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EVALUATION OF THE USE OF PLANT AND SYNTHETIC SUBSTRATES IN SPAWNING, EGG VIABILITY AND SURVIVAL OF FINGERLINGS OF *Chirostoma humboldtianum*

Evaluación del uso de plantas y sustratos sintéticos en el desove, viabilidad de huevos y supervivencia de alevines de *Chirostoma humboldtianum*

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ABSTRACT

Shortfin silverside *Chirostoma* is an endemic fish of Mexico, a member of the Family Atherinidae that requires strategies to facilitate its management in captivity and to increase its production, due to its economic importance and commercial demand. Therefore the selection of *C. humboldtianum* on different substrates of plant and synthetic origin in spawning, egg viability and survival of fingerlings of this species was compared. The study was conducted in circular ponds 3 m Ø pit provided with plants including *Ceratophyllum demersum, Eichhornia crassipes* and *Cassuarina equisetifolia,* and synthetic substrates such as nylon fibers, *Agave* spp and polyethylene fibers for spawning. The proportion of eggs and the viability of eggs and fingerlings on each repetition substrate was recorded. The results showed that the selection of spawning substrates was significant in *E. crassipes* and *C. equisetifolia* ($P < 0.05$), followed by the nylon fibers. With respect to the values of viability eggs and fingerlings were higher in *E. crassipes, C. equisetifolia,* and nylon fibers ($P < 0.05$). The alternative use of substrates in the reproduction, spawning and rearing of *C. humboldtianum* in captivity is possible, and aquatic vegetation could replace with synthetic substrates that are adequate and that perform the nesting and management of this species of commercial interest.

Key words: Shortfin silverside fish; reproduction; spawning; plants and synthetic substrates; breeding.

RESUMEN

El pescado blanco *Chirostoma humboldtianum* es un aterínido endémico de México que requiere de estrategias que faciliten su manejo en cautiverio y aumente la producción, debido su importancia socioeconómica y demanda comercial. Por lo cual, se comparó la selección de *C. humboldtianum* en diferentes sustratos de origen vegetal y sintético en el desove, viabilidad de huevos y supervivencia de alevines en esta especie. El estudio se realizó en estanques circulares de 3 m Ø a cielo provistos de sustratos vegetales y sintéticos para el desove *Ceratophyllum demersum*, *Eichhornia crassipes* y *Cassuarina equisetifolia*; además de fibras de nailon, *Agave* spp y polietileno. La proporción de huevos y viabilidad de huevos y alevines en cada repetición por sustrato fue registrada. La selección de sustratos en el desove fue significativa *E. crassipes* y *C. equisetifolia* ($P<0,5$), seguida de las fibras de nailon. Los valores de viabilidad de huevos y alevines fueron superiores en *E. crassipes*, *C. equisetifolia* y fibras de nailon ($P<0,5$). El uso alternativo de sustratos en la reproducción, desove y crianza de *C. humboldtianum* en cautiverio es factible, así como el reemplazo de vegetación acuática por sustratos sintéticos se desempeñan adecuadamente en el anidamiento y manejo de esta especie de interés comercial.

Palabras clave: Pescado blanco; reproducción; desove; sustratos vegetales y sintéticos; crianza.

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INTRODUCTION

Fish may have different reproductive behaviors, including behavioral elements in spawning, ensuring adequate the successful incubation of eggs the survival offspring under hatching conditions. One of the most common essential elements is the selection of a suitable site and the preference of certain structures or vegetation in wich to spawn [3]. The interaction between fish and aquatic vegetation is highly variable due to differences in aquatic systems, plant type, and the composition of the fish community. These preferences may confer a suitable influence on the development of eggs and larvae, reducing offspring mortality, especially that exhibit no parental care behavior [6, 11, 21].

