

# Influence of palatability on the feeding preferences of the endemic Hawaiian tiger cowrie for indigenous and introduced sponges

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**ABSTRACT:** Kāne'ohe Bay has been invaded by at least 10 non-indigenous sponge species, some of which have become dominant over native sponges and even competitors against reef-building corals. We discovered the Hawaiian tiger cowrie *Cypraea tigris schilderiana* to be a voracious sponge predator, particularly on some of these non-indigenous sponges. This study sought to examine whether feeding preference for sponges by cowries was influenced by palatability of sponge chemical extracts. We quantified the consumption rate of 18 species of common native and non-native sponges in comparison to 3 native corals from Kāne'ohe Bay in flow-through aquaria. When offered a smorgasbord of prey species in 4 replicate week-long trials, cowries exclusively fed on sponges, which included several non-native species, in a selective gradient. Three *Dysidea* spp. were consumed entirely within 72 h, followed by *Mycale parishii*, *Haliclona caerulea*, *Halichondria coerulea*, and *Cladocroce burapha* within 96 h, and *M. grandis* and *Gelliodes wilsoni* in 144 h. Preferred sponges spanned the full range of sponge mineral composition, from those with densely packed spicules that measured >300 μm to those with a collagen fiber skeleton. Among avoided sponges, only *Monanchora clathrata* produced compounds that, when extracted, proved to be a deterrent to cowries and the whitespotted toby *Canthigaster jactator* in feeding trials. Our study highlights the previously unrecognized importance of cowrie predation on introduced sponges in Hawaiian reefs, and suggests that palatability alone does not influence feeding preference. We encourage conservation for this overharvested, native spongivore because of its potential use in biological control for future sponge introductions.

**KEY WORDS:** Spongivory · Management · Conservation · Invasive sponges · Selective feeding · Secondary metabolites

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## 1. INTRODUCTION

Historically, Kāne'ohe Bay and Pearl Harbor have been the focus of surveys for non-indigenous species in Hawai'i, which have reported at least 10 sponge introductions from the Indo-Pacific and the Caribbean (Bergquist 1977, Coles et al. 2002, Coles 2006). Although it is difficult to determine the timing or vector of nonindigenous sponge introductions, it is

believed that fouling of ship hulls and marine debris are major contributors (Cariton & Geller 1993, Barnes 2002). Protected embayments are relatively rare in Hawai'i (Bahr et al. 2015). Kāne'ohe Bay and Pearl Harbor are examples that offer shallow coastal reef protection from high wave energy and long water residence times, particularly in the southern portions of Kāne'ohe Bay (Lowe et al. 2009). In addition, runoff from streams surrounding Pearl Harbor and

Kāneʻohe Bay offer a stable source of refractory dissolved organic carbon, which is thought to be a preferred food source for sponges (Pawlik et al. 2016). These conditions appear to favor the proliferation of introduced sponge species over other exposed reef locations around Oʻahu, because high abundance and competitive dominance of a number of introduced sponges are confined to Pearl Harbor and Kāneʻohe Bay.

The first known introductions of sponge species to Hawaiʻi were observed by De Laubenfels (1950) and Bergquist (1967). Their list consisted of both Indo-Pacific (*Mycale* [*Zygomycala*] *parishii*) and Caribbean species (*Haliclona caerulea*, *Halichondria melanodacia*, *Terpios zeteki* [now *Suberites aurantiacus*], and *Tedania ignis*). In more recent years, *Biemna fistulosa*, *Gelliodes wilsoni*, *Mycale grandis*, *Monanchora clathrata*, and *Cladocroce burapha* were also added as potential introductions from the Indo-Pacific (Coles & Bolick 2007, Núñez Pons et al. 2017). Among these introduced sponges, *M. grandis* has become a dominant presence over the past few decades. This invasive, introduced sponge competes with scleractinian corals and is able to overgrow reef corals, with no evidence of a top-down control from any spongivorous predators (Coles & Bolick 2007). A number of native Hawaiian sponge predators such as Moorish idols *Zanclus cornutus*, whitespotted tobies *Canthigaster jactator*, and green sea turtles *Chelonia mydas* have been observed feeding on sponge species found in Kāneʻohe Bay (Hobson 1975, Bailey-Brock 1989, Vicente et al. 2019b). However, relative to other Hawaiian reefs, the abundance of fishes is quite low within Kāneʻohe Bay (Williams et al. 2008, Stamoulis et al. 2017) and these predators are unable to control introduced sponges due to a combination of the small size of *C. jactator*, commercial aquarium fishing pressure on *Z. cornutus* (Walsh et al. 2003), and minimal consumption by green turtles, which are mostly algae and seagrass specialists (Arthur & Balazs 2008).

Another potential sponge predator includes cowries of the genus *Cypraea*, of which 4 congeners are spongivorous specialists in the Hawaiian Islands (Hayes 1983). The largest of these species is the endemic Hawaiian tiger cowrie *C. tigris schilderiana*, which is a common spongivore in Kāneʻohe Bay (Cate 1961) that has repetitively been observed consuming large amounts of *M. grandis* both in the field and in captivity (see Video S1 at [www.int-res.com/articles/suppl/m647p109\\_supp/](http://www.int-res.com/articles/suppl/m647p109_supp/), all Supplements). Spongivory by other tiger cowrie species has also been reported in Zanzibar and the Gulf of Mannar (Helber et al. 2017, Raj et al. 2019). The Hawaiian tiger cowry is

significantly larger, warranting its reclassification from Indo-Pacific congeners as a subspecies of *C. tigris* (Foin 1972) and increasing its value in the curio trade due to both its size and endemism. Traditionally, *C. tigris schilderiana* (*Leho-kiko* in Hawaiian) was fished by Native Hawaiians for food and the shell was then used as a lure (*Leho heʻe*) to fish for deep-water *Octopus* species (Titcomb et al. 1978). Like many other shells in this genus, tiger cowries have been in high demand for over 1000 yr in the trade of Indo-Pacific shells (Reese 1991), but harvest of this endemic gastropod is unreported and unregulated by the state of Hawaiʻi.

Repeated field observations of cowries actively feeding on sponges motivated us to evaluate the feeding preferences of Hawaiian tiger cowries for endemic vs. native sponge species. The potential for these endemic cowries to be used as a native biocontrol agent for invasive sponges on Hawaiian reefs also motivated us to include several native coral species to confirm predation was specific to sponges. A second goal was to determine the rate of sponge consumption by cowries and whether chemical defenses were responsible for possible deterrence of predation by fishes and cowries. Collectively, these results could inform whether *C. tigris schilderiana* has potential to be used for biocontrol of current or future sponge introductions in Hawaiian waters.

## 2. MATERIALS AND METHODS

### 2.1. Collection of predator and prey species

A total of 11 *Cypraea tigris schilderiana* were collected from patch reefs within the Hawaiʻi Marine Laboratory Refuge surrounding Moku o Loʻe (Coconut Island) in Kāneʻohe Bay at a depth of 3–4 m in January 2017. Excreted sponge remnants of the orange keyhole sponge *Mycale grandis* were observed on the posterior end of collected individuals, so cowries were held in a wet table immediately after collection and fed the sponge *M. grandis* before running trials. A wet table holding 190 l of seawater was provided with sand, coral rubble, and circulating seawater in a flow-through system. The seawater turnover rate was maintained at 3.5 l min<sup>-1</sup>, and additional water movement was provided with 2 MP40 water pumps (Vortech; EcoTech Marine) placed diagonally at opposite ends of the tank for water circulation at a flow rate of 17 000 l h<sup>-1</sup>.

