Variation in partner benefits in an anemone shrimp symbiosis

C. Seabird McKeon, James L O'Donnell

Symbiotic interactions, where two species occur in close physical proximity for the majority of the participants' lifespans, may constrain the fitness of one or both of the participants. Host choice could result in lineage divergence in symbionts if fitness benefits vary across the interaction with hosts. Symbiotic interactions are common in the marine environment, particularly in the most diverse marine ecosystems: coral reefs. However, the variation in symbiotic interactions that may drive diversification is poorly understood in marine systems. We measured the fecundity of the symbiotic shrimp *Periclimenes yucatanicus* on two anemone hosts on coral reefs in Panama, and found that while fecundity varies among host species, this variation is explained largely by host size, not species. This suggests that shrimp on larger hosts may have higher fitness regardless of host species, which in turn could drive selection for host choice, a proposed driver of diversification in this group.

Variation in partner benefits in an anemone shrimp symbiosis

Abstract

Symbiotic interactions, where two species occur in close physical proximity for the majority of the participants' lifespans, may constrain the fitness of one or both of the participants. Host choice could result in lineage divergence in symbionts if fitness benefits vary across the interaction with hosts. Symbiotic interactions are common in the marine environment, particularly in the most diverse marine ecosystems: coral reefs. However, the variation in symbiotic interactions that may drive diversification is poorly understood in marine systems. We measured the fecundity of the symbiotic shrimp *Periclimenes yucatanicus* on two anemone hosts on coral reefs in Panama, and found that while fecundity varies among host species, this variation is explained largely by host size, not species. This suggests that shrimp on larger hosts may have higher fitness regardless of host species, which in turn could drive selection for host choice, a proposed driver of diversification in this group.

C. Seabird McKeon
Smithsonian Marine Station- National Museum of Natural History, Ft. Pierce, FL, USA

Jimmy O'Donnell
School of Marine and Environmental Affairs, University of Washington, Seattle, WA, USA

Introduction

Variation in partner benefits has been proposed as an engine of diversification in symbiotic systems (Schemske & Horvitz 1984; Thompson 1994) and can have important effects on the survival of associated organisms (McKeon & Moore 2014). The Palaemonid shrimps of the subfamily Potoniinae are one of the most diverse groups of reef animals (Bauer 2004). Pontoniines are well known for their many and diverse symbiotic associations, on both Indo-Pacific and Caribbean reef systems (Bruce 1976). Many *Periclimenes* and related genera can be found as commensals on hosts as varied as file clams, sponges, and echinoderms as well as cnidarian hosts (Li 1993). Several species are found in association with anemones and corals. The tremendous diversity of the pontoniines, and frequent symbiotic interactions with other taxa has spurred interest in the role of symbioses in diversification of the subfamily. Molecular studies have identified potential evolutionary groups and pathways to symbiosis with different marine organisms (Kou et al. 2015), but little work has been done on the ecological forces that might be at the root of such divergence.

The ecological interactions between Pontoniine shrimps and their hosts may be complex, incorporating feeding strategy, nitrogen transfer, as well as defense and secondary mutualisms, such as cleaning behaviors (Limbaugh et al. 1961; Spotte 1996). Among the best studied of the Pontoniines, *Periclimenes yucatanicus* (Ives, 1891), the ‘Spotted Anemone Shrimp’ (Figure 1), is a common exosymbiont of anemones in shallow marine habitats from the southern United States to the Caribbean coast of South America (reviewed in Spotte et al. (1991)). Notable differences in coloration and other morphological features exist across this taxon’s range (Spotte et al. 1991; Wicksten 1995). *P. yucatanicus,* as with several Caribbean Pontoniines, maintains host associations with many species of anemones and corallimorphs across a broad range of sizes and morphologies. Interactions of Anemone shrimps with this broad range of hosts has been examined in the context of rapid environmental change (Silbiger & Childress 2008) and host choice behavior (Mascaro et al. 2012). However, little work has been done to investigate the question of differential fecundity and fitness resulting from host variation (Ross 1983), and the implications of this on diversification in symbiotic lineages. This study investigated differences in size and fecundity of *P. yucatanicus* on two different anemone host species: the Sun Anemone *Stichodactyla helianthus* (Ellis, 1767), and the Corkscrew Anemone *Bartholomea annulata* (Lesueur, 1817).

