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PRIMARY RESEARCH PAPER

Microhabitat selection of axolotls, *Ambystoma mexicanum*, in artificial and natural aquatic systems

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Abstract Studies of habitat selection are crucial for the conservation of threatened amphibians. Wild salamanders are often distributed near rocks or vegetation, which provide shelter. However, nothing is known about habitat selection of the Mexican axolotl (*Ambystoma mexicanum*), an endangered salamander of great cultural and ecological value. This study aims to test the relationship between vegetation presence and the distribution of captive-raised axolotls in two systems: a closed canal in their native ecosystem ($n = 10$) and an artificial canal within a zoological park ($n = 6$). We used radio-telemetry to analyse the hourly distribution and movement patterns of axolotls in each study site during 72-h observational periods. We found that movement patterns and microhabitat selection were related to vegetation coverage and diurnal and nocturnal periods. Sex and age had no effect in habitat selection. Axolotls in both study sites preferred vegetated microhabitats, but in Xochimilco this preference was only significant during daytime when they were less active. These habitat-specific patterns of spatial distribution may represent behavioural strategies for reducing predation. The first approach of behavioural insights from this study will inform the construction of refuges to reduce the alarming depletion of axolotls in the wild.

Keywords Xochimilco · Restoration · Wetland · Aquatic plants · Amphibian conservation · Mexico city

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Introduction

Habitat loss caused by urbanization is perhaps the greatest threat to declining populations of amphibians worldwide (Ficetola et al., 2015; Grant et al., 2016). Amphibians need water for reproduction, and some neotenic salamander species, such as the Mexican axolotl *Ambystoma mexicanum* (Shaw and Nodder, 1798), spend their whole life in the aquatic environment. Yet, freshwater ecosystems have suffered some of the most extreme transformations from urbanization (Ricciardi & Rasmussen, 1999; Hamer & McDonnell, 2008). Understanding habitat selection of threatened and endangered amphibian species is now considered crucial for their successful

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conservation (Chalmers & Loftin, 2006; Mayor et al., 2009). Habitats vary in quality, even at a local scale, and individuals are expected to maximize their survival and reproductive success by choosing the best available habitat (Arlt & Pärt, 2007).

Studies in nature suggest that, when in water, wild salamanders prefer habitats with abundant rocks, vegetation, or both. For instance, the abundance of blackbelly salamanders *Desmognathus quadramaculatus* (Holbrook, 1840), in all its life stages, is positively associated with the density of pebbles and cobblestones (Davic & Orr, 1987). Similarly, the described optimal habitat for fully aquatic, giant Eastern hellbenders *Cryptobranchus alleganiensis* (Sonnini de Manoncourt and Latreille, 1801) are streams or rivers with large, flat rocks and crevices (Foster et al., 2009). Crested newts *Triturus cristatus* (Laurenti, 1768), in contrast, are found most often in shallow ponds with abundant plants and seem particularly attracted to broad-leaved pondweed *Potamogeton natans* Linnaeus, 1753 and square-leaved liverwort *Chiloscyphus pallescens* (Ehrh. Ex Hoffm.) Dumort (Gustafson et al., 2006). Rocks and vegetation supply shelter (Hartel et al., 2007; Foster et al., 2009), protection against potential predators (Denoël & Andreone, 2003) and safe breeding sites where salamanders can mate and lay eggs (Petranka et al., 1982; Miaud, 1993; Marco et al., 2001).

Habitat preferences in salamanders are not universal (see Lecis et al., 2004), and remain unstudied in the Mexican axolotl (*Ambystoma mexicanum*), a charismatic and world-famous salamander that is critically endangered in the wild but is easily reproduced in captivity (Voss et al., 2015). The natural habitat of the axolotl (*Ambystoma mexicanum*) provides a variety of conditions that may influence its habitat selection. Xochimilco, the last remaining area where the axolotl is naturally distributed (Contreras et al., 2009), is a managed but highly polluted wetland composed of a series of muddy, smooth canals that connect lakes of different sizes (Bojórquez & Villa, 1997; Armillas, 2017), which influences macrophyte distribution (Duarte et al., 1986; Capers et al., 2010; O'Hare et al., 2012; La Toya et al., 2013). Vegetation may serve as refuge against predators such as crayfish (Zambrano et al., 2015), fish, snakes and birds (pers. obs). In addition, areas of vegetation attract important axolotl food sources, such as invertebrates and small fish (Ward, 1992; Crump & Scott, 1994; Lehtiniemi et al., 2005; Zambrano et al., 2010).

