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Food and Conspecific Chemical Cues Modify Visual Behavior of Zebrafish, *Danio rerio*

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Abstract

Animals use the different qualities of olfactory and visual sensory information to make decisions. Ethological and electrophysiological evidence suggests that there is cross-modal priming between these sensory systems in fish. We present the first experimental study showing that ecologically relevant chemical mixtures alter visual behavior, using adult male and female zebrafish, *Danio rerio*. Neutral-density filters were used to attenuate the light reaching the tank to an initial light intensity of 2.3×10^{16} photons/s/m^2. Fish were exposed to food cue and to alarm cue. The light intensity was then increased by the removal of one layer of filter (nominal absorbance 0.3) every minute until, after 10 minutes, the light level was 15.5×10^{16} photons/s/m^2. Adult male and female zebrafish responded to a moving visual stimulus at lower light levels if they had been first exposed to food cue, or to conspecific alarm cue. These results suggest the need for more integrative studies of sensory biology.

Introduction

Aquatic environments are ideally suited for the transmission of olfactory information,[1,2] and therefore fish provide some of the best examples of this cross-modal interaction. Female swordtails (*Xiphophorus pygmaeus*),[3,4] sticklebacks (*Gasterosteus aculeatus*),[5] and Mexican pupfish (*Cyprinodon* spp.)[6] vary in their preference for heterospecific or conspecific mates, depending on which sensory modality, vision or olfaction, is stimulated. Similarly, darters appear to use olfactory cues to locate their invertebrate prey, but require the visual stimulus of movement to feed.[7,8] Alarm cue is released from damaged fish skin and provides reliable information about predation risk in the immediate environment,[9] but the response of the fish to it depends on a combination of chemical and visual cues. Hartman and Abrahams[10] found that fathead minnows, *Pimephales promelas*, were more likely to respond to an olfactory alarm signal in the absence of visual information about the risk of predation. If olfactory cues were able to 'prime' the visual sensory system,[11] individuals would be less likely to miss visual information, and more likely to act appropriately on receipt of visual cues.

Since the discovery that olfactory stimulation causes an electrophysiological response in fish retinae, it has been acknowledged among many physiologists that the olfactory and visual sensory systems are functionally linked.[12–14] The effect of an olfactory stimulus on visual sensitivity has to date only been tested using amino acids. Maaswinkel and Li,[15] using a similar method to that employed here, tested whether a range of isolated amino acids as olfactory stimuli enhanced visual sensitivity during behavioral trials. Their results suggest that although olfactory input does increase visual sensitivity, this effect depends on the amino acid used. As well as having different effects on vision, single amino acids can elicit a variety of behaviors in zebrafish.[16,17] How complex mixtures involving multiple amino acids and other classes of olfactory stimuli affect either vision or visual behavior is unknown, even though such mixtures inform most aspects of fish behavior.[2]

Zebrafish, *Danio rerio*, is an important model species for analysis of visual behavior[18–21] and olfactory behaviors,[22,23] as well as the possible link between them.[15] Zebrafish visual behavior is being used to demonstrate the effects of drugs (e.g., alcohol[24] and cocaine[25]). It is therefore important to characterize the factors that affect this behavior. In order to understand better the potential interactions between visual and olfactory sensory systems, we analyzed behavioral responses of adult zebrafish to food cue and conspecific alarm cue.[26] We show that the complex, ecologically relevant chemical mixtures of food cue and alarm cue affect visually-mediated behaviors, although the neuroanatomical basis for this interaction remains to be elucidated.

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Materials and Methods

Fish origin and husbandry

Zebrafish from the breeding population at the University of Valparaíso, Chile, were used during this work. The effect of food cue on visual sensitivity was tested using adult fish (Cornell/*HuC*:GFP; 15 months old). These are behaviorally wild-type fish that express GFP in new neurons during development. Due to stock limitations, the effect of conspecific alarm cue on visual sensitivity was tested using a different strain (Cornell or NWT; 23 months old). Both NWT and Cornell are wild-type lines were derived from the AB line (University of Oregon; see http://zfin.org/; Zebrafish International Resources Center, ZIRC).