Methods

We conducted our study in protected waters close to the Smithsonian Tropical Research Institution field station, Bocas del Toro, Panama, where *P. yucatanicus* are abundant, readily visible at close range, and easily captured. They are often found in groups on a single anemone host, with larger individuals bearing eggs externally, leading to the belief that the species is sequentially hermaphroditic. While *P. yucatanicus* can be found on a wide variety of anemone and anemone-like hosts in the Caribbean, we focused on their interactions with the two most common host species in the area: *Stichodactyla helianthus* and *Bartholomea annulata*. The two anemone host species are of different architecture: *Bartholomea* has a small oral disc with long, thin tentacles, while *Stichodactyla* has a very broad oral disc with short stubby tentacles.

Shrimp and anemones were surveyed and/or collected from shallow reefs (<2m) using snorkel equipment within a mixed seagrass/patch reef/mangrove habitat matrix. The tentacular spread of the anemone was carefully measured avoiding the disturbance and retraction of the anemone. The longest distance between the tips of two tentacles based at opposite sites of the oral disc was measured for both taxa of anemone as a proxy of anemone size. Other measures of anemone size (disc diameter, water displacement) were unsuccessful. All *P. yucatanicus* on an individual anemone were counted prior to disturbance, collected using a dip net or slurp gun, and returned to the lab in individual plastic bags. Shrimp were never removed from the water.

Each shrimp was measured for carapace length as a proxy of body size, and examined for the presence or absence of eggs on the pereopods. Eggs were staged by the presence of visible eyespots on the embryos within the eggs (Azofeifa-Solano et al. 2014). Older eggs with visible eyespots were not included in the analysis. Eggs were irrigated off of the pereopods of gravid shrimp using a syringe, and counted using a dissecting microscope and a hand counter. The day after collection, shrimp were returned alive to the site from which they were gathered. Sampling continued until 30 or more egg-bearing individuals were collected from each host species.

We tested for the effect of host anemone species on fecundity of *P. yucatanicus* while controlling for the effect of shrimp body size using an analysis of covariance (ANCOVA). All statistics were calculated in the statistical programing environment R (R Core Team 2014).

Results

A total of 32 gravid shrimp with eggs of appropriate size were gathered from *S. helianthus*, 34 were collected from *B. annulata*. There was a difference in the number of eggs carried by shrimp coming from the two species of anemone (*B. annulata* m = 98.97, sd = 42.35; *S. helianthus* m = 145.03, sd = 68.98; Welch two sample t (50.88) = -3.245, p = 0.002; Figure 2). As expected of decapod crustaceans (Corey & Reid 1991), there was a positive linear relationship between the number of eggs carried by an individual shrimp and its carapace length (F (1,64) = 81.71, $p < 0.0001$, $R^2 = 0.5608$, Figure 3). Anemones of the species *S. helianthus* (m = 16.03 cm, sd = 4.30) were larger than those of *B. annulata* (m = 13.56 cm, sd = 5.02; Welch two sample t (63.46) = 2.15, p = 0.035), and shrimp were larger on *S. helianthus* (m = 8.34, sd = 1.10) than on *B. annulata* (m = 7.19, sd = 0.862; Welch's two sample t (58.66) = 4.71, $p < 0.0001$), but we found no effect of anemone species on shrimp size when accounting for anemone size (*F (1,62)* = 0.0001, p = 0.993). Finally, we incorporated these variables into an ANCOVA model to understand the effect of anemone species, shrimp size, and their interaction effects on shrimp egg number. There was no effect of the interaction between anemone species and shrimp size on egg number (*F(1,62)* = 0.22, p = 0.64), indicating that the host anemone species had no effect on the slope of the relationship between shrimp size and egg size. A reduced, additive linear model found no effect of anemone species on egg number (t = -0.023, p = 0.982), a significant effect of shrimp size (t = 7.725, $p < 0.0001$), resulting in an overall positive effect of shrimp size on egg fecundity, independent of host anemone species (*F(2,63)* = 40.22, $p < 0.0001$, $R^2 = 0.547$). Interaction between anemone size and species suggest that the pattern observed with *P. yucatanicus* is driven largely by host size, with larger sized hosts capable of supporting larger and more fecund shrimp.

