



Does humic acid alter visually and chemically guided foraging in stickleback fish?

Robert B. Mobley¹ · Emily G. Weigel² · Janette W. Boughman¹

Received: 1 May 2019 / Revised: 11 September 2019 / Accepted: 4 October 2019 / Published online: 16 October 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Sensory systems function under the influence of multiple, interacting environmental properties. When environments change, so may perception through one or more sensory systems, as alterations in transmission properties may change how organisms obtain and use information. Humic acids, a natural and anthropogenically produced class of chemicals, have attributes that may change chemical and visual environments of aquatic animals, potentially with detrimental consequences on their ability to locate necessary resources. Here, we explore how environmental disturbance affects the way threespine sticklebacks (*Gasterosteus aculeatus*) use visual and olfactory information during foraging. We compared foraging behavior using visual, olfactory, and bimodal (visual and olfactory) information in the presence and absence of humic acids. We found evidence that humic acids reduced olfactory-based food detection. While visual perception was not substantially impaired by humic acids, the visual sense alone did not compensate for the loss of olfactory perception. These findings suggest that a suite of senses still may not be capable of compensating for the loss of information from individual modalities. Thus, senses may react disparately to rapid environmental change, and thereby push species into altered evolutionary trajectories.

Keywords Vision · Olfaction · Humic acid · Multimodal shift · Habitat change

Introduction

A single sensory system rarely provides an organism with all information necessary for survival and reproduction. Instead, sensory systems are frequently used in combination (Partan and Marler 1999), particularly in response to changing environmental conditions. Despite that signaling systems frequently work together in nature, sensory systems are often studied separately, which does not accurately represent the full set of sensory information that an individual must process to respond to local ecological conditions (Endler 1992). Since environments can be altered in ways that simultaneously affect multiple senses and signals to different extents, the potential exists for sensory systems to act in compensatory ways in the face of environmental change,

such as when olfactory information is prioritized over previously dominating visual information in the transition from light to dark conditions (Partan 2017; and references within). When evolutionary responses are inadequate, long-term and devastating effects on species and the communities to which they belong may result (Longcore and Rich 2004); thus, it is essential to determine whether and how sensory systems act in compensatory and robust ways.

One such agent of environmental change that has the potential to affect multiple sensory systems simultaneously are humic acids in water bodies. In addition to low level toxic effects of humic substances (Zhao and Zhu 2016), humic acids have the potential to disrupt visual communication by attenuating shortwave and UV light (Morris et al. 1995), resulting in red-shifted or “tea-stained” water. As many animals make use of UV and color signals that are suited to a broad-spectrum habitat, visual detection of these signals may be lost when humic acids are present.

When introduced to a body of water, humic substances are also known to disturb chemical communication in fishes. The impediment to olfaction may occur through several mechanisms. Organic acids may alter the pH of the environment, thereby altering the nature of chemical stimuli by

✉ Robert B. Mobley
mobleyro@msu.edu

¹ Department of Integrative Biology, Michigan State University, 288 Farm Lane, Room 203C, East Lansing, MI 48824, USA

² Biological Sciences, Georgia Institute of Technology College of Sciences, Atlanta, GA 30332, USA

producing involatile salts (Muller-Schwarze 2006). Humic substances also adsorb hydrophobic molecules such as steroidal pheromones, making them unavailable for chemical communication (Mesquita et al. 2003). Whatever the mechanism, the activity of olfactory receptor neurons can be impeded over a broad range of pheromone concentrations by even minute quantities of humic acids (Hubbard et al. 2002). The detrimental effects of humic acids extend to behaviors as well, demonstrated by the loss of chemically based mate preferences for conspecifics in swordtails (Fisher et al. 2006) and zebrafish (Fabian et al. 2007) in humic environments.

Given the potential of humic acids to alter these traits, it is perhaps ominous to note that this class of organic acids can arise from both natural plant degradation (Thomas 1997) and synthetic agricultural and gardening supplementation (Geyer et al. 1996). Thus, potential exposure could come through both subtle and predictable events like seasonal plant decay, and substantial, sudden events, like the introduction of agricultural runoff (Geyer et al. 1996; Hansten et al. 1996; Thomas 1997).

We assessed how humic acids modify visual and olfactory cues assessed alone and in combination by threespine stickleback (*Gasterosteus aculeatus*) to determine how visual and olfactory senses respond to altered sensory information. The threespine stickleback has radiated in multiple environments (reviewed in McKinnon and Rundle 2002), which vary in several attributes that may affect the transmission of visual and chemical stimuli (Ormond et al. 2011). As sticklebacks have adapted to these habitats, they have displayed divergence in their reliance on different sensory systems. For example, limnetic sticklebacks, which occupy pelagic regions, are more sensitive to, and have a greater behavioral preference for, bright nuptial colors than fish from benthic habitats (Boughman 2001). The behavioral and molecular (Rennison et al. 2016) diversification of the stickleback visual system demonstrates how the stickleback sensory system has adapted to the specific habitats in which the fish thrive.

