

Shoaling in zebrafish: what we don't know

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Abstract

Zebrafish have been gaining increasing popularity in behavioral neuroscience. However, the number of behavioral test paradigms specifically designed for zebrafish, and in general the amount of information available on the behavior of this species, is relatively small when compared with classical laboratory model organisms such as the mouse, the rat, and the fruit fly. A particularly typical behavioral feature of zebrafish is shoaling, i.e., group formation. Given the importance of social behavior in our own species and the fact that zebrafish possess several characteristics similar to those of other vertebrates, including humans, at many levels of biological organization (e.g., neuroanatomy, neurochemistry, biochemical processes, and amino acid sequence of proteins or nucleotide sequence of genes), the zebrafish is expected to be an excellent tool not only for basic research but perhaps also for translational research. Briefly, we propose that once social behavior of the zebrafish is better characterized and once appropriate behavioral methods have been developed, this species can be utilized for the analysis of the mechanisms of social behavior of other vertebrates including our own. In this review, we discuss general principles of shoaling and highlight what we know and what we do not know about this behavior as it pertains to zebrafish.

Keywords: *Danio rerio*; schooling; shoaling; zebrafish.

Introduction

Zebrafish (*Danio rerio*) are an important model organism in genetics and developmental biology (Driever et al., 1994). They are easy to breed and house in large numbers and mature quickly (see below) and have thus been suggested as an efficient complement to other vertebrate models such as rats and mice (Guo, 2004). The study of zebrafish behavior – which initially lagged far behind genetic, developmental, and pharmacological investigations – has increased dramatically over the past few years. Recently, researchers have examined genetic effects on behavior

(Burgess and Granato, 2008), especially as they relate to disease models (Guo, 2004; Lieschke and Currie, 2007), simple behavioral responses primarily of larval zebrafish (Chen et al., 2010; Rihel et al., 2010), the learning capabilities of adult zebrafish (Williams et al., 2002; Bilotta et al., 2005; Colwill et al., 2005; Al-Imari and Gerlai, 2008; Pather and Gerlai, 2009), and the effects of various drugs on both learned (Levin and Chen, 2004; Levin et al., 2006; Eddins et al., 2010) and innate (Levin et al., 2007; Gerlai et al., 2008; Speedie and Gerlai, 2008) behavior. Although findings on zebrafish behavior are accumulating rapidly (Miklosi and Andrew, 2006), much remains to be learned about zebrafish shoaling. The effects of environmental conditions, genetic differences between populations, and even the basic characteristics of ‘natural’ zebrafish shoaling (i.e., absent any laboratory manipulation) all have yet to be described in any detail. Here, we suggest that shoaling, a complex behavior in which zebrafish engage for most of their waking hours, could serve as a sensitive behavioral assay for a wide range of applications and expand the role of the zebrafish as a model organism into the realm of social cognition.

This article reviews what is known about zebrafish shoaling and draws on results from other fish species in an attempt to present a coherent narrative, highlighting potentially interesting areas for further research and suggesting that there is much to be gained from a detailed examination of the social behavior of this popular model organism.

Natural history of zebrafish

Zebrafish are small cyprinids, 2–4 cm long at maturity, found in warm slow-moving streams, ponds, and flooded rice paddies in southern and southeastern Asia (Engeszer et al., 2007b; Spence et al., 2008). They are found throughout the water column (Spence et al., 2006), usually in shallow areas close to banks (McClure et al., 2006). Zebrafish feed primarily on terrestrial insects that fall onto the surface of the water (McClure et al., 2006) and are preyed upon by a number of co-occurring fish (Engeszer et al., 2007b) – and possibly also avian – species.

Under laboratory conditions zebrafish spawn every few days throughout the year and can produce up to 200 eggs at one spawning. Mating most often takes place during the first hour after sunrise (Spence et al., 2006, 2008) during which time male zebrafish aggressively defend spawning territories (Spence and Smith, 2005). The fish remain active throughout the daylight hours (Hurd et al., 1998). Zebrafish display no parental care for their eggs or larvae. Larvae hatch at 72 h

post-fertilization and reach maturity at around 3 months in the lab but possibly as late as 6 months in the wild (Spence et al., 2008).

