Impacts of *Oreochromis mossambicus* (Perciformes: Cichlidae) upon habitat segregation among cyprinodontids (Cyprinodontiformes) of a species flock in Mexico

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Abstract: Five species of *Cyprinodon* in Laguna Chichancanab, Yucatan, Mexico comprise a young species flock whose ecology and evolution has not been thoroughly studied, but whose existence is threatened with extinction. Species flocks evolve in isolated areas where predators and competitors are absent. Since the description of the Chichancanab flock, *Oreochromis mossambicus*, a species introduced into the lake for which I examined habitat in the 1980's, has become common throughout the basin. I assessed relative abundance of flock species in the lake. examined habitat use and segregation among the three most common flock species and examined the affects of *O. mossambicus* upon flock species habitat use. *Cyprinodon beltrani* was the most abundant flock species in 1997, followed by *C. maya* and *C. labiosus*; *C. verecundus* and *C. simus* were rare. *Cyprinodon beltrani* was found in shallow water, nearshore, over thick beds of submerged *Chara*, and little emergent vegetation. *Cyprinodon beltrani* exhibited diurnal variation in nearshore habitat use. In the field, the habitat use of *C. beltrani* and *O. mossambicus* broadly overlapped. In aquarium experiments, three flock species exhibited habitat use segregation and *C. beltrani* and *C. labiosus* showed agonistic behaviors that strengthened segregation. *Cyprinodon maya* differed from *C. beltrani* and *C. labiosus* by its greater dispersion of individuals and use of areas higher in the water column. The presence of *O. mossambicus* caused a shift in habitat use by *C. maya* and *C. labiosus* that put these species into habitat occupied by *C. beltrani*. The presence of introduced species has caused a significant perturbation of the conditions that fomented speciation of the Chichancanab flock 8 000 years ago.

Key words: Habitat segregation, Laguna Chichancanab, *Cyprinodon*, species flock, Yucatan, Mexico.

The Laguna Chichancanab species flock is an exceptional group of species whose study provides insights into rapid speciation events but whose existence is threatened by an introduced exotic species. Species flocks are not formed from sequential invasions, basin capture or other aggregations of species into one lake, but rather result from speciation that occurs within a lake basin boundary (Greenwood 1984) in the absence of predators or competitors (Masters and Rayner 1993, Dieckman and Doekli 1999).

The five species of *Cyprinodon* in Laguna Chichancanab, (*C. beltrani*, *C. labiosus*, *C. maya*, *C. simus* and *C. verecundus*) constitute a young species flock thought to have diverged from its sister species as recently as 8 000 years ago and to have speciated sympatrically within the Chichancanab basin. When Humphries and Miller (1981) described the Chichancanab species flock, the sole constituents of the lake ichthyo-fauna were the five flock species, four of which were very abundant, and *Gambusia sexradiata*.

The recent introduction of Oreochromis mossambicus (Strecker *et al*. 1996, Schmitter-Soto and Caro 1997) threatens the continued existence of the flock.

Extinction of endemic flock species caused by the introduction of exotic species is well documented (Witte *et al*. 1992). Over 200 species were lost in Lake Victoria after the introduction of the Nile perch and an entire species flock in Lake Lanao went extinct in the wake of a number of introductions, including *O. mossambicus* (Witte *et al.* 1992, Cohen 1994). The status of the Chichancanab flock since the introduction of *O. mossambicus* in the 1980's, and the impacts of the introduction upon the flock species have not been determined. The impacts of *O. mossambicus* upon habitat segregation among flock species are of particular interest because habitat segregation may have played a important role in the evolution of the Chichancanab flock (Humphries 1981). If *O. mossambicus* overlaps in habitat use with flock species, detection of shifts in habitat use by flock species may provide insights into the mechanisms originally driving segregation among the species.

I surveyed fishes in Laguna Chichancanab in December, 1996 to: describe nearshore habitat use by flock species (pupfishes) and *O. mossambicus*, determine the degree to which these species overlapped in habitat use, and describe the status of the flock after the introduction of *O. mossambicus*. The results from this initial survey provided the framework for aquarium experiments that I conducted in November, 1997 to determine differences in microhabitat use among three of the flock species and to investigate the effects of *O. mossambicus* upon microhabitat use by these flock species.

