REACTIONARY BEHAVIOR OF A GOBIOID FISH, *VALENCIENNEA STRIGATA*, TO ANTHROPOGENIC AND NATURAL BURROW BLOCAKAGES

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Abstract. Human material pollution, especially plastic, is increasingly being introduced to marine habitats ranging from the deepest benthic trenches on our planet to isolated atolls. Studying the impacts this has on animal behavior will better inform societal pressures and management decisions surrounding global waste management. This study creates an ethogram, a framework for quantifying behavior, for the gobioid fish, *Valenciennea strigata*, and subjects mated pairs of these fish to burrow blockages of a range of materials to determine if there is a discriminatory reaction to materials originating from human sources. Shoreline macro plastic pollution, which is larger than 5mm, was quantified at specific sites on Mo'orea, French Polynesia, following methods from a previous study to compare the abundance of shoreline macro plastics between 2016 and 2018. This study found a clear difference in the reactionary behavior of *V. strigata* when subjected to burrow blockages of anthropogenic materials vs. their habitat substrate. Plastic pollution remains pervasive at these study sites in Mo'orea.

Key words: plastic; pollution; ethogram; reactionary behavior; burrow; goby

INTRODUCTION

Nesting behavior and monogamy is relatively rare among fish, having been described in only 18 fish families (Whiteman and Cote 2004). Information is relatively scarce on the burrowing, fossorial mode of life adopted by some fish, in particular, gobioid fish (Atkinson and Taylor 1991).

On the shallow reefs of Mo’orea, French Polynesia and along the shores of the Indo-Pacific lives a demersal, burrowing, monogamous fish in the Gobiidae family, *Valenciennea strigata*, also called the blue streak goby, or 'O'opu in Tahitian (Reavis 1997, Randall 1973). The adult male and female are often found swimming in tandem. These mated pairs of fish construct shelter for their eggs, which the female lays every 13 days beneath the coral rubble, while maintaining multiple burrow entrances within their territory. Sand-burrowing gobies carry loose sand in their mouths and large pieces of shell and coral in their jaws, ejecting this material beside the burrow opening (Hudson 1977). These fish seal themselves in their burrows at night, closing their primary burrow with rubble and algae approximately an hour before sunset, assumedly to avoid nocturnal predation. Animal burrows with entrance ornamentation have an anti-predatory function in nature (Williams et al. 2006). How often must these fish contend with burrow blockages? Coral rubble can change its accumulation by up to two meters in depth at specific localities over the course of a year due to tides, currents, and storms (Shannon et al. 2012). Moving water flowing through a reef displaces substrate and wave action removes algae from rocks (Stewart 2006). Burrow blockages must play a significant role affecting the daily behavior of *V. strigata*, especially as they leave their burrows exposed, often containing a brood of eggs, behind them as they forage for food in the reef sediment.

V. stingata divides parental labor between the female, who spends greater spans of time feeding to produce eggs, and the male, who may fast for days while protecting clutches of eggs and excavating and re-excavating their burrow (Pratchett et al. 2006). *V. strigata* has been observed excavating human closed burrow entrances indicating that they maintain burrow entrance fidelity under human observation. Animal burrow entrances provide a unique location to present animals with different materials obstructing their path, forcing a resulting behavior from the animal that has been separated from its dwelling. The distinct burrowing behavior of *V. strigata* and its monogamous approach to burrow maintenance make it ideal for study.

Anthropogenic refuse is being continuously introduced into the world's oceans and is rapidly accumulating (Critchell and Lambrechts 2016). Many studies have investigated the effects of plastic pollution on marine life, which have documented more than 50 species of marine fish ingesting micro plastics (Sevoca et al. 2017). How will marine species deal with the plastic and other non-biodegradable materials humans have dropped into the sea to circulate for eons?

Are some reef fish able to recognize and react differently to materials in their habitat that originate from human waste streams as opposed to materials that make up the natural substrate of their habitat? *V. strigata* is a reef fish whose habitat is highly localized, facilitating observations of fish behavior by confronting individuals with materials foreign to the fish's habitat.

