Variation in risk-taking and aggression in morphotypes of the beadlet anemone, *Actinia equina* (L.), and the green anemone, *Actinia prasina* (Gosse)

J.R. Collins[a], Emily L. Vernon[b], & Jack S. Thomson[a],*

[a] School of Environmental Sciences, University of Liverpool, L69 3GP, UK
[b] School of Biomedical and Biological Sciences, University of Plymouth, PL4 8AA, UK,

ABSTRACT

Anemones exhibit distinct between-individual differences in behaviours such as risk-taking and aggressiveness. The genus of anemone *Actinia* contains numerous morphotypes which occupy different locations on the shore and show different levels of aggressiveness. In this study we explored whether their propensity to take risks likewise differed between three groups of anemones: *Actinia prasina*; a low shore morph of *Actinia equina* characterised by a blue limbus around the pedal disc (described as ring-present, RP); and upper shore morphs of *A. equina* characterised by a lack of such a ring (ring-absent, RA). Risk-taking behaviour was measured using startle-response durations across two trials, separated by two weeks. In-between trials anemones were allowed to engage in pairwise contests with a randomly-selected opponent, during which the number of occurrences of behaviours related to movement of the body, tentacles and acrorhagi were counted and compared between groups to identify systematic variation in potentially offensive and defensive postures. Length of holotrich nematocysts, thought to be linked to aggressiveness, was also measured. Both risk-taking and inflation/deflation of acrorhagi significantly differed between groups: RA anemones were risk-prone and aggressive, *RP* anemones risk-averse and non-aggressive, *A. prasina* occupying a position in-between. Likewise nematocyst lengths varied between groups, shortest among RP anemones and longest in RA anemones. These data suggest that different morphotypes are associated with differences in risk-taking which may result from a complex interplay of various ecological factors linked to the habitats these morphotypes occupy, and add further credence to theories on speciation within the *Actinia equina* species complex.

1. Introduction

The extent to which individual animals take risks can have implications for their fitness that depend on internal and external factors (Sih et al., 2004). Risk-prone animals are likely to forage regardless of danger whereas risk-averse individuals favour safety above obtaining food (Wilson et al., 1993). This represents a trade-off since time spent avoiding dangers – such as predation, novelty, or periods of poor environmental quality leading to stress or harm – cannot be spent foraging, and successful strategies would limit risk-averse behaviours when not necessary. The relative success of risk-prone and risk–averse strategies largely depends on the environmental pressures to which the population is exposed; for example, risk-prone animals tend to excel where predation risk is low (e.g. Bell, 2005). Risk-taking behaviour is often strongly correlated with intraspecific aggression where risk-prone individuals are also likely to be more aggressive (Rudin and Briffa, 2012; Sih et al., 2004; although c.f. Bell, 2005). The link between (intra-specific) aggression and risk-taking (which, in the context of anti-

predator strategies, is considered inter-specific aggression; Huntingford, 1976) is at least partially driven by internal factors such as hormone or gene expression (Koolhaas et al., 1999). Multiple behaviours linked in such a manner are termed behavioural syndromes, particularly when correlated across contexts (Sih et al., 2004) and, though the evolutionary reasons for the development of behavioural syndromes is still unclear, functionally adaptive relationships are often observed. For instance, the link between risk-taking and aggression may allow for maintaining possession of a resource when under challenge or predation threat (Taylor and Lattanzio, 2016). The syndrome may also represent underlying genetic mechanisms which limit the extent to which these behaviours can evolve independently of the other (Sih et al., 2004).

Variation in behaviour – especially when linked in such a manner – has profound implications for fitness dependent upon the environment to which organisms are exposed. Behavioural plasticity allows individuals to modulate their behaviour (including risk-taking and aggressiveness) in accordance with internal state as well as epigenetic and

environmental factors (Dall et al., 2004; Frost et al., 2007). However, the extent to which animals can modulate their behaviour is limited (Briffa and Greenaway, 2011; Frost et al., 2007), especially when linked as a behavioural syndrome (Sih et al., 2004; Bell, 2005; Adriaenssens and Johnsson, 2012). Behavioural consistency (or consistent individual differences in behaviour) may be explained by limitations on plasticity or from selection favouring combinations of behaviour adaptive to a particular environment (the constraint and adaptive hypotheses, respectively; Bell, 2005). The net result, however, is that the behavioural profile of a population should be the most appropriate for the challenges present in the habitat (Adriaenssens and Johnsson, 2012; Hensley et al., 2012) and is likely to differ between discrete populations based putatively on differences in environmental challenges and the behavioural responses available (e.g. Bell, 2005; Dingemanse et al., 2007; Endler, 1995). Understanding these behaviours, how they're linked, and the mechanisms driving them may inform theories on how these behaviours evolve, either independently or together. Numerous studies compare populations from isolated patches but, even within a single community, behaviour may vary across an environmental gradient (Hensley et al., 2012) and this information can elucidate the relationship between behaviours within a syndrome.