MATERIALS AND METHODS

Laguna Chichancanab is located in the interior of the Yucatan peninsula on the border of the Mexican states of Quintana Roo and Yucatan (Fig. 1). Covich and Stuiver (1974)

and Humphries (1981) provide detailed descriptions of the lake basin. The following experimental methods have been abbreviated but a detailed description of methods is available from the author.
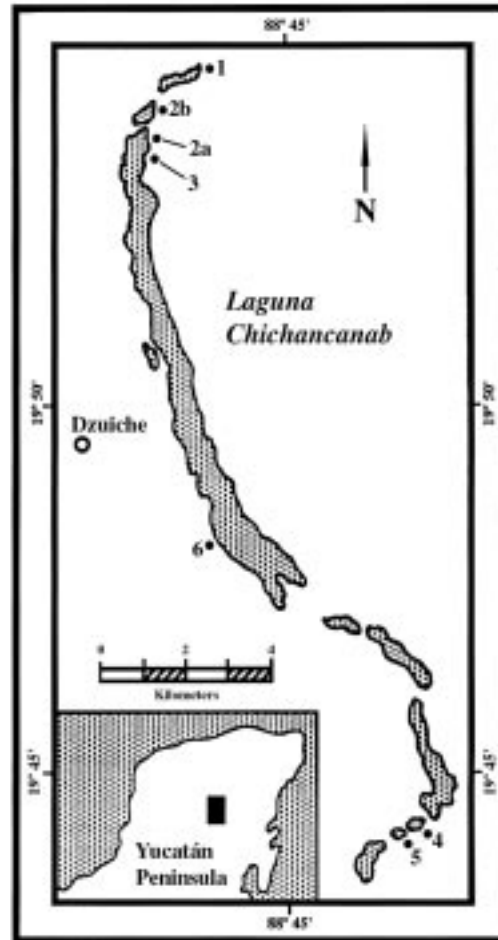

Fig. 1. Collection sites at Laguna Chichancanab on the border of the Mexican states of Quintana Roo and Yucatan. Modified from Humphries, 1981.

To assess relative abundances of lake fishes, I fished unbaited minnow traps at sites 1 - 4 sampled by Humphries (1981; Fig. 1). To assess abundance of *C. simus*, a limnetic dweller, I seined, dip-netted and snorkeled areas at site 2a and b. To describe differences in nearshore habitat

use among fishes, I fished 24 unbaited minnow traps, stacked to fill the vertical water column, at 0.5 m intervals on transects stretching from shore into the lake to a depth of 1 m. I checked traps every 3 - 4 hr throughout a 24 hr period then moved the transects to another location, and repeated the 24 hr survey at five different locations at station 2b (Fig. 1). The following variables were recorded for each trap set: water depth at trap opening, presence of emergent and submerged vegetation, and distance to nearest shore.

Aquarium Experiments: Experiments using *Cyprinodon beltrani*, *C. labiosus*, *C. maya*, and *O. mossambicus* were conducted in a continuous-flow system of four fiberglass tanks with an observation window marked with a 2-dimensional grid. Each trial and tank contained a different group of randomly selected fishes. I used *O. mossambicus* between 28 mm and 69 mm total length because this size was within the range of *O. mossambicus* most commonly captured with pupfishes in the lake. I conducted two trials of each of five experimental treatments to examine habitat use by single species of pupfishes, by a group of congeneric pupfishes and by flock species in the presence of *O. mossambicus*. A trial consisted of a 15 min observation period, during which a count of the number of fishes of each species within each tank grid location was recorded every minute. As a measure of dispersion, I recorded the number of different areas in which fish of the same species were found at the 1-minute observation mark. The treatments were as follows: the first treatment was a single-species control designed to show that fishes did not exhibit a tank-side preference. Only gravel substrate was present in a tank that included five individuals of a single species. Treatment 2 was a single-species treatment used to examine microhabitat use for the species and provided a "control" with vegetation present with which to compare multi-species treatments. There were five individuals of the same species in a tank with half gravel and half submerged vegetation (SV) substrate. Treatment 3 was an "intruder" treatment where five individual pupfish of the same species and one *O. mossambicus* were in a tank with half gravel and half SV substrate.

Treatment 4, a multiple-intruder treatment, included five conspecific cyprinodontids and five *O. mossambicus*.