The objectives of this study are to establish an ethogram for the behaviors of *V. strigata*, to use ethological categorization to determine if variation exists in reaction time and reactionary behavior of *V. strigata* when exposed to native substrate vs anthropogenic burrow blockages, and to determine if that variation correlates to the average size of parent fish and the average size of burrow entrances. In addition, a survey of anthropogenic non-biodegradable material pollution was completed.

METHODS

Blockage materials, used as treatments in this study, were collected from anthropogenic refuse found on beach surrounding the Richard B. Gump Research Station, located at (-17.490329°, -149.826226°). *Turbaria ornata* algae was taken from the mats it forms on the shoreline and coral rubble samples were taken from the benthos surrounding *V. stringata* burrows on Gump Reef. The materials representing the natural substrate in which *V. strigata* burrows, were collected and standardized to 15 grams ± 0.05 grams. The same standard was applied to blue plastic polyethylene bottle caps and steel metal bottle caps, representing common forms of trash found in this habitat. To account for the positive buoyancy of plastic, rocks were zip-tied with white polyethylene ties into the caps within the mass limit. Trail tests of other blockage materials were also performed, but not statistically tested, and are reported in the results section. Fish behaviors were recorded on an underwater slate and GPS data was recorded with a GoPro camera to prevent pseudo replication of data.

Procedure followed searching for mated pairs of *V. strigata* around the shallow reefs of Mo'orea at sites where they had been previously identified to inhabit in Reavis (1997) and by incidental encounters. Observations for fish behavior were done between 09:00 and 16:00, between 12 October 2018 and 14 November 2018, assuming a random distribution of reactionary behaviors throughout the day.

When encountered, the fish were observed and their behaviors were recorded, making note of immediate behaviors under human observation for a period of 5 minutes. Coral rubble, *Tubanaria ornata* algae, blue polyethylene plastic bottle caps, and steel metal bottle caps, were the four treatments of burrow blockages selected for their uniformity in volume and distribution of mass across the pieces expected to be moved by the fish. The sequence of application of blockage treatments to each burrow was randomized. The treatments were placed at the immediate entrance of each burrow, blocking entry for the fish, but not entering the burrow itself more than 2-3 centimeters. Timing of treatments and reactionary behaviors were recorded for each treatment, according to ethological categories for each treatment, see (Table 3). If the treatment was not touched by the fish after 10 minutes of observation, making sure to record events of burrow investigation by the mated pair, the treatment was removed and considered a null response.

Pictures were taken of burrows to calculate approximate proportions for the distribution of natural substrate on the reefs within a square meter surrounding the burrow and to measure approximate burrow entrance sizes and fish sizes to determine if the distribution of these metrics correlated to any observed behavior, both measured in the software, ImageJ (Schneider et al. 2012). A bottle cap of known size in the image was used as reference to calculate the size of the burrows and fish, see (Fig. 2; Appendix A). A quadrat was used in the image midway through data collection to facilitate image analysis. Sample videos were taken of categorized behaviors for assigning ethological categories to specific examples of behavior. While searching for the fish, anthropogenic plastics and other non-biodegradable refuse was recorded by presence or absence during the swim to reach each site. Along kilometer markers found on Mo'orea, 5 minute transects were walked along the shore to count pieces of plastic to evaluate the prevalence of plastic and other refuse exposed to the habitat of *V. strigata*. See (Fig 3; Appendix 3) for a map of these transects. These data were compared to the same abundance data collected by Elizabeth Connors two years prior (Connors 2017).

Study sites

GPS coordinates were taken for all 25 burrows studied with error of ± 5 meters on the island of Mo'orea, clustered around three localities: Ta'ahiamanu Public Beach, Piha'ena Public Beach, and Tema'e Public Beach (Table 4; Appendix A).

Data Analysis

Alpha α values for these analyses are set to 0.01. Chi squared tests were run for counts of overserved removals of burrow blockages by type of material using the software, PAST 3.0 (Hammer et al. 2001). A two sample paired t test was run between the plastic abundance data from 2016 and 2018. A pie charts of the proportions of observed ethologically categorized fish behaviors to total time observed was created. Average sizes of observed *V. strigata* and burrow entrances were found. Percentages of types of substrate within the square meter surrounding photographed burrows were tabulated.