Therefore, our main hypothesis is that axolotls prefer microhabitats with vegetation coverage in different environments. We therefore expected that the distributions of captive-raised axolotls introduced into a natural canal in Xochimilco or an artificial canal within the Chapultepec Zoo would not be random, but rather associated with the presence of vegetation. Furthermore, we explored whether other factors such as diurnal or nocturnal periods, axolotl age and sex, and the spatial distribution, either homogeneous (even) or heterogeneous (uneven), of vegetation influenced this preference. Understanding the importance of vegetation for habitat selection in this endangered salamander could help improve restoration efforts of their native ecosystem, including the construction of refuges, and provide valuable information for their management in captivity. Moreover, confirmation that captive-raised individuals use vegetation as refuges, an assumed adaptive behaviour documented in wild salamanders of several species, could suggest that reintroduction and relocation programs of the Mexican axolotl are feasible.

Methods

Study sites

The artificial study site was a circular, concrete canal located within the Chapultepec Zoo (ChZ) (19°25′N and 99°11′W) in Mexico City (see photograph in Appendix 1). The zoo is located within the original axolotl habitat in the Mexican basin and is visited by approximately 5.5 million people per year. The 1.2-m-deep water canal was 2.5 m wide and circled around a terrestrial island, together they measured 15 m in diameter (Fig. 1a). Aquatic vegetation was patchily ("uneven") distributed in this habitat in 23 quadrats (Fig. 1). This canal was provisioned with small fish and crayfish before the axolotls were released, and no additional food was given during the 72-h monitoring period. Axolotls could have also used alternative food sources, such as snails, aquatic larvae or insects that colonized the pond. As far as we know, there was no real risk of predation yet; during visiting and working hours, axolotls could have perceived zoo staff and visitors as predators.
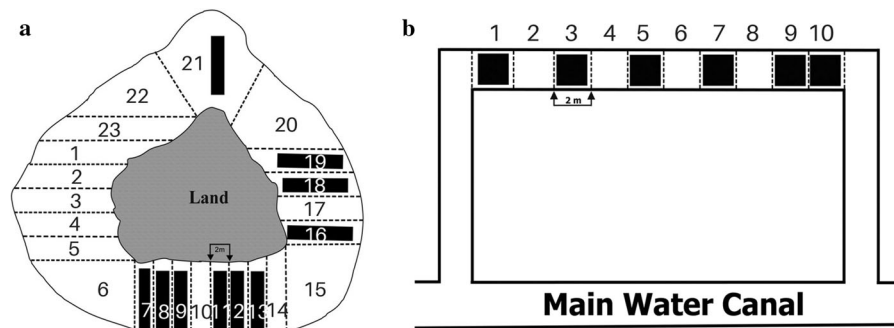
Fig. 1 Experimental plots for axolotl (*Ambystoma mexicanum*) tracking. **a** Chapultepec Zoo; **b** Xochimilco. Black squares and rectangles represent vegetated areas, whereas non-plants areas were left blank. Please note that in the Xochimilco site, vegetation was distributed evenly

The natural study site was a closed canal, or refuge, located in the south of Mexico City within Xochimilco (Xch) (19°15′N and 99°06′W), a 2200 ha wetland with the last remaining wild population of axolotls (Hinojosa & Zambrano, 2004; see photograph in Appendix 1). It measured 1.80 m × 20 m and was approximately 1 m deep (Fig. 1b). This canal had local elements of the wetland but was semi-isolated from the main channel by mesh water filters that were reinforced by a group of emergent plants, creating a barrier to increase water quality and exclude exotic fish (Valiente et al., 2010). Within this canal, macrophytes *Myriophyllum aquaticum* (Velloso) Verdcourt, 1973 and *Eichhornia crassipes* (Mart.) Solms-Laubach, 1883 were planted in five quadrats (1.8 × 2 m), alternated with five other non-plants quadrats of the same size. Therefore, the vegetated and non-plants areas were distributed "evenly" along the whole canal. We assume that axolotls in both canals had similar diets, composed mostly of small fish, crayfish and insects. Potential predators of adult axolotls included snakes and water birds; however, predation pressure on full grown axolotls is considered low. In this natural canal, human presence during the study was negligible.

