

## **A Methodological Review of Personality-Related Studies in Fish: Focus on the Shy-Bold Axis of Behavior**

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Personality research has begun to take hold in the animal kingdom as psychologists turn to animal models to investigate various aspects of personality. Similarly, behavioral ecologists and related fields have begun to explore the idea that individual variation in behavior is more than just noise around an average for a given population or group of interest. As a result, many have begun to turn to personality-related questions to explain individual differences in animal behavior. Collectively, psychologists, ecologists and related fields have created a boom in animal personality-related research. This interest has expanded to a variety of fish species, with many studies focused on an important axis of behavior in humans: the shy-bold axis. Unfortunately, there has been very little consideration for the methodology employed. We review both the experimental and statistical methodology found in a body of research on fish species, for which personality-related research has been conducted. Our aim is to shed light on many important considerations that are often overlooked in order to facilitate research concerned with the reliability and validity of the many methods used.

The classic approach to behavioral and evolutionary ecology seeks answers based on averaged behavior and fails to consider unique variation between individuals and the functional importance of such variability (Mather, 1998; Slater, 1981). The importance of finer scale investigations at the level of the individual is only beginning to emerge as researchers have begun explore Darwin's less accepted views that evolution acts on the individual and may not be limited to only physical traits (Darwin, 1998; Gosling, 2001). This burgeoning interest has given rise to research in areas of animal personality and temperament in a wide range of taxa and has led to recent recognition of the potential implications in ecological studies of animal behavior. If something similar to personality is found in animals and individual differences in behavior reflect more than just noise around an average, then traditional approaches to behavioral ecology, behavioral biology and how we approach questions about evolution may be challenged. The boom of interest in these new directions combined with the fear of anthropomorphism seems to have placed the search for "individual differences" at the forefront of investigations with little regard to ensuring adequate measures and methodology. In a complex and controversial topic such as animal personality, these considerations are vital to research in this area. This article aims to present some inconsistencies and considerations by reviewing a subset of studies focused on an important shy-bold axis, which has been a growing focus in research with many species of fish.

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### ***What is personality?***

There is much debate on definitions and the best approach to animal personality and temperament research. Personality and temperament are distinctly different in human theories but are used interchangeably in animal research in addition to other terms such as “behavioral syndromes,” “phenotypic expression,” “behavioral plasticity” and “individual differences.” The variation in terms used may be a result from the negative connotation associated with the term “personality” due to fear of anthropomorphizing (defined as projections of human characteristics onto other animals) (Gosling, 2001; Vazire & Gosling, 2004). Despite criticism, accumulating evidence suggests “personality” and “temperament” can be defined objectively and precisely in animals (see Gosling, 2001 for a review). For those that support this avenue of investigation in animals, there is a broad consensus that personality and temperament can be distinguished from moods and states by considering an individual’s distinguishing pattern of behavior which remains consistent over time and across situations/contexts (Budaev, 1997b; Gosling, 2001; Vazire & Gosling, 2004).

The bold-shy axis of behavior is categorized under the empirical indices of introversion and extroversion in humans and has been regarded by psychologists as one of the more distinctive, heritable and stable sources of behavioral variation (Coleman & Wilson, 1998; Kagan, Reznick, & Snidman, 1998). Although research on bold-type behaviors in animals is not a new area of investigation, there is growing acceptance for incorporating individual-based approaches to animal research. In turn, this has spurred new growth in research investigating a similar axis in many non-human species.

Several species of fish have become popular models for research in behavior, behavioral ecology, personality, and behavioral neuroscience (ex: guppies, sticklebacks, zebrafish, and cichlids). Fish models are economical (low maintenance, cost, and space required), easily manipulated for high experimental control and allow for large samples sizes (thus high-powered analysis). Many exhibit a broad range of behaviors and there are many shoaling species which offer a simple model for other social species (as opposed to primates with complex social systems). For ecologists, research in the natural environment is not impossible, and experimental re-creation of natural environments is conceivable. Genetic information is widely available for many species, including zebrafish (*Dani rerio*) and guppies (*Poecilia reticulata*), which can be used to facilitate utility and comparative power across fields such as biology, ecology, ethology, genetics, neuroscience and evolutionary research. Therefore it is not surprising that research in individual differences and personality, especially in regards to the shy-bold continuum, has been vastly expanded upon using fish models in recent years.

### **Common Approaches to Personality-Related Research**

The studies presented in Tables 1-4 are grouped with regards to methodological and analytical approaches. The authors do not intend for this to be an exhaustive literature review but instead to represent a solid body of literature

with respect to exploring boldness in fish species and from which to compare definitions, measures and methodology. Keep in mind that these are not absolute categories, they do not represent all available approaches and several studies potentially fit into either type of approach. However, we felt that most of these studies fit best into one of two major categories with regards to methodology and subsequent analyses. Table 1 includes studies that employ approaches similar to those used by psychologists; i.e. they test multiple traits and behaviors and use correlational or multivariate analysis (*Psychological or Correlational Approach*). Tables 2-4 have been separated due to length and convenience, but generally all include studies that investigate behavior with regards to predefined criteria and tend to be ecologically-based in nature (*Ecological and/or Evolutionary Approach*). The studies across these tables have been grouped with regards to the definitions employed for boldness: response to novel object (Table 2), response to novel environment (Table 3) and response under risk (Table 4). The following sections review the general methodology and analyses for psychological and ecological approaches.

### ***Psychologically -based approaches***

The approach to personality employed by animal researchers has varied widely. Those studying higher order species such as primates (as well as some lower order species) have taken routes more closely comparable with human psychologists which are based on hierarchical models of personality (Gosling, 2001; Itoh, 2002; Zuckerman, 1992). This approach could be considered more exploratory. It is common to decide on many variables that may be important and extract meaning for behavior through multivariate techniques, without solid, predefined, operational definitions for each trait. This is evident from Table 1, in which most studies provided neither an *a priori* definition of boldness nor explicit quantification of boldness. That is not to suggest that a set of behavioral variables can be thrown into analysis without any prior understanding. The researchers listed in Table 1 have described what kinds of behaviors may be expected to represent bold traits and have provided a rationale for their measures. There needs to be an understanding of the traits of interest and how they may be measured in order to choose variables which may best represent traits of interest. Furthermore, demonstrating validity of a measure is incredibly important and acceptable reliability needs to be obtained (see Gosling, 2001 for a review of acceptable reliability and validity considerations and see Burns, 2008 for a review on the validity of three commonly used tests and measurements).