Zebrafish were kept at 26–27°C in 40 L aquaria filled with de-ionized (reverse osmosis) water to which was added Instant Ocean® Sea Salt to reach a conductivity of 400 micro-Siemens/cm^2, and with sodium bicarbonate to reach pH 7.3 (hereafter referred to as 'fish water'). Fluorescent light provided a 13.5:11.5 (L:D) photoperiod (light phase 9 AM–10.30 PM). Fish were fed a mixture of dried chironimid larvae (hereafter referred to as 'bloodworms') and Tetramin® flakes at 9.30 AM and 4.00 PM during the experimental period.

Apparatus

The apparatus (Fig. 1) has been described elsewhere.[27] It briefly consisted of a cylindrical glass tank (diameter 18 cm, depth 10 cm) suspended from a steel frame. A 12 cm diameter post was placed in the center of the tank, leaving a 3 cm wide 'racetrack' around the edge of the tank. The tank was surrounded by a cardboard drum (diameter 28 cm, depth 14 cm), which could be rotated in either direction by a motor at a constant speed of 10 rpm. The cardboard drum was white, with a black segment covering 10% of the circumference. The base of the drum was also white, with a black wedge shape corresponding to the black segment. The whole base, including the black wedge, reflected in the infrared.

During the trials, chemical stimuli were introduced to the experimental tank as cues in water using separate funnels and silicon tubes. The tube to be used was fed through a covered hole in the screen surrounding the frame, and hung just above the surface of the water in the experimental tank.

The fish could be viewed and behavior recorded using an infrared-sensitive video camera (Siemens Pro-100L CCIR B/W camera) supported from the top of the frame. Three sides of the frame were covered in black cardboard, the top and one side were draped in two layers of blackout fabric. A 10 cm square hole was cut in the fabric covering the top of the frame, over which were laid 15 cm × 15 cm squares of neutral-density (ND) filters (LEE filters 209 and 299; nominal absorbances 0.3 and 1.2, respectively). A halogen light (Fig. 1) was positioned directly above the hole. With no filters in place, there was a total irradiance of 5.14×10^{18} photons/s/m^2 at the water surface.

To measure spectral irradiance, a cosine corrector (model CC-3; Ocean Optics Inc.) was placed at the level of the water surface and connected to a spectrometer (model QE65000; Ocean Optics Inc.) *via* a 400 micron solarization-resistant optical fiber (QP400-2-SR; Ocean Optics Inc.). The readings were analyzed using the Spectrasuite program (Ocean Optics Inc.) between 380 and 780 nm for the experimental set up with no filters in place. These measurements were converted to absolute spectral irradiance by reference to similar measurements of a calibrated light source (Ocean Optics DH2000-CAL). The absorbance of both filters (Lee ND filter 209 and 299) was measured using a spectrophotometer (model UV2101PC; Shimadzu Corp.), and the absorbance between 380 and 780 nm of each filter was used to calculate the irradiance under each filter combination.

Stimulus preparation

Food cue was made by mixing 0.1 g of food (dried bloodworms and Tetramin® flakes, used as feed) into 1 liter of fish water. Visible food particles settled to the bottom overnight and stimulus water was extracted from the top of the container.

