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REPORT

Some anemonefish lack personality: a comparative assessment of behavioral variation and repeatability in relation to environmental and social factors

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Abstract Determining the extent of repeatable differences in the behavior of animals and the factors that influence behavioral expression is important for understanding individual fitness and population processes, thereby aiding in species conservation. However, little is known about the causes of variation in the repeatability of behavioral differences among species because rarely have comparative studies been undertaken to examine the repeatability of behavioral differences among individuals within their natural ecological settings. Using two species of endemic subtropical anemonefishes, *Amphiprion mccullochi* and *A. latezonatus* at Lord Howe and North Solitary Islands, Australia, we conducted an in situ comparative analysis of personality traits, examining the repeatability of boldness, sociability and aggression as well as the potential role of environmental and social factors on behavioral expression. For *A. mccullochi*, only boldness and aggression were highly repeatable and these behaviors formed a behavioral syndrome. For *A. latezonatus*, none of the three behaviors were repeatable due to low-inter-individual variation in behavior. We suggest that the harsher and more variable environmental and social conditions experienced by *A. latezonatus* have resulted in reduced repeatability in behavior, in contrast to *A. mccullochi* which typically inhabits a more stable lagoonal reef environment. Additionally, group size and size rank, rather than nearest-neighbor distance and anemone size, influenced the expression of these behaviors in both species, suggesting that behavioral variation was more sensitive to social than environmental factors. Overall, differences in repeatability between these closely related species likely reflect adaptations to contrasting environmental and social conditions, although alternative explanations must be considered. The differences in behavioral consistency between these two endemic anemonefishes could lead to disparity in their resilience to environmental or social change in the future.

Keywords Clownfish · *Amphiprion* · Coral Reef · Boldness · Aggression · Sociability

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Introduction

Consistent variation in the behavioral traits of individuals has been characterized in a number of animal taxa (Gosling and John 1999; Sih et al. 2004; Réale et al. 2007). Such 'personality traits' are defined as inter-individual differences in behavior that are consistent over time and across contexts (Réale et al. 2007). Understanding personality

traits is important because it informs the more general evolutionary question of why behavioral variation is maintained in the face of natural selection since one would expect the selection of one beneficial trait to the detriment of others (Dall et al. 2004). Variations in personality traits are often correlated at the individual level; these correlations are termed behavioral syndromes (Sih et al. 2004). For example, boldness is often positively correlated with aggression (Riechert and Hedrick 1993; Sih et al. 2004). Understanding these correlations is challenging from both proximate and ultimate perspectives, as personality traits might be expected to be more independent than morphological traits like size and weight (Sih et al. 2004). Therefore, the study of behavioral syndromes is important because it helps to determine why certain personality traits are linked, when flexibility is typically considered adaptive (Dall et al. 2004).

Although temporal consistency in personality traits and behavioral syndromes has been reported in a range of taxa, the effect of social and environmental context on consistency in personality traits has received relatively less attention. Social context may include variation in group size or composition (Webster and Ward 2010; Mainwaring et al. 2011; Pearish et al. 2013), and environmental context may include factors such as differing habitat structure, seasonality or temperature (Dingemanse et al. 2007, 2010; Martin and Réale 2008; Biro et al. 2010). Many studies have shown that the expression of personality traits can change in response to changes in context (van Oers et al. 2005; Martin and Réale 2008; Natarajan et al. 2009; Schuett and Dall 2009; Dingemanse et al. 2010; Shürch and Heg 2010; Webster and Ward 2010; Mainwaring et al. 2011). For example, zebra finches (*Taeniopygia guttata*) are bolder in solitary than group contexts (Mainwaring et al. 2011), while shoaling Eurasian perch (*Perca fluviatilis*) are bolder in group contexts with other bold individuals (Magnhagen and Bunnefeld 2009). In contrast, others have found that environmental context has little effect. Martin and Réale (2008) demonstrated that habitat type had no effect on inter-individual variance in behaviors of chipmunks (*Tamias striatus*), suggesting that the influence of environmental variation may not be universal. This highlights the importance of quantifying social and environmental contexts and how they influence species-specific personality traits because the fitness consequences of behavioral decisions play out under a variety of natural settings.

To better understand the role of variation in social and environmental context, field studies quantifying the consistency of behavioral traits are needed to provide a more complete picture and to address the potential for laboratory artifacts. For example, an examination of behavioral types in the cooperatively breeding cichlid (*Neolamprologus pulcher*) in the wild revealed decoupling of the behavioral syndrome that had previously been observed in the laboratory (Witsenberg et al. 2010). Additionally, comparative approaches are useful for addressing the role of context variation, whereby behavioral consistency of individuals from populations of the same species differing in key ecological variables are quantified and compared (Brown and Braithwaite 2004; Bell 2005; Dingemanse et al. 2007; Burns et al. 2016). Furthermore, while genetic differences may contribute to behavioral differences, comparisons of individuals from different species can also be useful for understanding the roles of social and environmental variation provided the species are closely related. Although interspecific comparisons are currently few (Rehage and Sih 2004; Erikkson et al. 2010), such studies have the potential to act as 'natural experiments' that can test a priori hypotheses regarding behavioral consistency in relation to social and environmental variation (Dall and Griffith 2014). So far, however, when comparative approaches have been employed, the behaviors of individuals have predominantly been scored under laboratory conditions (Bell et al. 2009), limiting the conclusions that can be drawn about the extent of behavioral consistency or plasticity. Finally, a better understanding of the role of social and environmental variation could be achieved by adopting a priori predictions regarding their effects, which could be tested through observation or experiments in natural contexts (Dall and Griffith 2014). This requires some advance knowledge of the ecology of the species in question, making the choice of model species an integral component of behavioral consistency and plasticity studies.

