AN ABSTRACT OF THE THESIS OF Summer A. Andrada for the Master of Science in Biology presented July 28, 2017.

Title: ARE COWRIES ECOLOGICAL CONDUITS FOR NATURALLY OCCURRING POLYBROMINATED DIPHENYL ETHERS TO ENTER MARINE FOOD WEBS?

Approved: ____

Jason S. Biggs, Chair, Thesis Committee

Abstract:

Every day, large volumes of chemical compounds are emitted into the environment by anthropogenic activities. Many of these environmental pollutants resist natural removal and reduce the fitness of organisms in the wild. One class of great concern are the persistent organic pollutants (POPs), which are chemically stable, lipophilic compounds that often accumulate in fatty tissue and become concentrated within food webs. Commonly used POPs include pesticides, industrial chemicals, and combustion by-products. This study evaluates polybrominated diphenyl ethers (PBDEs), which are chemical additives used to reduce the flammability of fabrics, electrical circuits, appliances and furniture. PBDEs have been detected in environmental compartments all over the world, including human breast milk and fetal livers. Studies have reported that naturally occurring PBDE analogs are created by a cyanobacterium, *Hormoscilla spongeliae*, in symbiosis within the marine sponge, *Lamellodysidea herbacea*, and that these natural PBDE analogs pose an even greater threat to wild organisms than their manmade counterparts. However, we have no idea how, or how much natural PBDEs enter marine food webs. Currently, no organisms have been identified as entrance points for these compounds. This study conducted a preliminary evaluation of PBDE uptake and clearance in the money cowry, *Monetaria moneta*, which could be one such point of entry. Results indicate that *M. moneta* readily consumes *L. herbacea*, and accumulates PBDEs as a result, suggesting that cowries might be one direct pathway for naturally occurring PBDEs to enter the marine food webs. By accumulating within the prey of species that humans consume, PBDEs can reach levels unfit for human consumption.

TO THE OFFICE OF GRADUATE STUDIES

The members of the committee approve the thesis of Summer A. Andrada presented July 28, 2017.

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ARE COWRIES ECOLOGICAL CONDUITS FOR NATURALLY OCCURING POLYBROMINATED DIPHENYL ETHERS TO ENTER MARINE FOOD WEBS?

BY

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ARE COWRIES ECOLOGICAL CONDUITS FOR NATURALLY OCCURING POLYBROMINATED DIPHENYL ETHERS TO ENTER MARINE FOOD WEBS?

INTRODUCTION

Environmental pollution continues to rank among the greatest challenges humans face today. Every year, anthropogenic chemicals increase in prevalence within coastal ecosystems and the deleterious effects they trigger are becoming more and more apparent. The biological effects often reach far beyond adjacent ecosystems, and some are beginning to enter humans through drinking water and food webs. With continuing improvements in analytical methods, new chemicals of environmental concern are being identified almost annually. Such discoveries are paralleled by an increasing number of potential consequences for ecosystems and the species which encounter them. This ultimately affects humans by destroying or diminishing valuable food resources, or by accumulating in edible species to levels that render them unfit for human consumption. In fact, the US National Library of Medicine maintains a database dedicated to the study of chemical pollutants and raising public awareness of the dangers associated with chemical biomagnification.

Chemical pollution, by definition, occurs when one or more manmade compounds enter an environment, resist natural removal, and cause damage to, or reduce the fitness of, one or more organisms (Rhind 2009; Potters 2013). Among them, concern regarding persistent organic pollutants (POPs) grows, as they are highly stable compounds found to be accumulating in the environment. POPs are highly recalcitrant, lipophilic compounds that are typically biomagnified by food chains, reaching especially alarming concentrations within the charismatic megafauna found at the top of tropical marine food webs (Jones and de Voogt 1999; Vallack et al. 1998; Siddiqi et al. 2003).

One persistent and commercially-produced class of POPs gaining global attention are the polybrominated diphenyl ethers (PBDEs). These compounds are widely used as industrial flame retardants added to a multitude of products, including several polymers and resins used to manufacture electronic circuits, plastics, and fabrics (Meerts et al. 2001; Lee and Kim 2015; Wiseman et al. 2011). Although US production of PBDEs started in the 1960s and peaked in the late 1990s, their detection in humans and the environment has only recently gained attention. PBDEs within the environment have the propensity to lose their bromine moieties, which not only creates more toxic congeners, but also causes them to be even more persistent and potentially able to disperse over greater distances (Siddiqi et al. 2003; Kimbrough et al. 2009). In response, many U.S. manufacturers phased out the production of commercial penta- and octa-BDEs in their products in 2004. Several states followed suit by banning and limiting the usage of commercial PBDE congeners. Though penta- and octa-BDEs are no longer in use, deca-BDEs are still being produced in the U.S. (U.S. EPA 2010).