Both male and female zebrafish form dominance hierarchies (Spence and Smith, 2006; Paull et al., 2010). Dominance may be independent of size (Spence and Smith, 2006), although female zebrafish do prefer larger males (Pyron, 2003). Dominance-related aggressive behavior may be limited to – or at least greatly increased during – mating and spawning times (early morning; Paull et al., 2010), as male-male aggression and shoaling are mutually exclusive in related species [at least in guppies, Magurran and Seghers (1991)].

Behavioral ecology of shoaling

The most widely accepted definition of a shoal is a group of fish that remain together for social reasons (i.e., not because of some attractive feature of their shared environment). A school, in contrast, is a shoal exhibiting synchronized, highly polarized collective motion (Pitcher and Parrish, 1993). This definition is problematic: whereas the definition of a school depends on easily observable characteristics, that of a shoal does not. What are ‘social reasons’ and how could the reasons for the formation of any given group be determined? What if both social and external reasons for aggregation exist? As the distinction between these two modes of collective motion has yet to be placed on a solid empirical footing, here all groups of fish are referred to as shoals, for simplicity.

Almost half of all known fish species shoal at some point in their lives (Shaw, 1978) and shoaling has been proposed to confer multiple advantages in reducing predation and facilitating foraging (Krause and Ruxton, 2002). These are reviewed below.

Anti-predatory advantages of shoaling

The anti-predatory advantages of shoaling operate via several distinct mechanisms [see Krause and Ruxton (2002) for a detailed discussion]. A large shoal might be more likely to detect an oncoming predator – or detect it sooner – than an individual fish and, assuming this information is rapidly disseminated within the shoal, might be more likely to escape the attack. This is known as the ‘many-eyes’ hypothesis (Pitcher and Parrish, 1993) and should allow an individual in a large shoal to spend less time on vigilance and more time foraging than a singleton (Magurran and Pitcher, 1983). Visually mediated social transmission of an alarm reaction has been demonstrated in zebrafish (Suboski et al., 1990), implying that they could benefit from the many-eyes effect. Even when attacked, assuming a predator cannot consume the entire shoal, a fish in a larger shoal is proportionally less likely to be targeted by the predator, an effect referred to as dilution (Krause and Ruxton, 2002). In addition, predators attacking a rapidly dispersing shoal may experience ‘confusion’, being unable to select and follow a single target. Predator confusion might be enhanced by phenotypical homogeneity among the fish (Landeau and Terborgh, 1986).

Foraging advantages of shoaling

Fish in shoals might be better able to locate food than singletons, as a large shoal might be more likely to detect food sources and observation of the behavior of individuals that have located them will attract other members of the group to the food [which is called local enhancement, Krause and Ruxton, 2002]. Fish may also rely on the behavior of conspecifics to determine when to forage and when to be vigilant for predators (Ryer and Olla, 1991). Predatory species that hunt in groups might be able to consume prey that would be too dangerous or fast for an individual fish (Krause and Ruxton, 2002).

Despite the possible foraging benefits described above, it has also been suggested that foraging can be enhanced by gaining some distance from a shoal and that distances between individuals in shoals are maintained by a tension between the competing demands of safety and hunger (Krause and Ruxton, 2002). Food-deprived zebrafish shoals are less compact in the presence of food (Miller and Gerlai, 2007), which suggests that foraging in zebrafish is enhanced by a relaxation of the shoal and that the typical inter-individual spacing seen under conditions that do not encourage foraging is determined by other, most probably anti-predatory, considerations. Conversely, forming larger shoals could be a response to a perceived threat of predation. Banded killifish (*Fundulus diaphanus*) form smaller or larger shoals, respectively, as they are presented with food- or predator-related cues (Hoare et al., 2004).