A total of 18 common shallow water sponge species from Kāneʻohe Bay were collected around Coconut

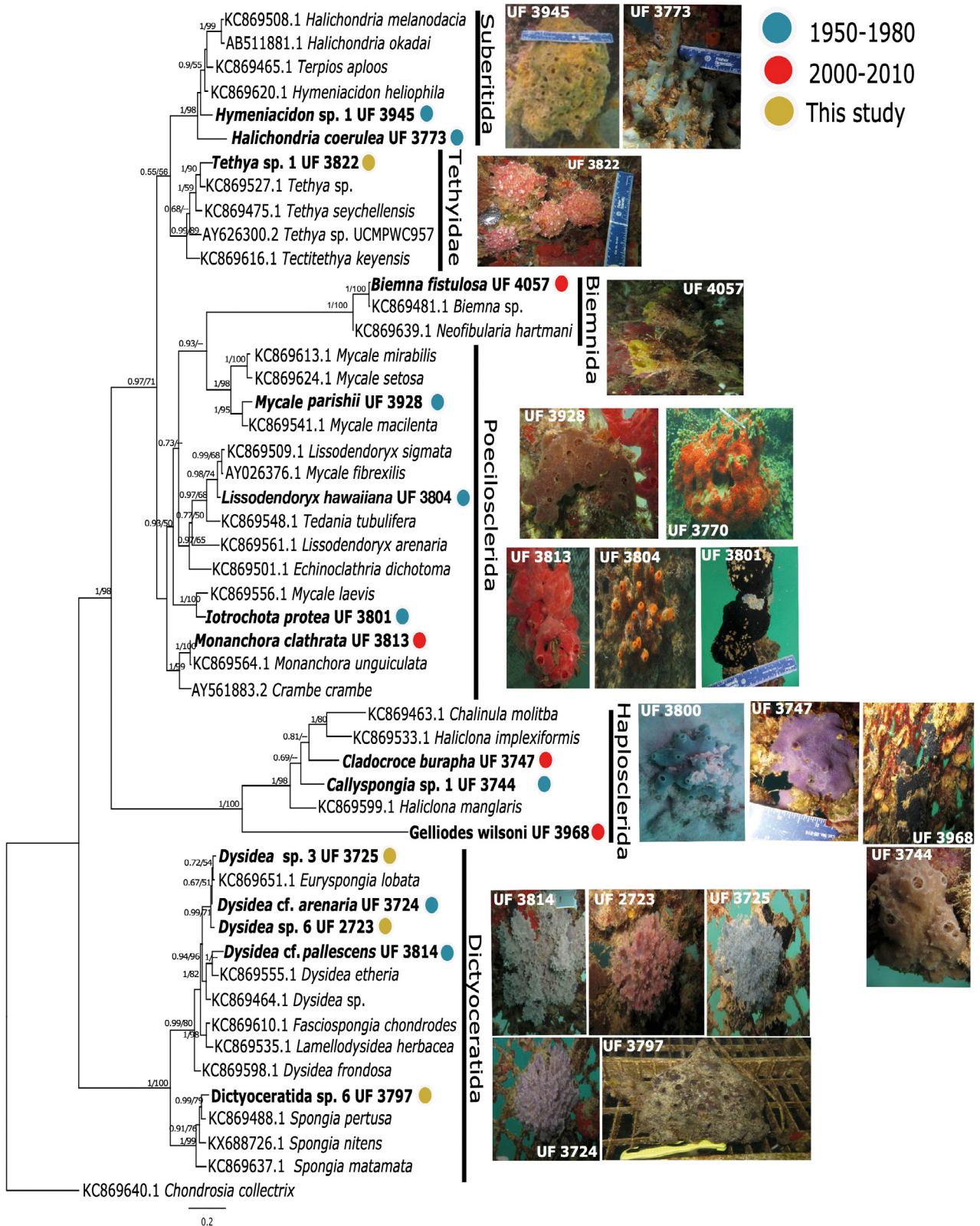


Fig. 1. Phylogenetic tree and *in situ* images of voucher specimens used in this study. Bayesian and maximum likelihood topologies were generated from partial sequences spanning 925 positions of the D1–D2 region of the 28S rRNA gene. With 2 exceptions, sequences in **bold** highlight those sponge species tested as a prey item for sponge predator *Cypraea tigris schilderiana* in this study. Tree is missing sponges *Haliclona caerulea* UF 3800 and *Mycale grandis* UF 3770, which are recent (2000–2010) introductions to Kāne’ohe Bay. The colors indicate the year when species were first reported in Kāne’ohe Bay



Island (Fig. 1). To evaluate whether cowries would also prey on corals, the 3 most common coral species found in Kāneʻohe Bay (*Porites compressa*, *Pocillopora acuta*, and *Montipora capitata*) were also added as potential prey items. It was difficult to collect prey species with exact identical weights across species and between individuals due to morphological variations in shape and density. Thus, 2 individual pieces (each 10–100 g) of each prey species were collected and acclimated in a wet table without predators for 48 h before the beginning of each prey consumption trial. Prey consumption trials were repeated 4 times. At the end of the prey consumption trials, an additional 4 replicate 10 ml samples of each species were collected and immediately frozen (–20°C) for the production of crude organic extracts that were used to make experimental foods from these prey species. All invertebrates were collected under special activities collection permit (SAP) nos. 2018-03 and 2019-16 (covering the period of 13 Jan 2017 through 10 Apr 2019) issued by the State of Hawaiʻi Division of Aquatic Resources. Vouchers of each sponge species were submitted to the Florida Museum of Natural History.

## 2.2. Identification of sponge species

Vouchered sponge species were identified by comparing morphological traits to species previously reported and described for Hawaiʻi (De Laubenfels 1950, 1951, 1954, 1957, Bergmann & Burke 1955, Bergquist 1967, 1977, Núñez Pons et al. 2017). Histological sections and the spicule composition of each species were observed under light microscopy following the methods of Boury-Esnault & Rutzler (1997). Visual morphological identifications were compared with genetic identifications using 28S rRNA, and cytochrome *c* oxidase subunit 1 (COI). The 28S rRNA gene sequences from each species were deposited in GenBank and COI sequences were deposited in The Barcode of Life Data System (Ratnasingham & Hebert 2007). Sequences were assigned accession numbers MT452531–MT452547 for 28S rRNA and MT586734–MT586744 and MT571643–MT571645 for COI. Sequences spanning the D1–D2 subunit of the 28S rRNA gene for each sponge species was generated using primer 28F63mod (5′-ACC CGC TGA AYT TAA GCA TAT HAN TMA G-3′) and 1072RV (5′-GCT ATC CTG AGG GAA ACT TCG G-3′) (Medina et al. 2001). COI sequences were generated using primer LCO1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO2198 (5′-TAA ACT TCA GGG

TGA CCA AAA AAT CA-3′) (Folmer et al. 1994). Polymerase chain reactions (PCR), PCR programs, sequencing reactions, and phylogenetic analyses were completed following methods described in Vicente et al. (2019a) using Geneious v.10 (Kearse et al. 2012).