Studies on fishes, particularly freshwater teleost fishes, have played a key role in the development of the field of personality traits and in identifying any influence of social and environmental variation (e.g., Moretz et al. 2007; Biro et al. 2010; Cote et al. 2010; Colléter and Brown 2011; Frost et al. 2013). However, most of these studies have been in laboratory settings where the context and stimuli were highly controlled. Anemonefish (genus *Amphiprion*) are one of the most extensively studied marine fish species (e.g., Allen 1972; Ross 1978; Moyer 1980; Fautin and Allen 1997; Buston 2003; Richardson 1999; Schmiege et al. 2016). Anemonefishes form an obligate symbiosis with habitat-forming sea anemones, and each anemone generally hosts a group of fish consisting of a breeding pair plus smaller non-breeding subordinates (Fautin and Allen 1997; Buston 2003). Much is known about these fishes' behavior, including the social organization and expression of personality traits (Buston 2003; Wong et al. 2013; Schmiege et al. 2016). Laboratory-based work has demonstrated the existence of personality traits and behavioral syndromes in the false clownfish (*Amphiprion ocellaris*) and a link between the personality traits of the

clown anemonefish (*Amphiprion percula*) and the growth of its host anemone (Wong et al. 2013; Schmiege et al. 2016, respectively). Anemonefishes are subject to considerable variation in their social and environmental context, both within populations of a given species (e.g., Moyer 1976) and among species (e.g., Moyer 1980; Richardson 1999). For example, some species tend to occur on isolated and discrete anemones and exhibit low population sizes, limited movement between anemones and strict monogamy (Allen 1972; Buston 2003). Others, particularly in temperate locations, live in hosts that are found in high densities and hence exhibit enhanced movement between anemones and polygynous mating systems (Moyer 1980; Richardson 1999). Social context can also vary greatly within and between species. For example, dominance rank within the group is established based on relative size (termed 'size rank') (Buston 2003; Buston and Cant 2006), and this can vary if individuals move between groups and as individuals ascend in the hierarchy. Therefore, quantification of the behavioral types of anemonefishes in their natural context is needed to expand our understanding not only of anemonefish behavior, but also of the consistency and plasticity of behavioral types in animals more generally.

To address these knowledge gaps, we used a comparative approach to investigate boldness, sociability and aggression in two anemonefishes, *Amphiprion mccullochi* (McCulloch's anemonefish) and *Amphiprion latezonatus* (wide-band anemonefish), in the wild. Specifically, we examined whether: (1) personality was present in both species and whether any behavioral differences were correlated with environmental and social conditions; and (2) variation in personality traits was correlated at the individual level in both species, thus forming a behavioral syndrome. *Amphiprion mccullochi* has a restricted subtropical distribution. It is found at Lord Howe Island (LHI) and Middleton and Elizabeth Reefs, New South Wales (NSW), Australia, where it associates with *Entacmaea quadricolor* (Fautin and Allen 1997; Hobbs et al. 2009) (Fig. 1). *Amphiprion latezonatus* is also endemic to subtropical eastern Australia and is found from Cook Island to South West Rocks, with high densities at North Solitary Island (NSI) (Richardson et al. 1997; Scott et al. 2011; Malcolm and Scott 2017) (Fig. 1). This fish occurs with a broader range of host anemones including *E. quadricolor, Heteractis crispa* and *Stichodactyla gigantea* (Fautin and Allen 1997; Scott et al. 2015).

Based on findings from a closely related anemonefish, *A. ocellaris* (see Wong et al. 2013), we predicted that individuals of both *A. mccullochi* and *A. latezonatus* would display consistent inter-individual differences in their behavior over time (personality traits) and that these behaviors would be correlated with each other (behavioral syndromes). However, because *A. latezonatus* experiences more variable social and environmental conditions at NSI (e.g., contiguous sea anemones allowing increased mobility and social interactions between groups, major swell events and large diel temperature variation), we predicted that there would be reduced repeatability of behavioral differences in *A. latezonatus* relative to *A. mccullochi* at LHI. We also predicted that environmental factors (anemone size and nearest conspecific neighbor distance) and social factors (size rank and group size) would influence the average expression of behavioral traits in both species. Finally, we predicted that we would find a behavioral syndrome incorporating boldness, sociability and aggression for both species, as has been previously reported for another species of anemonefish (Wong et al. 2013), although the magnitude of correlation between behaviors would be less for *A. latezonatus* as the more variable environmental and social conditions may favor greater behavioral de-coupling.

Materials and methods

Amphiprion latezonatus were observed at Elbow Cave and Monterey Mooring, NSI, NSW, Australia (29°55′31″S, 153°23′10″E, depth 13.3–25 m) and *A. mccullochi* were observed at Far Flats, LHI, NSW, Australia (31°31′46″S, 159°04′E, depth 1.5 m) (Fig. 1). Groups were filmed to quantify personality traits and their socio-ecological predictors in March 2014 and April 2015 (austral autumn), respectively. Using SCUBA, nine discrete social groups with two or more fish per group were located at each of the islands. All groups resided in *E. quadricolor* and were marked with dive weights and flagging tape, which were placed ~1–1.5 m away from the anemones.

Groups were filmed for 15 min across three consecutive days using GoPro Hero 3 cameras (Silver Edition, settings: 960 resolution, 60 frames s^{-1}, wide field of view) with BacPacs and housings mounted on weighted stainless steel tripods (height: 50 cm). Each group was filmed at the same time each day between 0900 and 1200 hrs, to maintain consistency and avoid dusk and dawn and the changes in behavior that may occur at these times. The same field of view was achieved daily by placing the tripods directly over the group's weighted marker. Before filming, a 30-cm metal ruler was placed on the substratum in front of each group for scale, so that standardized body size measurements could be estimated from the footage. After the recording started, the diver moved away from the social group and returned at the end of filming to minimize disturbance during this period. On the final day of recording, the length and width of each anemone habitat were measured using a 1-m tape and the distance from the focal