Alarm cue was produced every day (adapted from Wisenden et al.[28]) using adult female fish from genetic backgrounds and home tanks different from those of the trial fish. In accordance with local (FONDECYT) and NIH regulations (Animal Welfare Assurance Number: #A5823-01; awarded to

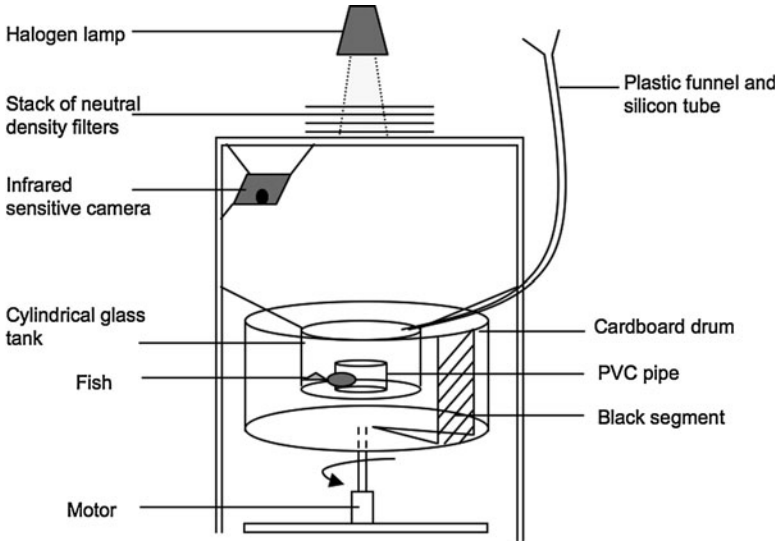

FIG. 1. Diagram of the experimental apparatus. We compared the light level at which the fish responded to the visual stimulus (*Black Segment*) between control groups and those exposed to food cue and conspecific alarm cue. The visual stimulus was moved by rotating the cardboard drum using the motor. Cues were added via silicon tubing and the behavior was recorded using an infrared sensitive camera.

KEW), fish were cold-anesthetized and sacrificed immediately. Five shallow scores were made on each flank and 15 mL fish water was washed over the body and into a beaker. The volume was then made up to 30 mL with fresh fish water and placed in an ice bath. In all experiments, fish water was used as a control and all containers were covered to prevent contamination from airborne particles.

Experimental protocol

Individuals to be tested were held overnight in individual transparent 1 L tanks. Fish were not fed during their isolation. One liter of fresh fish water was poured into the experimental tank, to a depth of 4 cm, and a naïve fish placed in the trial tank. Six layers of ND 299 filter, and 9 of ND 209 filter were put in place, (Fig. 1, stack of neutral density filters) providing a total irradiance of 2.3×10^{16} photons/s/m^2. Fish were left for 20 min to dark-adapt.[29] The experimental room was heated constantly to 26°–27°C.

Each trial began with one minute of recording during which the drum (Fig. 1) was rotated at 10 rpm at 15 sec intervals. The direction of the rotation was chosen such that the fish was confronted with the boundary between black and white in the shortest time. During the second minute, stimulus water (food cue, alarm cue, or control water) was injected into the tank. The sequence of rotations of the first minute was repeated in the third and subsequent minutes of the trial. After the third minute, the light level was increased by the removal of one ND 209 filter every minute. At the end of each trial the stimulus input tube was rinsed thoroughly with fish water.

Both the food cue and alarm cue experiments followed a blocked design. Each consisted of four treatments (sex (cue treatment; alarm cue experiment $n = 10$ per treatment, food odor experiment $n = 9$ per treatment) ordered within each block according to a Latin square design. Experimental trials were conducted between 9.30 AM and 5 PM over the course of 3 weeks.

Response variables

The response to the visual stimulus was scored as the number of times the fish turned within one body length of the black segment, divided by the total number of times it 'encountered' the black segment. An encounter is defined as the fish coming within one body length of the black segment. A very low score would be obtained by the fish repeatedly swimming past the black segment (Fig. 1) without turning, whereas a high scoring fish would turn several times in close proximity to the segment and rarely swim past it without turning. To the observer, this behavior looked as though the fish saw the segment as an attractive stimulus, rather than a threatening stimulus. Because these fish were raised in captivity and have learned to associate people near the tank with food, the fish may be drawn to the black stripe associating the shadow with the possibility of food.

