Size structure and behaviour shed light on the ecology of cryptic morays (Muranidae)

Tess A. Lyons

Supervised by:

Prof Jessica Meeuwig, Prof Dirk Zeller and Dr Shanta Barley

This thesis is submitted in partial fulfilment of the requirements for a

Masters of Biological Science (Marine Science)

BIOL5555 Masters in Biological Sciences Research Dissertation

School of Biological Sciences

Faculty of Science

The University of Western Australia

Submitted: May 2019

Word count: 5,416

Formatted in the style of the journal *Marine Ecology Progress Series*

ABSTRACT

Increasingly the role of predators in reef resilience is recognised and, as reef health declines in response to pressures related to climate change and overfishing, an improved understanding of cryptic predatory species, such as morays (Muraenidae) is vital. This study sheds light on two species of cryptic morays, the yellow-edged moray (*Gymnothorax flavimarginatus*) and the giant moray (*Gymnothorax javanicus*), and the role they play on the reef ecosystem through investigating the ecological fundamentals of each species. Specifically, through the use of stereo BRUVS, this study: 1) specifies the distribution of the species across the Indo-Pacific; 2) defines a relationship between head morphometry, specifically head height, and total length and considers the applications to trophic ecology and reproductive maturity; and 3) documents moray behavioural repertoire and the variation in the frequency and timing in which these behaviours occur by species and by size class. Morays were found to be opportunistic predators with a possibility of prey size varying ontogenetically, such that prey size range increases with body size. Calculation of a size distribution indicated approximately 25% of the population was reproductively mature thereby setting up a baseline against which changes relating to fishing mortality can be assessed. Behavioural analysis demonstrated potential existence of hierarchical dominance which implied morays may play a role as a high level predator, thus contributing to coral reef health.

Table of Contents

ACKNOWLEDGEMENTS

1. INTRODUCTION

Coral reefs support high levels of biodiversity, providing habitat to an estimated nine million species worldwide (Knowlton 2001). Coral reefs also provide direct economic services including for fisheries, tourism and costal protection with the contribution from the Great Barrier Reef alone estimated at AUD $56 billion. However, coral reefs are also highly vulnerable as a result of the consistent, rapidly increasing pressures of climate change and the direct impacts relating to ocean acidification and temperature rise (Hoegh-Guldberg et al. 2007, Halpern et al. 2008, Abdo et al. 2012) as well as overfishing (Knowlton & Jackson 2008). This vulnerability means that understanding reef ecology is vital. However, the understanding of these functions and processes has proved difficult due to the complex and sometimes cryptic nature of the many species that inhabit the reef.

Ecology is largely about connectivity, in that it explores how organisms interact with each other and the environment around them (May & McClean 2007). Conventionally, ecological research has largely focused on bottom-up processes such as primary production as structuring mechanisms of reef systems (Sale 1991, Lapointe 1997, Frederiksen et al. 2006, May & McClean 2007). However, there is increasing evidence of the roles that top-down predation can play in maintaining coral reef health (Ruppert et al. 2013) and thus the functional roles, both predatory and competitive, played by high trophic level species such as reef sharks (Barley paper). To understand these roles, we must first look at species level ecology and focus on basic ecological principles. Distribution, demography and behaviour are fundamentals of ecology and are the basis of shedding light on ecosystem function (Pelletier et al. 2007, Scheiner & Willig 2007). Distribution provides insight into the less visible aspects of community, demonstrating the relationship within and between species groups (Verberk 2011). Moreover, distribution is directly reflective of changes in the ecosystem including changes to reef health (Hourigan et al. 1988).[1] Body size lends hand to understanding demography, with body size influencing mortality rate, reproduction, diet, gape limitation, metabolic rate and competitive interactions, each of which is important in the structuring of populations (Peters 1983, Manuel et al. 2015, Robinson & Baum 2015, Robinson et al. 2016, Barley et al. 2017b, Dunic & Baum 2017). Insight into to predator behavioural repertoires

---

[1] Add https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0194537 if you have time

provides much information relating to the predatory and competitive roles as well as the maintenance of ecosystem function (Myrberg & Gruber 1974, Sperone et al. 2010).

Whilst generally much attention has been paid to understanding these ecological basics in reef sharks and predatory reef fish (Miller et al. 2010, Harasti & Malcolm 2013, Barley et al. 2017a, Tickler et al. 2017), less is known about the ecology of morays. Morays are cryptic in nature and hence difficult to study, with much of the current ecological information being obtained through extractive studies such as that completed by Reece et al. (2010). The moray family, Muraenidae, has more than 100 species worldwide with 59 of these distributed through the Indo-Pacific (Bohlke & McCosker 2001). The diet of morays largely consists of benthic fish and crustaceans, with trophic levels varying between 3.6 and 4.5 (Froese & Pauly 2019) suggesting they likely play role as scavengers and predator on the reef (Matić-Skoko et al. 2014). Little is known about their behaviour, however in recent years there has been documentation of cooperative hunting with coral trout and 'knotting' their bodies to compress and anchor prey (Chave & Randall 1971, Barley et al. 2016, Malcolm 2016). The need to further our understanding of morays is not only highlighted by our distinct lack of knowledge regarding their ecology, but recent research on other predators has shown they can have a major influence on the structure and function of ecosystems and that the removal of such predators can cause cascading changes to community composition (Barley et al. 2017b, Tickler et al. 2017).

Many studies of morays have relied on destructive sampling using traps. However, stereo baited remote underwater video systems (BRUVS) are a promising avenue for the study of cryptic morays. Stereo BRUVS typically focus on the characterisation of fish assemblages in terms of diversity, abundance, size and biomass (Cappo et al. 2006). The system overcomes many issues associated with other sampling techniques such as animal welfare, depth and the ability to sample behaviour, however, like other underwater sampling methods it does not overcome difficulties associated with visibility (Cappo et al. 2006, Whitmarsh et al. 2017). The use of bait also does not mimic a natural situation, although it does assist in obtaining adequate sample sizes and potentially allows for behavioural displays that otherwise may go undocumented (Cappo et al. 2006, Harvey et al. 2007). Whilst typically these systems have been utilised to report novel behaviours such as 'knotting' and paddling whilst feeding demonstrated by Barley et al. (2016), pairing BRUVS-derived video with an ethogram, a defined catalogue of behaviours, can allow for documentation of the frequency and timing in

which these behaviours occur (Jordan & Burghardt 1986, MacNulty et al. 2007), providing additional ecological insights.