Analyses: I used SAS for all statistical analyses (SAS Institute 1988) and adjusted the probability of acceptance to 0.05 divided by the number of tests examined. I used a general linear model (PROC GLM) for an unbalanced ANOVA and least squared means comparisons to identify differences in habitat use by fishes collected on lake transects. For aquarium experiments, I used t-tests to determine if fishes showed a tank side preference. I used analyses of covariance (ANCOVA) with time of day as a covariable and least-squared means to compare average locations of species within the tanks and to examine microhabitat preferences for the species. I used the log transformed values of the average number of occurrences of each species in each tank location for each trial for seven of the eight possible tank locations, and dispersion in an ANCOVA. The area nearest the water surface, on the non-vegetated tank side was omitted from analyses so the possible tank positions would be independent. I examined planned comparisons based on a priori hypotheses. I predicted that *C. beltrani* and *C. labiosus* would use areas nearest, or within vegetation more often when *O. mossambicus* were present, with the highest use coinciding with the highest density of *O. mossambicus*. I predicted that *C. maya* would use areas closer to the bottom of the tank on either tank side when *O. mossambicus* were present because *C. maya* were not commonly encountered in nearshore minnow traps.

Agonistic Behaviors: In separate aquarium trials, I quantified the numbers of interspecific and intraspecific "agonistic encounters" between two male-female pairs of *C. beltrani* and *C. labiosus* in tanks with gravel substrate that included two small stones (10 x 5 cm) as focal points (Kodric-Brown 1995). A "charge" was defined as an aggressive approach by one individual that resulted in the receiver moving away from the charger. I used a 2 x 2 contingency analysis and Chi-square distribution to compare the numbers of interspecific and intraspecific charges.

RESULTS

Relative abundance: *Cyprinodon beltrani* was the most abundant flock species, *C. labiosus*, the second and *C. maya*, was the third most abundant flock species in minnow trap samples from stations 1 - 4 (Table 1). *Oreochromis mossambicus* outnumbered all flock species except *C. beltrani*. Only one individual *C. simus* was collected in a minnow trap at site 2; a site where Humphries (1981) collected all five species in 1978. No *C. simus* were observed during snorkeling surveys or in seine hauls and no definitively identified *C. verecundus* were collected in systematic samples. The most abundant fishes observed in snorkeling surveys were *O. mossambicus* and large schools (30 - 80 individuals) of *Astyanax aeneus*. Both *G. sexradiata* and *A. aeneus* were underrepresented in minnow trap samples, but abundant in seine hauls. I considered *A. aeneus* the species of *Astyanax* present in the lake based on Schmitter-Soto (1998).

Nearshore habitat use: Transects were trapped for 1 244 trap-hours and 120 traps were fished. There were no *C. labiosus*, *C. simus*, *C. verecundus*, *G. sexradiata* or *A. aeneus* collected in traps on transects. *Cyprinodon beltrani* was found in shallow water, nearshore, over thick beds of SV, and little emergent vegetation. This species inhabited these areas early in the morning but moved out of the nearshore area in the evening. Traps that contained *C. beltrani* were: (1) in areas with less emergent vegetation (P=0.003); (2) were checked earlier in the day (P=0.004); (3) were closer to the nearest shore (P=0.01); and (4) had more *O. mossambicus* present in the same trap (P=0.01) compared to traps without *C. beltrani*. There were no differences in habitat characteristics in areas where *C. beltrani* was present versus where it was absent for two variables: presence of submerged *Chara* and trap depth.

Habitat use of adult *C. beltrani* and *O. mossambicus* between 30 - 81 mm total length broadly overlapped. Of the total traps set, twenty-seven percent contained both *C. beltrani* and *O. mossambicus*. Forty percent of the traps containing *C. beltrani* also contained *O. mossambicus* individuals, and both *C. beltrani* and *O. mossambicus* were observed together during snorkeling surveys. Low capture rates of flock species other than *C. beltrani* prevented the quantification of habitat use for most of the flock species. There were significantly more *O. mossambicus* captured in traps with *C. maya* (P=0.0001), but this was likely an artifact of small sample size because only 15 traps contained *C. maya*.

TABLE 1

Number of fishes captured in minnow traps in standardized samples, and number of trap-hours at five stations in Laguna Chichancanab, Yucatan, Mexico.