The turn rate of each fish was recorded as the total number of 180 degree turns a fish made during each minute of the trial. These data were used to test whether turn rate increased with increasing light level across the trials, and whether turn rate depended on the stimulus water used in the trial. Any large, light- or cue-dependent increase in turn rate may affect the interpretation of the reaction to the visual stimulus results.

Both response variables [i.e., (i) proportion of turns within one body length of the black segment, (ii) turn rate] were used to assess the effect of the input of stimulus water. The cue was introduced to the tank during the second minute, but the light level was not increased until the end of the third minute. Comparison of the mean response during the first minute to that during the third minute was used to test the hypothesis that the addition of stimulus water alone affected the behavior of the test fish.

Video recordings of the behavioral trials were scored by the same person, ignorant of the treatment.

Statistical analyses

The reaction to the visual stimulus data were arcsine square root transformed and the overall turn rate data were square root transformed before analysis. Of the transformed data, 95% were found to conform to the assumptions of general linear models, as tested using Kolmogorov-Smirnov and Levene tests. Although the analyses of the reaction to the visual stimulus were performed on transformed data, the proportional data are presented in the figures for clarity.

The data were analyzed using a repeated measures ANOVA in SPSS (SPSS, Inc.). The mean difference in response between minutes one and three in each trial was tested using a paired Student's *t*-test in Excel (Microsoft®) to test for a change in the baseline behavior after the addition of the stimulus water. A paired *t*-test was also used to assess whether any change in response between these 2 minutes differed between the sexes or genetic strains. A *t*-test for two groups was used in Systat (Systat Software, Inc.) as a *post hoc* test to identify the light levels at which treatment groups differed.

Results

Response to food cue

Exposure to food cue in itself did not significantly affect the reaction to the visual stimulus compared with the control ($F_{1,34} = 2.388$, $p = 0.132$). There was, however, a significant interaction between light level and the cue treatment ($F_{9,306} = 2.183$, $p = 0.023$). Those fish exposed to food cue (Fig. 2A, dashed line) showed an elevation in their response to a visual stimulus at lower light levels than those exposed to control water (Fig. 2A, solid line). The *post hoc* tests revealed that the group exposed to food cue were different from the control group at four light levels during the trial, as represented on Figure 2A.

Response to alarm cue

Exposure to alarm cue or control water affected a fish's reaction to the visual stimulus ($F_{1,34} = 11.827$, $p = 0.002$). *Post hoc* analyses revealed that the mean response of the group exposed to alarm cue (Fig. 2B, dashed line) was higher than that of the control group (Fig. 2B, solid line) across all light levels and significantly higher at four light levels, as illustrated by the asterisks on Figure 2B. There was, however, no significant light by stimulus interaction ($F_{9,306} = 0.842$, $p = 0.885$).

Fish in both experiments showed a significant increase in their reaction to the visual stimulus as the light level increased, regardless of the stimulus water to which they were exposed (food cue: $F_{9,306} = 17.236$, $p < 0.001$; alarm cue:

