Effects of religiously (—) motivated harvesting on the density and size composition of a sulfur cave-dwelling population of *Poecilia mexicana*

The cave molly, ~~poeciliid fish~~ *Poecilia mexicana*, successfully colonized a sulfur cave in Tabasco, Mexico and gained increasing importance as a model organism to examine evolutionary adaptations to extreme environmental conditions. Closely linked to the ecology of the cave molly population are the local Zoque people. Every year, they harvest cave fish in a religious ceremony called La Pesca, which consists of the introduction of 'barbasco' roots containing the fish toxicant rotenone. Basic ecological data on the endemic cave molly population is still missing; therefore, our first aim was to provide data on population densities, size class compositions and use of different microhabitats. We tested for a signature of La Pesca on populations that are annually exposed to the toxicant, and populations not exposed to barbasco. We found overall densities to be strongly increased at sites annually exposed to the ritual, along with a marked shift in size/age compositions towards an overabundance of juvenile fish. We tentatively argue the annual harvest locally diminishes competition (and possibly, cannibalism by large adults), which is followed by a phase of overcompensation of fish densities. This line of interpretation, however, needs to be discussed with caution, as environmental gradients inside the Cueva del Azufre (~~like~~ including higher ~~H2S~~ H_2S concentrations in deeper portions) may also contribute to the observed differences among populations.

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Introduction

Cave fishes are emerging as model systems to study regressive evolutionary processes like the reduction of eyes and pigmentation that typically accompany~~ing~~ the colonization of caves by previously surface-dwelling species (Jeffery 2009; Romero & Green 2005). ~~Beside the well-known model organism for EvoDevo studies~~ For example, the characid *Astyanax mexicanus*, is a model organism for EvoDevo studies ~~another cave fish that has received comparable attention in the study~~ of cave evolution. T~~t~~he cave form of a Mexican live-bearing fish, the so-called cave molly (*Poecilia mexicana*; Parzefall 1993; Parzefall 2001) presents another opportunity for study. *Poecilia mexicana* has adapted to the vastly divergent ecological conditions inside a South Mexican sulfide cave, the Cueva del Azufre (also referred to as Cueva Villa Luz or Cueva de las Sardinas; Gordon & Rosen 1962). The cave molly differs from its surface-dwelling ancestors in a distinct set of morphological, physiological, behavioral, and life-history traits; e.g., cave mollies have reduced eye size and reduced pigmentation (Parzefall 2001; Riesch et al. 2010; Tobler et al. 2008a; Tobler et al. 2011b). Cave environments are assumed to be energy limited compared to photosynthetically based epigean habitats (Hüppop 2005) and fish densities reported for several different cave systems are usually low, often with ~~often~~ less than one individual per m^2 (Trajano 2001). In contrast, the Cueva del Azufre is a sulfidic habitat and nutrient-rich due to chemoautotrophic primary production (Hose & Pisarowicz 1999; Summers Engel 2005). Although the cave molly has been established as a model to examine evolutionary adaptations to extreme environmental conditions (namely hydrogen sulfide and absence of light), population densities have not yet been quantified in the Cueva del Azufre system.

¶ ~~Moreover~~, Even less is known about the influence of the local indigenous Zoque people and an annual ceremony named 'La Pesca' on the cave molly's ecology. The Cueva del Azufre is sacred to Zoque people, and once a year, at the first Sunday of the Easter week, the Zoque enter the cave and introduce rotenone- and deguelin-containing barbasco roots (*Lonchocarpus* sp., Fabaceae) into the water. Rotenone is an inhibitor of the mitochondrial complex-I of the respiratory chain, causing reduced ~~thus depressin~~g cellular respiration (Singer & Ramsay 1994). Barbasco is introduced into the water in the middle portion of the cave, so only downstream cave chambers are affected, while (sub-)populations upstream of the release site—like the population inhabiting cave chamber X (nomenclature after Gordon & Rosen 1962)—are not affected (Fig. 1). Capture of poisoned cave fish is facilitated by the anesthetic effect of barbasco, as narcotized fish are flushed out of the cave, where they are harvested using wooden baskets, and afterwards cooked and eaten as part of a religious ceremony honoring the Rain Gods (Tobler et al. 2011a). The yield of the annual harvest is considered to be indicative of the quality of the subsequent crop harvest (Hose & Pisarowicz 1999; Tobler et al. 2011a).

Population genetic analyses using nuclear microsatellites not only detected strong population genetic differentiation between cave mollies and adjacent surface populations, but also some degree of genetic differentiation among populations from different cave chambers inside the Cueva del Azufre (Plath et al. 2007; Tobler et al. 2008a), and so it appears as if cave mollies do not migrate freely between different cave chambers. Intriguingly, Tobler et al. (2011a) found (sub-)populations living downstream of the barbasco-release site to show an increased rotenone tolerance (Tobler et al. 2011a). Annual harvests ~~are enormous and~~ amount to several thousand individuals, and the ceremony is likely to have taken place for centuries (Hose & Pisarowicz 1999), and it is likely to act as a strong selective pressure on ~~so it ought to have left further footprints on~~ *P. mexicana* populations annually exposed to it.

From a conservational point of view, knowledge about whether and how human activities affect teleost populations is especially pertinent in the case of locally adapted populations that are endemic to a small ~~distribution~~ area. Furthermore, the annually recurring anthropogenic disturbance may act as a constant selection factor in the system, removing a large proportion of certain age classes ~~small (and thus young) individual~~s from the affected populations.