Stereo BRUVS are also a powerful tool for estimating fish length (Harvey et al. 2001), a key demographic parameter (Peters 1993). However, morays present challenges due to both their undulating movements when swimming and frequent crypsis as cave dwellers where only their heads may be visible. Fish show strong morphometry with consistent relationships between total length and body depth and height, and head morphometry (Kulbicki et al. 2005). The strong morphometric relationships found by Kulbicki et al. (2005) combined with the consistent visibility of moray heads presents an opportunity to use head morphometrics as a proxy for total body size.

Specific knowledge relating to the distribution, demography and behaviour of morays is limited. This study aims to shed light on these ecological fundamentals with relation to understanding the ecosystem as a whole and demonstrate an appropriate methodology to do so through four main objectives: 1) defining the distribution of morays throughout the Indo-Pacific; 2) assessing the potential to use head morphometry as a proxy for total size in demographic studies; and 3) document the behavioural repertoire of morays and the frequency and timing in which they perform these behaviours.

2. METHODS

Video imagery used in this study was extracted from a global database of video collected between 2015 to 2018, yielding 2,977 samples from eight locations. All samples, including those with no moray observations, were utilised with respect to assessing distribution and to determine abundance analysis. Species with a minimum of 15 observations were the focus of morphometric and behavioural analysis.

2.1 Video imagery collection and conversion

All video imagery was collected with seabed stereo BRUVS over a total of 13 expeditions. Consistent with previous stereo BRUVS studies, the systems were composed of a rigid base bar mounting two high definition digital video cameras (GoPro Hero models 4 and 5) housed in pressure resistant housings and fixed at a distance of 80 cm apart (Harvey et al. 2007, Barley et al. 2016). Each camera was angled four degrees inwards. Perpendicular to the base bar and fixed between the cameras, was a 1.2-meter arm suspending a stiff plastic mesh bait
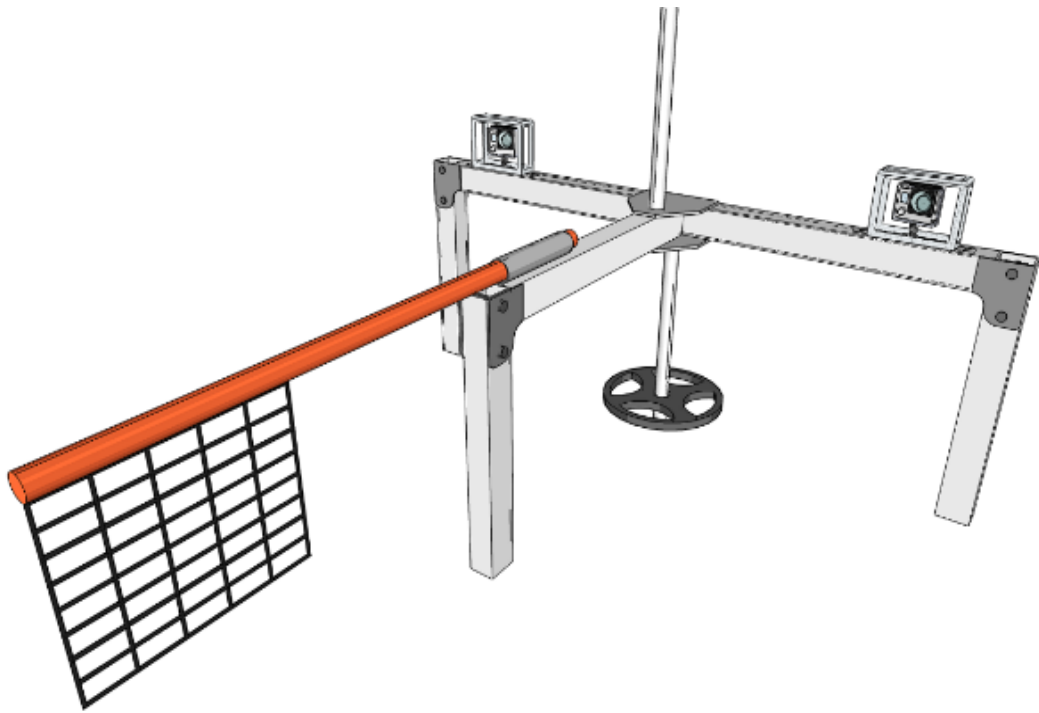
Figure 1. Schematic of a stereo BRUVS (baited remote underwater video system), showing the tripod frame, cameras and housing, bait arm, bait bag and weights (image from P. Bouchet).

bag filled with 800 grams of crushed pilchards (*Sardinops spp.*). Each stereo BRUVS had a tripod frame allowing stability by resting on the seabed (Figure 1). The stereo BRUVS were deployed based on a stratified random sampling plan based on reef structure and depth, between 0 – 50 m. Stereo BRUVS were deployed for at least an hour, allowing a standard 60 minute time period to be processed, commencing from time that the rig settled on the seabed. Whilst the imagery in the database had already been processed for species identification, abundance and total length measurements for the reef fish assemblage as a whole, it was reanalysed in this study for the collection of specific size and behavioural data on the morays. Imagery was initially converted to AVI format using Xilisoft video conversion software and analysed using EventMeasure (www.seagis.com.au/event.html). Samples were excluded from both the size and behavioural studies if visibility was inadequate or if footage was not available from both cameras.