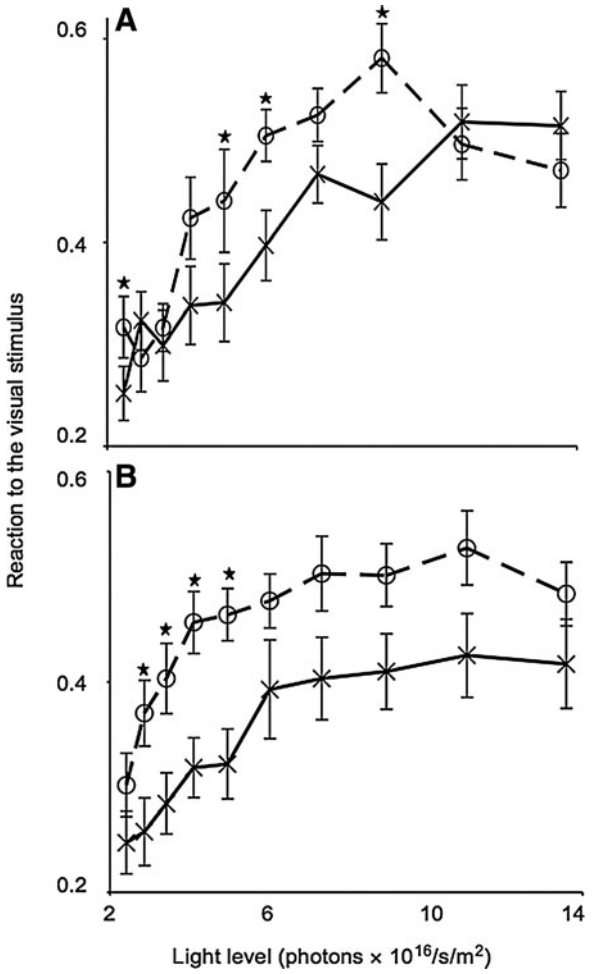

FIG. 2. The visual response of the fish at each light level, measured as the proportion of turns fish made on or at the black segment out of the total number of encounters with the segment. **(A)** Each line represents eighteen replicates of each of the control treatment (*solid line*) and the food cue treatment (*dashed line*). **(B)** Each line represents twenty replicates of each of the control treatment (*solid line*) and the alarm cue treatment (*dashed line*). *Error bars* are the standard error of the mean and *asterisks* denote light levels at which the difference between the responses of the groups to the visual stimulus was significant ($p < 0.05$).

$F_{9,261} = 11.341$, $p < 0.001$). This strongly suggests that the reaction to the visual stimulus is correlated with the visibility of the stimulus.

Activity levels

Turn rate increased slightly as light level increased in both experiments (food cue: mean difference in turn rate between the darkest and lightest minutes of a trial = 2.896 turns/min, 95% CI = 0.68 to 5.112; $F_{9,513} = 3.315$, $p = 0.001$; alarm cue: mean difference in turn rate between the darkest and lightest minutes of a trial = 2.575 turns/min, 95% CI = −0.01 to 5.17; $F_{9,162} = 2.726$, $p = 0.006$). In neither experiment was turn rate affected by cue (food cue: $F_{1,23} = 1.106$, $p = 0.304$; alarm cue: $F_{1,28} = 1.753$, $p = 0.196$). There was a significant difference between the turn rates of male and female fish in both experiments; males turned more than females across all light levels (food cue: mean difference = 3.75 turns/min $F_{1,57} = 7.732$, $p = 0.007$; alarm cue: mean difference = 4.20 turns/min $F_{1,29} = 4.146$, $p = 0.051$).

Discussion

Our results demonstrate that the complex chemical mixtures of food and conspecific alarm cue increase the response of zebrafish to a visual stimulus at low light levels. Unfortunately, the light levels at which these experiments and the similar one conducted by Maaswinkel and Li[15] took place cannot be directly compared as the cited article provides insufficient detail about the light environment used.

This study found that the input of the chemical stimuli had no effect on the recorded behaviors, in the absence of a change in light level, for one minute following input. An important consideration is whether such an effect went undetected in these experiments because it takes longer than one minute to take effect. The highly sensitive nature of the chemosensory system in fish,[30] and the potential fitness consequences of not responding to the messages it can convey,[31–33] argue against a time delay of more than a minute in chemically-induced behavioral changes in fish, although this has not been explicitly investigated.

In both experiments, fish made an average of about 3 turns per minute more during the lightest minute than during the darkest one. Although statistically significant, this small change in turn rate does not fully explain the much larger change recorded in the reaction to the visual stimulus as light level increased, and the latter increase cannot therefore be explained as a by-product of a light-induced increase in turn rate. Further evidence for the independence of the two response variables is that the turn rate of male fish was significantly higher than that of female fish across all light levels in both experiments, but there was no sex difference in the reaction to the visual stimulus.