Another essential aspect to consider in the reproduction of oviparous fish comprises knowledge about the media and the oviposition sites that they choose these in wildlife [29], in order to implement their captivity and promote the success or reproduction. The most common means of oviposition is aquatic vegetation through significant reciprocal functions with fish, such as the release of stimulus-response-inducing spawning. In some species of fish this behavior is highly specific because they particularity require flora to spawn [11, 27]. In small species such *Hyphessobrycon eques*, *H. marginatus*, *Serrapinus notomelas*, *Roeboides paranensis*, *Serrassalmus marginatus*, *Metynnis maculatus*, *Oligossarcus pintoi*, *Eigenmannia trilineata,* and *Satanoperca pappaterra,* submerged aquatic vegetation as *Egeria najas* and *E. densa* play a key role in their life cycle, due to that the *Elodea* is grouped in large masses, providing refuge and feeding areas for these fish [18]. However, in some cases, aquatic vegetation can adversely affect the gonadal development of fish, and the viability of spawning and breeding, when it releases organic substances during in the decomposition process [17]. As suggested experimentally, the use of synthetic materials or terrestrial vegetation may influenced the development of eggs and larvae [16].

Arboreal vegetation and synthetic materials to provide a spawning habitat for commercial fish in the absence of macrophytes, have been used successfully in different species, such as American pike (*Esox lucius*), Atlantic sturgeon (*Acipenser oxyrinchus desotoi*), European perch (*Perca fluvialis*), common bullhead (*Cottus gobio*), and various cyprinids (*Rutilus rutilus*, *Leuciscus leuciscus, L. cephalus, Abramis brama,* and *Cyprinus carpio*), which have been provided with nests, plastic mesh, polyester pads, ceramic tile, and, branches of spruce (*Picea abies*), juniper (*Juniperus com*munis), cypress (*Cupressus glabra*) and sycamore maple (*Acer pseudoplatanus*) [9, 12, 13, 16]. However, studies on the management of artificial or synthetic substrates in some freshwater fish species are scarce.

Shortfin silverside *Chirostoma humboldtianum* (Atherinidae: Valenciennes, 1835) is an endemic species of Mexico largely for use in fishing. It has formed over for centuries, along with other atherinids, in the artisanal fisheries of different ethnic groups in the Mexico's Central Plateau, because it is considered a food with high nutritional quality and one that is commercially attractive due to its the organoleptic characteristics of its meat [8, 14, 22]. The latest data of the production of fish of the genus *Chirostoma* reported around 3,381 tonnes per year [5], and although this figure is higher than that recorded for 2003, the wild populations of this group have been reduced due to overfishing, loss of habitat, water pollution, and the introduction of alien species [23]. In particular, the natural distribution of *C. humboldtianum* has been altered, and it is now considered as an extirpated species of lakes in the Valley of Mexico and in the state of Nayarit [2, 15]. The latter necessitates the establishment of aquaculture in this species for serving basics reproduction.

In the wild life, the females of the *Chirostoma* spp spawn on aquatic vegetation, which serves to fix their eggs, for their subsequent fertilization by several males. This process occurs in submerged aquatic vegetation, including floating or rooted vegetation, such as filamentous algae of *Spirigira* spp., coontail *Ceratophyllum* spp., the roots of the water hyacinth *Eichhornia crassipes*, cattail *Typha* spp. and curlyleaf pondweed *Potamogeton* spp. [26]. The structures in aquatic macrophytes, such as leaves, stems, or roots, in addition to the easy attachment of the egg through the interlovular filaments, can mantain an appropriate distance among the favoring oxygenation, thus incubation [23]. Artificial fertilization of *Chirostoma*, has been conducted in captivity, using the water hyacinth for collecting eggs [23, 25] or nylon fibers as oviposition substrate [14, 26]. However, no studies report the influence of the substrates employed used in the oviposition and incubation of *Chirostoma*, which provide alternatives for the reproduction and management of the spawning and early stages in captivity of this group of aterinids. Thus, the aim of this investigation was to compare the effect of the substrates of vegetable and synthetic origin spawning, egg viability and the survival of fingerlings of *C. humboldtianum* in captivity.