2.2 Size and demography

Calibration files were uploaded to allow length measurements for each stereo BRUVS rig following standard methods (Harvey & Shortis 1998, Harvey et al. 2001). The morphometric analysis was based on three standardised head measurements and the measurement of total length where possible. The three head measurements were 1) total head length, defined as the

distance between the anterior tip of the snout to the anterior edge of the parabranchial opening, 2) gill slit to mouth, referring to the mouth commissure to the anterior edge of the
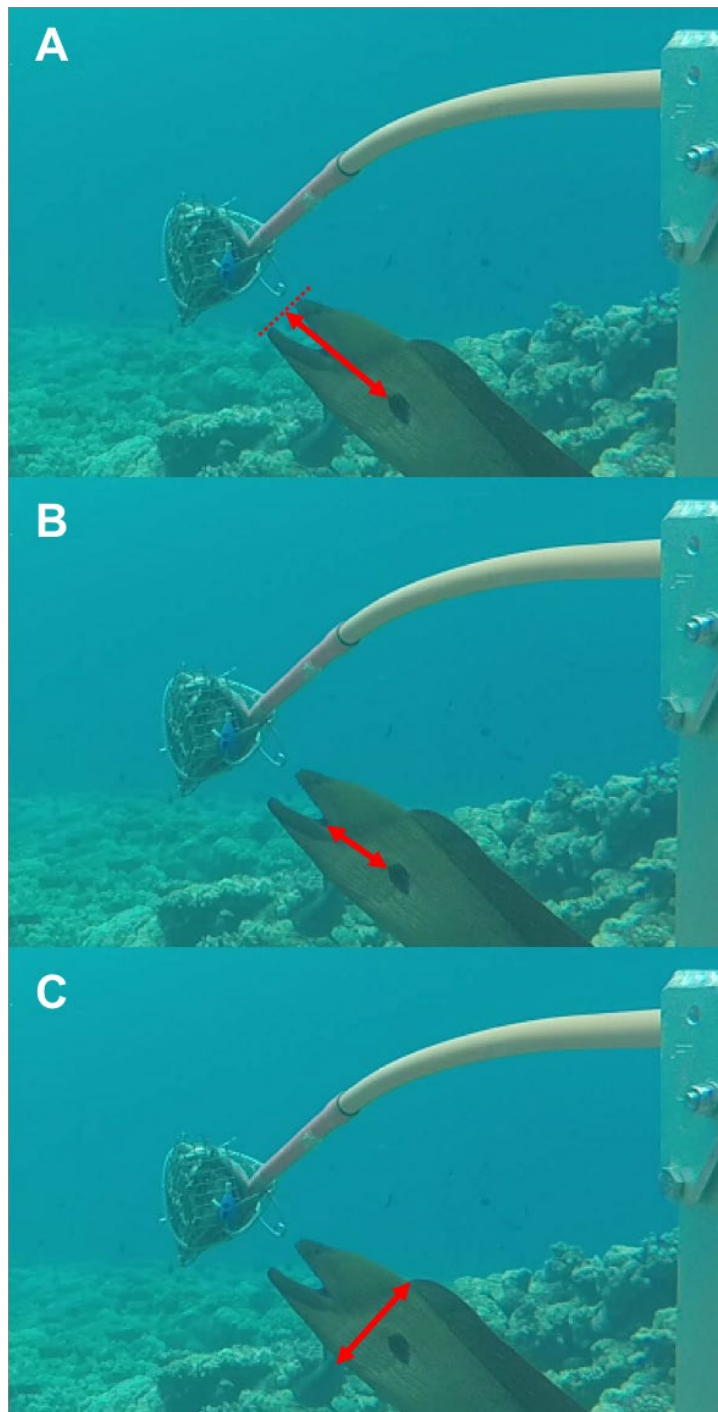
Figure 2. Diagram of the morphometric head measurements during data processing. A) head length; B) gill slit to mouth; and C) head height.

parabranchial opening and 3) head height, defined as the anterior edge of the elongated dorsal fin to the most direct inferior point (Figure 2). Total length was defined as the measurement between the anterior tip of the snout to the posterior tip of the tail.

The relationship between head morphometry and total length was tested using linear regression (Zar 1999) in R Studio (RStudio 2015), based on those individual eels for which head and total length measurements were successfully obtained. The head morphometric variable most strongly correlated to total length was used for subsequent analyses of the effect of size on behaviour. Additionally, this head morphometric variable was used to allocate individuals to small, medium and large size classes, dependent on its distribution. To assess the proportion of observed morays that are reproductively mature, available data relating size at maturity to maximum length was extracted from FishBase (Froese & Pauly 2019), The ratio of the length at maturity to maximum length was regressed against total length to predict length at maturity when this information for a species was absent.

2.3 Behaviour

An ethogram (Jordan & Burghardt 1986, MacNulty et al. 2007, Thompson 2014) was created to define the behaviours exhibited by morays, allowing for consistent and semi-quantitative analysis (Table 1). A preliminary review of the imagery and literature was completed to determine which behaviours were consistently observed and/or reported in the literature and therefore to be included in the ethogram. Further criteria for the inclusion of behaviours in the ethogram included: whether the behaviour could be defined objectively and whether the behaviour was ecologically relevant. The ethogram was broken into three groups; entries and exits, passive behaviours and active behaviours. Passive behaviours were those that occurred regardless of the presence of the bait bag or other fish, for example, pumping. Active behaviours included the behaviours such as biting and nudging, which involved interaction with the bait bag or other organisms.

The behavioural analysis incorporated two components: counts and timing of behaviours. Each individual that entered the field of view of either the left or right camera was logged and species identifications from the database checked. When multiple morays were present in frame, the second moray was marked as such and behaviours were logged independently. Where there were multiple entries and exits, morays were considered 'returning' individuals rather than 'new' individuals except if distinguishable by size or markings.

When a behaviour listed in the ethogram was observed, a mark was placed in the video and the behaviour was logged in the activity field, generating both a count and a time of occurrence relative to commencement of the image processing. The sum of these marks for

each behaviour for each individual were then used to estimate frequency. Additionally, the time of the entry and exit marks were used to calculate time of first arrival, total time spent in frame, mean time spent in frame on each entry and total time from first entry to last exit (Table 2). Averages of behaviour counts and timing metrics were calculated for each species and size class.

