Several parameters that influence body size in the sea anemone *Actinia equina* in rock pools on the Yorkshire coast

Bryony Carling[1*], Louise K. Gentle[1] Nicholas D. Ray[1]

[a] *School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Southwell, UK*

* *Corresponding author, e-mail:* bryonycarling@hotmail.co.uk

Despite being classed as an asocial species, aggregations of sea anemones can be common in abundant species. UK populations of the geographically common aggressive intertidal sea anemone Actinia equina, form clustered aggregations notwithstanding a violent nature towards neighbours and relatives. Smaller in body size, and more abundant than those found in warmer climates, little research has been undertaken to discover what factors affect body size. This study investigates whether aggregation, distance to neighbour, submergence at low tide or pH in rock pools affect body size of A. equina in their natural habitat. Populations were investigated at five sites on the Yorkshire coast during August and September 2016. A total of 562 anemones were recorded revealing that solitary anemones were significantly larger than those found in clustered aggregations. In addition, anemones found submerged in rock pools at low tide were significantly larger than those found on emergent rock, and smaller anemones were found in significantly higher pH conditions (8.5+) than larger anemones. Anemones submerged at low tide are constantly able to feed and not subject to harsh conditions such as wind exposure and temperature, hence they can achieve larger sizes. Consequently, the size of the anemones may reflect a trade-off between the benefits of aggregating in exposed environments and the costs of competition for a reduced food resource.

Keywords: intraspecific competition, distribution, aggregation, trade-off

INTRODUCTION

Species such as anemones, that are susceptible to desiccation and dislodgement, often aggregate for protection. However, this creates competitive living environments where individuals contend for food and space (Hanski & Ranta, 1983; Firth *et al.* 2014). For example, the British intertidal sea anemone *Metridium senile* lives in close-knit groups of the same genetic clones, and shares captured food within its community. Nevertheless, it will only engage in aggressive intraspecific competition with genetically different clones (Purcell & Kitting, 1982; Wood, 2005). Similarly, the sea anemone *Anemonia viridis* can be found in clusters, yet will only engage in intraspecific competition for space after rapid reproduction occurs, with younger anemones outcompeting older ones (Chintiroglou & Koukouras, 1992). Aggressive competition involves acrorhagi (fighting with nematocyst-armed tentacles that are separate to feeding tentacles) in sea anemones is considered to be more related to intraspecific competition for space than the usual roles of prey capture or predator defence in other aquatic organisms (Francis, 1973; Bartosz *et al.* 2008).

Body size of most organisms is usually significantly linked with the ability to win aggressive encounters (Brace *et al.* 1979; Just & Morris, 2003). Indeed, body size in sea anemones is directly linked to habitat quality (Sebens, 1982; Werner & Gilliam, 1984; Wolcott & Gaylord, 2002), whereby habitats with more environmental stress and less prey contain smaller sea anemones (Sebens, 1982; Wolcott & Gaylord, 2002). This suggests that although a large body size is beneficial in competitive environments, size is limited by food acquisition, where anemones in aggregations essentially have to ‘share’ the food source (Sebens, 1987). Moreover, as intraspecific fighting only occurs in certain situations, a small body size in clustered anemones may be a necessary trade-off compared to food acquisition (Sebens, 1982).

In addition, the pH of the surrounding environment affects intracellular pH (pH_i) which is crucial for controlling metabolic functions in sea anemones (Venn *et al.* 2009; Gibbin & Davy, 2014). Therefore, during aggressive encounters, damage afflicted on an opponent can be more or less severe, depending on the pH of the surrounding environment. For example, the haemolytic activity of *Actinia equina* reaches its optimum at 8.8 pH (Maček & Lebez, 1981). However, the relationship between pH and body size in British populations warrants further investigation.

The sea anemone *A. equina* is an ecologically important invertebrate, due to its high abundance, extensive range and great resilience (Haylor *et al.* 1984). Found in the intertidal zone, it inhabits a large geographical range including the Atlantic, Mediterranean sea, Japan and South Africa (Haylor *et al.* 1984; Chomsky *et al.* 2009; Gadelha *et al.* 2012). Abundance, colouration, reproductive strategies and distribution of this species have been known to vary geographically which has caused years of taxonomic debate as to whether geographical separation has resulted in different species, making it a focus for studies on different populations globally (Chomsky *et al.* 2009). Despite the many studies that have been conducted on this species, there has been little research into the relationship between body size and distribution. The UK *A. equina* populations have a broad basal diameter, up to 50mm, that enables the anemone to attach itself to substrate, and reproduce asexually, ejecting numerous polyps onto nearby substrata (Wood, 2005; Chomsky *et al.* 2009; Briffa & Greenaway, 2011). Due to the small size, high abundance and reproductive methods of *A. equina,* intertidal habitats such as rock pools contain clustered aggregations (Chomsky *et al.* 2009), creating intraspecific competition. This differs to populations of *A. equina* in the Mediterranean which reproduce sexually, grow larger and are less abundant (Chomsky *et al.* 2009), yet little research has been conducted on factors affecting anemone size.