To track axolotl movement within the study sites, radio-telemetry transmitters were surgically implanted inside the abdomen of 16 captive-raised axolotls, eight males and females. All of these organisms were bred and raised in the Laboratorio de Restauración Ecológica (LRE), Instituto de Biología, UNAM (Permit: FAUT: 0112) in minimalistic conditions (i.e. without live or artificial plants). The axolotls used in this study are third-generation captive raised in the LRE. The originals were donated by the Center for Biological and Aquatic Research (CIBAC).

Each animal was first anesthetized in a benzocaine (10%) bath (3 ml of benzocaine diluted in 1 l of water), and then a one-cm-long incision was made in the ventral area where the transmitter was inserted (full surgical details in Mena & Zambrano, 2016). The weight of the transmitters (930 mg) was less than 2% of the axolotls' total body weight (range 60–105 g), well below the maximum Acceptable 12% limit (Brown et al., 1999). After transmitter implantation, the axolotls recovered in laboratory tanks for 7 days. The LRE's veterinarian (DVM), specialized in *Ambystoma mexicanum*, examined the ability to feed, defecate and move of each axolotl daily and observed no adverse reactions to the transmitters.

Radio-telemetry tracking

Radio-tagged axolotls were introduced into the natural and artificial canals and were monitored with a directional Yagi antenna and TRX receptor. Every hour, during 72 consecutive hours, we registered their spatial location within numbered quadrats that were categorized as either "plants" or "non-plants". Neither of the two canals was artificially illuminated at night, and hand lamps were used to access both study sites. There were three monitoring periods: two in Xochimilco (March and June 2011), and one in the zoo (December 2011). We used six axolotls for the monitoring period in the zoo (females = 4, males = 2), and five axolotls for each of the monitoring periods in Xochimilco (March and June, females = 2, males = 3). No axolotl was used for more than one

monitoring period. The June monitoring period occurred during Mexico City's rainy season. Ten of the sixteen transmitters were Telenax BTX-OO3I, Playa del Carmen, Quintana Roo, Mexico; the rest were Wildlife HWSC materials SOPI-2011, Murphysboro, Illinois, USA.

Statistical analyses

We first performed Chi-square tests to test for equal distribution of axolotls between plants and non-plants quadrats within the natural and artificial canals. Then we used generalized linear mixed models, with binomial distribution and axolotl id as a random effect, to examine whether factors such as time (diurnal and nocturnal periods), and axolotl sex and age, influenced their preference for plants (= 1) or non-plants quadrats (= 0). Based on local sunrise and sunset times, we considered 7:00–18:00 h as diurnal and 19:00–06:00 h as nocturnal. Because personal observations in captivity suggested that juveniles might be more active, age was treated as a categorical variable divided into two groups: juveniles (< 1 year old), and adults (1 year or older).

We used a correspondence analysis (CA) to determine whether the presence of plants in each quadrant was associated with a differential use of refugees among axolotls. CA is a multivariate descriptive method that uses χ^2 tests to determine the relationships between categorical variables and provides factor scores (coordinates) to visualize the "best" two-dimensional (Dim 1 and Dim 2) representation of the data (Hill, 1974). We used the categorical variables: (a) axolotl identity, (b) the identity of each quadrant together with its relative use by the axolotls, and (c) the presence or absence of vegetation. In this study, Dim 1 was used as a scale of variation between habitat preference of individual axolotls, and Dim 2 as a combination of dispersion and presence or absence of vegetation and the results were displayed in a biplot (Zermeño et al., 2013). The points within the graph are the calculated scores for each categorical variable; the closer the distance, the higher the association (Rodriguez-Lara et al., 2014).