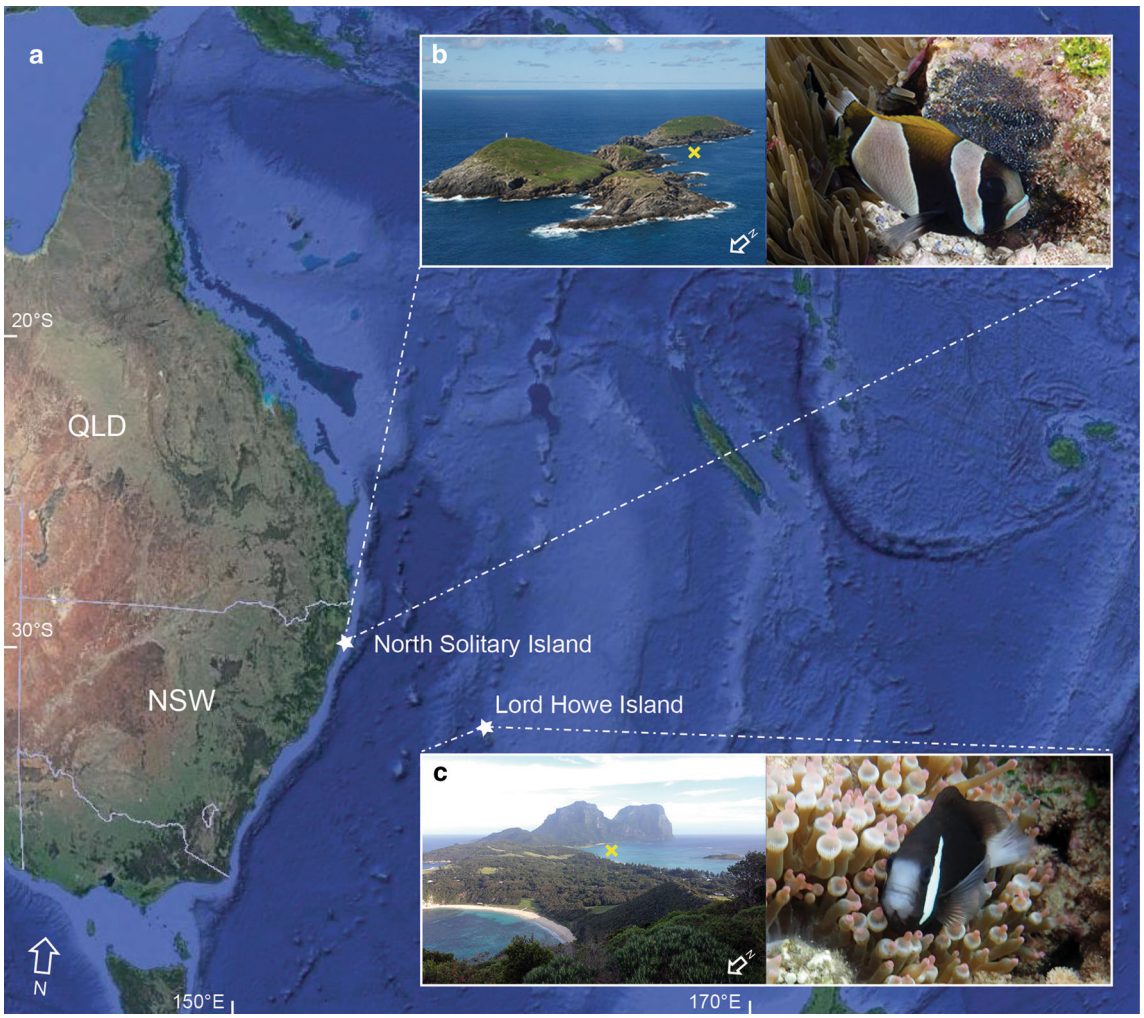

Fig. 1 **a** Study localities; **b** North Solitary Island (1.2 km long, 12.5 km offshore) and *Amphiprion latezonatus* (with eggs laid on substratum); and **c** Lord Howe Island (9.6 km long, 590 km offshore) and *A. mccullochi*. Photo credits: **a** Google Earth; **b** Adam Davey and Ian Shaw; **c** Anna Scott. Crosses indicate the approximate filming sites

group to the nearest conspecific and heterospecific anemonefish neighbor was visually estimated.

Recordings were viewed using VLC media player, and behavioral traits were scored using an ethogram developed by Wong et al. (2013) for *A. ocellaris*. The first 2 min of footage were disregarded to allow the fish to acclimatize to the placement of the camera, and the following 10 min were assessed for targeted behavioral traits. Each individual in a group was scored over the same 10 min of video recording owing to limitations on the time that could be spent taking recordings underwater at depth. Boldness was scored as the time (s) spent out of contact with the anemone. Sociability was measured as the number of non-aggressive acts performed with conspecifics and included meetings, soft touches of fins and submissive body shakes. Aggressive interactions were the number of antagonistic acts with conspecifics, and included biting and chasing. Due to camera placement, some videos required cropping in VLC to clearly visualize behaviors. This also allowed for recognition of individual fish over consecutive days based on their relative size and markings (Buston 2003). Body size was estimated via the video recordings using the ruler as a size standard, and this was used to assign a rank based on relative body size ('size rank'), with the largest individual being designated rank 1, the second largest as rank 2, etc. Whenever individuals could not easily be recognized from the videos, they were excluded from further analyses.

Statistical analysis

To compare behavior between the two species, a general linear model was run using JMP v.11 with species as the main effect, individual and group ID as random effects and time spent in the anemone (s), number of social acts and number of aggressive acts as response variables. Three

separate univariate models were conducted, one per response variable. The number of social and aggressive acts were corrected for the amount of time the fish was 'in sight' in the recording. To normalize the data, time spent outside the anemone (boldness) was reflected before log + 1 transformation, social acts 10 min^{-1} (sociability) was log + 1 transformed and aggressive acts 10 min^{-1} (aggression) was fourth root transformed.

The repeatability of boldness, sociability and aggression was determined across the three days for both species to compare the consistency of behavioral traits between species. Following Nakagawa and Schielzeth (2010), linear mixed effects models and restricted maximum likelihood methods were used to estimate variance components and repeatability. Individual ID was used as a random factor, with size rank (ordinal factor, ranks 1–6), group size (ordinal factor, 2–7 individuals per group), anemone size (continuous factor, 54–2340 cm^2) and nearest-neighbor distance (continuous factor, 1–7 m) as fixed effects. Sex was not accounted for as it could not be definitively confirmed. Factors were progressively removed from the models using backward stepwise elimination if they were not significant ($p > 0.05$).