RESULTS

Ten trials were run with blue polyethylene bottle caps and coral rubble, and 15 trials were run with plastic caps, coral rubble, *Turbanaria ornata* algae, and steel metal bottle caps. Of the 25 trails run with plastic caps and rocks, the rocks were moved 24 times and the plastic was moved only once by the pairs. The 15 trials run including algae and metal bottle caps resulted in 14 removals of algae and 5 removals of metal bottle caps. The removal of all burrow blockages was expected across all tests performed.

TABLE 1. Chi-Squared Test Results

Comparison	χ2	*p*-value
Rubble vs Plastic	15.571	< 0.001
Rubble vs Algae	0.0036	0.95
Rubble vs Metal	3.352	0.067
Algae vs Plastic	13.644	< 0.001
Algae vs Metal	2.701	0.1
Plastic vs Metal	4.459	0.034

Grouping these data to compare the anthropogenic burrow blockage materials and the natural substrate blockage materials gives the following: $\chi 2 = 16.086$, p-value <0.0001.

Both types of anthropogenic materials and both types of natural substrate removals were grouped together because the frequencies of removal for the plastic and metal bottle caps are not significantly different from each other, and the frequencies of removal for the coral rubble and algae are not significantly different from each other.

Multiple preliminary trials were run with 15 gram treatments of green fishing line, a fishing lure, and a yellow plastic bag. None of these treatments were touched by the fish within the 10 minute reaction time limit.

While studied, the pairs of *V. strigata* exhibited caution and constant awareness of being under observation. At just about all times, at least one of the parent fish kept this researcher in their line of sight. After burrow blockages were applied, every pair but one investigated each blockage within two minutes.

Over the course of this study, a few interspecific interactions between the burrows of *V. strigata* and other reef fish were recorded. *Balistapus undulatus*, the orange-lined triggerfish, was observed pecking at the blue plastic bottle caps three separate times after the parent fish had left the immediate area surrounding their burrow to feed after they had inspected and left the plastic burrow blockage. Perhaps these *B. undulates* are acting opportunistically to predate these burrows or to investigate the new brightly colored objects present in the territory they maintain. *Gomphosus varius*, the bird wrasse, was recorded circling and inspecting the burrows of *V. strigata* four times with multiple *G. varius* individuals seeming to specifically appear when the burrows of these gobies were potentially more vulnerable while under human observation, due to resulting distraction of the parent *V. stigata*. The snout of *G. varius* is elongated and aids the wrasse in eating small invertebrates that hide between the grooves of coral heads (Randall et al 1997). *G. varius* are also known to eat small fish. The size of the *V. strigata* burrow entrance and the depth of the burrow could be an evolutionary selection pressure affecting the length of the *G. varius* snout (Darwin 1871). These observations require further study to quantify these interspecific interactions.

The larger goby removed the burrow blockage in 21 of the 30, or 70%, of the burrow blockage removals recorded. The average *V. strigata* of the 15 pairs that were photographed was 153mm in length and had burrow entrances that were on average 93mm across. Neither of these metrics for size had any found correlation with behavior.

The average percent coverage of algae in a square meter around the burrows photographed was 15%, indicating how prevalent this material is as a potential burrow blockage for these fish.

TABLE 2. Abundance data from 5 minute plastic surveys conducted at sites from Connors (2017)

Latitude	Longitude	2016	2018
-17.491583°	-149.849983°	71	48
-17.482833°	-149.810150°	106	22
-17.507338°	-149.820670°	54	35
-17.491583°	-149.849983°	52	43
-17.485529°	-149.831277°	37	41
-17.490447°	-149.826035°	20	29

A two-sample paired t test for the plastic abundance data gives a t Statistic =1.4825 and p-value =0.1983.

