Site fidelity and homing abilities in Mediterranean anemonefish *G. bucchichi*

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Abstract:
The symbiotic relationship between anemonefish and anemones is well documented in many subtropical and tropical parts of the world (Fautin 1986). In the Mediterranean Sea, however, the only known anemonefish, *Gobius bucchichi*, is not well studied (Abel 1960). In this study I determine whether *G. bucchichi* and its host anemone, *Anemonia viridis*, have a symbiotic relationship. After determining that the two species do have an association, my goal was to find if *G. bucchichi* demonstrates site fidelity and homing abilities in regards to *A. viridis*. This is a poorly understood relationship that should be investigated, as studies have shown decreasing ocean pH impair the sensory organs of anemonefish (Munday et al. 2009). These sensory organs, namely olfactory senses, influence larval settlement, imprinting on host anemones, and homing abilities (Munday et al. 2009). In this study, it was determined that *G. bucchichi* demonstrates both site fidelity and homing abilities, therefore the species should be monitored as ocean acidification rises in the Mediterranean Sea (CIESM 2008).

Introduction / Background

Many factors drive a healthy ecosystem, but one fundamental component is species interactions. Whether it is mutualism, commensalism, or parasitism, each plays a critical role in the functionality of a system. Like many subtropical bodies of water, the Mediterranean Sea is rich in biodiversity, therefore high in species interactions (Bianchi, Morri 2002). One set of species in particular, a small goby, *Gobius bucchichi*, and a common anemone, *Anemonia viridis* (previously *Anemonia sulcata*), have an atypical association many are familiar with. Similar to clownfish, the goby takes refuge among the flowing, stinging tentacles of *A. viridis*. The goby’s skin is covered in a mucus that is immune to the harmful nematocysts of its host (Patzner 2005). Unlike other mutualist anemonefish, this relationship is commensal, meaning the goby gains something while the anemone is unaffected (Fautin 1990). This gain could be life-saving for a small fish in a rocky reef. Without the readily available protection from *A. viridis*, the entire species population of *G. bucchichi* could potentially decrease in size (Munday et al. 2009). Before any species protection can take place, more information is needed about the relationship.

Even though *G. bucchichi* is one of the most abundant fish species in the depth range 0-5m, little information is known about it (Abel, 1960; Bouchereau et al., 1992) The main goal of this study is to provide evidence that *G. bucchichi* has an association with *A. viridis*, and secondly to determine if the gobies have site fidelity and homing abilities if displaced. Site fidelity is defined as the tendency to return to a previously occupied location (Switzer 1993). In the case of *G. bucchichi*, this site would be an *A. viridis* individual where the goby resides. No previous research has been done testing the fidelity of this species, which is crucial for finding if they have homing abilities. Gerking (1959) defines homing as the choice that a fish makes to
return to a formerly occupied place instead of going to equally probable places. Homing can be accomplished in a number of ways, however, visual cues, chemicals, acoustics, and olfactory cues are most common. Losey (1978) found that anemonefish use visual cues and olfactory attraction to find their host anemone. Olfaction is the sense of smell in all organisms, and is highly utilized by fish. Fish can use smell to hunt, find a mate, escape predation, or in this case, find their way back home (Leduc et al., 2013).

While maintaining these olfactory senses is crucial for fish, they may be in trouble in the near future. The rise of ocean acidification due to anthropogenic causes has been found to negatively affect sensory abilities in many marine fish (Munday et al. 2009). Ocean Acidification is defined as the uptake of Carbon Dioxide, resulting in reductions in pH and changes in seawater carbonate chemistry (Godbold, Calosi 2013). With the Earth’s increasing CO₂ emissions and pollution, this emerging problem will have endless consequences if it is not slowed down. If ocean acidification impairs G. bucchichi senses needed for homing and protection, the species may be in trouble.

To begin my project, I hypothesize that G. bucchichi will have an association with A. viridis. Furthermore, G. bucchichi will associate with certain habitat types, particularly sandy/cobble areas. My third hypothesis is that G. bucchichi has site fidelity to A. viridis individuals. If that trait is shown, G. bucchichi will also display homing abilities when displaced from its original host anemone and placed at a different anemone.

