Color Pattern and Ecological Speciation in Coral Reef Fishes of the Genus *Hypoplectrus*

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There has long been a great amount of debate about how speciation might operate in the presence of gene flow. One promising system that might hold some answers to this question is a genus of Caribbean reef fish: *Hypoplectrus* – commonly called hamlets. We spent 3 weeks at the Smithsonian Tropical Research Institute's Bocas del Toro Station on Isla Colon where we assisted PhD candidate Oscar Puebla on preliminary field and experimental work related to his post-doctodoral research on several species belonging to this genus. We initially captured individuals of 2 species, *Hypoplectrus puella* and *Hypoplectrus nigricans*, in order to conduct mate choice experiments testing for the role of color pattern in assortative mating. The limitation of these first experiments was the very low frequency of individuals displaying mating behavior. During the second week we modified our design and used video footage we made of displaying individuals and played these back to subject fish. However no individual appeared to react to the clips whatsoever. Finally, during the third week, we used an aquarium to test the efficiency of the aggressive mimic *Hypoplectrus unicolor* – the butter hamlet – on predation of glass gobies (*Coryphopterus hylainus*) in the presence of the model fish: *Chaetodon capistratus* – the foureye butterflyfish. Furthermore we also explored several reefs accessible from the station in search of rare Hypoplectrus morphs. We marked rare these individuals and on subsequent dives, observed that that they appear to be swimming to a designated area on the deeper reef slope to spawn. This suggests that assortative mating of these rare morphs might, in fact, not be costly. In such an instance, previously-proposed models that have demonstrated rapid speciation through assortative mating in the presence of gene flow might hold strong, at least in case of certain marine ecosystems.

1. INTRODUCTION

Absolute barriers to the dispersal of organisms (e.g. mountains, large water bodies and canyons in terrestrial systems) are scarce in the marine realm even at very large geographic scales. This is because, for many marine organisms, the larval stage is planktonic and mobility is at the mercy of oceanic currents. This provides the potential for gene flow across large geographic distances (Veron 2006). As a result this poses a fundamental question in marine evolutionary biology, as to whether geographic and oceanographic barriers, on their own, can account for the high levels of species diversity one observes in certain marine ecosystems. This is particularly interesting for a high diversity marine ecosystem such as coral reefs where such a stunning array of colors, patterns, shapes and sizes characterize the fish communities that inhabit them. Speciation for marine organisms in these systems is most probably operating in the presence of gene flow between populations that are already in the process of diverging. Given this, there are most likely other mechanisms (i.e. besides physical barriers) driving speciation in such systems. One example of these is ecological speciation and influenced by behavioral differences. However, despite significant work attempting to explain the process of ecological speciation in freshwater fishes, comparatively little is known about how it might be operating in marine teleosts (bony fish). Certain models have shown that, under certain conditions such as assortative mating among others, rapid speciation, even in the presence of gene flow, is possible (Gavrilets 2004). The author suggested that this would likely be the case if the same trait under sexual selection (assortative mating) is naturally advantageous to the organism as well. However, few such examples have been documented to date (Schluter 1996, 1998). Furthermore these models have been criticized for their failure to take into account that assortative mating is likely costly for species that are rare. Rarity, in the context of assortative mating, implies that it is much more difficult to find a mate of the same species and as a result, must bring about some cost.

One group of coral reef fishes that provides a good opportunity to study and understand how speciation might be operating under the above-mentioned constraints is the genus *Hypoplectrus* – commonly called hamlets. Hamlets are small coral reef fish endemic to the Tropical Western Atlantic that belong to the Serranidae family (this family includes groupers) (Randall 1983). There are to date 10 described species (including one soon to be described – the tan hamlet) within this genus together displaying quite considerable variation in color pattern (Puebla et al 2007). Nevertheless there is still considerable debate among scientists and taxonomists as to whether these should be considered separate species or simply different varieties/morphs of the same species. Genetic analysis has shown that there despite clear genetic differences between color morphs, these are all closely related (Puebla et al 2007). Puebla et al (2007) observed 251 independent spawning events in the field (at several locations throughout the Caribbean), of which 247 were between individuals of the same color morph. In other words, 98.4% of spawning events were assortative with respect to color pattern. Hamlets are simultaneous hermaphrodites (Fischer 1980; Barlow 1975). This implies that each individual contributes both egg and sperm during courtship. This reduces an inherent cost to assortative mating that firstly requires an individual to find another of the opposite sex on top of searching for a mate of the same morph/species. During spawning period, every evening approximately one hour before dusk, potential mates begin displaying to each other and spawn several times. During courtship, one individual displays while the other watches. Spawning occurs and is followed by a reversal of roles in which the individual originally displaying will now observe the other display (Fischer 1980). There are two discernable behaviors that hamlets display when courting: head snapping and forward pitches. The former involves the displaying individual repeatedly snapping its head to its observing mate. The forward pitch involves the individual swiftly swimming away from the mate while slightly raising its underside as to expose the eggs it carries. Despite the fact that virtually all observed spawning events observed in the field by Puebla et al