Table 1. Ethogram used to define behaviours recorded in this study. Definitions were derived or adapted from existing literature where possible (D'Aout & Aerts 1999, Mehta & Wainwright 2007).

Class	Behaviour	Definition
Entries/Exits	Entry	Moray eel appears in either camera frame
	Exit	Last point moray eel appears in either camera frame
Passive	Pumping	Continuous (+2) pumping of water through gills indicated by the repeated opening and closing of mouth
	Jaw gaping	slow, exaggerated opening of the jaw (30-90º, measured or estimated as the angle formed at the mouth commissure)
	Locomotion	Sinusoidal movements when not engaging in other behaviours with both head and tail free of reef structure
Active	Bite	Jaw closure around an bait bag and contact with teeth
	Nudge	Contacting an object with the head (snout to gill slit)
	Body stroke	Contacting an bait bag with the body (gill slit to tail)
	Displaced	movement away when another animal moves toward their location
	Jerking	A sharp (3HL/sec) pull backwards when feeding on prey
	Rotating	Rotating rapidly (>4 rotations/ sec) along primary axes while holding prey in jaws
	Aggression	threat displays made toward another animal e.g. lunging, biting
	Snapping	Rapid opening and closing of mouth (<1.5 sec)

The counts of behaviours were analysed in two ways, both by species and by size class. First, for the most commonly observed behaviours, entries, pumping and nudging, chi-squared goodness of fit tests (Zar 1999) were used to determine whether a given behaviour was evenly distributed in its frequency across a) species and b) size classes. The expected distribution for the goodness of fit reflected the number of individuals within either the species or the size classes, in this way controlling for variable numbers of individuals. Second, behaviours were grouped by class (entries, active and passive) as per the ethogram and then a contingency chi-

squared test (Zar 1999) was used to determine whether the frequency of entries, passive and active behaviours varied by species and by size class.

Finally, using R Studio (RStudio 2015), the effect of species and size class on timing metrics were tested through ANOVAs. When a significant result of size was detected, pairwise Tukey tests were used to determine among which size classes differences existed.

Table 2. Definitions and calculations of behavioural metrics.

Metric	Definition	Calculation
Time of first arrival	Time taken from the deployment of the stereo BRUVS to the first observation of an individual	Time of first observation - time of deployment
Total time spent in frame	Total time spent in view	$\sum$(Exit from frame - related entry)
Mean time spent in frame on each entry	The average time spent in view	$(\sum$(Exit from frame - related entry))/ number of entries
Total time from first entry to last exit	The time from the first time an individual entered in to view until the last exit of the same individual	Time of last exit - time of first entry
Behaviour duration	The total time an individual is engaged in a particular behaviour	End time of behaviour - start time of behaviour

3. RESULTS

3.1 Focal species

A total of nine species of morays belonging to the family Muraenidae were observed across the 2,977 samples and eight locations. These species were the starry (*Echidna nebulosa*), laced (*Gymnothorax favagineus*), yellow-edged (*Gymnothorax flavimarginatus*), the giant (*Gymnothorax javanicus*), turkey (*Gymnothorax meleagris*), yellow (*Gymnothorax prasinus*), greyface (*Gymnothorax thyrsoideus*), undulated (*Gymnothorax undulatus*), and ribbon

(*Rhinomuraena quaesita*) morays. All species were tropical except the yellow moray which is temperate. Only the giant moray and the yellow-edged moray had more than 15 observations, 91 and 18 respectively. Thus, the analysis was restricted to these two species, with 109 observations over 105 deployments across 5 locations.

3.2 Distribution

The two focal species were tropically distributed with records of both species at the Chagos Archipelago and the Cocos (Keeling) Islands (Figure 3; Appendix 1). The giant moray was also found at the Rowley Shoals in northern Western Australia and at sites in far north Queensland. The yellow-edged moray was not observed at any locations inside the Australian Mainland's Exclusive Economic Zone.
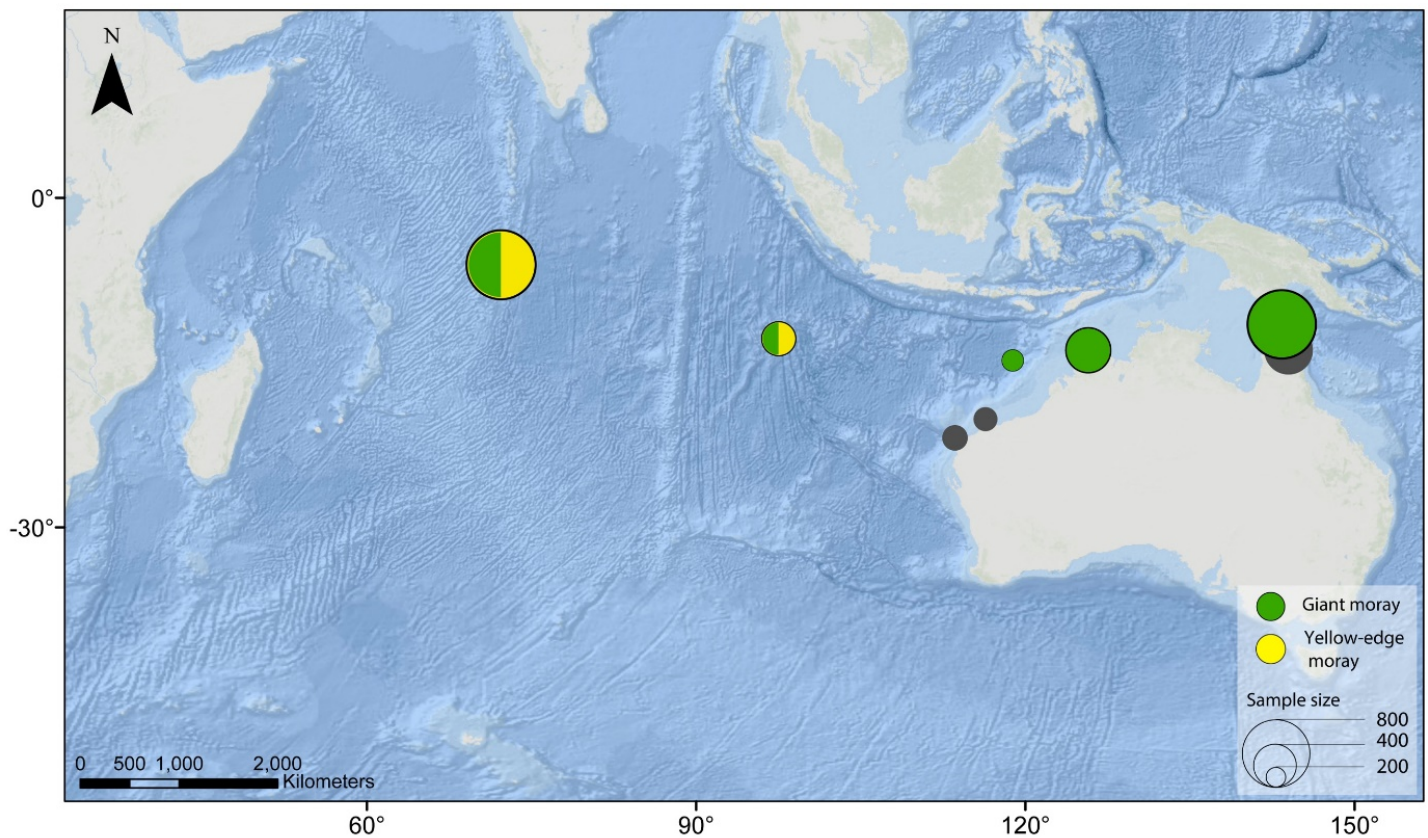
Figure 3. Map of all stereo BRUVS survey locations sampled between 2015 and 2018, indicating the presence or absence of each species. Grey indicates no morays of the two focal species were observed on the survey.

