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THE TWO PERCEPTUAL WORLDS OF AMPHIBIOUS FISHES: THE SENSORY ECOLOGY OF *PERIOPHTHALMUS BARBARUS*

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THE TWO PERCEPTUAL WORLDS OF AMPHIBIOUS FISHES: THE SENSORY ECOLOGY OF *PERIOPHTHALMUS BARBARUS*

A THESIS APPROVED FOR
SCHOOL OF BIOLOGICAL SCIENCES

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ABSTRACT

The evolutionary transition of vertebrates from water to land required extensive modifications to sensory systems originally adapted for aquatic environments, enabling them to function effectively in terrestrial settings. Mudskippers (Oxudercidae), amphibious fishes that live in both aquatic and terrestrial habitats, serve as a powerful model for investigating how animals manage two distinct sensory worlds. This study tested whether mudskippers experience separate sensory experiences in water and on land by measuring visual and olfactory acuity in each environment. Visual acuity was assessed using an optomotor assay, and olfactory sensitivity was measured by recording electro-olfactogram (EOG) responses to a panel of odorants. Results show that mudskippers have significantly higher visual acuity in air than in water, supporting prior predictions based on morphological adaptations for aerial vision. In contrast, olfactory responses were markedly stronger and more diverse in water. Together, these findings demonstrate that mudskippers possess distinct sensory capacities in air and water, supporting the hypothesis that they perceive land and sea through different senses. These results highlight the importance of sensory tradeoffs during the water-to-land transition and call for further research into the mechanisms underlying multimodal sensory adaptation in amphibious species.

INTRODUCTION

Approximately 375 million years ago, fishes began transitioning from life in the water to life on land (Clack, 2012; Coates et al., 2008; Ahlberg, 2018). This transition involved a suite of anatomical and physiological transformations, including modifications to the feeding, locomotor, and sensory systems (Coates, 1990, 1996; Ahlberg et al., 2005; Shubin et al., 2006; Pierce et al., 2013; Stewart et al., 2020; Lemberg et al., 2021; MacIver et al., 2017; Cloutier et al., 2020). Some hypotheses suggest that vision and chemoreception may have played an early role in the transition to terrestrial life, with gradual modifications increasing their effectiveness in new environments (MacIver et al., 2017; Mollo et al., 2014). This suggests that animals transitioning between water and land live in two perceptual worlds, or *umwelten* (von Uexküll, 1934), where they experience their environment differently in water than they do on land. In this study, I focus on mudskippers — amphibious fishes that serve as a present-day model for the evolutionary transition from aquatic to terrestrial life (You et al., 2014; Polgar et al., 2011) — to investigate whether they indeed experience two distinct perceptual worlds.

Mudskippers (Oxudercidae) are fishes in the order Gobiiformes, which evolved away from their ancestors' aquatic way of life and live most of their life on land (Clayton, 1993; Gordon et al., 1978). Previous studies have investigated several aspects of mudskipper adaptations to terrestrial life, including: physiological traits, such as cutaneous and buccal respiration (Graham, 1997); morphological traits, such as modified fins for terrestrial locomotion and raised eyes capable of blinking which supports aerial vision (Stebbins & Kalk, 1961; Aiello et al., 2023); and genetic adaptations that indicate mechanisms for desiccation resistance, ammonia excretion, vision, and olfaction (You et al., 2014; Hu et al., 2022). These studies highlight the integrated

evolutionary changes across multiple bodily systems that have enabled mudskippers to thrive in a terrestrial environment.

Among these adaptations, sensory systems present a unique challenge, as their function is tightly constrained by the physical properties of the surrounding medium. Given the fundamental physical differences between air and water, such as refractive index for vision (Thewissen & Nummela, 2008), odorant solubility and volatility for olfaction (Mollo et al., 2014), and medium density and viscosity for the lateral line system (Coombs & Montgomery, 1999), no organism can be fully optimized to meet the sensory demands of both environments simultaneously. This must mean that mudskippers face unique challenges compared to other animals, where some senses may be useless or dampened in one environment while necessary or effective in others. This could potentially lead to mudskippers having two separate *umwelten* where in one environment they prioritize one sense and, in another environment, prioritize information from another sense. While lateral line, hearing, and taste are also affected by the transition between water and air, vision and olfaction are particularly interesting because previous work suggests these senses may retain partial function on land. For example, mudskippers exhibit unique eye adaptations for aerial vision (Sivak, 1978; Aiello et al., 2023), and recent morphological analyses show adaptations to their olfactory system that potentially support the operation of this sense while on land (Kim et al., 2019; Kuciel, 2013). These findings raise the question of whether mudskippers use vision and olfaction effectively on land, and if so, how their sensory acuity compares between water and air.

The first sense I studied for my thesis is vision. Vision is a complex sensory system that can be broken down into several components (spectral sensitivity, acuity, and temporal resolution), each of which can be studied independently. My overarching question is: which aspects of mudskipper vision may already show adaptations to life on land? In this case, I focused on one

key visual trait, visual acuity, with the prediction that it may be enhanced in air relative to water. Visual acuity refers to how well an organism can resolve fine details or distinguish small differences between objects, and it plays a critical role in tasks such as detecting prey, finding mates, avoiding predators, and navigating the environment.