As Huntingford (1976) explains, Principle Component Analysis and related techniques can be used to determine and express the relationships between the original variables and provide a new set of independent components. In other words, those variables that are related are grouped together and separated from others that are independent. The components that are extracted are chosen in decreasing order according to how much of the total variance is accounted for (the first component would account for the most variance) (Huntingford, 1976). In this way, understanding how traits such as boldness, fear, exploration or aggressiveness

are expressed and related in a given species is accomplished through examining loadings from a variety of measures and with careful interpretation. This more exploratory approach is an important process when investigating new species, as traits of interest are not necessarily present across all species and measurements for one may not be applicable to another. One must keep in mind however, that as Zuckerman (1992) points out, “you cannot get more out of a factor analysis than you put into it” (p. 676). Thus it is suggested that several measures of any variable of interest should be used. It is important to note that labels for factor loadings found through these processes are somewhat subjective. Labels are, and should be, given with careful consideration of the behaviors that make up the factor, combined with well thought out rationale. However, they should be interpreted with caution and not considered as absolute. This approach provides a good starting point for investigating and understanding underlying traits and relationships between traits, as well as providing a framework from which to branch out to questions regarding ecological implications and the significance of emerging traits.

### ***Ecologically-based approaches***

The approach described above is important, yet challenging due to the number of subjects needed, experimental control required and detailed behavioral recording needed. As a result, often researchers attempt to identify or distinguish between only one or two behavioral axes of interest. This approach has been commonly employed by ecological biologists and behavioral ecologists with regards to questions concerning the ecological significance of personality and temperament and whether these concepts can be used to explain individual variation in behavior. These questions have only recently begun to gain acceptance among ecological researchers as a growing body of evidence points to the importance of investigating individual influences in populations instead of focusing on classically studied average optimal behavior. Consistency in behavior and personality at the individual level has wide-spread implications for populations with respect to social dynamics, fitness consequences and mate selection among other ecological consequences. For example, there is accumulating evidence that boldness is associated with predator inspection and anti-predator defenses, dispersion (killfish: Fraser, Gilliam, Daley, Le, & Skalski, 2001), activity (guppies: Budaev, 1997b; convict cichlid: Budaev, Zworykin, & Mochek, 1999b; three-spined stickleback: Harcourt, Sweetman, Johnstone, & Manica, 2009) and social preferences (three-spined stickleback: Harcourt et al., 2009), with growing support for influences in other ecological phenomena.

The rising interest of personality in ecological approaches has spurred research in a related topic: *behavioral syndromes*. Evolutionary ecologists have classically considered correlated behaviors in animals as syndromes and thus refer to “behavioral syndromes” as analogous to personality and temperament (Sih, Bell, Johnson, & Ziemba, 2004). From this perspective, behavioral syndromes refer to suites of correlated behavior in individuals expressed in a given context or across contexts (Sih et al., 2004). More specifically, this approach distinguishes between

behavioral syndromes and *behavioral types*. The former is a property of a population for which Bell (2007) specifically refers to as the “correlation between rank-order differences between individuals through time and/or across situations...” (p.755). The latter is a property of the individual referring to the particular configuration of individually expressed behaviors (Bell, 2007).

### **Terminology**

As Wilson, Coleman, Clark and Biederman (1993) point out, psychologists and ethologists tend to focus on structure, heritability, consistency and underlying mechanisms for personality and traits without investigating ecological consequences or the predictive power of traits in natural settings. This recognition has spurred rapid growth in important ecological approaches to personality, providing a promising avenue for obtaining a renewed understanding of population dynamics. Unfortunately, it has also resulted in growing inconsistencies in the use of many terms which should be addressed. In this section we use a psychology-based framework for presenting discrepancies since personality related research in animals generally began with this framework.

### ***Traits***

The majority of personality phenomena studied in animals is focused on traits, although across studies it has been used with different meanings, making comparative research challenging. In human and psychologically-based frameworks a personality *trait* is a “dimension along which consistent individual differences in a specific behavior or a group of behaviors can be quantitatively described” (Itoh, 2002, p.250). Examples of traits are terms such as *curious*, *bold*, *friendly*, or *aggressive*. To illustrate, if you refer to an animal as friendly, you likely mean that the animal is friendly not just now but also next week, in a different environments and with different people. This does not necessarily mean that the animal is *always* friendly but rather their pattern of behavior is generally friendly. In this manner, individual variation is distinct from temporal, situational and environmental changes and is more indicative of underlying characteristics of individuals. The hierarchical personality framework allows individual differences to be considered on several levels as related traits can be clustered into domains or dimensions that indicate a greater level of regularity and generalizability (Itoh, 2002; Pervin & John, 1997). Measurements in this fashion can be accomplished via recordings of trait ratings by experienced judges or objective behavioral recordings (Gosling, 2001). Basic characteristics or traits of individuality can be found through data aggregation and multivariate techniques as described earlier. This approach serves to extract information for multiple personality axes and reflect inherent differences between individuals (Budaev & Zworykin, 2002).

Sih et al. (2004) uses the term *trait* to mean behavior in any single context, which appears to remove the property of consistency in the definition. Reale, Reader, Sol, McDougall and Dingemanse (2007) give a definition for a behavioral trait (synonymous with *character*) as “...a characteristic of an organism shared by

all or some of the individuals of a species that can vary, although not necessarily, among these individuals...” (p. 293). Reale et al. (2007) separate behavioral traits from other trait categories such as life history, physiological or morphological traits. According to the definition above, which many psychologists employ, these categories do not classify as traits. They instead seem to be more related to *phenotype* which is another word commonly used interchangeably with trait. Traits are involved in behavioral phenotypic expression of individuals, but phenotypes encompass much more than just traits or behaviors, such that using them interchangeably is not necessarily appropriate. Furthermore, an example is given by Reale et al. (2007) suggesting that nursing, nest building and grooming, among others, are behavioral traits. These examples would be considered by psychologists and ethologists as behaviors that may vary irrespective of personality traits (although may certainly be influenced by them). Brown, Jones and Braithwaite (2005) state that shy-boldness traits are relatively flexible (citing Sih et al., 2004) and argue that we can't infer ecological behavior from laboratory experiments. While this is certainly a valid concern, if conclusions about shy-boldness change from captive to experimental settings, it may be more likely that our measures somewhere along the way are not measuring the same thing, or are not indicative of boldness. If boldness is representing a trait characteristic of personality, then in theory, it should be found similarly in both settings. This is, of course, based on the assumption that this continuum exists for a given species. Coleman and Wilson (1998) suggest that boldness and shyness may be context specific, although one could again make a case with the point just made; measures somewhere along the way may not have been measuring the same thing or were not indicative of boldness. It is obvious that these discrepancies have the potential to cause confusion for comparing and understanding research.

### ***Individual differences***

With a boom in personality and temperament research in animals and the recognition of important ecological considerations, the term *individual differences* seems to have become the new buzz word for animal behavior research. However, finding significant individual differences in behavior between animals in a given species, population or community does not automatically point to personality trait differences. Although observed individual differences in behavior may be related to underlying characteristics of the individual, there are a host of other biological or ecological variables that must be considered: these may be more directly involved in the behavioral outcome than personality variables. As Budaev and Zworykin (2002) point out, the behavior of an individual “depends on both its motivational state in a particular time and the immediate environmental stimuli” (p. S189) and it is challenging to “...extract stable characteristics of individuality from constantly changing overt behavior in response to the environment” (p. S190), even in controlled laboratory settings. Techniques developed in human psychology can be used in similar ways to help extract what may be representative of underlying characteristics in animals but as already described, results must be interpreted with caution. Thus behavioral differences do not automatically equate

to differences in what we've been discussing as *personality* or *individuality*. In uncontrolled settings and natural contexts where multiple environmental variables are impossible to control for, conclusions for individual differences in personality must be made even more cautiously.