3.3 Size and demography

Of the 109 individuals from the two focal species, total length estimates were available for only five individuals (4.6%), all of which were giant morays. The mean total length of the giant morays was 94.4 cm (± 7.2 SE) and ranged from 68.2 cm to 111.8 cm. Estimates of

head morphometrics including head height, head length and gill slit to mouth measurements, were estimated for 56, 55 and 51 individuals respectively (51.3%, 50.5%, 46.8%) across both

$$Log_{10} TL = 0.62(Log_{10} HH) + 1.73$$

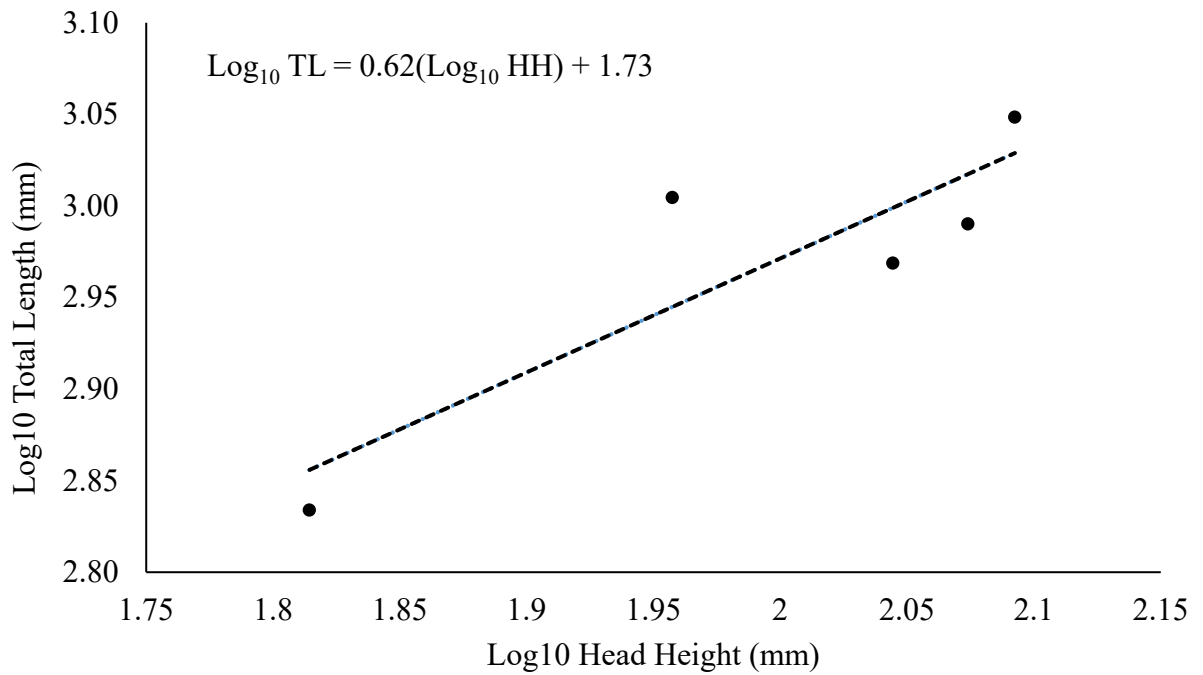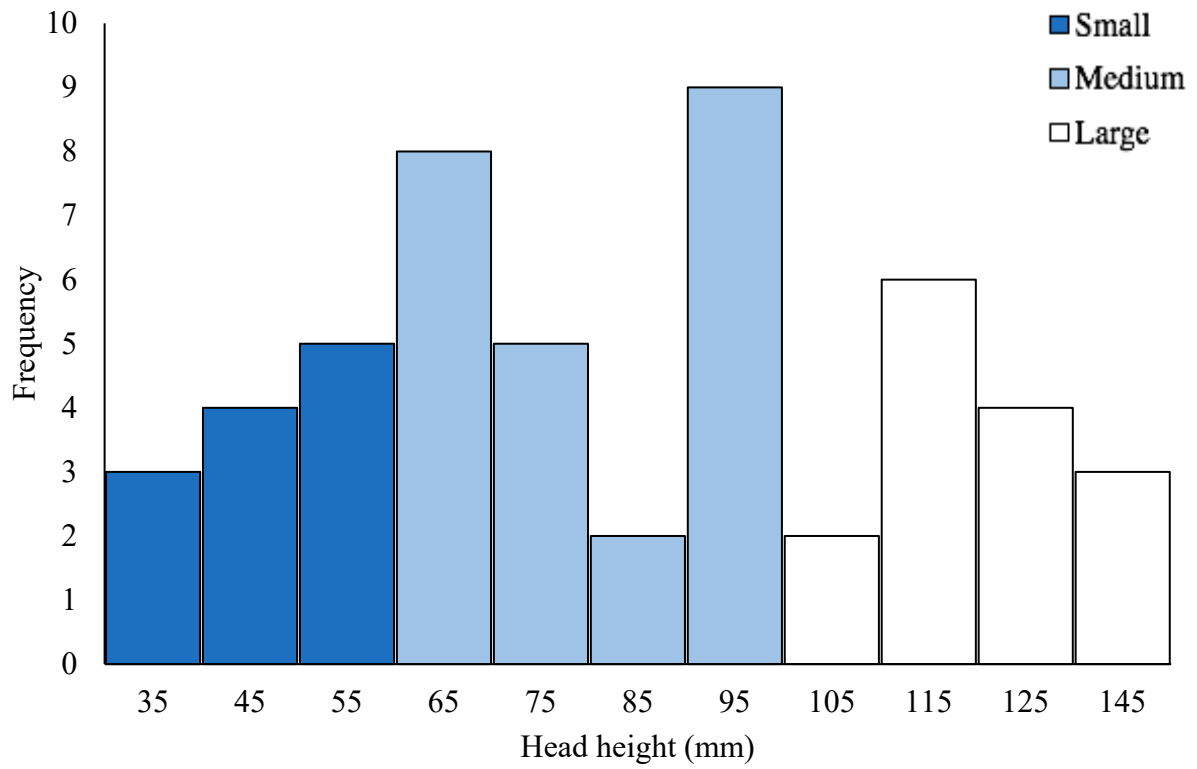*Figure 4. Correlation between the head heights (HH) and total lengths (TL) in mm on a Log10 scale for the giant morays where both lengths were successfully obtained.*

