

The Influence of Sex and Phenotype on Shoaling Decisions in Zebrafish

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Fish typically choose shoalmates with similar phenotypic characteristics to themselves, thus creating shoals for which predators have difficulty identifying and attacking one specific individual. And while shoaling should provide similar anti-predator benefits to both males and females, the two sexes do not always make the same shoaling decisions. Here we explore the effect of phenotype on sex-specific shoaling in three varieties of zebrafish (*Danio rerio*) and the closely related pearl danio (*Danio albolineatus*). We hypothesized that males and females of each type of zebrafish (wildtype, *golden* mutants and *leopard* mutants), as well as male and female pearl danios, would choose to shoal rather than be alone and, when given a choice of shoalmates, would shoal with fish of their own phenotype rather than dissimilar fish. As expected, our results show that most fish preferred to shoal rather than be alone. However, while both sexes of wildtype zebrafish responded identically to shoaling decisions, male and female mutant zebrafish and pearl danio fish differed in their response to such choices. When given a choice of shoalmates, wildtype zebrafish of both sexes showed no discrimination between different *D. rerio* strains, although they did choose to shoal with wildtype conspecifics rather than pearl danios. The shoalmate preferences of the mutant zebrafish revealed that males showed no discrimination between shoals of their own variety and wildtype shoals, while mutant females preferred shoals of their own strain. Similarly, male pearl danios showed no discrimination between shoals of their own species and shoals of wildtype zebrafish, while pearl danio females preferred their own species. These results demonstrate the complex influence of sex and phenotype on shoaling behavior.

Shoaling (forming loose social aggregations) is a behavior demonstrated by many species of teleost fish, providing individuals with benefits such as enhanced foraging opportunities, access to mates, and protection from predators (Krause & Ruxton, 2002). With respect to the anti-predator benefits, it has been suggested that it may be difficult for a predator to identify and attack any one specific individual within a group of phenotypically similar fish (Pitcher, 1986). This phenomenon, referred to as the confusion effect, causes predators to hesitate momentarily before attacking aggregated prey, leading to a lower capture success than when attacking solitary or dispersed prey (Milinski, 1979; Ohguchi, 1981). In a related phenomenon, the oddity effect, a phenotypically distinct individual within a shoal is more likely to be targeted by a predator (Landeau & Terborgh, 1986). It is not surprising, therefore, that shoaling fish typically choose to associate with

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fish that bear a resemblance to themselves rather than fish that are phenotypically different.

In studies on the confusion and oddity effects, a number of phenotypic attributes have been shown to affect shoaling preferences for a variety of fish species (reviewed in Krause, Butlin, Peuhkuri, & Pritchard, 2000). Such attributes include body size (Hauser, Carvalho, & Pitcher, 1998; Krause & Godin, 1994; McCann, Kohen, & Kline, 1971; Theodorakis, 1989), parasite load that causes black spots on the body (Barber, Downey, & Braithwaite, 1998; Krause & Godin 1996), body shape (Hauser et al. , 1998), body coloration (McRobert & Bradner, 1998) and stripe pattern (Engeszer, Ryan, & Parichy, 2004; McCann, et al., 1971; Rosenthal & Ryan, 2005). As predicted, fish are capable of discriminating between potential shoalmates on the basis of each of these phenotypic features, typically shoaling with fish that are similar in appearance. This assortative shoaling allows fish to potentially incur the benefits of the confusion effect and avoid the costs of the oddity effect.

One aspect of shoaling behavior that has been less well represented in the literature is the effect of sex. Many studies disregard sex, either by examining only one sex or by ignoring the sex of the fish altogether. Part of this omission may be a perception that the two sexes would make similar shoaling choices, especially in species in which males and females are phenotypically similar. In studies on a sexually dimorphic species such as the guppy (*Poecilia reticulata*), it was found that males are less likely than females to join shoals (Magurran, 1999), and female guppies stayed in their shoals (Griffiths & Magurran, 1998) while males spent more time moving between shoals (Magurran, 1998). In studies on another sexually dimorphic species, the Siamese fighting fish (*Betta splendens*), both males and females chose to shoal with larger groups of females, but females avoided males, while males did not (Snekser, McRobert, & Clotfelter, 2006a). Interestingly, sex-related shoaling differences have also been noted in species in which males and females are phenotypically similar. Female rainbow fish (*Melanotaenia eachamensis*) made shoaling choices based on relatedness of individuals within the shoal, while males did not (Arnold, 2000). Similarly, female zebrafish (*Danio rerio*) preferred to shoal with unfamiliar and unrelated males, while males showed no such preference (Gerlach & Lysiak, 2006). Zebrafish males and females also differed in their shoaling decisions when shoal sex and shoal size were varied (Ruhl & McRobert, 2005; Ruhl, McRobert, & Currie, 2009). Additionally, male zebrafish have been shown to be more sensitive than females to visual striping cues in shoaling assays (Engeszer, Wang, Ryan, & Parichy, 2008).