### ***Stability vs consistency***

There is a notable and fundamental difference between *stability* and *consistency* with regards to behavior, with the latter being the targeted concept in personality studies; however, these terms are commonly used interchangeably. Stability refers to behavior that does not change. Consistency is used to describe a behavioral measure that is predictable across time and/or contexts even if the degree or level of the behavior changes (Budaev & Zworykin, 2002). For example, an individual may be bolder compared to other individuals in the face of a predator and in a novel environment even though the degree of boldness between the two contexts may differ. This refers more to relative standing in the population, or group of interest, and whether that standing changes or not. Therefore it is important to keep this in mind when making conclusions regarding personality. By definition, we are looking for consistency in behavior across time and context in order to make conclusions about personality. Thus, as Budaev and Zworykin (2002) concluded, "...behavior may be situation specific whereas individual differences, consistent" (p. S190).

This concept is generally understood and implemented but the words, *stability* and *consistency*, are used interchangeably to mean the same thing which can lead to confusion. For example, Wilson et al. (1993) state that one of the goals of their research was to examine whether or not individual differences in shyness and boldness were phenotypically stable in pumpkinseed sunfish. They found that behavioral differences between trapped (bold) and seined (shy) individuals persisted over a 30-day field-experimental period. Conclusions appear to be made with regard to personality due to the fact that they discuss their findings in relationship to innate differences. Since personality is defined with respect to consistency, this may have been the more appropriate term in this and other similar cases. In addition, there may be many times when we are in fact interested in behavioral *phenotypic stability*; however it's important to note that there is a difference in the use of this term and *phenotypic consistency*, with respect to behavior, and they should be not used interchangeably.

In a major review of behavioral syndromes by Sih et al. (2004), the authors use the term *consistency* which appears to have the same meaning as defined above but they delineate between *within*-individual and *between*-individual consistency as distinct aspects of behavioral syndromes. Within-individual consistency is referred to as "...the tendency for any given individual to exhibit consistent behavior across observations", independent of other individuals' behavior (p. 246). They suggest an example is of an individual being generally aggressive. On the other hand, "between-individual consistency refers to consistent differences among individuals in behavior," for which they give the example of rank order consistency in a trait such as aggressiveness (p. 246). They further suggest that

finding between-individual consistency does not imply that there also exists within-individual consistency. Within-individual consistency is also described with regards to *behavioral plasticity*. High within-individual consistency suggests limited behavioral plasticity. Based on their descriptions and examples, we suggest that their definition of between-individual consistency is equated to the original definition of consistency presented above. Within-individual and behavioral plasticity both seem to be equated with stability. Thus, there can behavioral consistency between individuals across time or context but there need not be individual stability.

### **Defining and Measuring Boldness and Shyness**

As mentioned earlier, the shy-bold axis of behavior has received increasing attention, especially from ecological researchers. Individual variation along this axis may have important consequences in many contexts across an individual's lifespan (i.e. boldness may influence success in mating, competition, feeding, adjusting to environmental change and responding to predators), subsequently influencing individual fitness. In humans, individuals vary on a continuum from extreme shyness to extreme boldness, characterized by an individual's initial reaction to unfamiliar events (Kagan et al., 1998). This criterion has set the foundation for how many animal researchers investigate boldness. It is expected and generally accepted by animal researchers that shy individuals will respond to unfamiliar objects or situations by fleeing, retreating, becoming cautious, quiet or inactive. Bold individuals, on the other hand, do not show these responses or show the opposite behavior (i.e. moving towards, becoming active, exploring and investigating) with the same novel object or in the same novel situation (Wilson et al., 1993). Therefore *novelty* is key for investigating boldness. However, unlike concepts such as aggression or exploration that have been studied extensively, defining and measuring boldness in animals is much less straightforward. The following is based on information presented in the studies given in Tables 1-4.

#### ***Psychologically – based approaches***

There are several behavioral tests, listed in Table 1, that are used in a variety of applications in laboratory settings and have been implemented in many personality related studies on a variety of fish species. Their use has not been limited to studies only employing psychologically-based methodology. In fact they were used, or modified for use, in many of the “ecologically based” studies, but since these approaches tend to utilize several tasks in a single study, they are described here.

A mirror task is not used to measure bold traits but is regularly implemented along with several others in studies using correlational or multivariate techniques. It is commonly used to measure aggressive responses to mirror reflections at one end of an experimental tank. Validity for this task has been supported on several levels, including neurologically. For example, treatment



with 5-hydroxytryptamine (5-HT) synthesis inhibitor *p*-Chlorophenylalanine (PCPA) increases aggression in mammals and birds and has been shown to increase aggressive responses in this task with fire-mouth cichlids (Adams, Liley, & Gorzalka, 1996).

Predator inspection tasks have been used to investigate fear responses. This is a task in which a predator or model predator is placed at one end of the tank and responses to the predator are recorded. It is used by behavioral neuroscientists using zebrafish to model avoidance behaviors and examine neurological pathways associated with anxiety (as reported in Bass & Gerlai, 2008). Neurological studies suggest that there's a genetic predisposition for consistent alarm responses in the presence of a predator (Gerlai, Lahav, & Rosenthal, 2000; Hall & Suboski, 1995). More recently, this has been used as a potential avenue to examine bold behavior. Budaev (1997b) suggests that this test likely examines behavior that results from a trade-off decision between exploring a novel environment, fleeing or freezing such that the resulting behaviors might represent motivational systems that can be identified. Moretz, Martins, and Robison (2007) suggests that individuals with a higher frequency of predator approaches may be considered bolder, although results are mixed for the three strains of zebrafish examined (out of six Pearson correlations, two were significant and represented different strains: latency to feed after a disturbance,  $r = -0.42$ , and frequency to leave a shoal,  $r = 0.31$ ). A variation of this has been used by Budaev, Zworykin, and Mochev (1999a, 1999b). Instead of using a predator as a stimulus fish, a slightly larger, non-predator fish of a different species was used and subsequent inspection behavior recorded. Although no rationale was provided in either study for this measure, logically this could serve to measure bold behavior more directly without confounding it with fear responses. Although these constructs are often equated, they are not necessarily so. This is evident from Budaev's (1997b) study with guppies. Latency to emerge into a novel environment was highly correlated with exploratory measures but not with those of fear and escape.