(2007) were between similar morphs, it is not yet possible to fully determine respective color patterns as the responsible cue in driving assortative mating. Observations in the field are limited in their ability to control and thus rule out other possible cues (chemical or behavior) that might differ to some degree between morphs. We have thus attempted to design mate choice experiments that can control for these and test whether or not color pattern is the trait being selected for (sexual selection) when choosing a mate.

Randall and Randall (1960), as well as Thresher (1978) suggested that several hamlet morphs might have evolved as aggressive mimics. In other words, their color pattern resembles that of a non predatory/aggressive species in order to approach its prey more easily. These color patterns would presumably thus have been naturally selected for since they convey a selective advantage to the organism. One of several putative aggressive mimic morphs was the butter hamlet, *Hypoplectrus unicolor* (Figure 4). Puebla et al (2007) showed that, indeed, individual butter hamlets followed their model (*Chaetodon capistratus* – the foureye butterflyfish {Figure 5}) significantly more than the control morph – the barred hamlet. Furthermore, significantly more predatory strikes were performed by the butter hamlets in the presence of butterflyfish than when alone. Once more, although more detailed experiments are required to show this, color pattern appears to be the trait selected for (in this case natural selection).

In light of the above, the project around which our internship is built broadly seeks to observe, determine and explore the role color pattern plays in sexual and natural selection in hamlets as well as understand and record mating patterns in individuals of rare morphs. Understanding these processes and the way in which they operate in the *Hypoplectrus* genus will likely lead to greater insight into speciation and radiation in high diversity marine systems such as coral reefs.

Most of the described morphs are not common at a large proportion of locations throughout their range (Puebla et al 2007). Much work is thus needed to identify mating success and the identity of mates of these rare morphs to attempt to understand whether or not assortative mating for these is costly or not.

2. METHODS

(a) *Study site*

The field part of our internship was based at the Smithsonian Tropical Research Institute's Station in Bocas Del Toro. The station is located on Isla Colon near the town of Bocas del Toro. The station provided us with air tanks, dive lockers to store our dive equipment as well as, access to their boats, a computer lab, dormitory rooms and a kitchen to cook meals. During the first two weeks fully devoted to our internship, we used one of the station's boats to access nearby reefs where we used SCUBA to capture individuals of both species: *Hypoplectrus puella* and *Hypoplectrus nigricans*. Individuals were collected from the reef at Sunset Point/STRI Point (Figure 1). During the third week we captured, marked and observed individuals from STRI Point reef, Mangrove Inn reef and a patch reef near Punta Caracol, all off of Isla Colon, as well as Punta Juan reef off of Isla San Cristobal (Figure 1).

(b) *Capturing Methods*

In order to test for the relationship between visual cues, based on color pattern recognition, and assortative mating among different color morphs, we chose to focus our attention on two of these morphs: the barred hamlet (*H. puella)* and the black hamlet (*H. nigricans)* (Figures 3 & 4) These species are found throughout the Caribbean and are the most abundant hamlet morphs on the reefs near the station. Prior to our arrival at the station we obtained permission from STRI's Scientific Dive Officer, Edgardo Ochoa, to scuba dive under the institution's authority. Our supervisor also obtained permission to capture and individuals from the field and release them upon completion of experiments in the lab. Individual hamlets of both species were collected while scuba diving at STRI Point reef at depths between 15ft and 60ftTwelve hamlets (5 barred and 7 black) were captured over the course of 7 dives during the first week (February 2nd to 8, 2009). Most of the dives were performed in the morning although a few were done in the afternoon. Below is a list of the 3 distinct methods used to catch individuals during the first week. No individuals were caught using the first method and only one was caught using the second.

(1) Three divers (Oscar Puebla, Marie-Claude Cote-Laurin and Jose Benchetrit) used a weighted casting net and, together slowly descended over a patch of coral where an individual of either morph was spotted. Once the net was secured over the patch of coral, the two other divers held hand nets while waiting for the hamlet to emerge from a hole or crevice. However after many attempts, no individual ever emerged from the complex structure within the coral patch. Furthermore, the net was became entangled with coral patches dominated by *Agaracia tenufolia* resulting in many pieces of the coral colonies being broken.