Other advantages and disadvantages of shoaling

Shoaling can also facilitate locating potential mates and provide hydrodynamic advantages (although this is debated; Pitcher and Parrish, 1993). Fish in larger shoals have proportionally more sources of social information, making it probable that social learning is enhanced in shoaling fish (Brown and Laland, 2003). This could be especially beneficial for migratory species (Pitcher and Parrish, 1993). Additionally, information transfer in shoals – which has lately been the subject of intensive research (e.g., Conradt and List, 2009, and other articles from the same special issue on ‘Group decision making in humans and animals’) – could be facilitated in larger shoals leading to a better utilization of available information on, for example, local predator and food distributions. Conversely, fish in a shoal may suffer greater exposure to parasites (but might also be better able to detect parasitized conspecifics) and predators might target large shoals more than small shoals or singletons, thus reducing the advantages conferred by dilution (Krause and Ruxton, 2002).

What we know

Shoals of different species vary in their average membership from less than 10 [e.g., guppies, *Poecilia reticulata* (Croft et al., 2003)] to several hundreds of thousands [e.g., herring, *Harengula thrissina* (Parrish, 1992)]. Shoals are

usually elongated along their direction of travel (Bumann et al., 1997) and display a higher density towards the front of the shoal (Partridge et al., 1983; Hemelrijk and Hildenbrandt, 2008). Fish maintain a distance of between 0.6 and 2 body lengths (BLs) from their nearest neighbors (Partridge et al., 1983; Parrish et al., 2002), although spacing within the shoal depends on species, the size or speed of the shoal (Partridge, 1980; Partridge et al., 1980), and the age of the fish (van Olst and Hunter, 1970). Fish speeds and headings often correlate with those of their nearest neighbors (Partridge and Pitcher, 1980; Partridge et al., 1980) ensuring shoal cohesion. Most shoals disperse at night (Croft et al., 2003).

Shoal preferences

Several researchers have attempted to document those characteristics of shoals that influence individuals' preferences, usually by giving a test fish the option of joining one of two shoals that differ in some dimension of interest. The most common paradigm used involves dividing a tank into three sections separated by transparent barriers. The test fish is placed in the central compartment and different shoals (or video stimuli) are presented in the two side compartments. The amount of time the test fish spends in the vicinity of each side compartment is measured and is assumed to correlate with preference for one shoal over the other. Such studies have demonstrated that zebrafish prefer a shoal of conspecifics to an empty tank, even if the stimulus fish are of a different phenotype to the test individual (Sneckser et al., 2006) and different phenotypes of zebrafish prefer to shoal with conspecifics of a similar phenotype (Rosenthal and Ryan, 2005; Sneckser et al., 2010). These results suggest that zebrafish, although found in mixed species shoals in the wild (Spence et al., 2008), would display a preference for shoaling with conspecifics over heterospecifics. Indeed, detailed analysis of the distribution of zebrafish in mixed shoals has demonstrated that zebrafish swim closer to their conspecifics than to members of the other species comprising the shoal, under laboratory conditions (Saverino and Gerlai, 2008). Other cyprinids, also found in mixed-species shoals, might only segregate into single-species shoals when there is an immediate threat of predation (Pitcher and Parrish, 1993).

Individuals of many shoaling species preferentially shoal with conspecifics of a similar size (Krause et al., 2000; Croft et al., 2003) and coloration (McRobert and Bradner, 1998) which has the effect of increasing the visual homogeneity of the shoal and thus potentially increasing predator confusion (Krause and Ruxton, 2002). Both guppies and three-spined sticklebacks (*Gasterosteus aculeatus*) preferentially shoal repeatedly with the same similar-sized individuals (Croft et al., 2005).

Zebrafish, like other species (Breder, 1954), prefer to join more numerous shoals, although this preference also depends on the activity level of the stimulus shoal (Pritchard et al., 2001) and the sex of the fish [male zebrafish prefer to shoal with females; females display no preference; Ruhl and McRobert (2005)]. This preference could be due to the greater protection from predation that larger shoals are assumed to confer

(Landeau and Terborgh, 1986). European minnows (*Phoxinus phoxinus*) in smaller shoals are more likely to abandon shoaling and attempt to hide during a predator attack (Magurran and Pitcher, 1987). In addition, larger shoals may locate food more quickly than smaller shoals (Day et al., 2001). Shoal size might also depend on environmental factors: guppy shoal sizes depend on the level of predation [Magurran and Seghers (1991); but see Croft et al. (2003)] with the majority of fish in low-predation populations found as singletons and shoals of approximately 20 individuals being most common in high-predation areas. Minnows form larger shoals in the presence of a predator only in environments devoid of hiding places (Orpwood et al., 2008).