A shoaling/schooling task has also become commonly used in more social, schooling fish species. However, there's some variation in how it's used and what it is thought to represent. It seems to be more widely used as a measure of schooling tendency and preference for conspecifics. Budaev (1997b) placed a school of conspecifics at the end of a tank and examined behaviors associated with schooling tendency. Harcourt et al. (2009) first examined boldness using measures associated with the propensity to take risks and then used a shoaling task to determine individual preference of social groups (bold groups or shy groups). However, Moretz et al. (2007) used the task to measure the frequency of an individual *leaving* the stimulus group, rationalizing that separation from the group may be indicative of bolder individuals. There is potential for this measure, however, it's possible that the observed behavior is more closely related to social system mechanisms. With respect to the other potential bold measures, out of the 3 strains of zebrafish tested by Moretz et al. (2007) there were no significant correlations with *latency to feed after a disturbance* and only one out of three strains was significantly correlated with *predator approaches* ( $r = 0.31$ ). This

measure would need further support and validation before it should be considered as a shy-bold measure.

An open-field task places individuals into an open and novel environment and has been used to selectively breed small mammals for emotional reactivity and non-reactivity (Warren & Callaghan, 1975). It has been successfully modified for use in fish models to measure activity and exploration with some support for application in bold measures (Burns, 2008). Activity may indirectly measure boldness in a novel environment as freezing behaviors are a common occurrence and are thought to be related to the shy-bold continuum. Additionally, activity is found to be positively correlated with boldness in many species (e.g., guppies: Budaev, 1997a; three-spined stickleback: Bell, 2005; Harcourt et al., 2009; zebrafish: Moretz et al., 2007).

There was an important study using guppies conducted by Burns (2008) that investigated the validity of the open-field task in addition to a novel object and emergence task. Novel object tasks are designed to measure fear of novelty, and thus boldness and shyness, via responses to objects that are placed in the environment that have never been observed before. An emergence task (also referred to as a novel environment task by Budaev, 1997a) is designed to measure the propensity of an animal to leave a safe area and emerge into a novel and less safe area. Burns (2008) considers latency to emerge a conflict between shyness and exploratory behavior. There are several variations of this task. Budaev and colleagues used this task in guppies (1997b) and in convict cichlids (1999b) in a design measuring the latency of individuals to pass through a small opening into a larger, novel, open area. Brown et al. (2005) used a very similar design but the home compartment was small and completely enclosed. Furthermore, they marked a D-shaped area immediately in front of the box to aid in tracking movement and to potentially provide a dangerous background for fish to cross, as it was in stark contrast to their coloration and the rest of the environment. Note, however, that species such as zebrafish have been shown to have a preference for dark versus light environments which may or may not be related to boldness (see Serra, Medalha, & Mattioli, 1999). Others, such as Fraser et al. (2001), have used it in a tank with an enclosed “safe” area on one end and an open “less-safe” area on the other end measuring the latency to leave the “refuge.” Furthermore, Harcourt et al. (2009) combined the propensity of individuals to leave a refuge with the propensity to feed in an open area on the other end of the tank. However, some of these studies combine measures so that it is difficult to tease apart whether or not boldness is observed directly and as Burns (2008) points out none of these have been tested for internal, external or discriminant validity.

**Table 1***Psychological or Correlational Approach: Exploration & Extraction of Individuality*

Species	Purpose & Bold Definition	Bold Measure	Quantifying Boldness	Tasks Used	Main Findings	Consistency Across Time	Consistency Across Context	Authors
Guppy <sup>W</sup>	<b>P:</b> Identify characteristics of individuality, motivational systems and corresponding personality dimensions <b>D:</b> No <i>a priori</i> definition	1. Latency to emerge into novel compartment 2. Freezing 3. Frequency of predator inspection 4. Ambulation	<b>Principle Component Analysis</b>	Open-field, predator inspection, mirror task, schooling tendency task & emergence task	1. Two main factors extracted: Activity-Exploration & Fear-Avoidance 2. Activity measures in open field test was related to exploration and boldness 3. Latency to emerge was correlated with Activity/Exploration but not with Fear/Escape	Yes	Lab setting: Yes Natural setting: N/A	Budaev (1997b)
Lion-Headed Cichlid <sup>N</sup>	<b>P:</b> Consistency & ontogeny of IDs <b>D:</b> No <i>a priori</i> definition	1. % time inspecting novel fish 2. % time in proximity of novel fish	<b>Correlational</b> (Bold not explicitly defined)	Open-field, strange fish test, mirror test	1. Behavioral consistency at 12-13.5 months in strange fish and mirror test 2. Consistency only obvious in tests with discrete stress	Yes	Lab setting: some Natural setting: N/A	Budaev et al. (1999a)
Convict Cichlid <sup>N</sup>	<b>P:</b> Examine ecological significance of individuality (i.e. its role in mate choice & parental care) <b>D:</b> No <i>a priori</i> definition	1. Frequency of freezing, movements and escape attempts 2. % time inspecting novel fish 3. Latency to emerge into novel compartment 4. Latency to approach novel fish	<b>Factor Analysis</b> (Bold not explicitly defined)	Open-field, strange fish test, mirror test	1. Two main factors extracted: Activity & Activity-Exploration 2. 2 <sup>nd</sup> -order factor extracted: Boldness 3. Temperament influenced mate choice & food provisioning in males	Yes	Lab setting: Yes Natural setting: N/A	Budaev et al. (1999b)

Three- Spined Sticklebacks <sup>W</sup>	<b>P:</b> <i>Part a:</i> Identify IDs & consistency in response to territorial intruders during breeding season <i>Part b:</i> Identify IDs & consistency in aggression & response to predators outside breeding season. <i>Part c:</i> Followup to previous experiments <b>D:</b> <i>Part a:</i> No <i>a priori</i> definition <i>Part b:</i> Response to alarm stimulus <i>Part c:</i> Response to alarm stimulus	<i>Part a:</i> N/A <i>Part b:</i> 1. Duration of jerky swim type 2. Latency to swim after predator exposure 3. Frequency of approaches to predator <i>Part c:</i> Proportion of jerky swim type	<i>Part a:</i> N/A <i>Part b:</i> <b>Factor Analysis</b> <i>Part c:</i> <b>Principle Component Analysis</b>	<i>Part a.</i> Variation of predator inspection <i>Part b.</i> Variation of predator inspection <i>Part c.</i> Variation of open field task	<i>Part a:</i> Extracted four factors from response to intruders during breeding season (Aggression, Curiosity, Nest Activity & Sex) <i>Part b:</i> Extracted two factors from response to predator outside breeding season (Precaution- Investigation & Boldness-Timidity) <i>Part c:</i> Extracted two factors from response to novel environment (Activity & Timidity- Boldness)	<i>Part a:</i> Yes <i>Part b:</i> No (Inconsistency in predator fish behavior. Sticklebacks were not repeatedly measured in Part b) <i>Part c:</i> Yes	Lab setting: <i>Part a:</i> Yes <i>Part b:</i> No <i>Part c:</i> Yes Natural setting: N/A	Huntingford (1976)
Zebrafish <sup>N</sup>	<b>P:</b> Examine behavioral syndromes across three strains of zebrafish <b>D:</b> No <i>a priori</i> definition	1. Leaving shoal group (frequency) 2. Predator approach (frequency) 3. Latency to feed after disturbance	<b>Correlational</b> (Bold not explicitly defined)	Used variations of: open-field, predator inspection, mirror task & schooling tendency task	Evidence for several syndromes: Activity, Bold-Aggression, & Domestication. Boldness-Aggression syndrome depended on behavioral measure and most results differed between strains.	N/A	Lab setting: Some Natural setting: N/A	Moretz et al. (2007)