Figure 5. Size distribution of the giant moray eel population sampled in this study where head heights were obtained, catergorised by 10mm bins such that 35 mm relates to those individuals with head heights between 30 mm and 40 mm.

species. Head height was strongly correlated to total length for giant morays (Figure 4; n = 5 p < 0.05; $r^2 = 0.77$), however neither head length nor gill slit to mouth were correlated with total length (p = 0.85 and p = 0.51 respectively). Head heights of the giant moray (n = 51) ranged between 3.24 cm and 15.93 cm with an average height of 8.46 cm (± 4.35 SE). As a result of the strong relationship between total length and head height for the giant moray, individuals of this species were classified by head height as small (< 6 cm), medium (6-10 cm) and large (> 10 cm) and a size distribution was built using head heights (Figure 5). There was also a strong relationship between head length and head height (n = 50, p < 0.05, $r^2 = 0.80$). Using head height as a proxy for total length, the majority of the giant morays were in the 'medium' size category (47.1%), followed by the 'large' category (29.4%), with the fewest individuals in the 'small' category (23.5%).

The ratio of the length at maturity relative to maximum length is strongly negatively correlated to maximum length, for which both lengths are reported (Froese & Pauly 2019)(Figure 6; n = 9, p < 0.05; $r^2 = 0.78$). Thus, while there is no estimate of length at maturity for the giant moray, based on this regression, it becomes mature at approximately 69 cm total length, which corresponds to a head height of approximately 11 cm. Based on the distribution of head heights, approximately 25.5% of the observed animals were reproductively mature.
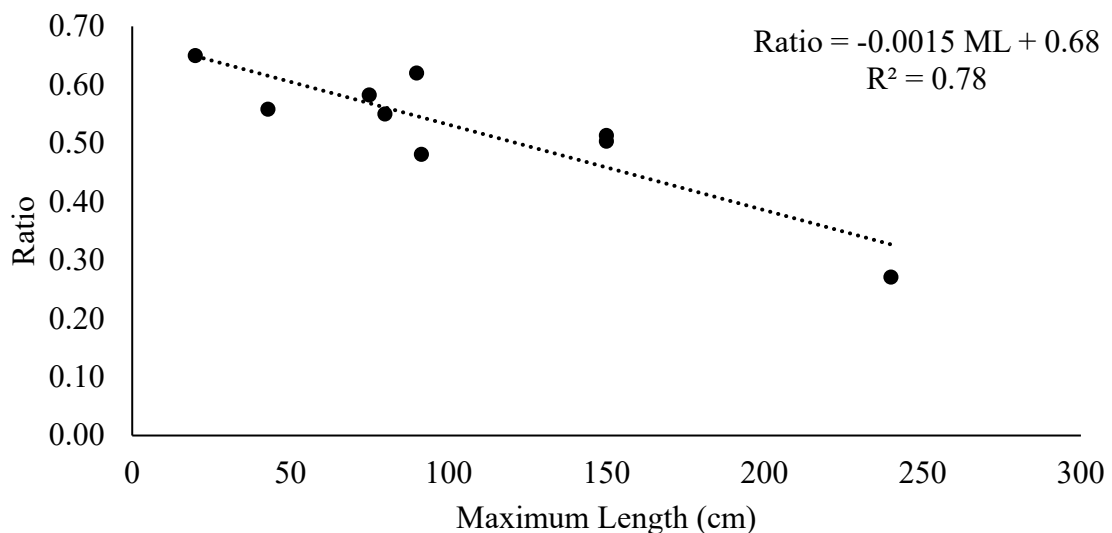

Figure 6. The ratio of length at maturity relative to maximum length (ML) in cm, for 9 species of morays.