Ontogeny of shoaling

Some fish species shoal only as juveniles, others throughout their life (Shaw, 1978). Zebrafish larvae begin to display a preference for conspecifics around the post-flexion stage [approximately 12 days post-fertilization; Engeszer et al. (2007a)] and continue to shoal into adulthood. Engeszer et al. (2004) have shown that the preference for a particular phenotype (see above) in zebrafish depends on the rearing environment of the fish [see also McCann and Carlson (1982)] and is possibly learned during a crucial period when the fish are juveniles [Engeszer et al. (2007a); but see Moretz et al. (2006)]. The spacing of individuals within a shoal can also change as a function of the age of the fish (van Olst and Hunter, 1970).

Positions within shoals

In addition to preferring a particular shoal, fish can display a preference for certain positions within the shoal. The front or edges of a shoal may be more dangerous than the interior, as they are more exposed to predators (Bumann et al., 1997) but may also provide better opportunities for foraging. Food-deprived roach (*Rutilus rutilus*) are more likely to take up positions in the front of a shoal than fed fish and frontal fish consume more food than other members of the shoal (Krause, 1993a). In shoals of chub (*Semotilus atromaculatus*), frontal fish are more likely to be attacked by a predator than their more central shoal-mates (Bumann et al., 1997) and minnows prefer positions in the center of a shoal over peripheral positions only after being frightened [by the application of alarm substance, Krause (1993b)].

Positional preferences may also reflect individual differences between fish within the same population. Roach found in the front of a shoal are more likely to retain their position than fish further back in the shoal (Krause, 1993a). Preferred shoal position, where it is a more-or-less constant trait, may correlate with other behavioral traits. Shoal leadership in sticklebacks correlates with boldness in non-social situations (Harcourt et al., 2009). More timid sticklebacks (as determined by the time taken to recover from a simulated predator attack) are more likely to remain close to a stimulus shoal and less likely to take up frontal positions in a shoal (Ward et al., 2004).

In general, different positions within a shoal offer different opportunities and present different risks. Even assuming

that each fish always occupies its preferred position, positions might not be constant for all fish and can vary with internal state (e.g., hunger) and environmental conditions [e.g., predation, Krause (1994)]. Individuals may, in addition, be forced into certain positions in the shoal by the more strongly expressed preferences of their conspecifics.

Environmental effects on shoaling

As discussed above, the distances between members of a shoal may represent a trade-off between foraging and protection from predation. As such, shoaling might be expected to be sensitive to changes in environmental conditions relating to the immediacy of predation threat and the availability of food and to internal factors such as hunger. Although zebrafish can be attracted to food sources by the actions of conspecifics [as demonstrated by Pitcher et al. (1982) for goldfish, *Carassius auratus*, and minnows], they can also compete for food and interfere with each other's foraging (Wright et al., 2006b). Hamilton and Dill (2002) have shown that dominant zebrafish will monopolize a reliable stationary food source. Because zebrafish feed primarily on insects (McClure et al., 2006), they probably have little to gain from being alerted to the location of food as it is being consumed by a conspecific.

Hunger increases distances between members of juvenile Pollock (*Theragra chalcogramma*) shoals and this effect is modulated by the presence of a predator (Sogard and Olla, 1997) and the distribution of available food [i.e., whether it is clumped or dispersed, Ryer and Olla (1998)]. Similarly, in many other species, the presence or threat of predation tends to increase shoaling tendency and shoal cohesion and hunger and/or the availability of food tends to decrease shoaling and loosen shoals (Krause and Ruxton, 2002). Zebrafish, like other fish species, rapidly disperse when a predator is detected [in what is called a flash expansion, Pitcher and Parrish (1993)] and then reform a more tightly packed shoal than before the detection of the predator (Miller and Gerlai, 2007).

