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Estimates of sponge consumption rates on an Indo-Pacific reef

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ABSTRACT: Determining predator diets is essential for understanding the strength of top-down processes and how they cascade through food webs. This is especially important for sponges, key members of benthic communities, whose dominance has increased in recent years on some coral reefs. However, the diversity of spongivorous fishes and the sponges they consume are relatively unknown. Here, we estimated sponge consumption by spongivorous fishes in the Wakatobi Marine National Park, Indonesia. We deployed cameras to identify fish biting at the dominant reef sponge *Xestospongia* spp. and then used gut content analysis and fish abundance estimates to quantify sponge consumption. In total, 33 species from 10 families of reef fish were identified taking bites from *Xestospongia* spp.; however, the 2 most prolific sponge-grazers, *Ctenochaetus binotatus* and *Chaetodon kleinii*, had no sponge in their guts, showing that for some fish, bites on sponge surfaces are not reliable evidence of sponge consumption. Gut contents indicated that *Pygoplites diacanthus* was an obligate spongivore, while *Pomacanthus imperator*, *P. xanthometopon*, *Zanclus cornutus* and *Siganus punctatus* regularly consumed sponges. Sponge consumption by these 5 spongivores was estimated at 46.6 ± 18.3 g sponge 1000 m^{-2} d^{-1}. Molecular approaches developed to sequence the 18S gene for sponges consumed by angelfishes led to the successful amplification of 14 consumed sponges representing 6 orders of Porifera. We provide the first estimate of sponge consumption in the Indo-Pacific and are the first to successfully sequence partially digested sponges from fish stomachs, identifying several sponges previously unknown to be consumed by spongivores.

KEY WORDS: Spongivory · Angelfishes · Diet · DNA gut contents

1. INTRODUCTION

Accurate quantification of a species' functional role is essential to estimate its contribution to ecosystem function (Bellwood et al. 2019). On tropical coral reefs, with the exception of well-studied fish groups such as herbivores and corallivores (Bellwood & Choat 1990, Bellwood et al. 2004, Pratchett 2005, Cole et al. 2011), our understanding of the range of functional roles performed by individual species remains limited, impeding our ability to make informed decisions on ecosystem management (Bellwood et al. 2019). Heightened interest in the interactions between sponges and other organisms (Bell et al. 2018a, Pawlik et al. 2018) has been driven by evidence that sponges may, at least initially, be less affected by this period of unprecedented environmental change than some other benthic organisms (Bell et al. 2013, 2018a,b, Pawlik et al. 2018). Experimental studies indicate that some sponges may have a greater tolerance to environmental shifts than calcifying fauna (Vicente et al. 2016, Bennett et al. 2017). Furthermore, several studies have reported recent increases in sponge abundance (Ruzicka et al. 2013, McMurray et al. 2015, de Bakker et al. 2017), with transitions to sponge-dominated states being report-

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*Corresponding author: james.bell@vuw.ac.nz ed in the Caribbean, Atlantic, Indo-Pacific and Pacific (reviewed in Bell et al. 2013). Increasing sponge abundance will likely have profound impacts on food chain dynamics (Bell 2008, Bell et al. 2013, 2018b), but our ability to predict these changes is restricted by limited data on the taxonomic breadth of spongivores and their preferred prey (Wulff 2017). These knowledge gaps need to be addressed to better understand the future functioning of sponge-dominated reefs (Bell et al. 2018a).

Sponges have structural and chemical defences that deter predation (Hay 1996, Hill et al. 2005, McClintock et al. 2005), but they are thought to rely mainly on secondary chemistry (Pawlik et al. 1995, Pawlik 2011). On Caribbean reefs, angelfishes (Family Pomacanthidae) are well-known spongivores that are thought to reduce the impact of chemical substances produced by sponges by adopting a 'smorgasbord' approach to foraging (Randall & Hartman 1968), whereby small amounts of many sponge species are consumed (Wulff 2006). For example, Randall & Hartman (1968) recorded >40 species of sponge in the gut contents of *Holacanthus ciliaris* and >20 species from other spongivorous angelfishes. Applying existing data on sponge palatability (determined by feeding choice experiments using pelletised extractions of secondary metabolites) to gut content data from Randall & Hartman (1968) demonstrated that the majority of sponges consumed by the angelfishes *Pomacanthus arcuatus* and *P. paru* were chemically undefended (Pawlik et al. 1995, 2018, Pawlik 2011). Two palatable species (*Callyspongia vaginalis* and *Niphates erecta*) made up approximately one-third of gut contents of the 2 angelfish, suggesting a preference for these 2 species in particular (Pawlik et al. 2018).

Indo-Pacific reefs exhibit some of the highest sponge diversity in the world (van Soest et al. 2012); however, few studies have considered the ecological role of spongivory (but see Powell et al. 2014, 2015). In a study conducted in the Wakatobi Marine National Park (MNP), Powell et al. (2014) found that spongivore abundance was highest at Sampela reef, a site that has undergone a coral–sponge regime shift (Powell et al. 2014, Biggerstaff et al. 2015). However, in the study by Powell et al. (2014), spongivores were identified from the literature and from *in situ* observations of feeding behaviour, with only 11 of the 21 species listed having gut content data verifying sponges were an ingested prey item. Importantly, fish may bite at sponges but have no predatory impact (Bell et al. 2017, Pawlik et al. 2018), which seems likely for 2 surgeonfishes included in the list of 21 spongivores compiled by Powell et al. (2014) as they likely lack the necessary dental morphology to remove sponge tissue (Bellwood et al. 2014). Combining species that likely fulfill different functional roles on reef systems into a single spongivore category could inadvertently bias our understanding of reef functioning (Wulff 2017). Hence, when the diet of a species remains ambiguous, a multi-method approach can prevent functional misclassifications and provide more detail on a specific diet (Nagelkerken et al. 2009, Bell et al. 2017, Nielsen et al. 2018).

Whilst gut content data provide an unambiguous record of consumed prey (Wulff 2006), it may be biased towards items with structural components that are not easily digested, and taxonomic resolution can be hindered by digestion (Nielsen et al. 2018). Sponges range from having highly dense to very diffuse tissue, which likely affects digestibility and hence tissue available for identification. Over the past decade, molecular methods have emerged as a valuable tool to overcome some of the challenges associated with diet identification (see Nielsen et al. 2018). Polymerase chain reaction (PCR) can detect small amounts of DNA, providing greater resolution to diets that are hard to visually characterise because they lack hard parts, are heavily degraded or are liquid remains (Symondson 2002, Vestheim & Jarman 2008, Nielsen et al. 2018). However, gut content DNA can be degraded and fragmented by digestion (Deagle et al. 2009), and PCR can be inhibited by substances found in digestive tracts (Schrader et al. 2012). Additionally, gut contents contain a mixed profile of samples, and predator DNA may be preferentially amplified over degraded prey DNA (Vestheim & Jarman 2008). Where group-specific primers are not an option, methods that block predator DNA from amplification, e.g. predator-blocking primers (Vestheim & Jarman 2008, Leray et al. 2013, Leray et al. 2015) can be developed to successfully resolve the diets of marine animals.

Here, we aimed to improve our understanding of the ecological significance of spongivory in the Central Indo-Pacific. Our specific objectives were (1) to identify sponge-grazing fishes using cameras to record fish taking bites from *Xestospongia* spp., one of the most abundant and conspicuous reef sponges in the Wakatobi MNP; (2) to determine diet composition of key sponge-grazing fish using gut content analysis; and (3) to estimate sponge consumption rates using the mean weight of sponge in the gut contents of each spongivore and spongivore density data. Ingested sponges recovered from gut contents could not be easily identified due to a lack of reliable field guides and a species-rich environment (Bell & Smith 2004, Rovellini et al. 2019). Additionally, homogenisation of sponges within the digestive tract limited traditional identification methods using spicules; therefore, our final objective was (4) to develop molecular methods to identify ingested sponges from the gut contents of spongivorous angelfish from the Indo-Pacific.

