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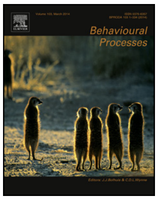

Sex differences in a shoaling-boldness behavioral syndrome, but no link with aggression

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ABSTRACT

A behavioral syndrome is observed in a population when specific behaviors overlap at the individual level in different contexts. Here, we explore boldness and aggression personality spectra, the repeatability of shoaling, and possible associated correlations between the behaviors in a population of lab-reared zebrafish (*Danio rerio*). Our findings describe a sex-specific boldness-shoaling behavioral syndrome, as a link between boldness and shoaling behaviors is detected. The results indicate that bold males are likely to have a stronger shoaling propensity than shy males for unfamiliar conspecifics. Conversely, bold females are more likely to shoal than shy females, but only when presented with heterospecific individuals. Additionally, aggression does not correlate with boldness or shoaling propensity for either sex. A positive relationship between boldness and shoaling that differs by sex is contrary to most of the present literature, but could help to explain population dynamics and may also have evolutionary implications.

1. Introduction

Behavioral syndromes are an increasingly popular area of research in behavioral science, reflecting recognition that syndromes are important in the understanding of individual and population-wide behavior. Conceptually, a behavioral syndrome is a suite of correlated behaviors consistent within a population along two (or more) behavioral axes (Budaev, 1997a; Conrad et al., 2011; Gosling, 2001; Huntingford, 1976; Réale et al., 2007; Sih et al., 2004). The most common behaviors investigated (i.e., axes of measurement) are activity, boldness, aggression, and sociability (Réale et al., 2007). Within each of these distinct behavioral axes, variability exists, giving individuals a 'personality' of their own (Wolf and Weissing, 2012). For instance, some individuals are bold, while others are shy. Some are relatively aggressive, while others are passive. When there is a correlation between two of these behavioral measures (such as between boldness and aggression) that is consistent across contexts, it is suggestive of the presence of a behavioral syndrome (Sih et al., 2004).

Contradictory evidence exists pertaining to the evolutionary and ecological formation and maintenance of behavioral syndromes. Animal personality appears to be somewhat malleable, with individuals responding to external cues, however, there may also be sources of behavioral constraint (Smiseth et al., 2008; Snekser et al., 2008). Two dichotomous hypotheses have been proposed and explored regarding behavioral syndrome maintenance: the constraint hypothesis and the plasticity hypothesis. The constraint hypothesis suggests that syndromes are commonplace and an individual's correlated behaviors are a result of an internal constraining force, believed to be gene pleiotropy, a hormonal influence, a physiological constraint, or a combination of the preceding (Stamps, 1991). This constraint may also be external, or influenced by environmental factors such as predation levels (Gabriel et al., 2005) and experience (Smith and Blumstein, 2012). Conversely, the plasticity, or adaptive hypothesis refutes behavioral syndrome perpetuity and describes individuals as altering their behavior according to the situation (Bell, 2005; Neff and Sherman, 2004). The literature is rife with evidence in support of each hypothesis, which strongly suggests that the real world lies between the two maxima.

Numerous examples of behavioral syndromes have been documented across different fauna (Brodin, 2008; Brown et al., 2005; Budaev, 1997b; Colleter and Brown, 2011; Dingemanse et al., 2007;

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Logue et al., 2009), and behavioral syndromes in fish have been well studied (Bell, 2005; Cote et al., 2010; Moretz et al., 2007; Pike et al., 2008). Zebrafish (*Danio rerio*) have been a particularly useful model organism to explore behavioral syndromes due to their ever-increasing popularity as a model system in other biological disciplines (Kiesel and McRobert, 2013) and previous studies have indicated syndromes may play a role in the behavior of this species (Ariyoma and Watt, 2012; Kiesel et al., 2012; Moretz et al., 2007; Wisenden et al., 2011).

Zebrafish, like many other social fish, commonly form shoals (loosely organized groups). Individuals who participate in shoals benefit by experiencing decreased predation, increased foraging success, and more mating opportunities (Hoare et al., 2004; Krakauer, 1995; Tosh et al., 2006; Turner and Pitcher, 1986). Several phenotypic and physical aspects influence shoaling decisions. In zebrafish and other closely related species, factors such as body pattern (Snekser et al., 2010), coloration (McRobert and Bradner, 1998), shoal size (Ledesma et al., 2010), sex (Ruhl and McRobert, 2005), nutritional state, and parasite load of conspecifics (Krause and Godin, 1996), familiarity (Ward and Hart, 2003), early experience (Ledesma and McRobert, 2008), and circadian rhythms (Paciorek and McRobert, 2012) are all important in driving social decisions.