**Material and Methods**

**Species and site description**

_G. bucchichi_ is a species from the family Gobiidae that is native to the Eastern Atlantic Ocean, the Mediterranean Sea, and the Black Sea (Patzner 2005). It is generally found in depths from 1-30m, in sandy or muddy substrate (Patzner 2005). It can grow up to 10 cm in length, and feeds largely on small crustaceans (Wood 2012). As of now, it is the only known anemonefish in the Mediterranean Sea (Wood 2012). It can be found living near the tentacles of _A. viridis_, and will dart behind the anemone when threatened (Patzner 2005).

_A. viridis_ is most commonly known as the Snakelocks Anemone. This species ranges in the Atlantic Ocean from Great Britain to Spain, as well as the Mediterranean Sea (Eaker 2003). This anemone can reach up to 19 cm in diameter, and is usually found in shallow waters up to 12m (Eaker 2003). Unlike most anemones, _A. viridis_ cannot retract its tentacles, for they are too long and numerous (Wood 2002). These tentacles make it possible for _A. viridis_ to feed on plankton as well as large gastropod mollusks (Eaker 2003).

This study was conducted in October 2014 at Station de Recherches Sous-Marines et Oceanographiques (STARESO) near Calvi, Corsica in France (Fig. 1, coordinates 42°34'48.0"N 8°43'27.3"E). All observational data and field experiments were done using SCUBA in the station’s harbor. This harbor is a sheltered area of water with a breakwater on the North side, and natural rocky cliffs on the South side (Fig. 2). The habitat in the harbor is composed largely of sand patches, boulders, cobble, and some _Posidonia oceanica_, an endemic Mediterranean seagrass. The harbor and surrounding water has little human impact and low wave exposure.
General approach

This study was conducted along two permanent transects, placed in the south end of the STARESO harbor (Fig.3). The depths of these transects were ranged from about 2 to 5 meters, along sand, cobble, and boulders. This is the general habitat where both *A. viridis* and *G. bucchichi* can be found. Transect 1 was 12 meters long, and was placed over an area that was primarily cobble and sand. Transect 2 was 14 meters long, and was placed over an area of boulders with cobble patches. Each transect was initially surveyed, noting *A. viridis* individuals in 2.5m to the left and right of the transect line. Because *A. viridis* reproduces primarily by asexual longitudinal fission, clumps of anemones were counted as one individual (Hiscock 2008). During this initial survey, the presence of *G. bucchichi* was also noted at each anemone.

An X,Y coordinate map of all the anemones in the area was created by noting which meter mark each anemone was located at, as well as the distance from the transect. Along with noting the location of each anemone, I took measurements of their diameter, and also noted the type of habitat surrounding each individual. Habitat types included: Sand (S), Cobble (C), Boulder (B), Cobble/Sand (C/S), and Cobble/Boulder (C/B).

After all information about the anemones was collected, they were each labeled by sticking a metal stake with a duct tape flag nearby. These flags were labeled A-Z, AA-AZ, BA-BZ, CA-CZ, and DA-DE. 109 individuals were labeled in total across both transects.
Goby/Anemone Association

For 10 consecutive days, these 109 anemones were surveyed, while noting which individuals had a goby nearby. When noting a goby’s presence, I surveyed a 30 cm diameter range around the anemone. Because this species of anemonefish is typically found at the tips of the anemone, they weren’t always in direct contact with *A. viridis*. To test association, a control experiment was done in an area adjacent to both transects. This area consisted of similar habitat, although *A. viridis* was nearly absent from the site. To represent the area around an anemone that a goby could be present in, a wire hoop with the same 30 centimeter diameter was constructed. This hoop was then dropped at 50 points along a 10 meter tape in the previously stated habitat. The hoop was dropped a meter to the left and right of the tape every 0.5 meters, then on the meter tape every meter. At each of the 50 random points, *G. bucchichi* presence was noted. A bar graph using JMP (Fig. 5), and then a Chi-square Fisher’s exact test was performed to see the probability of goby presence between the transects and the control site.

Habitat Association

As previously stated, the habitat characteristics were initially observed for each *A. viridis* individual on transect 1 and 2. The same five categories for habitat (S, C, B, C/S, and C/B) were also stated for each of the 50 random points in the control plot. The proportion of goby presence on each substrate was then compared to the overall habitat distribution. A bar graph was made in JMP to illustrate these differences (Fig. 6), and a Chi-Square analysis was run to test habitat association.