One such gradient occurs across the rocky shore, which is naturally exposed to temporal and spatial variations in submergence with knock-on effects to the ecology of littoral organisms (such as on feeding regimes, desiccation and predation risk). Community structure on the shore is considered to be driven by an interaction of biotic and abiotic challenges: upper limits of species are defined by their physiological tolerance to emersion whilst lower limits are defined by their ability to compete for space, avoid predators and obtain sufficient food (Southward, 1958). Differences in competitiveness and responses to risk may, therefore, directly influence the distributions of animals and the potential for individuals of a particular behavioural type to aggregate (Hensley et al., 2012; Wolf and Weissing, 2012). Animals without the requisite physiology or behavioural repertoire for a set of environmental conditions are likely to move or are otherwise unlikely to survive within that habitat (Huey, 1991). On rocky shores, in particular, this has resulted in the formation of discrete zones containing particular assemblages of organisms most suited to (and best capable of competing for) that shore height. However, the distribution of particular species across these vertical gradients is likely to reflect intraspecific behavioural and physiological variation (Hensley et al., 2012). Risk-taking animals trade off safety for greater opportunities to utilise available resources, which may manifest as a more rapid recovery from a disturbance (Réale et al., 2010). At the top of the shore, where submersion time is already limited, risk-taking behaviour may be a more appropriate strategy to minimise time lost after a disturbance; in contrast, on the lower shore where organisms are expected to spend the majority of their time submerged and predators may be more abundant, time lost to protection is less important. Aggressiveness, however, is more likely to increase towards the lower shore where competition for space is a significant component of community structure (Southward, 1958). Potentially, where the distribution of animals is enforced through behavioural and physiological limitations relative to their environment, reproductive isolation may occur between individuals with one likely outcome being sympatric speciation (Hensley et al., 2012).

Though morphologically simple, cnidarians are capable of exhibiting behaviours which consistently differ between individuals i.e. are not random responses to environmental challenges (e.g. Ayre and Grosberg, 1995; Briffa and Greenaway, 2011). In *Actinia* a common and cosmopolitan intertidal anemone, interclonal contests are commonplace and involve individuals fighting off unrelated anemones using specialised tentacles, acrorhagi, containing high densities of nematocysts (Turner et al., 2003; Williams, 1991). During contests acrorhagi are inflated and brought down on an opponent in a process called overtopping; in the most aggressive interactions acrorhagial tissue, termed a peel, is left behind on the opponent (Turner et al., 2003). The

purpose of aggression appears linked to territoriality, as anemones do not attack clonemates (Turner et al., 2003); instead, aggression appears most prevalent in high density aggregations, often arising as individuals migrate to suitable microhabitats during colder seasons (Brace and Quicke, 1986; Brace and Reynolds, 1989). Individual *Actinia* also appear to consistently differ in their response to perceived predation threat; termed startled responses, feeding tentacles are withdrawn when disturbed and only re-extended after a certain period of time (Briffa and Greenaway, 2011).

The species *Actinia equina* is known for its numerous morphotypes, varying in colour and pattern of the column wall and in the colour of the pedal disc (Quicke and Brace, 1984; Watts and Thorpe, 1998). Some of these morphotypes have been reclassified as new species based on genetic evidence, such as the strawberry anemone *Actinia fragacea* (Carter and Thorpe, 1981). More recently a green-coloured morphotype was putatively described as heterospecific and described as the green anemone *Actinia prasina* (Solé-Cava and Thorpe, 1987). However, among the remaining morphotypes of *A. equina* there remains debate over whether the morphotypes constitute multiple variants of a single species or numerous separate species (Watts et al., 2000; Perrin et al., 1999) and to what extent they are capable of interbreeding (Perrin et al., 1999). Three morphotypes have been previously described and are found in discrete zones on the intertidal: the Low (L) morph, Mid (M) morph and Upper (U) morph, each named after the zone on the shore in which they are usually found. U and M morphs are indistinguishable by gross morphology and only reliably separated through genetic analysis (Perrin, 1993). Whilst these morphs are often identified by colour this may not always be appropriate since colour is likely assimilated from the diet and therefore environmentally determined (Watts et al., 2000); however, lower shore varieties have a blue ring around the limbus of the pedal disc, which is absent in higher shore varieties. The genetic relationship between these morphotypes is, furthermore, unclear; sexual reproduction among *A. equina* appears limited with most offspring produced asexually, and young brooded within the enteron are most frequently of the same morphotype (Perrin et al., 1999). Thus, though these morphotypes overlap in distribution (with the magnitude of overlap defined largely by shore topography) the degree of gene flow between the morphs is not known. Distributional differences may be a result of individual differences in behaviour, with those morphotypes positioned higher on the shore tending to exhibit high levels of aggression (Brace and Reynolds, 1989). In contrast, low shore morphs tend to show very little aggression, often not even attempting to attack their opponent (Brace et al., 1979; Brace and Reynolds, 1989). *A. equina* are able to adjust their behaviour dependent on their environment (e.g. Brace & Quicke, 1986) but the extent to which their distribution relies on evolved responses to their environment rather than phenotypic plasticity are unclear (Briffa and Greenaway, 2011). Furthermore, even after being held under laboratory conditions for up to 6 months behavioural differences in aggressiveness are maintained (Brace et al., 1979). Lengths of holotrich nematocysts within the acrorhagi also differ between morphs (Watts et al., 2000), and may be important in determining contest winners and losers (Rudin and Briffa, 2011). Further understanding of the distribution and behaviour of these morphotypes has been cited as key in understanding both their ecology and their relatedness (Perrin et al., 1999).

Whilst variation in aggressiveness across morphotypes is well characterised, as yet no studies have attempted to determine whether this behavioural response to an environmental challenge likewise varies between morphotype/with shore height. Linking discrete behavioural types to the different morphotypes may help explain why the morphs aggregate at particular shore heights, and may provide evidence that explains speciation among the *Actinia* group based on habitat-derived reproductive isolation (Ingley and Johnson, 2014). Furthermore, evidence for the presence of a risk taking-aggressiveness behavioural syndrome has been found within this species overall (Rudin and Briffa, 2012) but how this relationship varies with ecological factors