The aims of this non-invasive study are to establish whether aggregation, distance to closest neighbour, submergence and pH have a significant effect on body size in *A. equina* in its natural habitat. Considering the species' behaviour and ecology, it is predicted that solitary anemones will be larger than those in clustered aggregations as they do not have to share food, submerged anemones will be larger than emergent anemones as they have greater access to food, and rock pools of higher pH will contain larger anemones as they are capable of inflicting more damage in a contest.

MATERIALS AND METHODS

Study sites

Five sites were chosen at random along the Yorkshire coast, picked from destinations that were known to have *A. equina* populations. Sites investigated were Runswick Bay (NZ 81209 16250), Robin Hoods Bay (NZ 95453 04776), Scalby Ness Rocks (TA 03793 90953), Old Quay Rocks (TA 13148 81424) and South Landing (TA 22926 69113). All sites were open to access by the public.

Methodology

Transects were conducted at all sites between August and September, 2016, to determine the number and size of *A. equina* at each location. Five transects were carried out at each of the five different sites, totalling twenty five transects across the sites. Each transect was repeated three times over the course of five weeks as anemones are partially sessile and conditions such as strong wind could severely affect visibility in submerged rock pools.

Transects were 10m in length and 2m wide, perpendicular to the sea, and conducted during low tide. During each transect, *A. equina* were searched for on top of, beneath and in the crevices of, the rockpools. When an anemone was found within the transect, the basal diameter of the individual was measured to the nearest mm, using a Mitutoyo 530 312 Vernier calliper. Distance between anemones was measured in cm using a measuring tape. The solitary/clustered status of the anemone was also recorded: an anemone was considered to be clustered if it was less than 5cm away from its nearest neighbour and solitary if not. Information on whether anemones were either exposed (on emergent rock) or submerged (in a rock pool) at low tide was also collected. Anemones were defined as submerged if completely covered or more than half of the body was submerged and tentacles were showing. Tentacle status (displayed or not displayed) was also recorded. The pH of the water surrounding the submerged anemones was measured using a Hanna HI-98130 pH meter at the same time of day for each replication.

Statistical analyses

The effect of both aggregation status (solitary or clustered) and shore placement (submerged or emergent at low tide) on the size of anemone was investigated using a Poisson regression model with aggregation status and shore placement as categorical predictors of size. Interactions between the terms were also included in the model. The effect of pH on the size of submerged anemones only was assessed by producing a further Poisson regression model, with pH as a continuous predictor of size. The effect of distance to nearest neighbour on the size of anemone was assessed by producing a final Poisson regression model, with nearest neighbour distance as a continuous predictor of size.

To determine under what circumstances tentacles were more likely to be displayed, a binary logistic regression was undertaken using pH, size of anemone and distance to nearest neighbour as continuous predictors, and aggregation status (clustered or solitary) as a categorical predictor. Interactions between the terms were also included in the model. Insignificant terms and interactions were removed via a stepwise backwards elimination. All data were analysed using Minitab version 17.3.1.

RESULTS

A total of 562 anemones were measured across all sites, comprising 210 (37%) clustered and 352 (63%) solitary anemones. Exactly half of the anemones were found submerged in rock pools at low tide. Of all anemones found on emergent rock, 41% were clustered, the remaining 59% solitary. Whereas those submerged in rock pools, only 33% were clustered, the remaining 67% solitary.

Findings from the first Poisson regression model revealed that solitary anemones were significantly larger than clustered anemones ($\chi^2_{1,559}$=9.14, P<0.003) and anemones found submerged in rock pools at low tide were significantly larger than those found on emergent rock ($\chi^2_{1,559}$=8.25, P=0.003) (Figure 1). There was no significant interaction between the terms.