The use of olfaction, although widespread in this fish family (McLennan 2003), also seems to have diverged in many populations. Interestingly, reliance on olfaction tends to be strongest when visual cues are likely reduced: fishes from tea-stained environments are able to discriminate populations based on olfactory cues (Hiermes et al. 2015), and benthic sticklebacks which live in more red-shifted habitats (Boughman 2001) have been shown to use olfaction to discriminate con and heterospecific potential mates (Rafferty and Boughman 2006). In contrast, sticklebacks from clear water and limnetic populations have not been shown to use olfaction in these ways (Mobley et al. 2016).

The reliance on different sensory systems has also been shown to be context dependent across populations of *G. aculeatus*. Increases in turbidity result in increased use of olfactory cues over visual cues during mate choice (Heuschele

et al. 2009). Increases in pH also promote female attraction to male olfactory cues (Heuschele and Candolin 2007) and are likely to affect sensation of other cues, such as those used in foraging, in similar ways.

Here, we measured sticklebacks' response in the presence or absence of humic acid under three conditions: only visual, only olfactory, or both visual and olfactory (bimodal) information available. We evaluated association with a food stimulus, as well as coughing, an olfactory sampling behavior analogous to sniffing in mammals (Nevitt 1991). We predicted that humic acids would have a detrimental effect on responding to the stimulus through either visual or olfactory perception alone. In this assay, both presence and location of the stimulus were redundantly conveyed through each sensory modality (Partan and Marler 1999), and we predicted the combined use of vision and olfaction would enhance perception, and could thus reduce the deleterious effects of acid. While humic acids have demonstrated consequences for visual and chemical perception in fish (Hubbard et al. 2002; Fisher et al. 2006; Fabian et al. 2007), we add to this knowledge base by exploring how the interaction of the senses affect responses to this substance. By controlling access to visual and olfactory information, we examine how the diverse and complex stickleback sensory system reacts to the potentially multimodal disruptive elements of humic acids and demonstrate how a single ecological perturbation can alter perception of stimuli through different sensory channels in this system.

Methods

Fish collection and housing

Our experiments used female benthic sticklebacks collected from Priest Lake, British Columbia, in the spring of 2014. To our knowledge, humic acids have not been quantified in this lake, but visual properties of the lake and measures of dissolved organic carbons (Ormond et al. 2011) indicate they are in lower abundance than treatments in experimental trials. Fishes were fed a mixture of bloodworms (Chironomidae) and brine shrimp (*Artemia*) ad libitum daily, but were fasted for 24 h prior to behavioral trials to increase motivation to respond to a food stimulus (bloodworms) in the experiments.

Fishes were housed in 284 or 110-L tanks at approximately equal densities before and between behavioral trials. Two weeks before trials began, we uniquely tagged each fish in the experiment with elastomer (Northwest Marine Technology, Inc., Shaw Island, WA). Fish experienced two trials: one without humic acid, and one with humic acid, in each of three experimental conditions (see below). Following the first trial, fishes were placed back into their housing

tank for 1 week until the second trial was conducted, after which they were housed separately.

Experimental apparatus

Behavioral trials took place in a 73.5 cm by 30 cm tank, filled to a height of 11 cm with 24.5 L of water. Water flow was generated by a 38-L tank on a shelf 60 cm above the trial tank, which fed water via gravity into the trial tank through two, 6.35-mm diameter polyvinyl hoses. Water flowed out of the tank at the rate of inflow (1250 mL/min) through three hoses attached to the front of the trial tank, maintaining a constant water level. Markings outside the trial tank divided it into lateral regions, demarcating a 15-cm neutral zone in the middle of the tank, from the outer regions that possessed containers with (Food Association Zone) or without (Control Association Zone) a food stimulus (Fig. 1).