Evidence from a broad range of taxa exemplifies how anthropogenic ~~man-made, novel~~ selection factors can profoundly alter the evolutionary trajectories of populations (Smith & Bernatchez 2008; Tuomainen & Candolin 2011). Well-known examples include industrial melanism in moths ~~butterflies~~ (Kettlewell 1961), pesticide- and heavy metal-resistance in insects (Antonovics et al. 1971; McKenzie & Batterham 1994), and convergent morphological shifts in different stream-dwelling fishes as a consequence of altered flow regimes following the construction of impoundments (Franssen et al. 2013). Today, teleost fishes are increasingly under selection resulting from commercial or recreational fisheries (Jørgensen et al. 2007), both of which are known to have various effects on teleost populations, including the loss of genetic variability and (plastic or heritable) alterations of the morphology ~~morphological phenotype~~ (Hutchings & Fraser 2008). Moreover, several studies demonstrated heritable changes in behavioral, physiological and life-history traits in fish populations that had been harvested experimentally (Biro & Post 2008; Walsh et al. 2006).

In the present study, we provide first data of local densities within different chambers of the Cueva del Azufre and further tested the hypothesis that La Pesca affects local (sub-)population densities as well as age/size class distributions. We predicted to find generally lower population densities, and especially fewer large-bodied individuals downstream of the barbasco-release site. We used a non-invasive technique to repeatedly assess fish densities and size-distribution patterns (as a proxy for age) inside the Cueva del Azufre (up- and downstream of the barbasco-release site) and in the sulfidic creek leaving the cave (El Azufre). Moreover, given the high structural heterogeneity of the water course inside the Cueva del Azufre with respect to water depth and flow velocity (Hose & Pisarowicz 1999), and because Croft et al. (2004) reported on size-specific preferences regarding water depth in another poeciliid, the Trinidadian guppy (*P. reticulata*), we combined our assessment of fish densities with an investigation of microhabitat use by ~~of~~ different size classes of cave mollies.

Material and Methods

Study system

Locally adapted subterranean populations of *P. mexicana* can be found in at least two different limestone caves in the vicinity of the southern Mexican city of Tapijulapa (state of Tabasco, México): the Cueva del Azufre (Gordon & Rosen 1962) and the much smaller, non-sulfidic Cueva Luna Azufre (Tobler et al. 2008c). La Pesca, however, affects only the Cueva del Azufre, which is about 500 – 600 m deep and divided into 13 different cave chambers (I-XIII), with the innermost chamber being XIII (Gordon & Rosen 1962). Several springs discharge water with high concentrations of hydrogen sulfide (H_2S) into the creek draining the cave (Tobler et al. 2006). The cave creek forms a highly heterogeneous mosaic of shallow pools and backwaters that are partially divided by swift flowing riffle passages (Gordon & Rosen 1962; Hose & Pisarowicz 1999). While the front cave chambers receive some dim light through cracks in the ceiling, the inner parts of the cave are lightless. Consequently, (sub-)populations experience divergent selection regimes regarding light exposure, with populations from the innermost chambers living under perpetually dark conditions, while those from front chambers are exposed to dim sunlight through ~~a number of sky lights~~ cracks (Fontanier & Tobler 2009).

Upon leaving the underground, the sulfidic creek draining the Cueva del Azufre is ~~henceforth~~ called 'El Azufre'. After meandering for approximately 1.5 km, it eventually drains into the Río Oxolotán, which is part of the Río Grijalva drainage system. Despite the gradual oxidation of H_2S to sulfate and elemental sulfur with increasing distance from the sulfide sources —which increasi~~ngly rendering~~es the water turbid~~,~~ity and despite the influx of some smaller clear water affluents, El Azufre still has a remarkably high H_2S concentration of up to ~40 μMol (Schlupp et al. 2013; Tobler et al. 2006).

Study sites and data collection

We compared the abundance and distribution of different size classes of *P. mexicana* among different sampling sites along a transect starting at chamber X in the Cueva del Azufre, and following the water flow outside the cave to the confluence of El Azufre with the first freshwater influx, ~~namely~~ from the Clear Creek (Fig. 1A). This transect, therefore, cover~~s~~ed sample sites located upstream of the release point of barbasco (sample point in chamber X), several sites directly downstream of the release point of barbasco that are strongly affected by the annual ceremony (three sites in chamber V; CV-1, CV-2, and CV-3; Fig. 2B-C), and sites outside of the cave that are not ~~under the~~ influenced ~~of~~ by barbasco [clear creek (CC) and its confluence with EA (EA-con; Fig. 2A)], as well as two surface sites of El Azufre that are annually exposed to barbasco (EA-ex, EA-mf), even though concentrations are probably considerably lower than inside the cave (Table 1).

Field work was conducted in January 2010, i.e., about 9 month after the last La Pesca ceremony in 2009 (L. Arias-Rodriguez, pers. obs.). At each of the eight sample sites, we defined sampling grids consisting of 50 × 50 cm quadrants with wooden sticks fixed in the ground (or stones where a grid angle fell on the shore). The number of quadrants was mostly 25 per sampling grid (i.e., 5 × 5 quadrants). In the narrow non-sulfidic surface creek (CC), however, the arrangement of quadrants was more longitudinal (4 × 7 = 28 quadrants), and in chamber V, where a particularly high degree of structural heterogeneity precluded defining larger grids, one sampling site of 5 × 5 quadrants and two smaller ones (15 and 8 quadrants, respectively) ~~could be~~ were defined (Table 1). The grids reflected the natural variation in water depth, flow velocity, and substrate types, thus covering the range of different microhabitats inhabited by mollies (an example is shown in Fig. 3).