Site Fidelity

Once all data were collected for anemone location, goby presence, and habitat, I began to capture and tag *G. bucchichi* individuals found along either transect. To capture the fish, I used two small aquarium nets. Once captured, another diver would hold the goby’s head within the net, while keeping their tail-end exposed. A variety of elastomer colors was used to mark each individual that was captured. The mark (one, two, or three colors) was placed on the top of the fish’s back, towards the tail (Fig. 4). A total of 25 fish were tagged; 21 on transect, four off transect. A Poisson distribution was used to determine how the gobies would be distributed if there was no site fidelity. A Pearson Chi-Square analysis was then run comparing the random distribution to the collected data.
Homing Ability

To test homing abilities in *G. bucchichi*, a final experiment was done with both transects. Tagged gobies were recaptured and transplanted onto a different anemone with similar habitat ~3 meters away. The goby tag, the original anemone the goby was found at, and the new anemone were noted. This was done for five individuals. To rule out the chance that a goby did not return to its original anemone due to trauma, a control experiment was also done in which a different set of five tagged gobies were recaptured with an aquarium net and placed back at the same anemone they were found at. To simulate swimming to a different anemone ~3 meters away, I swam with the goby in the net the same distance. The following days after the displacement, the anemones where gobies were removed were checked to see if the goby had returned. The control goby’s anemones were also checked. If the gobies returned to their original anemone, it would show that they have site fidelity and homing abilities. If the gobies did not return to their original anemones but fled from the new anemone they were placed at, it would show that they have site fidelity but lack homing abilities. And finally, if the gobies stayed at the new anemone they were placed at, it would suggest that they lack site fidelity to a certain anemone. An equation was then created to calculate the probability of each goby returning to their original anemone by chance.

Results

Goby/Anemone Association

To ensure that *G. bucchichi* does in fact have an association with *A. viridis*, I compared the overall presence of *G. bucchichi* between both of the transects combined to the control plot, where anemones were nearly absent. Figure 5 shows the difference in presence between the treatments. A Chi-Square analysis found that the difference between the two treatments was significant (p = 0.0097), indicating that a true association exists.
After assessing substrate types across both transects and the 50 random points on the control plot, I compared the sum of goby sightings per habitat type (S, C, B, C/S, and C/B). In order to find out if *G. bucchichi* preferred a particular habitat, it was necessary to determine if the collected data differed from the values that would have been expected in a random distribution. Figure 6 shows the overall percentage of habitat type that was available to gobies compared to where gobies were found. A Chi-Square analysis indicated that the results found were significant (ChiSq = 49.1, df = 4.0, P < 0.0001).

**Site Fidelity**

To measure site fidelity among *G. bucchichi* individuals, it was necessary to show that their distribution among the transects was not random. When the survey data from 10 days of observation were analyzed, it was determined that a goby was proximal to an anemone an average of 1.77 days during this period. A Poisson distribution was calculated to view what the data would look like if gobies had no preference for anemones, and were just random. I then compared this distribution with the actual data I collected. Using a Pearson Chi-Square test, I found that my results were significant (ChiSq = 112.7, P < 0.0001), indicating there is a preference for anemones.
Homing Ability:

When each of the five tagged gobies were displaced, they all returned to the anemones they were originally associated with. To find out the probability of this happening, the number of anemones the goby had to pass to get to their original anemone was examined. This was accomplished using the map created with the values determined in the initial anemone survey described above. The X-axis represents the meter mark on the transect line, and the Y-axis represents how many meters the anemone was to the left or right of the transect line (Fig. 8). Each dot in this map represents one of the 109 anemones, and the thickness of the point represents how frequently there was a goby associated with that individual. After calculating how many other anemones each tagged goby had to cross over to get back to its original anemone, these values were multiplied together. The following equation was then arrived: 1/6 x 1/5 x 1/4 x 1/9 x 1/6 = 0.0001543209. The final value is the calculated p-value, which is much less than the critical p-value of 0.05. This result indicates that *G. bucchichi* is capable of homing.
Discussion

The first result I found in my study answered the question; does *G. bucchichi* have an association with *A. viridis*? After comparing their presence on transect (with anemones) and off transect (no anemones), I found a statistically significant difference (p=0.0097), therefore I can reject the null hypothesis and state that *G. bucchichi* does have an association with *A. viridis*. This result is consistent with almost all previous research about *G. bucchichi* and *A. viridis*. By finding this, I was able to move forward with testing the rest of my hypotheses.