In the absence of predator cues, shoaling may also be a response to more generally fear-inducing conditions. Al-Imari and Gerlai (2008) have shown that the tendency to approach a stimulus shoal decreases in zebrafish as they habituate to a testing environment. Delaney et al. (2002) have demonstrated that 2–3 h after being placed in a large novel tank, presumably an aversive environment, zebrafish cease shoaling altogether and disperse, spending most of their time individually or in pairs near artificial plants in the tank.

Genetics of shoaling

If shoaling is a response to environmental pressures (such as resource patterning and predator distribution) then populations from different ecological regimes might vary in their willingness to shoal or the manner in which they do so. Populations of guppies, for example, from different rivers have been shown to vary in shoaling tendency; guppies from populations facing high predation pressure shoal more tightly (Huizinga et al., 2009) and are more likely to be found in

larger shoals (Magurran and Seghers, 1991) than individuals from low-predation populations.

Differences in shoaling tendency have also been found between different strains of zebrafish (Wright et al., 2003; Moretz et al., 2007). Boldness, which has been shown to affect shoaling decisions (Wright et al., 2003), varies between lab-reared and wild strains of zebrafish (Wright et al., 2006a), as do many other behaviors (Robison and Rowland, 2005). As more sensitive measures of shoaling become available, it might be possible to uncover in greater detail the role that genes undoubtedly play in fine-tuning the expression of shoaling to match varying environmental conditions.

Mechanisms of shoaling

To shoal effectively, fish must be aware of the locations (and possibly the speeds) of their shoal-mates. In those species examined so far, shoaling is maintained through both vision and the lateral line (Partridge and Pitcher, 1980) and, in most species, shoals disband at night (Croft et al., 2003). Hunter (1969) showed that jack mackerel (*Trachurus symmetricus*) react to a change in direction by one shoal member within 0.5 s when the group is in a highly polarized state. Nearest neighbors of the focal fish reacted first. Speeds and headings of minnows correlate best at a lag of approximately 0.3–0.6 s (Partridge, 1980), although this value varies with the number of fish in the shoal. Partridge (1981) demonstrated that heading and speed in schools of saithe (*Pollachius virens*) correlate with those of at least their three nearest neighbors [but see Partridge and Pitcher, 1980]. Larger shoals (more than approximately 10 individuals, for saithe) may consist of several intermixed subgroups that move somewhat independently of each other (Partridge, 1981).

Locating a shoal to join may be facilitated by odor cues. Bloom and Perlmutter (1997) showed that under some conditions zebrafish display a preference for water in which other zebrafish have been living. In juvenile zebrafish, this preference extends to kin over non-kin and familiar kin over unfamiliar kin (Gerlach and Lysiak, 2006). Thus, zebrafish might preferentially shoal with kin or with familiar conspecifics, as has been demonstrated for guppies (Lachlan et al., 1998), although this effect could be modulated or even overridden by environmental factors (Morell et al., 2007).

When injured, zebrafish skin (like that of other cyprinids) releases an alarm substance (Waldman, 1982). Zebrafish shoals exposed to alarm substance initially exhibit an anti-predatory 'flash expansion', followed by a period of increased shoal density [relative to a pre-exposure baseline; Speedie and Gerlai (2008)], behaviors similar to those observed when predator cues are detected (Miller and Gerlai, 2007). Similar results have been reported for guppies (Huizinga et al., 2009). Alarm substance has also been shown to increase erratic (possibly escape-related) movements in zebrafish in a dose-dependent manner (Speedie and Gerlai, 2008; Parra et al., 2009).

Some of the neural mechanisms underlying shoaling in zebrafish have been studied using various drugs that modify or disrupt shoaling. Both an NMDA antagonist (MK-801) and a dopamine agonist (SKF 38393) have been shown to disrupt

shoaling in zebrafish (Echevarria et al., 2008) and ethanol delivered acutely at medium concentrations (around 0.5% v/v) also increases the spacing between members of zebrafish shoals [although this effect varies across different zebrafish strains, Gerlai et al. (2008)].