When repeatable behaviors were found, we defined possible personality trait dimensions (i.e., behavioral syndromes) using principal components analyses (PCA). PCA was conducted on the three variables (boldness, sociability and aggression) for *A. mccullochi* only (as there was no evidence for consistencies in any of the three behaviors for *A. latezonatus*). The correlation matrix for *A. mccullochi* indicated that sociability was weakly correlated with boldness and aggression ($r < 0.3$) and therefore sociability was eliminated from further analysis. The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy indicated that sampling was acceptable (KMO = 0.5) and KMO terms for individual terms were at the acceptable limit of 0.5.

Results

A total of 57 *A. mccullochi* from nine natural social groups and 75 *A. latezonatus* from the same number of groups were recorded. The median group size for *A. mccullochi* was two (range 2–4) and four for *A. latezonatus* (range 2–7).

Overall levels of boldness, aggression and sociability

On average, *A. mccullochi* and *A. latezonatus* spent 525.2 ± 17.2 s (SE) (87.5%) and 505.5 ± 11.3 s (84.3%) of time outside their anemones, respectively; the difference in boldness was not statistically significant ($F_{1,16} = 2.51$, $p = 0.13$). The number of aggressive acts by *A. mccullochi* (1.7 ± 0.42 acts 10 min^{-1}) and *A. latezonatus* (2.9 ± 0.48 acts 10 min^{-1}) were also not significantly different between species ($F_{1,16} = 1.45$, $p = 0.25$), including after group size correction ($F_{1,14} = 0.11$, $p = 0.73$). *Amphiprion mccullochi* was less social (9.9 ± 1.71 acts 10 min^{-1}) than *A. latezonatus* (18.9 ± 1.94 acts 10 min^{-1}) ($F_{1,17} = 6.85$, $p = 0.02$), but this difference was not apparent after correcting for group size ($F_{1,15} = 0.03$, $p = 0.86$).

Repeatability of behavior

For both species, the behavioral variance attributed to intra-individual variation (Residual) was always significant (95% confidence intervals did not overlap with zero; electronic supplementary material, ESM, Tables S1 and S2). On the contrary, behavioral variance attributed to inter-individual variation (Individual ID) was not significant with the exception of boldness and aggression for *A. mccullochi* (ESM Tables S1 and S2). Concomitantly, repeatability estimates for *A. mccullochi* showed high-adjusted repeatability for boldness and aggression (Table 1; boldness: $F_{16,38} = 2.47$, $p < 0.01$; aggression: $F_{18,38} = 4.42$, $p < 0.001$) but not for sociability (Table 1; $F_{8,38} = 0.84$, $p = 0.58$). *Amphiprion latezonatus* showed low-adjusted repeatability for boldness, sociability and aggression (Table 1; boldness: $F_{21,49} = 1.02$, $p = 0.46$; sociability: $F_{21,49} = 0.65$, $p = 0.86$; aggression: $F_{16,49} = 0.91$, $p = 0.56$).

Influence of environmental and social factors

Amphiprion mccullochi was bolder in smaller groups ($F_{2,16} = 3.55$, $p = 0.05$). Specifically, individuals in groups of four spent less time outside the anemone than individuals in groups of two (Fig. 2a). Individuals were more sociable in larger groups ($F_{2,8} = 6.28$, $p = 0.02$), and individuals in groups of three and four were significantly more sociable than in pairs (Fig. 2b). Sociability declined among lower-ranked individuals ($F_{3,8} = 25.4$, $p < 0.001$), with individuals of size ranks 3 and 4

Table 1 Repeatability scores (after backward stepwise elimination of non-significant factors) for three behaviors (boldness, sociability and aggression) for *Amphiprion mccullochi* and *Amphiprion latezonatus*

Species	Boldness	Sociability	Aggression
Amphiprion mccullochi	32.8*	0.0	53.3*
Amphiprion latezonatus	0.87	0.0	0.0

* Indicates repeatability score is significantly different from zero ($p < 0.05$)

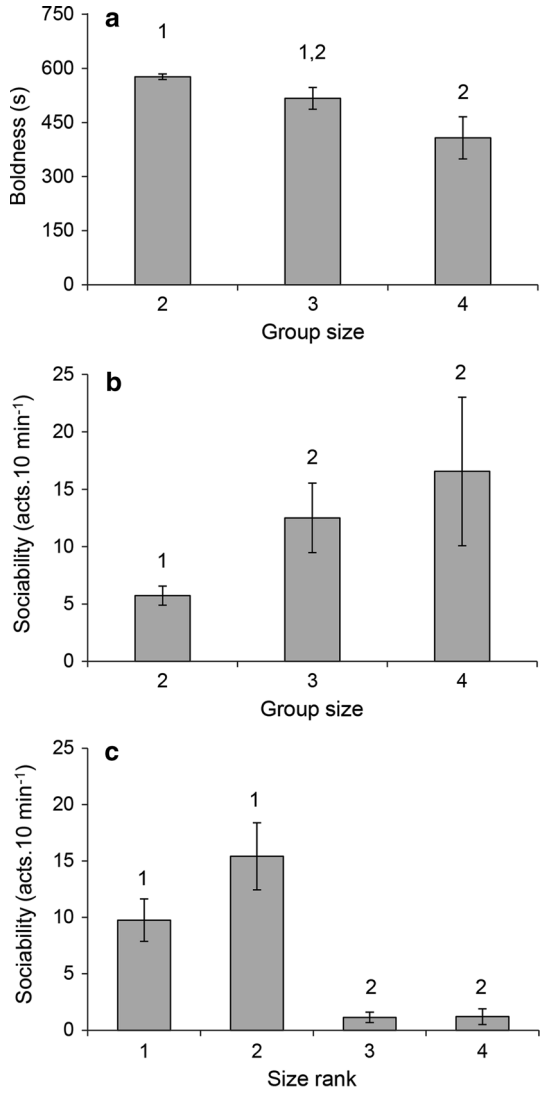

Fig. 2 Significant relationships between social and environmental factors on **a** boldness (average time spent outside the anemone 10 min^{-1}), **b**, **c** sociability (average number of non-aggressive acts performed with conspecifics 10 min^{-1}) for *Amphiprion mccullochi*. Values are corrected for amount of time in sight, error bars are standard errors, numbers above bars correspond to significant differences ($p < 0.05$)

significantly less sociable than ranks 1 and 2 (Fig. 2c). Fish were less sociable as the distance to their nearest neighbor increased ($F_{1,8} = 8.32$, $p = 0.02$). In contrast, aggression of *A. mccullochi* was unrelated to any social or environmental factor (Table 2; ESM Table S3).