Daily measurements took place between 11:00 a.m. and 4:30 p.m. Each site was visited at least 5 times (mean ± SD = 6.25 ± 1.16) on consecutive days. During the counts, we slowly approached a site while trying to avoid any movements that would cause the resident fish to flee, and ~~thus~~ we counted juveniles (< 10 mm standard length (SL)), sub-adults (10-30 mm) and adults (> 30 mm) in each quadrant. The observer was standing motionless at least 1.5 m downstream from the respective quadrant. Sizes were estimated qualitatively, aided by a prior training session that used wooden sticks of known size as a reference. Our definition of adults roughly followed Riesch et al. (2010), who determined the mean (± SD) standard length of reproducing females to be 31.44 ± 4.40 mm (El Azufre) and 36.97 ± 4.59 mm (Cueva del Azufre, chambers V and X).

Habitat parameters were assessed after the last fish count. For each quadrant, we determined water depth using a makeshift wooden ruler stuck vertically into the water ~~on~~ at five random locations and calculating the mean from those five measurements. Flow velocity was measured on the water surface by scoring the time a small wooden stick of about 3 cm length took to float through the whole length of a quadrant (measurement was repeated five times per quadrant). Mean surface flow velocity was then expressed as $m*s^{-1}$. Research follows the authorizations from CONAPESCA-DGOPA.09004.041111.3088 and Tacotalpa, Tabasco municipality.

Statistical analysis

Our first question was whether population densities differed ~~between~~ among sampling sites. We used data for the different quadrants per site (averaged from the repeated measurements) and expressed density as total numbers of individuals per square meter. Density estimates per quadrant were used as the dependent variable in a univariate general linear model (GLM) with 'sampling site' as a fixed factor. We initially entered 'mean water depth' ($F_{1,162} = 0.12$, $P = 0.98$) and 'mean flow rate' ($F_{1,162} = 0.22$, $P = 0.64$) as covariates, but removed them from the final ~~model~~ analysis since neither had a significant effect (also none of the interaction terms were significant). We used Fisher's LSD tests for pairwise *post hoc* comparisons ~~between~~ among sites.

A further question was whether size-class compositions differed among sample sites and whether distribution patterns would be stable ~~between~~ among repeated sampling days. We used the Bray-

Curtis index (Bray & Curtis 1957) to estimate pairwise similarities ~~between~~ among each sampling point (calculated with the R-package 'ecodist 1.2.7'; Goslee and Urban 2012; R Development Core Team 2008), and used these for non-metric multidimensional scaling ('NMDS PROXSCAL' function in SPSS 21). To detect clusters, we used the 'two step cluster analysis' function based on Euclidian distances and the Bayesian information criterion.

Our first analysis detected pronounced variation in population densities and size distributions (see results), and thus, ~~so~~ we decided to analyze potential effects of water depth and flow velocity (i.e., microhabitat choice) in a site-wise fashion. We concentrated on sites inside the Cueva del Azufre (CV-1, CV-3 and CX) where (*a*) fish densities were sufficiently high and (*b*) sufficient variability of those environmental variables was found to allow for a meaningful analysis. For each site, fish density per quadrant was entered as the dependent variable in repeated measures (rm) GLMs with 'size class' (three levels) as the repeated measurement. We grouped water depth (< 5 cm, 5 – 10 cm, > 10 cm) and flow velocity (< 10 cm*s^{-1}, 10 – 50 cm*s^{-1}, > 50 cm*s^{-1}) into three classes each and used these habitat parameters as fixed factors. However, neither the main factor 'flow velocity' nor any interaction term involving 'flow velocity' had a significant effect in any of the three site-specific models (CV-1: $F_{4,38} = 1.27$, $P = 0.30$; CV-3: $F_{2,4} = 3.28$, $P = 0.14$; CX: $F_{2,20} = 0.44$, $P = 0.65$), and so we subsequently removed this term from all models.

Results

Local population densities

When comparing mean densities per quadrant across sites we detected a significant difference ~~between~~ among sampling sites (GLM; $F_{7,164} = 32.49$, $P < 0.001$; Fig. 1B). *Post hoc* pairwise LSD tests found most pairwise comparisons to be statistically significant; qualitatively, densities increased from surface sites (mean ± SE across sites = 21.0 ± 5.0 individuals*m^{-2}) towards the cave (119.5 ± 12.7 individuals*m^{-2}). Also, sites downstream of the barbasco release-site (chamber V; 162.3 ± 16.1 individuals*m^{-2}) had considerably higher densities than the site in chamber X that lies upstream of the release-site (37.4 ± 4.8 individuals*m^{-2}).

Differences in size-class compositions

The NMDS based on Bray-Curtis similarities found data from repeated sampling days to cluster together, suggesting that the observed size-class compositions were stable over ~~time~~ the period of this study (Fig. 4). There were three distinct clusters, and in only two cases were single sampling days of a given sampling site assigned to the 'wrong' cluster. The first cluster comprised the three sample sites in cave chamber V and EA-con. Samples had high overall densities and were composed mostly of small individuals. Cluster two comprised the rearmost cave site CX. Samples in this cluster were characterized by intermediate densities but a particularly high proportion of large individuals. Cluster three comprised all surface sites except EA-con and was characterized by overall low densities and mostly intermediate-sized fish (Fig. 1C).

Microhabitat use of different size classes

In the rmGLMs treating the different size-classes as the repeated measurement, the interaction term 'size-class × water-depth' had a significant effect for two of the three sampling sites included in this analysis—notably, those sites with the most variation in water depth (Fig. 5). This result is indicative of differences in microhabitat use ~~between~~ among different size classes of cave mollies: generally, larger fish were found in deeper areas, while smaller fish resided in shallow parts. A significant main effect of the repeated measurement ('size class') in all three analyses confirms the overabundance of small-sized fish in cave chamber V, and of large-bodied fish in chamber X (Fig. 5).