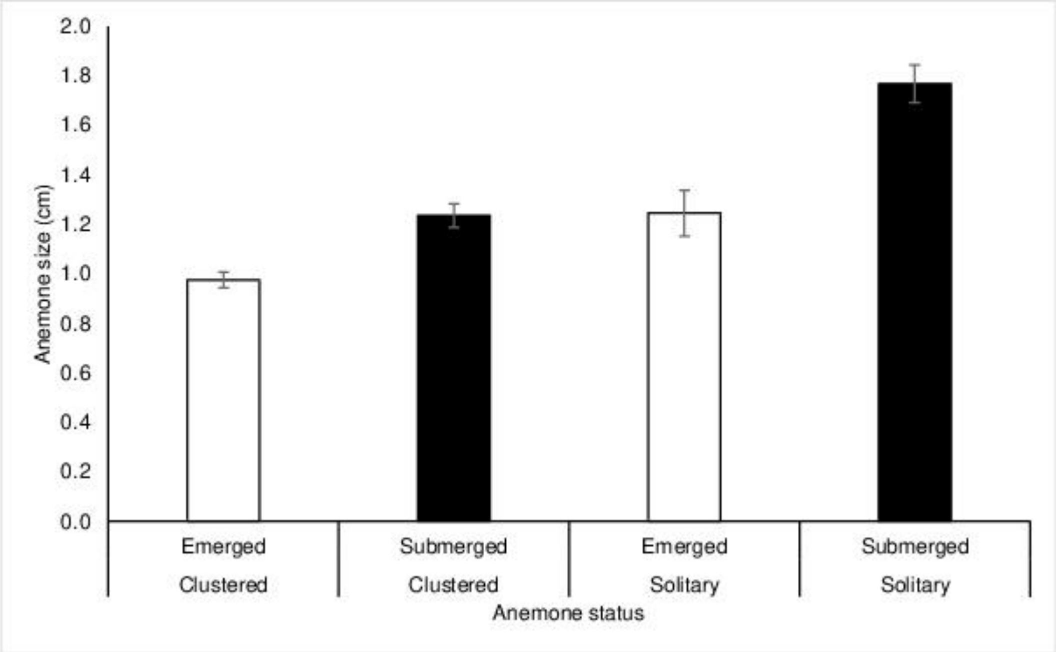

Fig. 1. Mean (±1 SE) anemone size in relation to aggregation status (clustered or solitary) and shore placement (emerged or submerged).

There was a significant positive effect of distance to nearest neighbour on anemone size ($X^2_{1,539}$=12.85, P<0.001; Figure 2) where an increase in distance to nearest neighbour showed an increase in size. In addition, there was a significant negative effect of pH on anemone size ($X^2_{1,231}$=8.41, P=0.004; Figure 3) where an increase in pH showed a decrease in size. pH ranged from 7.35-9.46.

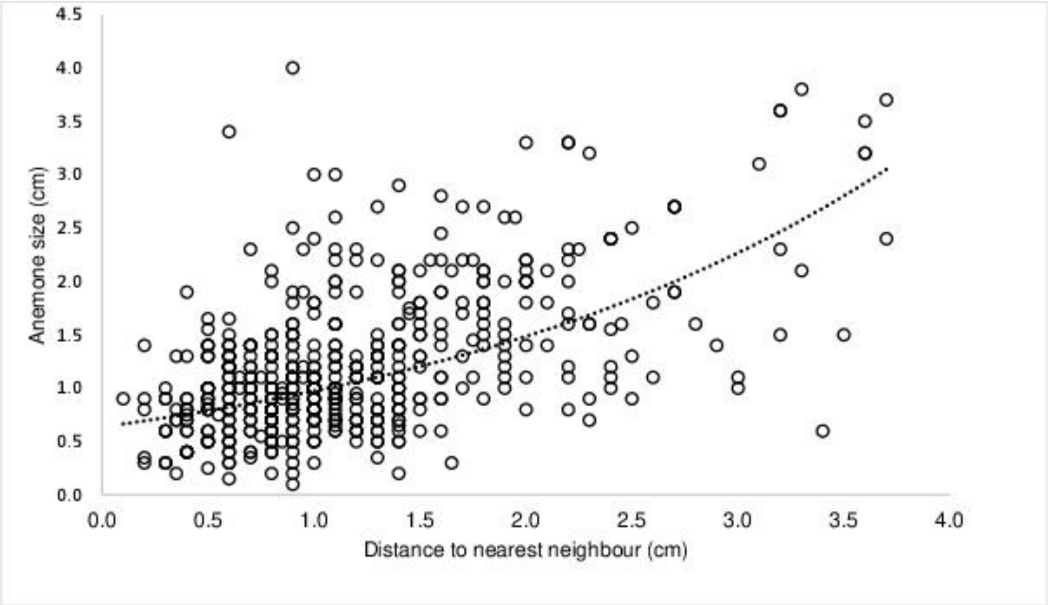

Fig. 2. The effect of distance to nearest neighbour on size of *A. equina* ($X^2_{1,539}$=12.85, P<0.001). Dotted line represents fitted trend line.