Mudskippers are hypothesized to have better aerial vision than aquatic vision (Stebbins and Kalk, 1961). This is because they are known to have an elliptical lens, instead of a spherical lens, which would benefit aerial vision (Sivak, 1978). Additionally, some of their most important behaviors associated with survival and reproduction occur on land, such as mating displays, foraging, and resource guarding (Clayton and Townsend, 2017; Michel et al., 2015). Considering that the most vital behaviors for survival are carried out in a terrestrial setting, it would be adaptive to have better aerial vision. To test this, I ran an optomotor experiment to investigate aerial and aquatic vision in mudskippers. The optomotor response, an innate reflex in which an animal follows the movement of a surrounding visual pattern, has been used for decades to measure visual acuity (McCann and MacGinitie, 1965). Since then, this response has been observed across a wide variety of taxa, including fishes (Caves et al., 2017).

The other sense I examined in my thesis is olfaction. Like vision, olfaction is a complex sensory system composed of multiple components that can be studied independently, such as olfactory processing, the different types of olfactory receptors, and the accessory structures that work with the neurons. My overarching question is: is the olfactory system of mudskippers adapted to life in water and on land? In this case, I focused on the basic sensory function of odor detection, specifically, whether olfactory receptor neurons (ORNs) respond to odorants in both air and water. Understanding this is important because olfaction plays a critical role in essential behaviors such as foraging, social communication, and predator avoidance.

Mudskippers have multiple olfactory adaptations that may support this sense while they are on land. Their external nasal morphology consists of two sets of nostrils; their excurrent pair is directly below their eyes, and their incurrent pair is on the edge of their upper jaw (Kuciel, 2013). All members of Oxudercidae have lost the typical olfactory rosette seen in all other teleost fish and instead have a continuous sensory epithelium throughout their olfactory canal with an olfactory bulb near the excurrent nostril (Kim et al., 2019). For olfactory behavior, when these fish raise their head, accessory nasal sacs within the canal collapse (Kuciel, 2013). When lowering their head, these sacks are no longer compressed. This change of pressure allows the animal to draw in fluids, which has the proposed function of sucking water into their olfactory canal as means of keeping the epithelium moist (Kuciel, 2013). In addition to this behavior, their olfactory canal consists of glycogen-producing mucosal cells which is proposed to help in mucosal production to keep the epithelium moist (Kim et al., 2019) this could help with chemoreception as a moist epithelium is necessary for compound detection (Getchell and Getchell, 1992). I hypothesize that mudskippers maintain olfactory function in both aquatic and terrestrial environments due to their well-developed olfactory system. I investigated whether they can detect sensory cues on land and in water by measuring their ORNs response to various odorants

Overall, in my thesis, I am testing the visual acuity and olfactory abilities of mudskippers, both in the water and on land, using an optomotor assay and recording the summated generator potential of the olfactory epithelium. The purpose of these experiments is to test whether mudskippers experience distinct umwelten in water and on land, and to determine how their visual and olfactory systems are adapted to function in each habitat.

METHODS

Animals

This study was conducted on 26 wild-caught *Periophthalmus barbarous* individuals received from a local pet shop, Wet Pets by Steve, in Norman, OK. The fish were housed separately in individual tanks since knowledge from mudskipper aquarists suggests that aggressive behaviors occur when housed together. Aquarists also recommended a 12-hour light and dark cycle since this matches their year-round light conditions. Each experimental tank was designed to mimic a semi-terrestrial environment. Approximately one-half of the tank contained a mud substrate platform for the mudskippers to emerge onto (collected from Clinton Lake, KS and sterilized by baking and rinsing with excess hydrogen peroxide), while the other half was filled with brackish water (specific gravity 1.005–1.010). The water level was maintained at about one-quarter of the tank's height, leaving the upper three-quarters filled with air. The temperature in the room was kept between 24–29 degrees Celsius with a humidity higher than 50%. All individuals had total lengths between 98–120mm, with an average total length of 111 mm. Individuals were sexed according to specifications provided in Udo et al. (2016). [Care for the fish and experimental protocols were approved by the University of Oklahoma's Institutional Animal Care and Use Committee - protocol numbers 2023-0266 and 2024-0298].

VISION

Testing chamber

The maximum spatial frequency that a mudskipper can resolve was tested using an optomotor drum, which consists of a stationary platform that is surrounded by a rotating visual stimulus. As the drum rotates, the animal's reflexes make it turn or move its body, head or eyes to track the rotation (McCann and MacGinitie, 1965). The optomotor drum and visual stimuli used in this study were constructed using accepted methods outlined in Caves et al. (2020). Dimensions

of the optomotor drum were adjusted from the original study according to the dimensions of *P. barbarus*. A standard circular acrylic fish tank (24.5 cm in diameter, 30 cm in height) housed a 10 cm in diameter clear acrylic tube, which maintained a consistent viewing distance between the fish and the visual stimuli. An LED lamp was placed overhead with a diffusion filter on the chamber lid to provide consistent lighting.