2. MATERIALS AND METHODS

2.1. Study sites

This study was conducted at 4 sites within the Wakatobi MNP, the third-largest MNP in Indonesia (Clifton & Unsworth 2013) (Fig. 1). Four sites with different benthic cover, topographical and environmental conditions and fish assemblage compositions (Curtis-Quick 2013, Powell et al. 2014) were chosen to maximise the number of different reef fish species that could be recorded as potential spongivores. All sites were separated by at least 3 km, aside from Sampela and B1, which are separated by a channel that is approximately 1.5 km wide and extends to a depth of 40 m. Sampela reef was the most degraded of the 4 sites, with sedimentation rates reported as 4 times higher than the other 3 sites (Crabbe & Smith 2005) and with the lowest abundance of live coral cover (~10%) and high abiotic cover (<50%) (Mortimer 2020). This site has experienced a coral–sponge regime shift and has a high abundance of the phototrophic encrusting sponge *Lamellodysidea herbacea* (Powell et al. 2014, Biggerstaff et al. 2015, Mortimer 2020). KDS, B1 and The Ridge both had higher coral cover (~30%; Mortimer 2020, Operation Wallacea unpubl. data) but The Ridge had a higher percentage of soft coral (~13%) than Sampela and B1 (~3%; Mortimer 2020). Sponge percentage cover was similar between Sampela, B1 and The Ridge, covering on average 11.9 ± 1.4% of the substrate. The Ridge had the highest number of standardised sponge species recorded (9.2 ± 1.3 m^{-2}), followed by B1 (6.2 ± 0.8 m^{-2}) and Sampela (5.3 ± 0.5 m^{-2}); no data were recorded for KDS (Mortimer 2020).

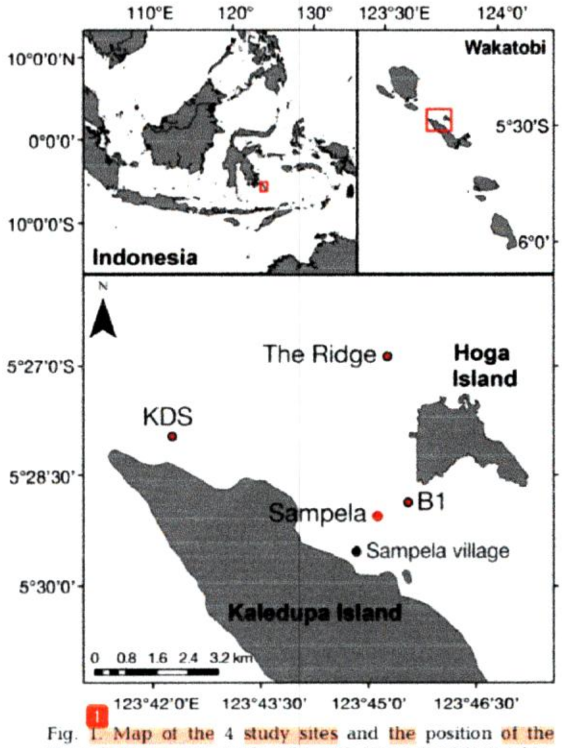

Fig. 1. Map of the 4 study sites and the position of the Hoga/Kaledupa channel within the Wakatobi Marine National Park (MNP) and Indonesia

2.2. Videos of sponge-grazing fishes

To identify sponge-grazing fish that would be targeted for gut content analysis, videos of the giant barrel sponge *Xestospongia* spp. were conducted at all 4 sites from June to August 2016. We use the term 'sponge-grazing' to describe feeding behaviour whereby fish bite at sponge surfaces but biomass removal (i.e. spongivory) has not been verified. Barrel sponges in the Wakatobi MNP are considered to comprise a species complex including *X. testudinaria*, *X. bergquistia* and at least one undescribed species (Bell et al. 2014). *Xestospongia* spp. was chosen because an initial trial suggested this genus incurs significant grazing activity and was present in sufficient densities across the 4 study sites (McGrath 2018). Additionally, the Caribbean congener *X. muta* was used by Dunlap & Pawlik (1998) to monitor predation by parrotfishes, suggesting that members of this genus attract predators in a variety of reef locations. A GoPro camera was deployed approximately 1 m away from the sponge and set to record from the end of diving activities to maximise the amount of footage in which diving activities did not disrupt fish behaviour (Emslie et al. 2018). To avoid any effect of depth or microhabitat on fish assemblages (Depczynski & Bellwood 2004, Stefanoudis et al. 2019), sponges were filmed on the open reef slope (8–14 m, depending on the site). At the beginning of the video, a measuring stick was placed next to the sponge to generate screenshots from which the 2-dimensional area of sponge captured in the frame was estimated to standardise grazing intensity by area. All cameras were deployed during the morning (~08:00–11:00 h) and collected on a subsequent dive in the afternoon, often yielding >2 h of uninterrupted footage.

2.3. Video analysis

The first 5 min of video footage were discarded to account for disturbance from divers leaving the site. The remaining footage was analysed by counting all bites made by fish on *Xestospongia* spp., with bites determined as individual 'strikes' on the sponge surface. Fish were identified to species level except for *Ctenochaetus* spp., as it was not always possible to distinguish between *C. binotatus* and *C. striatus*. Distinguishing individual fish that may have fed repeatedly from the same sponge was not possible using these methods; however, the aim was to measure the grazing intensity of each species rather than individual bite rates. To calculate the area of sponge captured in video footage, screenshots generated from footage containing the scaling device were imported into ImageJ (Abràmoff et al. 2004).

2.4. Sponge-grazing fish densities

Sponge-grazing fish densities were quantified at all 4 sites by underwater visual census (UVC) using 6 replicate 50 × 5 m belt transects deployed on the reef slope at 10 m depth, with each transect separated by 15 m. For each transect, the lead diver recorded fish data and a second diver followed behind, deploying the transect tape to ensure the area covered was undisturbed by diver presence (Emslie et al. 2018). In total, 14 species were recorded by UVC (Table 1). This included 9 of the 11 species recording >95% of total bites on *Xestospongia* spp. (see Table 2) and 4 additional pomacanthids (*Centropyge vroliki*, *Pomacanthus imperator*, *P. xanthometopon* and *P. sexstriatus*) that have been recorded grazing on sponges in other studies (Powell et al. 2015) and as part of an initial trial conducted in the present study (see Appendix).

2.5. Gut content analysis

To quantify the amount of sponge consumed by sponge-grazing fishes, 5 species (*C. binotatus*, *Chaetodon kleinii*, *Pygoplites diacanthus*, *Zanclus cornutus*, *Forcipiger flavissimus*) identified from *Xestospongia* spp. video footage as frequent sponge grazers were spear-fished at The Ridge and Sampela over 2 wk in July 2017 by local fishers (see Table 3 for sample sizes). Some species that were identified in video footage as frequent sponge grazers (e.g. *Coradion melanopus*) were not targeted due to low population size. We also captured one *P. imperator* from Sampela, and 3 *P. xanthometopon* and 5 *Siganus punctatus* from The Ridge; these species were not observed in videos of *Xestospongia* spp. but were observed feeding on other sponges in an initial trial (see Appendix). All fish were placed on ice immediately after spearing and kept at −20°C until dissection. During dissection, fish were weighed (precision: 0.1 g) and total length (mm) was recorded. The entire digestive tract was removed and preserved at −20°C in 96% ethanol to prevent further digestion. Preserved digestive tracts were returned to Wellington and dissected at the Victoria University Coastal Ecology Laboratory (VUCEL). During dissection, contents from the stomach (or entire digestive tract for *C. binotatus* and *S. punctatus*) were examined under a dissecting microscope and separated into identifiable

Table 1. Sponge-grazing fish densities (mean ± SE) per 250 m^2 surveyed by underwater visual census (n = 6) at 4 sites in the Wakatobi Marine National Park

Family / species	Sampela	B1	The Ridge	KDS
Acanthuridae				
Acanthurus pyroferus	4.8 ± 1.1	3.2 ± 0.4	5.0 ± 0.7	1.8 ± 0.7
Ctenochaetus binotatus	4.8 ± 1.0	4.3 ± 1.0	2.5 ± 0.5	3.3 ± 0.7
Chaetodontidae				
Chaetodon kleinii	2.8 ± 0.8	4.3 ± 1.0	4.2 ± 1.5	2.8 ± 0.7
Coradion melanopus	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Forcipiger flavissimus	1.3 ± 0.5	8.0 ± 0.9	5.5 ± 0.6	6.0 ± 1.4
Pomacanthidae				
Centropyge bicolor	2.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
Centropyge nox	0.3 ± 0.2	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0
Centropyge tibicen	2.3 ± 0.9	0.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Centropyge vroliki	0.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3
Pomacanthus imperator	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Pomacanthus sexstriatus	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5
Pomacanthus xanthometopon	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.3
Pygoplites diacanthus	0.2 ± 0.2	0.8 ± 0.4	2.7 ± 0.6	1.2 ± 0.4
Zanclidae				
Zanclus cornutus	3.0 ± 0.5	2.8 ± 0.5	5.0 ± 0.8	4.8 ± 0.5

and unidentifiable portions. For *P. diacanthus*, *Pomacanthus* spp., *S. punctatus* and *Z. cornutus*, food items in the identifiable portion were separated into 13 major categories (see Table 3), blotted to remove gastric juices and weighed (wet weight: 0.01 g) to estimate diet proportions. Due to low stomach/digestive tract volumes, the diet composition of *C. kleinii*, *F. flavissimus* and *C. binotatus* was estimated visually via relative volumetric quantity (after Nielsen & Johnson 1992). Samples of sponges recovered from gut contents were stored in separate microcentrifuge tubes at −20°C in 96% ethanol for subsequent DNA extraction. All dissecting instruments were ethanol-cleaned and rinsed 3 times with double distilled water (DDW) between stomachs to prevent cross-contamination.