Boldness and sociability of *A. latezonatus* were significantly related to group size (boldness: $F_{4,20} = 6.43$, $p = 0.002$; sociability: $F_{4,20} = 17.3$, $p < 0.001$), although no consistent patterns emerged (Fig. 3a, b). Aggression was significantly related to size rank and group size (size rank: $F_{4,15} = 15.2$, $p = 0.001$; group size: $F_{5,15} = 15.8$ $p = 0.008$), also with no consistent patterns (Fig. 3c, d; Table 2; ESM Table S4).

Table 2 Component loadings of each of the two included variables (boldness and aggression) and total variance explained by the first principal component (eigenvalue = 1.42) for *Amphiprion mccullochi*

Behavior (*Amphiprion mccullochi*)	Principal component PC1 (boldness-aggression)
Boldness	−0.811
Aggression	0.814
Total variance (%)	71.1

Sociability was omitted owing to weak correlation with the other variables

Behavioral syndrome analysis

For *A. mccullochi*, one factor had an eigenvalue greater than Kaiser's criterion of 1 and explained 71.1% of the total variance, and therefore, this component was retained and the two other components eliminated (Table 2). The variables that clustered on the same principal component were boldness and aggression.

Discussion

Determining the extent and factors that influence the repeatability of behavioral differences among animals is critical to understanding individual fitness and population processes, and thereby aids species conservation (Wolf and Weissing 2012). In anemonefishes, a previous study investigating the repeatability of behavioral differences in *A. ocellaris* showed high repeatability in all behaviors measured under a laboratory setting (Wong et al. 2013), contributing to the growing consensus that consistent individual differences in behavior are the norm in a wide range of taxa (Bell et al. 2009). Our comparative field study shows that some anemonefish lack personality. For both species, there was behavioral consistency at the intra-individual level. For *A. latezonatus* however, there was low inter-individual variation in behavior which resulted in no significant repeatability for any of the behaviors scored. In contrast, *A. mccullochi* also showed inter-individual differences for boldness and aggression, as well as a behavioral syndrome between these two behaviors. This study has, therefore, addressed the relatively unexplored area of in situ comparative analysis of personality traits and raises the following intriguing questions: (1) why are certain behaviors of species more repeatable than others? (2) why are there differences between laboratory and field estimates of repeatability? and (3) what social and environmental factors influence variation in behaviors?

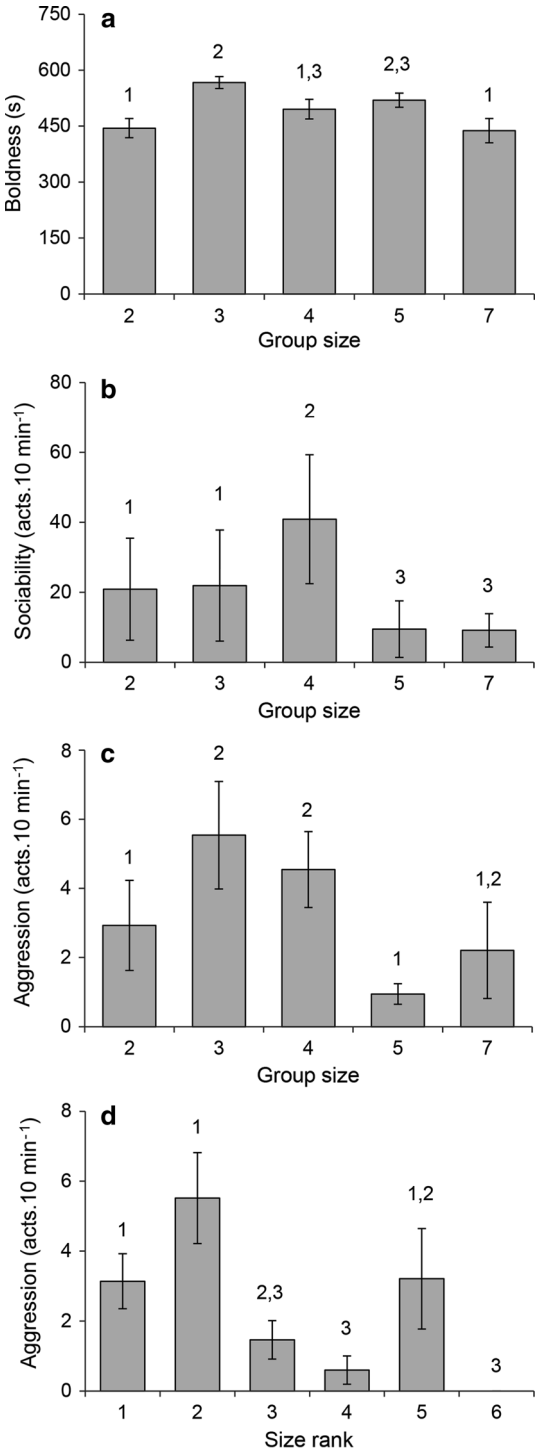

Fig. 3 Significant relationships between social and environmental factors on **a** boldness (average time spent outside the anemone 10 min^{-1}), **b** sociability (average number of non-aggressive acts performed with conspecifics 10 min^{-1}) and **c**, **d** aggression (average number of the number of antagonistic acts with conspecifics 10 min^{-1}) for *Amphiprion latezonatus*. Values are corrected for amount of time in sight, error bars are standard errors, numbers above bars correspond to significant differences ($p < 0.05$)