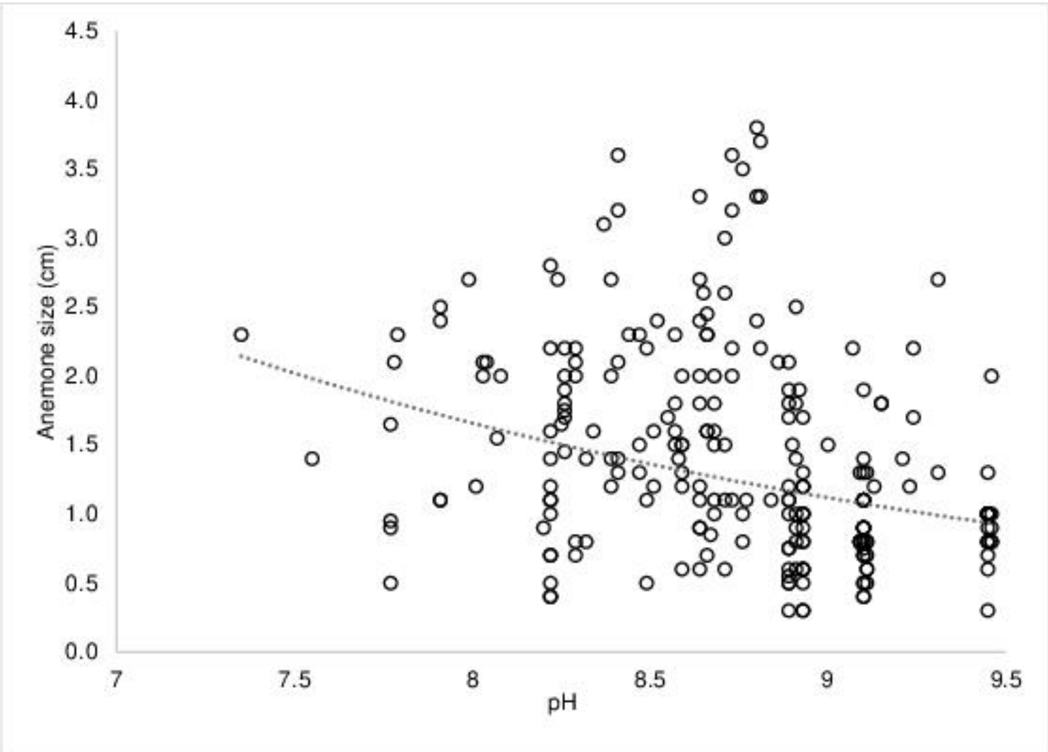

Fig. 3. The effect of pH on size of *A. equina* ($X^2_{1,231}$=8.41, P=0.004). Optimum pH for effectiveness of toxin=8.8 (Maček & Lebez, 1981). Dotted line represents fitted trend

Findings from the binary regression on the likelihood of displaying tentacles revealed that there was no significant effect of aggregation status or distance to nearest neighbour, and no significant interactions between any of the terms. However, there was a significant positive effect of size ($X^2_{1,223}$=6.40, p=0.011) and a significant negative effect of pH ($X^2_{1,223}$=9.55, p=0.002), whereby larger anemones situated in pools with a lower pH were more likely to show tentacles (Figure 4).