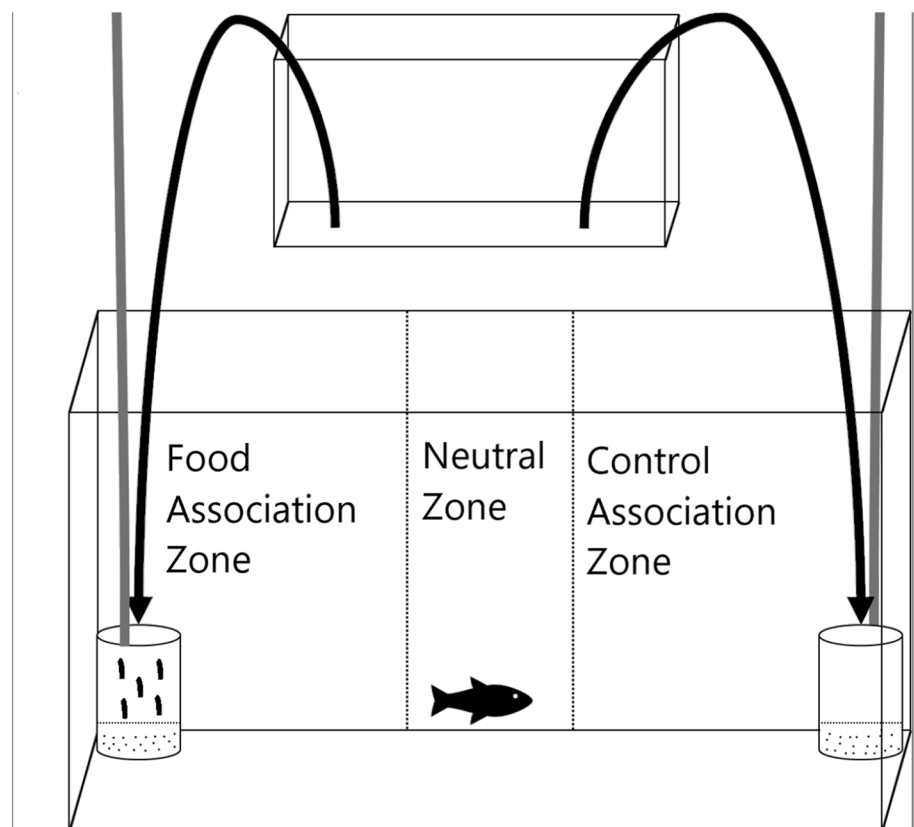
We used three types of plastic containers to control the senses that fish could potentially use to perceive the food stimulus. All containers were divided into upper and lower sections by a thin mesh glued to the interior, approximately 5 cm from the bottom of the container. The sides of each container were perforated below the mesh divide. The mesh served as a platform to prevent the food stimulus from escaping the container through the perforations, while still permitting the intended transmission of sensory cues during

trials. The container was covered with a lid with three holes: one to allow an aquarium bubbler to generate movement of the food stimulus; one to allow an escape for the excess air supplied by the bubbler; and one by which the hoses from the top tank fed water through the containers into the trial tank. Bimodal (visual and olfactory) trials used clear containers as described above. In trials that only allowed the use of olfactory cues, black containers were used so that the contents of the container could not be seen. For visual-only trials, a small jar was placed within an unpainted container, to prevent chemical cues from entering the arena. Tests with dye confirmed that water from containers on each side did not mix on opposite ends of the tank, and reached the center of the tank within the acclimation period prior to data collection (described below). Two containers of the same type were used in each trial and placed on opposite ends of the tank, one of which held a food stimulus, while the empty container served as an object control.

Food stimulus preparation

Each trial used 5 g of bloodworms, a standard food given to the fish in the laboratory, as a stimulus. Frozen bloodworms were thawed prior to the trial and placed on the mesh in one of the containers inside the experimental tank. Bloodworms were rinsed for the visual-only trials because preliminary

Fig. 1 Diagrammatic setup of experiment. Cylinders indicate stimulus containers, which contained a mesh (horizontal dashed line) below which the sides of the container were perforated (dots). Black lines in containers indicate presence of food stimulus (bloodworms). Vertical dotted lines show the demarcations that divide the tank into Food Association, Neutral and Control regions. Black arrows show water flow from top tank into experimental arena through each stimulus container. Grey lines represent airlines into containers



trials revealed the frozen bloodworms emit red coloration when added to water, which obstructed the view of the bloodworms in the visual-only container. In other trials, water from the top tank washed over the stimulus throughout the trial, and the dye dissipated quickly in the larger tank.

Behavioral trials

To test humic acid's effects, 30 fishes were randomly divided into 1 of 3 sensory treatments: visual only, olfactory only, and visual and olfactory (bimodal) (10 fishes per treatment). Trials were paired such that each fish was exposed to a stimulus in the absence and presence of humic acids. For a fish's initial trial, the end of the tank on which the food-bearing container was placed was randomly assigned. Since the mechanistic and long-term effects of humic acids on stickleback sensory systems are unknown, trial order was not randomized; the humic acids exposure always followed the non-humic acids exposure.

At the beginning of each trial, the containers and appropriate food contents were placed in the tank and the water flow started before the fish was placed into an acclimation chamber in the neutral zone of the trial tank for 3 min. The acclimation chamber was transparent and allowed water to enter, permitting exposure to the food cues during the acclimation period.