**Note:** Studies that were methodologically or analytically similar to psychological approaches. The category title of “Boldness Measure” was not changed for the sake of consistency, but many of these studies were not directly measuring an *a priori* definition of boldness. Instead, measures and /or tasks were employed and statistical analyses and results led to conclusions inferring some label or measure(s) of boldness. Conclusions for this table with regards to consistency across time and context were not limited to bold variables (i.e. responses given are with respect to any behavioral measure that were examined cross-time or context). P: Purpose; D: Definition; ID(s): Individual difference(s); <sup>W</sup>: Indicates sample was wild or wild-caught; <sup>N</sup>: Not wild or wild-caught (fish came from aquarium, breeder or some other distributor); <sup>WN</sup>: Indicates both types were used in the study. See text for descriptions on “Task Used.”

**Table 2***Ecological and/or Evolutionary Approach: Response to Novel Objects*

Species	Purpose & Bold Definition	Bold Measures	Quantifying Boldness	Main Findings	Consistency Across Time	Consistency Across Context	Authors
Pumpkinseed Sunfish <sup>W</sup>	<b>P:</b> Identify boldness and shyness & examine ecological consequences <b>D:</b> Measures based on the assumption that bold individuals become actively exploratory with novel objects or in novel situations	1. Reaction to novel object in natural environment	<b>Bold</b> = individuals that explored & were captured in novel wire traps <b>Shy</b> = Those left behind	1. Shy-bold continuum exists for this population 2. Differences between groups are not significant in lab environment 3. Differences between groups are stable in nature only	Yes	Lab setting: No Natural setting: Yes	Wilson et al. (1993)
Pumpkinseed Sunfish <sup>W</sup>	<b>P:</b> Examine context specificity of shyness & boldness <b>D:</b> Measures based on the assumption that bold individuals become actively exploratory with novel objects or in novel situations	1. Distance and behavioral response to threatening object 2. Distance and behavioral response to novel food source 3. Behavioral response post - introduction of predator to system	1. <b>Bold</b> = approached/allowed object within 5cm <b>Shy</b> = retreated immediately <b>Intermediate</b> = allowed object proximity no closer than 5 cm 2. <b>Bold</b> = moved within 5cm of food <b>Shy</b> = failed to approach <b>Intermediate</b> = approached but not within 5cm 3. <b>Bold</b> = no behavioral change pre vs. post predators	1. Consistent IDs found independently in both contexts 2. IDs did not correlate cross context 3. Intermediate individuals from novel object-context behaved more boldly post-predator introduction	Yes	Lab setting: N/A Natural setting: No	Coleman & Wilson (1998)

Zebrafish <sup>W</sup>	<p><b>P:</b> Analyze inter-population differences in shoaling tendency and boldness &amp; estimate heritability of shoaling tendency</p> <p><b>D:</b> Exploration of a novel object</p>	<ol style="list-style-type: none"> <li>1. Total time spent within 1.5 body-lengths of novel object (10-min sample period)</li> </ol>	<p><b>Bold</b> = those that spent a longer duration within 1.5 body-lengths of object (This measure was not directly specified in the article, but inferred by current authors)</p>	<ol style="list-style-type: none"> <li>1. Evidence for genetically-based differences in boldness</li> <li>2. Increase in boldness over trial exposure</li> </ol>	<p>Boldness: No</p> <p>Shoaling: Yes</p>	<p>Lab setting: N/A</p> <p>Natural setting: N/A</p>	Wright et al. (2003)
Zebrafish <sup>WN</sup>	<p><b>P:</b> Investigate shoaling and boldness using OTL analysis (Quantitative Trait Loci)</p> <p><b>D:</b> Exploration of a novel object</p>	<ol style="list-style-type: none"> <li>1. Total time spent within 1 ½ body-lengths of novel object (10-min sample period)</li> <li>2. Latency to enter stimulus zone</li> <li>3. Frequency of entering stimulus zone</li> </ol>	<p><b>Principle component analysis</b> (used to combine info from 3 measures)</p>	<ol style="list-style-type: none"> <li>1. Difference in shoaling and boldness between wild and lab strains</li> <li>2. Demonstrate potential for QTL mapping of behavioral traits</li> </ol>	<p>Not reported</p>	<p>Lab setting: N/A</p> <p>Natural setting: N/A</p> <p>Genetic mapping consistent for all 3 boldness measures</p>	Wright et al. (2006)

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**Note:** Studies that were methodologically or analytically similar to Ecological Approaches and quantified boldness as a response to novel objects. Conclusions for this table with regards to consistency across time and context were not limited to bold variables (i.e. responses given are with respect to any behavioral measure that were examined cross-time or context). P: Purpose; D: Definition; ID(s): Individual difference(s); <sup>W</sup>: Indicates sample was wild or wild-caught; <sup>N</sup>: Not wild or wild-caught (fish came from aquarium, breeder or some other distributor); <sup>WN</sup>: Indicates both types were used in the study.

**Table 3***Ecological and/or Evolutionary Approach: Response to Novel Environment*

Species	Purpose & Bold Definition	Bold Measures	Quantifying Boldness	Main Findings	Consistency Across Time	Consistency Across Context	Authors
European Wrasse <sup>W</sup>	<b>P:</b> Examine boldness-related schooling tendency in natural and experimental settings <b>D:</b> Response to novel environment	1. Frequency of hiding in natural shelters 2. Latency to emerge into novel environment 3. Frequency of freezing, moving, escaping	<b>Bold</b> = active in novel environment <b>Shy</b> = retreats or freezes in same novel environment	1. Solitary individuals demonstrated higher degree of bold behaviors compared to schooling individuals	No (Repeated measures only implemented in novel environment task with “solitary” individuals)	Lab setting: N/A Natural setting: some with regards to activity	Budaev (1997a)
Trinidad Killfish <sup>W</sup>	<b>P:</b> Determine if boldness is a source of the observed natural leptokurtic dispersal pattern (i.e. does boldness predict dispersal?) <b>D:</b> Propensity to move through & explore unfamiliar space	1. Latency to leave refuge (Leaving defined as 50% of body crossed the edge of refuge) 2. Latency to cross intervening gap to refuge on opposite side of the tank (Crossing gap = tail fin crossed midway mark in tank) 3. Time spent in front brick contained within refuge	Two indices of bold created: 1. <b>Bold</b> = High ratio of time spent in front of brick to total test time 2. <b>Bold</b> = High rank order with respect to: • Latency to cross gap (1 = shortest time) • Latency to leave refuge (1 = shortest time) • Time spent in front of brick (most to least) • Those never leaving refuge (all received same rank)	1. Field Release/Recapture: Individuals placing high on ranked indices dispersed farther in the river, controlling for length, sex and cohort group 2. Experimental-Stream Release/Recapture: Bolder fish dispersed farther as measured by either indices, controlling for length, sex and cohort group 3. At both sites, boldness and fish length were both significant predictors of distance moved, albeit independent from each other	Yes	Lab setting: N/A Natural setting: N/A  Consistency demonstrated via lab experiments predicting field observations	Fraser et al. (2001)