To describe movement within the canals, the following parameters were used: total distance travelled, average distance travelled during the day, and average distance travelled during the night (Janowsky-Bell & Horner, 1999; Faccio, 2003). To evaluate

hourly movement patterns, we assumed that axolotls used the shortest distance between two points. A Kruskal–Wallis ANOVA was used to test for differences in movement patterns.

Results

The efficiency of detecting the axolotls by telemetry was high. We were able to track axolotls 85% of the time that effort was expended during the three monitoring periods.

Axolotls within the artificial zoo canal were much more likely to be in plants than non-plants quadrats ($\chi^2 = 126.21$, $P < 0.0001$). Though distribution within the natural Xochimilco canal appeared random (March, $\chi^2 = 0$, $P = 1$; June, $\chi^2 = 0.0015$, $P = 0.973$), when taking time into account we found that, during daytime, there was a high probability of axolotls being within vegetated quadrats (glmer: $P = 0.039$; Table 1; Fig. 2). The preference for vegetated quadrats observed in the axolotls from the artificial zoo canal did not vary between diurnal and nocturnal periods (glmer: $P = 0.9$), nor did we find any significant effect of axolotl sex or age on their distribution within plants or non-plants quadrats in either canal (Table 1).

As depicted in Fig. 3a, in the artificial zoo canal (ChZ) where plants were heterogeneously distributed the axolotls had a percentage of use significantly lower than expected ($\chi^2 = 8.08$, $P < 0.05$). Meanwhile, in

Table 1 Generalized linear mixed models, of the natural Xochimilco canal ($n = 626$) and the artificial zoo canal ($n = 432$), showing the relationship between the probability of an axolotl being distributed within vegetated quadrats and its age, sex and time (diurnal and nocturnal periods)

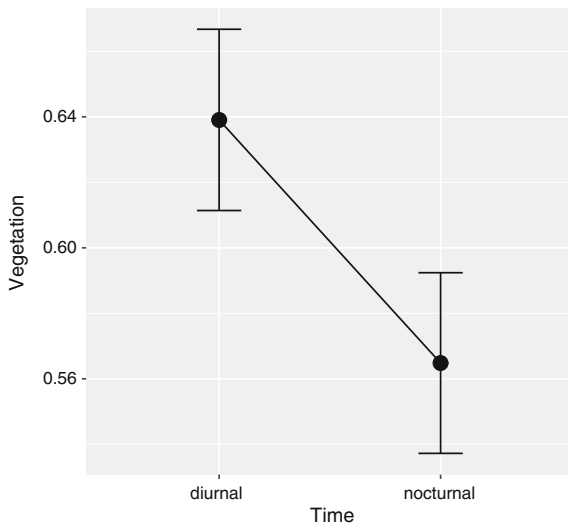|	Estimate ± SE	P
Natural Xochimilco canal		
Intercept	1.101 ± 0.35	0.0018
Age	− 0.171 ± 0.33	0.61
Sex	− 0.58 ± 0.34	0.092
Time	− 0.35 ± 0.17	0.039
Artificial zoo canal		
Intercept	1.47 ± 0.21	< 0.001
Age	0.35 ± 0.29	0.23
Sex	− 0.37 ± 0.27	0.18
Time	− 0.03 ± 0.24	0.90

Fig. 2 Probability of axolotls (*Ambystoma mexicanum*) in the Xochimilco canal being within vegetated quadrats according to diurnal and nocturnal time periods