(2) Basing ourselves on a paper in which the authors caught reef fish using clove oil, a mild anesthetic reportedly benign to fish and other reef fauna, we opted to attempt catching individuals using this method. One diver used a syringe to inject clove oil in and around an individual over the reef. The other two divers attempted to scoop out the sedated individual using hand nets. However getting close enough to effectively inject the

anesthetic near the individual proved extremely difficult and only worked once. Furthermore, this individual appeared to never fully-recover from the anesthetic and displayed abnormal behavior during the remainder of the week while in the aquarium.

(3) The third method involved each diver using a reel of line, a small hook, a weight and bait (chopped sardines caught off the station pier using a casting net) to fish individuals while hovering above the reef. Each reel, hook and weight was set up on the boat just prior to the dive and each diver carried extra bait in a zip-lock bag during the dive. As soon either a barred or black hamlet was spotted, we hovered above while dangling the bait in front of the fish. Once caught, we removed the hook and placed the individual in a mesh bag carried by Oscar Puebla. After completing the dives, all individuals that were captured were placed in a large cooler filled with seawater until we returned to the station. Some of the individuals caught exhibited trouble maintaining buoyancy suggesting that they might have been brought to the surface too fast. However, this usually subsided after a day or two in the aquaria.

(c) EXPERIMENTAL METHODS

First week

The first week of our internship was largely spent getting a feel for and an understanding of a good potential experimental design to test our hypothesis: that visual cues (i.e. recognition of color pattern in potential mate) are driving assortative mating in both barred and black hamlet morphs. Each of the 12 individuals captured was placed in a separate aquarium. This made for a total 12 aquaria. Each of these was set up with a tube supplying a constant flow of new seawater as well as a tube supplying oxygen connected to porous limestone rock to ensure that the water remained well-oxygenated. Furthermore, in an attempt to reproduce a more natural setting, we covered the bottom of each aquarium with a layer sand and placed a rock or coral skeleton (collected in the field snorkeling and on the shore near the station) in the middle of each aquarium. This was important given the fact that hamlets are a strictly coral reef species and live almost exclusively on reefs with significant coral cover. They firmly defend their territories that are made up of a patch of coral reef approximately $1m^2$, excluding conspecifics and other hamlet species (Barlow, 1975). The twelve aquaria were arranged in four sets. Each set comprised 3 aquaria (Figure 2) For each set, the individual in the middle tank was the tester fish. In one of the 2 adjacent aquaria of the set, we placed an individual of the same morph and, in the other, an individual of the opposite morph. A cardboard divider was placed in between and behind each of the aquaria to prevent fish from seeing other individuals in adjacent tanks. We delineated (using string) each tank into three sections: left and right, and middle (neutral). Between 16:50 and 18:45, when individuals spawn in the wild, we removed the cardboard separators and measured the amount of time the tester fish spent in either the right or left section of the aquarium. This was don using a stopwatch. Each of the three observers was measuring the time for the tester fish in 3 of the sets. This was done for 15 minutes upon which we switched sets as to include all 4. The order and person measuring each set changed each evening to avoid any potential bias. Furthermore, each of the side individuals was swapped every evening to control for the tester fish's potential inherent preference of one section of the aquarium. We would also mark and count every mating display (forward pitch and/or head snap) that any of the individuals exhibited while we measured the amount of time spent on each section.

However, only one individual (a barred hamlet) displayed clear and obvious mating behavior (head snapping). This was repeated for the remainder of the week until the release of captured individuals back on the reef where they were collected. We then cleaned all the aquaria, tubing and oxygen rocks using water and clorox.

Second week

During the second internship week from March 14^{th} to 20^{th}, we modified the design of our experiment testing for the role visual cues play in assortative mating in barred and black hamlets. The new experiments involved obtaining video footage of hamlets displaying in aquaria, editing the color of the displaying fish footage with Photoshop Extended, and then playing back this footage to fish on a computer screen during spawning time.

Capturing Methods and Experimental Set-up

We used the same technique to capture hamlets during the second week as during the first week. We devoted the first 3 days of the week (1 dive per day each morning) to capturing individuals in the field. All individuals were captured on the reef at STRI. In total we captured 22 individuals were captured and put in the same aquariums than the one used during the first week. We set up 13 aquaria, 10 of which held a single individual and 3 of which held 2 individuals of the same morph (2 with 2 barred and 1 with 2 black hamlets). The 6 hamlets remaining were kept in a larger pool in the event that some individuals in the aquaria were shy and, as a result, not displaying mating behavior. We placed a white sheet of shiny paper as a light background behind each tank to ensure that the individual being filmed stood out in the footage.