**Note:** Studies that were methodologically or analytically similar to Ecological Approaches and quantified boldness as a response to a novel environment. Conclusions for this table with regards to consistency across time and context were not limited to bold variables (i.e. responses given are with respect to any behavioral measure that were examined cross-time or context). P: Purpose; D: Definition; ID(s): Individual difference(s); <sup>W</sup>: Indicates sample was wild or wild-caught; <sup>N</sup>: Not wild or wild-caught (fish came from aquarium, breeder or some other distributor); <sup>WN</sup>: Indicates both types were used in the study.

**Table 4**  
*Ecological and/or Evolutionary Approach: Response Under Risk*

Species	Purpose & Bold Definition	Bold Measures	Quantifying Boldness	Main Findings	Consistency Across Time	Consistency Across Context	Authors
Three-Spined Stickleback <sup>W</sup>	<b>P:</b> Investigate boldness-aggressive syndrome & examine evidence for evolutionary constraints <b>D:</b> Response under risk	1. Time foraging after simulated predator strike 2. Latency to forage after simulated predator strike	<b>Principle Component Analysis</b> (Standardized scores from -3 to 3)	1. There are population differences in the correlation of boldness & aggression. Thus traits within behavioral syndromes may be free to evolve independently	N/A	Lab setting: some Natural setting: N/A	Bell (2005)
<i>Brachyraphis episcopi</i> <sup>W</sup>	<b>P:</b> Examine shyness-boldness continuum and ecological factors <b>D:</b> Propensity to take risks	1. Latency to leave refuge (variation on emergence task) 2. 'Hesitancy' = time to cross the black arc in front of refuge, minus the time to emerge	<b>Bold</b> = Scored based on latency measure	1. Relationship between boldness and standard length: Potential for metabolic influence on emergence from a refuge 2. Fish caught in high predation areas were bolder than those in low predation areas	N/A	Lab setting: N/A Natural setting: N/A	Brown et al. (2005)
Three-Spined Stickleback <sup>W</sup>	<b>P:</b> Examine relationship between boldness & social association <b>D:</b> Propensity of individuals to take risks	1. Amount of time spent foraging in open end of tank (compared to 'safe' area on opposite side under foliage)	<b>Bold</b> = Foraging for 40% of observation time. <b>Shy</b> = Foraging for 5% of the time (representing upper 34% and lower 27% of the distribution)	1. Bold and shy fish preferred bold social groups, i.e. matching phenotype is not a considerable factor for choosing social groups 2. Bold fish were more active 3. Significant interaction between focal personality type and hunger	N/A (Repeated measure was pooled for analysis)	N/A	Harcourt et al. (2009)

**Note:** Studies that were methodologically or analytically similar to Ecological Approaches and quantified boldness as some measure with regards to response under risk. Conclusions for this table with regards to consistency across time and context were not limited to bold variables (i.e. responses given are with respect to any behavioral measure that were examined cross-time or context). P: Purpose; D: Definition; ID(s): Individual difference(s); <sup>W</sup>: Indicates sample was wild or wild-caught; <sup>N</sup>: Not wild or wild-caught (fish came from aquarium, breeder or some other distributor); <sup>WN</sup>: Indicates both types were used in the study.



A quick summary of the Burns (2008) article is important for considering measures of boldness. Definitions given here are those defined by Burns (2008) directly. The three tasks were examined for face validity (professional judgment on whether test appears to measure variable), internal validity (consistency over time and context as judged through reliability), convergent validity (high correlations of measures of the same construct across different methods) and discriminant validity (low correlations between variables that should not be measuring the same construct). The emergence test was conducted using a tank with one end covered to create a refuge, with a sliding door that could be lifted. The latency for a fish's head to appear outside the door was measured. For the novel object task, the measures included latency to approach object within 4 cm and proportion of time spent within 4 cm. Open field and the emergence tasks demonstrated moderate levels of reliability ( $r > 0.25$ ) and had good discriminant validity. The novel object task did not display adequate internal or discriminant validity. The author points out that for this task, movement in general may have propelled fish in towards the object instead of movement resulting from some propensity to explore it. Also, many times an individual did not approach the novel object, which may indicate a need to increase observation time above 300 seconds. It is important to consider that the emergence task does not necessarily demonstrate that guppies view the "refuge" as a safe environment. The observation of several individuals rapidly darting into the open area may indicate a fear response more than exploratory behavior indicative of boldness. In conclusion, the author agrees with others that have recommended open field tests to be used for measures of boldness and exploratory behavior.

Evident from these tasks is that researchers have come up with many ways of measuring bold behaviors, yet Burns (2008) is the only article we've seen that directly addresses the validity and utility of these measures. Reale et al. (2007) recommends additionally testing biological and ecological validity whenever possible which can help to support the types of validity already discussed. Hopefully it is apparent that there is a need to address these issues. Additionally, it is important to note that these tasks need not be used in isolation, nor are researchers limited to the measures discussed. Many psychological based approaches use several tasks and attempt to create measures that can be used across several of these tasks. For example latency to enter a novel environment can be compared with latency to approach a mirror or conspecifics and latency to approach predator. We must keep in mind that these measures are not necessarily measuring the same trait, however consistency in measures across these contexts would greatly strengthen the argument that they were (or at least measuring related traits).

It is also important to recognize that there is a growing body of literature using these tasks in zebrafish which have become a favored model organism for many genetic and behavioral studies. Most of that literature is primarily concerned with brain mechanisms, influences of drugs and genetic analysis. Although they do provide more evidence of uses and applications of these tasks, fish are not often tested individually and personality and temperament are not addressed. Thus, these studies are not presented here.