Zebrafish are an ideal species to further explore the factors that influence shoaling decisions. Zebrafish have long been used in genetic and developmental studies and a recent surge in research has focused on their ecology and behavior (reviewed by Spence, Gerlach, Lawrence, & Smith, 2008). The availability of a large number of phenotypic variants (Detrich, Westerfield, & Zon, 1999; Nüsslein-Volhard, 1994; Parichy, 2003) and closely related species (Fang, 2003; Mayden et al., 2007) means that many naturally occurring variables exist for examining the

relative importance of specific physical traits on shoalmate choice. Thus far, studies on shoalmate choice of wildtype zebrafish have taken advantage of closely related species (Engeszer et al., 2008; McCann & Carlson, 1982; Spence & Smith, 2007), pigment mutants and transgenics (Engeszer et al., 2004; Snekser, McRobert, Murphy, & Clotfelter, 2006b; Spence & Smith, 2007; Engeszer et al., 2008), and fin mutants (Kitevski & Pyron, 2003). Less attention has been focused on the shoaling behavior of mutant zebrafish or closely related species, despite evidence for strain differences in other behavioral contexts (Gumm, Snekser, & Iovine, 2008; Itzkowitz & Iovine, 2007; Moretz, Martins, & Robison, 2006, 2007; Robison & Rowland, 2005; Wright, Butlin, & Carlborg, 2006; Wright, Rimmer, Pritchard, Krause, & Butlin, 2003).

The objective of this study was to explore possible sex differences in the shoaling behavior of wildtype zebrafish, two mutant zebrafish strains (*leopard* and *golden*) and the closely related pearl danio (*D. albolineatus*). In each case, we predicted that males and females of each strain would prefer to associate with other fish, regardless of phenotype, over an empty chamber and would prefer to associate with fish of similar phenotypic characteristics in dichotomous choice assays.

Method

Adult fish were obtained from commercial suppliers (World Wide Aquarium and Seven Star Tropical Inc., Philadelphia, PA). Focal fish (whose behavior was observed) were housed in separate tanks from stimulus fish (used in groups of three to elicit responses from focal fish). Fish were further separated by variety and sex; sex was determined by general body shape (with females being more rotund than males) and the presence of a genital papilla rostral of the anal fin in females. All tanks were covered on three sides with opaque white paper to visually isolate the fish from other varieties and the opposite sex. Tanks were maintained under fluorescent lighting on a 12L:12D cycle at 23° C. All fish were fed commercial flake food once per day.

Three varieties of zebrafish (*D. rerio*) as well as the closely related pearl danio (*D. albolineatus*) were used as both focal and stimulus fish. Wildtype zebrafish have five to seven dark stripes of melanophores and iridophores alternating with light stripes of xanthophores and iridophores (Parichy, 2006a,b; Quigley & Parichy, 2002). The mutant, *golden*, lacks melanophore pigmentation resulting in a yellow coloration with faint yellow stripes (Lamason et al., 2005). The mutant, *leopard*, has interrupted melanophore stripes, giving the fish a spotted appearance (Johnson, Africa, Walker, & Weston, 1995). Pearl danios (*D. albolineatus*), a close relative of zebrafish, have the same general body shape and size as *D. rerio*, but lack dark stripes altogether (Fang, 2003).

Test tanks were constructed by dividing aquaria (74 x 31 x 31 cm) into three compartments (Fig. 1) with clear glass partitions sealed with silicone caulk 18 cm from each end. The central compartment was further divided into left and right sides by opaque Plexiglas partitions (25 x 18 cm). These partitions allowed focal fish to swim to either side of the central compartment but prevented fish on one side from seeing the stimulus fish on the opposite side.

Each test session consisted of a 600 s observation period during which the time the focal fish spent on each side of the central compartment was recorded. Twenty fish of each sex (chosen at random from a holding tank) were tested in each assay and the same fish was not used more than once in any assay. Prior to each test, a single focal fish was placed into the central compartment and allowed one hour to acclimate. During this acclimation period, the stimulus shoals were held in their end compartments. Stimulus fish were, in all assays, the same sex as the focal fish. An equal number of tests were run with stimulus shoals in the left and right end compartments to reduce the risk of 'tank effects'.