After 3 min, we slowly lifted the acclimation chamber, allowing the fish to swim freely in the tank. For the following 5 min, we used the behavior monitoring software JWatcher to record what tank region the fish was in. Trials were scored as they occurred, as visual glare accentuated by humic acid prevented reliable scoring from video. We also tracked instances of coughing, a chemical sampling behavior in fish (Nevitt 1991). In fish with a non-ciliated olfactory epithelium, such as sticklebacks (Bannister 1965; Honkanen and Ekström 1992), water is drawn across sensory cells via changes in water pressure brought about by changing the volume of the olfactory sac (Nevitt 1991). This coughing behavior should, therefore, provide increased olfactory information about the environment. Characteristic movements of the gill opercula allowed this behavior to be recorded regardless of a fish's orientation. At the end of the trial, the fish was placed back in its original tank. The experimental tank was cleaned with ethanol and rinsed between trials.

A week later, the fish was used in a second trial. These trials included the assigned stimulus treatment and a concentration of 15 mg/L of humic acids (TeraVita SP-90, Lancaster, PA). To prevent responses due to familiarization made in the first exposure, the food-bearing container was placed on the end of the tank opposite to its position during that individual's first trial.

An additional group of ten fishes served as a control for the effect of repeated exposure to stimuli. These

fishes, designated the acid control group, experienced the bimodal stimulus treatment, but were never exposed to humic acids, and instead went through a second bimodal trial, with the food-bearing container on the side opposite to its position in the previous trial.

Statistical analysis

For each group of subjects, we analyzed the proportion of the 5-min monitoring period spent in the food association zone and the number of chemical sampling behaviors ("coughs") observed during trials. Trials were not forced choice, such that subjects that spent all their time in the neutral zone and/or the control association zone were included in analyses, and we tested differences between subjects' first and second trials, resulting in many zeros in the dataset. We initially evaluated food association with a zero-inflated, negative binomial mixed model, but due to the small sample size of each treatment group, and as transformations of the dataset did not improve distributional assumptions of parametric models, we ultimately used nonparametric tests (Wilcoxon Signed Rank Tests) to compare fish in their first and second trials. Tests were performed in R version 3.4.2 (R Core Team 2017). Due to the zeros and resulting ties in the response variables, we used the package "coin", which uses Pratt's method for handling zeroes to provide an asymptotic approximation of the p value (Pratt 1959). However, as these procedures did not affect statistical significance of results, estimates, confidence intervals, and significance levels reported are those calculated from R's built in "stats" package. Additionally, a Fisher's Exact Test was performed on the number of trials in which coughing did or did not occur in the presence and absence of humic acids.

Results

Food association

We found that humic acid influenced food associations based on olfactory information. Specifically, when using only olfactory cues, fishes were more likely to associate with the food stimulus when humic acid was absent, but not in the chemical's presence (Wilcoxon signed rank test; $V=6$, $p=0.03$; Fig. 2). This was not seen with other sensory treatments. Although fish tested with bimodal cues tended to reduce association with food when humic acids were introduced, this change was modest and not statistically significant (Wilcoxon signed rank test; $V=16.5$, $p=0.28$) (Fig. 2).

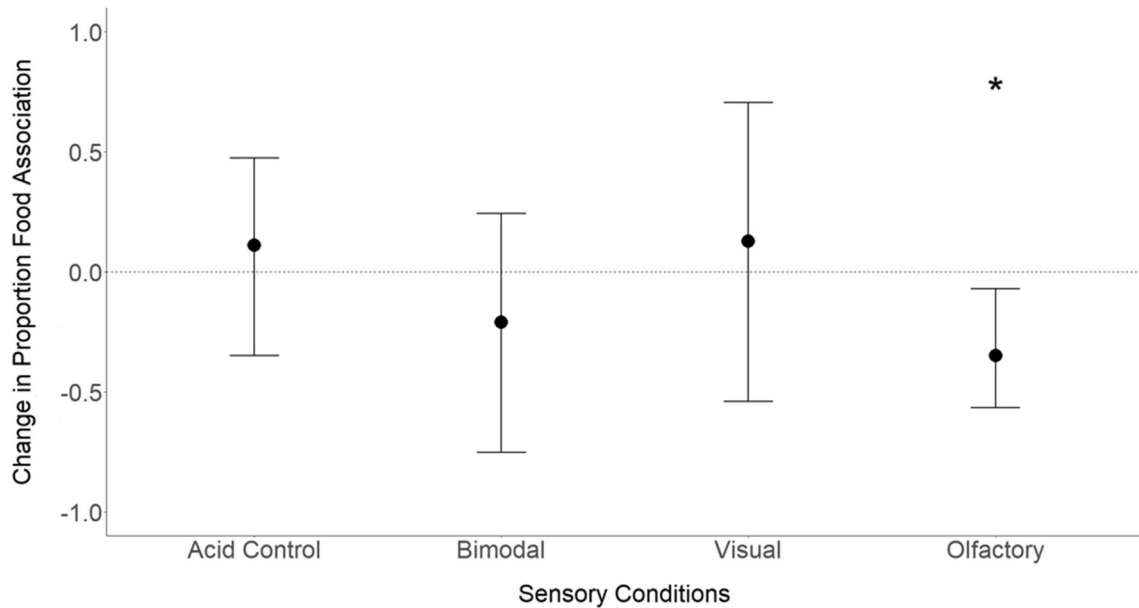


Fig. 2 Change in the median proportions of time associating with food stimulus for first (no acid present) and second (humic conditions, except in acid control) trials under different sensory conditions.