Discussion

be consistant
cave molly
or P. mexicana

Here, we provide detailed information on population densities of cave-adapted *P. mexicana* in the Cueva del Azufre. Repeated measurement in different cave chambers uncovered very stable patterns of high densities, confirming qualitative estimates provided by Parzefall (1993). Density estimates of *P. mexicana* in the cave are extraordinarily high and exceed those of other cave fishes which are usually low, with often less than one individual per m^2 (Trajano 2001). Furthermore, densities are higher inside the cave compared to adjacent surface populations.

The annual 'La Pesca' ceremony leads to a strong temporary reduction of local fish densities in those cave chambers that are situated downstream of the barbasco release site (Tobler et al. 2011a). Our study was conducted approximately nine months after the last ceremony, but given rather long generation times in *P. mexicana* (roughly 3–6 months for males and 7–10 months for females from birth until reaching maturation under common-garden rearing conditions; Riesch, Reznick, Plath & Schlupp, submitted), we predicted to find lower (sub-)population densities and especially fewer large-bodied individuals downstream of the site in the Cueva del Azufre where barbasco is annually released. Instead, while fish densities were generally high in the cave, they were highest downstream of the barbasco release site. However, in line with our prediction, sample sites affected by the release of barbasco had population structures that were strongly shifted towards an overabundance of the smallest size classes (i.e., juveniles). These patterns were stable when repeated samplings from subsequent days were compared.

Migration within the Cueva del Azufre is unidirectional, out of the cave, and migration among different cave chambers occurs ~~just~~ only to a small extent, which results in population genetic differentiation as shown based on nuclear microsatellites (Plath et al. 2007), and is also reflected in morphological differences ~~between~~ among fish from different cave chambers (Fontanier & Tobler 2009). Hence, re-colonization of the affected sites from other parts of the cave (i.e., source-sink dynamics) is unlikely, and the observed recovery of the respective populations likely represents an autochthonous effect. After the temporal decline in population density, the few surviving animals benefit from reduced intraspecific resource competition. ¶ ~~While~~ Detritus and green algae are the dominant food sources of surface-dwelling *P. mexicana* from non-sulfidic streams, while diets of conspecifics in the sulfidic surface and cave streams are dominated by chemoautotrophic (sulfur) bacteria and aquatic invertebrates (like larvae of the dipteran *Goeldichironomus fulvipilus* and small snails; Roach et al. 2011). In particular, access to invertebrate prey could be favored not only by the absence of competing fish species, but also by relaxed competition among the surviving adult *P. mexicana*. Generally, relaxed competition translates into higher growth rates, faster maturation, and increased adult fecundity (Stearns 1976) which may lead to stage specific biomass overcompensation thereby compensating for the removal of individuals from the population (de Roos et al. 2007; Schröder et al. 2009; Werner & Gilliam 1984). This idea received support from empirical harvesting experiments that found the negative relationship between adult mortality and abundance/density to be reversed if mortality does not affect a certain portion of the population. Experimental studies on laboratory populations of the poeciliid fish *Heterandria formosa* showed that biomass of the juvenile size class increased in response to intermediate adult mortality rates (Schröder et al. 2009). Another study showed that a pathogen outbreak in a wild perch population (*Perca fluviatilis*) was followed by a biomass overcompensation of the juvenile stage ~~in response to increased~~ as a result of adult mortality. Age-specific adult fecundity and body mass of one- and two year old perch increased after the disease outbreak, suggesting that mortality released adult perch from competition and cannibalism, thereby increasing somatic and reproductive growth (Ohlberger et al. 2011). In the Cueva del Azufre, the stage-specific biomass overcompensation ~~leads~~ may lead to increasing population densities, with high numbers of juvenile fish. This ~~will soon~~ would result in consistent cave molly populations regaining the high densities seen before La Pesca, again leading to increased competition. This is consistent with earlier observations of cave mollies showing reduced body condition (measured, e.g., as fat content) compared to fish from ~~benign~~ surface sites (Riesch et al. 2010; Riesch et al. 2011).

We are aware of potential caveats of our discussion, as not all differences reported here may be due to the annual La Pesca ceremony; e.g., environmental heterogeneity may also contribute to population differences. The highest H_2S concentrations (> 300 μM) are found in parts of chamber X, where most large sulfide sources are located, while concentrations in chamber V are lower (2 – 32 μM), as H_2S is increasingly ~~oxygenated~~ bound with oxygen with increasing distance from the sulfide sources (Tobler et al. 2006). However, ecotoxicological experiments repeatedly found small adults to have higher H_2S-resistance than large-bodied adults, possibly reflecting senescence effects (Plath et al. 2013; Tobler et al. 2011b). Hence, we would expect fish in chamber X to actually be smaller than fish from chamber V if different H_2S concentrations—but not the annual La Pesca—~~are~~ was the main driver of population differences.

Furthermore, cave chambers may differ in predation regimes. Inside the Cueva del Azufre, aquatic water bugs of the genus *Belostoma* prey upon cave mollies and *Belostoma* prefer large over small cave mollies as prey (Plath et al. 2011; Tobler et al. 2008b; Tobler et al. 2007). Mark-recapture analysis found individual densities of water bugs to be approximately one individual per m² in chamber V (Tobler et al. 2007), and while empirical data are as ~~yet~~ still lacking, observational evidence over several years of field work suggests that densities are much lower in the innermost chambers. Hence, human-induced selection and predation by *Belostoma* ought to have very similar effects on the populations exposed to them. We are inclined to argue, however, that the relative influence of *Belostoma* predation is considerably lower than the effects of the massive annual fish harvest. Previous reports of increased rotenone-resistance in fish from

~~size~~ preference by Belostoma.

chamber V, but not chamber X (Tobler et al. 2011a), confirm that La Pesca undoubtedly has a strong selective influence on populations annually exposed to it.