Stimuli

Outside of the stationary platform, a ring of visual stimuli (25 cm diameter, 30 cm height) was constructed using copy paper with laser-printed patterns. Patterns consisted of alternating black and white stripes with varying cycles per degree (CPD), or the number of black and white stripes in 1 degree of vision. Preliminary trials showed that mudskippers have worse vision in water than on land, so the water trials' CPD range that was tested was adjusted to accommodate their likely visual acuity. For water trials, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 CPD were used, while 0.8, 0.9, 1.0, 2.0, 3.0, and 4.0 CPD were used for land trials. All stimuli were presented in a random order.

Experimental Design

The visual response to stimuli was recorded in *P. barbarus* (n=12). During aquatic trials, the drum was filled with approximately 15 cm of water that matched the salinity and temperature of their housing tanks prior to subject insertion. During aerial trials, no water was placed in the chamber. Fish were placed into the chamber via a small entry point at the top of the drum, which was then followed by an acclimation period of five minutes. The stimuli rotated around the stationary platform at a speed of 1 RPM for two 45-second intervals (one clockwise and one counterclockwise rotation) separated by a 2 minute period. A rotation speed of 1 RPM was chosen

because this elicited the strongest optomotor response in preliminary trials. A 45-second interval was chosen because it can take mudskippers 30 seconds to respond to visual stimuli. A camera placed overhead the chamber opening was used to record subjects.

Video recordings were scored to determine whether the mudskipper responded to the stimulus. Preliminary data showed that mudskippers did not have a strong optomotor response; therefore, a positive response was characterized as the mudskipper moving its head and/or eyes in only the direction of the stimulus and at the same speed as the stimulus. If a mudskipper moved with the stimulus for both the clockwise and counterclockwise rotation, then this was counted as a positive response. While no response to the stimuli was characterized as no movement of the head or eyes in the direction of the stimulus for at least one of the two presentations per trial. At the end of each trial, fish were returned to their enclosures, and the drum was rinsed with water to reduce the possibility of pathogenic transmission between individuals. This was done for both terrestrial and aquatic trials. Each fish was used only once per stimulus, but individuals participated in multiple trials across the course of the experiment.

Vision Analysis

The analysis was based on that of Dobberfuhl et al. (2005). To quantify the animals' ability to detect visual stimuli, we defined the response threshold as the spatial frequency (CPD) at which animals respond positively 50% of the time. This threshold was estimated separately for each environment (air and water) by fitting a binomial logistic regression model to the response data. Only trials with non-zero spatial frequency (i.e., excluding control trials with CPD = 0) were included in threshold calculations, as control trials did not represent true visual stimuli.

To evaluate overall trends and interactions between stimulus and environment, an additional logistic regression model was fit using all data combined (still excluding trials of 0

CPD). This model included spatial frequency (CPD), environment (air vs. water), and their interaction (CPD × environment) as predictors.

Model coefficients and statistical significance were obtained from the fitted model summaries. All analyses were performed in R (version 2024.12.0) using the package dplyr and visualized with ggplot2.

OLFACTION

Pre-experimental setup

Randomly selected mudskippers (n=10) that were used in the vision experiment were anesthetized by immersion in water (1.005 specific gravity) containing 200 mg/L MS222 (3-aminobenzoic acid ethyl ester) buffered with 400 mg/L NaHCO3 and immobilized with 10 mg/kg gallamine triethiodide (Sigma–Aldrich) injected intracoelomically. Once anesthetized and immobilized, the fish were wrapped in a damp cloth and placed in a V-shaped Styrofoam holder with aerated water containing 50 mg/L MS222 being poured over the gills (∼100 ml/(kg)●(body weight)●(min)) via a gravity-fed tube inserted into the mouth. The olfactory system in P. barbarus lacks a typical rosette structure (Kuciel et al., 2011), so the olfactory canal, which contains the sensory epithelium, was exposed by cutting the overlying skin.

EOG recordings

An electro-olfactogram (EOG) represents the summated generator potential of the olfactory receptor neurons (Scott and Scott-Johnson, 2002). This is done by recording the electrical potential in the water, directly above the fish's olfactory epithelium, as odorants are applied (Ottoson, 1955). The recording and reference electrodes were Ag/AgCl electrodes insulated with

petroleum jelly. The recording electrode was placed directly in the center of the dissected area, between the incurrent and excurrent nostrils. The reference electrode was placed under the scales of the head. The fish was grounded with an electrode placed under the scales at the peduncle. The EOG responses were AC amplified with an AM Instruments Model 1700 (USA) with low-cut filter set at 0.1 Hz and a high cut filter set at 500 Hz. The recordings were digitized on a USB-6001 data acquisition device (National Instruments, Austin, TX, USA) running LabVIEW software (National Instruments, Austin, TX, USA) and stored on a hard drive for later analysis. At the end of the experiment the fish were euthanized with an overdose of MS222, then were measured for mass, total length, and maximum eye lens diameter.