Number of fishes captured in minnow traps

Site	Trap-hours (# traps)	*Cyprinodon beltrani*	*C. labiosus*	*C. maya*	*C. simus*	*Oreochromis mossambicus*	Total
1	96 (3)	5	0	0	0	10	15
2	374 (20)	456	7	1	1	41	506
2B	360 (18)	325	2	9	0	27	362
3	72 (6)	117	3	0	0	69	189
4	342 (18)	6	2	1	0	65	74
Totals	1244	909	14	11	1	212	1146
CPUE		0.73	0.011	0.009	0.0008	0.17	

Trap-hour = one trap set for one hour; CPUE = catch-per-unit-effort as number of individuals/total number of trap-hours.

Single-species treatments: There was no difference in tank side use for either of the three species (P > 0.20). Presence of submerged vegetation affected pupfish habitat use in single-species trials and species dispersed differently within tanks (Table 2). *Cyprinodon maya* used areas nearer the water surface more than the other two flock species and used the area just above the vegetation less often than *C. beltrani*. Both *C. labiosus* and *C. beltrani* were found more often on the unvegetated substrate and just above the SV substrate more often than *C. maya* (P < 0.006). *Cyprinodon maya* tended to remain more widely dispersed within a tank than *C. labiosus* (P = 0.005) and *C. beltrani*, though not significantly in the latter case.

TABLE 2

Results from ANCOVA used to compare habitat use in aquariums among three species of Cyprinodon *collected from Laguna Chichancanab in November, 1997.*

Tank section and side	Species comparisons	F	P
1-V (surface)	M > B L	8.78	*
2-G (mid-column)	M > B L	12.71	*
2-V (mid-column)	M > B > L	24.22	**
3-G (above substrate)	M L B	0.16	n.s.
3-V (above substrate)	B > L M	8.24	*
4-G (substrate)	L > B > M	24.05	**
4-V (substrate)	M B L	0.12	n.s.
Dispersion	M B > L	7.07	*

L=*C. labiosus*, B=*C. beltrani*, M=*C. maya*, V=submerged vegetation; G=gravel substrate, n.s.=not significant. *p<0.005, **p=0.0001. Relationships of mean values are illustrated with < > signs and in all cases, n=8. Dispersion=the number of quadrants occupied by a species within a tank at an observation time.

Intruder treatments: Contrary to my predictions, neither *C. beltrani* nor *C. labiosus* used areas in or near the SV more often in the presence of *O. mossambicus*. *Cyprinodon labiosus* was found more often on the gravel substrate in intruder trials compared to single-species trials and had higher dispersion values in the single-intruder compared to single-species and multiple-intruder treatments (F = 3.83, $r^2 = 0.31$, P = 0.03). *Cyprinodon beltrani* showed no differences in dispersion pattern in single species and intruder treatments.

Cyprinodon maya shifted its habitat use from lower in the water column in single-species treatments to higher in the water column in the single-intruder treatment, and changed its dispersion pattern. *Cyprinodon maya* was more often at the water surface above the gravel substrate in the multi-intruder treatment compared to the single-intruder and single-species treatments (F = 7.07, $r^2 = 0.49$, P = 0.005) and less often at the water surface above the vegetation in single-species compared to single-intruder treatments. *Cyprinodon maya* also had significantly lower dispersion values in the multi-intruder treatment compared to its single-species and single-intruder treatments (F = 7.11, $r^2 = 0.43$, P = 0.005).

Agonistic behaviors: More charges (59.9%) were initiated by *C. labiosus* ($\bar{x}$ = 11.5 per trial) and received by *C. beltrani* than any other combination of interactions between and within species in 19 trials. The Chi-square statistic was significant for the 2 x 2 contingency analysis (X^2 = 11.142, P = 0.001) and indicated there were more interspecific charges (n = 264) delivered compared to intraspecific (n = 112) charges.