Few studies have linked behavioral syndromes and social behavior by tracking individual preferences across contexts in order to explore how individual personality may effect shoaling decisions (Kiesel et al., 2012; Piyapong et al., 2009). In a previous study (Kiesel et al., 2012), we approached this question from a unique perspective, asking whether three distinct populations of closely related *Danio* fish species differed in their personalities and if those differences impacted shoaling tendencies. After conducting this population-level study, we suspected that a behavioral syndrome might account for specific shoaling behaviors. Therefore, we present here a similar study focused on individual-level comparisons in zebrafish. Specifically, we tested lab-reared wildtype zebrafish for a boldness-aggression behavioral syndrome and continued tracking those individuals through three distinct shoaling assays in order to investigate the possibility of personality-driven shoaling decision-making. We also considered sex differences in our analysis to determine if sex influences trends in personality and shoaling decisions.

2. Materials and methods

2.1. Animal care and housing

Adult wildtype zebrafish (*D. rerio*) were obtained from Seven Star Tropical Fish, Philadelphia, PA, USA and housed in 76 L aquarium tanks at 27.5–28.5 °C according to IACUC standards. Focal fish (individuals tested in the five assays) and stimulus fish (individuals that occupied the end chambers in each shoaling assay) were housed separately and in segregated groups based on sex. Each tank was maintained at a 12:12 light/dark cycle. The fish were fed flake food daily and at least one hour prior to testing, in the morning. Assays were performed between the hours of 9:00 and 13:00 in order to avoid interference with circadian cycles (Paciorek and McRobert, 2012).

2.2. Tracking individuals

Individual fish were tracked through a series of five behavioral assays measuring boldness, aggression, and shoaling. The assays were modified from previously established protocols (Cachat et al., 2010; Gerlai, 2003; Kiesel et al., 2012). Individual fish were randomly selected from the segregated focal tanks and subjected to each of the five assays in a random order. Before the first assay and between each subsequent assay, individual fish were allowed to acclimate for 10 min in a separate isolated tank to minimize potential sequence effects carried over from previous assays. In total, 18 males and 21 females were examined as focal fish in each of the five assays. Upon completion of each assay, fish were housed separately from naïve focal fish.

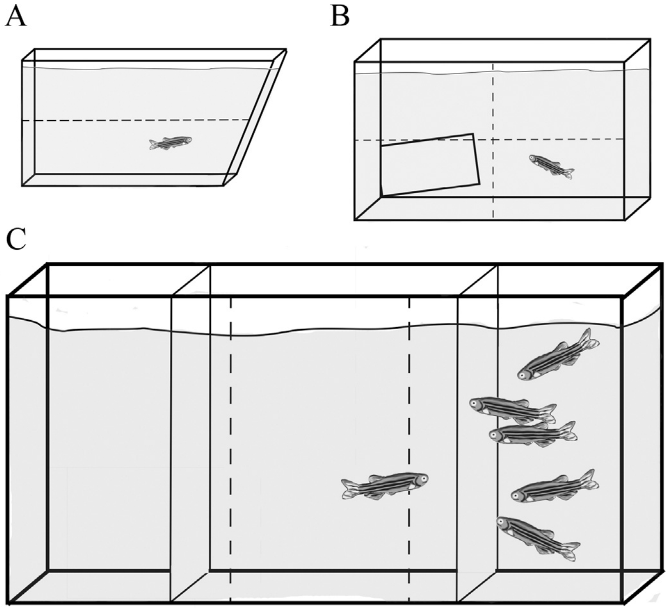

Fig. 1. Experimental tanks. (A) Boldness assay: a novel, open tank. Dashed line indicates top portion and bottom portion (B) Aggression assay: a 22.5° inclined mirrored tank. Dashed lines indicate four quadrants. (C) Shoaling assay: a divided tank with two shoaling choices on opposite ends. Dashed lines indicate shoaling preference zones.

2.3. Boldness – activity in a novel tank

A standard 1.5 L trapezoidal tank, measuring 15 cm tall × 26.5 cm top × 22.5 cm bottom × 6 cm width, was used to quantify boldness (Aquatic Habitats, Apopka, FL, USA). Opaque partitions externally surrounded three sides of the boldness tank, leaving the front exposed for viewing, to reduce undesirable outside stimuli. A single strip of tape, equally demarcating the top from the bottom portion according to the height of the trapezoid, was added externally (Fig. 1A). To isolate a novelty response, individuals were allotted a 30 s acclimation period before scoring commenced. The behaviors were quantified for 480 s and included time near surface, latency to enter upper portion, number of transitions, and number of erratic movements (Cachat et al., 2010). We note that the behavioral measurements of boldness and exploratory behavior are inextricably linked. Both are responses to novelty but their distinction is not clear in practice. Papers citing similar methods made similar conclusions based on the boldness axis (Cote et al., 2010) or exploratory behavior axis (Fraser et al., 2001; Dingemanse et al., 2007). Because of the novelty of our tank design and measurement protocol, we considered our measured behavior “boldness”. Boldness assays were recorded using an external video camera and scored at least twice by a single viewer to ensure accuracy.

2.4. Aggression – a mirror test

A 19 L rectangular tank, measuring 30 cm × 15 cm × 10 cm, was used to quantify aggression. Opaque partitions externally surrounded three sides of the aggression tank, leaving the front