Levins (1968) standardised diet breadth was calculated as:

$$B = 1 / \Sigma p_j^2 \qquad (1)$$

where B is niche breadth and p is the proportion of overall diet made up by the food category j. This can then be standardised and expressed on a scale of 0–1 according to the following:

$$BA = (B - 1) / (n - 1) \qquad (2)$$

where BA is the standardised niche breadth and n is the number of food categories. Low values indicate a specialised diet based on few dietary items.

2.6. Sponge consumption estimates

To estimate sponge consumption, we used the mean weight (g) of sponge in the gut contents of each species and assumed that stomachs were filled at least twice daily following Harmelin-Vivien & Bouchon-Navaro (1983). This weight was multiplied by the total number of fish of each species observed at each site and scaled to 1000 m^{-2}. All sponge consumption estimates are reported as mean ± 95% CI.

2.7. DNA analysis of gut content sponges

Total genomic DNA was extracted from gut content sponges recovered from the angelfishes *P. diacanthus*, *P. imperator*, and *P. xanthometopon* using a DNeasy Blood and Tissue Kit (Qiagen) following the protocol of the manufacturer. PCR was performed on extracted sponge DNA using custom 18S primers designed during this study (see Texts S1–S3 in the Supplement at www.int-res.com/articles/suppl/m672p123_supp.pdf for full details on primer design and trials) alongside a sponge control, a predator control and a negative control. PCR was conducted in 25 µl reactions consisting of 12.5 µl MyTaq Red Mix (Bioline), 0.5 µl of each universal primer (10 µm), 0.5 µl of each blocking primer (100 µm), ~25 ng of template DNA and a volume of distilled water to reach 25 µl. An additional 5 µl of BSA was added to the reaction to improve PCR yield. PCR cycling conditions were 94°C for 5 min; then 35 cycles of 94°C for 30 s, 48°C for 55 s, 72°C for 45 s and a final extension step of 72°C for 7 min. In total, 32 samples were successfully amplified and sent for purification and sequencing on an ABI 3730 capillary sequencer (Macrogen).

2.8. Data analysis

2.8.1. Video observations and fish densities

The difference in overall grazing intensity (count of bites; all species combined) between sites was tested using negative binomial regression using the MASS package (Venables & Ripley 2002) for R studio (RStudio Team 2015) with footage length (min) and area of sponge (m^2) included as offsets in the model. A difference in the density of sponge-grazing fishes recorded by UVC between sites was analysed using a 1-factor ANOVA, with test assumptions verified by Shapiro-Wilks test of normality and Levene's test of the equality of variances. Multivariate analyses were conducted with the PERMANOVA+ add-in to PRIMER v.6.1 (PRIMER-E). Differences in fish species contributing to grazing intensity (total bites) and in the community composition of sponge-grazing fishes between sites were assessed using Bray-Curtis similarity matrices with the unrestricted permutation of raw data and subsequent pair-wise comparisons. Data were square-root transformed prior to analysis and checked for multivariate homogeneity of group dispersions using PERMDISP. We used SIMPER (PRIMER-E) to calculate the contribution of each species (%) to the dissimilarity between sites.

2.8.2. Sequence data

Generated sequences were screened for contamination from other targets (e.g. fungus) and for duplicate samples (e.g. the same sponge sequence from the same stomach). Remaining sequences were quality checked using CodonCode Aligner (www.codoncode.com/aligner) by clipping bases until there were fewer than 3 bases with a phred quality score of less than 20 in a 25 bp window and removing any sequences shorter than 200 bp in length. A demosponge data set was created using 18S rDNA sequence data downloaded from the GenBank database; the minimum inclusion was 500 bp and downloaded sequences were limited to the 6 orders represented by sequences obtained by this study. All sequences were aligned using the ClustalW function in BioEdit v.7.0.5.3 (Hall 1999). A phylogenetic tree was constructed of aligned sequence data using ModelFinder in IQ-TREE multicore v.1.6.11 (Kalyaanamoorthy et al. 2017), which calculates the log-likelihoods of an initial parsimony tree for many different models, choosing the model that minimises the Bayesian information criterion (BIC). The glass sponge *Rhabdocalyptus dawsoni* was used as an outgroup (accession number AF100949) and branch supports were obtained using the SH-aLRT test (Guindon et al. 2010) and ultrafast bootstrap (Hoang et al. 2018). The best-fit model according to BIC was TIM2+F+I+G4. Branch supports are reported as SH-aLRT support (%) / ultrafast bootstrap support (%), and high support for the existence of a clade was determined by SH-aLRT ≥ 85 % / UFBoot ≥ 95 %.

3. RESULTS

3.1. Video observations

In approximately 50 h of video footage, 28 477 bites were recorded on *Xestospongia* spp. across the 4 sites. In total, 33 species from 10 families of coral reef fish were identified grazing on *Xestospongia* spp. (Table 2). Grazing intensity (total bites) was significantly different between sites, with barrel sponges at Sampela experiencing fewer bites (mean ± SE: 12.2 ± 2.6 bites min^{-1} m^{-2}) than barrel sponges at The Ridge (34.9 ± 10.8 bites min^{-1} m^{-2}) ($Z = 2.85$, $p = 0.004$) and barrel sponges at B1 (28.4 ± 6.0 bites min^{-1} m^{-2}) ($Z = 2.59$, $p = 0.009$) but not barrel sponges at KDS (21.7 ± 6.0 bites min^{-1} m^{-2}). Despite the large number of species observed grazing on *Xestospongia* spp., over half of the total bites recorded were taken by *Ctenochaetus* spp. (of the fish that could be identified to species, >95% of bites were attributed to *C. binotatus*) and *Chaetodon kleinii*, and 95% were taken by 11 of the 33 species recorded (Table 2). Species contributing to grazing intensity were significantly different between sites (pseudo$F_{3,25}$ = 1.73, $p = 0.012$), with pairwise tests indicating significant differences between B1 and Sampela ($p = 0.001$) and B1 and The Ridge ($p = 0.038$); all other pairwise comparisons showed no significant differences ($p > 0.05$). The highest average within-group similarity was recorded at B1 (49.0%), followed by The Ridge (36.6%), KDS (34.8%) and Sampela (30.7%). SIMPER showed that the top 3 species driving the differences in predation between B1 and Sampela (average dissimilarity 68.7%), and B1 and The Ridge (average dissimilarity 63.4%) were *Coradion melanopus*, *Ctenochaetus* spp., and *C. kleinii*. At B1, *C. melanopus* took 18.8% of bites, in contrast to 1% at The Ridge and none at Sampela. At B1, *Ctenochaetus* spp. took 50.4% of bites, compared to 18.4% at Sampela and 19% at The Ridge. At B1, *C. kleinii* took 7.4% of bites, compared to 49.1% at Sampela and 33.7% at The Ridge (Fig. 2).

3.2. Sponge-grazing fish densities

Sponge-grazing fish densities (all species combined) were not statistically different between sites, with overall 24 ± 1 (mean ±SE) fish observed per 250 m^2. There was also no difference in the densities of the top 2 species observed grazing on *Xestospongia* spp. (Table 2), *C. binotatus* and *C. kleinii* (Table 1). However, there was a difference in sponge-grazing fish species composition between sites (pseudo$F_{3,20}$ = 3.95, $p = 0.001$). Pairwise comparisons revealed that the sponge-grazing fish species composition at Sampela was significantly different from that at B1 ($t = 2.38$, $p = 0.003$), The Ridge ($t = 2.93$, $p = 0.003$) and KDS ($t = 2.19$, $p = 0.001$) and there was also a significant difference between The Ridge and B1 ($t = 1.55$, $p = 0.033$) (Fig. 3). SIMPER indicated that spatial variation in sponge-grazing fish species composition was mainly driven by variation in the abundance of *Forcipiger flavissimus*, *Centropyge bicolor* and *C. tibicen* (Table 1). At Sampela, densities of *C. bicolor* were 2.8 ± 0.9 fish per 250 m^2 but *C. bicolor* densities were only 0.3 ± 0.3 fish per 250 m^2 at KDS, and *C. bicolor* was not recorded at The Ridge or B1. Similarly, *C. tibicen* densities were 2.3 ± 0.9 per 250 m^2 at Sampela, but only 0.5 ± 0.3 per 250 m^2 at B1, and *C. tibicen* was not recorded at The Ridge or KDS. Densities of *F. flavissimus* were much lower at Sampela (1.3 ± 0.5 per 250 m^2) than at B1 (8 ± 0.9 per 250 m^2), The Ridge (5.5 ± 0.6 per 250 m^2) and KDS (6.0 ± 1.4 per 250 m^2).

