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CAVE MOLLY MALES (*POECILIA MEXICANA*) DO NOT RECOGNISE RECEPTIVE FEMALES WITHOUT BODY CONTACT

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We investigated the preference of Cave molly males to associate with a receptive or a non-receptive female while body contact was prevented but water-borne cues were allowed to reach the male. Both in light and in darkness, males did not prefer the receptive female. Water-borne chemical cues (pheromones) seem to be absent or they are not detected/used by males, respectively.

Key words: Poeciliidae, sexual cycle, pheromones, cave fish

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Istraživali smo način na koji mužjak špiljske ribe *Poecilia mexicana* odabire receptivnu odnosno nereceptivnu ženku, pri čemu je tjelesni kontakt bio onemogućen, ali su čimbenici nošeni vodom mogli doprijeti do mužjaka. Niti na svjetlu niti u tami mužjaci nisu preferirali receptivne ženke. Proizlazi da vodom nošenih tvari (feromona) nema, ili ih mužjaci ne raspoznaju, odnosno ne koriste.

Ključne riječi: Poeciliidae, spolni ciklus, feromoni, špiljske ribe

INTRODUCTION

The colonisation of lightless subterranean habitats requires that all behaviour patterns must be released by non-visual cues. In the context of reproductive behav-

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iour, this means that potential mating partners can only be detected by using chemical, acoustical, vibrational/tactile or electro-sensitive senses.

The cavernicolous population of the Atlantic molly (*Poecilia mexicana* Steindachner 1863) offers a unique tool to study sensory and behavioural adaptations of a formerly surface dwelling, diurnal species to life in darkness (PARZEFALL, 2001; PLATH *et al.*, 2004). The Cave molly inhabits a dark sulphurous limestone cave in South Mexico (GORDON & ROSEN, 1962).

In viviparous fishes (Poeciliidae), females have a sexual cycle and Cave molly females become receptive for roughly 2–3 days per month (PARZEFALL, 1973). Males touch the female gonopore before attempting to copulate, thereby achieving information concerning gender and a female's stage in the sexual cycle (PARZEFALL, 1973). Receptive females are most attractive for males (PARZEFALL, 1973). However, molly males almost constantly try to mate with any available female.

Previous studies have suggested that males need direct body contact to obtain the necessary chemical information (ZEISKE, 1968). In the present paper, we tested this hypothesis. Do males prefer to associate with receptive females on the basis of water borne far-field signals (chemical cues) when they cannot nip at the female gonopore?

METHODS

Test fish came from light reared laboratory stocks of Cave mollies originating from the hindmost isolated cave chamber of the Cueva del Azufre in Tabasco, Mexico (chamber XIII after GORDON & ROSEN, 1962; PLATH *et al.*, 2003). Fish were maintained as randomly outbred large aquarium stocks at the Biozentrum Grindel in Hamburg in several tanks of 100–200 l at a temperature of 25–30 °C under a 16:8 hour light-dark regime.

To determine the receptivity of the stimulus females, three females were housed in 25 l aquaria with one male for two months. We daily counted a male's nipping frequency for each female during five minutes. Nipping frequency increased approximately each 28 days when females became receptive (PARZEFALL, 1973).

In the choice tests, males were given the opportunity to associate with a receptive female and a non-receptive female, matched for size, which were confined to wire mesh cylinders (12 cm diameter, 5 mm mesh-width, 1 mm wire-diameter). The cylinder material allowed non-visual cues from the females to pass through (PLATH *et al.*, 2004). Cylinders were placed in the two outer preference zones (1/3 of the tank) of a test tank (100 × 35 × 35 cm), filled up to 2/3 with aged tap-water of 28–30 °C (Fig. 1). The bottom was covered with black gravel.

When the two stimulus females were swimming calmly in their cylinders, a male was gently introduced into the central neutral zone. We measured the time the male spent in both preference zones during 10 minutes. Then, the positions of the cylinders were reversed and measurement was repeated. Times from both parts of a trial were added. We decided *a priori* to exclude trials in which the males spent more than 80 % of their time in only one preference zone as side biases and trials with low response (<50 % time spent in the preference zones).

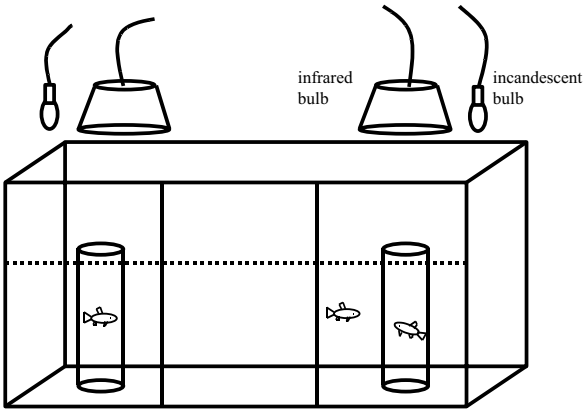

Fig. 1. Experimental set-up. For details see text.

Seven side biases occurred. In neither case trials had to be discarded due to low response. We tested for differences in the association times near either males using paired *t*-tests.

Cave mollies still posses functional eyes (PETERS *et al*., 1973). Hence, we tried to detect whether visual cues from the females provide additional information about females' receptiveness. Therefore, experiments were carried out both in darkness (two 500 W infrared bulbs 28 cm above the test tank; Fig. 1) and in light (the test tank was illuminated by two 60 W incandescent bulbs) in random order. Cave mollies do not see in the infrared (KÖRNER 1999; KÖRNER *et al*. in prep.). During the tests in light, the observer was sitting quietly approximately 2 m from the test tank. During the tests in darkness, the trials were recorded by an infrared-sensitive camera and monitored in a neighbouring room, where trials were scored directly (PLATH *et al*., 2004).

RESULTS

In light males did not discriminate between receptive and non-receptive females (paired *t*-test: t=0.43, n=11, p=0.67, power=0.05). They spent 52.43±18.57 % of their time near the receptive female and 47.57±18.57 % near the non-receptive female.

Males also showed no preference in darkness (paired *t*-test: t=–0.60, n=11, p=0.56, power=0.05). They spent 48.55±8.02 % of their time with the receptive female and 51.45±8.02 % near the non-receptive female. In both experiments only five of 11 males spent more time near the receptive female.

DISCUSSION

Our study confirms the assumption that near-field communication plays a major role in the mating behaviour of Cave mollies (ZEISKE, 1968; PARZEFALL, 1970). However, seven trials had to be discarded due to side bias. Hence, sample sizes are rela-

tively small in this study, resulting in low power of the statistical analyses and the results are discussed with caution.

Cave molly males obtain information concerning gender when they nip at the female gonopore before copulating (PARZEFALL, 1973). Sex recognition appears to be impossible when the male cannot touch the female (PLATH *et al*., 2003). Males can also determine a female's sexual attractiveness when nipping (PARZEFALL, 1973). Here, we demonstrate that female receptiveness cannot be determined by males on the basis of non-visual (i.e. chemical or water displacement) cues. The high H_2S content of the water inside the cave may make chemical communication via water borne pheromones impossible. Furthermore, Cave mollies occur at high density in some parts of the cave and often gather in shallow areas. Selection pressure to improve the ability of far-field recognition of female receptiveness might be low, because males can attempt to touch neighbouring fish. Even in light, no discrimination was found, indicating that visual cues do not indicate females' receptiveness.

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