## 2.3. Prey consumption trials

Three of the most similarly sized cowries, 281–347 g wet weight with a volume of 354–407 cm<sup>3</sup>, were selected from the 11 samples and acclimated for 2–3 wk before sponge feeding trials began. Acclimated sponge and coral pieces were weighed with an analytical scale before the beginning of each trial. An experimental wet table was prepared by placing one of 2 sponge and coral pieces in the presence of cowries. The other prey pieces were kept in an adjacent control wet table without predators. Prey exposure trials with tiger cowries were observed every 24 h for a total of 144 h. Each prey individual in experimental and control wet tables was weighed and photographed at 0 h. Prey items were then photographed every 24 h to measure daily consumption by % surface area loss (surface area consumption, SAC). Surface area measurements were completed by tracing prey species tissue using the freehand selection tool in ImageJ (Abràmoff et al. 2005). This approach was used instead of weighing to prevent stressing sponges by exposure to air on a daily basis. Daily observations allowed us to record initial preferential consumption of some sponge species over others. Remaining experimental and control prey items were weighed after 144 h to calculate weight consumption (WC) by subtracting the wet weight of prey remains from the initial wet weight taken at 0 h. Trials were successfully run 4 times with the same 3 cowrie individuals, from which the weight consumption rate (WCR) after 144 h and daily SAC were calculated. WCR was calculated at each 24 h interval as:  $WCR = (Wt_{2,i} - Wt_{1,i}) / (\text{time} \times C_i)$ , where  $Wt_{2,i}$  is the wet weight of each prey species  $i$ ,  $t_1$  is the first time point at which species  $i$  started showing a decrease in surface area,  $t_2$  is the second time point at which surface area showed that the entire sponge was consumed, time is the time between collections (h), and  $C_i$  is either the total volume (1124 cm<sup>3</sup>) or the total wet weight (952 g) of all 3 cowries. It is important to note that sponges can grow within these trials and so WC and SAC calculated in this way may be negative or exceed 100% (see Tables S1 & S2 in Supplement 3, all supplementary tables).

#### 2.4. Palatability assay of crude organic extracts of prey species

Tiger cowries and the Hawaiian whitespotted toby *Canthigaster jactator* were used as spongivorous model organisms to determine the palatability of crude extracts generated from each potential prey species in this study. Several studies have used *Canthigaster* spp. as model organisms to study the palatability of sponge extracts (Pennings et al. 1994, Slattery et al. 2016, Helber et al. 2017, Marty et al. 2017). Field observations of sponge consumption by the whitespotted toby (Bailey-Brock 1989) further motivated our use of this fish as a model spongivore. A total of 13 tobies were collected in April 2018 under SAP no. 2018-03, 100 m North of Lilipuna Pier in Kāneʻohe Bay and were immediately transported to the Hawaiʻi Institute of Marine Biology. Approval of protocol no. 18-2793 by the Institutional Animal Care and Use Committees at the University of Hawaiʻi at Mānoa allowed us to keep the tobies in 13, labeled, individual 2 gallon (ca. 7.5 l) tanks supplied with flow-through seawater ( $0.5 \text{ l min}^{-1}$ ) for these experiments. The remaining 8 tiger cowries were isolated into 8 individual  $25 \times 37.5$  cm cells in a wet table (190 l) separated by industrial vexas mesh to keep the cowries from moving in between cells.

Control and experimental foods for cowries and tobies was prepared following Marty & Pawlik (2015). Control foods consisted of a sodium alginate and squid mantle mixture suspended in distilled water with a minimal amount (50  $\mu\text{l}$ ) of methanol (MeOH) used for suspending extracts of experimental foods. A 3 ml aliquot of the food mixture was loaded onto a 5  $\text{cm}^3$  BD™ slip tip dispensing syringe and purged into a calcium chloride ( $\text{CaCl}_2$ ) solution which hardened the mixture into 1 mm diameter noodles (following Pawlik et al. 1995). Food noodles were cut into 3–5 mm pellets for feeding tobies. Tobies were fed 15 pellets every 2 d before the start of the experimental trials. For the cowries, 200  $\mu\text{l}$  of the food mixture was dispensed onto a screen mesh fixed to a  $2.5 \times 2.5$  cm tile. The food mixture was hardened by adding 200  $\mu\text{l}$  of the  $\text{CaCl}_2$  solution. Tiles holding control and experimental food for cowries were placed next to a small (1–2 g) piece of sponge preferred by the cowries (Video S2). The small piece of preferred sponge was used to bait the cowries to the tiles. Tiles were monitored for consumption at 24 and 48 h.

Feeding assays using experimental food began once tobies and cowries showed consistent consumption of control food. In total, 4 replicate 1:1 dichloro-

methane/MeOH extracts per prey species were dried and used to create experimental treatment foods. Experimental foods were prepared by suspending the dried crude extract from prey items in 50  $\mu\text{l}$  of MeOH and 5 ml of the control food mixture. This method generated an extract at twice (2 $\times$ ) the volumetric natural concentration for our initial feeding trials. Experimental foods at 1 $\times$  were prepared and assayed when <60% of pellets at 2 $\times$  were accepted by tobies or cowries. At the beginning of each trial control pellets were offered to individual tobies. Experimental pellets were offered only when control pellets were consumed by an individual. Experimental pellets were scored as either rejected or accepted after a subsequent control pellet was accepted by the tobies. An experimental pellet was considered rejected if the pellet was ignored or repetitively regurgitated by the tobies (following Pawlik et al. 1995).

Percent acceptance was calculated based on the number of pellets accepted using 10 individual tobies. Trials with cowries were monitored for food consumption at 24 and 48 h. Trials were scored as a rejection if the control was consumed but experimental tiles remained uneaten after 48 h. Trials of experimental foods were scored as accepted whenever the experimental food was completely ingested. Trials were repeated when control and experimental foods were ignored. Percent acceptance was calculated based on the number of tiles consumed using 8 individual cowries.

#### 2.5. Data analysis: WC and SAC

Data were first transformed to % WC and % SAC, then analyzed using R v.3.6.3 (R Core Team 2020) for the 2 data sets (Tables S1 & S2); 'rmarkdown' (Allaire et al. 2020) was used to generate Supplement 4. Values of % WC were corrected by subtracting the difference in weight loss of control prey species throughout 144 h from the experimental % WC value (Table S3), following Prince et al.'s (2004) methods for correcting feeding preference data for sea urchins. We applied the same correction method for % SAC by subtracting surface area loss of controls at 144 h from experimental % SAC values (Table S4). We used the 'stats v.3.6.2' package to run a 1-way ANOVA using prey species as factor to test for differences in % WC after 144 h ( $\text{lm}(\% \text{WC} \sim \text{species})$ ). Plotted residuals vs. % WC values showed several outliers but only *Mycale parishii* in trial 4 was removed in order to meet the assumption of homogeneity of variance (HOV). The modified data set

met HOV assumptions (Levene's test,  $p = 0.092$ ). Normal distribution of residuals was confirmed by linearity between residuals and quantiles. Although some residuals were slightly non-linear, multiple tests (Anderson-Darlington,  $p = 0.306$ ; Cramer-von Mises,  $p = 0.508$ ; Lilliefors [Kolmogorov-Smirnov],  $p = 0.549$ ; and Pearson chi-squared,  $p = 0.880$ ) failed to reject normality (pages 1–6 in Supplement 4). Significant differences ( $p < 0.05$ ) among Tukey's pairwise comparisons of % WC between species revealed preferred prey species (prey species showing a mean WC  $> 75\%$  after 144 h).