3.3. Gut content analysis

Pygoplites diacanthus had stomach contents containing >90% sponge (% wet weight); it also had the

Table 2. Total number of bites taken by 33 reef fish species filmed grazing on *Xestospongia* spp. at 4 sites in the Wakatobi MNP. Frequency of occurrence in videos (FO) calculated from the total number of *Xestospongia* spp. pooled across all sites (n = 30). X: fish was observed biting *Xestospongia* spp. at a particular site; (*) species sampled for gut contents

Species	Family	Total bites	Cumulative (%)	FO (%)	Sampela	B1	The Ridge	KDS
Ctenochaetus spp.*	Acanthuridae	10771	37.8	80	X	X	X	X
*Chaetodon kleinii**	Chaetodontidae	7829	65.3	86.7	X	X	X	X
*Zanclus cornutus**	Zanclidae	1835	71.8	36.7	X	X	X	X
Coradion melanopus	Chaetodontidae	1687	77.7	26.7		X	X	X
*Forcipiger flavissimus**	Chaetodontidae	1545	83.1	40		X	X	X
Centropyge tibicen	Pomacanthidae	1167	87.2	13.3	X	X		X
Scarus flavipectoralis juv.	Scaridae	996	90.7	26.7	X	X		X
*Pygoplites diacanthus**	Pomacanthidae	595	92.8	20	X	X	X	X
Heniochus singularius	Chaetodontidae	282	93.8	16.7		X	X	
Centropyge bicolor	Pomacanthidae	259	94.7	10	X			X
Centropyge nox	Pomacanthidae	225	95.5	10	X	X	X	
Zebrasoma scopas	Acanthuridae	163	96.1	13.3		X	X	X
Thalassoma lunare	Labridae	156	96.6	23.3	X	X		X
Chaetodon punctatofasciatus	Chaetodontidae	138	97.1	10		X		X
Balistapus undulatus	Balistidae	105	97.5	33.3	X	X	X	X
Centropyge vroliki	Pomacanthidae	102	98.2	10	X			X
Chromis xanthochira	Pomacentridae	102	97.8	10	X		X	
Anampses meleagrides	Labridae	89	98.5	13.3	X	X		X
Scarus niger	Scaridae	83	98.8	13.3		X	X	X
Halichoeres prosopeion	Labridae	69	99.0	26.7		X	X	X
Neoglyphidodon melas	Pomacentridae	65	99.2	13.3		X	X	
Diproctacanthus xanthurus	Labridae	51	99.4	6.7		X	X	
Canthigaster valentini	Tetraodontidae	38	99.6	3.3				X
Heniochus pleurotaenia	Chaetodontidae	37	99.7	3.3		X		
Scarus dimidiatus	Scaridae	22	99.8	6.7	X	X		
Pomacentrus adelus	Pomacentridae	13	99.8	3.3	X			
Scarus ghobban	Scaridae	11	99.9	3.3				X
Paracentropyge multifasciata	Pomacanthidae	10	99.9	3.3		X		
Siganus vulpinus	Siganidae	9	99.9	3.3				X
Chaetodon ulietensis	Chaetodontidae	8	99.9	3.3			X	
Sufflamen bursa	Balistidae	6	99.9	10	X		X	
Melichthys vidua	Balistidae	6	99.9	3.3				X
Odonus niger	Balistidae	3	100.0	3.3		X		
Other species grazing on sponges where bites could not be accurately determined:								
Acanthurus pyroferus	Acanthuridae			63.7	X	X	X	X
Acanthurus auranticavus	Acanthuridae			10	X			
Acanthurus nigricauda	Acanthuridae			3.3	X			
Acanthurus thompsoni	Acanthuridae			3.3	X			

narrowest diet breadth of all sampled species (diet breadth = 0.005) (Table 3). Soft coral was found in the stomach of one individual, and small amounts of tunicates and mobile invertebrates including polychaetes and small bivalves were also observed. The one *Pomacanthus imperator* stomach examined contained >90% sponge (% wet weight).

Sponge made up approximately half of the gut contents (% wet weight) of *Pomacanthus xanthometopon* stomachs, but soft coral (genus *Dendronephthya*) occurred in equal quantities (Table 3). Less frequent prey items included gastropods, polychaetes, and one stomach contained several juvenile starfish. Sponges were also found to be a major prey item of *Zanclus cornutus*, making up approximately 70% of total weighed contents. Other items observed included macroalgae and calcareous sediments. *Siganus punctatus* contained approximately one-third sponge (% weight), with the most dominant dietary item identified as the red alga *Laurencia* spp. Seagrass was present in all *S. punctatus* stomachs examined, as were other types of algae and small mobile invertebrates were occasionally observed.

C. kleinii had one of the widest diets observed of all the fish sampled for gut contents (Table 3). Although video observations of *Xestospongia* spp. suggested this species was spongivorous, sponge tissue was not observed, although sponge spicules were

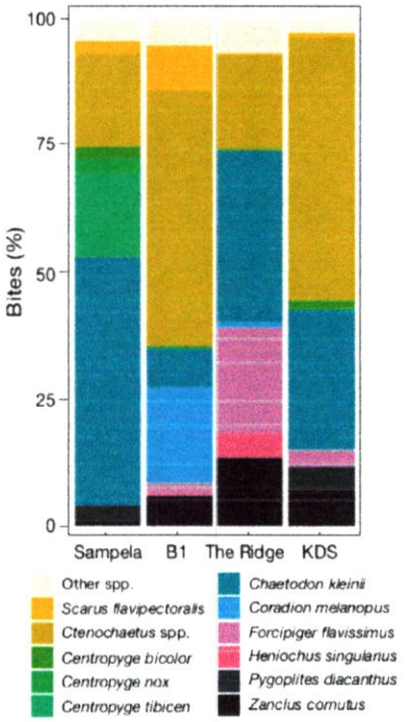

Fig. 2. Percentage of total bites on *Xestospongia* spp. taken by 11 species of sponge-grazing fishes filmed at 4 sites in the Wakatobi MNP

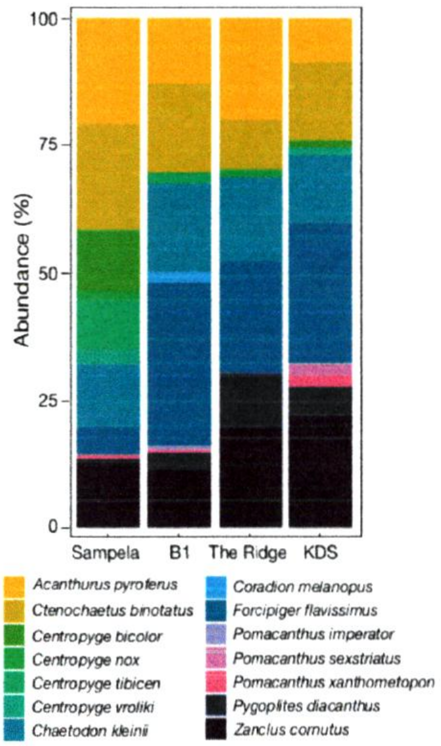

Fig. 3. Percentage of total abundance of sponge-grazing fishes surveyed by underwater visual census at each of the 4 study sites in the Wakatobi MNP

seen in 9 of 12 stomachs. Most of the organic matter in *C. kleinii* appeared to be of soft coral origin, and a variety of crustacea were observed. Small volumes of green filamentous algae were also present in most stomachs. *F. flavissimus* appeared to have a much more specialised diet than *C. kleinii*, with the majority of stomachs containing tentacles of *Serpulidae* worms (possibly *Spirobranchus* as distinctive toothed opercula were visible in many samples). Sponge tissue remains were sighted in 2 *F. flavissimus* stomachs, visually estimated to be <5% of the total volume. Other minor prey items for *F. flavissimus* included amphipods, isopods and decapod shrimps. The gut contents of *C. binotatus* were dominated by calcareous sediments and unidentifiable organic material. Sponge spicules were sighted in 9 of 12 gut contents but in very low numbers (1 or 2 gut^{-1}).

3.4. Sponge consumption

Estimates of sponge consumption were highest at the 2 outer reef sites, with 73.3 ± 21.5 g sponge 1000 m^{-2} d^{-1} (mean ± 95% CI) consumed at The Ridge and 52.9 ± 25.6 g sponge 1000 m^{-2} d^{-1} consumed at KDS (Table 4). The lowest estimate was at Sampela, with 27.6 ± 15.3 g sponge 1000 m^{-2} d^{-1}; 32.5 ± 10.9 g sponge 1000 m^{-2} d^{-1} was consumed at B1. Across all reefs, the 5 spongivorous fishes identified by this study consumed 46.6 ± 18.3 g sponge 1000 m^{-2} d^{-1}.