DISCUSSION

The introduction of *O. mossambicus* and its subsequent dispersal throughout the Chichancanab basin has significantly negatively impacted the flock of species of *Cyprinodon* in the basin. Compared to descriptions of large schools of flock species observed in 1978 (Humphries and Miller 1981, Stevenson 1992), four of the five flock members have decreased in abundance since their initial description. Humphries and Miller (1981) described schools of ranging from 50-1 000 adult *C. simus* at station 2b in 1978. In contrast to my difficulty in collecting most flock species, in 1978 in one 10-m seine haul at station 2b, Humphries (1981) collected 33 *C. beltrani*, 26 *C. labiosus*, 11 *C. maya* and 2 *C. simus*. Of the four readily identifiable cyprinodontids, *C. beltrani* was abundant during my study, but *C. labiosus* and *C. maya* and *C. simus* were rare. Very few *C. verecundus* were confidently identified in this study but their abundance was assumed to also have declined. I quantified abundance of fishes in only one season but because flock species breed year-round (Strecker and Kodric-Brown 1999) and additional, unpublished visits to the lake yielded the same low numbers, I assume that the low abundances are not seasonal in nature. The pattern of decreased abundance of Chichancanab flock species parallels that observed in Lake Victoria (Witte *et al.* 1992) where the less abundant species disappeared first (Cohen 1994). In Chichancanab, the originally less abundant species have experienced declines since 1978 whereas *Cyprinodon beltrani* remained abundant.

The nature of the negative impacts of *O. mossambicus* upon flock species was investigated here by examining microhabitat use of pupfishes alone and in the presence of *O. mossambicus*. Initial characterization of habitat use by flock species was important because hypotheses of flock speciation involve ecological segregation. Although Humphries and Miller (1981) and Stevenson (1992) qualitatively described habitat used by flock species, they did not quantify microhabitat differences among them. Results of my field surveys and laboratory experiments indicated that species of *Cyprinodon* in Chichancanab were segregated by microhabitat and behavioral differences and that the presence of *O. mossambicus* caused a shift in microhabitat use by *C. maya* and *C. labiosus*.

In nearshore habitats of the lake, *O. mossambicus* overlapped in habitat use with *C. beltrani* and possibly *C. maya*. Characters that typified habitat used by *C. beltrani* in nearshore areas included presence of submerged vegetation, proximity to shore and time of day. Diel variation in nearshore habitat use by *C. beltrani* was likely related to water temperature fluctuations (Barlow 1958, Humphries and Miller 1981) and may also correspond to foraging periods (Stevenson 1992).

In aquarium experiments, *C. maya* was most distinct in microhabitat use compared to *C. beltrani* and *C. labiosus*. Generally, *C. maya* used areas higher in the water column rather than just above or within vegetation beds. This species exhibited higher dispersion compared to *C. beltrani* and *C. labiosus*, which tended to travel in groups. These results were consistent with observations made by Humphries and Miller (1981) who described *C. maya* as a lone forager compared to the other species of *Cyprinodon* that moved in large schools.

Cyprinodon beltrani and *C. labiosus* overlapped in habitat use more so than either overlapped with *C. maya*, but distinctions in habitat use between *C. beltrani* and *C. labiosus* were evident. *Cyprinodon labiosus* used areas higher in the water column less than *C. beltrani*, and *C. beltrani* individuals showed greater dispersion than did *C. labiosus*.

Segregation among species of the Chichancanab flock may have a behavioral component as well as a habitat use component. *Cyprinodon beltrani* and *C. labiosus* displayed more interspecific agonistic behaviors compared to intraspecific encounters. Humphries and Miller (1981) observed males of both *C. beltrani* and *C. labiosus* guarding territories and described *C. beltrani* defense of feeding pits. The construction and defense of feeding pits has been observed in other cyprinodontids

(Barlow 1961) and may result in spatial segregation of individuals.

In the presence of *O. mossambicus*, *C. beltrani* did not differ in its habitat use or dispersion in the tank. However, *C. labiosus* and *C. maya* both shifted habitat use via increased dispersion, and use of areas near the substrate, respectively. These shifts forced *C. labiosus* and *C. maya* out of preferred habitat an in closer proximity to *C. beltrani*, which was found more often in groups near the substrate.

Differences in microhabitat use among flock species that I observed are concordant with genetic (Strecker *et al*. 1996) and dietary (Stevenson 1992) differences among the species. *Cyprinodon labiosus* and *C. beltrani* shared a mtDNA haplotype whereas, *C. maya* exhibited a unique haplotype (Strecker *et al*. 1996). The genetically most distinct flock species, *C. maya*, was also distinct in its ability to recognize congeneric mates (Strecker and Kodric-Brown 1999), its diverse diet (Stevenson 1992) and among congeners in its microhabitat use. This species consumed less detritus than *C. labiosus* or *C. beltrani* and uniquely included large arthropods and fish in its diet (Stevenson 1992). *Cyprinodon beltrani* fed mainly on blue-green algae and slightly less so on those organisms that constituted the largest percentage of the diet of *C. labiosus*.