Once again, spawning observations were conducted between 16:50 and 18:45 every evening. The rocks and coral placed in the middle of the tanks were removed approximately one hour before the beginning of observations (around 16:00) to ensure that individuals did not hide behind them while recording their mating behavior. Whenever a fish was observed displaying obvious mating behavior, one person slowly and carefully approached the aquarium to record footage. Two cameras were used to record mating displays: a Canon Powershot SD1000 and a Canon Powershot A75. Most of the video clips were recorded at 30 frames per seconds (fps) and some at 60 fps. Mating displays were recorded for both black and barred individuals. To reduce reflectivity against the front of glass face of the aquarium during recordings 2 large panels were placed behind the person filming the displays. After recording the video clips were transferred to a MacBook X laptop in order to edit and play back the footage to the fish. The computer used to manipulate and present the videos to the fish was a MacBook X. Unedited footage was first presented to the hamlets the following evening. This was to observe whether or not the fish would at all react to the footage of a displaying fish presented to them on a laptop computer screen. The 30-second video clip was played back over and over. This was first done for a clip recorded at 30 fps and the following evening for a clip recorded at 60 fps. The screen was placed vertically (the footage displayed in the same fashion) and placed against the side of the aquarium. We presented the footage to many aquariums, including some of those holding 2 individuals. After these trials we attempted to use a CTR screen to present the footage. We did not end up using Photoshop Extended version to manipulate the color of displaying individuals since there was no individual that appeared to react to or acknowledge the presence of the fish displaying on the computer screen.

Third week

We returned to the Station for the third and final week dedicated to the project from 14/04/2009 until 23/04/2009. We dived at the Mangrove Inn reef (Figure 1) and used the weighted cast net to capture foureye butterflyfish – *Chaetodon capistratus*. When a group of individuals were spotted swimming around a small patch of coral surrounded by sand, the 3 divers lowered the net quickly over the patch while directing the butterflyfish inwards. Once the net was secured, we used our hands to push the fish into the net where they became trapped. We then untangled the individuals and placed them in a mesh bag. We collected 6 individuals over the course of 2 dives and placed these in a large aquarium. We placed 2 sponges in the aquarium as a source of food for the fish. *Chaetodon* species are non-predatory omnivorous fish that feed on sponges, coral tissue, and tunicates (Lieske & Myers 2001). We conducted several dives to attempt to capture glass gobies - *Coryphopterus hylainus*. We searched for a small, isolated and not structurally complex patch of coral over which many gobies were observed swimming. The three divers then gathered around the coral patch preventing the gobies from escaping to another. Oscar Puebla then used a wash tube containing a mixture of 50% ethanol and 50% clove oil to inject the anesthetic into and all around the patch. We waited for approximately 20 – 30 seconds and then began using our hands to push water over and into the crevices, flushing out the sedated gobies. These were then placed in a fine mesh bag before returning to the surface. Many of the captured individuals ended up dying. We thus used plastic box to place the sedated individuals we captured on subsequent dives to try and counter this. Only one individual died using the box instead of the fine mesh bag on subsequent dives. In total we captured 17 gobies, 10 of which we placed in the large aquarium. The others were placed in a separate aquarium. All gobies were fed with fish food. We then dove at Punta Juan reef off of Isla San Cristóbal. We captured 2 butter hamlets – *Hypoplectrus unicolor* with the above-mentioned hook and line method while using SCUBA. We placed one of the individuals alone in an aquarium and the other in the large aquarium. We then placed a wooden board separation with several small holes drilled through the upper surfaces to allow for adequate water circulation and oxygen flow. In the evening we removed the separation and returned the following morning and counted the number of glass gobies in he aquarium. We also captured 4 individuals of each black and barred morphs on reefs 1 and 2 (Figure 1). We placed one individual of each morph in 4 aquaria. During spawning period we observed each of the 4 aquaria to determine if the two morphs would spawn with each other.

We then focused our attention on the intital aspects of a long-term and larger-scale project that our supervisor is set on conducting during the next several months. This involves tagging all individuals of every Hypoplectrus morph on as many reefs as possible near the station. We began by exploring a reef not previously visited by our supervisor Oscar Puebla, located southeast from the Punta Caracol Lodge (this reef will hereon be referred to as Punta Caracol reef). We conducted 2 consecutive dives every morning searching for rare hamlet morphs. Once these were spotted, we captured them using the hook and line method. Oscar Puebla then used a syringe in needle to inject a dye underneath the first layer of skin and into he fin of the individual. This was to ensure that the tag be visible from either side. Each individual was tagged at 3 out of 5 locations.