3.5. DNA analysis of consumed sponges

Out of 32 samples sent for sequencing, 7 amplified fungus, 2 amplified crinoid and 23 amplified sponge. Of the 23 sponge sequences, only 14 met the stringent quality-control standards employed by this study. The identity of one of these samples could not be resolved beyond Porifera and was removed from the data set. The remaining 13 sponges represented 6 orders of Porifera (Fig. 4). Six sponges were placed into Order Haplosclerida and came from *P. diacanthus* specimens collected at both Sampela and The Ridge (Fig. S2). The 18S data provided strong support that one sponge (Ridge RA2 S10) was placed into a clade with the Indo-Pacific sponge *Chalinula hooperi* (Bakus & Nishiyama 2000) (91.7/100) and another sponge (Ridge RA5) had strong support for placement within a clade of *Petrosia* sponges (98.6/96). The sponge Ridge RA1 S4A also formed a highly supported clade (97.8/99) with the species *Haliclona curacaoensis* and *Niphates erecta*. For the remaining samples in this order, identities could not be resolved beyond Haplosclerida. The Haplosclerida sponges formed 4 weakly supported major clades. All of the sponges placed in Order Haplosclerida were placed within a weakly supported major clade containing mainly Petrosiidae and Niphatidae sponges, with some interspersed *Haliclona*.

Table 3. Relative gut content (%) of 8 species of sponge-grazing reef fish caught in the Wakatobi MNP in 2017. *P. diacanthus* to *S. punctatus* are given in % wet weight (%W) and *C. kleinii* to *C. binotatus* are relative volumetric quantity (% volume, %V) due to low volume of stomach/gut contents. Frequency of occurrence (%F) of food items indicated. Bold values indicate sponge content >20%. TL: total length

	Pygoplites diacanthus		*Pomacanthus imperator*	*Pomacanthus xanthometopon*		*Zanclus cornutus*		*Siganus punctatus*		*Chaetodon kleinii*		*Forcipiger flavissimus*		*Ctenochaetus binotatus*	
Gut samples (n)	13		1	3		15		5		11		11		10	
TL range (mm)	122–188		102	204–272		82–150		236–285		82–104		122–158		122–136	
Levins' diet breadth	0.005		0.004	0.046		0.036		0.095		0.097		0.032		N/A	
	%W	%F	%W	%W	%F	%W	%F	%W	%F	%V	%F	%V	%F	%V	%F
Annelida															
Polychaete	<1	69	<1	<1	33	1	53	<1	60	15	92	75	100		
Crustacea															
Amphipod			<1			<1	13	<1	60	14	58	7	27		
Copepod									20	4	50				
Decapod						<1	7	<1	20	<1	17	9	18		
Isopod						<1	7			4	33	2	9		
Bryozoa															
Bryozoan						1	20								
Porifera															
Sponge	**94**	100	**96**	**53**	100	**73**	100	**32**	100	<1	75	2	18	<1	70
Chordata															
Ascidian	<1	38	1	<1	33	3	73	17	100						
Echinodermata															
Asteroidea				<1	33										
Ophiuroidea								<1	20						
Cnidaria															
Hydrozoa										2	42				
Anthozoa — hard coral				<1	100	<1	13			2	33				
Anthozoa — soft coral	5	8	3	46	100	10	7	<1	20	51	83	<1	9	<1	50
Mollusc															
Gastropod				<1	33	<1	20			<1	17				
Bivalve	<1	46				1	60	<1	60	<1	17	1	18		
Algae															
Calcareous algae				<1	33	1	73	1	20						
Red macroalgae								42	100						
Green filamentous	<1	46								4	58			5	80
Green turfing								<1	20						
Brown macroalgae						2	60	<1	40						
Seagrass															
Seagrass								6	80					4	90
Eggs															
Eggs						<1	6			3	50	<1	9		
Sediments															
Calcareous deposits	<1	15				6	67	1	60					90	100
Fluid															
Fluid/mucus												2	9		

Table 4. Estimates of sponge consumption for 5 species of spongivorous reef fish calculated from mean sponge weight in gut contents and fish densities. NB: *Siganus punctatus* was not included in the underwater visual census of sponge-grazing fishes; therefore, density was conservatively estimated from video observations

Site / spongivore	Total fish in 1500 m^2	Sponge weight (g) (mean ± 95% CI)	Sponge consumption (mean ± 95% CI) (g 1000 m^{-2} d^{-1})
Sampela			
Pygoplites diacanthus	1	2.05 ± 0.38	2.7 ± 0.5
Pomacanthus imperator	0	2.83	0.0 ± 0.0
Pomacanthus xanthometopon	1	4.68 ± 4.54	6.2 ± 6.1
Zanclus cornutus	18	0.39 ± 0.10	9.4 ± 2.4
Siganus punctatus	2	3.49 ± 2.35	9.3 ± 6.3
B1			
P. diacanthus	5	2.05 ± 0.38	13.7 ± 2.5
P. imperator	1	2.83	3.8
P. xanthometopon	1	4.68 ± 4.54	6.2 ± 6.1
Z. cornutus	17	0.39 ± 0.10	8.8 ± 2.3
S. punctatus	0	3.49 ± 2.35	0.0 ± 0.0
The Ridge			
P. diacanthus	16	2.05 ± 0.38	43.7 ± 8.1
P. imperator	0	2.83	0.0 ± 0.0
P. xanthometopon	0	4.68 ± 4.54	0.0 ± 0.0
Z. cornutus	30	0.39 ± 0.10	15.6 ± 4.0
S. punctatus	3	3.49 ± 2.35	14.0 ± 9.4
KDS			
P. diacanthus	7	2.05 ± 0.38	19.1 ± 3.5
P. imperator	0	2.83	0.0 ± 0.0
P. xanthometopon	3	4.68 ± 4.54	18.7 ± 18.2
Z. cornutus	29	0.39 ± 0.10	15.1 ± 3.9
S. punctatus	0	3.49 ± 2.35	0.0 ± 0.0

The second order that was represented by more than one sponge was Verongiida, and all of these sponges came from stomachs of *P. diacanthus* collected at Sampela (Fig. S3). There was strong support for all 3 sponges being placed within a clade containing Pseudoceratinidae and *Verongula* sponges (86.5/97). The remaining sponges were all classified as separate orders and were sequenced from the stomachs of *P. diacanthus* and *P. xanthometopon* collected at The Ridge. There was strong support for the placement of one sponge (Ridge RA6 S3) within a clade of Agelasidae sponges (87.5/95) and another sponge (Ridge RA4 B) had strong support for placement within a clade of *Aaptos* sponges from the order Suberitida (98.7/100). The sponge Ridge RA3 S1 had strong support for placement within a clade containing both Clionaidae and Spirastrellidae genera (97.2/99) and the only sponge that was successfully sequenced from the gut contents of *P. xanthometopon* (Ridge PX2 S13) was grouped in Order Poecilosclerida and had strong support for placement into a clade containing *Guitarra* sp. (88.1/100).

4. DISCUSSION

Resolving predator–prey dynamics is essential to understand the structuring of ecological communities (Leray et al. 2015, Nielsen et al. 2018). Our study provides the first estimate of sponge biomass consumption on coral reefs and is the first to successfully sequence sponges recovered from the stomach contents of spongivorous fishes. Five important spongivores were identified through a combination of video observations and gut content analysis and were estimated to consume 46.6 ± 18.3 g sponge 1000 m^{-2} d^{-1} (mean ± 95% CI). Interestingly, the most prolific sponge-grazers (*Chaetodon kleinii* and *Ctenochaetus* spp.) had no sponge tissue remains in gut contents, highlighting the importance of using direct methods of diet quantification to prevent functional misidentifications (see Bellwood et al. 2019). Fourteen unique sponge sequences were amplified from sponges found in angelfish stomach contents, suggesting that *Pygoplites diacanthus* exhibited high dietary plasticity within Phylum Porifera similar to the diets of angelfishes in the Caribbean (Randall & Hartman 1968) and the Pacific (Verdín Padilla et al. 2010).