In 2003, global estimates of PBDE production totaled 56,400 metric tons (US EPA 2010). Although considerable amounts are released during the production, use, and disposal of PBDE-treated furniture and appliances (Lee and Kim 2015), there are several other ways in which PBDEs will continue to enter the environment. Federal law requires that all furniture be sprayed with PBDEs to lessen their combustibility, so when household wastes are deposited into landfills or incinerators, their PBDEs become released into leachates and evaporate into the atmosphere. Once airborne, PBDEs can travel long distances, including the Arctic where they threaten marine mammals as endocrine disruptors (Siddiqi et al. 2003; de Wit et al 2005; Jenssen 2006). This problem is so real that PBDEs have now been detected in all environmental compartments, all over the world (Agrawal and Bowden 2005; Lee and Kim 2015; Zhang et al. 2016).

Although PBDEs do not appear to be acutely toxic to humans, they are known to potentiate liver tumors, inhibit neurodevelopment, cause thyroid dysfunctions and gastrointestinal problems, and lead to endocrine disorders (Siddiqi et al 2003). PBDEs have also been detected in human tissue, including lipocytes (Fernandez et al. 2007; McDonald 2005), blood (Schecter et al. 2007), and most alarming of all: breast milk and fetal livers (Schecter et al. 2003). For these reasons, PBDEs have been designated to be of major global concern. Aquatic systems seem to be the main recipients of many POPs, and such habitats are now becoming recognized as the predominant global environmental PBDE sinks (Wiseman et al. 2011). Although routes for PBDE absorption in humans include inhalation of contaminated air and dust, and absorption through the skin, PBDE concentrations found in human plasma and breast milk seem to correlate most closely with the dietary consumption of marine organisms (Sjödin et al. 2000; Schecter 2003). Therefore, understanding the major pathways by which PBDEs gain entry into marine food webs, and how that ultimately affect their prevalence in human breast milk and fetuses, are key to addressing the ecological and human health concerns of many POPs.

But environmental PBDEs are not simply consequences of the industrial/chemical revolution. It has long been known that PBDEs are also created by a symbiotic cyanobacterium, *Hormoscilla spongeliae* (formely *Oscillatoria spongeliae)*, in its association with the marine sponge family Dictyoceratida (Sharma and Vig, 1972;

Faulkner et al. 1994; Hinde et al 1994; Unson et al. 1994; Ridley et al. 2005; Hanif et al. 2007; Agarwal et al. 2017). Naturally produced PBDEs are analogs of artificial compounds, taking the hydroxylated- (OH-BDE) and methoxylated- (MeO-BDE) BDEs structural forms, which incidentally, are more commonly detected in animal tissues (Wiseman et al 2011). Manmade PBDEs have also been reported to undergo biotransformation processes yielding OH-BDEs products, yet these observed rates are exceedingly slow (Wiseman et al 2011). Currently there are many speculations as to the toxicity of OH-BDEs, but studies conducted thus far demonstrate an even greater threat to organisms due to the hydroxyl moiety increasing their affinity for biological receptors (Li et al. 2010). Structural and molecular docking assays using human receptors clearly demonstrate that hydroxylated-BDEs have greater affinity for thyroid receptors than thyroid hormones, which suggests that OH-BDEs potentially interfere with thyroid hormone homeostasis, metabolism, and transport (Meerts et al. 2000). However, investigations into if and how sponge-symbiont-derived hydroxylated and methoxylated BDEs may be entering marine food webs, and whether or not they contribute to the overall human PBDE load is sparse. Given the growing concerns regarding how these OH-BDEs compounds are getting into humans and disrupting our physiology, understanding if and how sponge-derived PBDEs enter marine food webs will be an important factor in mitigating this global threat to human health.

On Guam, only an opisthobranch, *Sagaminopteron bilealbum*, has been documented to feed upon *Lamellodysidea herbacea* (Carlson and Hoff 1973). Not much else is known about the ecology of these animals, but other opisthobranchs are known to use diet-derived chemicals as defensive compounds. Although it is possible that these small (~1 cm) organisms are one avenue for PBDEs to enter marine food chains, other organisms known to prey upon sponges without employing these diet-derived compounds as feeding deterrents (Carlson and Hoff 1973) and thus, would presumably be more readily consumed by predators. One such example are the mollusks within superfamily Cypraeoidea, which are more commonly referred to as cowries (Ponder and Lindberg 1997). Compared with other gastropods, cowries are significantly understudied in terms of their ecological roles in marine ecosystems (Hayes 1983; Osorio et al. 1993), and very little is known regarding the ecological niches they fill.