Only sponges that were preferred prey species were selected to measure differences in % SAC every 24 h during the first 72 h. We used the R package 'nlme' v.3.1-143 (Pinheiro et al. 2020) with repeated-measures ANOVA (Mangiafico 2016) and multilevel modeling (Borowski 2017) for these analyses. First, we fit a linear model using generalized least squares with fixed effects and excluded random effects: ( $gls(\%SAC \sim \text{species} + \text{hours} + \text{species} \times \text{hours}, \text{data} = \text{SAC})$ ). A second model using linear mixed effects with random 'trial' effect: ( $lme(\%SAC \sim \text{species} + \text{hours} + \text{species} \times \text{hours}, \text{random} = \sim 1|\text{trial}, \text{data} = \text{SAC})$ ), and a third model fitting an autoregressive lag 1 (corAR1) covariance structure: ( $lme(\%SAC \sim \text{species} + \text{hours} + \text{species} \times \text{hours}, \text{vrandom} = \sim 1|\text{trial}, \text{correlation} = \text{corAR1}(\text{form} = \sim 1 | \text{trial}, \text{value} = 0.5550), \text{data} = \text{SAC})$ ) were all tested to determine the model with the best description of the data. An ANOVA of the 3 models showed that including a random 'trial' effect and fitting an autoregressive lag 1 (corAR1) covariance structure produced the best model (lowest AIC; page 9 in Supplement 4). This model showed a random distribution of plotted residuals vs. % SAC values and met the HOV assumption (Levene's test,  $p = 0.962$ ). Normal distribution of the residuals was confirmed by linearity between residuals and quantiles. Significant Tukey's post hoc pairwise comparisons using the R package 'emmeans' v.1.4.3.01 (Lenth et al. 2019) was used to measure significant differences in % SAC of sponge species at different time points. Box plots and correlation heatmaps were generated using 'ggplot2' (Wickham 2016).

## 2.6. Data analysis: palatability assay of crude extracts from prey species

Significant differences in consumption of experimental vs. control foods were evaluated using a modified version of Fisher's exact test. In this analysis, the

marginal totals for experimental and control foods were fixed and treated as random samples (Marty & Pawlik 2015, Marty et al. 2017). Experimental foods were considered a deterrent if the mean consumption of 4 replicate extracts per prey species was  $\leq 60\%$  ( $p < 0.05$ ). An independent 2 sample  $t$ -test was used to evaluate significant differences in mean acceptance of experimental food between cowries and tobies.

## 3. RESULTS

### 3.1. Prey consumption trials

Preliminary consumption trials confirmed that a 1 wk period was sufficient to observe clear distinctions between sponges that were preferred and those that were avoided. For example, cowries began repetitively feeding on preferred sponges within the first 15 min after species were deployed and avoided less preferred species throughout the entire 144 h (Video S1). Less preferred species did not change in % SAC after 120 h, which allowed us to terminate the feeding trials after 144 h. Feeding preference was not influenced by the initial amount of sponge tissue, because sponges were either preferred or rejected regardless of the initial wet weight.

ANOVA on % WC showed a significant prey species effect ( $F_{20,62} = 42.09$ ,  $p < 0.001$ ) (Table 1, Fig. 2a). Tukey's post hoc pairwise comparisons revealed significant differences ( $p < 0.05$ ) in consumption between species showing a mean WC  $> 75\%$  and species showing a mean WC  $< 25\%$  (blue squares Fig. 2b, Table S5). Among the preferred prey sponges (i.e.  $> 75\%$  WC), *Mycale grandis* and *Dysidea* cf. *arenaria* were most consumed, with a mean weight loss of  $55.80 \pm 9.34$  and  $68.11 \pm 4.25$  g respectively. These were followed by *Cladocroce burapha*, *Gelliodes wilsoni*, *Haliclona caerulea*, *Dysidea* sp. 6, *Dysidea* sp. 3, *Mycale parishii*, and *Halichondria coerulea* (Table 2). The uncorrected WC and SAC for all these species showed 100% consumption at the end of the trial (Figs. S1 & S2 in Supplement 5). Less preferred prey species were Dictyoceratida sp. 6, *Biemna fistulosa*, *Hymeniacion* sp. 1, *Tethya* sp. 1, *Callyspongia* sp. 1, *Iotrochota protea* and *Lissodendoryx hawaiiiana* (Fig. 2a, Table 2). Prey species showing minimal consumption (i.e.  $< 25\%$ ) included the 3 coral species *Porites compressa* ( $0.13 \pm 0.36$  g), *Pocillopora acuta* ( $-0.05 \pm 0.36$  g), and *Montipora capitata* ( $0.16 \pm 0.78$  g lost). The change in mass for all 3 coral species are within the 1 g error of the analytical balance.

Table 1. Results of the 1-way ANOVA on % weight consumption and the repeated-measures ANOVA on % surface area consumption by Hawaiian tiger cowries on prey sponge and coral species. Significant p-values (<0.05) are in **bold**

ANOVA	Factor	df	SS	MS	F	p
% Weight consumption	Species	20	12.96	0.648	42.09	<b>&lt;0.001</b>
	Residuals	62	0.96	0.015		
Linear mixed effects ANOVA		df <sub>num</sub>	df <sub>den</sub>		F	p
% Surface area consumption	Species	8	159		11.66	<b>&lt;0.001</b>
	Hours	5	159		70.2	<b>&lt;0.001</b>
	Species × hours	40	159		2.56	<b>&lt;0.001</b>

Sponges with the least WC were *Dysidea* cf. *pallescens* (1.47 ± 4.69 g consumed) and *Monanchora clathrata* (2.17 ± 3.89 g consumed) (Fig. 2a, Table 2). Across these trials, an average of 567.96 ± 65.47 g of sponge tissue was consumed by a total of 952 g of wet cowrie weight wk<sup>-1</sup>.

Preferred species (those showing >75 % WC) were selected for the repeated-measures ANOVA on % SAC to evaluate cowrie preference for individual sponge species. The results showed significant species ( $F_{8,159} = 11.66$ ,  $p < 0.001$ ) and hours ( $F_{8,159} = 11.66$ ,  $p < 0.001$ ) main effects, and hours × species interactive effects ( $F_{40,159} = 2.56$ ,  $p < 0.001$ ) (Table 1). Tukey's post hoc pairwise comparisons of % SAC de-

tected significant differences among prey species at 24, 48, and 72 h but no significant differences for the remaining 72 h thereafter. Each time interval in the first 72 h revealed a feeding preference hierarchy of prey species (Table S7). *D. cf. arenaria* and *Dysidea* sp. 3 were the most preferred species, showing