4.1. Identified spongivores

Angelfishes are well-known spongivores in the Caribbean and the Pacific (Randall & Hartman 1968, Hobson 1974, Verdín Padilla et al. 2010), with at least 5 species thought to rely on sponges for the majority of their diet (Randall & Hartman 1968, Hobson 1974). The present study confirms that *P. diacanthus* may also be reliant on sponges as stomach contents contained >80% sponge. Our findings are consistent with previous *in situ* observations of *P. diacanthus* feeding behaviour (Powell et al. 2015). *P. diacanthus* had the largest predatory impact on sponges, accounting for almost half of the total sponge consumption. The one *Pomacanthus imperator* stomach dissected contained >90% sponge, suggesting a similar reliance on sponges to *P. diacanthus*, but more samples would be required to determine if *P. imperator* is a true obligate spongivore on Wakatobi reefs. The *P. imperator* stomach examined was dominated by sponges that appeared to be highly spiculose, whereas *P. diacanthus* stomachs contained both highly silicified sponges and fewer but noticeable aspiculate sponges with organic fibres present. Gut content data also confirmed *Pomacanthus xanthometopon* was a spongivore, but it appeared to have a lower reliance on sponges than *P. diacanthus*, consuming an equal amount of predominantly Dendronephthya soft corals, with one stomach containing 3 juvenile starfish.

Sponges were the dominant dietary item for *Zanclus cornutus*, although its diet also consisted of algae and other sessile invertebrates, including bryozoans and ascidians. The rabbitfish *Siganus punctatus* also regularly consumed sponges, which were found in the gut contents of all sampled individuals, although in widely varying amounts (2–76% wet weight, g). Our results agree with data from the Great Barrier Reef and Japan, where sponges represented 20 and 72% of the gut contents of adult *S. punctatus* (Pitt 1997, Sano 1989). Our data indicate that sponges and the red alga *Laurencia* spp. made up the majority of the diet of adult *S. punctatus* on Wakatobi reefs. Seagrass was also present in small amounts (6% wet weight, g) in 4 out of 5 individuals. This may have been ingested in reef areas as detritus, but could also indicate movement between intertidal seagrass beds and shallow reef areas.

It is a commonly held belief that spongivorous angelfish feed rotationally in a 'smorgasbord' fashion (Randall & Hartman 1968), perhaps to reduce the impact of secondary metabolites (Wulff 2006). Video observations in the present study agree with observations noted in Pawlik et al. (2018) that spongivores can demonstrate repetitive and focused feeding on palatable sponges. *P. imperator*, *P. diacanthus* and *Z. cornutus* were recorded taking numerous bites from the exposed choanosome of *Petrosia corticata* (Fig. A1), with many successive bites taken in single feeding events (e.g. 34 bites for *P. imperator*). Our observations also suggest that the cyanobacterial ectosome of *P. corticata* may have been conferring some degree of anti-predator defence as it was avoided by the fish, which took repetitive bites from the inner exposed tissue.

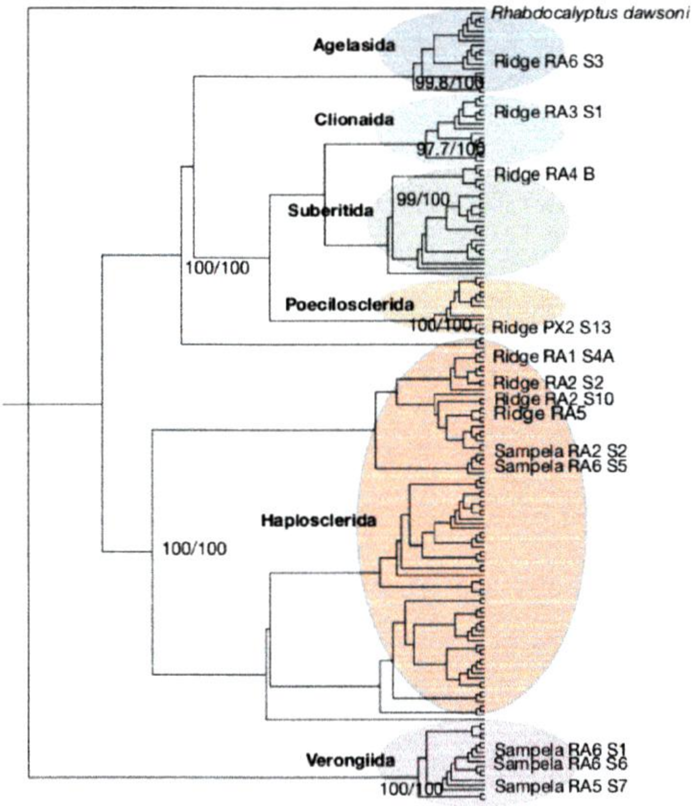

Fig. 4. Cladogram showing the placement of 13 sponges within 6 orders of Porifera (shown by colored ellipses) dissected from the stomach contents of the angelfishes *Pygoplites diacanthus* (RA) and *Pomacanthus xanthometopon* (PX) caught at 2 sites in the Wakatobi MNP and based on 18S sequence data. The outgroup is the glass sponge *Rhabdocalyptus dawsoni* AF100949). Branch supports reported as SH-aLRT support (%) / ultrafast bootstrap support (%)

4.2. Key sponge-grazing fishes not consuming sponge tissue

Despite recording >7500 bites on *Xestospongia* spp. sponge tissue, remains were not found in *C. kleinii* stomach contents, although spicules were sighted in 9 of 12 stomachs. Similarly, *Forcipiger flavissimus* recorded >1500 bites on *Xestospongia* spp. but out of 11 stomachs analysed, only 2 contained sponge tissue remains, both amounting to 2% of the total stomach content. Our findings indicate that for some fishes (e.g. butterflyfishes), bites on sponge surfaces do not provide reliable evidence of sponge consumption. Nagelkerken et al. (2009) made similar observations on a fringing reef in East Kalimantan, Indonesia, where *C. kleinii* was observed taking approximately one-third of bites from sponges but stomach contents contained only small volumes of sponge (<8% by volume of identifiable contents). The small volumes of sponge in the gut contents of chaetodontids detailed in this study and other studies from the Indo-Pacific (Sano 1989, Nagelkerken et al. 2009) suggest that butterflyfishes are likely targeting sponge-associated macrofauna. Spongivorous polychaetes (e.g. *Haplosyllis spongicola* and *Branchiosyllis oculata*) can directly penetrate sponge tissues where canals are small (Martin & Britayev 1998) and have been found in extremely high densities on both the surface and inside sponges (Pawlik 1983). Observations of the internal tissue of *Xestospongia* spp. in the Wakatobi MNP have found very high densities of an unidentified white polychaete (Family Syllidae) (579 ± 44 ind. g^{-1} [dry weight]) (C. Mortimer unpubl. data), which may explain the high grazing rates by chaetodontids and other invertivores.

Ctenochaetus binotatus likely lacks the necessary dental morphology to remove sponge biomass, instead appearing to 'brush' the surface of the sponge with comb-like teeth (Purcell & Bellwood 1993). Members of this genus are generally considered detritivores (Choat et al. 2002); however, other studies have indicated that *C. striatus* can also remove large quantities of algal turf (Marshell & Mumby 2015). Despite numerous observations of *Xestospongia* spp. grazing, gut content data presented here supports the conclusion that *C. binotatus* in the Wakatobi MNP is a detritivore that has no predatory impact on sponges. Sponge-grazing by detritivorous fishes may relate to the presence of mucus-bound sediment as some sponges, including *Xestospongia* spp., produce mucus as a sediment-clearing mechanism (McGrath et al. 2017). The sponge-associated holothuroid *Synaptula lamperti* ingests mucus directly from the sponge *Ianthella basta*, incorporating metabolised compounds into body wall tissues (Hammond & Wilkinson 1985). Little is known about sponge mucus; however, coral mucus is known to be energy- and nutrient-rich (Wild et al. 2004) and is consumed by some reef fishes (Cole et al. 2010). The feeding behaviour of *C. binotatus* could also be explained by the 'sponge loop', whereby sponges take up dissolved organic carbon, which is converted into cellular detritus and readily ingested by sponge-associated invertebrates (de Goeij et al. 2013, Rix et al. 2018). However, the ubiquity of the sponge loop has rarely been proven beyond observations of cryptic, encrusting sponges (but see Rix et al. 2018), and the extent to which the sponge loop supports higher trophic levels across global reef ecosystems is currently unknown (Bell et al. 2018b).

4.3. Sponge consumption

Quantitative data on the role of sponges in the diets of spongivorous fishes combined with density data made it possible to estimate sponge consumption at each location. Considering these are the first known estimates of sponge consumption, comparisons with studies from other regions are not possible. However, following the same methodology as Harmelin-Vivien & Bouchon-Navaro (1983) allows for a comparison with coral consumption in the Central Pacific. The maximum coral consumption reported was 62.5 g coral 1000 m^{-2} d^{-1} at the outer barrier reef slope (Harmelin-Vivien & Bouchon-Navaro 1983), which is similar to the estimates of daily sponge consumption reported for the outer reef sites in this study (mean: 52.9–73.3 g sponge 1000 m^{-2} d^{-1}), suggesting that the biomass transferred from sponges to spongivores may be of a similar magnitude to the coral to corallivore pathway, although this comparison is limited by large geographic differences. Nevertheless, data presented here suggests that spongivory may be an important but relatively overlooked trophic link on Wakatobi reefs.