the natural Xochimilco canal (XochM and XochJ), where vegetation distribution was homogeneous (even), the axolotls had a percentage of use that did not differed from the expected value ($\chi^2 = 0.32$, $P > 0.05$). Distributions of individual axolotls within canals are shown in Appendix 2. Multivariate analysis (CA) revealed that in each study site the axolotls were differentially and unevenly associated to each one of the quadrats (Fig. 3). Both in the artificial canal (Fig. 3c) and the two experiments from the natural canal (Fig. 3b and d), the axolotls were distributed into 2–4 clusters, of which only one cluster was composed by two or more axolotls that used preferentially the parts of the quadrats covered with vegetation. On the contrary, those cluster formed by only one axolotl showed that each individual preferred a single quadrat, in which each axolotl was mainly associated to the plants-covered parts of the quadrats. Interestingly, in all three experiments the axolotls were highly associated to the parts of the quadrats that had plants, as judged by the closer distance between the data points representing each axolotl and the data points representing vegetation within a quadrat (Fig. 3). In all cases, Dim 1 (individual axolotls) showed the maximum discrimination for habitat preference and explained between 35.1 and 59.7% of the total variation. In the artificial canal, the two factors from the CA (axolotl and quadrant) explained only 62.7% of

the total variation (ChZ, Fig. 3c), probably because there was one axolotl that showed no particular preference for any quadrat, whereas the remaining individuals had a preference pattern for the different quadrats. In regard to the natural canal, during the first experiment (XochM, Fig. 3b) the CA explained 85.1% of the total variation and 75.2% in the second experiment (XochJ, Fig. 3d).

The total distance that axolotls moved ranged from 0 to 23 m during the first hour of monitoring (Fig. 4A). Axolotls moved between 60 and 320 m over the complete 72 h, with longer (but not significantly so) total distances recorded for axolotls in the zoo canal than in Xochimilco (Fig. 4B). The average distance travelled per hour by each axolotl was between 2 and 2.8 m/h and was similar across all three monitoring periods, although more variability in distance travelled was observed for axolotls in the zoo canal (Fig. 4C). Significant differences in movement were observed between diurnal and nocturnal periods. The distance recorded during the nocturnal phase for both Xochimilco monitoring periods was significantly greater than that observed during diurnal phase, and it accounted for 68–71% of the total distance travelled. For axolotls in the zoo canal, there was no significant difference ($P > 0.05$) between nocturnal and diurnal travelled distances (Fig. 5).