If habitat segregation was integral to the evolution of the Chichancanab flock, displacement of flock species into marginal habitats may impede the divergence process by disrupting life history characteristics involved in maintenance of segregation of the species. This is not to suggest habitat use was the only factor that contributed to divergence of the flock species. Behaviors related to species recognition and postmating isolation are also implicated as driving divergence of sympatric species. Small scale spatial segregation, home territory affinity (Owen *et al*. 1990, Ribbink 1991) and cues involved in sexual selection (Seehausen *et al*. 1997) were considered influential in the speciation processes that led to the divergence of numerous trophic morphs within the African Great Lakes. In Chichancanab, *C. beltrani* showed little interspecific mate recognition (Strecker and Kodric Brown 1999). If *O. mossambicus* forces *C. maya* and *C. labiosus* into habitat occupied by *C. beltrani*, this shift may result in genetic introgression and loss of rare phenotypes through the breakdown of premating segregation mechanisms.

Other changes in the Chichancanab basin not studied here may contribute to the decline in abundance of flock species outside of the habitat displacement caused by *O. mossambicus*. First, *O. mossambicus*, if not a scavenger on, may be direct predator of the flock species. I found remains of cyprinodontids in gut contents of *O. mossambicus* collected in seine samples from the lake and observed agonistic encounters between *C. beltrani* and *O. mossambicus* during snorkeling surveys. Secondly, compared to alcohol preserved specimens from 1978, over 90% of all flock species collected in 1997 were heavily infested with nematode parasites. Finally, the presence of *A. aeneus* may be involved in shifts of habitat use or other interactions that affect segregation among flock species. This species is common in interior cenotes of Yucatan (Scholz *et al*. 1995, Schmitter-Soto and Gamboa-Pérez 1996) and was likely artificially introduced by local aquarists (Schmitter-Soto, pers. comm.).

In the Chichancanab basin, precarious conditions of an ichthyofauna devoid of predators and competitors that fomented the evolution of the species flock has been significantly altered. Based on impacts caused by exotic species in similar systems (Stiassny 1996), further declines in cyprinodontid populations in Laguna Chichancanab are likely.

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RESUMEN

Cinco especies de *Cyprinodon* en la Laguna Chichanca-nab, Yucatán, México comprenden un cardumen jóven de especies cuya ecología y evolución no ha sido estudiada a fondo, pero cuya existencia está siendo amenazada con la extinción. Los cardúmenes de peces evolucionan en áreas aisladas donde los depredadores y los competidores están ausentes. Desde la descripción del cardumen de Chichan-canab, *Oreochromis mossambicus*, una especie introduci-da al lago de la cual yo examiné el hábitat en los 80's, se ha vuelto común en toda la laguna. Yo evalué la abundan-cia relativa de especies del cardumen en el lago y uso de hábitat y segregación entre las tres especies del cardumen más comunes y examiné los efectos de *O. mossambicus* en el uso de hábitat de las especies del cardumen. *Cyprinodon beltrani* fue la especie del cardumen más abundante en 1997, seguida por *C. maya* y *C. labiosus*; *C. verecundus* y *C. simus* fueron raras. *Cyprinodon beltrani* fue encontrada en aguas someras, cerca de la costa, sobre lechos densos de *Chara* y poca vegetación emergente. *Cyprinodon beltrani* exhibió variación diurna en el uso de hábitat cerca de la costa. En el campo, el uso de hábitat de *C. beltrani* y *O. mossambicus* se traslapaban ampliamente. En experimen-tos en acuarios, tres especies de cardumen exhibieron se-gregación de uso de hábitat y *C. beltrani* y *C. labiosus* mostraron comportamientos agonísticos que fortalecen la segregación. *Cyprinodon maya* se diferenciaba de *C. bel-trani* y *C. labiosus* por su mayor dispersión de individuos y uso de áreas más altas en la columna de agua. La presen-cia de *O. mossambicus* causó un cambio en el uso de hábi-tat de *C. maya* y *C. labiosus* que puso a estas especies den-tro del hábitat ocupado por *C. beltrani*. La presencia de es-pecies introducidas ha causado una perturbación signifi-cante de las condiciones que fomentaron especiación del cardumen de Chichancanab hace 8 000 años.

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