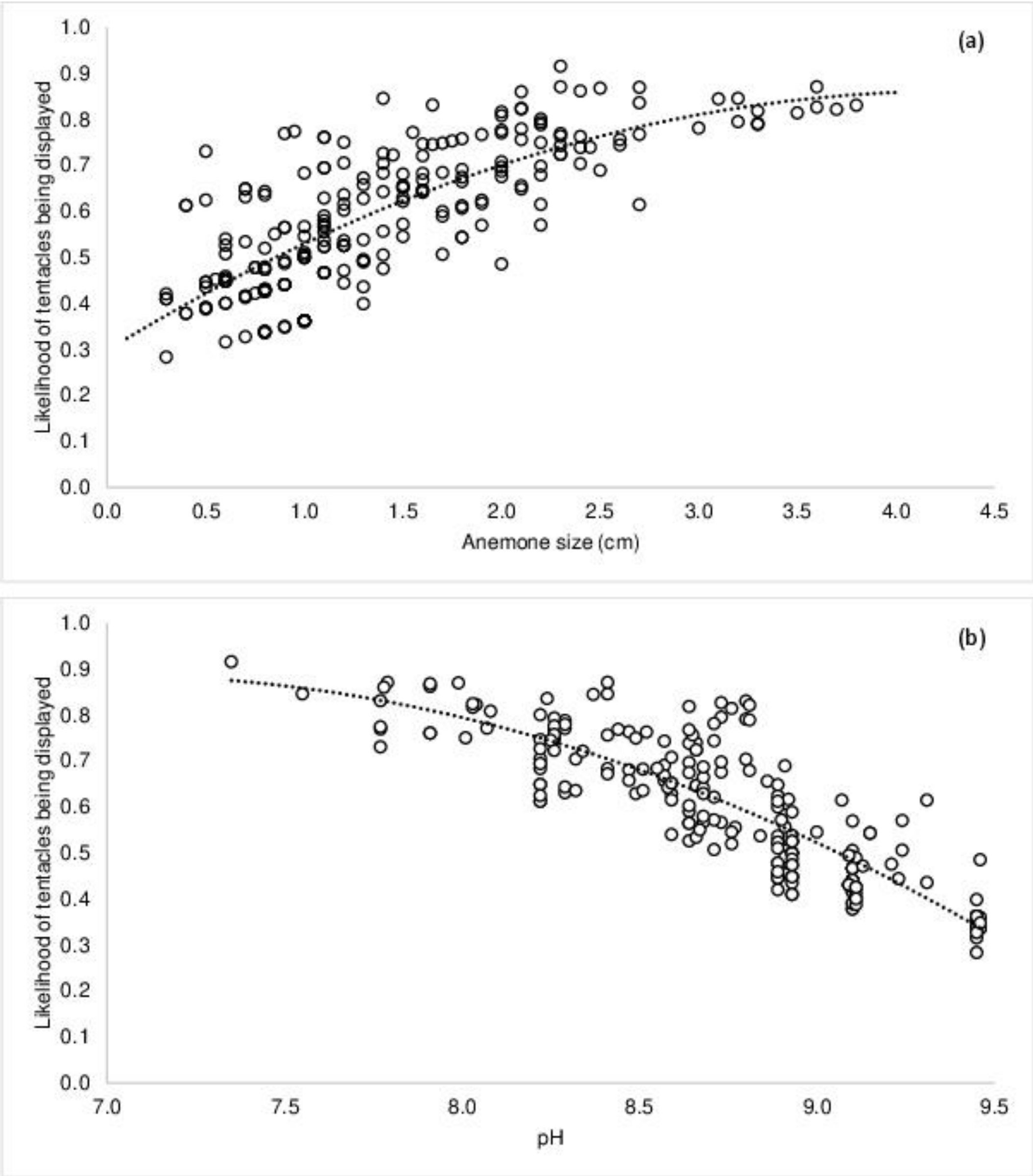

Fig. 4. The effect of (a) anemone size and (b) pH on the likelihood of tentacles being displayed in *A. equina*, (a: $X^2_{1,223}$=6.40, p=0.011; b: $X^2_{1,223}$=9.55, p=0.002). Dotted lines represent fitted trend lines.

DISCUSSION

The close proximity of British *A. equina,* due to its asexual reproductive methods of ejecting young polyps onto nearby substrata (Orr *et al.* 1982; Brace & Quicke, 1986), was apparent throughout this study as 37% of all anemones measured were found in clustered aggregations. Findings showed that there was a significant difference in aggregation status in relation to body size (*Figure 1*), where solitary anemones were around 0.6cm larger than clustered anemones. This finding is consistent with that of Chomsky *et al.* (2009) who note that in populations where density is higher, anemones tend to be smaller. Findings also showed that larger anemones were more distanced from their closest neighbour (*Figure 2*). Indeed, the association between size and aggregation may potentially be due to intraspecific competition for resources and space as *A. equina* is an aggressive species that often participates in contests for dominance against other anemones and its relatives (Bartosz *et al.* 2008; Foster & Briffa, 2014). For example, Rudin and Briffa (2011) discovered that body size was the primary determinant of assessing whether to engage in an aggressive encounter, with larger weapon size of nematocyst as the determining factor of the victor once engaged in fighting. Larger anemones are therefore less likely to be challenged or engaged in an encounter, hence one explanation for why larger anemones were significantly more solitary in this study. Consequently, larger anemones are able to access more and larger food sources (Brace *et al.* 1979; Robinson *et al.* 2009; Rudin & Briffa, 2011). However, the exact relationship between anemone size and aggregation needs further investigation as it is unclear whether large anemones are solitary because they have increased fighting ability, or whether solitary anemones are large because they have better access to food, or a combination of the two.