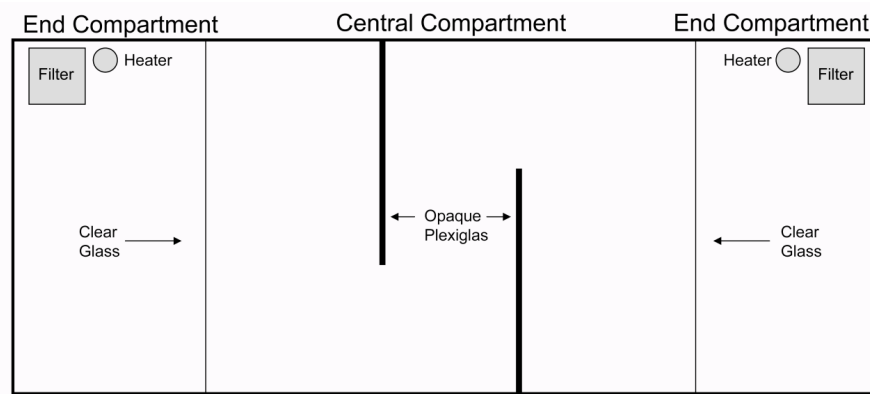


Figure 1. Diagram of aerial view of dichotomous choice testing tank.

For wildtype zebrafish, we ran two types of behavioral assay. In the first set, wildtype zebrafish (males or females) were given the choice of an empty chamber and a shoal of three fish of one variety: wildtype, *golden*, *leopard*, or pearl danios. In the second set of assays (simultaneous choice tests), wildtype zebrafish were given the choice between a shoal of three wildtype zebrafish and another shoal of three fish: wildtype, *golden*, *leopard*, or pearl danios.

Similar behavioral tests were performed with the mutant zebrafish (*golden* and *leopard*) and the pearl danios. In the first set of assays, male and female mutant zebrafish or pearl danios were given the choice of an empty chamber and a shoal of three fish of the same variety or a shoal of three wildtype zebrafish. In the second set of assays, male and female mutant zebrafish or pearl danios were given the choice of a shoal of three wildtype zebrafish and a shoal of three fish of the same variety as the focal fish.

The mean time spent on either side of the central compartment was calculated for each assay. We assumed that if there was no preference for either shoal, the focal fish should spend 300 s (or 50% of their time) on each side of the test tank. For this reason, we compared the association times to the null expectation of 300s using one-sample *t* tests for each assay (see Bradner & McRobert, 2001; Ruhl & McRobert, 2005; Ruhl et al., 2009; Sneksner et al., 2006a,b).

Results

When given the choice between an empty compartment and a compartment containing three fish of the same sex, male and female wildtype zebrafish always shoaled regardless of the variety of stimulus fish. In simultaneous choice tests, neither male nor female wildtype zebrafish showed a significant preference when given the choice between shoals of conspecifics but did spend significantly more time near wildtype zebrafish than near the heterospecific pearl danios (Table 1 and Fig. 2).

When *golden* zebrafish were given a choice between a shoal of three fish of the same variety and an empty compartment, both males and females spent significantly more time near the shoal of *golden* zebrafish. However, when given a choice between three wildtype zebrafish and an empty compartment, *golden* males shoaled while *golden* females did not demonstrate a preference. In simultaneous choice tests, *golden* females spent significantly more time near a shoal of *golden* females than near a shoal of wildtype females, while *golden* males did not exhibit a preference between *golden* males and wildtype males (Table 2 and Fig. 2).

Table 1

Mean association time \pm SE exhibited by male and female wildtype zebrafish for each behavioral choice test.

Sex of Focal Fish	Shoal 1	Mean time (s)	Shoal 2	Mean time (s)	SE	<i>t</i>	<i>p</i>
M	3 Wildtype	430	Empty	170	39.90	3.26	0.004
M	3 golden	402	Empty	198	29.75	3.43	0.003
M	3 leopard	415	Empty	185	16.72	6.87	<0.001
M	3 pearl	401	Empty	199	34.53	2.92	0.009
M	3 Wildtype	346	3 Wildtype	254	31.36	1.48	0.16
M	3 Wildtype	313	3 golden	287	27.08	0.48	0.64
M	3 Wildtype	312	3 leopard	288	29.79	0.39	0.70
M	3 Wildtype	362	3 pearl	238	28.74	2.14	0.04
F	3 Wildtype	476	Empty	124	20.97	8.39	<0.001
F	3 golden	390	Empty	210	27.94	3.22	0.005
F	3 leopard	447	Empty	153	22.33	6.59	<0.001
F	3 pearl	435	Empty	165	29.94	4.54	<0.001
F	3 Wildtype	288	3 Wildtype	312	23.88	0.49	0.63
F	3 Wildtype	355	3 golden	245	30.21	1.83	0.08
F	3 Wildtype	334	3 leopard	266	25.91	1.31	0.21
F	3 Wildtype	401	3 pearl	199	37.49	2.68	0.01

Note: $n = 20$ for each behavioral choice test. *t*-statistics (One-sample *t* test ($H_0 = 0.5$)) and corresponding *p*-values are given for each test. Statistically significant preferences are indicated in **bold**.