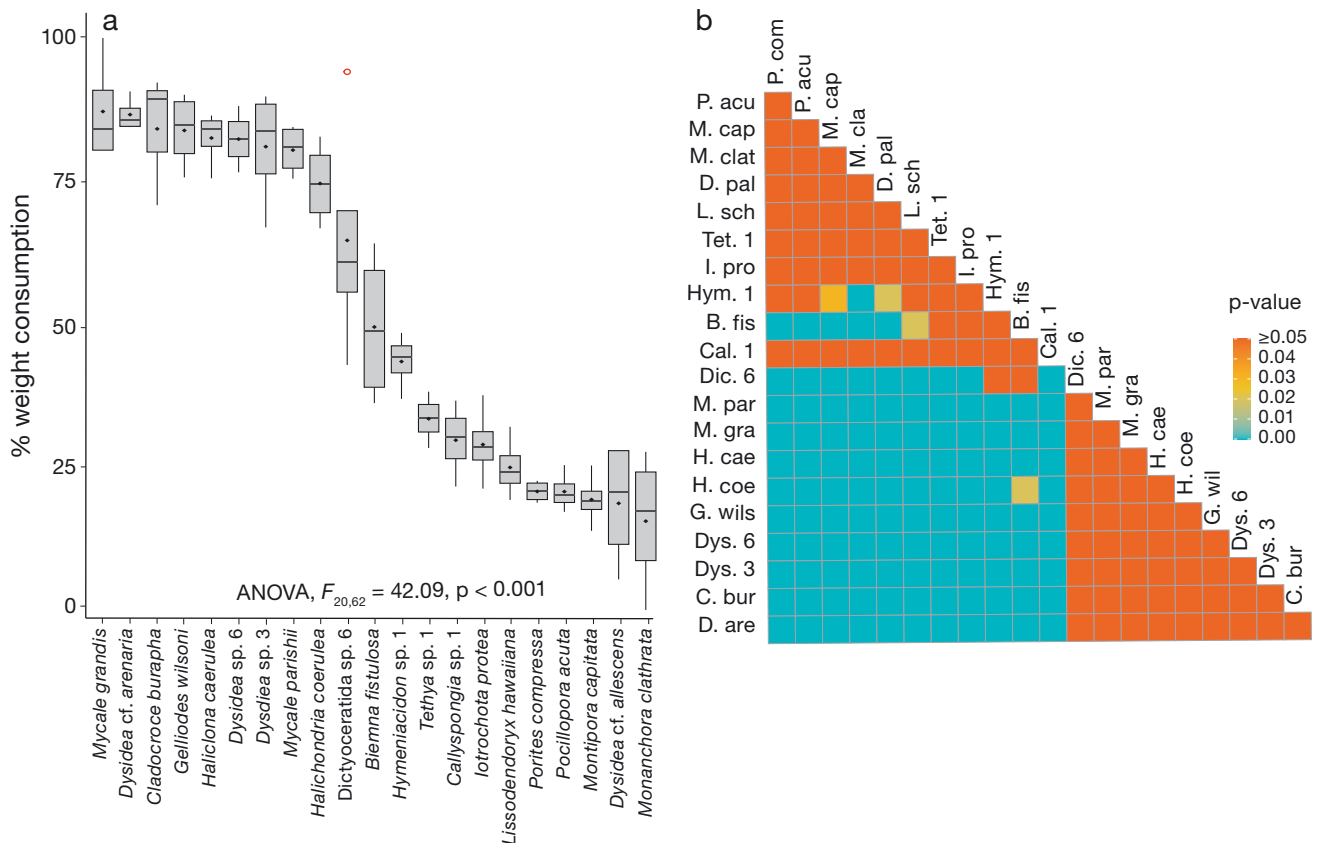


Fig. 2. (a) Boxplot of % weight consumption (% WC) of experimental prey species by Hawaiian tiger cowries after 144 h. Black dots: mean values for each species; red circle: one outlier for Dictyoceratida sp. 6. The % surface area consumption values were normalized to a scale from 0–100 %. ANOVA shows a significant prey species effect on % WC, which was corrected by subtracting the difference in % weight loss of control prey species from the % weight loss of experimental prey species after 144 h. Bars are median values for each species; boxes show the upper and lower quartile; whiskers indicate the least and greatest value excluding outliers. (b) Correlation heatmap of Tukey's post hoc pairwise comparisons between prey species. Significant pairwise comparisons ( $p < 0.05$ ) are indicated by light orange- to blue-colored boxes. Species showing a mean WC > 75 % were all significantly different from those showing a mean WC < 25 %. Exact p-values for pairwise comparisons can be found in Table S5

Table 2. Initial weight of prey tissue and weight consumption (WC) by 952 g or 1124 cm<sup>3</sup> Hawaiian tiger cowries. Weight consumption rate (WCR) is provided as mg of prey species cm<sup>-3</sup> of cowrie h<sup>-1</sup> and g<sup>-1</sup> of cowrie h<sup>-1</sup>. Sponge species are ordered by preference as indicated by the results of a Tukey's post hoc analysis on surface area consumption. Hours varied according to the amount of time it took for cowries to completely consume a given prey species. All values are reported as mean ± SE. Tabulated data are archived in Table S6. WC was based on the corrected % WC in Table S3. NA: not applicable since measurements were within the 1 g error of the analytical balance

Species	Initial weight of prey tissue (g)	WC (g after 144 h)	WCR (mg cm <sup>-3</sup> cowrie h <sup>-1</sup> )	WCR (mg g <sup>-1</sup> wet cowrie h <sup>-1</sup> )
<i>Dysidea</i> cf. <i>arenaria</i>	72.78 ± 3.88	68.11 ± 4.25	2.03 ± 0.75	1.72 ± 0.63
<i>Dysidea</i> sp. 3	68.42 ± 16.03	55.11 ± 13.73	1.40 ± 0.68	1.65 ± 0.80
<i>Dysidea</i> sp. 6	76.53 ± 11.14	60.32 ± 12.71	1.06 ± 0.19	1.25 ± 0.23
<i>Mycale parishii</i>	37.29 ± 10.72	32.84 ± 11.87	0.25 ± 0.13	0.22 ± 0.11
<i>Haliclona caerulea</i>	83.99 ± 17.42	73.96 ± 16.83	0.99 ± 0.18	1.17 ± 0.22
<i>Cladocroce burapha</i>	32.04 ± 7.57	25.84 ± 6.88	0.30 ± 0.09	0.36 ± 0.11
<i>Halichondria coerulea</i>	42.62 ± 5.02	38.56 ± 3.84	0.61 ± 0.20	0.72 ± 0.24
<i>Mycale grandis</i>	58.05 ± 9.22	55.80 ± 9.34	0.58 ± 0.24	0.49 ± 0.20
<i>Gelliodes wilsoni</i>	56.2 ± 4.89	41.66 ± 8.93	0.31 ± 0.09	0.37 ± 0.11
Dictyoceratida sp. 6	86.97 ± 16.23	37.01 ± 6.69	0.23 ± 0.04	0.27 ± 0.05
<i>Biemna fistulosa</i>	33.25 ± 5.1	13.42 ± 1.61	0.08 ± 0.01	0.10 ± 0.01
<i>Hymeniacion</i> sp. 1	47.7 ± 17.38	21.56 ± 12.45	0.13 ± 0.08	0.16 ± 0.09
<i>Callyspongia</i> sp. 1	70.02 ± 6.34	16.98 ± 4.77	0.11 ± 0.03	0.12 ± 0.04
<i>Tethya</i> sp. 1	25.42 ± 7.64	4.39 ± 2.56	0.03 ± 0.02	0.03 ± 0.02
<i>Iotrochota protea</i>	56.04 ± 15.11	10.26 ± 1.53	0.06 ± 0.01	0.08 ± 0.01
<i>Dysidea</i> cf. <i>pallescens</i>	64.69 ± 14.25	1.47 ± 4.69	0.12 ± 0.12	0.15 ± 0.14
<i>Lissodendoryx hawaiiiana</i>	43.18 ± 3.11	4.98 ± 1.07	0.03 ± 0.01	0.04 ± 0.01
<i>Monanchora clathrata</i>	41.43 ± 9.85	2.17 ± 1.94	NA	NA
<i>Porites compressa</i>	26.76 ± 9.79	0.13 ± 0.18	NA	NA
<i>Montipora capitata</i>	10.96 ± 2.3	0.16 ± 0.39	NA	NA
<i>Pocillopora acuta</i>	20.30 ± 2.11	NA	NA	NA
Total	1048.72 ± 96.04	567.96 ± 65.47	8.76 ± 1.58	10.35 ± 1.87

105.12 ± 10.8 and 87.88 ± 18.6% consumption respectively within the first 24 h of exposure. At 72 h, *Dysidea* sp. 6 was 102.56 ± 11.7% consumed and *H. caerulea* was 91.96 ± 4.15% consumed. At 96 h *M. parishii* was 93.82 ± 6.1% consumed, *C. burapha* was 96.63 ± 5.56% consumed, and *H. coerulea* was 92.29 ± 7.00% consumed (Fig. 3). The remaining preferred species, *M. grandis* and *G. wilsoni*, were 99.00 ± 16.1 and 101.5 ± 6.9% consumed at 144 h respectively.