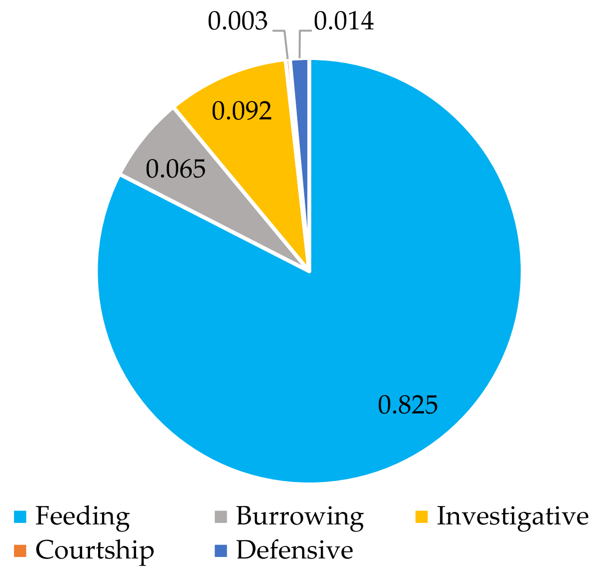

FIG 1. Proportions of Observed Grouped Behaviors across All Trials

TABLE 3. Ethogram of Observed *V. strigata* behaviors

Behavior Category	Grouped Behavior	Definition
Gill Filtering Sand	Feeding	The mated pair swim in tandem, never separating more than two meters, and alternate dipping their mouths into the sand every 10-20 seconds. The mouthful of sediment is then filtered through the fish's gills, extracting small invertebrates for ingestion.
Attempt to Access Burrow	Investigative	One or both fish approach a blocked burrow entrance and hover as close as a centimeter or less away from the blockage.
Lunged at Blockage	Defensive	After inspecting a burrow blockage, a single fish will quickly lunge at a burrow blockage from up to 30 centimeters away, dodging just before impact.
Flaring gills	Defensive	Gills are quickly and rhythmically flared while the mouth is open, causing the reflective blue and yellow coloring of the fishes jaw to flash.
Triggered Dorsal Fin	Defensive	A dorsal fin that is normally lowered, flush with the goby's spine, is raised, resulting in a display. Often seen at the same time as gill flaring.
Paired Circling	Courtship	Paired gobies bend their bodies into an "S" shape and swim, circling each other, following the other's tail.
Burrow Excavation	Burrowing	A single goby will enter the pair's burrow and emerge seconds later with a mouthful of sand that is then ejected from the fish's mouth up to 50 centimeters from the burrow entrance.
Remove Burrow Blockage	Burrowing	Pairs of goby will alternate grasping objects blocking the entrance to their burrow with their mouths and flinging their body to send the blockage away from the entrance.
Evening Burrow Sealing	Burrowing	At roughly an hour before sunset, the mated pair will pull pieces of *Padina spp*. algae and coral rubble around their burrow entrance, both gobies will enter the burrow, and they will close their burrow with them inside of it, assumedly until morning. No nocturnal behaviors have been documented.

Discussion

These data indicate that there is a statistically significant difference between the observed rate at which *V. strigata* interacts with burrow blockages of materials originating from their habitat substrate vs anthropogenic materials. Several variables across the material qualities of plastics, coral rubble, algae, and metals; including color, physical texture, and chemical cues, likely notify the gobies that these materials are different. As plastics are exposed to UV radiation and salt water, "degradation of plastic polymers can lead to low molecular weight polymer fragments, like monomers and oligomers, and formation of new end groups, especially carboxylic acids" that leach into marine habitat (Gewert et al. 2015). These gobies could use chemosensory tissues to detect the polyethylene used as treatments that were left in plastic bags containing salt water, sometimes in the sun, between trials (Whitear 1992). The decision that the gobies make to remove the blockage could be informed by the degree of familiarity the fish have with the material in question. Coral rubble and algae are ubiquitously present in the habitat of these fish, algae was found to make up 15% of the coverage in a square meter surrounding these burrows. Plastics and other forms of anthropogenic trash have been shown to be present in the habitats sampled, specifically quantified in 2016 and 2018. The *V. strigata* that were observed likely only rarely encounter anthropogenic material pollution actually inside of the immediate habitat in which they burrow. The mated pairs were observed closely inspecting the plastic blockages, but all except one pair of gobies determined that not moving the plastic was preferred to regaining access to their burrows.