Discussion

The distances travelled by axolotls within each monitoring period were sufficiently long to allow individuals to familiarize themselves with the different microhabitats in each study site. We observed that axolotls tended to locate to specific patches, supporting the idea that axolotls can perceive differences in microhabitats and choose particular areas more frequently than others (Simonetti, 1989). Our results suggest that axolotls in the natural Xochimilco canal preferred vegetated microhabitats during daytime, when they were less active. Similarly, in the artificial zoo canal, axolotls preferred microhabitats with plants cover, but this preference occurred regardless of diurnal and nocturnal cycles. The use of rocks and vegetation within aquatic environments for shelter, protection against predators and egg-laying has been documented in several species of wild salamanders (Davic & Orr, 1987; Gustafson et al., 2006; Foster
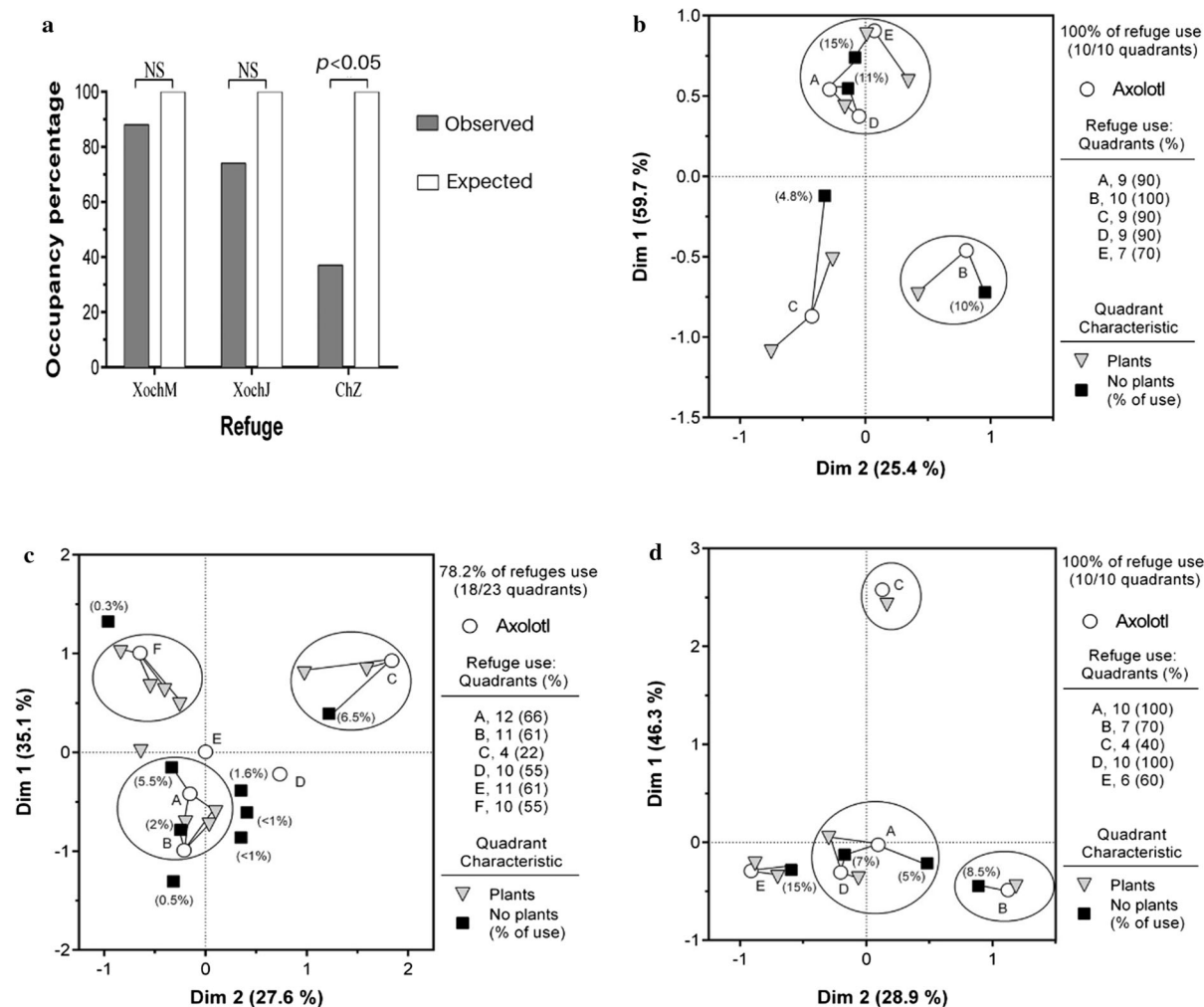
Fig. 3 **a** χ^2 test of the percentage occupation of patches in the three experiments. Correspondence analysis of the pattern of association among individual axolotls (*Ambystoma mexicanum*) and the different quadrants according to the presence or absence of vegetation in **c** Zoológico de Chapultepec, **b** the first experiment in Xochimilco and **d** the second experiment in Xochimilco. In **b–d**, the biplots show the relationship among the categories for each variable: the closer the adjacent points are, the higher the preference between an individual axolotl and a quadrant with or without plants. The Dim 1 and Dim 2 are the factors (axolotls and quadrants) that segregate data points and explain the variation associated to the observed data. In each biplot, we draw arbitrary ellipses to help interpretation: data points within ellipses showed closer distance and thus higher association. Data points lying in the centre of the biplot (Dim1 = 0, Dim 2 = 0) showed no particular preference for any particular quadrant

et al., 2009), but studies of captive-raised animals, which often lack behavioural skills associated with predator-avoidance and foraging efficiency (Kelley & Macías-Garcia, 2010), reintroduced into natural environments are rare.