MATERIAL AND METHODS

Breeding and breeding ponds

The study was conducted at the Center for Biological Research and Aquaculture Cuemanco of the Autonomous Metropolitan University Xochimilco (CIBAC, UAM-X) with a colony *of C. humboldtianum* of two years of age originating in the State of Mexico, which was conditioned and selected in opencast ponds for breeding in captivity. The average size and weight of fish recorded was 2.5 ± 15 centimeters (cm) and 150 ± 19 grams (g), respectively. For playback, it was utilized three geomembrane circular ponds with PVC 3.0 meters (m) in diameter and 0.80 (m) deep, supplied with water from the CIBAC-UAM Xochimilco artificial wetland; with constant aeration and with anti-aphids covered with mesh to prevent the entry of insects and bird predation. The water temperature recorded was 18.0 ± 2 ° C, pH 8.5 ± 0.5 (Hanna Instruments Model Model HI-98103, USA), dissolved oxygen of 6.0 ± 0.5 milligrams (mg) / L (Hanna Instruments Model HI-9172 Oximeter, USA), and NH4 + 0.2 ± 0.15 (Hanna Instruments Model HI83203 multiparameter photometer, USA). The *Chirostoma* diet was provided *ad libitum*, consisting of the in forage of fish of the family Poecilidae and zooplankton such as isopod crustaceans, amphipods, copepods and, cladocerans.

Breeding experiment with substrates

In each of the ponds, it was introduced and fixed in quadruplicate beds for spawning, a 30 cm area of foliage of aquatic macrophytes *Ceratophyllum demersum* (coontail), *Eichhornia crassipes* (water hyacinth), and Australian pine *Cassuarina equisetifolia* foliage and; the addition of nylon, *Agave* spp (agave) and polyethylene fibers (raffia), which were randomly placed and alternately on the perimeter of the fishpond (FIG. 1). The vegetation was previously washed with saline to 30% in order to reduce the entry of pathogenic pond microorganisms, to avoid predation of eggs, the invertebrates were manually deleted their foliage. In the case of fibers, they were washed with phosphate free germicidal soap (Bactium® Biodegerm). The substrates were removed from the ponds five days (d) after placement, to verify the presence and viability of spawnings, and the latter were observed through a stereoscopic microscope (OLYMPUS SZ40, USA) and counted with a hand counter.

