examining it first and then going away towards the more remote areas of the tank. On the contrary, on the appearance of the predator, the subjects in the biotope tank fled confusedly, thus immediately escaping in the lower layers and among the gorges of the tank. Such a reaction of avoidance to the predator has remained constant in all exposures.

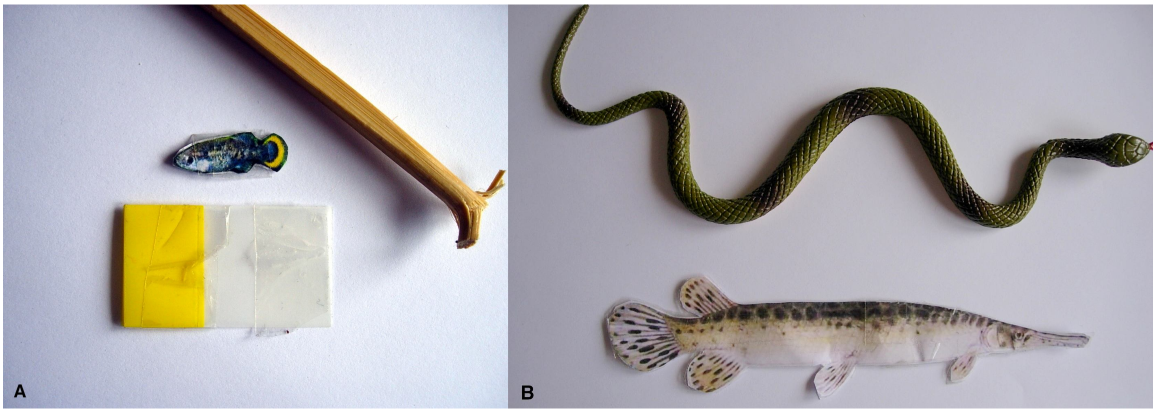

Figure 1: A) above, male dummy of *Z. tequila* with a clear yellow stripe on the caudal fin; below, plastic strip with a yellow band used to test the "supernormal stimulus" effect. The stick on the top (typical of the Chinese cuisine) was used to help the dummies' movement, staying out from fish' visual range. B) Predatory dummmies: above, *Thamnophis sp.;* below, *Esox sp.*

Bibliographic data on the various reproductive behaviors in the family Goodeidae are rare and, above all, very different from each other; this could be caused by the fact that the species of this family colonize extremely diversified environments on the one hand and that the geographic isolation may have led to the development of specific sexual behaviors on the others; so, our research can play an important role in the knowledge of behaviors regarding this species.

During the observation period, *Z. tequila* showed a fairly simple courtship ritual, characterized by a prolonged approach of the male to the female (the male swam practically almost united to the female), but with any particular mating dances such as the "juddering", used in other species of the family Goodeidae. In the case of *Skiffia (Neotoca) bilineata* (Bean 1887 cited in Lyons et al 1998) for example, the male places on the side of the female during the rituals of courtship and mating, and the female responds by starting a vigorous sideways vibration (juddering) and, using the strength of back muscles, moving fast flippers and tail. Such a movement seems to hyperextend the animal that, during the juddering, comes in a position, characterized by a dorsal concavity, with the head and the top of the fin that form a sort of "U", tilting at the same time the position of the body as to the water column. We could not even record the so-called "figure of eight dance" behavior, described in other species of Goodeidae

(Fitzsimmons 1976 in *Xenotoca sp*.; Macias Garcia et al 1994), including *Skiffia multipunctata* (Pellegrin 1901, quoted in Lyons et al 1998; Kelley et al 2006).

The available data show that the achievement of sexual maturity occurs between 6-10 weeks of age, in an aquarium at 26-28° C (Lambert 1990), and that the sexing of males can be made with certainty a few weeks after birth (Lambert 1990; Loiselle 1991).

Reproduction occurs in summer (Webb & Miller 1998); however, pregnant females have been caught in winter into the source as well (De La Vega-Salazar et al 2003b). According to our data, pregnancies had a floating duration both in biotope aquaria and in the enriched ones, and deliveries fall within the range of 6-8 weeks with a maximum duration peak of 259 days as to the biotope aquarium (Table 2, female B), and 178 days as to the enriched tank (Table 2, female D).

Due to the daily observations, it is inconceivable that we could have missed some birth; alternatively, it is possible that a phase of reproductive arrest of unknown origin has occurred, but it did not affect the following reproductive performance in any case. In contrast to what reported by Loiselle (1991) and Webb & Miller (1998), although we monitored a small number of animals, the number of fry born from the females studied in their first year of life was always higher apart from a case only (female D). During the entire observation period, 81 births happened in the biotope aquarium and 105 in the enriched tank. No data are available as regards populations in the wild, due to the shortage of such a species; some important reproductive parameters were obtained only from a similar species – i.e. *Zoogoneticus quitzeoensis* (Bean 1898 cited in Lyons et al 1998): in fact, young specimens were fished out from January to April and this can be seen as a sign of an active breeding season (Webb & Miller, 1998); moreover, adult females were sighted in April in Camècuaro Lake in the Mexican state of Jalisco (Kingston 1979).