The difference in turn rate between the sexes cannot easily be explained, although it may correlate with the greater ability of males than females to evade capture in a tank (KEW, personal observation). There is little empirical evidence in the literature to suggest a disparity in the activity level of male and female zebrafish. Plaut[34] offers a possible explanation for the difference: fin size in this species is an important determinant of swimming behavior. Although not dramatically sexually dimorphic, zebrafish females have a deeper body shape and slightly smaller fins than males.[35] For further discussion of sex differences in fish, see the review by Magurran and Marcías-Garcia.[36]

The control fish exposed in each experiment required quite different amounts of light to reach 50% of their maximum response (food cue experiment = 6.96×10^{16} photons/s/m^2; alarm cue experiment = 4.91×10^{16} photons/s/m^2). There are several interpretations for these results, one is that there are differences in visual sensitivity and visual behavior between the different strains used in these experiments. Behavioral and morphological differences in laboratory and wild zebrafish have been reported,[37,38] as well as background genetic differences between strains of zebrafish[39]. Because the fish from different strains were also of different ages, however, it is not possible to distinguish the effects of these factors using these data.

No evidence of a time of day effect was detected during this study, despite a wealth of evidence suggesting that visual sensitivity is controlled by a circadian rhythm (reviewed by Fleisch and Neuhauss[40]). This may have been because visual sensitivity does not vary much during the period within which all trials were conducted (9.30 AM—5 PM, lights on at 9 AM), although the similar study by Maaswinkel and Li[15] did find an effect over the course of the first few hours of the subjective day. The light levels over which this study was conducted were on a finer scale than those used by Maaswinkel and Li.[15] If there had been an effect of time of day, it is likely, therefore, that these results would have reflected it. That there was no time of day effect recorded during this study argues that the effect observed was a behavioral, rather than a physiological one.

Neither the results of this study, nor those of the experiments by Maaswinkel and Li,[15] can be used to demonstrate conclusively that chemical stimulation increases visual sensitivity of zebrafish. Fish perceive chemical information through both gustatory and olfactory sensory systems.[2] The finding of Maaswinkel and Li[15] that the effect they observed was much diminished upon severing the olfactory bulb from the epithelium suggests that olfaction, rather than gustation, mediated the increase in visual sensitivity observed in their study, as well as that reported here. Other sensory systems may also have been stimulated, however. That chemosensory systems were stimulated does not eliminate the hypothesis that chemical stimulation simply alters fish behavior and not visual sensitivity, however. Fish may be able to see the visual stimulus, but not respond behaviorally until a certain light level is reached, a light level that changes depending on information from other senses. For example, fish do not overtly respond to threatening stimuli in some contexts.[41–43] These zebrafish have been raised in entirely transparent containers, with frequent movement outside their tanks. They may, therefore, have become accustomed to visual stimuli moving close by. The difference between the control fish and those exposed to chemical stimuli may simply be that the context is too unnatural to elicit a response at low light levels unless more information about the moving object is detected using other senses. In the case of alarm cue, for example, because vision is likely to be an important sense to the majority of their predators,[44] zebrafish may be relatively safe in the dark. Indeed, they can demonstrate a preference for dark environments,[45] although this is dependent on other factors.[27] Thus, in situations of low perceived risk, zebrafish may be less likely to respond to visual stimuli, as has been demonstrated in fathead minnows (*Pimephales promelas*[46]).

More work is needed to elucidate the mechanisms, physiological or behavioral, by which olfactory sensory system can influence visual behaviors. Whether similar patterns are observed in other species, and with other chemical stimuli, remains to be tested. Nevertheless, the idea of senses priming each other[47] must be considered an important aspect of fish sensory behavior in at least feeding and alarm behavior.

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Disclosure Statement

No competing financial interests exist.