Table 2

Mean association time \pm SE exhibited by mutant zebrafish (golden and leopard) for each behavioral choice test.

Sex of Focal Fish	Shoal 1	Mean time (s)	Shoal 2	Mean time (s)	SE	<i>t</i>	<i>p</i>
golden zebrafish							
M	3 Wildtype	391	Empty	209	32.07	2.83	0.011
M	3 golden	422	Empty	178	37.57	3.24	0.004
M	3 Wildtype	265	3 golden	335	41.34	0.85	0.41
F	3 Wildtype	344	Empty	256	21.56	2.04	0.06
F	3 golden	431	Empty	169	31.11	4.21	<0.001
F	3 Wildtype	240	3 golden	360	21.85	2.72	0.013
leopard zebrafish							
M	3 Wildtype	417	Empty	183	22.33	5.23	<0.001
M	3 leopard	445	Empty	155	34.20	4.25	<0.001
M	3 Wildtype	313	3 leopard	287	44.63	0.30	0.77
F	3 Wildtype	430	Empty	170	28.51	4.55	<0.001
F	3 leopard	437	Empty	163	30.67	4.46	<0.001
F	3 Wildtype	239	3 leopard	361	25.35	2.40	<0.001

Note: $n = 20$ for each behavioral choice test. *t*-statistics (One-sample *t* test ($H_0 = 0.5$)) and corresponding *p* values are given for each test. Statistically significant preferences are indicated in **bold**.

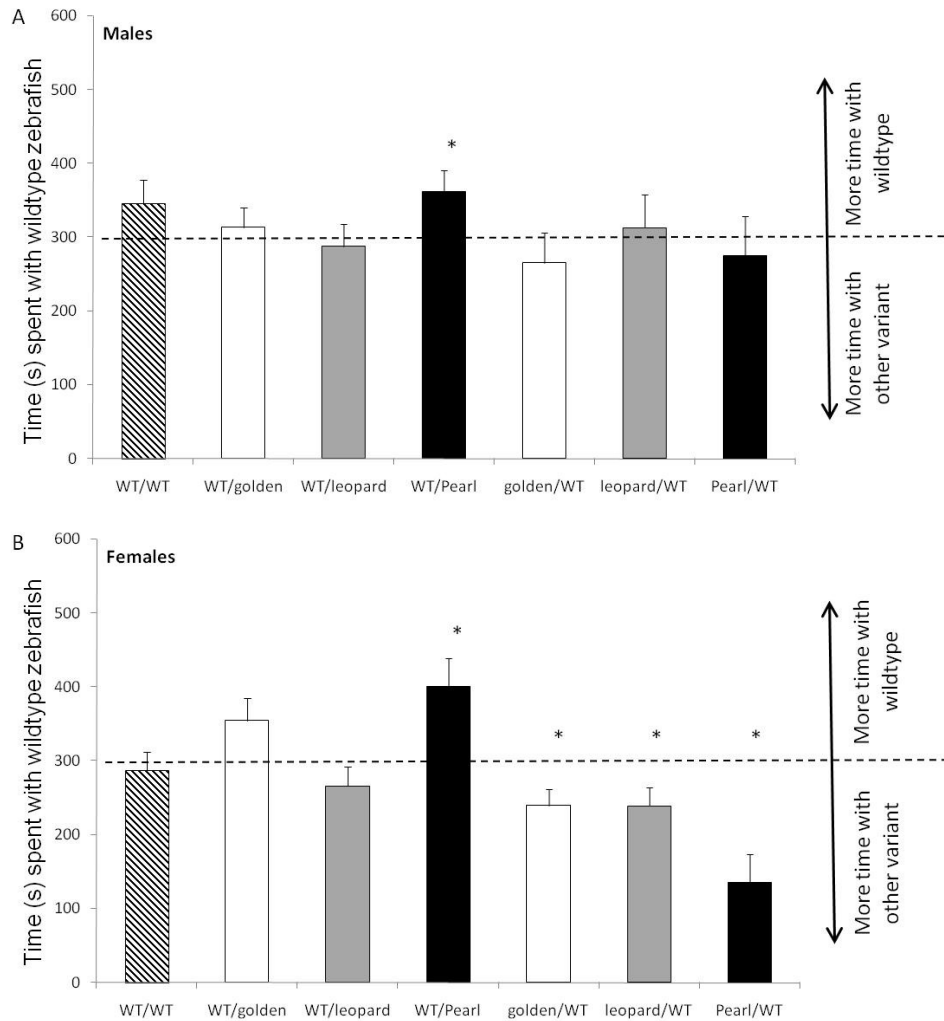


Figure 2. Mean + SE time spent with wildtype zebrafish in each of the dichotomous choice tests for males (A) and females (B). The strain of fish listed first is that of the focal fish; WT = wildtype. * $p < 0.05$

When *leopard* zebrafish were given a choice between a shoal of three *leopard* zebrafish and an empty end compartment, or between a shoal of three wildtype zebrafish and an empty compartment, both males and females spent significantly more time near the shoal of fish. In simultaneous choice tests, *leopard* females spent significantly more time near a shoal of *leopard* females than near a shoal of wildtype females while *leopard* males did not exhibit a preference between *leopard* males and wildtype males (Table 2 and Fig. 2).