This results in 86 possible combinations per morph per site (reef). Once the individual was tagged, its size was determined by measuring the length from the tip of the snout to the caudal peduncle using a fish-measuring caliper. The individual was then photographed using a Canon Powershot SD 1000 placed in a WP-DC13 underwater housing and released back on the reef. At approximately 17:15 every evening we returned to the reef to search for the individuals we tagged and observe spawning behavior. During the morning dives during which we searched for and tagged rare morphs, 2 of the divers (Jose Benchetrit and Marie-Claude Cote-Laurin) also recorded every individual observed of each morph on a slate to get an idea of the relative abundances of morphs at this particular reef. Also, Oscar Puebla and Marie-Claude Cote-Laurin conducted nine 100 m transects at 3 different locations of the Punta Caracol reef. Transects were surveyed at 3 locations along the edge of the reef near the shallow sandy reef flat, 3 locations on the reef at depths of approximately 25ft and 3 locations on the deeper reef slope at about 35 – 40ft. Each of the 2 divers counted the total number of individuals of each morph observed within 2m one side of the total length of the transect.

RESULTS AND DISCUSSION

During the first week we spent working, we encountered several limitations and obstacles that served to reshape and fine-tune our initial experimental design. These ultimately allowed us to get a better idea of a design that could really test the question we were originally interested in answering. During the spawning observations conducted during the first week, only one individual, a barred hamlet, was observed displaying clear mating behavior. The reasons for this are unclear. However, this is likely attributable to the stress of being in an unfamiliar environment such as an aquarium. There also might be considerable variation in propensity to display mating behavior among individuals within each morph. In order to account for the former, we made sure to place a rock or piece of dead coral in the middle of each aquarium behind which the fish can hide much like it would in its natural setting. We did not consider the 2 evenings of data collected measuring the amount of time each subject fish spent on either side of the aquarium since we realized that data obtained from this experiment was irrelevant with respect to testing for actual mate choice preference. Since, in all 4 sets, neither of the object fish in aquaria on either side of the subject fish were displaying mating behavior then the subject fish is not really presented with a choice of the amount of time the latter is spending in each side of the aquarium. Nevertheless, given the fact that at least one individual was observed displaying mating behavior to another located in an adjacent aquarium, it suggested that having 2 individuals in the same medium was not necessary to induce spawning behavior. This would still allow us to test our hypothesis all while controlling for other factors (i.e. chemical cues) that might, in part, explain their assortative mating. Several studies on fish behavior have made use of video tools including video-editing software (Rowland 1999). McDonald et al (1995) used video playback with edited footage of male threespine sticklebacks to show that females were choosing their mates based on color contrasts. Other studies similarly manipulated footage of male threespine sticklebacks and played these back to other males. The results showed that nuptial coloration in these was driving aggression (Rowland et al 1989a, 1989b; Bolyard and Rowland 1996). In the case of highly visual coral reef species, one study on blackbordered damselfish showed that these reacted to a computer animation of conspecifics (Shashar et al 2005). As can be seen by the work conducted in these studies, many scientists have been successful in using video playback to study various aspects of fish behavior, even with coral reef fish. However, the individuals of both barred and black morphs to which we presented video playback footage of displaying individuals do not at all reacted to the footage being presented to them. It appeared that these failed to even realize that there was a video image of another fish being presented to them. There are several reasons why this might have been so. The technology used to record and playback video sequences is designed for viewing by humans and thus uses imaging systems based on human perception. This presents two potential problems. Firstly, the frame rate of the sequence of images being played exceeds the flicker rate of humans as to cause us to see a continuous moving image. For any organism that has a flicker rate equal to or lower than that frame rate (usually 30 fps), the screen will likely appear to be playing a series of interrupted still images. Many fish studied by ichthyologists have a flicker rate lower than 30 – 35 fps (Rowland 1999). We attempted presenting video recordings filmed at both 30 and 60 fps. However in neither instance did an individual react to the footage. For recordings filmed at 60 fps, it must be noted that resolution was automatically (a feature of the camera) reduced by half. As a result, for these clips, low resolution might have been responsible for the absence of reaction or response on behalf of individuals. The flat screen of a laptop also presents a potential problem for the viewing fish. For flat screen images perception of color varies depending on the angle of perception of the subject viewer (Baldauf et al 2008). This is not the case for the older Cathode-ray tubes (CRT) screens. We attempted to use a CTR screen to display the same footage, but the results were the same – no reaction from any individual.