What we do not know

A shoal of zebrafish may consist of a dozen or more individuals, constantly twisting, turning, and changing positions relative to each other. Speeds, headings, and distances between the fish vary widely on very short timescales (Viscido et al., 2004; Miller and Gerlai, 2008). There are a great number of possible ways to quantify such movement and authors disagree on which measures best describe the underlying regularities of shoaling (Clark and Evans, 1954; Christman and Lewis, 2005). Most current measures are averaged over several minutes and might not accurately capture the dynamics of shoal structure. Measures of shoaling tendency often rely on test individuals that are physically separated from the stimulus shoal (see above) and, although these tests provide important information on forced-choice preferences, their relationship to shoaling can be questioned. In addition, many of the measures currently used to quantify shoaling vary widely between different individuals (Toms et al., 2010) and populations (Wright et al., 2003) and thus might be less than ideal for describing the effects of any drug or genetic manipulations. Identifying the constants of natural zebrafish shoaling would permit detection of more subtle external effects which are more likely to be relevant for comparisons to other species.

Shoal size

To the best of our knowledge, no-one has determined what size shoals zebrafish will spontaneously form, either in the wild or the lab (e.g., if 100 zebrafish are placed in a sufficiently large tank, will they form one or several shoals?). In the only published estimate we know of for zebrafish, Pritchard et al. (2001) suggested that zebrafish in the wild form shoals of 2–10 individuals but provided no data. Group size distributions in several taxa, including fish, have been found to follow truncated power laws (Bonabeau et al., 1999) and thus might not have a typical size (see also Krause and Ruxton, 2002). Freshwater fish species may form smaller shoals than marine species owing to the presence of vegetation in which to hide which, presumably, provides similar anti-predatory advantages to shoaling (Pitcher and Parrish, 1993).

Determining the size of a shoal depends on the criterion used to define membership in the shoal. Although most pelagic species form large clearly delineated and separated shoals, the same cannot be said of zebrafish. Many authors have adopted Pitcher et al.'s (1983) elective group size (EGS) criterion, which considers fish within 4 BLs of each other members of the same shoal, under the assumption that members of a shoal must be in communication with each other. Some authors have substituted other, equally arbitrary, thresholds

[e.g., Viscido et al. (2004): 5 BLs; Budaev (2007): 7 BLs]. We (Miller and Gerlai, 2008) recently suggested a measure of shoal membership based on the mean inter-individual distance which takes into account the overall distribution of the fish. However, this measure also relies on an arbitrary distance criterion. Aoki (1980) demonstrated a method for identifying shoals that have split based on the distances between nearest neighbors but this measure has not been adopted by later authors. Although EGS distributions for several species under varying environmental conditions have been reported (Pitcher and Parrish, 1993), neither it nor the other measures described here have been experimentally validated. For instance, no study that we are aware of has examined how shoal size distributions vary under different threshold values for the EGS, nor whether Pitcher et al.'s (1983) often-cited 4 BL threshold has any empirical basis as a limiting distance for communication. Nor has the question whether different species might have different thresholds (owing, for example, to different perceptual capabilities) been addressed.

The dynamics of shoaling

Despite the relative scarcity of detailed empirical trajectory data, a great number of theoretical models of shoaling – and other forms of collective motion – exist [review in Mirabet et al. (2007)]. Most models allow each member of a virtual shoal (an agent) to follow a set of simple 'traffic rules' (Parrish and Turchin, 1997) and demonstrate that certain characteristics of real shoals emerge from the interactions between the agents. In many cases, each agent is attracted towards distant agents, repulsed by agents that are too close, and attempts to match orientations with agents in the middle range (Couzin et al., 2002). Recently, several researchers have begun to test their models against the empirical data that are now becoming available (Viscido et al., 2004), revealing the range of ecologically valid values for the parameters of the models and, more interestingly, the paucity of existing data with which to compare model results.

Most authors of theoretical studies of shoaling consider the equilibrium state of their models where, after an initial settling-down period, agents remain in the same position within the moving shoal. Some data exist to refute the view that real shoals behave in this manner. As Aoki (1980) showed for field gudgeon (*Gnathopogon elongatus*) and, more recently, we (Miller and Gerlai, 2008) showed for zebrafish, the internal spacing between fish in a shoal oscillates. Distances between members of zebrafish shoals display regular fluctuations with a period of between 10 and 15 s (Miller and Gerlai, 2008). Shoals of giant danios (*Devario aequipinnatus*, a close relative of zebrafish) display persistent fluctuations in polarization and speed (Viscido et al., 2004).