Discussion

Considerable theoretical and empirical research has focused on tradeoffs and correlations among critical components of reproductive effort, such as age and size at first reproduction, schedule of reproduction, egg size and clutch size (Stearns 1992). Such focus has rarely been applied to symbiotic taxa, and even less frequently to marine symbiotic taxa. In many taxa, there is a positive correlation between clutch size or ovarian egg number and body length (Corey & Reid 1991; Salthe 1969), as seen in *P.yucatanicus*. The differences in clutch size seen in shrimp on the two host species represent a facet of life history theory of possible evolutionary importance.

As discussed by Mascaro et al. (2012), previous efforts to examine host choice in Pontoniine shrimps (Guo et al. 1996; Silbiger & Childress 2008; Spotte et al. 1991) have yielded complex results demonstrating across and within taxon variation, while Stanton (1977) noted that anemones with larger fleshy tentacles were more likely to host commensal crustaceans. The work of Silbiger & Childress (2008) demonstrated that *P. yucatanicus* in Florida strongly preferred a third species of anemone, *Condylactis gigantea*, over the two species presented in this work. It is unknown if this pattern is also true in Panama.

The work presented here suggests host preference may be determined by host size, via its effect on fecundity in *P. yucatanicus*. Shrimps of this species are capable of attaining larger sizes and greater fecundity in larger host anemones. Increased fecundity of *P. yucatanicus* on the larger *Stichodactyla* hosts may imply a fitness benefit to host selection. The third species of anemone surveyed by Silbiger and Childress, *Condylactis gigantea*, is larger still than *S. helianthus*, offering a hypothesis for the preference for that taxon they noted.

In the absence of host specificity, can differential reproductive success among hosts help to the explain patterns of diversification that we observe in Pontoniine shrimps? Social (Intra-specific) competition for resources other than mates has been invoked as a driver of speciation (West-Eberhard 1983), though most experimental examples of differential fecundity come from highly specific relationships (De Marisco & Reboreda 2008). Indeed, it remains unclear whether host specificity is a basal or derived trait (Lanyon 1992; Rothstein et al. 2002). The work of Kou et al. (2015) provides an intriguing hypothesis that the level of specialization in *P. yucatanicus* may be representative of a intermediate placement in the pattern of divergence of the group. While the variation in host-shrimp partnerships and resultant differences in fecundity meet the 'precondition for mutualism specialization' proposed by Schemske & Horvitz (1984), further information on host specificity and the placement of the Atlantic Pontoniine fauna within an evolutionary context is needed to see if the Atlantic fauna follows a similar route to divergence and specialization as the Pacific fauna.

Acknowledgements:

We would like to thank the Smithsonian Tropical Research Institute and the Organization for Tropical Studies for their support through the STRI/OTS scholarships and fellowships. Travel funding through East Carolina University and The Rivers Foundation was greatly appreciated. Editorial comments and support from Candy Feller and Kyle Summers were critical to the success of this work, as was the friendship of Tom Oliver and Rita Steyn during one crazy summer in Panama.

Literature Cited:

Azofeifa-Solano J, Elizondo-Coto M, and Wehrtmann I. 2014. Reproductive biology of the sea anemone shrimp *Periclimenes rathbunae* (Caridea, Palaemonidae, Pontoniinae), from the Caribbean coast of Costa Rica. *ZooKeys* 457:211-225.

Bauer R. 2004. *Remarkable Shrimps: Adaptations and Natural History of the Carideans*. Norman, Oklahoma: University of Oklahoma Press.