This work represents a preliminary evaluation of PBDE uptake and clearance in the money cowry, *Monetaria moneta*. Locally, these shelled mollusks have been observed feeding on the encrusting sponge, *Lamellodysidea herbacea* (Biggs, *personal obs*), which harbors extremely high levels of the PBDEs created by its cyanobacterium symbiont, *Hormoscilla spongeliae* (Hinde et al 1994; Unson et al. 1994; Agarwal et al. 2017). On Guam, spatial differences in PBDE presence among *L. herbacea* congeners are known to exist, which tightly correlates with *H. spongeliae* (symbiont) abundance (Agarwal et al. 2017). These site-specific differences in PBDE production within *L. herbacea* present a unique opportunity to explore PBDE pharmacokinetics in marine organisms using cross-treatment feeding trials. In other words, cowries from Piti Bomb Holes, a site where *L. herbacea* is loaded with PBDEs, and Pago Bay, a site harboring *L. herbacea* that is completely devoid of PBDEs, were collected and transferred to the University of Guam Marine Lab (UOGML) flow-through seawater system, where they were fed *L. herbacea* from each location for two months and then analyzed for the presence or absence of PBDEs. By doing so, this study has become the first to demonstrate an ecological pathway for naturally-occurring PBDEs to enter marine food webs.

MATERIALS AND METHODS

Study Organisms

The money cowry, *Monetaria moneta* (Figure 1), is a small sea snail with a porcelain-like, egg-shaped shell with bulbous edges, a flat underside, dark green transverse margins, and a golden yellow brim. Like all cowries, this shell, along with a cryptic lifestyle, serves as its primary defense against predation. The two shell morphologies are known to exist on Guam, a "smooth" and a "knobby" morph, both of which were used in this study. Both morphs are known to feed on algae and sponges, and have been observed hiding under rocks that often also contain, it is just that the smooth morphs are found in intertidal habitats, whereas the knobby morphs are subtidal (Renaud 1976; Hayes 1983; Osorio et al. 1993).

Figure 1. *Monetaria moneta* can be found during the day nestled into the underside of rocks and coral heads. Note the presence of *Lamellodysidea herbacea* on the left.

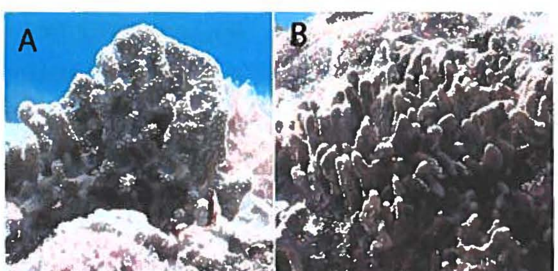

Figure 2. Photographs of representative *Lamellodysidea herbacea* sponges of clades (A) 1b and (B) III, *in situ*. Although these two growth forms closely resemble eacho other, they differ drastically in both symbionts, and the presence of PBDEs.

Agarwal and colleagues (2017) described four clades of *L. herbacea*, two of which (Clades 1b and III; Figure 2) were offered to cowries used within this study. All morphologies of *L. herbacea* are found attached to solid substrates in benthic shallow habitats (Thacker and Starnes 2003; Thomas et al. 2010); however, Clade 1b has only been observed at Piti Bomb Holes and Clade III appears to be restricted to Pago Bay.

Sample Collections and Study Sites

During low tides, *L. herbacea* and *M. moneta* were collected by hand, often in close proximity to one another (i.e. under corals and rocks). Both organisms were collected from two reef flats on Guam (Figure 3). Pago Bay, where Clade III can be found, is located immediately behind the University of Guam Marine Laboratory, and is subjected to minimal anthropogenic and industrial activities. Here cowries were collected from crevices in large boulders with *L. herbacea* growing around them. Piti Bomb Holes, where Clades 1a, 1b, and IV can be found, is a very popular diving spot for locals and tourists. As its name might imply, Piti reef flat is transected by several craters of varying sizes. Specimens belonging to Clade 1b were collected from the tops of large live corals and dead branching corals. Depths where both of these clades were collected ranged from 1-2 m (i.e., subtidal). Once separated from the substrate, each sponge was stored in Ziploc® bags, and transported to the UOGML where they were immediately placed into a flow-through seawater tank. Transit times between Pago Bay and Pit Bomb Holes to the UOGML were approximately five and fifteen minutes, respectively.

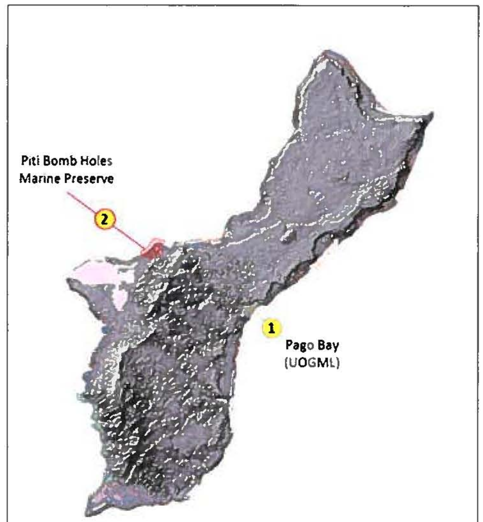

Figure 3. The two sample collection sites are located on opposite sides of the island center. Pago Bay (site 1) faces the Pacific Ocean; whereas Piti Bomb Holes (site 2) faces the Philippine Sea.