Table 3. Species information; number of individuals is the total number of individual animals observed of each species; number of observations is the total number of behavioural observations per species; number of behaviours is the total number of different behaviours observed of each species; mean values with associated standard errors and ranges for derived timing metrics for both priority species included in the study, all values are presented in minute.

	Number of individuals	Number of Observations	Number of Behaviours	Time to First Arrival		Total Time in Frame		Time In frame per Entry		Time of First Entry to Last Exit	
				Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
Yellow-edged	18	812	11	28.5 ±5.07	0.67 - 59.37	8.01 ±3.09	0.27 - 46.44	0.61 ±0.18	0.04 - 3.24	15.23 ±4.43	0.46 - 50.80
Giant	91	3210	13	29 ±1.75	1.56 - 59.03	7.81 ±1.15	0.03 - 52.75	1.24 ±0.19	0.03 - 10.32	17.56 ±41.85	0.03 - 56.97
Total	109	4022									

Table 4. Size class information giant morays as described for species in table 2; mean values with associated standard errors and ranges for derived timing metrics for both all size classes included in the study, all values are presented in minute.

	Number of individuals	Number of Observations	Number of Behaviours	Time to First Arrival		Total Time in Frame		Time In frame per Entry		Time of First Entry to Last Exit	
				Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
Small	12	530	10	16.43 ±3.45	5.12 - 45.44	10.05 ±3.04	0.42 - 13.36	0.75 ±0.18	0.09 - 2.10	32.72 ±4.02	0.54 - 47.03
Medium	24	1447	12	28.77 ±3.26	1.56 - 54.85	14.3 ± 3.30	0.08 - 52.75	1.9 ±0.47	0.08 - 9.05	23.66 ±3.81	0.08 - 56.65
Large	15	405	13	35.97 ±4.5	3.03 - 59.03	6.54 ±1.28	0.43 - 31.11	1.63 ±0.38	0.40 - 5.06	14.44 ±3.88	0.42 - 56.97
Total	36	2382									

18

3.4 Behavioural analysis

There were 4,022 occurrences recorded of the 13 behaviours defined in the ethogram (Table 4). Three behaviours occurred most frequently: entries (46.4%), pumping (22.2%) and nudging (21.2%). Each of these dominant behaviours were related to a different behavioural class in the ethogram, entries, passive and active respectively. Entries were the most dominant behaviours with the high proportion of these behaviours largely representing the repeated movements of individuals in and out of the field of view. The next most common behaviour, passive pumping, related to the animal breathing followed by nudging which was a method of actively freeing bait from the bag and feeding on it.

For the three most common behaviours (entries, pumping, and nudging), we found that there was an effect of species with the yellow-edged moray consistently showing higher frequency of entries ($\chi^2_{[0.05,1]} = 3.841$; $\chi^2_c = 13.79$, p < 0.001), pumping, ($\chi^2_{[0.05,1]} = 3.841$; $\chi^2_c = 10.73$, p <0.001) and nudging ($\chi^2_{[0.05,1]} = 3.841$; $\chi^2_c = 9.80$, p < 0.005) behaviours (Figure). There was also an effect of size class (Figure), with the largest morays entering less frequently than medium and small morays ($\chi^2_{[0.05,2]} = 5.991$; $\chi^2_c = 24.02$, p < 0.001), but medium morays were more active than large and small morays in terms of the frequency of pumping ($\chi^2_{[0.05,2]} = 5.991$; $\chi^2_c = 51.64$, p < 0.001) and nudging ($\chi^2_{[0.05,2]} = 5.991$; $\chi^2_c = 48.72$, p < 0.001). Considering all behaviours summed across their respective classes of entries, passive and active behaviours, there was no effect of species ($\chi^2_{[0.05,2]} = 5.991$; $\chi^2_c = 1.01$, p < 0.75) or size class ($\chi^2_{[0.05,4]} = 9.488$; $\chi^2_c = 7.04$, p < 0.25).