When pearl danios were presented with a choice between an empty chamber and either a shoal of three pearl danios or a shoal of wildtype zebrafish,

both males and females spent significantly more time near the shoal of fish. When presented with a shoal of three pearl danios and a shoal of three wildtype zebrafish, however, pearl danio females spent significantly more time near the pearl danio shoal while males showed no significant preference (Table 3 and Fig. 2).

Table 3
Mean association time \pm SE exhibited by pearl danios for each behavioral choice test.

Sex of Focal Fish	Shoal 1	Mean time (s)	Shoal 2	Mean time (s)	SE	<i>t</i>	<i>p</i>
M	3 Wildtype	482	Empty	118	36.42	4.99	<0.001
M	3 pearl	422	Empty	178	27.32	4.48	<0.001
M	3 Wildtype	275	3 pearl	325	53.27	0.47	0.641
F	3 Wildtype	407	Empty	193	37.99	2.82	0.011
F	3 pearl	464	Empty	136	34.30	4.78	<0.001
F	3 Wildtype	136	3 pearl	464	37.45	4.37	<0.001

Note: $n = 20$ for each behavioral choice test. *t*-statistics (One-sample *t* test ($H_0 = 0.5$)) and corresponding *p* values are given for each test. Statistically significant preferences are indicated in **bold**.

Discussion

Both sexes of wildtype zebrafish made similar shoaling decisions. Males and females each chose to shoal rather than be alone, regardless of the strain of shoal presented. Additionally, in simultaneous choice assays, neither sex showed a preference when given a choice between a shoal of wildtype zebrafish and a shoal of mutant zebrafish with atypical body coloration or stripe pattern. This lack of shoaling preference is in concordance with a number of studies in which wildtype zebrafish failed to discriminate between their own variety and other phenotypic mutants, including *leopard* (Spence & Smith, 2007), and *golden* (Saverino & Gerlai, 2008). However, these studies did not specifically examine the behavior of both male and female zebrafish. In one study in which sex was considered, neither male nor female wildtype zebrafish distinguished between wildtype shoals and shoals of transgenic RFP Glofish (Snekser et al., 2006b).

However, the literature also contains studies in which wildtype zebrafish demonstrate an ability to discriminate between phenotypically similar and phenotypically distinct shoalmates. In studies utilizing altered photographs (McCann et al., 1971), video playback models (Rosenthal & Ryan, 2005), or stripe-less *nacre* zebrafish mutants (Engeszer et al., 2004), wildtype zebrafish demonstrated a preference for shoals of fish with body color and stripe pattern similar to their own. These studies, however, did not separate the sexes and specifically compare the behavior of males to females.

In contrast to their lack of discrimination between different zebrafish strains, both male and female wildtype zebrafish showed a significant preference for conspecific shoals over shoals of the heterospecific pearl danio. It is difficult, however, to determine whether the avoidance of the pearl danios was due to differences in phenotype (i.e., lack of stripes), or some other difference between the species, such as swimming behavior. Nonetheless, when taking all of the

assays into account, body coloration and stripe pattern did not have a significant impact on shoalmate choice for wildtype zebrafish, contrary to our initial predictions. Perhaps the phenotypic differences need to be as dramatic as “stripes” versus “no stripes” (i.e., Engeszer et al., 2004) to make a difference in terms of shoaling benefits such as the confusion effect. In keeping with our initial predictions, however, wildtype males and females made similar shoaling choices.

In contrast, the tests with zebrafish mutants (*leopard* and *golden*) and the closely related pearl danio, body coloration and stripe pattern did appear to affect shoalmate choice, but only for females. Males of these three varieties behaved similarly to wildtype zebrafish, while females showed greater discrimination in shoalmate choice. When presented with two shoals (wildtype fish vs. the same variety as the test fish), males showed no significant preference for either shoal. Males also chose to associate with shoals rather than be alone. Like males, females, for the most part, preferred to shoal rather than be alone regardless of the phenotype of the shoal. But unlike males, female pearl danios, *leopard* zebrafish, and *golden* zebrafish preferred to shoal with phenotypically similar fish over dissimilar fish.