References

1. Wisenden BD. Olfactory assessment of predation risk in the aquatic environment. Philos Trans R Soc Lon B Biol Sci 2000;355:1205–1208.
2. Derby CD, Sorensen PW. Neural processing, perception, and behavioral responses to natural chemical stimuli by fish and crustaceans. J Chem Ecol 2008;34:898–914.
3. Ryan MJ, Wagner WE. Asymmetries in mating preferences between species: Female swordtails prefer heterospecific males. Science 1987;236:595–597.
4. de Caprona M, Ryan M. Conspecific mate recognition in swordtails, *Xiphophorus nigrensis* and *X. pygmaeus* (Poeciliidae): Olfactory and visual cues. Anim Behav 1990;39: 290–296.
5. Rafferty NE. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. Behav Ecol 2006; 17:965–970.
6. Kodric-Brown A, Strecker U. Responses of *Cyprinodon maya* and *C. labiosus* females to visual and olfactory cues of conspecific and heterospecific males. Biol J Linnean Soc 2001; 74:541–548.
7. Roberts N, Winn HE. Utilization of the senses in feeding behaviour of the Johnny darter, *Etheostoma nigrum*. Copeia 1962;1962:567–570.
8. Daugherty CH, Daugherty LB, Blair AP. Visual and olfactory stimuli in the feeding behavior of darters (Etheostoma) inhabiting clear and muddy water. Copeia 1976;1976:380–382.
9. Chivers D, Smith RJ. Chemical alarm signaling in aquatic predator-prey systems: A review and prospectus. Ecoscience 1998;5:338–352.
10. Hartman E, Abrahams M. Sensory compensation and the detection of predators: The interaction between chemical and visual information. Proc R Soc Lon B Biol Sci 2000; 267:571–575.
11. Rowe C. Receiver psychology and the evolution of multicomponent signals. Anim Behav 1999;58.
12. Weiss O, Meyer D. Odor stimuli modulate retinal excitability in fish. Neurosci Lett 1988;93:209–213.
13. Behrens U, Wagner H. Terminal nerve and vision. Microsc Res Tech 2004;65:25–32.
14. von Bartheld CS. The terminal nerve and its relation with extrabulbar "olfactory" projections: Lessons from lampreys and lungfishes. Microsc Res Tech 2004;65:13–24.
15. Maaswinkel H, Li L. Olfactory input increases visual sensitivity in zebrafish: A possible function for the terminal nerve and dopaminergic interplexiform cells. J Exp Biol 2003;206: 2201–2209.
16. Vitebsky A, Reyes R, Sanderson M, Michel W. Isolation and characterization of the *laure* olfactory behavioral mutant in the zebrafish, *Danio rerio*. Dev Dyn 2005;234:229–242.
17. Hara TJ. Feeding behaviour in some teleosts is triggered by single amino acids primarily through olfaction. J Fish Biol 2006;68:810–825.