An understanding of the selective advantages of behavioral consistency or behavioral plasticity is needed to explain why interspecific differences in repeatability exist. The lack of inter-individual behavioral variation in *A. latezonatus* (and hence low behavioral repeatability) may be related to the more variable environment at NSI and hence, the more variable social conditions experienced by *A. latezonatus*. Specifically, *A. latezonatus* are found on more exposed sites at NSI and are subject to large swell and storm events that cause fluctuations in density (Richardson et al. 1997), whereas *A. mccullochi* mainly inhabit sheltered lagoonal habitats at LHI. While both islands are subject to considerable seasonal temperature variation relative to tropical climes and have similar annual temperature ranges (average ~17.5–26 °C), diel fluctuations at LHI are less extreme than those at NSI (maximum daily variation 2.8 vs. 4.2 °C, respectively) (Australian Institute of Marine Science Data Centre, www.aims.gov.au/docs/data/data.html). Therefore, we suggest that the environmental fluctuations at NSI could erode inter-individual variation in behavior, as all individuals would benefit from responding in a similar way to the harsher environmental conditions to survive and reproduce effectively. Conversely, the more stable environment inhabited by *A. mccullochi* could allow for the expression and maintenance of greater inter-individual behavioral variation. Clearly, this explanation would require experimental manipulations, including possible translocations, to provide conclusive validation of this hypothesis. In any case, variation in the extent of inter-individual behavioral differences between taxa are not uncommon, with variation in repeatability occurring among species (Bell et al. 2009), among populations of the same species (Archard and Braithwaite 2011), among individuals within a population (Thomson et al. 2012) and among ontogenetic stages (Polverino et al. 2016a). Furthermore, the causes of variation in the repeatability of behavioral differences relate to factors such as temperature (Biro et al. 2010), size of captive enclosures (Polverino et al. 2016b) and microhabitat (Pearish et al. 2013), highlighting the importance of comparing the behavior of species inhabiting different environments when determining repeatability.

Additionally, variation in social stimuli within the environment could explain the differences in behavioral repeatability between the two species to some extent. The extraordinarily high density and semi-contiguous distribution of host anemones at NSI (Richardson et al. 1997; Richardson 1999; Scott et al. 2011) is likely to increase the likelihood that individual *A. latezonatus* interact with conspecifics from outside their immediate social group, disrupt social hierarchies and potentially displace individuals from other groups, as seen in another subtropical anemonefish species (*A. clarkii*; Moyer 1980). Indeed,

reduced stability of hierarchies has been shown to reduce the repeatability of some personality traits (such as exploration and activity) and disrupt behavioral syndromes (Rudin et al. 2016). Thus, the enhanced mobility of *A. latezonatus* could have reduced within-group hierarchy stability, eroding the consistency of inter-individual behavioral differences. Therefore, we hypothesize that relative to tropical anemonefishes, which reside in very stable social groups (Allen 1972; Ross 1978; Buston 2003), the clustering anemones generally inhabited by subtropical anemonefishes may have pre-disposed these species to reduced consistency in inter-individual behavioral differences. Indeed, both subtropical species spent most of their time outside their anemone habitat, opposite to the general pattern found in the laboratory for the tropical species, *A. ocellaris* (Wong et al. 2013). Comparison of the repeatability of behavioral differences between tropical and subtropical anemonefishes under natural ecological settings is therefore needed to provide a more definitive understanding of interspecific variation in personality traits in these fishes.

Intuitively, we expected repeatability estimates to be higher for fish under laboratory conditions than in the field, owing to the relatively stable conditions. The repeatability of behavior of the congener *A. ocellaris* in laboratory conditions (Wong et al. 2013) was higher than or similar to repeatability in field conditions (sociability of *A. ocellaris* was 50.2 vs. 0% for *A. latezonatus* and *A. mccullochi*; boldness of *A. ocellaris* was 30.4% relative to 0.9% for *A. latezonatus* and 32.8% for *A. mccullochi*). Given that different species were assessed, it is hard to make inferences about the effect of laboratory versus field environments. However, a previous field study on the freshwater cichlid *Neolamprologus pulcher* (Witsenberg et al. 2010) demonstrated reduced repeatability and altered behavioral syndromes relative to a laboratory study on the same species (Bergmuller and Taborsky 2007). The within-context behavioral variability of *N. pulcher* in the field was attributed to their experience of daily variation in ecological conditions (Witsenberg et al. 2010), lending support to the suggestion that specific behaviors are sensitive to the stability of the prevailing environment and, more generally, that behavioral plasticity is tightly linked with environmental stability. In contrast, a meta-analysis incorporating results from a range of taxa found that behavior was more repeatable under field conditions, a result that was attributed to an increase in the number of micro-niches in realistic ecological settings. These micro-niches were thought to promote greater between-individual behavioral variation (as opposed to greater intra-individual consistency) and hence the repeatability of behavioral differences among animals (Bell et al. 2009). However, this meta-analysis did not compare laboratory and field study estimates of repeatability within a given species, and therefore, predictions could be further refined if more research was done on species responses to these different settings (but see Laskowski et al. 2016).

Certain social and environmental factors are thought to cause greater variability in behaviors than others (Bell et al. 2009). In this study, the behaviors of both species were more sensitive to variation in social than environmental factors. The two social factors, group size and size rank explained significant variation in behavior for both species. However, the directionality of the significant effect of group size and size rank differed between the species. For *A. mccullochi*, the trend was very clear, with individuals being bolder and more sociable in larger groups, and more sociable if they were more dominant within the group. One potential reason for enhanced sociability of bolder individuals could be that the increased time spent outside the anemone enabled them to interact with more conspecifics from outside their current group. For *A. latezonatus*, there was no obvious directional trend between group size and size rank on behavior. The two environmental factors measured, nearest-neighbor distance and anemone size, largely did not explain variation in behaviors of the two species, except for a decrease in sociability of *A. mccullochi* with increasing distance to their nearest neighbor. It is perhaps not surprising that behaviors of both species were less responsive to environmental variation, given that sea anemones are typically not as limiting as they are for tropical anemonefishes (Buston 2003), and may therefore potentially decouple any associations between behavior and habitat availability.