Belostoma predation might also explain microhabitat use of different size classes of cave mollies. *Belostoma* are typically found on rocks at the water's edge (Tobler et al. 2008b), and so large cave mollies—being preferred by the water bugs (Plath et al. 2011)—could use deeper parts of the water column to avoid predation risk. The preference for large size-classes was confirmed for another belostomatid preying on mosquitofish (Schumann et al. 2012). On the other hand, small fish could avoid filial cannibalism, which is known from other poeciliids (Loekle et al. 1982; Nesbit & Meffe 1993), by using shallow parts of the water column.

In summary, we found remarkable fish densities of more than 200 individuals per m^2 in some parts of the cave. The annual La Pesca appears to have multiple effects on the population ecology and evolutionary trajectory of cave mollies. While human influences on highly endemic, locally adapted populations (or, in terms of conservation biology, evolutionary significant units; no overline Moritz 1994) generally are to be evaluated as highly problematic, management plans for cave mollies ought to consider the ~~important~~ role La Pesca plays in the religion of the local human population. Carried out in the traditional way, fish populations in downstream cave chambers can obviously recover after the ceremony. However, we wish to highlight the necessity to critically review that those practices do not affect deeper parts of the cave and that no commercially available, more efficient fish toxins ~~are being~~ will be employed in the future.

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Table 1(on next page)

List of samling sites

Sampling sites, their abbreviation code as used throughout the article, numbers of quadrants examined, and details regarding barbasco release.

Tables

Table 1

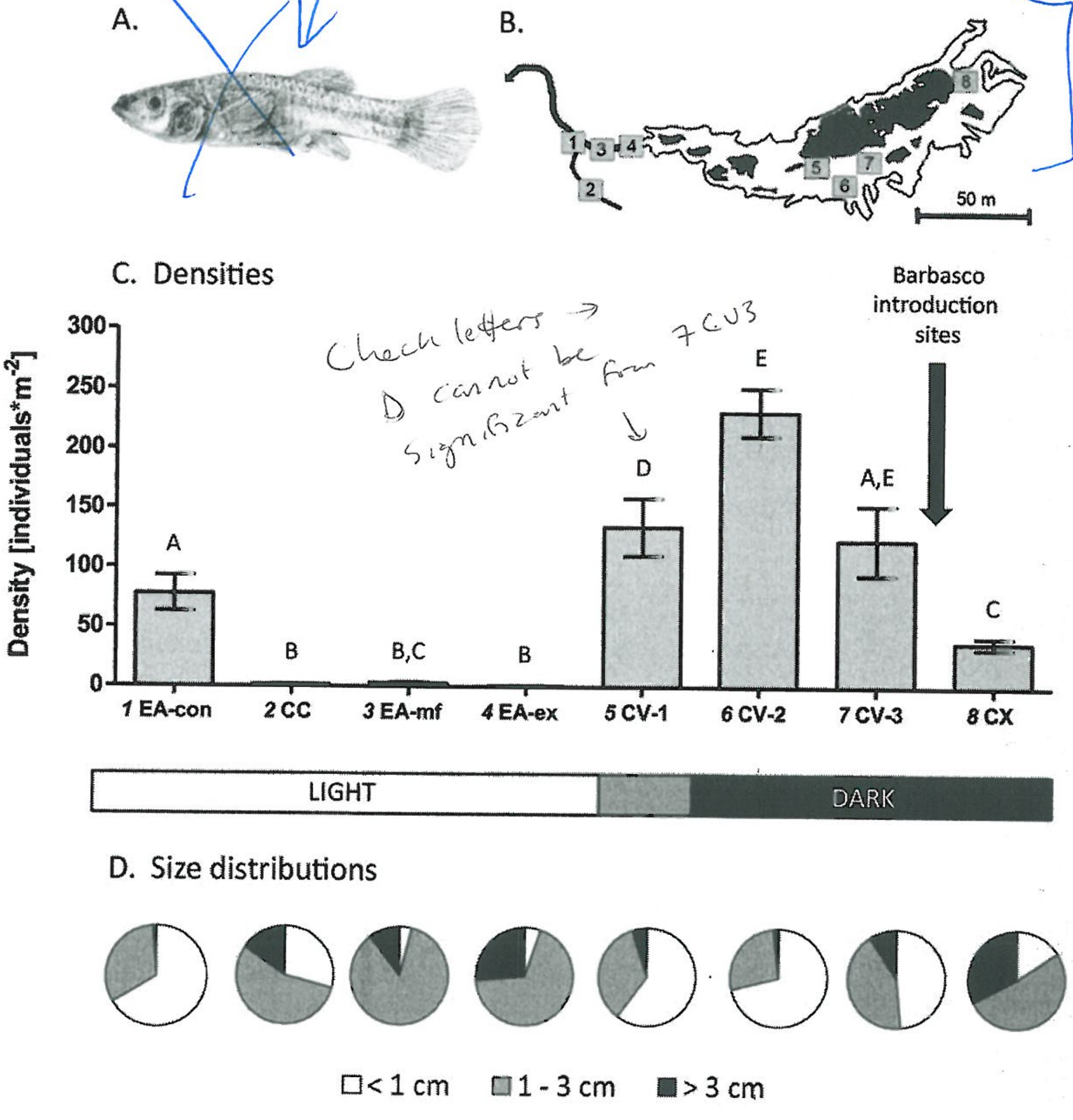
Sampling sites, their abbreviation code as used throughout the article, numbers of quadrants examined, and details regarding barbasco release.