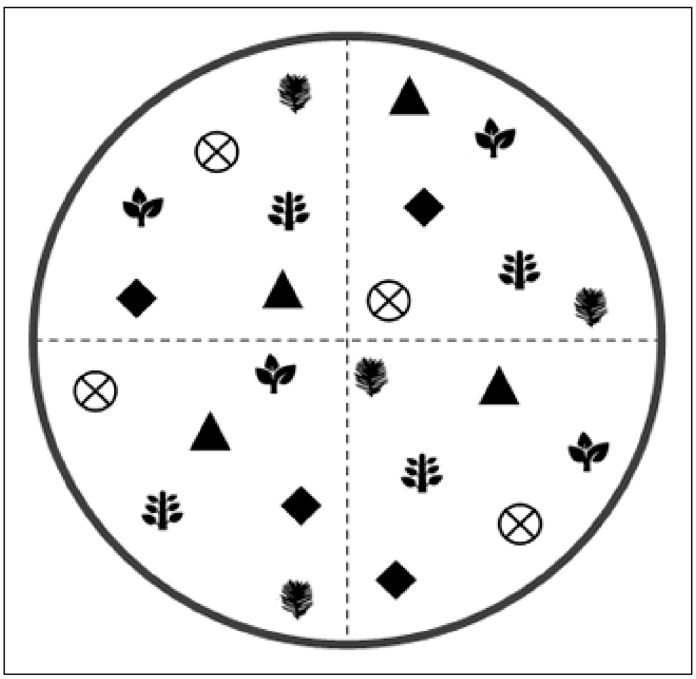

FIGURE 1. DIAGRAM OF THE DISTRIBUTION OF PLANTS AND SYNTHETIC SUBSTRATES IN THE POND FOR THE BREEDING AND SPAWNING OF FISH SHORTFIN SILVERSIDE. Chirostoma humboldtianum. Plants substrates: Coontail, Ceratophyllum demersum; water hyacinth, Eichhornia crassipes; Australian pine; Cassuarina equisetifolia. Synthetic substrates: ▲ Naylon fibers; ⊗Agave spp fibers; ◆Polyethylene fibers.

Incubation and egg viability

For the incubation of spawning, plastic boxes were employed for the substrate type, with 10 Liters (L) of reproduction pond water, both in the breeding and in the incubation the eggs, the cultures was maintained with constant aeration at a temperature of 20 °C and water salinity of to 5 g/L [10]. Viable eggs are well considered, by the presence of blastomeres or discoblastula, larval sketches, the presence of pigmented eyes or the heartbeat of the embryos, while eggs that opaque, white and that lacked the presence of an embryo or any of the characteristics described previously were recorded as non-viable. Once hatching of the fingerlings took place, they were transferred to their respective container substrates with the aforementioned features and body a count of the bodies obtained in each of the substrates was carried out. Exogenous feeding of fingerlings was started by inoculating the water with rotifers *Brachionus plicatilis* species and a combination of microalgae *Chlorella vulgaris* and *Tetraselmis suecica* comprised their, diet *ad libitum* for four weeks in order to continue recording the survival of the fingerlings.

Data analysis

The preference of *C. humboldtianum* to spawn in a substrate was evaluated as P (%) = [(Hs * 100) / Ht]; where Hs = total number of eggs in a substrate and, Ht = total number of eggs in all substrates. Egg viability was calculated as E (%) = [Ls * 100) / Hs] where Ls = total number of viable eggs in a substrate and, Hs = Total number of eggs laid on a substrate. Fingerlings survival was estimated as S (%) = [(Lvf * 100) / Ni], where Lvf = number of live fingerlings at the end of the trial and where, Lvi = Initial number of fingerlings obtained in a substrate.

Statistical analysis

Data normality and homoscedasticity was revised later to analyze the nonparametric Kruskal-Wallis test (**α** = 0.05) and a comparison of means Nemenyi [30], with SYSTAT Version 10.2 statistical software (SYSTAT Software Inc., IL, Chicago, USA).

RESULTS AND DISCUSSION

The results indicated the significant use of *C. humboldtianum* to spawn on substrates of vegetable origin such as water hyacinth *E. crassipes* (35.48%) and branches of australian pine *C. equisetifolia* (28.31%), whereas as , the synthetic substrates, the nylon fibers were favorable for oviposition (22.1%) (P<0.05). The substrates that exhibited a trend in less than 7% for use as spawning beds were submerged vegetation includig coontail *C. demersum* and agave fibers and polyethylene (TABLE I). The values of preference for spawning substrates justifiying *C. humboldtianum* are mentioned by Pitts [20], Sazima and Zamprogno [25], and Snickars and *et al*. [27], reported that the aquatic vegetation habitat comprises is one of the factors involved directly in the ecology and ethology of fish, which is not

TABLE I

AVERAGE VALUES IN PERCENTAGE OF THE SELECTION PRESENTED OF SHORTFIN SILVERSIDE *Chirostoma humboldtianum* TO SPAWN IN DIFFERENT SUBSTRATES, PERCENTAGE OF EGG VIABILITY, AND THE SURVIVAL OF FINGERLINGS