With regard to predatory behaviors, the cannibalism of which we had evidence was observed both in the biotope and in the enriched tank, with a perfectly equal distribution of cases. Such behavior, well known in many Teleostean fish (Fitzgerald & Whoriskey 1992; Smith & Reay 1991), is very common among various ovoviviparous species of the family Poecilidae. The knowledge of such a behavior is certainly a cornerstone in the management of captive fish, especially referring to those species that are in strong demographic decline in the wild and that are reared under controlled conditions in order to be protected and in light of a future, possible resettlement in their original habitats, as demonstrated by some works carried out on Mekong catfish *Pangasianodon (Pangasius) gigas* (Mattson et al 2002).

Referring to *Z. tequila* in particular, there is no information from the observation of its behavior in the wild and even scientific official literature does not report cases of cannibalism in a controlled tank. A mild-to-moderate intraspecific predatory activity has been noticed (Dr. Zimmerman personal communication) at the London Zoo. The behavior of fry at birth has never been described in literature, apart from that case where fry were described as follows: "weak, awkward in swimming, moving forward with difficulty" (Diego Montanari AFAE-Associazione Ferrarese Acquariofilia Erpetofilia, head of Poecilidi and Goodeid section, personal communication). According to the hypothesis of the AFAE President, "fry would attract adult fish' attention and would be attacked because mistaken for small crustaceans or insects". In this case the management of fry had a key-role, and the occurring of cannibalistic phenomena has led to a consequent change in the rearing technique of newborn fry, which were firstly placed in floating delivery rooms made of plastic or net and then moved to a purpose-made hatchery.

In our study, the cannibalized individuals were only newborn fry. This may be related to the newborn fry's small size (0.75 mm on average), that makes these subjects the favorite preys of adults; moreover, during the first hours after birth, they often rest on the bottom and are awkward due to the burden caused by the presence of degenerating trophotaeniae. Since their body position is not stable, these newborns barely move in the aquaria, even if such ability improves steadily over the next few days.

Data on cannibalism cast a shadow on the methods and the amount of food to be given. The maintenance of behaviors such as foraging is possible only by avoiding the administration of food *ad libitum*, so that the typical phenomenon observed in most aquaria, i.e. the fish moving towards the front wall when someone appears, in order to get higher amounts of food, does not take place. In the study we have carried out, such an element has been firmly avoided and, just for this reason, the administration of food has always been made with the laboratory's light off and remaining outside the visual field of the subjects.

In some species – e.g. *Stegastes rectifraenum* (Hoelzer 1992) and *Aidablennius sphynx* (Kraak 1996) – a persisting cannibalism has been recorded in overfed fish and laboratory results show that *Gasterosteus aculeatus* (Banister 1986) does not change the consumption of eggs following the increasing amount of food administered (Belles-Isles & FitzGerald 1991); the same has been observed in males of *Etheostoma flabellaris* (Lindstrom & Sargent 1997). The only contrary data regard *Pomatoschistus* (Kvarnemo et al 1998): in fact, reared within controlled conditions, it has shown a decreasing cannibalism in overfed males, in comparison with the specimens kept in a strict diet.

The reaction to predatory dummies, allowed the monitoring of some behavioral factors related to the ability of these subjects to react to surrounding, environmental stimuli. Such ability is essential, above all, if the animals are reared in captivity, in sight of a future release into the original habitat. The releases, in fact, may result in a failure if the fish are unable to adapt themselves successfully and to meet the environmental stimuli in the correct way (Beck et al 1994; Snyder et al 1996); among such stimuli, the most important are the potential native or foreign predators.

In this work we have used both a two-dimensional (*Esox sp.*) and a three-dimensional (*Thamnophis sp.*) model. In the first case just the females have carried out the inspection of the predator. The control of potential predators by preys is extremely important, and it represents a common behavior in wild fish. In fact, even in the case of shoaling fishes, there are few subjects that approach the intruder as soon as it appears (Magurran & Pitcher 1987; Milinski 1987; Dugatkin & Godin 1992; Brown & Schwarzbauer 2001). By approaching the predators, potential preys can consider – keeping their own proper distance – their position, their satiety status and their physical characteristics (Smith & Belk 2001).