18. Baier H. Zebrafish on the move: Towards a behaviour-genetic analysis of vertebrate vision. Curr Opinion Neurobiol 2000;10:451–455.
19. Bilotta J, Saszik S. The zebrafish as a model visual system. Int J Dev Neurosci 2001;19:621–629.
20. Rinner O, Rick JM, Neuhauss CF. Contrast sensitivity, spatial and temporal tuning of the larval zebrafish optokinetic response. Invest Opthamol Visual Sci 2005;46:137–142.
21. Ren JQ, McCarthy WR, Zhang H, Adolph AR, Li L. Behavioral visual response of wild-type and hypopigmented zebrafish. Vision Res 2002;42:291–299.
22. Whitlock KE. The sense of scents: Olfactory behaviours in the zebrafish. Zebrafish 2006;3:203–313.
23. Harden M, Newton L, Lloyd R, Whitlock K. Olfactory imprinting is correlated with changes in gene expression in the olfactory epithelia of the zebrafish. J Neurobiol 2006;66: 1452–1466.
24. Gerlai R, Lahav M, Guo S, Rosenthal A. Drinks like a fish: Zebra fish (*Danio rerio*) as a behaviour genetic model to study alcohol effects. Pharmacol Biochem Behav 2000;67: 773–782.
25. Darland T, Dowling J. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. Proc Natl Acad Sci 2001;98:11691–11696.
26. Speedie N, Gerlai R. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behav Brain Res 2008; 188:168–177.
27. Stephenson JF, Whitlock KE, Partridge JC. Zebrafish preference for light or dark is dependent on ambient light levels and olfactory stimulation. Zebrafish 2011;8:17–22.
28. Wisenden BD, Vollbrecht KA, Brown JL. Is there a fish alarm cue? Affirming evidence from a wild study. Anim Behav 2004;67:59–67.
29. Li L, Dowling JE. Zebrafish visual sensitivity is regulated by a circadian clock. Visual Neurosci 1998;15:851–857.
30. Hansen A, Reutter K. Chemosensory systems in fish: Structural, functional and ecological aspects. In: *The Senses of Fish: Adaptations for the Reception of Natural Stimuli.* Von der Emde G, Mogdans J, Kapoor BG, (eds). Kluwer Academic Publishers, Boston, 2004.
31. Smith RJF. Alarm signals in fishes. Rev Fish Biol Fish 1992; 2:33–63.
32. Valentinčič T, Caprio J. Visual and chemical release of feeding behavior in adult rainbow trout. Chem Senses 1997;22:375–382.
33. Milinski M, Griffiths S, Wegner K, Reusch T. Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. Proc Natl Acad Sci 2005;102:4414–4418.
34. Plaut I. Effects of fin size on swimming performance, swimming behaviour and routine activity of zebrafish *Danio rerio*. J Exp Biol 2000;203:813–820.
35. Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, *Danio rerio*. Biol Rev 2008;83: 13–34.
36. Magurran A, Macías Garcia C. Sex differences in behaviour as an indirect consequence of mating system. J Fish Biol 2000;57:839–857.
37. Wright D, Nakamichi R, Krause J, Butlin R. QTL analysis of behavioral and morphological differentiation between wild and laboratory zebrafish (*Danio rerio*). Behav Genet 2006;36: 271–284.
38. Moretz J, Martins E, Robison B. Behavioral syndromes and the evolution of correlated behavior in zebrafish. Behav Ecol 2007;18:556–562.
39. Sanders LH, Whitlock KE. Phenotype of the zebrafish *masterblind* (*mbl*) mutant is dependent on the genetic background. Dev Dyn 2003;277:291–300.
40. Fleisch V, Neuhauss S. Visual behavior in zebrafish. Zebrafish 2006;3:191–201.
41. Magurran A, Irving P, Henderson P. Is there a fish alarm pheromone? A wild study and critique. Proc R Soc B Biol Sci 1996;263:1551–1556.
42. Brown GE, Adrian Jr. JC, Patton T, Chivers DP. Fathead minnows learn to recognize predator odour when exposed the concentrations of artificial alarm pheromone below their behavioural-response threshold. Can J Zool 2001;79:2239–2245.
43. Brown GE, Poirier J-F, Adrian Jr. JC. Assessment of local predation risk: The role of subthreshold concentrations of chemical alarm cues. Behav Ecol 2004;15:810–815.
44. Engeszer RE, Alberici Da Barbiano L, Ryan MJ, Parichy DM. Timing and plasticity of shoaling behaviour in the zebrafish, *Danio rerio*. Anim Behav 2007;74:1269–1275.
45. Serra E, Medalha C, Mattioli R. Natural preference of zebrafish (*Danio rerio*) for a dark environment. Braz J Med Biol Res 1999;32:1551–1553.
46. Hartman EJ, Abrahams MV. Sensory compensation and the detection of predators: The interaction between chemical and visual information. Proc R Soc Lond B Biol Sci 2000; 267:571–575.
47. Rowe C. Receiver psychology and the evolution of multicomponenet signals. Anim Behav 1999;58:921–931.

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