The two collection sites were selected due to their differences in polybrominated chemistry found within *L. herbacea.* A recent study was conducted on sponges (*L. herbacea* and *Dysidea granulosa*) from multiple sites on Guam and was able to discover the gene clusters that encode the enzymes responsible for PBDE production (Agarwal et al, 2017). Interestingly, both collections, from Pago Bay (Clade III) and Piti Bomb Holes (Clade 1b) harbored vast amounts of the cyanobacteria symbiont *H. spongeliae*, but the symbiont inhabiting sponges in Pago Bay (Clade III) lacked the gene cassette responsible for PBDE synthesis, resulting in the complete lack of PBDEs within these samples (Agarwal et al. 2017).

Feeding Treatments

Each sponge was blotted dry and weighed at the beginning of each feeding trial. Approximately 3.0 g (wet weight) of *L. herbacea* were placed in each compartment that housed a cowry randomly assigned to one of four feeding treatments. Two treatments consisted of cowries consuming *L. herbacea* from their original habitats; e.g., Piti cowries eating Piti sponges. The other two treatments were cowries that fed from the opposite reef locale; e.g., Pago Bay cowries eating Piti sponges. Each treatment had a total of 10 cowries (n=10). Sponges offered were replenished every three to four days, and the entire trial lasted for two months. Immediately after the trial, cowries were euthanized by placing each in a 20-dram vial, and then into a -20C freezer for four hours. Specimens were then freeze-dried for 24 hr, and sent to Georgia Institute of Technology School of Chemistry and Biochemistry for chemical analyses using LC/MS/MS techniques described elsewhere (Agarwal et al. 2017). This entire experiment was repeated for a second time.

Housing, Maintenance and Preparation for Analyses

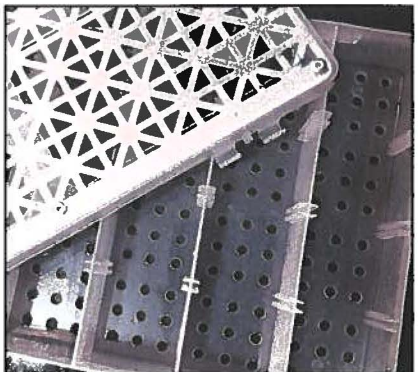

Figure 4. Caged design used to compartmentalize cowries and sponges.

Within the University of Guam Marine Laboratory flow-through seawater system, cowries were stored in two durable Husky® containers, each having 24 cells (1.5" x 1.5" x 1.5"). These plastic containers were modified: an aluminum screen was riveted to the top, and holes were drilled through the bottom of each container (Figure 4). This compartmentalized design directs seawater vertically through each cell, allowing constant exchange of water and removal of debris (e.g., fecal material), while preventing cowries from physically contacting one another. In addition, cages were drained and scrubbed every three to four days (i.e., immediately prior to replacing a sponge) to prevent algae and residual waste from accumulating. It is also important to note that within this experimental design all cowries and sponges all shared the same flow-through seawater tank and thus, the same seawater. This was done to account for the slight chance that PBDEs might leach into the seawater and become passively absorbed by the cowries. If this were to occur, then PBDEs would be detected within all cowries within these trials, regardless of collection site or sponge offered. Given that PBDEs have an extremely high octanol:water partition coefficient (US EPA 2010) and they occur as visible crystals within the wild, it is however highly unlikely that passive diffusion could/would be a factor.

Cowries from both locales were haphazardly distributed among cells, where they remained for the duration of a trial. Treatments (local or foreign sponge) were randomly assigned to each cell. A template was created and used to track what treatment occupied each compartment for each trial.

Control Experiment A: Mass Change

An experiment was conducted to observe how sponge mass would be impacted by the presence or absence of a cowrie. For the first three days of each trial, each cowrie was offered three grams of sponge (wet weight) for a period of 24 hours. At the same time, 3 gm sponges from Pago Bay (Clade III; n = 10) and Piti Bomb Holes (Clade 1b; n = 10) were haphazardly distributed within cells of an identical container without cowries, again for 24 hours. Weights were recorded for each offering by removing a sponge from the water, blotting it dry, and immediately weighing to the nearest hundredth of a gram. Similar, repeated measures studies were conducted for periods of three and fourteen days, respectively (unpublished results). Average mass change of offered specimens were compared among feeding treatments and those without cowries present.