If we compare all works available on this subject with the data obtained from our research, one of the main outcomes is that females in both the tanks have shown an inspecting attitude of the predator *Esox sp*. and reacted to its unexpected behavior (e.g. sudden movement of the tail). We do not know why males have always kept at a greater distance. Unfortunately, the data we have collected are not comparable with those of other authors, since there are still few studies carried out about the shoals of Goodeid in general and on this specific topic in particular. The only bibliographic data refer to *Skiffia multipunctata* and *Ameca splendens*, where the authors have verified that the subjects reared in the lab usually spend more time close to the predator in comparison with those reared outdoors (Kelley et al 2004; Kelley et al 2006).

The use of a three-dimensional *Thamnophis sp.* dummy led to slightly different reactions of avoidance in the two tanks: an early inspecting phase and a subsequent quick leaving by the parties took place in the non-biotope tank; on the contrary, in the biotope tank with no surface protection areas, fish acted fleeing into the deepest areas of the tank, among the narrow gorges in the lower part of the water column. Such behavior is described as regards almost all shoaling species and is called "flash expansion" (Pitcher 1983): it consists of a seemingly disorderly flee, probably used in order to give no fixed references to the predator. In our case, the lack of a plant cover in the surface areas of the tank recreating the native habitat of this species may have led fish to flee quickly rather than inspect the snake in open water.

The present study has also tried to examine the behavioral features related to exploration (by females) and to territoriality (by males).

The exposure of a male adult specimen of *Z. tequila* led the females to have an important reaction: in fact, they got closer the glass – firstly inspecting the subject and then placing sideways or slightly before it, with a behavior similar to that observed during courtship. They continued to follow the dummy, even if its direction had been changed. The use of a single yellow stripe did not elicit the same effect; therefore, it seems unlikely that such an ornament serves as a stimulus releaser for mating or, at least, for the female when shows its availability to courtship. On this point, the iridescent scales on the side of the animal may have a more important role: they are present in adult and larger *Z. tequila* mostly and are exhibited during courtship, as demonstrated by the attraction exerted by the intact dummy.

As regards the males' reaction into the non-biotope (enriched) tank, we have recorded a series of complex behaviors against the competitor *Z. tequila*, interpretable as territorial defense behaviors (following the dummy side by side and darkening of the coat). No inspective approach was apparently seen in the biotope tank, neither toward the conspecific nor the yellow stripe. Actually, it would be possible that guest males have behaved in such a way, but on a greater distance, using the larger open spaces (the enriched, non-biotope tank is lacking in such spaces due to the great amount of plants) offered by the layout. The lack of similar studies concerning Goodeid does not help in supposing the causes of the different reactions described above, but it is undeniable that the main interactions occur among males and that they are characterized by a hierarchy, whose linearity has not yet been dealt with by scientific studies.

Conclusions. Biodiversity on Earth is a treasure that has been conquered over millions of years, and the thousands of species evolved in environments very different from each other are realities that deserve to be protected – as the General Assembly of the United Nations also recognized by the declaration of 2010 as the "Year of Biodiversity". Over the last century, thousands of animal and plant species have become extinct as the result of a failure in adapting themselves to the rapid environmental changes caused by humans – so that Prof. William Steffen (University of Canberra) gave the name "Anthropocene" to the current era, just due to the significant impact that man has made on the planet and its ecosystem (Steffen et al 2007). In fact, a world population of 6.4 billion people (see FAO 2009), combined with an increasing population growth, leads to a massive exploitation of environmental resources, in spite of plant and animal species that inhabit natural environments. According to the data issued from the Living Planet Index – a database that analyses the trends of animal populations, including over 1400 species – we have lost 25% of terrestrial species, 28% of marine species and 29% of those living in fresh water between the years 1970 and 2007.

It is estimated that 2,400 species become extinct in the world every day (Life 2009) and that many of them are still unknown to science. If ordinary people, when they talk about conservation of endangered animal species, usually think of symbolic animals, mostly mammals, actually many freshwater fish species are among the most endangered species, because their ecosystem is the first to suffer from the consequences of direct human-induced environmental changes (Magurran 2009). *Zoogoneticus tequila* – so far only enjoyed in the hobby world of fishkeeping and, paradoxically, not yet included in the lists of species protected under the Washington Convention (CITES) – strongly and urgently needs of *ad hoc* protection programs carried out by the authorities of its native countries.

Acknowledgements. The authors wish to thank Professor John Lyons, Curator of the Museum of Zoology at the University of Wisconsin; they also thank Professor Francesca Rosati (University of Teramo) for editing this paper. The work was supported by the University of Teramo, financial support ex 60% 2009.

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Received: 19 July 2011. Accepted: 03 September 2011. Published online: 15 December 2011.
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How to cite this article:
Arbuatti A., Trentini R., Bernabò N., Lucidi P., 2011 Contribution to the biology of the endangered Mexican fish, *Zoogoneticus tequila*, and suggestions for its indoor management. AACL Bioflux **4**(5):670-683.