Negative values indicate a reduction in food association in the second trial, relative to the first. Error bars denote 95% CI (Wilcoxon signed rank test; * $p < 0.05$)

Coughing

Coughing, although it occurred infrequently in trials, decreased in the presence of humic acid [Fisher’s exact test; Odds ratio (95% CI)= 15.42 (3.97, 73.22), $p < 0.001$; Fig. 3]. When using a single sensory system coughing occurred less

often in the presence of humic acids than in their absence (Wilcoxon signed rank tests; for vision: $V = 36$, $p = 0.01$; for olfaction: $V = 45$, $p = 0.009$). When using both sensory systems, the amount of coughing was the same in humic and non-humic conditions (Wilcoxon signed rank tests; acid control: $V = 27$, $p = 0.64$; bimodal: $V = 28$, $p = 0.55$; Fig. 3).

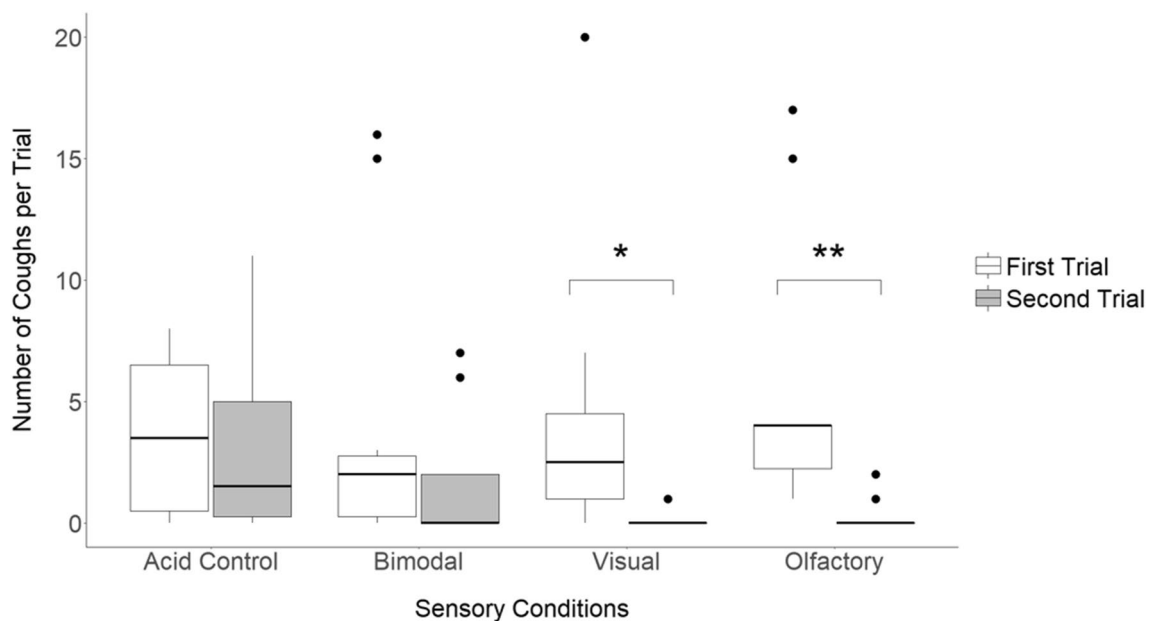


Fig. 3 Number of coughs for first (no acid present) and second (humic conditions, except in acid control) trials under different sensory conditions (Wilcoxon signed rank test; * $p < 0.05$, ** $p < 0.01$)

Discussion

Humic acid appears to modify the use of olfactory cues in responding to a food stimulus in this assay. When humic acid was absent and olfactory cues were available, fish associated with the food stimulus more than the control container for olfactory cues alone. Vision used without olfactory cues, however, did not produce a preference for the stimulus. When humic acid was introduced, there was no preference for the food stimulus regardless of the sensory treatment, suggesting that humic acid interferes with detection. These results show an asymmetry, i.e., a greater effect on olfactory cues than visual, in perception by sticklebacks for this task and their response to environmental change. These outcomes align with those found in swordtails, which exhibited reduced association to conspecific chemical cues, but not visual cues, in presence of humic acids (Fisher et al. 2006).