In Xochimilco, there are predators that feed on axolotls at different life stages. For example, exotic fish, primarily tilapia *Oreochromis niloticus* (Linnaeus, 1758), of which there are large populations in

this wetland (Zambrano & Mazari, 2011), feed on axolotl larvae and juveniles and harass adults (unpubl.) Native crayfish *Cambarellus montezumae* (Saussure, 1857) also predates on them at larvae stages, but they become food of adult axolotls (Zambrano et al., 2015). Though the study canal was enclosed and no predatory fish could enter, the semipermeable barrier allowed water flow and axolotls could have behaviourally reacted to predator

Fig. 4 Axolotl
(*Ambystoma mexicanum*)
movements throughout the
experiment. **A** Metres
travelled by axolotls in the
first hour of the experiment.
B Average total distance
(metres) covered by the
axolotls in each
experimental set. **C** Average
distance travelled by the
axolotls per hour. XochM is
the first experiment, and
XochJ is the second
experiment. ChZ is the zoo
experiment. The bars in the
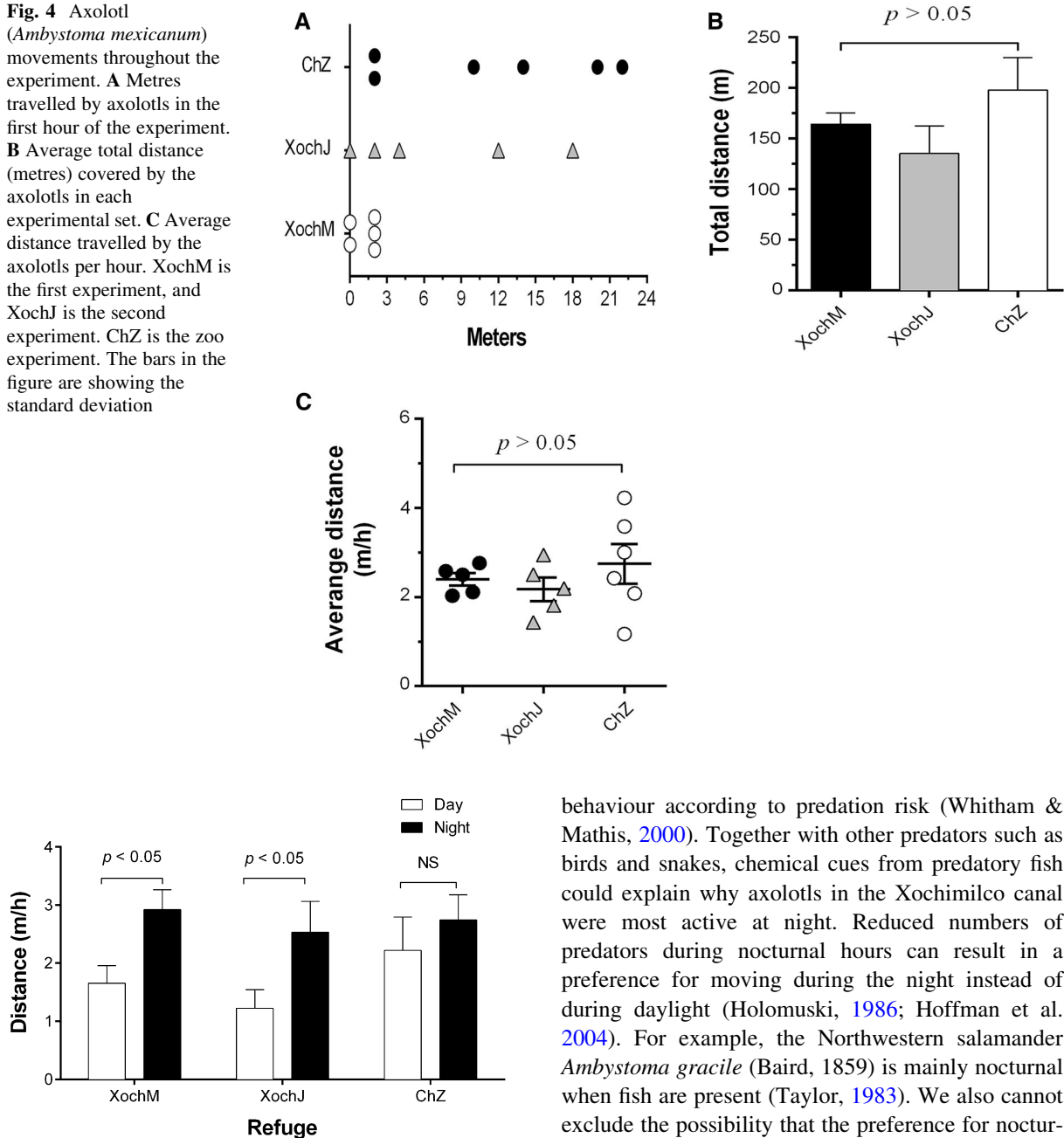figure are showing the
standard deviation