Odorant delivery and stimuli

Stimulus delivery to the olfactory epithelium for aerial trials was conducted using standard methods outlined in Burton et al (2019). Aerial stimuli were delivered at a distance of 8 cm from the animal for a duration of 2 seconds, with a carrier flow rate of 6L/min, and a channel pressure of 30kPa. For the aquatic stimulus, an IV bag containing water (1.005 specific gravity) slowly introduced water (5 mL min-1) over the olfactory epithelium. For stimulus delivery, 0.1 mL of the odorant liquid was injected into the IV tube near the olfactory epithelium through an injection port.

All stimuli were dissolved in 1.005 specific gravity (SG) water or multi-chain triglyceride (MCT) oil at a 1:5 dilution, depending on whether they were for aerial (MCT oil) or aquatic (1.005 SG water) trials. This is because the olfactometer, built for aerial odor delivery, works best with oil-dissolved odorants (Burton et al., 2019). There were 5 presentations for each odorant, with at least 1 minute elapsed between successive stimuli presentations. The stimuli, including 1.005 specific gravity (SG) water, MCT oil (aerial control), coffee, liquid aminos, garlic, ginger, and 4 concentrations of ammonium hydroxide (7 M, 350 mM, 17.5 mM, 875 μM), were applied in a

randomized order. Odorants were chosen because they each have a wide variety of olfactory and gustatory chemicals associated that activate a wide range of potential chemosensory receptors.

Olfaction Analysis

EOG responses are low frequency, so prior to analysis, signals were mean-centered and processed using a second-order low-pass Butterworth filter with a cutoff frequency of 30 Hz. With the filtered EOG response, the peak-to-peak voltage was calculated and then averaged for all 5 trials of an odorant per animal.

To assess the effects of different odorants on the response magnitude, we used linear mixed-effects models (LMM). In these models, each odorant was treated as a fixed effect, and each animal was included as a random effect to account for repeated measures within individuals. This approach allowed us to model the non-independence of observations arising from each animal being tested across multiple odorants.

The peak-to-peak averaged responses were then rank-transformed to stabilize variance and reduce the influence of outliers, following the method of Conover & Iman (1981). A linear mixed-effects model was fit to the ranked data with Odorant as a fixed factor and Animal (fish identity) as a random intercept. Treatment (odorant) effects were assessed by an ANOVA on the mixed model. Post hoc pairwise comparisons between odorants were performed using estimated marginal means (least-squares means) with Tukey's adjustment for multiple testing. All statistical tests were two-sided with significance evaluated at $\alpha = 0.05$. All analyses were performed in R.

RESULTS

Mudskipper visual acuity

The binomial logistic regressions for modeling air and water gave a threshold of response for the mudskippers at 1.83 CPD in air and 0.39 CPD in water (Figure 1). The interaction term for

the second binomial logistic regression showed the water trials had a significantly greater slope than the air trials (Estimate = –13.02, *SE* = 3.74, *z* = –3.48, *P*=0.0005). For trials with a positive response, behavioral results show head movement occurred 82% of the time in air and 100% in water. The fish tracked the stimulus via eye movement 94.9% of the time in air and 8.5% in water.

Olfaction response in air and water

Ammonia elicited a significant EOG response in air, while the other odorants did not (Figure 2A) and were indistinguishable from the control and each other (Table 1). Ammonia elicited the strongest (largest peak-to-peak value) response across animals. Minimal variation was observed among the remaining odorants.

In contrast, responses in water (Figure 2D) were markedly stronger and more differentiated across odorants. The odorants tested had pronounced responses compared to the control (Figure 2B,D). The linear mixed model shows that all odorants showed significantly different responses to the control (Table 2).

Dose dependent response to olfactory stimuli

There was a concentration-dependent effect of ammonia on minimum signal amplitude for both aerial and aquatic odorants (Figure 3). For air trials, the strongest response (i.e., largest peak-to-peak values) was seen in ammonia 7M with a quickly attenuated response in further concentrations (Figure 3, Table 3). For aquatic trials, the strongest responses were observed in the ammonia 7M (N=10) and ammonia 350 mM (N=5) conditions, with progressively attenuated responses in ammonia 17.5 mM (N=5) and ammonia 875 µM (N=5) (Figure 3, Table 4).

ORNs respond differently to ammonia than other odorants

The EOG responses for ammonia and the other odorants are essentially flipped where initially, ammonia has a hyperpolarizing effect followed by a depolarizing effect while the other odorants have the opposite (Figure 2 A,B).

DISCUSSION

Mudskippers see better on land

The results of the optomotor assay support my hypothesis, with significantly higher threshold responses observed under aerial conditions (Figure 1). Higher visual acuity on land is likely because of their flattened lens morphology, which is more similar to land vertebrates than aquatic animals' lens morphology (Sivak, 1978). This adaptation likely provides correction for aerial vision but hinders aquatic vision.

Behaviorally quantified spatial acuity yielded a CPD threshold of 1.83 on land and 0.39 in water (Figure 1), while morphological estimates yielded a theoretical acuity estimate of 2.79 CPD for water (Caves et al., 2017). A study of 69 species of ray-finned fishes found that visual acuity in fishes ranged from 0.87 to 31.58 CPD (Caves et al., 2017), putting mudskippers at the bottom for spatial acuity when using their aquatic vision capabilities.