Behaviors that promote sampling of chemical cues are common in many taxa, such as puffing by octopods (Chase and Wells 1986), nose tapping in urodeles (Jaeger et al. 1986), tongue flicking in reptiles (Cooper 1998), antennal flicking in arthropods (Berg et al. 1992), and coughing in fish (Nevitt 1991). Such behaviors may be initiated by a number of stimuli, including non-chemical sources (Muller-Schwarze 2006). That we found reduced coughing in the presence of humic acids may be surprising, as fish could be expected to increase sampling under impeded sensory conditions, to improve perception of a difficult to detect stimulus. However, increased sampling may only be initiated after detection of the stimulus. If exposure to humic acids precedes or prevents the initial detection of food cues, then efforts to improve cue sampling would not be expected to occur. In addition to the alteration of the sensory environment, humic acids may have detrimental effects to organismal health that modifies an organism's physiology (Zhao and Zhu 2016), and thus behavior (Santonja et al. 2017).

That coughing in fish using bimodal cues was also not significantly affected by humic acids is also surprising. The presence of humic acids may not eliminate detection of either visual or olfactory cues, but instead may reduce detection enough to eliminate the coughing behavior, indicating a “knockdown” rather than a “knockout” of both types of stimuli. If each cue is still available in the bimodal condition, the combined inputs may equal or surpass the threshold needed to engage in this chemical sampling, even in the inhibitory presence of humic acids. Such threshold changes have been demonstrated in parts of the nervous system that receive visual and olfactory information (Dalton et al. 2000).

The nature of the visual-olfactory interaction depends on how stimuli are utilized. When stimuli are temporally

or spatially displaced, one modality may serve an alerting function, providing information on the presence of a stimulus, while another modality is used to assess information such as location or quality (Rowe 1999; Rowe and Guilford 1999). In our study, olfaction may have served as an alerting behavior that also prompted the search and localization for food. Without the olfactory cue, the food may never have been noticed in the environment, as found by Webster et al. (2007); sticklebacks foraging in low turbidity environments foraged equally well in high turbidity, until the chemical environment was manipulated.

Our results indicate vision likely plays a subordinate role to olfaction in this task. In the face of an environmental change, such as the introduction of humic acids, subordinate modalities may be unable to preserve behaviors. While using multimodal cues potentially protects against ecological traps (Madliger 2012; Hale et al. 2015), the way animals process different sensory stimuli may nullify this potential. As in the case of humic acids, other environmental pollutants may affect multiple sensory modalities, adding additional complexity to enduring environmental change (Halfwerk and Slabbekoorn 2015). These types of habitat changes may challenge organisms to acquire additional types of information, such as properties of cues that are not altered by the environmental change, or may promote the rapid adaptation of affected senses, to withstand modification of the sensory environment.

Sensory systems serve in multiple behavioral tasks, and multiple environmental factors influence how sensory systems function and evolve (Endler 1992). These connections of sensory abilities evolving to meet different tasks are exemplified by sensory biases for courtship characters that resemble food sources, as in the response of carotenoid pigments by guppies (Rodd et al. 2002). While our results demonstrate that humic acid affects perception in a foraging context, such manipulations of the environment are likely to carry over into other contexts. Copepod evasion of predators is also reduced in humic environments (Santonja et al. 2017) although this may be due to deteriorated physiological condition brought on by humic substances, in addition to or instead of perceptual impairment. In newts, species recognition and mating preferences are altered by humic acids, apparently due to the alteration of visual (Secondi et al. 2014), but not chemical information (Secondi et al. 2015). Alterations of the light environment in at least one threespine stickleback population have previously been implicated in the collapse of reproductive barriers between sympatric benthic and limnetic populations (Taylor et al. 2006) and collapse of cichlid species (Seehausen et al. 1997). But changes in the visual environment due to turbidity (Engstrom-Ost and Candolin 2007), eutrophication (Seehausen et al. 1997), and dissolved organic acids including tannins (Scott 2001) and humic substances also alter the chemical

environment, raising questions of whether these are primarily visual or also olfactory effects on sensory divergence and speciation. These studies highlight that perturbation of the sensory environment can have pervasive damaging effects on adaptation and the maintenance of biodiversity. We need more studies of how organisms integrate information from different sensory modalities in the face of changing sensory environments.

Acknowledgements We are grateful to Marquita Tillotson and Savannah Foster for their assistance in carrying out behavioral trials and data collection, as well as the members of the Boughman lab who provided animal care. We thank Courtney Larson, Miranda Wade, Scott Warner, Nikki Cavalieri, Murielle Ålund, the BEACON Chemical Communication Group, and two anonymous reviewers for useful comments in preparation of this manuscript. Nicole Jess, Andrew Denhardt and MSU CSTAT provided valuable statistical consultation. This work was supported by grants from the National Science Foundation to JWB.