### 3.2. Palatability assay of crude organic extracts from prey species

Tobies and cowries showed similar responses towards accepting experimental foods from all prey species (Fig. 4). Most experimental foods were consumed at rates that were not significantly different from the control foods. Only experimental foods prepared at 2× the volumetric concentration of crude extracts from *L. hawaiiiana* and *M. clathrata* showed significant decreases in consumption by both predators relative to the control foods. At 1×, *M. clathrata* was still a deterrent to both predators at naturally

occurring volumetric concentrations. At 1×, experimental foods with extract from *L. hawaiiiana* showed no significant difference for cowrie predation (75.00 ± 17.68%) but was marginally deterrent against puffers (50.00 ± 28.87%), which is the only time these 2 spongivores differed in their response to a potential prey species in these trials.

## 4. DISCUSSION

Evaluating prey preferences of tiger cowries using corals and sponges not only confirmed previous anecdotal observations that tiger cowries are spongivores (Helber et al. 2017, Raj et al. 2019), but also that tiger cowries are sponge specialists that clearly prefer some sponge species over others. We observed consumption rates among sponge species ranging from undetectable amounts to as high as 2.67 ± 0.47 mg of sponge g<sup>-1</sup> wet weight cowrie h<sup>-1</sup> (Table 2). Further, we observed specialized feeding behavior and preference even among different tissue types within a single species, where cowries cut out a small (1 cm) opening through the tough ectosome of



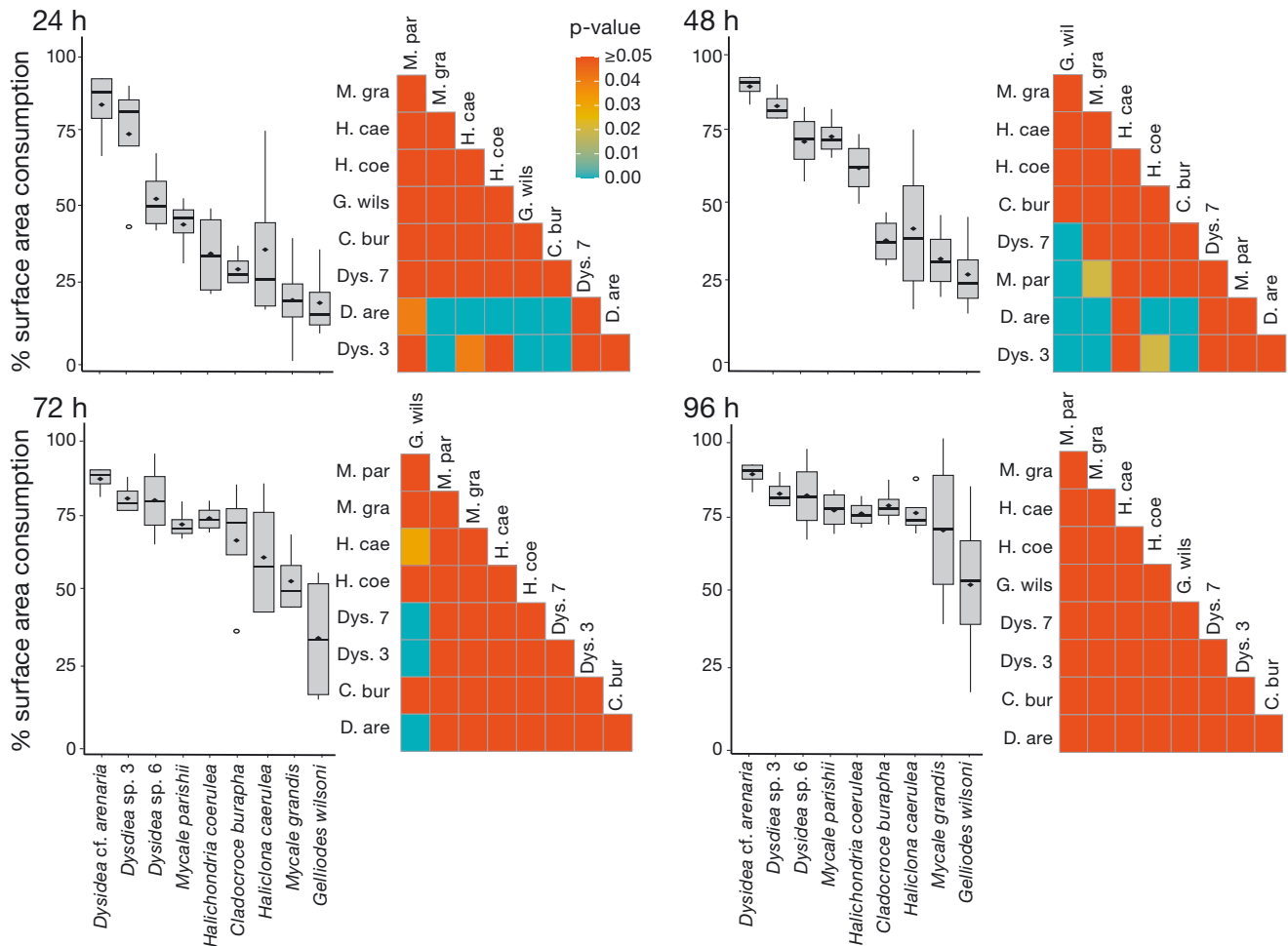


Fig. 3. Boxplot of % surface area consumption (SAC) of sponge species that show >75% weight consumption over a 144 h period. % SAC values were normalized to a scale from 0–100%. Correlation heatmap of Tukey's post hoc pairwise comparisons between prey species are associated with each subplot. Significant pairwise comparisons ( $p < 0.05$ ) are indicated by light orange to blue colored boxes. % SAC was corrected by subtracting the difference in % SAC of control prey species from the experimental SAC throughout 144 h. Exact p-values for pairwise comparisons can be found in Table S7. See Fig. 2 for explanation of boxplots

*Tethya* sp. 1, and then exclusively consumed the interior of the sponge.