Control Experiment B: Presence/Absence of Polybrominated Phenolic Chemistry

This was done to account for spatial difference of PBDEs in *L. herbacea* from the two sites. And if the presence of PBDEs were detected, the nature of these polybrominated phenolic compounds were observed in terms of whether changes in the chemistry of the sponge is altered or remained constant from before and after 24 h of consumption by the cowrie. Sponges (Clade 1b and III) from both sites were collected (n=10 for each feeding trial) and brought to the UOGML. From the same sponge, a piece was immediately placed in the freeze-dryer while the remainder was pre-weighed and offered to its designated cowrie. After 24 h, the piece of sponge left in the presence of the cowrie was weighed, frozen, and then freeze-dried for 24 h. All "before" and "after" pieces of sponge were labeled and sent to Georgia Institute of Technology School of Chemistry and Biochemistry for further analysis.

Polybrominated Diphenyl Ether (PBDE) Analysis

The specimens were placed in 1:1 dichloromethane:methanol for 24 h. Cowries were submerged with an intact shell, which was never compromised throughout the extraction process. PBDEs were detected using a Bruker amaZon SL ion trap mass spectroscopy and PBDE standards at the Georgia Institute of Technology School of Chemistry and Biochemistry.

Statistical Analysis

All data normalization and analyses were conducted using R (Crawley 2013). A Student's t-test was used to test whether significant differences exist between both control groups; whereas a one-way ANOVA with post-hoc Tukey HSD (honest significant differences) was used to test whether or not the means of several groups were significantly different. The Tukey HSD test compares every possible pairs of means based on a Studentized range distribution – a built-in function in R Studio. This function automatically adjusts sample sizes to produce intervals based on the range of sample means (Crawley 2013). Then a nonparametric binomial distribution test was used to test the likelihood for the uptake and loss response of PBDE in the *M. moneta* within treatment groups.

RESULTS

Preliminary Data (The first cowrie discovered in Piti Bomb Holes)

On June 2016, one cowrie was collected near *L. herbacea* at Piti Bomb Holes and immediately transported to the UOGML. It was then freeze dried for 24 h and sent to Scripps Institution of Oceanography University of California San Diego for analysis. The mass spectra (Figure 5) showed evidence of PBDEs in the cowrie tissue that were similar in molecular structure from a sponge extract that was earlier documented (Unson et al. 1994).

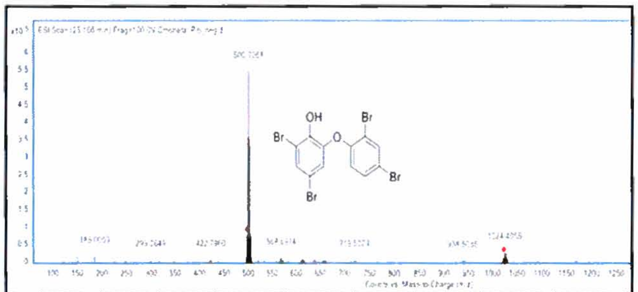

Figure 5. LC-MS mass spectra of a cowrie from Piti Bomb Holes showing the most abundant PBDE present, 2'-OH-BDE-68, with a retention time at 25 mins and with a mass at 500m/z.

In 1969, Sharma et al. reported the first detection of PBDEs from *L. herbacea* collected from the Western Caroline Islands. Then a similar study was done on *L. herbacea* and related species collected from Palau that also documented PBDE structural elucidation of major and minor metabolites (Carte and Faulkner, 1981). Ultimately, Carte and Faulkner gave rise to the idea that many metabolites (i.e. PBDEs) from *L. herbacea* were somehow associated with the presence of its cyanobacteria symbiont in the ectosome of the sponge.

With concerns about PBDEs detected in the marine sponge, it sparked the field in understanding the interrelationship between cyanobacteria and *L. herbacea*. In 1994, the first successful isolation of the cyanobacteria from *L. herbacea* was reported (Hinde et al. 1994). Subsequently, Unson and colleagues were the first to observe that PBDEs were present as crystalline material within *L. herbacea* tissues, and in extremely high proportions (~12%) of the sponge's dry weight (Unson et al. 1994).

Control Experiment A: Mass Changes

All sponge specimens increased in mass in the absence of a cowrie. Mean mass increase was slightly higher in the Control Group Piti Bomb Holes 0.12 ± 0.018 than in Control Group Pago Bay 0.10 ± 0.015 (t=0.961, p=0.3501, d.f.=18). However, the means of both groups were ruled insignificantly different (Figure 6).