Funding This study was funded by a National Science Foundation Career Grant (Career Grant deb-0952659) and a National Science Foundation Dimensions of Biodiversity Grant (deb-1638778), awarded to JWB.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Michigan State University Institutional Animal Care and Use Committee permit number 04/13-092-00).

References

- Bannister LH (1965) The fine structure of the olfactory surface of teleostean fishes. *J Cell Sci* 106:333–342
- Berg K, Voigt R, Atema J (1992) Flicking in the lobster *Homarus americanus*: recordings from electrodes implanted in antennular segments. *Biol Bull* 183:377–378. <https://doi.org/10.1086/BBLv183n2p377>
- Boughman JW (2001) Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–948
- Chase R, Wells MJ (1986) Chemotactic behaviour in octopus. *J Comp Physiol A* 158:375–381. <https://doi.org/10.1007/BF00603621>
- Cooper WE (1998) Prey chemical discrimination indicated by tongue-flicking in the eublepharid gecko *Coleonyx variegatus*. *J Exp Zool* 281:21–25. [https://doi.org/10.1002/\(SICI\)1097-010X\(19980501\)281:1%3c21:AID-JEZ4%3e3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-010X(19980501)281:1%3c21:AID-JEZ4%3e3.0.CO;2-E)
- Dalton P, Doolittle N, Nagata H, Breslin PAS (2000) The merging of the senses: integration of subthreshold taste and smell. *Nat Neurosci* 3:431–432. <https://doi.org/10.1038/74797>
- Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139:S125–S153
- Engstrom-Ost J, Candolin U (2007) Human-induced water turbidity alters selection on sexual displays in sticklebacks. *Behav Ecol* 18:393–398. <https://doi.org/10.1093/beheco/arl097>
- Fabian NJ, Albright LB, Gerlach G et al (2007) Humic acid interferes with species recognition in zebrafish (*Danio rerio*). *J Chem Ecol* 33:2090–2096. <https://doi.org/10.1007/s10886-007-9377-z>
- Fisher HS, Wong BBM, Rosenthal GG (2006) Alteration of the chemical environment disrupts communication in a freshwater fish. *Proc R Soc B Biol Sci* 273:1187–1193. <https://doi.org/10.1098/rspb.2005.3406>
- Geyer S, Fischer M, Wolf M, et al (1996) Agriculture and its impacts on the isotope geochemistry and structural composition of dissolved organic carbon. In: *Isotopes in water resources management. V. 1. Proceedings of a symposium*
- Hale R, Trembl EA, Swearer SE (2015) Evaluating the metapopulation consequences of ecological traps. *Proc R Soc B Biol Sci* 282:20142930. <https://doi.org/10.1098/rspb.2014.2930>
- Halfwerk W, Slabbekoorn H (2015) Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. *Biol Lett* 11:20141051. <https://doi.org/10.1098/rsbl.2014.1051>
- Hansten C, Heino M, Pynnönen K (1996) Viability of glochidia of *Anodonta anatina* (Unionidae) exposed to selected metals and chelating agents. *Aquat Toxicol* 34:1–12
- Heuschele J, Candolin U (2007) An increase in pH boosts olfactory communication in sticklebacks. *Biol Lett* 3:411–413. <https://doi.org/10.1098/rsbl.2007.0141>
- Heuschele J, Mannerla M, Gienapp P, Candolin U (2009) Environment-dependent use of mate choice cues in sticklebacks. *Behav Ecol* 20:1223–1227. <https://doi.org/10.1093/beheco/arp123>
- Hiermes M, Mehliis M, Rick IP, Bakker TCM (2015) Habitat-dependent olfactory discrimination in three-spined sticklebacks (*Gasterosteus aculeatus*). *Anim Cogn* 18:839–846. <https://doi.org/10.1007/s10071-015-0850-8>
- Honkanen T, Ekström P (1992) Comparative study of the olfactory epithelium of the three-spined stickleback (*Gasterosteus aculeatus*) and the nine-spined stickleback (*Pungitius pungitius*). *Cell Tissue Res* 269:267–273
- Hubbard PC, Barata EN, Canario AVM (2002) Possible disruption of pheromonal communication by humic acid in the goldfish, *Carassius auratus*. *Aquat Toxicol* 60:169–183
- Jaeger RG, Goy JM, Tarver M, Márquez CE (1986) Salamander territoriality: pheromonal markers as advertisement by males. *Anim Behav* 34:860–864. [https://doi.org/10.1016/S0003-3472\(86\)80071-9](https://doi.org/10.1016/S0003-3472(86)80071-9)
- Longcore T, Rich C (2004) Ecological light pollution. *Front Ecol Environ* 2:191–198
- Madliger CL (2012) Toward improved conservation management: a consideration of sensory ecology. *Biodivers Conserv* 21:3277–3286. <https://doi.org/10.1007/s10531-012-0363-6>
- McKinnon JS, Rundle HD (2002) Speciation in nature: the threespine stickleback model systems. *Trends Ecol Evol* 17:480–488
- McLennan DA (2003) The importance of olfactory signals in the gasterosteid mating system: sticklebacks go multimodal. *Biol J Linn Soc* 80:555–572
- Mesquita RM, Canario AV, Melo E (2003) Partition of fish pheromones between water and aggregates of humic acids. Consequences for sexual signaling. *Environ Sci Technol* 37:742–746
- Mobley RB, Tillotson ML, Boughman JW (2016) Olfactory perception of mates in ecologically divergent stickleback: population parallels and differences. *Evol Ecol Res* 17:551–564
- Morris DP, Zagarese H, Williamson CE et al (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnol Oceanogr* 40:1381–1391. <https://doi.org/10.4319/lo.1995.40.8.1381>
- Muller-Schwarze D (2006) *Chemical ecology of vertebrates*. Cambridge University Press, Cambridge
- Nevitt GA (1991) Do fish sniff? A new mechanism of olfactory sampling in pleuronectid flounders. *J Exp Biol* 157:1–18