Site code	Site	Number of quadrants	Affected by deposition of rotenone?	Approximate distance to upstream rotenone release site [m]
1 EA-con	El Azufre, confluence with Clear Creek	25	No (only partly)	150
2 CC	Clear Creek	28	No	-
3 EA-mf	El Azufre, mudflat	25	Yes	120
4 EA-ex	El Azufre, exit of the Cueva del Azufre	25	Yes	110
5 CV-1	Cueva del Azufre, Chamber V, site 1	25	Yes	0
6 CV-2	Cueva del Azufre, Chamber V, site 2	15	Yes	0
7 CV-3	Cueva del AzufreChamber V, site 3	8	Yes	0
8 CX	Cueva del Azufre, Chamber X	25	No	-

Figure 1

Study system and population densities.

(A) ~~Drawing of a female cave molly.~~ (B) Map of the study area showing the different sampling sites (*numbers*). *1* EA-con, *2* CC, *3* EA-mf, *4* EA-ex, *5* CV-1, *6* CV-2, *7* CV-3, *8* CX. With the exception of sampling site CV-1 all sampling sites inside the Cueva del Azufre are completely dark. Barbasco is released annually between chamber V (CV) and chamber X (CX). Three sampling sites inside chamber V were defined (CV-1 to CV-3). Downstream of the exit of the Cueva del Azufre (EA-ex), a rather homogeneous mudflat (EA-mf) was sampled. Further sampling sites were a small non-sulfidic creek (Clear Creek; CC) and its confluence with El Azufre (EA-con). (C) Mean (± SE) densities of mollies at each sampling site. Letters above the error bars signify statistically different groups (Fisher's LSD tests). (D) Size class compositions of mollies at the different sampling sites.



C. Densities

D. Size distributions

drawing not relevant

Make larger

Check letters →
D cannot be
significant from 7 CV3
↓

Figure 2

Pictures of probing sites

(A) El Azufre confluence with Clear Creek (*1* EA-con), (B) Cueva del Azufre chamber V (*6* CV-2) and (C) site *7* CV-3.

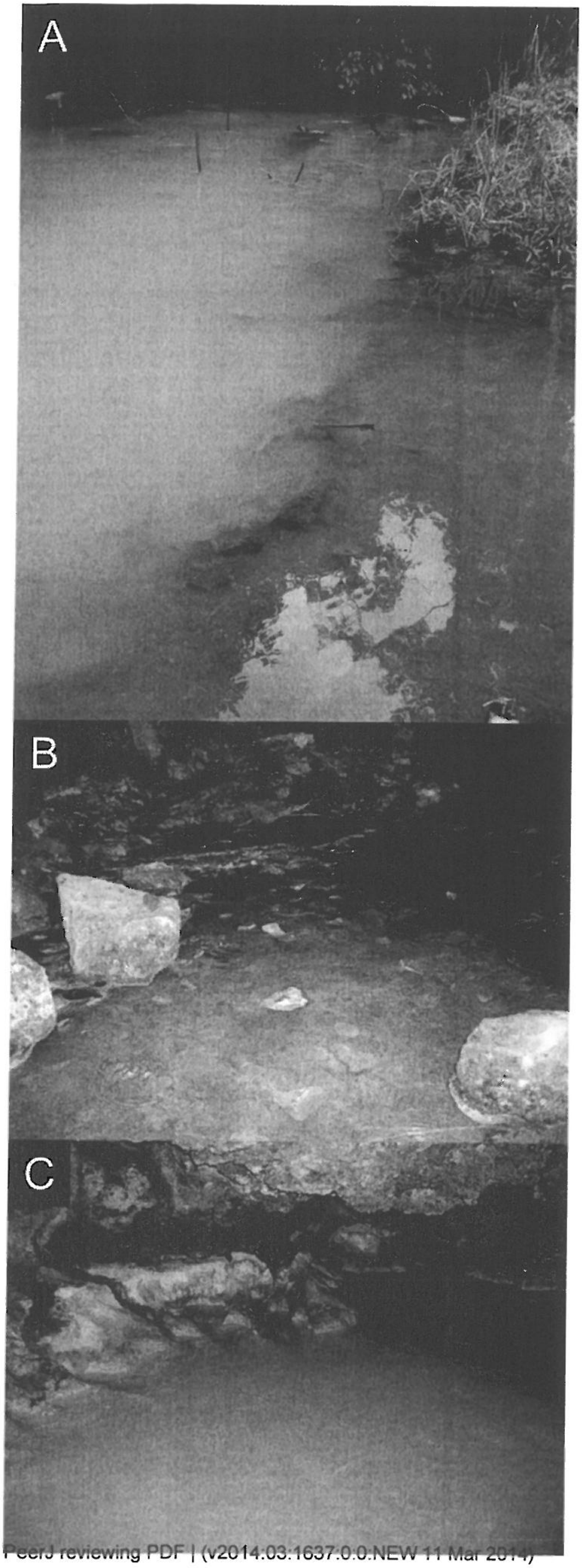

A
B
C

Figure 3

~~Exemplary~~ S~~s~~ketch of site *5* CV-1

showing the high degree of heterogeneity in flow regimes, water depth, substrate types, and (in this case) light regime.