Submerged habitats were found to contain significantly larger anemones than those in emergent habitats (*Figure 1*). Again, this finding can be explained partly in terms of food resources as submerged habitats, in the form of rock pools, are home to a larger diversity of prey organisms, such as the mussel *Mytilus edulis*, crustaceans and fish eggs, allowing the anemone to feed frequently and attain a large body size (Goss-Custard *et al.* 1979; Shick, 1991; Davenport *et al.* 2011). This supports the findings that larger submerged anemones were significantly more likely to show their tentacles than smaller anemones (*Figure 4a*). Conversely, emergent habitats are associated with lower food resources, as anemones will not feed for risk of desiccation (Sebens, 1987; Chomsky *et al.* 2004). In addition, emergent habitats are subjected to harsh conditions such as increased temperature, wind exposure and potential dislodgement via tidal movement (Navarro & Ortega, 1984; Shick, 1991; Tomanek & Helmuth, 2002) that many intertidal species cannot tolerate at low tide (Sebens, 1982; Wolcott & Gaylord, 2002). For example, anemones have been found to shrink in higher temperatures as they are unable to balance energy input and metabolic requirements

(Chomsky *et al.* 2004). Consequently, the size of the anemones may reflect a trade-off between the benefits of aggregating in exposed environments and the costs of competition for a reduced food resource. Alternatively, *A. equina* may feed in a similar manner to *Metridium senile*, where smaller anemones feed in areas of high velocity, in contrast to larger anemones that feed more efficiently in slower flow conditions (Shick 1991; Anthony, 1997). This may be directly linked to the size of the anemone's tenticular surface area, as larger prey may be more common in rock pools in contrast to smaller nutrients that are found in high velocity areas (Sebens, 1981; Anthony, 1997). Nevertheless, this indicates that shore placement can be an important factor determining body size as smaller anemones found on emergent rock would experience the high velocity of the incoming tide, whereas the larger anemones that were found submerged in rock pools would remain somewhat sheltered.

A significant negative regression showed that smaller anemones were more likely to be found in rock pools of a higher pH than larger anemones (*Figure 3*), despite initial predictions that larger anemones would be found in areas of higher pH due to its probable greater fighting ability (Rudin & Briffa, 2011). When attacking another organism with its stinging nematocysts, haemolytic activity from the toxin in *A. equina* has an optimum pH of 8.8 (Maček & Lebez, 1981). Smaller anemones that were found in a higher pH content may prefer these habitats as they are found in large aggregations and may have to engage in aggressive encounters for territory more frequently than larger anemones. As these pools have a higher pH, their attacks will be more damaging due to the haemolytic activity having a more alkaline optimum pH (Maček & Lebez, 1981). However, although smaller anemones were found in rock pools with a higher pH, there was a significant negative effect of pH on the likelihood of displaying tentacles (*Figure 4b*). This suggests that despite close proximity in an environment where fighting could cause more damage, anemones were not displaying aggressive behaviour. Alternatively, higher pH can be linked to shallower water with less wave exposure (Middelboe & Hansen, 2007) providing a reduced area for obtaining prey items and a consequent small size in anemone. Therefore, smaller *A. equina* may reside in environments with a higher pH as a trade-off between a lower habitat quality but a reduced frequency of aggressive encounters and interspecific competition for resources. Many factors modify pH in rock pools, such as the rates respiration and photosynthesis (Newcomb *et al*. 2011), therefore smaller body size in submerged pools may also be determined by competition with other organisms such as neighbouring invertebrates and algae. Further investigation into rock pool pH should incorporate depth of pool, measure potential food availability and establish which organisms reside within the pool to determine how habitat quality differs between pools containing larger anemones and why.

CONCLUSION

The findings of this preliminary study show aggregation status, neighbour distance, shore placement and pH all influence size in *A. equina*. Larger anemones adopted a solitary lifestyle on rocks that stayed permanently submerged in water of a low pH. These habitats appear to contain more favourable conditions such as greater access to food. Conversely, smaller anemones resided in harsher conditions, perhaps trading-off the advantages of size for less interspecific competition.

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