Mudskippers smell better in water

The results of this study show that mudskippers exhibited stronger olfactory responses in water than in air, suggesting that their sense of smell remains more effective in the aquatic environment. While terrestrial olfaction in vertebrates depends on a mucus layer that can dissolve volatile airborne odorants and contains accessory proteins to facilitate receptor access (Getchell & Getchell, 1992), such proteins are absent or poorly characterized in fish (Weiss et al., 2021). This may explain that although the same odorants were tested in air and in water, none were detected in air, other than ammonia. The reason that mudskippers may have been able to detect ammonia

on land (only at high concentrations) is likely because of ammonia's volatile nature and ability to readily dissolve into any medium, potentially including fishes' mucus.

Overall, the weaker olfactory responses observed in air may reflect a mismatch between the physical properties of mudskipper olfactory mucus and the demands of aerial odorant detection. These results suggest that although mudskippers engage in key behaviors on land, their olfactory system may still be primarily adapted for underwater chemoreception, with aerial olfaction being minimal in their sensory ecology.

Ammonia responds differently

Ammonia had a hyperpolarizing effect on the ORNs while all other odorants had a depolarizing effect (Figure 2). This difference suggests that ammonia engages a fundamentally different mechanism of olfactory transduction compared to typical odorants. Previously, it has been found that certain odors inhibit the firing of olfactory sensory neurons (OSNs) in many animals (Morales et al., 1994; Gesteland et al., 1965; Duchamp-Viret et al., 1999; Kang and Caprio, 1995; Cao et al., 2017). In the context of ammonia, this response may reflect activation odorant-sensitive K^+ channels (Morales et al., 1994) in the olfactory epithelium, leading to a rapid efflux of K^+ ions and resulting in the observed hyperpolarization. Suppression of ORNs during odorant stimulation, although more rare than positive stimulation, is a well-known phenomenon (Akers, 1992; Fadool et al., 1993; Michel and Ache 1994; Michel et al., 1991, Dionne, 1992; Dubin and Dionne, 1993; Morales et al., 1994; Kang and Caprio, 1995). Given the consistency of this pattern across all trials and the literature precedent for odorant-evoked inhibition, it is likely that ammonia functions as an inhibitory, hyperpolarizing stimulus in the mudskipper olfactory system.

Dose dependent response to ammonia

The results of the experiment testing different concentrations of ammonia on the EOG response showed that there is a dose-dependent suppression of signal minima with increasing dilution, consistent with stronger sensory activation at higher ammonia concentrations. This strongly suggests that the response to all odorants was in fact biological and was not an artifact due to pH change at the olfactory epithelium.

Future directions

Future research should investigate other aspects of mudskippers sensory ecology in order to determine how they fully navigate the aerial and aquatic environment. Specific experiments that should be done are investigations of the temporal and spectral resolutions of their eye to understand inter- and intra-species relationships. Olfaction should also be expanded on, where future research is needed to discover the exact mechanism by which ammonia causes hyperpolarization of the ORNs. Due to the fact that airborne molecules are more likely to interact with a fish's gustatory system (Mollo et al., 2014), this sense should be studied to determine whether mudskippers can detect airborne chemicals using the gustatory system. This hypothesis is supported by work on walking catfish, in which aerial chemoreception may occur via extraoral taste buds (Bressman et al., 2020). While mudskippers likely have fewer extraoral taste buds than walking catfish, as in many other fish species, these could provide a basis for detecting chemicals in the air.

Possible limitations

Due to materials available, an AC amplifier was used instead of a DC amplifier which is typically used for this type of experimentation. AC coupling removes the baseline (DC) component of the signal and can distort slow voltage shifts, potentially converting what is physiologically a monophasic response into a biphasic one. Therefore, part of the observed signal could reflect a recording artifact rather than a true biological response. Additionally, sex differences, age, and the

developmental stage of each fish were not considered due to low sample size. These variables should be explored in future studies.

CONCLUSION

Amphibious animals face many unique challenges (Wright & Turko, 2016). Of these challenges, they must be able to navigate both the terrestrial and aquatic habitats through means of senses that can each be optimized only for one environment. The current study shows that mudskippers have two perceptual worlds in which they live. In water, their vision is greatly reduced, but their sense of smell is intact. On land, their sensory ecology is different, where their sense of smell is almost absent and vision is better. This must mean that they process their senses differently in different environments and likely rely on different senses in water than they do on land. This needs to be studied more to investigate exactly how they weigh their senses in different environments to navigate either one.

Data availability

All data is available upon request.

Condition 1	Condition 2	p.value	Significance
Ammonia 7M	**Coffee**	**4.641E-05**	*
Ammonia 7M	**Garlic**	**3.363E-05**	*
Ammonia 7M	**Ginger**	**5.481E-06**	*
Ammonia 7M	**LA**	**1.4E-06**	*
Ammonia 7M	**MCT**	**1.88E-05**	*
Coffee	**Garlic**	**0.9999984**	
Coffee	**Ginger**	**0.9845513**	
Coffee	**LA**	**0.8817802**	
Coffee	**MCT**	**0.999735**	
Garlic	**Ginger**	**0.992679**	
Garlic	**LA**	**0.9182421**	
Garlic	**MCT**	**0.99997**	
Ginger	**LA**	**0.998108**	
Ginger	**MCT**	**0.9988297**	
LA	**MCT**	**0.9642557**	

Table 1: Pairwise comparisons for all combinations of aerial odorants tested. Experiment-wise alpha is maintained at 0.05 by Tukey's hsd. LA = liquid aminos and MCT = multi-chain triglyceride oil.