Bruce AJ. 1976. Shrimps and prawns of coral reefs, with special reference to commensalism. In: Jones OA, and Dean RM, eds. *Biology andGeology of Coral Reefs*. New York: Academic Press, 37-94.

Corey S, and Reid D. 1991. Comparative Fecundity of Decapod Crustaceans: I. The Fecundity of Thirty-Three Species of Nine Families of Caridean Shrimp. *Crustaceana* 60:270-294.

De Marisco M, and Reboreda J. 2008. Differential reproductive success favours strong host preference in a highly specialized brood parasite. *Proeedings of the Royal Society, Series B* 275:2499-2506.

Guo CC, Hwang JS, and Fautin DG. 1996. Host selection by shrimps symbiotic with sea anemones: A field survey and experimental laboratory analysis. *Journal of Experimental Marine Biology and Ecology* 202:165-176.

Kou Q, Li X, Chan T, and Chu K. 2015. Divergent evolutionary pathways and host shifts among the commensal pontoniine shrimps: a preliminary analysis based on selected Indo-Pacific species. *Organisms, Diversity & Evolution* 15:369-377.

Lanyon S. 1992. Interspecific brood parasitism in blackbirds (Icterinae): a phylogenetic perspective. *Science* 225:77-79.

Li X. 1993. The associations between pontoniines and other marine invertebrates I. *Marine Sciences* 6:43-48.

Limbaugh C, Pederson H, and Chace F. 1961. Shrimps that clean fishes. *Bulletin of Marine Science of the Gulf and Caribbean* 11:237-257.

Mascaro M, Rodriguez-Pestana L, Chiappa-Carrara X, and Simoes N. 2012. Host selection by the cleaner shrimp *Ancylomenes pedersoni*: Do anemone host species, prior experience or the presence of conspecific shrimp matter? *Journal of Experimental Marine Biology and Ecology* 413:87-93.

McKeon CS, and Moore JM. 2014. Species and size diversity in protective services offered by coral guard-crabs. *PeerJ* 2:e574.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Ross DM. 1983. Symbiotic Relations. In: Bliss D, ed. *The Biology of Crustacea*. New York: Academic Press, 163-211.

Rothstein S, Patten M, and Fleischer R. 2002. Phylogeny, specialization, and brood parasite-host coevolution: some possible pitfalls of parsimony. *Behavioral Ecology* 13:1-10.

Salthe SN. 1969. Reproductive modes and the number and sizes of ova in the urodeles. *The American Midland Naturalist* 81:467-490.

Schemske D, and Horvitz C. 1984. Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science. p 519.

Silbiger N, and Childress M. 2008. Interspecific variation in anemone shrimp distribution and host selection in the Florida Keys (USA): Implications for Marine Conservation. *Bulletin of Marine Science* 83:329-345.

Spotte S. 1996. Supply of regenerated nitrogen to sea anemones by their symbiotic shrimp. *Journal of Experimental Marine Biology and Ecology* 198:27-36.

Spotte S, Heard R, Bubucis P, Manstan R, and McLelland J. 1991. Pattern and Coloration of Periclimenes rathbunae from the Turks and Caicos Islands, with Comments on Host

Associations in Other Anemone Shrimps of the West Indies and Bermuda. *Gulf Research Reports* 8:301-311.

Stanton G. 1977. Habitat partitioning among associated decapods with *Lebrunia danae* at Grand Bahama. Third International Coral Reef Symposium. Miami, Florida. p 169-175.

Stearns SC. 1992. *The evolution of life histories*. Oxford, New York: Oxford University Press.

Team RC. 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Thompson J. 1994. *The Coevolutionary Process*: University of Chicago Press.

West-Eberhard M. 1983. Sexual Selection, Social Competition, and Speciation. *The Quarterly Review of Biology* 58:155-183.

Wicksten M. 1995. Within-species variation in *Periclimenes yucatanicus* (Ives), with taxonomic remarks on *P. pedersoni* Chace (Crustacea: Decapoda: Caridea: Palaemonidae). *Proceedings of the Biological Society of Washington* 108:458-464.