Despite several obstacles and limitations encountered during these first two experimental attempts, there are nonetheless several accomplishments worth noting. Firstly, we were able to determine an effective, efficient and low impact method to capture individual hamlets from the field. We also were able to keep and maintain individuals in aquaria. We were also able to get some individuals to display mating behavior to individuals in separate aquaria and even to spawn several times when in the same aquarium.

During the third week, we first attempted to test the efficiency of *Hypoplectrus unicolor* (butter hamlet) on glass gobies in the presence of the mimic's model – *Chaetodon capistratus*, the foureye butterflyfish. During the three days we ran the experiment, the butter hamlet ate only 2 out of the 10 gobies in the aquarium. We did not focus a great amount our efforts on this experiment since we had already begun working on the marking project. However, one limitation of this experiment was that the gobies did not appear to be comfortable in the aquarium and did not hide in and around the sponge's structure. Furthermore the butterflyfish were often observed chasing the hamlet from their section of the aquarium. Such behavior is atypical in the fish's natural setting. We had also captured four individuals of both black and barred morphs and placed one of each of these in a separate aquarium. Interestingly we did observe one spawning event between two of these distinct color morphs. Although the significance of such an observation is debatable, given that this was a forced situation, it nevertheless demonstrates that individuals of different morphs will mate together. It is unclear whether there are strong pre or post-zygotic mechanisms preventing hybrids from being viable. We collected the fertilized eggs from the surface of the aquarium and attempted to keep these in the lab. However these never ended up developing into larvae.

The most interesting findings came about during the second part of the final week. We tagged an individual *Hypoplectrus gummigutta* (orange/golden hamlet). We then returned to the reef during the evening and observed the same individual again one the deeper (approx. 35 – 40ft) slope of the reef nears its edge. Soon afterwards, another orange hamlet appeared and these two displayed mating behavior to one another. At the same site, a few meters away, we then observed an individual *Hypoplectrus aberrans* (yellowbelly hamlet). Similarly, this individual was then joined by another conspecifics shortly afterwards. We then observed 8 spawning events between these two. The following morning we returned to the reef and observed the same tagged orange hamlet at the opposite end of the reef. We also came observed and tagged a quite distinct variety of hamlet. This individual was significantly larger than all other hamlets, irrespective of morph, encountered on the reefs in Bocas del Toro to date. Its color pattern resembled somewhat that of the yet described tan hamlet. This individual, like the other rare morphs observed at this reef, was extremely mobile, moving quickly across the reef unlike the highly sedentary and territorial black and barred hamlets. In fact, these individuals were often observed being chased by black and barred individuals. For three consecutive evenings when returning to the spot (we had marked it with an underwater buoy) where we observed the spawning of the rare morphs, we observed the arrival, one by one, of both *H. gummigutta* and *H. aberrans* individuals (those we marked and the same mate). With respect to the transects conducted, these showed that throughout the reef at Punta Caracol, barred hamlets and black hamlets are by far the most common morphs. There were several butter hamlets and 1 – 2 orange and yellowbelly hamlets observed. This rough estimate of the relative abundances of morphs at this particular reef suggests that it is representative of reefs in the area of Bocas del Toro (Puebla et al 2007).

What is so interesting about these findings is that these rare morphs appear to have a set mate and a designated spawning site towards which the fish seem to be able to navigate across wide expanses of reef. There is the potential argument that these individuals are not orienting themselves and navigating to this site and are instead inadvertently being chased there by the territorial morphs. However the reef has a large area of edge and the fact that these individuals return to the same exact spot, and are even spotted at the opposite end of the reef with respect to the former is strong evidence that they indeed do have a designated meeting spot to spawn. Of course, with further work related to this early project, it would be possible to determine, by tagging all individuals, if in fact these are mating with the same mate. In any event, these findings would show that assortative mating in the case of rare morphs might not be as costly as initially proposed by the critics of Gavrilets' (2004) model. In addition, it is interesting to take note of the increased mobility of these rare morphs. The fact that these morphs are rare at present time is perhaps resting in the fact that they are unable to establish themselves alongside the extremely common black and barred hamlets. This might be a factor working to keep these morphs rare. In any event, the findings that these particular works have shown have put together a few more pieces of the puzzle that is the story of this fascinating genus of coral reef fish. More broadly, they also piece together part of the puzzle that is the mechanism of speciation in the most biologically diverse of marine ecosystems- coral reefs.