The eggs that the pairs of *V. strigata* lay every 13 days certainly act as incentive for these gobies to maintain burrow entrances that were repeatedly closed in this study and over the course of a normal day for these fish. No eggs were ever seen during this study because human excavation would have likely resulted in the abandonment of the eggs and the burrow by the mated pairs. One observation was made of five juvenile fish that were no longer than 5 centimeters swimming in the entrance to a burrow that appeared to be dug by a nearby pair of adult fish because of the burrow's size. These young fish were already exhibiting the paired swimming behavior. One pair of adult fish was observed spending more time than average following the courtship behavior described in the ethogram and did not remove either rocks or plastic burrow blockages. This might have been because this pair had not yet laid eggs in their burrow. The one pair of gobies that removed the plastic blockage did so at 9m50s, exposing the possibility that more removals could potentially be seen if the time until "null response" was extended. The observation period for each blockage was changed to 15 minutes for the remaining 13 trials. There were no additional removals of the plastic blockages and all other overserved burrow blockage removal occurred in under 10 minutes. Whether or not *V. strigat*a eggs have been laid, or how developed these eggs are, likely act as a variable controlling how committed the parent gobies are to maintaining specific burrow entrances.

Considering the interactions observed with *G. varius* and *B. undulatus* in the field as attempts at predation, another way to frame the decision making that the *V. strigata* parents must make in this experiment is to apply landscape of fear terminology. The landscape of fear is the fluctuating perceived predation risk associated to a defined space that a prey species inhabits and that correlates to changes in the behavior of that prey species (Catano et al. 2016, see also Laundré et al. 1999). It can vary with changing abiotic and biotic environmental conditions. Here the burrow blockages seem to have had different effects on the landscape of fear localized to each burrow entrance. The blue plastic caps increasing the parent goby's cautionary response and resulting in temporary burrow abandonment, while the blockages made of substrate materials seem to have not altered the landscape of fear surrounding the burrow resulting in immediate removal of these blockages by the fish. Additionally, the online database, FishBase, reports *V. strigata* as occupying a higher trophic level than either *G. varius*, or *B. undulatus* which these observations seem to refute (Capuli 2018).

Plastic pollution in the ocean is a growing problem with no tangible solutions in sight. "Over three-quarters of the GPGP, Great Pacific Garbage Patch, mass was carried by debris larger than 5 cm and at least 46% was comprised of fishing nets." (Lebreton et al. 2018). "Plastics will continue to be input into the sedimentary cycle over coming millennia as temporary stores – landfill sites – are eroded," (Zalasiewicz et al. 2016). The micro plastics generated from our waste will likely serve as the geologic marker for the Anthropocene, the geologic epoch of time in which humans have come to dominate many surface geological processes." Life on Earth will have to contend with plastics present in the environment for eons to come. This study only quantified macro plastics present at specific field sites and these sites have multiple anthropogenic factors influencing the real amount of pollution these gobies might encounter and may not be representative of all of the habitat that *V. strigata* occupies across the Indo Pacific. The presence of these plastics is pervasive in areas specifically set aside as Marine Protected Areas and in a country that places a high priority on environmental awareness and holds regular beach cleaning days (Te mana o te moana 2004). It is assumed that much of this pollution originates from the tourism industry present on Mo'orea.

Future research could attempt to prove that *V. strigata* can ingest micro plastic if it is made available within the sediment that these fish filter feed on. Testing other materials at the entrance to *V. strigata* burrows could give us a better understanding of how these fish perceive objects in their habitat. Research into how plastics are effecting many levels of ecology both on land and in the sea is surely going to compose a growing field of literature until we are able to curtail our demand for single use plastics and remove the waste that we have already left in our oceans.

Acknowledgments

Mãuruuru roa to the Gump Station, the Atitia Center, Dr. Stephanie Carleson, Dr. Cindy Looy, Dr. Ivo Duijnstee, Dr. Vincent Resh, Renske, Jim, Clay, and my friends both on the island and who housed me in Berkeley before and after leaving for French Polynesia.

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APPENDIX A

TABLE 4. Locations of *V. strigata* burrows ± 5m, reported in the sequence they were found.