When determining if *G. bucchichi* associates with a particular habitat type, I found that their distribution among my transects was disproportionate to the Poisson random distribution that was calculated. If *G. bucchichi* do not have any preference in substrate, one would expect them to be found proportionally to what habitats were available. In figure 6, the red bars represent the proportions of available habitat. If the gobies did not prefer any particular substrate, the blue bars (where the gobies were actually found), would be equal to the red bars. However, this is not the case. Only the cobble/sand and cobble/boulder categories were proportional to each other. Sand, cobble, and boulder were not equal to the random distribution of habitat. After finding a p-value of <0.0001 in a Chi-Square test, it could be concluded that *G. bucchichi* does prefer certain habitats. Prior to this study, I hypothesized that gobies would be found primarily on sandy/cobble areas, due to their light skin coloration. I believed they would choose anemones surrounded by sand to camouflage themselves. However, according to the results, *G. bucchichi* preferred residing on areas with cobble or boulder substrate. Anemones that grow against rock may provide even more protection for *G. bucchichi* if threatened. One weakness in testing the habitat association hypothesis was that I was the only person categorizing each anemone’s
habitat. Although I was consistent with what I labeled as S, C, B, C/S, and C/B, a stronger study might have used the opinions of more researchers.

When testing for site fidelity in *G. Bucchichi*, I had to conclude that their distribution in my transects was not random. If the gobies did not have site fidelity, one would expect to find that their distribution among the 109 anemones on my transect was random. When I found that their distribution did not match up with the Poisson distribution I ran (Fig. 7), I could conclude that their distribution was not random. This result is consistent with site-fidelity and is further proved by the Chi-Square analysis I ran (p<0.0001). With theses results, it could be concluded that *G. buchichi* individuals use certain anemones as a home, and some anemones never even have a goby on them. Due to my project’s short duration, I was unable to do any further research behind this site fidelity. Gerlac and Atema (2012) state that site recognition processes might be based on olfactory imprinting that some anemonefish acquire in early development.

For my last hypothesis, I tested homing abilities in these gobies. After each tagged goby I displaced came back to its original anemone, I found that this species does in fact have homing abilities (p=0.000154). The fact that each individual had to navigate through a number of other suitable anemones showed that there is a definite preference to their host anemone. However, the mechanism behind this homing ability is also unknown. More studies are needed to fully understand these processes in this particular goby.

Since most other anemonefish and reef fish use olfactory senses to find suitable sites, it is likely the same mechanism that *G. buchichi* uses (Leduc et al., 2013). If this is the case, their homing abilities and settlement may be affected with the rise of ocean acidification (Leduc et al., 2013). As recent studies have shown, the Mediterranean Sea’s potential pH change is relatively large compared to that of the Atlantic Ocean (CIESM 2008). This potential decrease in pH is due to the higher alkaline state that the Mediterranean Sea has, which allows I to absorb more anthropogenic CO\textsubscript{2} than the open ocean (CIESM 2008). According to Munday et al. (2009), this pH change affects a complex range of olfactory stimuli that help anemonefish orient themselves and also choose a host anemone. Without these senses, the life histories of certain fish will be disrupted and populations will not be properly replenished (Munday et al. 2009). Bianchi and Morri (2000) address the fact that Mediterranean biodiversity is undergoing rapid alteration due to increasing climate change and human impact. However, protection measures for both species and individual ecosystems are still scarce (Bianchi, Morri 2000). These are the reasons why studying the site fidelity and homing abilities in *G. buchichi* are important. Hopefully this study done on *G. buchichi* will be useful in the future if this species ever needs to be protected.

**Possible studies for the future**

Possible studies for the future could test if changes in pH do in fact affect the olfactory senses of *G. buchichi*. One could determine if decreasing pH affects the homing abilities in the goby. This would support or reject the idea that *G. buchichi* uses olfactory cues to find/settle at its host anemone.

Another study could look at the range *G. buchichi* individuals roam from host anemone during foraging periods. The study could test if the goby has a maximum distance away from the anemone that wouldn’t allow them to navigate back to their original site.

If the study took place during breeding season, it could be done on larval gobies and if they settle on *A. viridis* immediately.
Literature cited