Figure 1. *Periclimenes yucatanicus* in *Stichodactyla helianthus*

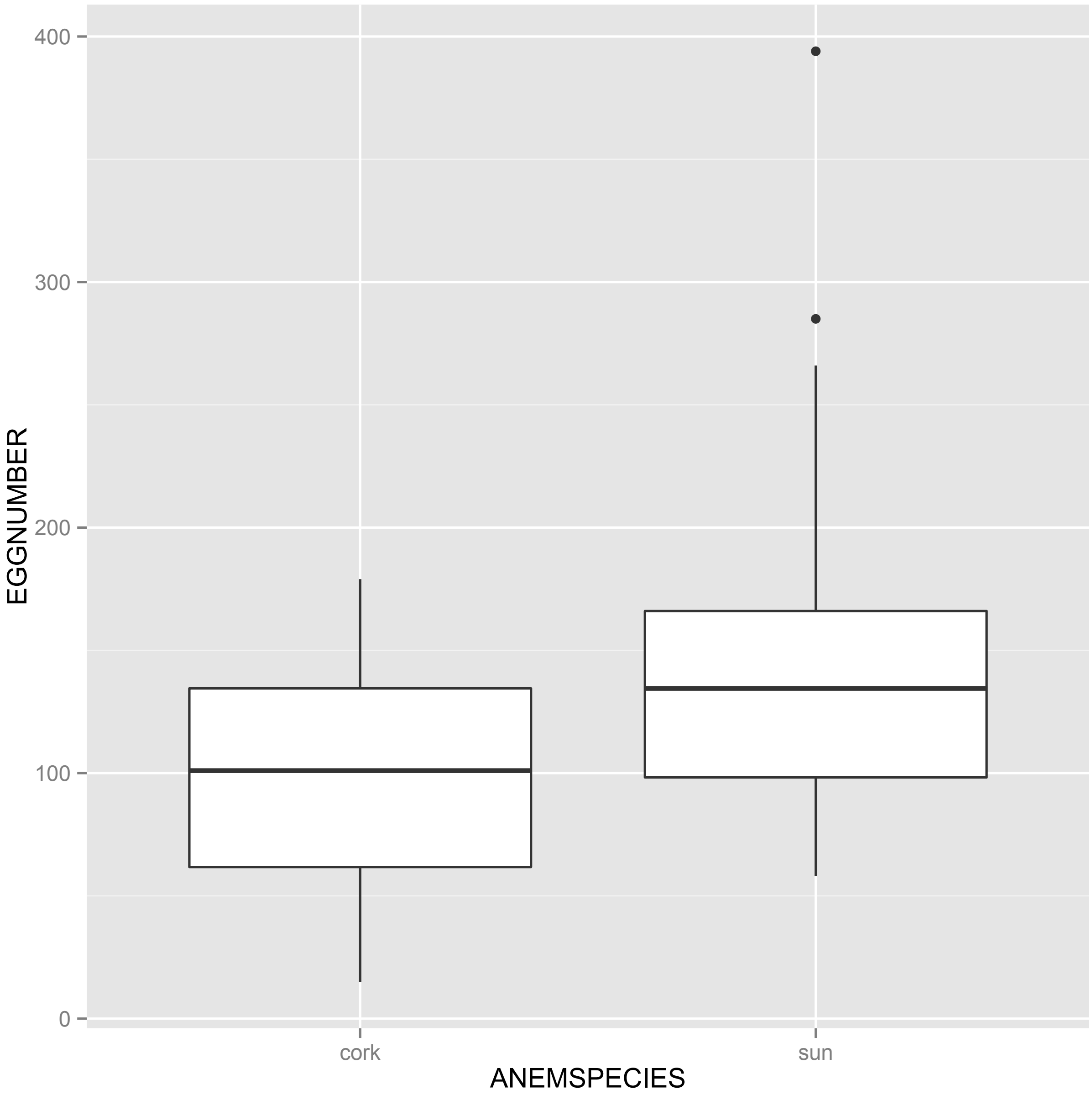