Previous studies that focused on the shoaling tendencies of zebrafish mutants found that *leopard* zebrafish did not discriminate between shoals of *leopard* or wildtype zebrafish (Spence & Smith, 2007) or shoals of striped and stripe-less video models (Rosenthal & Ryan, 2005). Similarly, the *golden* mutant zebrafish showed no significant preference for striped or stripe-less models (Rosenthal & Ryan, 2005) and did not sort themselves from shoals of wildtype zebrafish (Saverino & Gerlai, 2008). Again, these previous studies ignored the sex of the fish in analysis and it is thus difficult to draw complete comparisons between these studies and the work presented here.

Overall, the results presented here show that sex can be a critical factor in the shoaling behavior of zebrafish (*D. rerio*) and the pearl danio (*D. albolineatus*). It also appears that stripe pattern and body coloration are phenotypic features that impinge on shoalmate choice in these species. However, the results are complex and, in some ways, create more questions than they answer. For example, why was the behavior of wildtype zebrafish so different from that of the mutant zebrafish? And why, within the mutant zebrafish strains and the pearl danio strain, did females make different shoaling choices than males? Perhaps the sex differences reflect a difference in the benefits provided by shoaling. Shoaling in males, in more natural situations, may be influenced by potential mate associations, with any shoal of zebrafish, regardless of body color or stripe pattern, providing an opportunity for increased reproductive success. Conversely, females may be making shoaling decisions based primarily on maximizing predator defense, and thus choose to associate with phenotypically similar fish. Previous studies on guppies indicate similar trends, with male strategies seemingly directed toward reproduction and females strategies toward increased foraging efficiency and survival (reviewed in Magurran & Garcia, 2000). Additional zebrafish shoaling experiments are necessary to better understand the evolutionary impetus of each sex to shoal.

It is important to note that the results presented here may be limited due to the use of captive-bred animals obtained from commercial suppliers. The fish we studied may have been in captivity for many generations and therefore have not been subject to the same selective pressures as wild fish. Despite this limitation, fish from commercial suppliers will obviously continue to be a staple in research and have already contributed substantially to our knowledge of zebrafish behavior (e.g., Bass & Gerlai, 2008; Colwill, Raymond, Ferriere, & Escudero, 2005; Gerlach & Lysiak, 2006; Kitevski & Pyron, 2003; Larson, O'Malley, & Melloni, 2006; McCann et al., 1971; McCann & Carlson, 1982; Miller & Gerlai, 2007; Pyron, 2003; Rosenthal & Ryan, 2005; Ruhl & McRobert, 2005; Saverino & Gerlai, 2008; Sneker et al., 2006b; Spence, Ashton, & Smith, 2007; Spence & Smith, 2005, 2006, 2007, 2008). Furthermore, access to mutations and transgenic fish available only in captive strains provide an incredibly valuable scientific resource.

Finally, while we can design experiments aimed at identifying the specific factors that mediate behavior, our interpretation of these studies is only speculation. We will never be able to see through the eyes of a fish and therefore we can only make suggestions about which factors influence behaviors such as shoalmate choice and the potential differences in decision-making between male and female fish. It is always possible that factors unseen by humans are responsible for the behaviors we study and we must remember that the perceptual worlds of our study organisms may be very different from our own (see Engeszer et al., 2008; von Uexkull, 1909).

References

- Arnold, K. E. (2000). Kin recognition in rainbowfish (*Melanotaenia eachamensis*): Sex, sibs, and shoaling. *Behavioral Ecology and Sociobiology*, *48*, 385-391.
- Barber, I., Downey, L. C., & Braithwaite, V. A. (1998). Parasitism, oddity and the mechanism of shoal choice. *Journal of Fish Biology*, *53*, 1365-1368.
- Bass, S. L. S., & Gerlai, R. (2008). Zebrafish (*Danio rerio*) responds differentially to stimulus fish: The effects of sympatric and allopatric predators and harmless fish. *Behavioural Brain Research*, *186*, 107-117.
- Bradner, J., & McRobert, S. P. (2001). Background coloration affects body colour segregation in fish. *Journal of Fish Biology*, *59*, 673-681.
- Colwill, R. M., Raymond, M. P., Ferriere, L., & Escudero, H. (2005). Visual discrimination learning in zebrafish (*Danio rerio*). *Behavioural Processes*, *70*, 19-31.
- Detrich III, H. W., Westerfield, M., & Zon, L. I. (1999). Overview of the zebrafish system. *Methods in Cell Biology*, *59*, 3-10.
- Engeszer, R. E., Ryan, M. J., & Parichy, D. M. (2004). Learned social preference in zebrafish. *Current Biology*, *14*, 881-884.
- Engeszer, R. E., Wang, G., Ryan, M. J., & Parichy, D. M. (2008). Sex-specific perceptual spaces for a vertebrate basal social aggregative behavior. *Proceedings of the National Academy of Science*, *105*, 929-933.
- Fang, F. (2003). Phylogenetic analysis of the Asian cyprinid genus *Danio* (Teleostei, Cyprinidae). *Copeia*, *4*, 714-728.