APPENDIX

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USA

C. *Host Institution: The Smithsonian Tropical Research Institute*

The Smithsonian Tropical Research Institute (STRI) is a bureau of the Smithsonian Institution seated in Washington D.C. The main purpose of STRI is to promote research aimed at better understanding tropical ecology and conservation both in terrestrial and marine ecosystems of Panama. As an isthmus, Panama presents a unique environment for studying the point of passage and meeting between two different types of fauna and flora: one from North and the from South America. Furthermore, the isthmus provides the ideal setting for studying marine environments given the proximity of both Pacific and Caribbean coasts as well as the numerous offshore archipelagos that dot both of these.. Although the STRI is based in Panama, the institution also conducts some research in several other locations within the tropics. The Institute's scientists are working approximately 40 tropical countries around the world. The STRI was created after the Smithsonian Institute began operating in Panama during the creation of Gatun Lake and Barro Colorado Island. A small field station was established on this island in 1923. The Institute has since become a world-wide leader in the field of tropical ecology and biology. The STRI employs more than 38 staff scientists. Every year, more than 900 scientists come to work, study or visit STRI facilities in Panama as part of their research. The STRI provides many facilities to scientists, fellows and visitors. These include among others, laboratories equipped with molecular and genetic analysis tools, access to cars and boats, access to canopy cranes, on-site dormitories as well as access to diving equipment. The STRI holds several research stations throughout the country, a central library in Panama City, four marine laboratories as well as fellowships and other sources of funding to scientists and/or visiting students. STRI objectives are also to disseminate the research results, encourage public education programs and train future tropical biologists through fellowships, internships, field courses, seminars and workshops. Research conducted at STRI includes not only biological sciences, but also social sciences, in order to build a better understanding the human aspect in tropical environments. Areas of research include animal behavior, plant ecology, canopy biology, paleoecology, archaeology, evolution, genetics, marine ecology, anthropology and conservation science. The institution is also involved extensively in conservation efforts in Panama and in other locations outside the country. For example, they are directly involved in the applied conservation research for the Biological Dynamics of Forest Fragments Project near Manaus, Brazil. Furthermore they are also implicated with other such initiatives aimed at protecting forests of Central Amazonia. More specifically related to our field of study (marine environments) the institution is greatly involved in the protection of coastal marine environments of the country, notable through the work of Dr. Hector M. Guzman, a staff scientist. A key contribution to the conservation of marine and terrestrial environment was also the establishment and management of marine protected areas, including Coiba National Park and the Las Perlas Archipelago, in cooperation with Panama's national environmental agency (ANAM).

As visiting students, we had the opportunity to use the facilities provided by the Bocas del Toro Research Station located on Isla Colon. This station primarily provides access to marine environments such as coral reefs, sea grass beds and mangrove areas. Nevertheless, it also provides access to adjacent tropical wet forests and the diverse plant and animal communities that comprise them. This station also provides tours, courses to student groups and educational activities, such a comprehensive program in research and education on both marine and terrestrial environments. Bocas is geographically located in a rich area of islands, mainland bays, forested mountains slopes an rivers, but is also actually actively concerned with socio-political issues of agriculture, growing tourism, fisheries, endangered species such as sea turtles. Bocas is therefore an ideal site to study issues of sustainable multiple uses. Since the region is rich in rocks and fossils, the history of the formation of the isthmus is of interest for paleoecological scientists. STRI researchers topics have included mass coral spawning, turtle nesting, the impact of fisheries on conch and sea cucumbers, chemosymbiotic clams, and mangrove productivity[1].

D. *Summary of the activities: project and field work*

Week 1: Capturing individual hamlets and preliminary behavioral observations

We captured individuals of both barred and black hamlets and attempted to carry out mate choice experiments using aquaria. We placed each individual in a separate aquarium and measured the amount of time spent on either side of the tank.

Week 2: Experiments using video playback and measuring reactions of individuals of H. puella and H. nigricans

The same activities carried out during week 1 were carried during the second week of internship. However behavioral observations were not made for one individual choosing between 2 others, but rather between of one hamlet’s reaction to video playback of another displaying hamlet (for more detail, see Methods section).

Week 3: Determining the efficiency of the butter hamlet on its prey (glass gobies) in the presence of the model foureye butterflyfish and tagging hamlets

The clove oil, weighted casted net and fishing methods were used to collect foureye butterflyfish, gobies and black, barred and butter hamlets. We also observed and tagged individuals of rare morphs at Punta Caracol reef and returned every evening to try and observe spawning behavior in the field. We also conducted transects at this same reef to determine relative abundances for each morph.