We tested the hypothesis that cowries prefer to consume sponges lacking chemical defenses relative to those with deterrent chemical compounds. This hypothesis was rejected for all less preferred prey species (<25% WC) except for *Monanchora clathrata*, which was highly deterrent with only  $2.17 \pm 1.94$  g consumed out of  $41.43 \pm 9.85$  g offered. This species appears to be chemically defended, because cowries and tobies only accepted  $40.63 \pm 13.9$  and  $12.5 \pm 7.50\%$  respectively of experimental food containing extracts from *M. clathrata* at the natural volumetric concentration (Fig. 4). From sponges that were less preferred, *Dysidea* cf. *pallenscens* produced extracts that were readily consumed by both tobies and cowries. These

results were surprising, because *D. pallenscens* from the Mediterranean and Micronesia are known to produce avarol, which significantly deters predation by a congeneric toby (Müller et al. 1985, Pennings et al. 1994). These contrasting results could be explained by intraspecific geographical variation of secondary metabolite production across *Dysidea* spp. conspecifics (Erpenbeck et al. 2012a) or that the Hawaiian specimens are heterospecifics that produce different secondary metabolites. Geographic variation of secondary metabolite production is known to influence variability of chemical defenses among sponges across a wide taxonomic range (Loh & Pawlik 2014) and provides fertile scope for future study.

Cowries have been previously reported feeding on sponges that were chemically deterrent towards fish

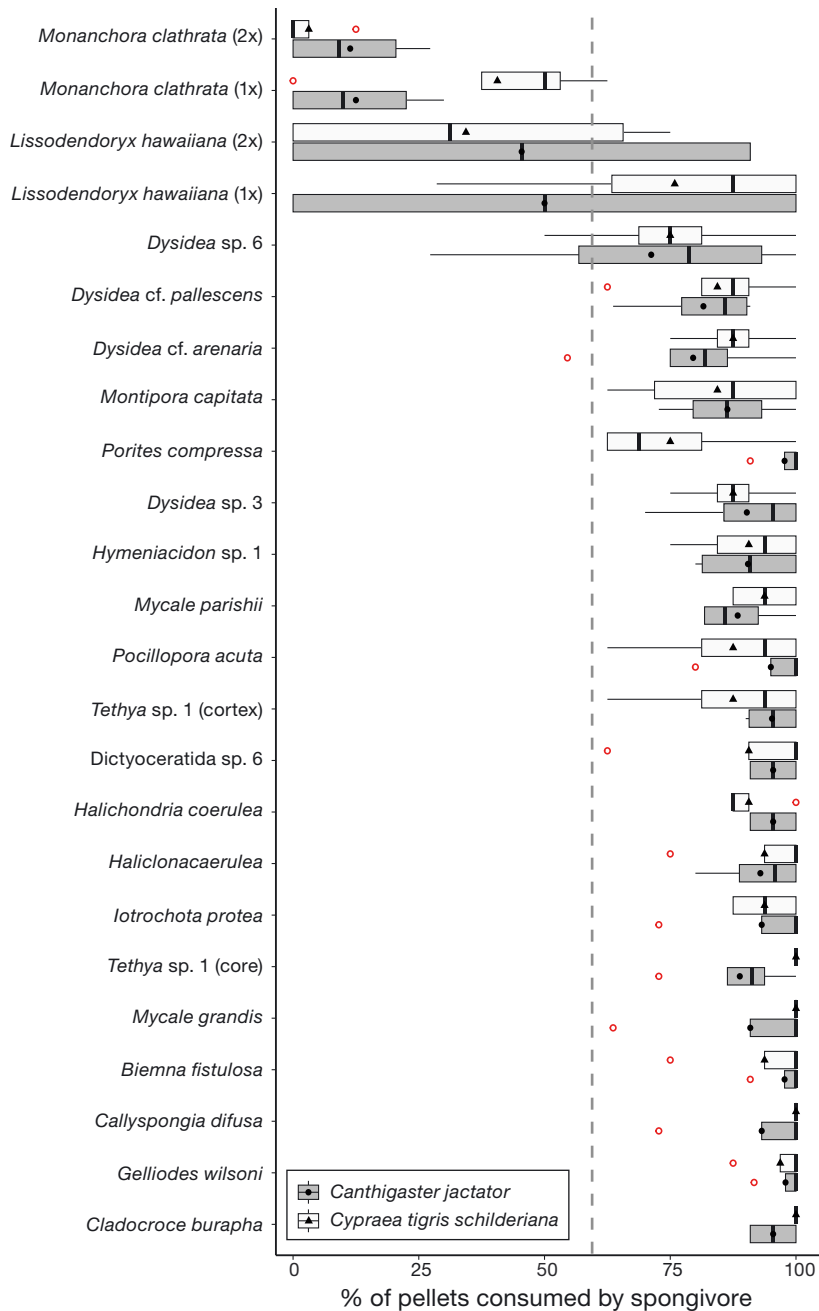


Fig. 4. Boxplot of % of experimental foods ( $n = 4 \text{ species}^{-1}$ ) consumed by cowries *Cypraea tigris schilderiana* in the form of tiles and consumed by tobies *Canthigaster jactator* in the form of pellets, in which crude organic tissue extracts from prey items incorporated at a 2x volumetric concentration into artificial food pellets were offered to each predator. Trials were repeated at 1x when <60% of pellets at 2x were accepted by tobies or cowries. Percent was calculated based on the number of tiles (out of 8 food tiles offered to 8 individual cowries) and pellets (out of 10 pellets offered to 13 tobies). In every case either 8/8 control food tiles for cowries or 10/10 control pellets for tobies were consumed. Mean values are shown as either a triangle (*C. tigris schilderiana*) or a circle (*C. jactator*). Red circles: outliers for each species; dotted line across the boxplot: threshold for statistical significance as determined by a modified Fisher's exact test where a sample is significantly deterrent ( $p < 0.05$ ) if 6 or fewer pellets are consumed. Tabulated data are presented in Tables S8 & S9. See Fig. 2 for explanation of boxplots

predators (Pawlik & Deignan 2015, Helber et al. 2017). This observation motivated our use of the Hawaiian whitespotted toby as an additional model predator to test whether these sponges were deterrent to predators spanning different phyla or whether cowries are able to tolerate defensive chemicals produced by the sponges. Acceptance and rejection of experimental foods were remarkably consistent between cowries and tobies across 19 of the 21 sponge and coral species (Fig. 4). Additional extracts from a broader range of chemically defended sponges may reveal additional discrepancies or whether there is general congruence in sponge palatability across predators in Hawai'i. Specifically, sponges in the order Verongida would be of interest given the clear foraging disparity observed between cowries and fish in other locations (Pawlik et al. 2013, Pawlik & Deignan 2015, Helber et al. 2017).

The assortment of prey sponge species spanning 6 taxonomical orders of Porifera, including a variety of spiculate and aspiculate (Order Dictyoceratida) species, suggests that physical defenses exert weak influence on feeding preference by cowries based on the range of mineral composition of preferred sponge species. Physical defenses of sponges have never been tested on gastropods but have previously been tested using generalist fishes (Burns & Ilan 2003, Jones et al. 2005). For example, the hypothesis that sponge skeletal composition influences palatability was tested on 8 Caribbean sponges with a variety of spicule morphologies and densities from both palatable and unpalatable species, using the bluehead wrasse *Thalassoma bifasciatum* (Chanas & Pawlik 1995). The authors prepared experimental foods by embedding spicules from different species in a squid matrix at volumetrically equivalent natural concentrations and found no significant differences in acceptance rate by *T. bifasciatum* based on