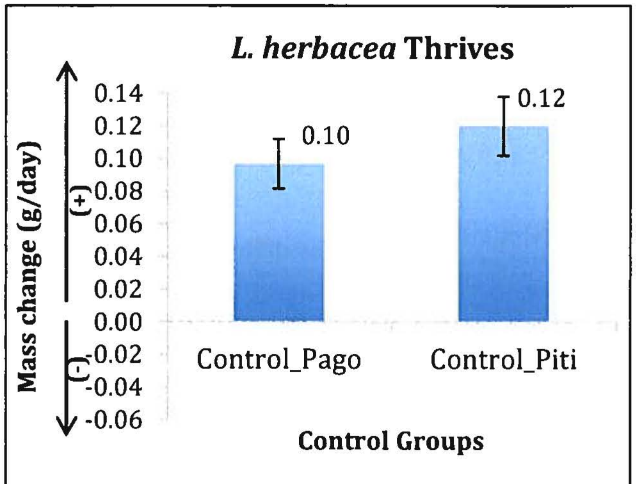

Figure 6. *Lamellodysidea herbacea* thrives after being taken from their reef localities and placed in the UOGML flow-through seawater system. Both forms exhibit daily increases in mass over a 3 d observation. Data bars represent mean values (n=10)±SE (bars).

All sponge specimens decreased in mass in the presence of a cowrie. The four feeding treatments demonstrated that each cowrie fed on its designated sponge. Physical feeding markers on *L. herbacea* were observed as evidence of consumption leading to mass loss. A one-way ANOVA with post-hoc Tukey HSD test was used to reveal any significant

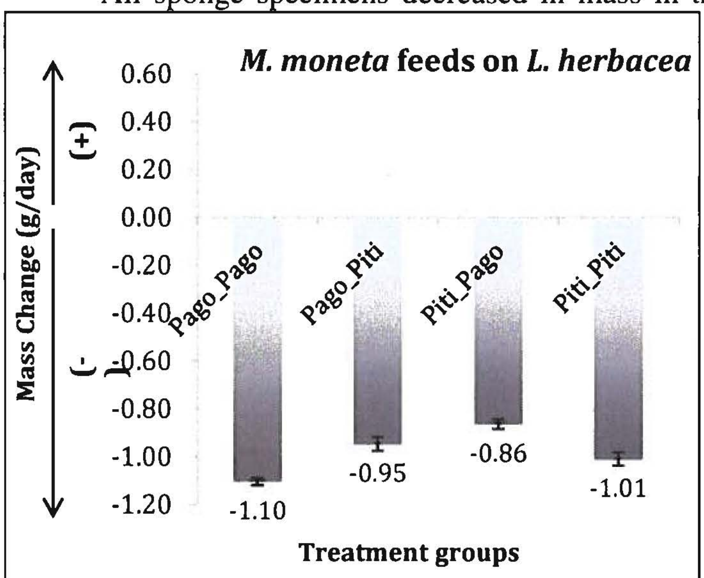

Figure 7. *Lamellodysidea herbacea, Monetaria moneta.* Four feeding treatments demonstrated consumption (mass change) of *L. herbacea* by *M. moneta*. Data mean values (n=10)± 0.015, 0.028, 0.021, 0.028 SE (bars).

differences between treatments. The p-value (**p<0.01) corresponding to the *F*-value (*F*=18.65) is lower than p=0.05 which suggests that one or more treatments are significantly different. Tukey's HSD test was applied to 6-paired treatments to find which showed statistical differences. Pairs with 95% confidence interval were found within Pago_Pago vs Pago_Piti (**p<0.01), Pago_Pago vs Piti_Pago (**p<0.01), Pago_Pago vs Piti_Piti (*p< 0.05), and Piti_Pago vs Piti_Piti (**p<0.01).

To reveal differences when comparing treatments and control groups of the same *L. herbacea* clade, a one-way ANOVA with post-hoc Tukey HSD test was used. The Control Group-Pago Bay paired with Pago_Pago or Piti_Pago all exhibited significant differences among one another (*F*-value= 1,374, ***p*<0.01, d.f.= 27). The Control Group-Piti Bomb Holes paired with Pago_Piti or Piti_Piti (*F*-value=640, **p<0.01, d.f.=27) showed significant differences. However, when treatments were paired, Pago_Piti vs Piti_Piti, they were found insignificantly different (Figure 8).