The days not spent in the field were devoted to the search for ways to obtain replica hamlet models among artisans in Panama City. However this was unsuccessful, since most of the carved fish encountered came from abroad and none could be custom-carved on-site in Panama. Our supervisor ordered the material to make lifelike replicas from another country, but this material was only set to arrive in June. The remainder of time were used to research and read relevant literature. We also used this time to write and prepare the various reports, presentations and papers required for the associated course (ENVR 451).

Table 1: Summary of the number of day dedicated for the project and in the field

	Days in the field	Days spent on the project
1st Internship week	7	7
2nd Internship week	6	6
3rd Internship week	9	9
Other weeks	0	12
TOTAL	**22**	**34**

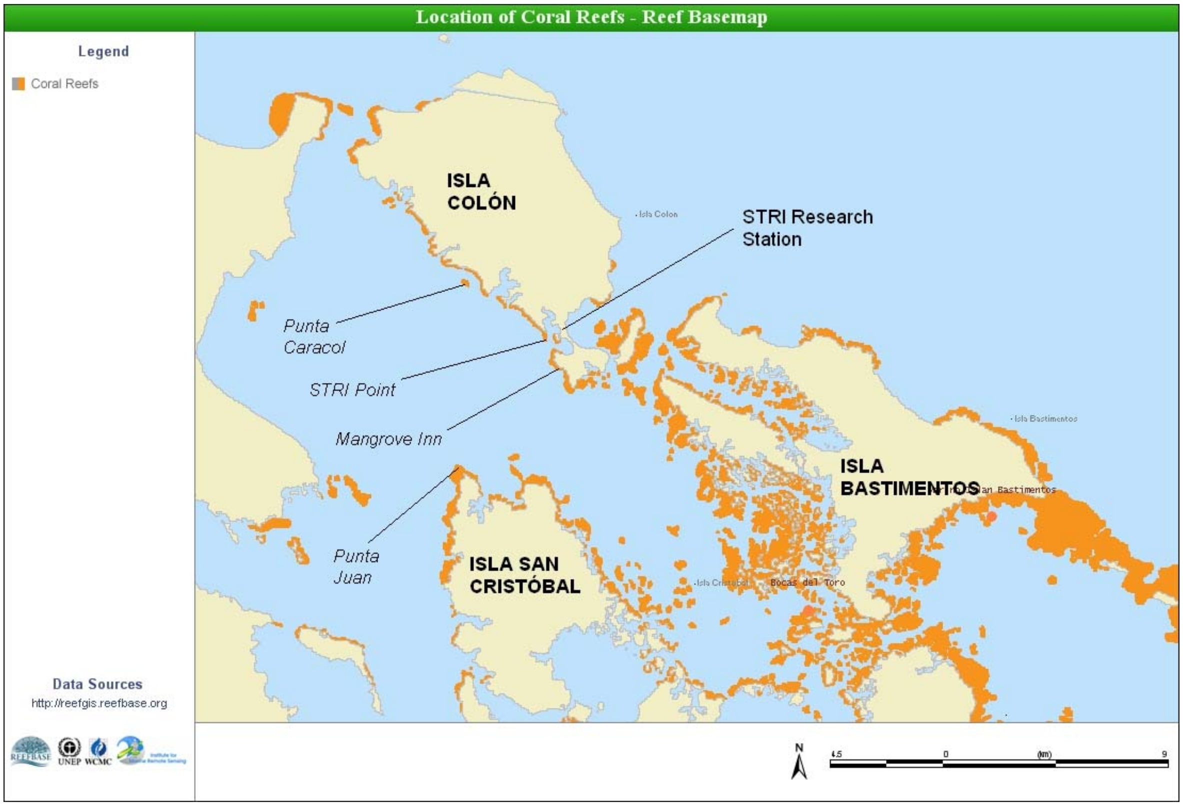

Fig.1 ReefBase-generated map of areas where coral reefs are found in the western archipelago of Bocas Del Toro, Panama. Coral reefs are represented in orange and those visited for the purpose of this study are listed and indicated.

Fig. 2 Barred Hamlet – *Hypoplectrus puella*

Fig. 3 Barred Hamlet – *Hypoplectrus nigricans*

Fig 4 Butter Hamlet – *Hypoplectrus unicolor*

Fig. 5 Foureye Butterflyfish – Chaetodon capistratus

Fig. 6 Orange Hamlet: *Hypoplectrus gummigutta*

Fig. 6 Yellowbelly Hamlet: *Hypoplectrus aberrans*

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