	Plants substrates			Synthetic substrates				
Pond	Coontail *C. demersum*	Water hyacinth *E. crassipes*	Australian pine *C. equisetifolia*	Naylon fibers	*Agave* spp fibers	Polyethylene fibers	SEM	*P*
	Selection of substrate to spawn							
1	6.81^{c}	29.76^{ab}	34.44^{a}	20.24^{b}	5.46^{c}	3.26^{c}	2.26	0.0012
2	7.22^{c}	41.26^{a}	21.29^{b}	20.80^{b}	7.67^{c}	1.73^{c}	2.35	0.001
3	4.68^{c}	35.41^{a}	29.20^{ab}	25.60^{b}	2.39^{c}	2.70^{c}	1.9	0.0016
	6.24	35.48	28.31	22.21	5.17	2.56		
	Egg viability							
1	3.25^{c}	22.18^{a}	28.50^{a}	21.04^{a}	3.59^{b}	2.08^{b}	2.08	0.0023
2	3.73^{c}	33.05^{a}	16.96^{b}	21.62^{b}	4.82^{c}	0.98^{c}	1.82	0.001
3	2.12^{b}	26.49^{a}	23.61^{a}	22.90^{a}	1.03^{b}	1.85^{b}	1.79	0.0027
	3.03	27.24	23.02	21.85	3.15	1.64		
	Fingerling survival							
1	7.75^{c}	34.25^{ab}	38.75^{a}	23.25^{b}	6.50^{c}	3.75^{c}	2.62	0.0013
2	8.75^{c}	48.25^{a}	24.50^{b}	24.50^{b}	8.75^{c}	2.00^{c}	304	0.0011
3	5.00^{b}	35.25^{a}	29.25^{a}	25.75^{a}	2.25^{b}	3.00^{b}	2.34	0.002
	7.17	39.25	30.83	24.50	5.83	2.92		

^{abc} Different letters indicate differences in line, Kruskal-Wallis (P <0.05); 5 degrees of freedom.
SEM = Standard Error of the Mean; average values of the ponds.

only related to the signals - stimuli influencing reproduction, but is also is involved in egg viability and success in pup survival, due to the availability of food and shelter offered by the foliage of aquatic plants. The latter is addition to that the, fish, through evolution, have developed different levels of specialization in terms of choosing specific plants for oviposition or conversely, they have acquired plasticity for the use of different types of vegetation or inert substrates during playback.

C. humboldtianum prefers for oviposition in water hyacinth *E. crassipes*, wich was similar to that reported by Dayal and *et al.* [7]. These authors that found adequate egg collection from the snakehead murrel fish (*Channa striatus*) with the combination of different vegetable substrates such as *Eichhornia crassipes* and pennywort *Hydrilla verticillata*, because this species spawns a mass with non-adherent eggs, wich remain freely within in the space of floating and submerged vegetation, in turn can enable greater egg survival.

In particular, aquatic macrophytes play a key role in the adhesion of the eggs of different fish species that spawn in *Myriophyllum* spp (watermilfoil or coontail), *Elodea* spp, *Fontinalis* spp, *Sagittaria*, and *Scyrpus* spp (bulrush). However, when this type of vegetation is absent some fish such as *Esox lucius* and the cyprinid *Rutilus rutilus* come to nest on the shoots or roots of *Salix* spp (sallow), *Phragmites* spp (common reed), *Nuphar* spp (spatterdock), *Rorippa* spp (watercress), *Butomus* spp (Flowering-rush), E*quisetum* spp (Common Scouring Rush) and some grasses of the Family Poaceae [6, 19].

Among substrates that are not specific to the aquatic environment, but they work to obtain seed or fish eggs, as observed in this study with *C. humboldtianum.* It was find the branches of Australian pine (*Cassuarina* spp), whose foliage has been routinely used as a collector of eggs and a structure for pens during spawning in the aquaculture production of various species of carp including *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, silver carp, *Ctenopharyngodon* idella, and *Cyprinus carpio* [1]. This is due to the resistance to water and to the disposition of the foliage itself, which plays a role in facilitating nest for spawning. Another aspect that exerts an influence concerning the choice of Australian pine as bedding for spawning is its easy availability and safety in use, since only the bark of the trunk has a low tannin content (6-8%), while the branches are even utilized as green forage [4].