In this study, there was a negative relationship between boldness and aggression in *A. mccullochi*. The directionality of this correlation differed from expected, that is, bolder individuals are usually also more aggressive (Huntingford 1976; Garamszegi et al. 2009; Colléter and Brown 2011). Although positive relationships between boldness and aggression have been reported in many species, other studies have revealed that there can also be no or a negative relationship between these traits (Bell 2005; Dingemanse et al. 2007). While the 'constraint hypothesis' posits that boldness and aggression should be positively related, the 'adaptive hypothesis' predicts either no or a variety of possible correlations (Bell 2005). Hence, an explanation for our finding is that the negative correlation represents an adaptive response to the social and environmental conditions faced by *A. mccullochi*. For instance, it may be beneficial for anemonefish that are bold (and hence spending more time at risk of predation outside the anemone) to engage in less conspecific aggression while outside their anemone (which may detract from predator avoidance or vigilance). In any case, elucidating the selective pressures favoring a negative correlation is

clearly beyond the scope of the current study, but certainly represents a promising area of future investigation in anemonefishes.

Overall, this study has shown interesting and unexpected results when repeatable inter-individual differences in behavior were compared between two taxonomically similar yet ecologically different subtropical anemonefish species. We suggest that differences in repeatability between the species reflect contrasting environmental and social conditions, and similarly, that differences in repeatability in certain behaviors likely reflect a less rigid social structure owing to more contiguous anemone distribution than for tropical anemonefishes. Even so, alternative explanations should not be ruled out given the correlative nature of the study. For example, since smaller and presumably younger individuals lived with the larger breeding pairs in *A. latezonatus* groups (average group size of *A. latezonatus* was larger than for *A. mccullochi*), and given that age is related to behavioral repeatability (Polverino et al. 2016a), the lower average age of fish in *A. latezonatus* groups could account for some variation in behavior. Another alternative is that life-history or physiological differences between the two species, which have been shown to influence the expression of personality traits (Careau et al. 2008; Biro and Stamps 2008), could underlie these differences; future work would be invaluable for assessing these options. However, it is also possible that environmental and social variation could have influenced the evolution and potential divergence of life-history and physiological traits, which in turn could influence behavior (Tremmel and Müller 2013). Regardless of whether the link between environmental and social variation and behavior is direct or indirect, our findings are important as the presence or absence of repeatability of behavioral differences in a species may affect its ecology and evolution, as well as persistence in the face of environmental change. Traits such as boldness, for example, are likely to influence mortality risks and fecundity (Biro and Stamps 2008), which in turn may influence interactions with mutualistic anemones (Schmiege et al. 2016) or population growth rates and persistence (Wolf and Weissing 2012). Thus, the profound differences in behavioral consistency in these two endemic subtropical Australian anemonefishes could lead to a disparity in their resilience to environmental or social changes. Therefore, further in situ comparative investigations of the repeatability of anemonefish behavior would help to elucidate the selective advantages of behavioral consistency versus variability in relation to social and environmental variables. Additionally, experimental manipulations and phylogenetically controlled comparisons of personality traits among multiple anemonefish species would enable a better understanding of their potential responses to variations in their natural environment.

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References

Allen GR (1972) Anemonefishes: their classification and biology. TFH Publications Inc, Neptune City, NJ, USA, p 288

Archard GA, Braithwaite VA (2011) Variation in aggressive behaviour in the poeciliid fish *Brachyrhaphis episcopi*: population and sex differences. Behav Processes 86:52–57

Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. Anim Behav 77:771–783

Bell AM (2005) Differences between individuals and populations of three-spined stickleback. J Evol Biol 8:464–473

Bergmüller R, Taborsky M (2007) Adaptive behavioural syndromes due to strategic niche specialization. BMC Ecol 7:12–13

Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity? Trends Ecol Evol 23:361–368

Biro PA, Beckmann C, Stamps JA (2010) Small within-day increases in temperature affect boldness and alters personality in coral reef fish. Proc R Soc Lond B Biol Sci 277:71–77

Brown C, Braithwaite VA (2004) Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. Anim Behav 68:1325–1329

Burns JG, Price AC, Thomson JD, Hughes KA, Rodd FH (2016) Environmental and genetic effects on exploratory behavior of high- and low-predation guppies (*Poecilia reticulata*). Behav Ecol Sociobiol 70:1187–1196

Buston PM (2003) Social hierarchies: size and growth modification in clownfish. Nature 424:145–146

Buston PM, Cant MA (2006) A new perspective on size hierarchies in nature: patterns, causes, and consequences. Oecologia 149:362–372

Careau V, Thomas M, Humphries M, Reale D (2008) Energy metabolism and animal personality. Oikos 117:641–653

Colléter M, Brown C (2011) Personality traits predict hierarchy rank in male rainbowfish social groups. Anim Behav 81:1231–1237

Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A (2010) Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). Proc R Soc Lond B Biol Sci 277:1571–1579

Dall SRX, Griffith SC (2014) An empiricist guide to animal personality variation in ecology and evolution. Front Ecol Evol 2:3

Dall SRX, Houston AI, McNamara JM (2004) The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecol Lett 7:734–739

Dingemanse NJ, Kazem AJN, Réale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol 25:81–89