Latitude	Longitude
-17.492283°	-149.850913°
-17.485505°	-149.833104°
-17.490012°	-149.825833°
-17.498922°	-149.760996°
-17.498775°	-149.760792°
-17.490285°	-149.825780°
-17.499130°	-149.760990°
-17.498838°	-149.761234°
-17.499012°	-149.761175°
-17.499286°	-149.760689°
-17.491499°	-149.851056°
-17.499458°	-149.760797°
-17.499110°	-149.757578°
-17.499121°	-149.757218°
-17.499263°	-149.757918°
-17.491656°	-149.851088°
-17.491048°	-149.850738°
-17.491193°	-149.850857°
-17.491169°	-149.850802°
-17.499090°	-149.760379°
-17.499551°	-149.760516°
-17.491975°	-149.851067°
-17.489759°	-149.825712°
-17.498974°	-149.760562°
-17.489093°	-149.825945°

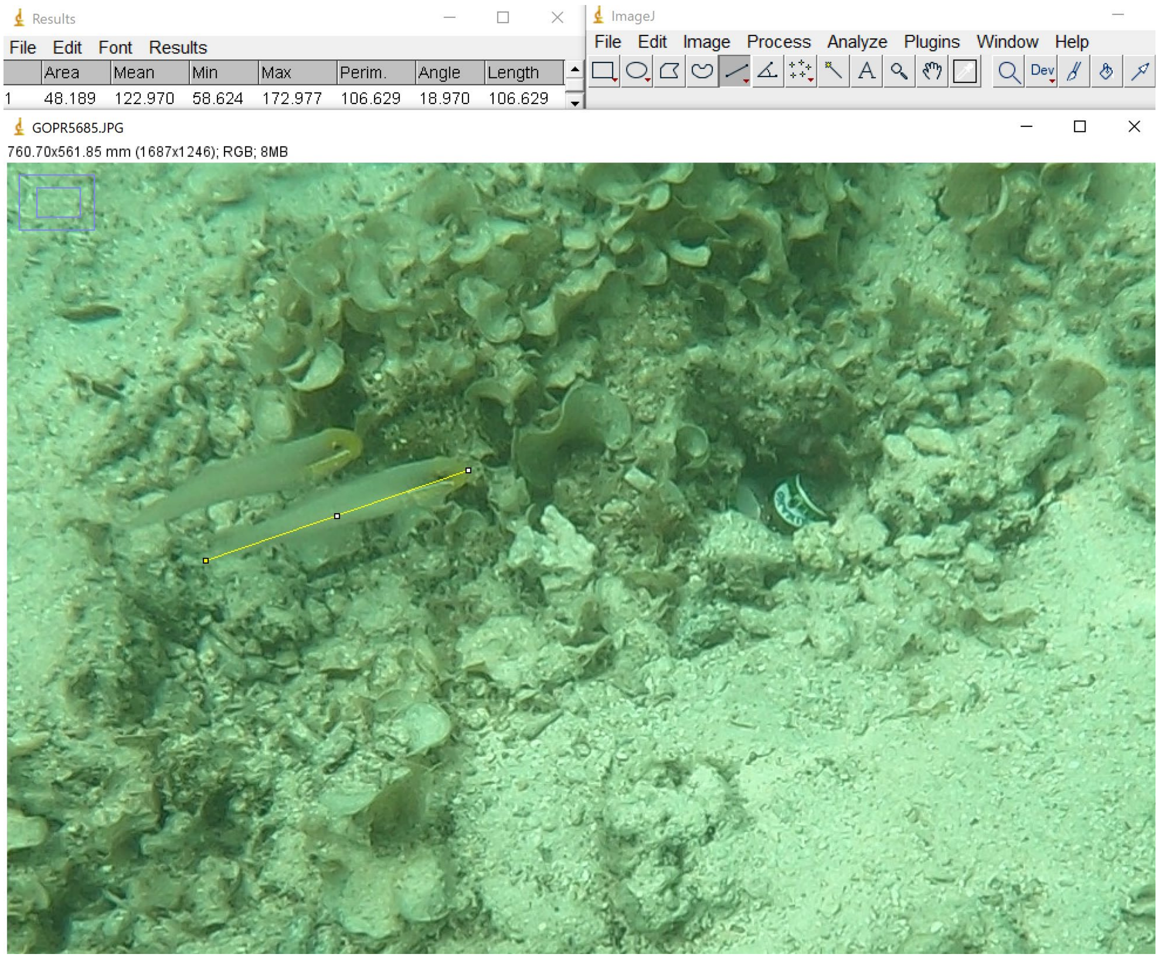

FIG. 2. Example ImageJ analysis of fish and burrow size metrics

FIG. 3 Map of macro plastic survey sites following locations defined in Connors (201