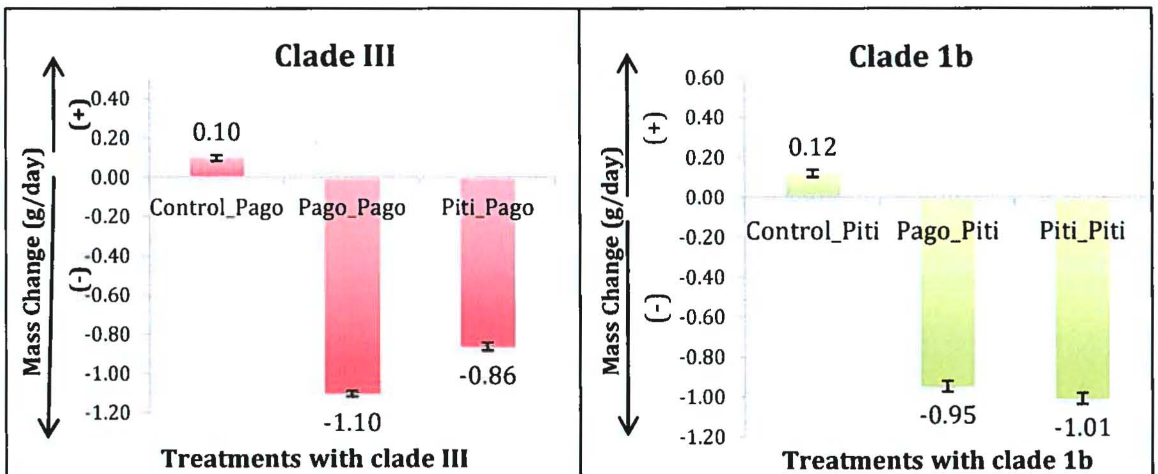

Figure 8. Growth and consumption of *L. herbacea* grouped by the same clade. Clade III (Pago Bay; shown here in red) showed significant differences among all groups at a 95% confidence interval. Clade 1b (Piti Bomb Holes shown here in green) showed significant differences among control and treatment groups**p<0.01, but not among treatment pairs. Data points represent mean values (n=10)±SE (bars).

Control Experiment B: Presence/Absence of Polybrominated Phenolic Chemistry

Samples of *L. herbacea* were investigated to see whether the polybrominated phenolic chemistry was either altered or remained constant before and after consumption. This experiment validated the chemical integrity of the sponges offered.

Feeding Treatments

A binomial test was used to test for a positive and negative response.

DISCUSSION

The marine sponge family Dictyoceratida has been the focus of several metabolome investigations. Of the sponge species, *Lamellodysidea herbacea* has been the most studied, serving as a model system for addressing the origins of secondary metabolites in sponge microbes (Hanif et al. 2007, Thomas et al 2010). *L. herbacea* occurs in two chemotypes: one is sesquiterpenes and polychlorinated amino acid while the other produces polybrominated diphenyl ethers. Both chemotypes have been reported to originate from the symbiotic cyanobacteria *Hormoscilla spongeliae* (Agarwal and Bowden 2005). It was suggested that these compounds may serve a role in the chemical defense of the sponges against predators and bacterial invasion, however more studies are needed to prove this claim (Faulkner et al. 1994).

The money cowry *Monetaria moneta*, which was used in this feeding assay, is an ecologically relevant predator in the Indo-Pacific benthic ecosystem (Renaud 1976; Hayes 1983). *M. moneta* have been reported to feed primarily on two kinds of algae *Jania capillacea* and *Schizothrix calcicola* (Renaud 1976). To date, however, *M. moneta* was observed feeding on *L. herbacea* on Guam (Biggs, *personal obs*). Although only anecdotal observation of the *M. moneta* feeding on *L. herbacea* existed, it was reported that several species of cowries generally prefer sponges to other food items (i.e. algae, diatoms, coralline algae, hydroids, and foraminifera) (Hayes 1983). Our study helped enhance the knowledge of *Lamellodysidea herbacea* predators and hopefully ignite more research to explore the interrelationship between these specimens and what roles polybrominated phenolic chemistry might play in predator-prey interactions.

PBDEs have been detected in many marine organisms but if and how these products enter food chains has not been shown before. This study is the first to demonstrate an ecological pathway for naturally-occurring PBDEs to enter marine food webs. The experimental design intended to cover all the possibilities that could happen in the marine environment be allowed to also occur in a laboratory setting such as diseases, lack of sunlight and oxygen, overgrowth of macroalgae, or change in symbiotic communities over time.

Foremost, evidence of spatial differences on Guam's coastal waters needed confirmation. In 1976, Renaud reported two morphologies of *M. moneta* at Enewetak, Marshall Islands. He discovered that both morphologies were associated with particular habitats: "smooth" with intertidal domains and "knobby" with subtidal domains. With the supervision of chemoreceptors, cowries of the same morph aggregated towards one another when triggered by the mucous trails produced (Renaud 1976). This suggested chemical differences in the mucous production of the two morphs of *M. moneta*, which also played a role in the distribution of these cowries. On Guam, similar findings of both existing morphs were found at the two collection sites: Pago Bay, an intertidal habitat where only smooth cowries were found and Piti Bomb Holes, a subtidal habitat where only knobby cowries were found.