Condition 1	Condition 2	p.value	Significance
Ammonia 7M	**Coffee**	**0.001104**	*
Ammonia 7M	**Garlic**	**0.000352**	*
Ammonia 7M	**Ginger**	**1.52E-09**	*
Ammonia 7M	**LA**	**0.215657**	
Ammonia 7M	**Water**	**2.5E-13**	*
Coffee	**Garlic**	**0.999144**	
Coffee	**Ginger**	**0.002709**	*
Coffee	**LA**	**0.342722**	
Coffee	**Water**	**1.13E-09**	*
Garlic	**Ginger**	**0.007808**	*
Garlic	**LA**	**0.181733**	
Garlic	**Water**	**3.73E-09**	*
Ginger	**LA**	**3.62E-06**	*
Ginger	**Water**	**0.000833**	*
LA	**Water**	**2.01E-12**	*

Table 2: Pairwise comparisons for all combinations of aquatic odorants tested. Experiment-wise alpha is maintained at 0.05 by Tukey's HSD post hoc tests. LA = liquid aminos and MCT = multi-chain triglyceride oil.

Condition 1	Condition 2	p.value	Significance
Ammonia 7M	**Ammonia 350 mM**	**0.087311**	
Ammonia 7M	**Ammonia 17.5 mM**	**0.000169**	*
Ammonia 7M	**Ammonia 875 µM**	**1.11E-05**	*
Ammonia 350 mM	**Ammonia 17.5 mM**	**0.063787**	
Ammonia 350 mM	**Ammonia 875 µM**	**0.005448**	*
Ammonia 17.5 mM	**Ammonia 875 µM**	**0.574628**	

Table 3: Pairwise comparisons for all combinations of aerial ammonia concentrations tested.

Experiment-wise alpha is maintained at 0.05 by Tukey's HSD post hoc tests.

Condition 1	Condition 2	p.value	Significance
Ammonia 7M	**Ammonia 350 mM**	**0.995272**	
Ammonia 7M	**Ammonia 17.5 mM**	**0.000211**	*
Ammonia 7M	**Ammonia 875 µM**	**2.18E-07**	*
Ammonia 350 mM	**Ammonia 17.5 mM**	**0.000948**	*
Ammonia 350 mM	**Ammonia 875 µM**	**2.85E-06**	*
Ammonia 17.5 mM	**Ammonia 875 µM**	**0.006316**	*

Table 4: Pairwise comparisons for all combinations of aquatic ammonia concentrations tested.

Experiment-wise alpha is maintained at 0.05 by Tukey's HSD post hoc tests.