For viability of the eggs, higher values were observed in those deposited in vegetation such as *E. crassipes* (27.24%) and *C. equisetifolia* (23.02%), as shown in TABLE I. In the synthetic substrates, the record for the highest viability was obtained in nylon fibers (21.85%), while for the substrates coontail, polyethylene, and agave fibers, lower viability values (P <0.05) were obtained.

As for the survival of fingerlings, this was significantly higher in vegetable substrates *E. crassipes* (39.25%), and *C. equiseti*folia (30.83%), while the synthetic substrates and, nylon fibers had a higher number of live fingerlings (24.50%) compared with the remainder of the substrates (<8%) (P <0.05) as depicted in TABLE I. This behavior of the eggs and hatchlings of *C. humboldtianum* is similar to that found by Woynarovich and Horváth [28], who reported that the submerged aquatic macrophytes *Ceratophyllum* spp and *Hydrilla* spp have been used as shelter and rest areas for newly hatched fingerlings of the giant gourami *Osphronemus goramy*, where the former increased the survival of the species during the rearing period; at the same time, it serves as a *Ceratophyllum* feeding area for fingerlings because it is a habitat composed of various macroinvertebrates. A similar case has been observed for the floating vegetation water hyacinth *Eichhornia crassipes*, which plays a key role in the life cycle of the piranha *Serrasalmus spilopleura,* in that it provides shelter and food for hatchlings and juveniles of this species; in addition to functioning as a transportation route for of breeding piranhas, thus avoiding greater predation at this vulnerable stage of development of the species [25].

Regarding the use of synthetic materials for nesting in fish, nylon fibers are suitable for breeding and fish spawning due to the arrangement of the fibers, wich simulate the foliage of submerged aquatic vegetation, thereby stimulating the courtship or oviposition in the fish [28]. In the *Chirostoma estor* silverside shortfin the use of nylon fibers has been reported during oviposition in captivity, so that the eggs do not stick together, preventing fungal infection diseases [14, 24]. Similarly, nylon fibers have been employed as nests in the pike perch *Stizostedion lucioperca,* which also lays its eggs in various inert fibrous materials [28].

According to Petr [19], aquatic vegetation plays a key role as nursery habitat for at least 12 families of fish in North America. Various groups of fish fauna, such as Cyprinidae, Percidae, Esocidae and, including family Atherinidae reported, as behavior reproductive behavior, the obligatory deposition of eggs in specific plants; however, in the work of Sasso [26], the use of different materials for fixing eggs coincided with the results of this study, in which the breeding, spawning, and rearing of *C. humboldtianum* in different vegetation types was achieved with a reported preference in the selection of the water hyacinth, branches of Australian pine, and synthetic substrates such as nylon fibers, with high values in the survival of eggs and fingerlings.

CONCLUSIONS

In captivity, the alternative use of aquatic vegetation in the breeding, spawning and rearing *C. humboldtianum* presented significant values in the selection of substrates for nesting, highest percentages were obtained in the water hyacinth *Eichhornia crassipes* and in causarina *C. equisetifolia*; addtionally the replacement of aquatic vegetation with synthetic substrates was feasible, with better results for nylon fibers. On the other hand, e highest values of egg viability and fingerling survival was obtained with the use of *E. crassipes*, *C. equisetifolia,* and nylon fibers. The management of both of these synthetic materials and plants for the stimulation of breeding and selection during the oviposition of *C. humboldtianum* in captivity represents an important aspect for establishing the production of this endemic species with great potential for aquaculture. Likewise, this latter activity would help to reduce the pressure in artisanal fisheries and rational utilization without compromising the natural populations of this group of fish.

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