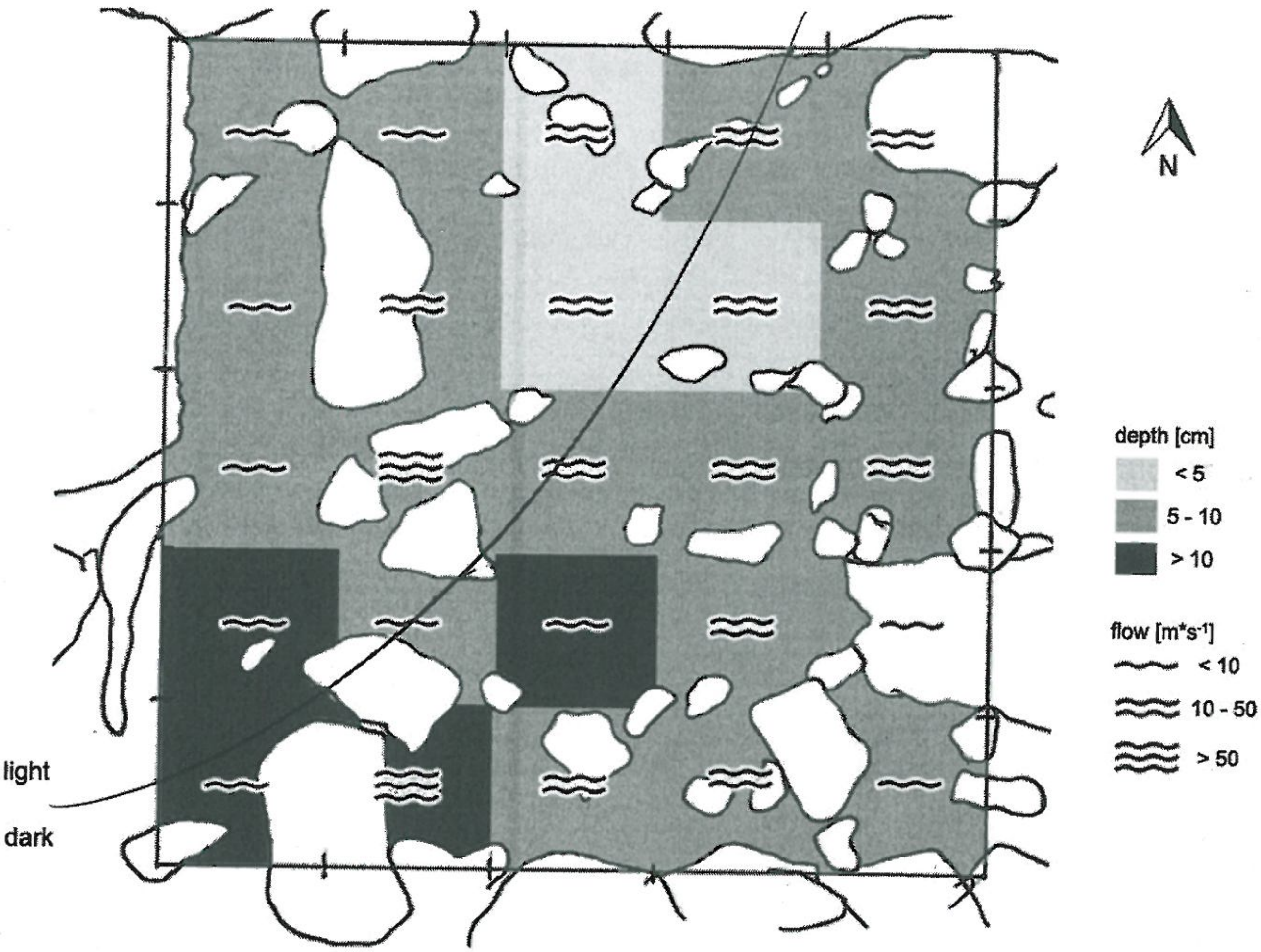


Figure 4

Differences in size-class compositions of *Poecilia mexicana* in the Cueva del Azufre system.

Non-metric Multi-Dimensional Scaling (NMDS) plots based on Bray-Curtis similarities for each sampling site and day.