Dingemanse NJ, Wright J, Kazem AJN, Thomas DK, Hickling R, Dawnay N (2007) Behavioural syndromes differ predictably

between 12 populations of three-spined stickleback. J Anim Ecol 76:1128–1138
Erikkson AC, Booth DJ, Biro PA (2010) 'Personality' in two species of temperate damselfish. Mar Ecol Prog Ser 420:273–276
Fautin DG, Allen GR (1997) Anemonefishes and their host sea anemones. Western Australian Museum, Perth, Western Australia
Frost AJ, Thomson JS, Smith C, Sneddon LU (2013) Environmental change alters personality in the rainbow trout, *Oncorhynchus mykiss*. Anim Behav 85:1199–1207
Garamszegi LZ, Eens M, Török J (2009) Behavioural syndromes and trappability in free-living collared flycatchers, *Ficedula albicollis*. Anim Behav 77:803–812
Gosling SD, John OP (1999) Personality dimensions in nonhuman animals: a cross-species review. Curr Dir Psychol Sci 8:69–75
Hobbs J-PA, Neilson J, Gilligan JJ (2009) Distribution, abundance, habitat association and extinction risk of marine fishes endemic to the Lord Howe Island region. Report to Lord Howe Island Marine Park, Lord Howe Island, Australia
Huntingford FA (1976) The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Anim Behav 24:245–260
Laskowski KL, Monk CT, Polverino G, Alós J, Nakayama S, Staaks G, Mehner T, Arlinghaus R (2016) Behaviour in a standardized assay, but not metabolic or growth rate, predicts behavioural variation in an adult aquatic top predator *Esox lucius* in the wild. J Fish Biol 88:1544–1563
Magnhagen C, Bunnefeld N (2009) Express your personality or go along with the group: what determines the behaviour of shoaling perch? Proc R Soc Lond B Biol Sci 276:3369–3375
Mainwaring MC, Beal JL, Hartley IR (2011) Zebra finches are bolder in an asocial, rather than social, context. Behav Processes 87:171–175
Malcolm HA, Scott A (2017) Range extensions in anemonefishes and host sea anemones in eastern Australia: potential constraints to tropicalisation. Mar Freshw Res 68:1224–1232
Martin JGA, Réale D (2008) Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*. Anim Behav 75:309–318
Moretz JA, Martins EP, Robison BD (2007) Behavioral syndromes and the evolution of correlated behavior in zebrafish. Behav Ecol 18:556–562
Moyer JT (1976) Geographical variation and social dominance in Japanese populations of the anemonefish *Amphiprion clarkii*. Japanese Journal of Ichthyology 23:12–22
Moyer JT (1980) Influence of temperate waters on the behavior of the tropical anemonefish *Amphiprion clarkii* at Miyake-jima, Japan. Bull Mar Sci 30:261–272
Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev 85:935–956
Natarajan D, de Vries H, Saaltink D-J, de Boer SF, Koolhaas JM (2009) Delineation of violence from functional aggression in mice: an ethological approach. Behav Genet 39:73–90
Pearish S, Hostert L, Bell AM (2013) Behavioral type–environment correlations in the field: a study of three-spined stickleback. Behav Ecol Sociobiol 67:765–774
Polverino G, Cigliano C, Nakayama S, Mehner T (2016a) Emergence and development of personality over the ontogeny of fish in absence of environmental stress factors. Behav Ecol Sociobiol 70:2027–2037
Polverino G, Ruberto T, Staaks G, Mehner T (2016b) Tank size alters mean behaviours and individual rank orders in personality traits of fish depending on their life stage. Anim Behav 115:127–135
Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution. Biol Rev Camb Philos Soc 82:291–318
Rehage JS, Sih A (2004) Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. Biol Invasions 6:379–391
Riechert SE, Hedrick AV (1993) A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae, Agelenidae). Anim Behav 46:669–675
Richardson DL (1999) Correlates of environmental variables with patterns in the distribution and abundance of two anemonefishes (Pomacentridae: *Amphiprion*) on an eastern Australian sub-tropical reef system. Environ Biol Fishes 55:255–263
Richardson DL, Harriott VJ, Harrison PL (1997) Distribution and abundance of giant sea anemones (Actiniaria) in subtropical eastern Australian waters. Mar Freshw Res 48:59–66
Ross RM (1978) Reproductive behavior of the anemonefish *Amphiprion melanopus* on Guam. Copeia 1978:103–107
Rudin FS, Tomkins JL, Simmons LW (2016) Changes in dominance status erode personality and behavioral syndromes. Behav Ecol 28:270–279
Schmiege PF, D'Aloia CC, Buston PM (2016) Anemonefish personalities influence the strength of mutualistic interactions with host sea anemones. Mar Biol 164:24
Schuett W, Dall SR (2009) Sex differences, social context and personality in zebra finches, *Taeniopygia guttata*. Anim Behav 77:1041–1050
Schürch R, Heg D (2010) Life history and behavioral type in the highly social cichlid *Neolamprologus pulcher*. Behav Ecol 21:588–598
Scott A, Malcolm HA, Damiano C, Richardson DL (2011) Long-term increases in abundance of anemonefish and their host sea anemones in an Australian marine protected area. Mar Freshw Res 62:187–196
Scott A, Rushworth KJW, Dalton SJ, Smith SDA (2015) Subtropical anemonefish *Amphiprion latezonatus* recorded in two additional host sea anemone species. Mar Biodivers 46:327–328
Sih A, Bell AM, Johnson JC, Ziemba RE (2004) Behavioral syndromes: an integrative overview. Q Rev Biol 79:241–277
Thomson JS, Watts PC, Pottinger TG, Sneddon LU (2012) Plasticity of boldness in rainbow trout, *Oncorhynchus mykiss*: do hunger and predation influence risk-taking behaviour? Horm Behav 61:750–757
Tremmel M, Müller C (2013) Insect personality depends on environmental conditions. Behav Ecol 24:386–392
van Oers K, Klunder M, Drent PJ (2005) Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. Behav Ecol 16:716–723
Webster MM, Ward AJ (2010) Personality and social context. Biol Rev Camb Philos Soc 86:759–773
Witsenburg F, Schürch R, Otti O, Heg D (2010) Behavioural types and ecological effects in a natural population of the cooperative cichlid *Neolamprologus pulcher*. Anim Behav 80:757–767
Wolf M, Weissing FJ (2012) Animal personalities: consequences for ecology and evolution. Trends Ecol Evol 27:452–461
Wong MYL, Medina A, Uppaluri C, Arnold S, Seymour JR, Buston PM (2013) Consistent behavioural traits and behavioural syndromes in pairs of the false clown anemonefish *Amphiprion ocellaris*. J Fish Biol 83:207–213