In addition, shoal membership is not constant, as shoals fission and fuse on several different timescales concurrently. Krause et al. (2000) showed that approximately 36% of encounters between shoals of several freshwater species end in fusion of the shoals. Individual guppies may remain in any given shoal only for about 10 s (Croft et al., 2003). Almost no studies have employed either sufficient numbers of fish or

sufficiently large testing enclosures to examine interactions between shoals under laboratory conditions.

Comparative studies of shoaling

Few studies have addressed comparative questions about shoaling. Obviously, many features of the shoaling of a large marine fish will be different from the shoaling of zebrafish. However, even within small freshwater species, there could be important and interesting variations in shoaling tendency and manner. For instance, Magurran and Pitcher (1983) showed that shoal size has different effects on foraging tactics in minnows and goldfish, which could be related to the generally greater shoaling tendency of the former species. As an excellent example of what can be done with detailed data, Partridge et al. (1980) compared the shoaling of cod (*Gadus morhua*), herring (*Clupea harengus*), and saithe and showed consistent species-specific differences in the regularity of spacing, density, and shape of the shoals.

The domestication of many species of fish might have led to important changes in their shoaling behaviors when compared to their wild-type progenitors (Wright et al., 2006b). For example, the constant food supply and greatly reduced level of predation in the lab might explain why a laboratory strain of zebrafish (AB) spends less time shoaling than a wild-type derived strain (Wright et al., 2006a). Lab strains of zebrafish also spend more time close to the surface of the water than wild-type populations and are less fearful (Robison and Rowland, 2005).

Positional preferences

No published study to date has examined positional preferences within the shoal in zebrafish, although we have observed that some, but not all, zebrafish display a preference for either the front or the back of the shoal (unpublished data). Similar results have been reported for golden shiners [*Notemigonus crysoleucas*, Leblond and Reeb (2006)] and saithe (Partridge, 1981). In addition, positional preferences (and other characteristics of shoaling) in zebrafish have not been related to their ecology. Differential survivability of different positions within shoals may depend on the level and type of predation that shoals are exposed to [and could be, for instance, less important in relation to aerial than aquatic predators; Bumann et al. (1997) and Krause (1994)].

Conclusion

Shoaling is a complex behavioral trait exhibited by a wide range of fish species. Living and foraging in groups is, of course, not limited to fish but extends throughout the animal kingdom (Krause and Ruxton, 2002). To the extent that animal groups serve similar functions – primarily reducing the risk of predation and enhancing foraging success – it is probable that some of the characteristics and perhaps even the mechanisms of group formation and maintenance are similar across multiple taxa. Only when detailed descriptions of the collective motion of multiple species are available will it

become possible to separate those mechanisms that are species-specific from more general group behaviors underlying many or all animal groups, from ants to humans.

Zebrafish occupy a uniquely advantageous niche in this endeavor: they are easy to house and test in large numbers; they display – as detailed above – complex long-term group behaviors on several temporal and spatial scales (as other common model organisms such as mice, for instance, do not) including both social dominance and cooperation; and a large body of genetic, developmental, and pharmacological research exists for them, making it easier – at least in theory – to relate their behavior to its neural underpinnings. By examining the shoaling behaviors of zebrafish under different genetic or pharmacological manipulations, insight might be gained into the neural mechanisms of shoaling and, perhaps, aspects of social cognition in other species. However, zebrafish shoaling has not yet been characterized in sufficient detail. A better understanding of how, when, and with whom zebrafish shoal will lead to improved (and more easily automated) behavioral assays that will be able to detect more subtle departures from typical social behaviors as a result of mutagenesis or drug administration. Examining the dynamics of shoals is one, as yet mostly untried, way to uncover the structure of the behavior. In addition, testing for differences in the characteristics of shoaling between different species, or between different strains of the same species, will lead to a better understanding of the adaptive functions of shoaling and the genetics behind it.

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