### *Ecologically – based approaches*

Unlike the studies represented in Table 1, most of those presented in Tables 2-4 specify a definition of boldness from which the subsequent design is based on (although not for all). The definitions used are similar, but not necessarily the same. Fraser et al. (2001) defines boldness as the “propensity to move through and explore unfamiliar space.” Budaev (1997a) considers boldness based on Wilson and colleagues’ (1993, 1994) definition that a fish is considered bold if it’s active in a novel environment (presumably explores it) rather than responding by retreating or freezing (indicative of shyness). Harcourt et al. (2009) define bold as “the propensity of individuals to take risks” and Bell (2005) measures response under risk as an indication of boldness. Reale et al. (2007) go as far to say that shyness-boldness is based on an individual’s reaction to any risky behavior but *not* to new situations, which they reserve for their definition of exploration. These definitions do not all mean the same thing. In some cases it seems to have led to some misconceptions or redefining what boldness is. Reale et al.’s (2007) definition completely removes the aspect of novelty, the condition under which boldness has been classically defined.

Most notably, defining boldness as the propensity to take risks or measuring responses under risk may lead to spurious conclusions. It seems that this definition resulted from research in ecological settings, as it logically makes sense that risky behavior may be directly influenced by bold traits. However, this conclusion may not be entirely adequate. As defined earlier, boldness is classically considered with respect to novelty (i.e. responses to novel events and environments). Although potentially related, risky behavior may be something different. Consider the suggestion given by Wilson et al. (1993) in which one may conclude that fish take more risks when they are hungry. Thus, in a social environment, shy individuals that lack competitive advantages may actually be more likely to explore novel (and potentially risky) environments in search of resources. With this evidence, some researchers caution that boldness may be context-specific and that results from one context may not be applicable to or predictive of other situations (Coleman & Wilson, 1998; Wilson, Clark, Coleman & Dearstyne, 1994). While this may certainly be true, we must be careful to argue from this angle. It is possible that the *behavior* observed in this case is context-specific and not actually representative of *boldness*. It is important to keep in mind that behaviors resulting from ecological pressures are more likely to reflect situational circumstances as opposed to underlying traits unique to that individual. In the above example, the individual that moves to explore a novel environment may still be considered a shy individual overall, but situational circumstances (competition for limited resources versus exploring a new and risky habitat) resulted in a behavior that may not be indicative of the underlying trait.

This difference may provide an alternative explanation of the results found by Wilson et al. (1993). They found differences between groups of pumpkinseed sunfish classified as bold and shy in measures conducted in the wild (with respect to swimming proximity with conspecifics, stomach context of potentially risky

prey species and responses to human presence) but did not find differences between those same groups when conducting subsequent laboratory studies. It is possible that laboratory studies did not invoke the same type of risky environment and subsequent choices such that behavioral differences were not reflected in the same way. Results appear to be only analyzed with respect to originally defined groups of bold and shy. However it would be interesting to determine if bold and shy individuals emerged from laboratory measures, irrespective of the original classification, and if they remained consistent across experiments. Therefore measuring the “propensity to take risks” is valuable for investigating ecological systems, but does not necessarily reflect behaviors which are representative of underlying traits.

The group of studies in Tables 2-4 provide a mixture of approaches which all incorporate novelty but vary in what is considered bold behavior and how it should be measured. It is important to take into consideration the species of interest, what behaviors might be representative of boldness and what environments might elicit the observation of bold traits. However, the variety of approaches found in these studies do not seem to necessarily stem from these considerations.

The first category (Table 2) measures behavior with respect to responses to novel objects. Wilson et al. (1993) was the only study to set the criterion for which fish were expected to bold vs. less bold (or shy) *prior* to data collection. They presented a novel wire trap to pumpkinseed sunfish in their natural environment and labeled those that approached (and were caught) as bold and those that remained as shy. All subsequent measures were based on this initial criterion. The study by Coleman and Wilson (1998) was an expansion of this initial study and instead measured responses to a novel object (as did Wright and colleagues, 2003 & 2006) for which bold individuals were classified based on behavioral responses. Between these three studies, novel objects that were used varied from an arbitrary black plastic object (Wright, Rimmer, Pritchard, Krause & Butlin, 2003; Wright, Nakamichi, Krause & Butlin, 2006) to a more specifically selected novel food source and potentially threatening object (i.e. a net with invertebrates contained within aquatic vegetation and a red-tipped meterstick, respectively) (Coleman & Wilson, 1998). In all three cases object choices differed in general content and intended target behaviors.

The testing apparatus and features used across the studies that tested boldness in laboratory settings varied widely. Fraser et al. (2001), Budaev (1997a) both measured responses to novel environments, but in different ways (Table 3). Fraser et al. (2001) created a covered refuge with a brick containing an artificial plant in each of three holes and measured behaviors and movement out of this area and into the novel and open space beyond the refuge. Budaev (1997a) measured response to a novel environment via an emergent task with no structural components in either the home or novel compartments. Behaviors were measured with regards to moving through a small opening and exploring the open space beyond. Harcourt et al. (2009), Bell (2005) and Brown et al. (2005) considered their measures to be with response under risk, although Brown et al.’s design was similar to the design by Budaev (1997a) just described. An emergence task was

used with a completely enclosed starting compartment, as described earlier. They measured the time it took for the fish's snout to emerge from the refuge and *hesitancy* (defined as the time it took to cross the black arc minus the time it took to emerge from the box, p. 1005). Harcourt et al.'s (2009) and Bell's (2005) measures both included feeding components but again, preceded in a different manner. Harcourt et al. (2009) used a single plastic plant to create a refuge at the deep end of the tank (note the difference between this and the refuge created by Fraser et al., 2001), created a shallow end on the opposite side with gravel and measured the time spent out of the refuge. Bell (2005) included several objects in the tank as refuges but included an additional component; a predator skull (great egret) was attached to the tank and attacks were simulated from above, measuring behaviors based on feeding responses after simulated attack. Thus it is evident that methodology varies widely and is not necessarily due to the difference in species being investigated (for example, studies by Bell (2005), Harcourt et al. (2009) and Huntingford (1976) are all conducted on three-spined sticklebacks, yet vary greatly in methodology).

Of the approaches, it might be most difficult to disentangle underlying traits when incorporating feeding in the measures of boldness. Harcourt et al. (2009) were interested in whether or not the state of hunger in three-spined sticklebacks interacted with personality. However the measures of boldness used for subsequence analysis may have been confounded by feeding. First, the fish were trained in the experimental tank to a prey item (bloodworm) located on the opposite end of a deep, "safe" environment in the tank before any trials were conducted. Secondly, they measured the amount of time fish spent out of the safe resting area (full body out of artificial weed) without administering food and yet chose to delineate bold versus shy individuals based on percent feeding time when food was administered. One must consider that the initial training for three days in the experimental tank likely eliminates the element of novelty which is important for measuring bold behavior. Additionally, the training may have reduced the element of risk associated with the experimental tank (which is what the definition of bold in this study was based on). Bell (2005) measured the time spent foraging within one body length of the food dispenser and the latency to take a bite after a simulated attack by a predator. Coleman and Wilson (1998) measured responses to novel objects but used a net with food as the presented object. Moretz et al. (2007) measured the latency to feed after being netted and moved which was expected to be a measure of stress recovery or boldness. As with Harcourt et al. (2009), in all of these examples the feeding component may have influenced subsequent behavior to reflect hunger motivation instead of boldness traits. In wild-based studies such as Coleman and Wilson (1998), incorporating feeding into boldness measures may introduce social and competitive variables influencing subsequent behavior. Furthermore Bell (2005) introduced a predator in addition to the feeding measure and Moretz et al. (2007) exposed fish to a predator immediately prior to measurements of feeding latency. Both have the potential to further confound the resulting behavior. In the study by Bell (2005), there were additional measures that were not considered as part of the boldness measures: *activity in an unfamiliar environment*. However, based on what has been discussed so far, these measures

(*latency to move* under divider and explore novel space and *frequency of freezes*) may also be indicative of bold traits. This approach may be more straightforward and have fewer potential confounds. Thus, it might be more informative to measure boldness with less complex measures and approach questions regarding the influence of boldness on foraging success and other ecological consequences via separate measures that can be later related.