- Ormond CI, Rosenfeld JS, Taylor EB (2011) Environmental determinants of threespine stickleback species pair evolution and persistence. *Can J Fish Aquat Sci* 68:1983–1997. <https://doi.org/10.1139/f2011-113>
- Partan SR (2017) Multimodal shifts in noise: switching channels to communicate through rapid environmental change. *Anim Behav* 124:325–337. <https://doi.org/10.1016/j.anbehav.2016.08.003>
- Partan SR, Marler P (1999) Communication goes multimodal. *Science* 283:1272–1273
- Pratt JW (1959) Remarks on zeros and ties in the Wilcoxon signed rank procedures. *J Am Stat Assoc* 54:655–667. <https://doi.org/10.1080/01621459.1959.10501526>
- R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rafferty NE, Boughman JW (2006) Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. *Behav Ecol* 17:965–970. <https://doi.org/10.1093/beheco/arl030>
- Rennison DJ, Owens GL, Heckman N et al (2016) Rapid adaptive evolution of colour vision in the threespine stickleback radiation. *Proc R Soc B Biol Sci* 283:20160242. <https://doi.org/10.1098/rspb.2016.0242>
- Rodd FH, Hughes KA, Grether GF, Baril CT (2002) A possible non-sexual origin of mate preference: are male guppies mimicking fruit? *Proc R Soc Lond B Biol Sci* 269:475–481. <https://doi.org/10.1098/rspb.2001.1891>
- Rowe C (1999) Receiver psychology and the evolution of multicomponent signals. *Anim Behav* 58:921–931
- Rowe C, Guilford T (1999) The evolution of multimodal warning displays. *Evol Ecol* 13:655–671
- Santonja M, Minguez L, Gessner MO, Sperfeld E (2017) Predator–prey interactions in a changing world: humic stress disrupts predator threat evasion in copepods. *Oecologia* 183:887–898. <https://doi.org/10.1007/s00442-016-3801-4>
- Scott R (2001) Sensory drive and nuptial colour loss in the three-spined stickleback. *J Fish Biol* 59:1520–1528. <https://doi.org/10.1006/jfbi.2001.1806>
- Secondi J, Okassa M, Sourice S, Théry M (2014) Habitat-dependent species recognition in hybridizing newts. *Evol Biol* 41:71–80. <https://doi.org/10.1007/s11692-013-9248-1>
- Secondi J, Rodgers G, Bayle F et al (2015) Mate preference, species recognition and multimodal communication in heterogeneous environments. *Evol Ecol* 29:217–227. <https://doi.org/10.1007/s10682-014-9744-5>
- Seehausen O, van Alphen Jacques J M, Witte Frans (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808–1811. <https://doi.org/10.1126/science.277.5333.1808>
- Taylor EB, Boughman JW, Groenenboom M et al (2006) Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Mol Ecol* 15:343–355. <https://doi.org/10.1111/j.1365-294X.2005.02794.x>
- Thomas JD (1997) The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshw Biol* 38:1–36
- Webster MM, Atton N, Ward AJW, Hart PJB (2007) Turbidity and foraging rate in threespine sticklebacks: the importance of visual and chemical prey cues. *Behaviour* 144:1347–1360
- Zhao Q, Zhu L (2016) Effect of humic acid on prometryn bioaccumulation and the induction of oxidative stress in zebrafish (*Danio rerio*). *RSC Adv* 6:16790–16797

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.