- Gerlach, G., & Lysiak, N. (2006). Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Animal Behaviour*, *71*, 1371-1377.
- Griffiths, S. W., & Magurran, A. E. (1998). Sex and schooling behaviour in Trinidadian guppies. *Animal Behaviour*, *56*, 689-693.
- Gumm, J. M., Sneksner, J. L., & Iovine, M. K. (2008). Fin-mutant female zebrafish (*Danio rerio*) exhibit differences in association preferences for male fin length. *Behavioural Processes*, *80*, 35-39.
- Hauser, L., Carvalho, G. R., & Pitcher, T. J. (1998). Genetic population structure in the Lake Tanganyika sardines *Limnothrissa miodon*. *Journal of Fish Biology Supplement A*, *53*, 413-429.
- Itzkowitz, M., & Iovine, M.K. (2007). Single gene mutations causing exaggerated fins also cause non-genetic changes in the display behavior of male zebrafish. *Behaviour*, *144*, 787-795.
- Johnson, S. L., Africa, D., Walker, C., & Weston, J. A. (1995). Genetic control of adult pigment stripe development in zebrafish. *Developmental Biology*, *167*, 27-33.
- Kitevski, B., & Pylon, M. (2003). Female zebrafish (*Danio rerio*) do not prefer mutant longfin males. *Journal of Freshwater Ecology*, *18*, 501-502.
- Krause, J., Butlin, R. K., Peuhkuri, N., & Pritchard, V. L. (2000). The social organization of fish shoals: A test of the predictive power of laboratory experiments for the field. *Biology Reviews*, *75*, 477-501.
- Krause, J., & Godin, J. G. J. (1996). Influence of parasitism on shoal choice in banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae). *Ethology*, *102*, 40-49.
- Krause, J., & Godin, J. G. J. (1994). Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): Effects of predation risk, fish size, species composition and size of shoals. *Ethology*, *98*, 128-136.
- Krause, J., & Ruxton, G. D. (2002). *Living in groups*. New York: Oxford University Press.
- Lamason, R. L., Mohideen, M.A., Mest, J.R., Wong, A.C., Norton, H. L., Aros, M.C., et al. (2005). SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science*, *310*, 1782-1786.
- Landeau, L., & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour*, *34*, 1372-1380.
- Larson, E. T., O'Malley, D. M., & Melloni Jr., R. H. (2006). Aggression and vasotocin are associated with dominate-subordinate relationships in zebrafish. *Behavioural Brain Research*, *167*, 94-102.
- Magurran, A. E. (1999). The causes and consequences of geographic variation in antipredator behavior, perspectives from fish populations. In S. A. Foster & J. A. Endler (Eds.), *Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms* (pp. 139-163). New York: Oxford University Press. 336pp.
- Magurran, A. E. (1998). Population differentiation without speciation. *Philosophical Transactions of the Royal Society B*, *353*, 275-286.
- Magurran, A. E., & Garcia, C. M. (2000). Sex differences in behaviour as an indirect consequence of mating systems. *Journal of Fish Biology*, *57*, 839-857.
- Mayden, R. L., Tang, K. L., Conway, K. W., Freyhof, J. R, Chamberlain, S., Haskins, M., et al. (2007). Phylogenetic relationships of *Danio* within the order Cypriniformes: A framework for comparative and evolutionary studies of a model species. *Journal of Experimental Zoology (Mol Dev Evol)*, *308B*, 642-654.
- McCann, L. I., & Carlson, C. C. (1982). Effects of cross-rearing on species identification in zebrafish and pearl danios. *Developmental Psychology*, *15*, 71-74.