Interestingly, we found that sponge consumption was lowest on the sponge-dominated reef site of Sampela (27.6 ± 15.3 g sponge 1000 m^{-2} d^{-1}), mainly driven by low densities of the 5 identified spongivorous fishes. It has been suggested that increasing sponge dominance may support a greater abundance of spongivorous fishes (Bell et al. 2013), and Powell et al. (2014) recorded a higher density of spongivorous fishes at Sampela relative to surrounding reefs (Powell 2013, Powell et al. 2014). Our results likely differ from Powell et al. (2014) due to the differences in the species categorised as spongivores. We used gut content analysis to confirm 'true' spongivores (i.e. those removing sponge biomass), whereas Powell et al. (2014) used *in situ* observations to categorise several species as spongivores that we suggest have no quantifiable predatory impact on sponge assemblages (e.g. *C. binotatus*). Results from the present study suggest that spongivores may not necessarily benefit from shifts to sponge dominance that are driven by a decline in corals and involve the increase of 1 or 2 competitive sponge species (e.g. Aronson et al. 2002, Powell et al. 2014). *Lamellodysidea herbacea* accounts for approximately 40% of total sponge abundance at Sampela based on area occupied (Powell et al. 2014, Biggerstaff et al. 2015) but does not appear to be preferred or readily consumed by spongivores. The only spongivorous fish filmed taking bites from *L. herbacea* was *Z. cornutus* (see the

Appendix), although tissue removal could not be verified by video analysis. Additionally, DNA analysis did not identify *L. herbacea* in the gut contents of spongivorous angelfishes. *L. herbacea* has abundant cyanobacterial symbionts that produce polybrominated diphenyl ethers that may function as antipredator compounds (Becerro et al. 2003), which could be another factor leading to the proliferation of *L. herbacea* at Sampela.

4.4. Taxonomy of consumed sponges

From 13 angelfish stomachs, 14 unique sponge sequences were retrieved spanning 6 orders of Porifera, suggesting substantial dietary plasticity, similar to the diets of angelfish in the Caribbean (Randall & Hartman 1968) and the Pacific (Verdín Padilla et al. 2010). The majority of sequences were placed into Order Haplosclerida, an extremely diverse order of Porifera (van Soest & Hooper 2002). There was strong support for the placement of one sponge into a clade with the Indo-Pacific sponge *Chalinula hooperi* (Bakus & Nishiyama 2000), which is commonly found in the shallow waters of pristine, offshore reefs (Cleary & de Voogd 2007). There was also strong support for the placement of one sponge into a clade containing sponges from the genus *Petrosia*, and another sponge had high support for placement alongside 2 Caribbean sponges *Niphates erecta* and *Haliclona curacaoensis*. *Haliclona* spp. have been reported from the gut contents of spongivorous fishes in the Caribbean (Randall & Hartman 1968, Hourigan et al. 1989) and the Pacific (Verdín Padilla et al. 2010). Similarly, *N. erecta* makes up a substantial proportion of the diet of Caribbean angelfishes as it is undefended by secondary metabolites or structural components and invests heavily in growth to compensate for biomass removal (Pawlik 2011).

Three sponges in the Verongiida group had high support for placement into a clade alongside sponges from the genera *Pseudoceratina* and *Verongula*. Verongiida sponges lack spicules; instead, they are structurally supported by 3-dimensional internal scaffolds made of fibrous chitin (Żółtowska-Aksamitowska et al. 2018), which may lessen their digestibility and cause them to persist in stomach contents at the expense of sponges with more diffuse tissue. Whilst this is quite possible, an unknown species of *Verongula* was also observed in the stomach contents of *Holacanthus tricolor* in the US Virgin Islands (Hourigan et al. 1989), and Verongiida sponges appear to form some part of the diet of *P. diacanthus* as these sponges were amplified by 2 fish caught at Sampela reef. Of the sequences amplified from fish caught at Sampela reef, 3 were Verongiida sponges and 2 were placed in Order Haplosclerida, whereas at the outer reef site, sequences were amplified from 5 orders of Porifera. This could be indicative of differences in sponge assemblage composition, which can vary substantially over short spatial scales in the Wakatobi MNP (Powell et al. 2014, Mortimer 2020). It may also reflect differences in diversity, as Sampela reef has substantially lower sponge diversity than The Ridge (Powell et al. 2014, Mortimer 2020). There was strong support for one sponge belonging to a clade with 2 species of *Aaptos*. *A. suberitoides* is commonly found on Wakatobi reefs and has been found to produce numerous compounds with antitumor, antimicrobial and antiviral activity (reviewed in Cleary et al. 2018). Meylan (1988) documented the closely related *A. aaptos* as one of the 10 most important sponges eaten by the hawksbill turtle, even though it produces toxic secondary metabolites (Nakamura et al. 1982). There was also strong support for the placement of one sponge into a clade of sponges from the family Agelasidae that are known for producing bromopyrrole alkaloids (Wilson et al. 1999, Lindel et al. 2000) that deter predation (Pawlik et al. 1995). An unknown species of *Agelas* represented the largest volume of sponge in the gut contents of the angelfish *Holacanthus ciliaris* and was also consumed by a filefish (Randall & Hartman 1968). The presence of sponges with substantial chemical defence is interesting because angelfishes are thought to mainly consume chemically undefended, palatable sponges (Pawlik 2011, Pawlik et al. 2018). However, without robust dietary proportion data, the extent of predation on particular Indo-Pacific sponges by angelfishes remains unknown.

There was strong support for one sponge belonging to a clade containing the bio-eroding families Clionaidae and Spirastrellidae. This is consistent with observations from the Caribbean which have recorded bio-eroding sponges from the genera *Spirastrella*, *Cliona* and *Spechiospongia* in angelfish gut contents (Randall & Hartman 1968). The only sponge that could be amplified from the gut contents of *P. xanthometopon* had strong support for placement into a clade of sponges from the genus *Guitarra* (Carter 1874) from the Guitarridae family. To date, there are 15 described species of *Guitarra*, but none have a documented distribution in the Indo-Pacific (https://www.marinespecies.org). Finally, the identity of one sample could not be resolved beyond the higher classification of Porifera. This is likely due to poor data coverage for sponges from the Indo-Pacific, one of the most speciose regions of Porifera on the planet (van Soest et al. 2012).

4.5. Limitations of sponge consumption estimates

Sponge consumption estimates provided were conservatively based on stomach contents rather than total gut contents, and on the assumption that the spongivores identified (*P. diacanthus*, *P. imperator*, *P. xanthometopon*, *Z. cornutus* and *S. punctatus*) filled their stomachs twice daily. Coral material has been estimated to pass through the intestinal tract of the butterflyfish *Chaetodon unimaculatus* in 1.5–2 h, indicating that stomachs may be filled at least 6 times daily (Cox 1986, Cole et al. 2011). Considering that reported gut length to body length ratios for *C. unimaculatus* (Berumen et al. 2011) and *Pomacanthus* spp. (Pérez-España & Abitia-Cárdenas 1995) are similar, it seems likely that angelfishes may also fill their stomachs up to 6 times daily. However, we chose to follow the same methods as Harmelin-Vivien & Bouchon-Navaro (1983) to facilitate a comparison with coral consumption. Other methods of estimating consumption were considered, such as the method employed by Cole et al. (2011); however, retaining fish in aquaria to conduct feeding trials was not feasible at this study site.

This study was limited to vertebrate sponge predators that are thought to have the biggest predatory impact on sponge assemblages on shallow coral reefs (Randall & Hartman 1968, Meylan 1988). However, tropical sponges also have a range of invertebrate predators including echinoderms, molluscs, crustaceans and annelids (see Bell et al. 2020 for review), although the effect of invertebrate predation on tropical sponge populations and species diversity is largely unknown. Additionally, other spongivorous vertebrates considered to exhibit top-down control on sponge populations in the Caribbean were not considered in our estimates of sponge consumption. Hawksbill turtles are found in extremely low abundances on Wakatobi reefs (only one turtle was sighted during the 3 mo study period), hence their impact on sponge assemblages in the Wakatobi MNP is likely negligible. Nevertheless, before populations were decimated by over-exploitation, they likely had a strong structuring effect on sponge populations as they are known to feed almost exclusively on a narrow range of available sponges (Meylan 1988, von Brandis et al. 2014).