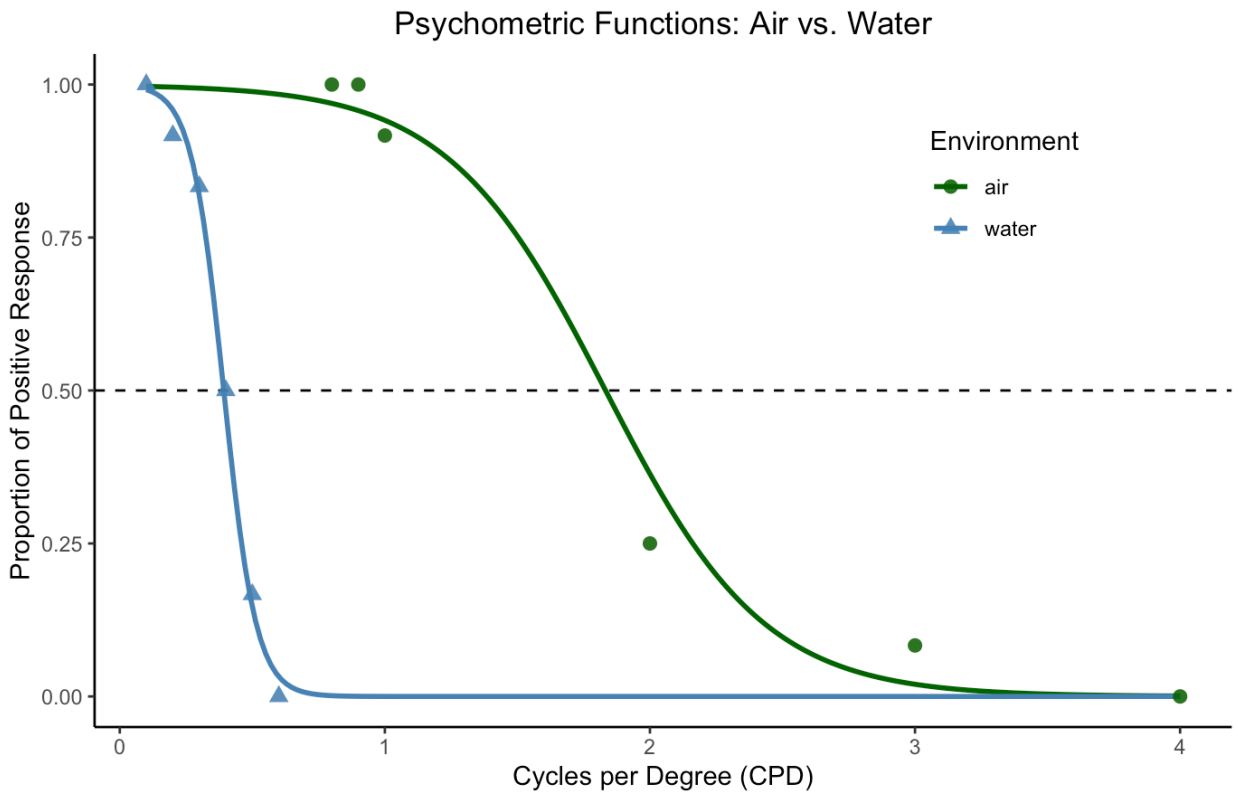

Figure 1: Psychometric functions of visual detection performance in air and water. Binomial logistic regression curves depict the predicted probability of a response across spatial frequencies (cycles per degree, CPD) for trials conducted in air (green) and water (blue). The dashed line represents the 50% response level. Points along the lines indicate the probability of response found through experimentation. There was a significant interaction between CPD and environment (Estimate = –13.02, SE = 3.74, z = –3.48, P=0.0005), meaning the slopes are significantly different from each other.

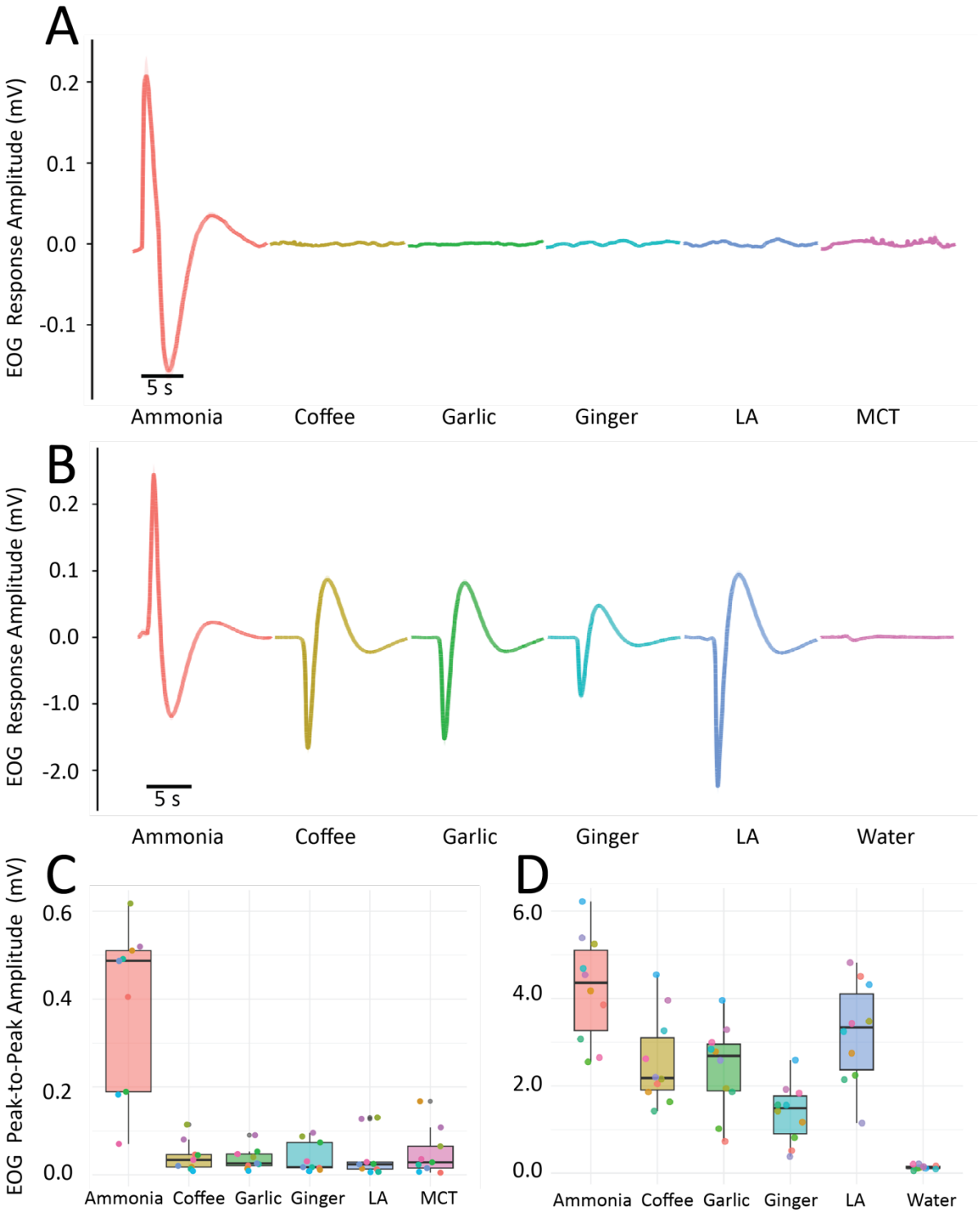

Figure 2: EOG responses of 10 mudskippers to different odorants in air and in water. A) Mudskippers' averaged EOG response to aerial odorants. B) Mudskippers' averaged EOG