spicule type or length. In contrast, similar trials produced a different result in the Red Sea, where the Red Sea wrasse *T. klunzingeri* was deterred by physical defenses in 4 out of 6 sponge species with spicules >250  $\mu\text{m}$  (Burns & Ilan 2003). Although we did not test the physical defense hypothesis experimentally by embedding spicules in a squid matrix, our study shows no obvious role for physical defenses influencing preference, because cowries were unbiased in feeding preference regardless of the mineral composition of sponges. For example, among preferred palatable species, 5 sponge species are spiculate, with species producing megascleres averaging 138–147  $\mu\text{m}$  (*Cladocroce burapha*), 174–200  $\mu\text{m}$  (*Haliclona caerulea*), 380–600  $\mu\text{m}$  (*Halichondria coerulea*), 527–583  $\mu\text{m}$  (*Mycale grandis*), and 300  $\mu\text{m}$  (*M. parishii*) (Bergquist 1967, Núñez Pons et al. 2017). Cowries also preferred 4 dictyoceratid (aspiculate) sponge species, from which 3 *Dysidea* spp. were the quickest to be consumed (Fig. 3). Less preferred species included mostly spiculate species and one dictyoceratid species (*D. cf. pallescens*). Time lapse footage did reveal anecdotal differences in the time it took for cowries to digest sponge species with a high spicule content vs. dictyoceratid sponges, however. Cowries began to defecate digested tissue of the aspiculate *D. cf. arenaria* within 15–25 min after coming in contact with the sponge, whereas that of high spicule density sponge *M. grandis* (Vicente et al. 2016) took 40–65 min after contact before defecation began (Video S1). Previous studies provide similar anecdotal support for physical defenses having little deterrence on sponge feeding by cowries. For example, Raj et al. (2019) observed cowries in India feeding on *Rhabdastrella globostellata* which produces megascleres >600  $\mu\text{m}$  (Lim et al. 2008) and Helber et al. (2017) observed cowries feeding on an aspiculate sponge *Pseudoceratina* sp. in Zanzibar.

It is well known that carnivorous gastropods rely on olfactory sensors to find specific prey (Wyeth 2019) and could perhaps influence prey selection by tiger cowries in our study. For example, olfactory sensors help gastropod scavengers find dead fish up to 25 m away (Lapointe & Sainte-Marie 1992). Likewise, gastropod generalists use olfaction to hunt bivalves and barnacles (Ferner & Weissburg 2005), and opisthobranch specialists navigate by olfaction to find their exclusive soft coral prey (Wyeth & Willows 2006). Determining olfactory attraction or repellent properties of sponge species as prey have never been tested on cowries, but based on our observations of the speed and directionality of cowries in response to addition of a preferred sponge prey to the aquarium, we be-

lieve that olfaction is likely an important proximate mechanism of prey selection. Future studies using flow-through Y- and T-mazes with water plumes from less preferred sponge species might help determine if olfactory cues influence prey preference.

Given that physical and chemical defenses of the sponges seem to have little predictive power on the selectivity of cowries in this study, one might hypothesize that prey are being chosen on the basis of nutritional quality. While we have no direct estimates of nutritional quality of the sponge species assayed here, there are 2 primary reasons that we do not consider this hypothesis likely. First, previous studies have shown very little evidence to support that selectivity of spongivorous predators is driven by nutritional quality of preferred sponge species (McClintock 1987, Waddell & Pawlik 2000). For example, the seastar *Echinaster echinophorus* was shown to prefer both the nutrient-rich sponge *Geodia neptuni* as well as nutrient-poor *Callyspongia vaginalis* (Chanas & Pawlik 1995, Waddell & Pawlik 2000). In some cases nutrient-poor sponges (e.g. *Tedania ignis*) were selected by *E. echinophorus* over nutrient-rich sponges (*Chondrilla nucula* and *Chondrosia collectrix*). Second, we surveyed a wide taxonomic range that likely differed substantially in nutritional quality, and there was no obvious patterns among preferred and rejected sponges in this study. Thus, we believe that sponge selection by cowries is unlikely to be a consequence of nutritional value.

One additional capability of cowries revealed by this study is their ability to discern between closely related *Dysidea* congeners, which are difficult to identify due to the lack of a mineral skeleton and incongruence between molecular and morphological characters (Erpenbeck et al. 2012b). Phylogenetic analysis of the 28S rRNA showed that 3 closely related *Dysidea* spp. share similar morphological features, detectable in the field only by texture and color (Fig. 1) which are taxonomically unreliable characters for Dysideidae. Although challenging for humans to identify, cowries showed a clear preference among them within the first 24 h, consuming first *D. cf. arenaria*, followed by *Dysidea* sp. 3, and then *Dysidea* sp. 6 (Fig. 3). Similar distinctions between closely related sympatric congeners of *Tedania* spp. have also been observed in feeding assays using starfish in the Caribbean (Wulff 2006). The order of feeding preference by cowries in such assays provides a potential chemotaxonomic assessment among cryptic sponge species.

The only sponge we tested that showed clear evidence for being a deterrent was *M. clathrata*. Such a

low occurrence of chemically defended sponge species is unprecedented among broad taxonomic surveys to date (Pawlik et al. 1995, Becerro et al. 2003, Burns & Ilan 2003, Ruzicka & Gleason 2008, Helber et al. 2017). One possible hypothesis is that palatable species which grow and reproduce more rapidly are quicker to foul on ship hulls and marine debris than are chemically defended sponges (Pawlik et al. 2008, Leong & Pawlik 2010). Previous work found the sponge community of a shipwreck dominated by palatable sponge species, despite being in close proximity to a reef with a variety of both chemically defended and undefended sponges (Pawlik et al. 2008). Although international commercial vessels are more prone to acquire sponges from harbors rather than coral reefs, it seems likely that fouling on these vessels is similarly dominated by fast-growing and undefended species. Insofar as this assumption is correct, we hypothesize that species that have successfully invaded Hawai'i tend to be quick to grow and reproduce rather than producing chemical defenses against predators.

A biocontrol strategy using cowries has recently been proposed for coral reefs facing devastating bleaching events and increased competition with the sponge *R. globostellata* in the Gulf of Mannar, India (Raj et al. 2019). The fact that some of the most preferred prey species of the endemic tiger cowrie *Cypraea tigris schilderiana* are non-native raises the question of whether these endemic cowries could be used as a potential biological control agent for non-native sponges on Hawaiian coral reefs. *C. tigris schilderiana* is both large and a voracious predator, consuming up to 60% of their body weight in sponges each week. In Kāne'ohe Bay, competition between an introduced sponge (*M. grandis*) and native coral species has increased substantially through time such that there is concern about the spread of *M. grandis* (Coles & Bolick 2007, Coles et al. 2007). Historically, tiger cowries were far more common in Hawaiian waters and particularly in Kāne'ohe Bay (kupuna J. Kaluhiwa pers. comm.) where they may have exerted top-down control on preferred sponge species. Cowries have been overharvested throughout the Pacific and have experienced a precipitous decline in many locations (Lim & Chin 1982, Aiken & Leigh 1985, Appukuttan & Ramadoss 2000). Cowries are extremely unlikely to control sponges at very low population densities. Given that *M. grandis* is a preferred prey sponge of these cowries, it might be possible to control this alien species through breeding and release of cowrie predators that replenish the historical population abundance. Hawai'i has already under-

taken population enhancement of native urchins through captive breeding as part of an effective management strategy to control alien invasive algae in Kāne'ohe Bay (Westbrook et al. 2015, Neilson et al. 2018). A similar strategy using tiger cowries may prove effective in controlling non-native sponges in Kāne'ohe Bay, but appropriate field experiments would be needed to accurately estimate foraging pressure and feeding preference variability of cowries in a natural setting.

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