### **Conclusions & Considerations**

As is evident, there are numerous possibilities on how to go about researching the concept of boldness. One must consider incorporating a novel object, a novel environment or both and what those details might entail for a given species. Boldness is commonly associated or even equated with fearlessness, exploration or activity which may be very difficult to tease apart. Thus, many choose to control for as many variables as possible in a simple task of exploring a novel environment. However, understanding ecological implications is important, so examining responses to predators may be a better choice. Unfortunately in either situation, it is difficult to determine if any given measure is actually “tapping into” boldness. It is extremely challenging to determine whether a particular behavior of interest is reflecting a situational response, the underlying trait of interest or a closely-related trait.

So which approaches, definitions or techniques are better? Which should be considered for further use? For a number of reasons, the answers to these questions remain unclear; however, this review aims to shed light in important areas that should be well thought out when designing these types of studies. An important consideration to any design regarding personality or personality traits should be to incorporate methods that will help increase the internal validity of the constructs of interest. There is great controversy regarding the application of personality studies in animals in general, that this point becomes especially critical. Some efforts have been made to do this in some ecological studies. For example, Fraser et al. (2001) demonstrated ecological validity of their measures by finding that boldness predicted distance of dispersal in killfish for both natural and simulated environments. Huntingford (1976) found behaviors loaded similarly on two separate behavioral tests for individuality (Boldness-Timidity and Timidity-Boldness factors in predator and open field tests respectively) and that responses of individuals were correlated between the two tests, providing evidence in favor of the validity of these tasks for this species. However, this important aspect is often overlooked as most of the studies presented here fail to address or report any type of validity or reliability for their measures. Based on the literature presented, we suggest using several simple measures of boldness and looking for converging and diverging validity to further support relationships found with other variables. Both Wilson et al. (1993) and Wright et al. (2003) acknowledge the potential limitations from only having one measure of boldness. As Wright et al. (2006) recognized, combining information from multiple measures collected during a behavioral task may facilitate the analysis of underlying traits. These are important consideration and may be especially important for researchers that want to investigate ecological

implications. For example, if one wants to look at the relationship of bold behavior on social relationships in natural settings, one needs to first demonstrate some degree of internal validity of the bold measures before comparisons and conclusions can be made with any confidence for social relationships.

Approaches based on human personality are criticized due to their nature of describing personality structure rather than investigating the underlying mechanisms. It may be however, that a foundation needs to be set by first investigating the structure of individuality in a particular species. Mechanistic and functional aspects may be more challenging to investigate and interpret without first having a structural starting point.

The advantage to psychological approaches is that some of this ambiguity is resolved with multivariate statistics. This approach has been criticized for its limited comparability as behaviors may not load the same way into the same kinds of factors across species. However, the upside is that if behaviors load similarly across studies for a single species, much more confidence can be placed on those factors, their meaning and the potential structure of personality for that species. Furthermore, this allows for species-specific measures that can be used to summarize overall factors. For example, Huntingford (1976) commented on a type of swimming that is highly characteristic of three-spined sticklebacks. Irregular jerky movements are common in normal swimming but change to more traditional continuous swimming in the face of a predator. This was used as a potential indicator for boldness along with other measures. This behavior is obviously not applicable across species but loaded into a factor with measures that have been used with other species (i.e. *latency for swimming after predator exposure & frequency of approaches*) so that comparability is not unfeasible (note that these behaviors could have been related to fear as discussed earlier but that is still something that has not been teased apart for any study).

One of the most important take home messages from this review is that research conclusions in personality and temperament studies should be made with utmost care for a multitude of reasons. First, as just pointed out and emphasized by Burns (2008), “evidence is first required that the test be valid within a context before a test should be used to measure the consistency of temperament between contexts” (p. 344). We must also consider that domestication can remove selection pressures found in natural populations and intensify others, resulting in the potential for decreased ecological validity (Moretz et al., 2007; Wright et al., 2006).

Secondly, there are suggestions that personality may become more stable with age (similar to that distinguished in humans as the difference between temperament and personality), which is supported in one example by Budaev et al.’s (1999a) study in lion-headed cichlids. Thus, ecological studies must either include this variable in research or be aware of the potential influence when making conclusions, as it may often be difficult to determine the age of individuals in a natural setting.

Third, there is accumulating evidence that consistent individual differences become more pronounced with mildly stressful situations (Budaev et al., 1999a). Budaev et al.’s (1999a) results demonstrated consistency in temporal stability of

behaviors only in situations involving mild stress. Behaviors scored with little or no stress (such as locomotion) were not significantly consistent over two exposures, regardless of the task or age. Thus, behavioral complexity in non-threatening situations could mask the presence of consistent individual differences (Budaev et al., 1999a).

Finally, although it's become common practice to study animal behavior over context and time in order to make comments on potential personality traits, inconsistency in behavior across context does not necessarily equate to inconsistency in a personality trait of interest. When only including a couple of contexts in which to measure a particular trait such as boldness, inconsistency may be more a result of our measurement and the contexts we chose. Consider an example with an aggressive human. This individual may consistently show higher aggression over time and in many relevant situations (at a bar, in a heated discussion, driving in traffic, etc). These are contexts in which aggressive behavior would be expected to emerge in an aggressive individual. However, if you were to include many other contexts (going to church, eating lunch, getting ready for work, etc) and pick points of time throughout the day to measure behavior, your results would likely reflect a greatly reduced measure of aggressiveness for that individual, masked by context for which aggression would be less likely to emerge. Sih et al. (2004) summarizes this point well with the following statement: "the insight for behavioral ecologists is that expression of [personality] might depend on the situations studied, and that our goal should be to understand which situations allow [personality] to emerge" (p. 267).

In conclusion, it is absolutely necessary to build a strong methodological foundation for any personality-related research of interest. We hope that this point will help to facilitate studies specifically examining the reliability and validity concerns for the variety of methodological approaches that have been employed.

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