- McCann, L. I., Kohen, D. J., & Kline, N. J. (1971). The effects of body size and body markings on nonpolarized schooling behavior of zebrafish (*Brachydanio rerio*). *Journal of Psychology*, *79*, 71-7-5.
- McRobert, S. P., & Bradner, J. (1998). The influence of body coloration on shoaling preferences in fish. *Animal Behaviour*, *56*, 611-615.
- Milinski, M. (1979). Can an experienced predator overcome the confusion of swarming prey more easily? *Animal Behaviour*, *27*, 1122-1126.
- Miller, N., & Gerlai, R. (2007). Quantification of shoaling behavior in zebrafish (*Danio rerio*). *Behavioural Brain Research*, *184*, 157-166.
- Moretz, J. A., Martins' E. P., & Robison' B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology*, *18*, 556-562.
- Moretz, J. A., Martins, E. P., & Robison, B. D. (2006). The effects of early and adult social environment on zebrafish (*Danio rerio*) behavior. *Environmental Biology of Fishes*, *80*, 91-101.
- Nüsslein-Volhard, C. (1994). Of flies and fishes. *Science*, *266*, 572-574.
- Ohguchi, O. (1981). Prey density and selection against oddity by three-spined sticklebacks. *Zeitschrift fur Tierpsychology*, *23*, 1-79.
- Parichy, D. M. (2006a). Homology and the evolution of novelty during danio pigment pattern development. *Journal of Experimental Zoology*, *306B*, 1-13.
- Parichy, D. M. (2006b). Evolution of danio pigment pattern development. *Heredity*, *97*, 200-210.
- Parichy, D. M. (2003). Pigment patterns: Fish in stripes and spots. *Current Biology*, *13*, R947-R950.
- Pitcher, T. J. (1986). Functions of shoaling behavior in teleosts. In T. J. Pitcher (Ed.), *The Behavior of Teleost Fish* (pp. 294-337). London: Chapman & Hall..554pp.
- Pyron, M. (2003). Female preferences and male-male interactions in zebrafish (*Danio rerio*). *Canadian Journal of Zoology*, *81*, 122-125.
- Quigley, I. K., & Parichy, D. M. (2002). Pigment pattern formation in zebrafish: A model for developmental genetics and the evolution of form. *Microscopy Research and Techniques*, *58*, 442-455.
- Robison, B. D., & Rowland, W. (2005). Zebrafish (*Danio rerio*) as a model organism in conservation biology: Behavioral variation among wild and domesticated strains. *Canadian Journal of Fisheries and Aquatic Science*, *62*, 2046-2054.
- Rosenthal, G. G., & Ryan, M. J. (2005). Assortative preferences for stripes in danios. *Animal Behaviour*, *70*, 1063-1066.
- Ruhl, N., McRobert, S. P., & Currie, W. J. S. (2009). The effect of sex ratio on shoaling, spawning and aggression in small laboratory populations of zebrafish (*Danio rerio*). *Lab Animal*, *38*, 264-269.
- Ruhl, N., & McRobert, S. P. (2005). The effect of sex and shoal size on shoaling behavior in *Danio rerio*. *Journal of Fish Biology*, *67*, 1318-1326.
- Saverino, C., & Gerlai, R. (2008). The social zebrafish: Behavioral responses to conspecific, heterospecific and computer animated fish. *Behavioural Brain Research*, *191*, 77-87.
- Snekser, J. L., McRobert, S. P., & Clotfelter, E. D. (2006a). Social partner preferences of male and female fighting fish (*Betta splendens*). *Behavioural Processes*, *72*, 38-41.
- Snekser, J. L., McRobert, S. P., Murphy, C. E., & Clotfelter, E. D. (2006b). Aggregation behavior in wildtype and transgenic zebrafish. *Ethology*, *111*, 1-10.

- Spence, R., Ashton, R., & Smith, C. (2007). Oviposition decisions are mediated by spawning site quality in wild and domesticated zebrafish, *Danio rerio*. *Behaviour*, *114*, 953-966.
- Spence, R., Gerlach, G., Lawrence, C., & Smith, C. (2008). The behaviour and ecology of the zebrafish, *Danio rerio*. *Biological Reviews*, *83*, 13-34.
- Spence, R., & Smith, C. (2008). Innate and learned colour preference in the zebrafish, *Danio rerio*. *Ethology*, *114*, 582-588.
- Spence, R., & Smith, C. (2007). The role of early learning in determining shoaling preferences based on visual cues in the zebrafish, *Danio rerio*. *Ethology*, *113*, 62-67.
- Spence, R., & Smith, C. (2006). Mating preferences of female zebrafish, *Danio rerio*, in relation to male dominance. *Behavioral Ecology*, *17*, 779-783.
- Spence, R., & Smith, C. (2005). Male territoriality mediates density and sex ratio effects on oviposition in the zebrafish, *Danio rerio*. *Animal Behaviour*, *69*, 1317-1323.
- Theodorakis, C. W. (1989). Size segregation and the effects of oddity on predation risk in minnow schools. *Animal Behaviour*, *38*, 496-502.
- von Uexkull, J. (1909/1985). Environment (Umwelt) and the inner world of animals (C.J. Mellor and D. Gove. Trans.). In G. M. Burghardt (Ed.), *Foundations of Comparative Ethology* (pp. 222-245). New York: Van Nostrand Reinhold. (Reprinted from J. von Uexkull (1909). *Umwelt and Innenwelt der Tiere*. Berlin: J. Springer). 441pp.
- Wright, D., Butlin, R. K., & Carlborg, O. (2006). Epistatic regulation of behavioural and morphological traits in the zebrafish (*Danio rerio*). *Behavioral Genetics*, *36*, 914-922.
- Wright, D., Rimmer, L. B., Pritchard, V. L., Krause, J., & Butlin, R. K. (2003). Inter and intra-population variation in shoaling and boldness in the zebrafish (*Danio rerio*). *Naturwissenschaften*, *90*, 374-377.