Figure 2. Egg Number by Anemone Species

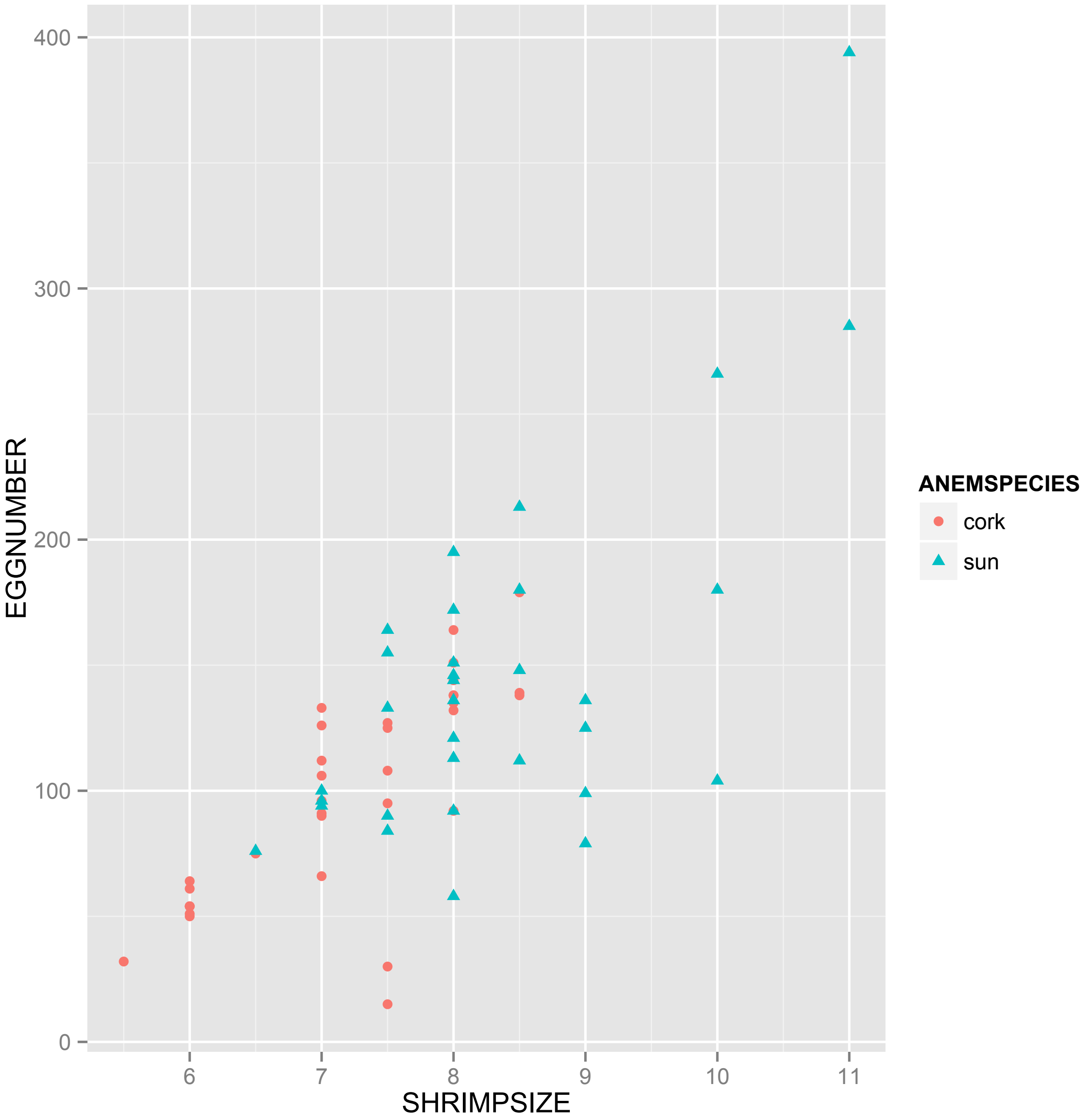

Figure 3. Egg Number by Shrimp Size