response to aquatic odorants. For both A and B, ORNs initially respond to ammonia with a hyperpolarizing wave while other odorants initially respond with a depolarizing wave. C) Peak to peak averages of EOG responses from aerial presentations of odorants. D) Peak to peak averages of EOG responses from aquatic presentations of odorants.

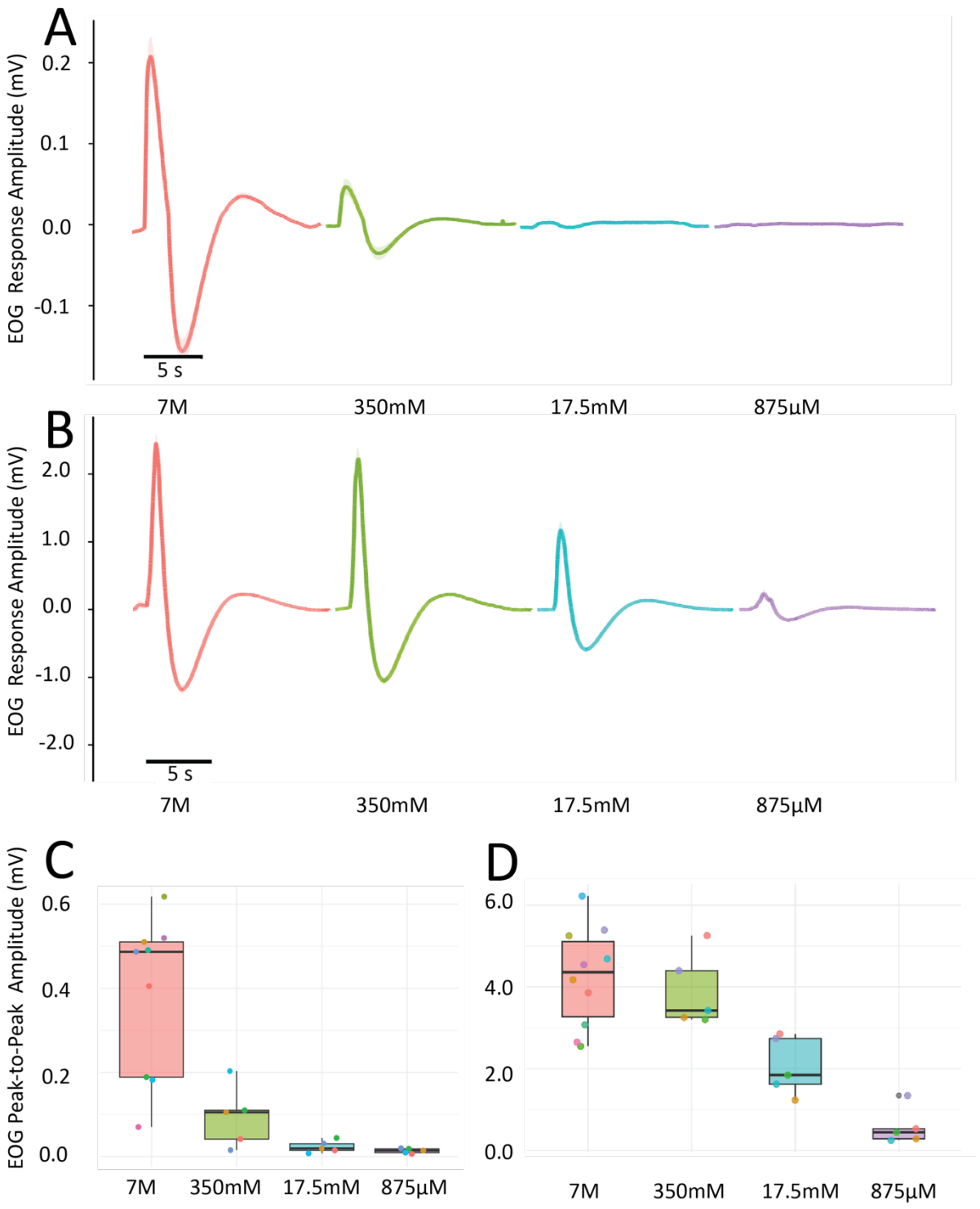

Figure 3: EOG responses to different concentrations of ammonia in air and in water. A) Mudskippers' averaged EOG response to aerial presentations of various ammonia concentrations.

B) Mudskippers' averaged EOG response to aquatic presentations of various ammonia concentrations. For both A and B, as ammonia concentration decreases, EOG amplitude decreases. C) Peak to peak of EOG responses from aerial presentations of ammonia concentrations. D) Peak to peak of EOG responses from aquatic presentations of ammonia concentrations.

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