Both collection sites were reported to have their own exclusive *L. herbacea*: clade III at Pago Bay and clade 1b at Piti Bomb Holes as previously described (Agarwal et al. 2017). In the 2017 study reported by Agarwal and colleagues, they discovered a difference between the polybrominated phenolic chemistry within these two clades. Symbionts inhabiting clade 1b (Piti Bomb Holes) harbor the gene cassette encoding PBDE biosynthesis; whereas clade III (Pago Bay) lacked the gene cassette, and were entirely absent of PBDEs. These studies showing evidentiary support led to the deduction of the existence of spatial differences in Guam's coastal waters. However, room for validation was mentioned to placate others concern whether differences in *L. herbacea* PBDE concentration in Guam's coastal waters do exist. So the experiment that controlled for the absence and presence of polybrominated phenolic chemistry were able to provide true-negative and true-positive control groups. All collected clade 1b specimens did have PBDEs present "before" and "after" consumption and the phenolic chemistry was not altered, meaning the OH-BDE structure remained the same. All collected clade III specimens did not have any PBDEs present "before" and "after" consumption from a cowrie.

Secondly, in order to deduce cross-feeding treatments, it was sought out whether *M. moneta* do feed on *L. herbacea.* The control experiment that accounted for sponge mass was able to show its nature influenced by the presence or absence of a cowrie within the same cell. All *L. herbacea* without a cowrie present showed that they were capable of remaining intact and not degrade when placed in the tank. Unexpectedly, all sponge samples were able to flourish. Between the two sponge morphs observed, Clade 1b (Piti Bomb Holes) deemed more resilient by expressing a slightly higher growth rate. However, insignificant differences were inferred after statistically testing both control groups, as a result, concluding growth rates is rather premature.

After observing treatment groups, cowries from their natural habitat consumed more sponge from the same habitat compared to the other. This led to the assumptions of feeding preferences or perhaps more time is needed to get used to the type of sponge clade being eaten. When observed for 14 d (unpublished results) the treatment Piti_Pago slightly consumed more than Pago_Piti (this may continue to fluctuate but differences remained insignificant). Overall, treatment groups with cowries and sponge clades from the same habitat remain the lead in consumption and more mass loss.

Significant differences were evident among control groups when paired with a treatment group. This was an obvious observation through the positive (growth) and negative (consumption) outcomes of all groups. Our data indicate that treatment groups are significantly different from the control groups, but there is little to suggest that some treatment groups are significantly different from one another. The later information is not relevant to this study rather the understanding as to whether these cowries consume sponges is what was sought out. More importantly, it is acceptable to say that *M. moneta* do consume *L. herbacea* however, the role of OH-BDE in *M. moneta* needs further investigation.

Lastly, our findings of spatial differences in the PBDE distribution in *L. herbacea* on Guam and additionally reporting *M. moneta* feeding on these sponges allowed for the possibility of cross-feeding treatments. The Pago_Piti treatment group was able to show

OH-BDE uptake while depuration was shown with the Piti_Pago treatment group. With bioaccumulation taking place, "smooth" cowries may have developed an adaptive digestive system that enables them to consume these compounds without damaging any organs (Dewi et al, 2016). For the Piti_Pago showing depuration of OH-BDEs may lead to speculations that these "knobby" cowries are able to metabolize these compounds even at low concentration intervals. However, these postulated conclusions need further studies.

Alternative Discussion

Lastly, our findings of spatial differences in the PBDE distribution in *L. herbacea* on Guam and additionally reporting *M. moneta* feeding on these sponges allowed for the possibility of cross-feeding treatments. However, the Pago_Piti treatment group was not able to clearly show an uptake of OH-BDEs. Knowing the recalcitrant nature of PBDEs and with no traces of phenolic chemistry in these cowries raise questions of xenobiotic metabolism activities. Perhaps the digestive system of the cowries has the capability to metabolize these compounds.

CONCLUSION

With *M. moneta* from Pago Bay showing bioaccumulation of PBDEs in their tissues and are capable of surviving in a laboratory setting, gives opportunities to investigate the potential of this marine organism in ecological studies. With a growing concern for naturally made PBDEs, more of these studies need to be geared towards OH-BDEs which have recently been proposed to be of even higher threat to humans than man-made PBDEs.

First, because PBDEs bioaccumulated in the cowries, this may also occur with other naturally derived halogenated compounds from other marine producers, such as algae (Hayes 1983; Cabrita et al., 2010). Next, this study will make possible the detection of toxic compounds of a particular ecosystem of interest, however more studies and experimental designs are needed to move forward. Another direction this study can gear towards is to discover whether PBDE and their analogs can be detected in eggs, a similar study that reported PBDE detection in *Sagaminopteron nigropunctatum* egg masses on Guam (Becerro et al. 2006). This will enhance the understanding as to whether maternal transfer of these compounds is possible and at what concentration based on the adult cowrie. Finally, because there exist an uptake of PBDEs over time, future research can discover acute toxicity levels within cowries and lethal doses to define the ultimate threshold within these marine organisms.

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