Fig. 5 Distance covered during day and night in each
experimental set. XochM is the first experiment, and XochJ is
the second experiment. ChZ is the zoo experiment. The bars in
the figure are showing the standard deviation

odours (Wisenden, 2000; López, 2012). Neotenic
graybelly salamanders *Eurycea multiplicata* (Cope,
1869) rely more on chemical than visual cues to detect
predators (Hickman et al., 2004), and can modify their

behaviour according to predation risk (Whitham &
Mathis, 2000). Together with other predators such as
birds and snakes, chemical cues from predatory fish
could explain why axolotls in the Xochimilco canal
were most active at night. Reduced numbers of
predators during nocturnal hours can result in a
preference for moving during the night instead of
during daylight (Holomuski, 1986; Hoffman et al.
2004). For example, the Northwestern salamander
Ambystoma gracile (Baird, 1859) is mainly nocturnal
when fish are present (Taylor, 1983). We also cannot
exclude the possibility that the preference for noctur-
nal behaviour in these neotenic salamanders is a
adaptive trait conserved in adult ambystomatids
(Keen, 1984; Petranka, 1998) even though they
remained in an aquatic environment.

Axolotls within the artificial zoo canal showed a
preference for vegetated (plants) quadrats indepen-
dently of diurnal and nocturnal time periods. Further-
more, their activity levels did not vary between night
and day. But, the hypothesis that predation risk

influences daily patterns (Taylor, 1983; Semiltch, 1987; Hoffman et al. 2004) seems to be supported by our finding of higher nocturnal movements in Xochimilco (where predators and their chemical cues were present) than in the zoo (where predators were completely absent).

Another factor that may influence microhabitat selection is environmental seasonality. Salamanders modify their activities with respect to climate conditions based on changes in temperature or sunlight (Orser & Shure, 1975). Lower temperatures (below 17°C) are related t2007o the reproductive season in *A. mexicanum* (Ferreira et al. 1993; Marín,). The coldest months (November to March) coincide with the highest rates of reproduction. Considering that vegetation coverage could be especially important for reproduction, as female axolotls require vegetation to lay their eggs in protected sites (Salthe, 1969; Marín, 2007), seasonality could have generated the preference for vegetated quadrats independent of diurnal and nocturnal cycles observed in the zoo canal. Axolotls were introduced into the artificial zoo canal in December—well within the reproductive period, and in March and June in Xochimilco.

In the Xochimilco canal, vegetation and axolotls - were uniformly distributed and individuals were spatially separated, with no grouping, whereas in the heterogeneous (uneven) zoo canal, axolotls seemed to form groups in close association with vegetation coverage and remained in groups. By comparing the results from both study sites, it is possible to suggest that the heterogenous habitat in the zoo canal resulted in the grouping of organisms within high-quality patches. However, other factors that could have caused grouping, like seasonality, are impossible to tease out. Though a study with *Ambystoma tigrinum* (Green, 1825) found that, in a shaded pond, females and males selected vegetated and non-plants areas, we found no difference in habitat preferences according to axolotl sex (Madison, 1998).

Our study confirms that vegetation is an important habitat component for axolotls. We believe this knowledge will be important for the maintenance and management of this species, and it will also aid restoration efforts in its native ecosystem (Voss et al., 2015). Many reintroduction programs of endangered animals fail, often because captive animals lack behavioural skills needed for survival (Kelley & Macías-Garcia, 2010). That captive-bred axolotls, raised in minimalistic conditions (i.e. without live or artificial plants), used vegetation as shelter in both artificial and natural environments gives hope for the future success of reintroduction and relocation programs of this species. However, further questions arising from this project need to be studied to better understand the habitat selection and daily activities of axolotls.

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