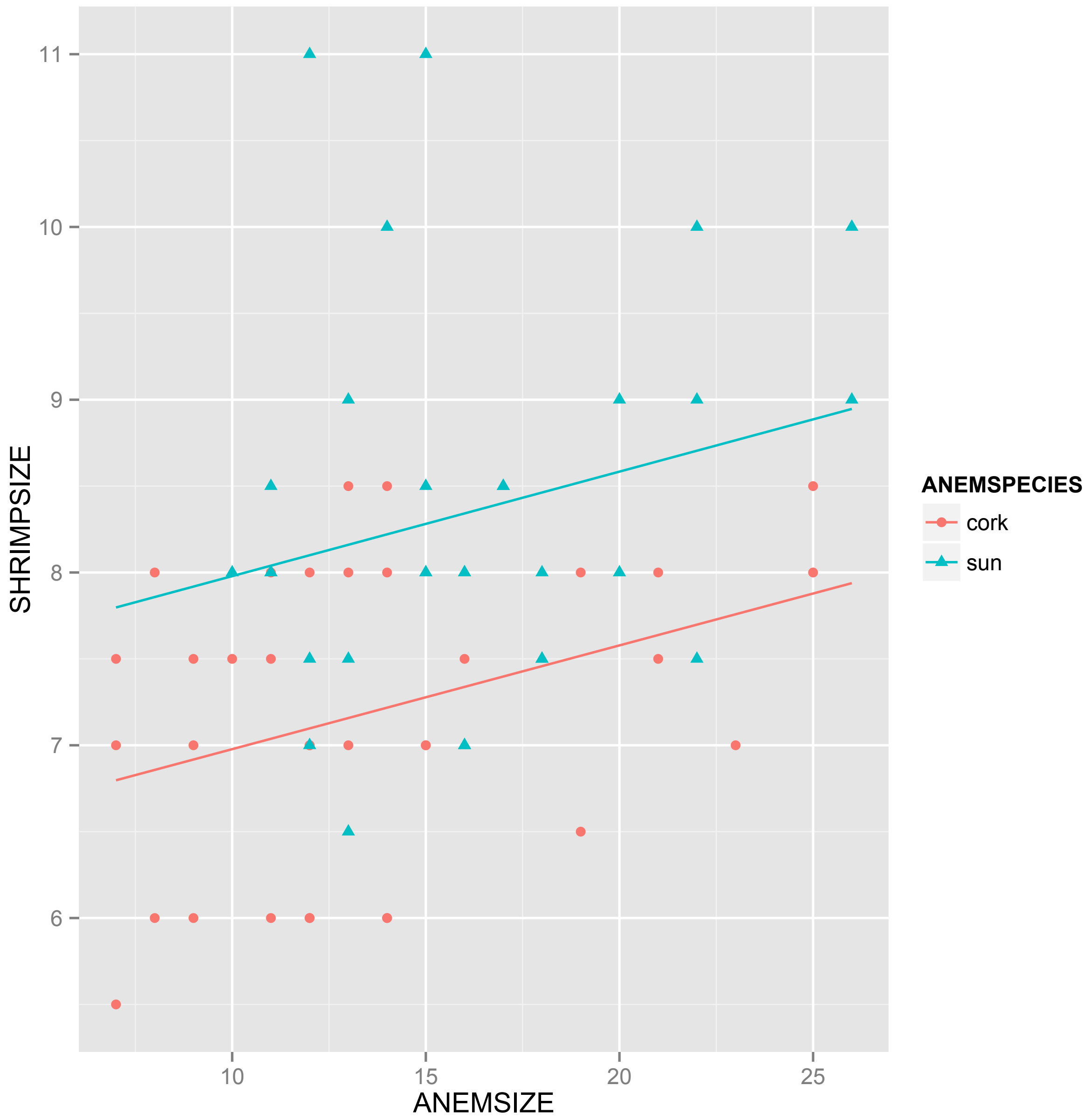

Figure 4. *P. yucatanicus* carapace size on anemone size by species