On Caribbean reefs, parrotfishes are important spongivores (Dunlap & Pawlik 1996, Dunlap & Pawlik 1998). Consumption of sponges by parrotfishes has been observed following the exposure of cryptic species (Wulff 1997), through inter-habitat transplants (Dunlap & Pawlik 1996) and by juveniles (Bruggemann et al. 1994, Dunlap & Pawlik 1998, Pereira et al. 2016). In comparison to the Caribbean, evidence of sponge predation by parrotfish on Indo-Pacific reefs is scarce. In our study, 4 species of parrotfish were observed in video footage but not sampled for gut contents due to logistical difficulties encountered whilst attempting field collections. Two *Scarus ghobban* individuals were bought from the local fish market, and their gut contents were examined for evidence of sponge predation but found to contain mainly algal fragments. However, it is important to note that although parrotfish are found at the local fish market, they are not commonly targeted by local fishers but instead are caught in non-selective fish traps and fish fences, and, although we have no specific time series data regarding parrotfish biomass, a recent MSc research project found that herbivorous fish biomass increased from 2013–2017 (Sing Wong 2019). Of the 4 scarids recorded, the most numerous bites were taken by juvenile *S. flavipectoralis* (3.5% total bites), suggesting that sponge predation could be more prevalent amongst juvenile parrotfish on Wakatobi reefs. However, these juveniles appeared to be targeting mucus-bound sediment on the sponge surface as grazing scars were not evident following feeding events. This was especially apparent in the additional observations of *A. aaptos* (see the Appendix) as this sponge has a vibrant yellow interior which would be exposed following the removal of outer tissue by predation. With the exception of juvenile *S. flavipectoralis*, parrotfish represented <1% of total bites recorded on *Xestospongia* spp. Lack of representation in our data set could be explained by our choice to film open reef sponges, whereas parrotfish have been observed to rapidly consume cryptic sponges exposed by researchers (Wulff 1997). Anecdotal evidence of this type of spongivory by parrotfishes was reported from the Marshall Islands by Bakus (1967), when cryptic sponges living underneath a table *Acropora* were exposed and apparently fed upon by scarids.

Other likely spongivorous fishes were not included in the estimates of sponge consumption due to low abundances on Wakatobi reefs (e.g. pufferfishes) and time and logistical constraints (e.g. filefishes and triggerfishes). Additionally, the true diet of some prevalent sponge-grazing fishes recorded in the present study remains ambiguous due to lack of gut content data. Four species of *Centropyge* (*bicolor, nox, tibicen, vroliki*) were recorded taking numerous bites from *Xestospongia* spp., and Powell et al. (2015) also recorded similar feeding behaviour in the Wakatobi MNP. Sponge was recorded as the dominant dietary item (>40% by volume) in the stomach of *C. vroliki* (Eagle & Jones 2004), and morphological studies indicate that *Centropyge* spp. are adapted to feed on attached colonial invertebrates (Konow & Bellwood 2011). However, other studies list *Centropyge* as epilithic algal grazers that are important and numerous herbivores (Green & Bellwood 2009). Similarly, *Coradion melanopus* in particular stands out as a prevalent *Xestospongia* spp. grazer, taking 18.8% of total bites but representing only 2% of sponge-grazing fish abundance. *In situ* feeding observations of *C. melanopus* at B1 suggested extremely selective grazing on barrel sponges, with 6 individuals observed to take bites almost exclusively from *Xestospongia* spp. (C. Mortimer unpubl. data). Unfortunately, gut content samples could not be collected due to small population size so sponge biomass removal by *C. melanopus* could not be verified. However, the gut contents of the congener *C. altivelis* contained 18% sponge (Nagelkerken et al. 2009), indicating that members of the *Coradion* genus may receive some nutritional benefit from associating with sponges. Future studies in the Indo-Pacific should prioritise the gut content analysis of these species to resolve their potential impact on sponge assemblages.

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Appendix. Additional video observations

***Petrosia corticata* at The Ridge**

During the 148 min of footage recorded on the exposed inner tissue of *Petrosia corticata*, 888 bites were recorded giving a grazing intensity rate of $112.6\ \text{min}^{-1}\ \text{m}^{-2}$. The majority of bites were directed at an area of exposed choanosome, and sponge tissue was observed being removed by all species. The most voracious predator was *Zanclus cornutus* which took 59.5% of bites, followed by *Pygoplites diacanthus* taking 25.5% and *Siganus punctatus* at 6.6%. Other species observed taking bites included *Pomacanthus imperator*, *Heniochus singularius*, *Centropyge nox* and *Scarus ghobban* (terminal phase). *P. imperator* took the highest no. of bites in a single feeding event (34).

***Lamellodysidea herbacea* at Sampela**

In the 130 minutes of footage recorded on *L. herbacea*, 68 bites were observed on the sponge surface giving a total grazing intensity rate of $4.4\ \text{min}^{-1}\ \text{m}^{-2}$. *Scarus flavipectoralis* juveniles took the most numerous bites (45.6%), followed by *Ctenochaetus binotatus* (29.4%). *Z. cornutus* and *Centropyge kleinii* took 8 and 7 bites respectively and 2 bites were made by *Centropyge tibicen*. Although bites were recorded on sponge surface, it was not immediately evident that sponge tissue was being removed.

***Aaptos suberitoides* at Sampela**

In the 230 min of footage recorded on *Aaptos suberitoides*, 133 bites were recorded giving a grazing intensity rate of $4.6\ \text{min}^{-1}\ \text{m}^{-2}$. The most numerous bites recorded by the angelfish *Centropyge bicolor* (76.7%) followed by the juvenile parrotfish *S. flavipectoralis* (9.8%); ≤5% of bites were recorded by *Scarus niger*, *C. tibicen* and *Z. cornutus*. *Canthigaster valentini*, *C. kleinii*, *Halichoeres prosopeion* and *Sufflamen bursa* all took only one bite in the whole video.

Tubular sponge (possibly *Theonella* sp.) at KDS

In the 33 min of footage recorded on the green tubular sponge, a total of 385 bites were recorded by 6 species. The majority of bites were taken by *P. diacanthus* (295) and were directed towards an exposed bit of choanosome which made up approximately 4.8% of total sponge area. This area of exposed inner tissue had a grazing intensity rate from *P. diacanthus* of 265.8 bites $\text{min}^{-1}\ \text{m}^{-2}$ whereas the undisturbed sponge surface had a grazing intensity rate of 10.3 bites $\text{min}^{-1}\ \text{m}^{-2}$. *Z. cornutus* took the second highest number of bites (47) all of which were directed towards the white exposed inner tissue giving a grazing intensity rate of 74.8 bites $\text{min}^{-1}\ \text{m}^{-2}$. *Pomacanthus xanthometopon* was also sighted feeding from the sponge, taking 4 bites on the white exposed inner tissue. Other species noted were *C. tibicen*, *C. kleinii* and *C. binotatus*, taking 25, 9 and 4 bites respectively, all from the undisturbed sponge surface.

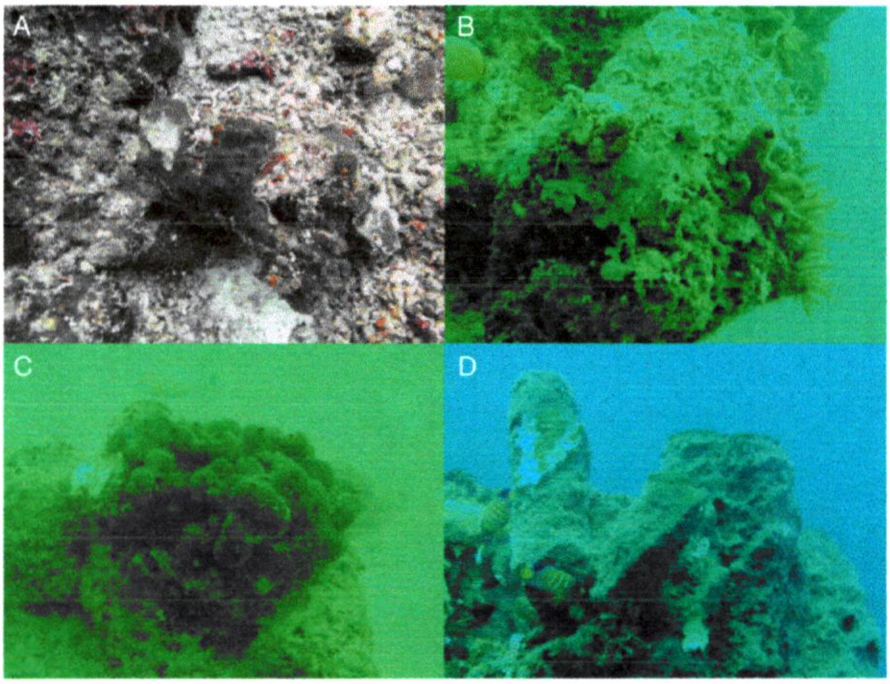

Fig. A1. Screenshots of (A) *Petrosia corticata*, (B) *Lamellodysidea herbacea*, (C) *Aaptos suberitoides* (D) green tubular sponge

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