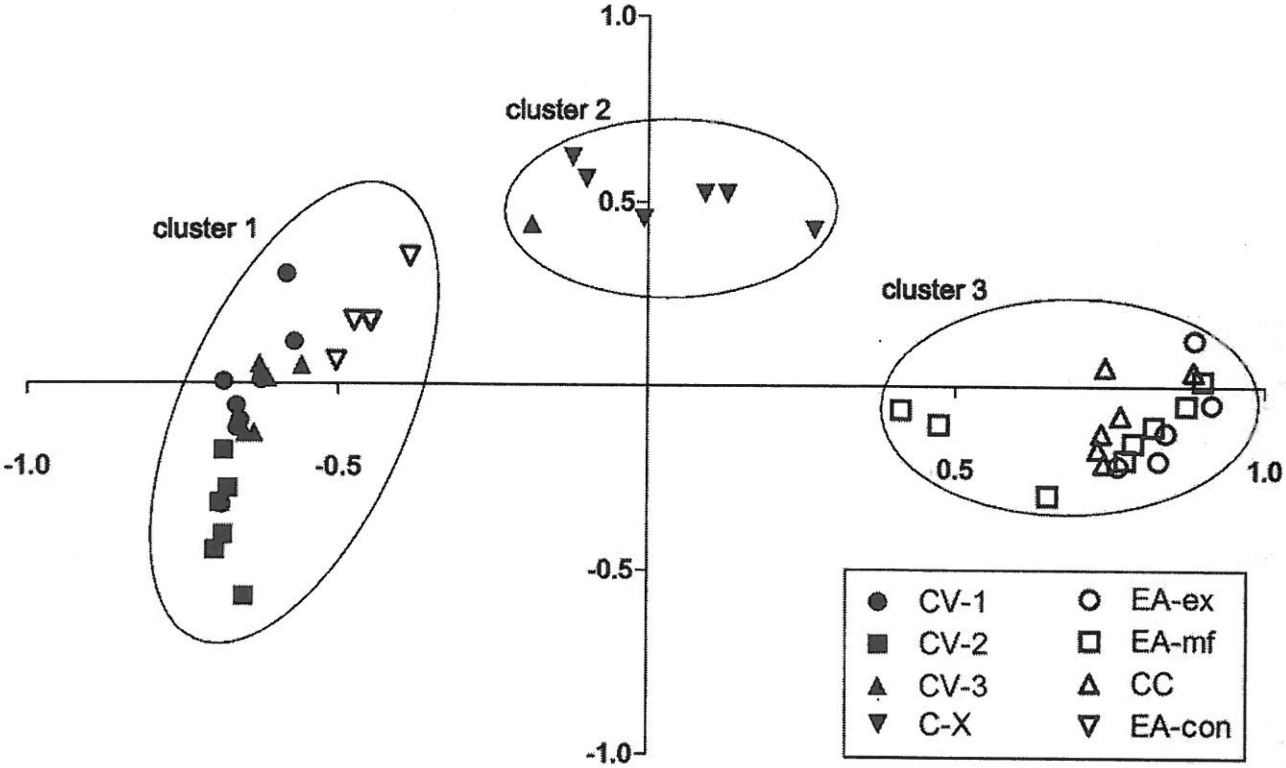
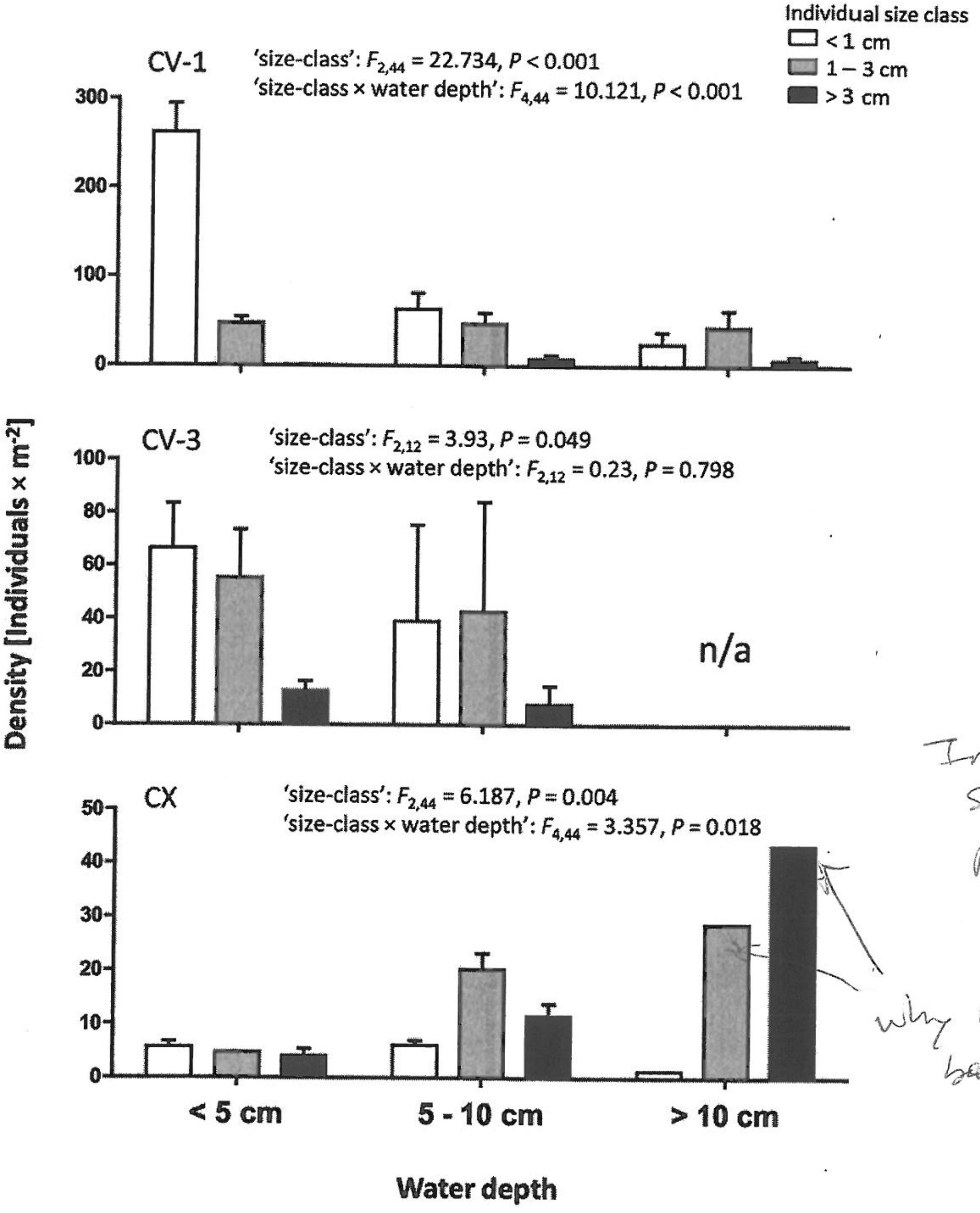


Figure 5

Population densities of cave mollies in the Cueva del Azufre.

Mean (± SE) densities of mollies, categorized in three size classes (< 1 cm, *white*, 1 – 3 cm, *gray*, and > 3 cm, *black*) in three water depths (< 5 cm, 5 – 10 cm, and > 10 cm). Results of rmGLMs are inserted. Note the different y-axis scales.



Indicate significance post hoc test

why no error bars?