3.5 Timing and duration

Time of first arrival varied between 0.7 and 59.4 minutes post deployment with an average of 28.7 minutes (±1.7 SE, Table 4), and varied by size class (Table 5; df = 2, p= 0.01). This difference was driven by small morays entering the field of view earlier than medium and large morays (Fig 6). Total time in frame varied from 0.03 to 52.75 minutes with an average presence in frame of 7.77 minutes (± 1.06 SE). No effect of species or size was detected on the total time in frame. Time in frame per entry ranged between 0.03 and 10.3 minutes with an average time of 1.1 minutes (± 0.16 SE) spent in frame per each entry. This metric too, detected no significant effect of species or size. Time from first entry to last exit varied between 0.03 and 57.0 minutes with an average of 17.0 minutes (± 1.68 SE), similar
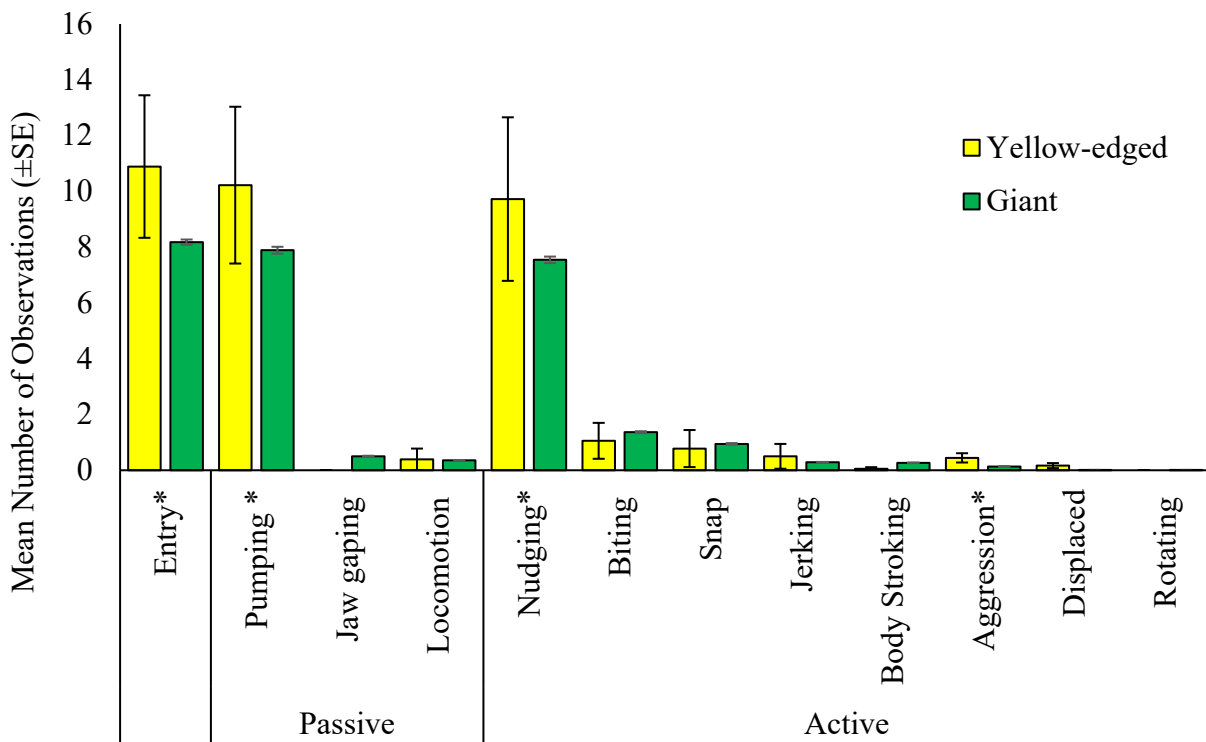
Figure 7 Mean number of observation observed per individual for both priority species. Significance indicated by *. Entry (chi squared with 1 df =13.79, p < 0.001); Pumping (chi squared with 1 df =10.73, p < 0.001); Nudging (chi squared with 1 df =9.80, p <0.005 time of first arrival, time from first entry to last exit also varied by size (df = 2, p = 0.03), with differences driven by large morays spending the less amount of time in the area then small morays (small – large, p = 0.008).
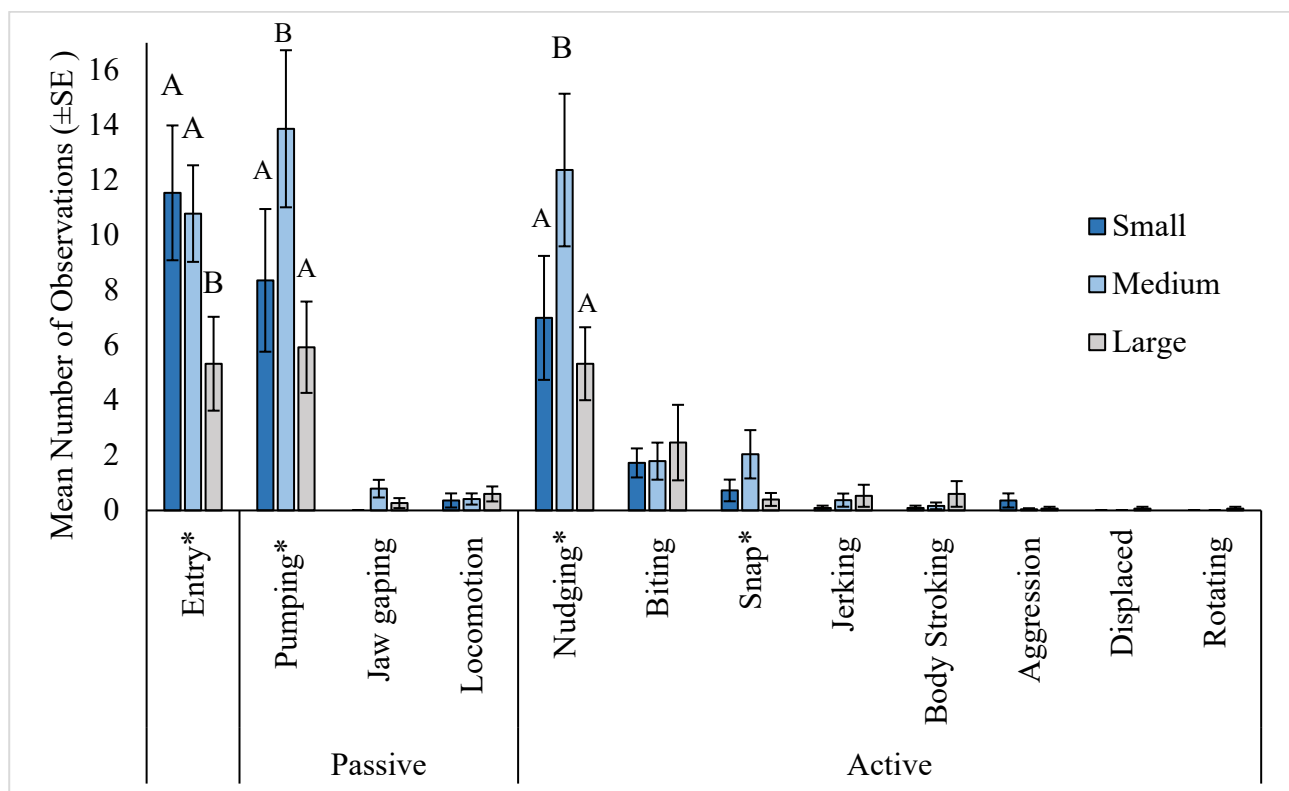

Figure 8. Mean number of observation observed per individual by size class. Significance indicated by * with letters indicating direction. Entry (chi squared with 2 df =24.02, p < 0.001); Pumping (chi squared with 2 df =51.64, p < 0.001); Nudging (chi squared with 2 df =48.72, p < 0.001).
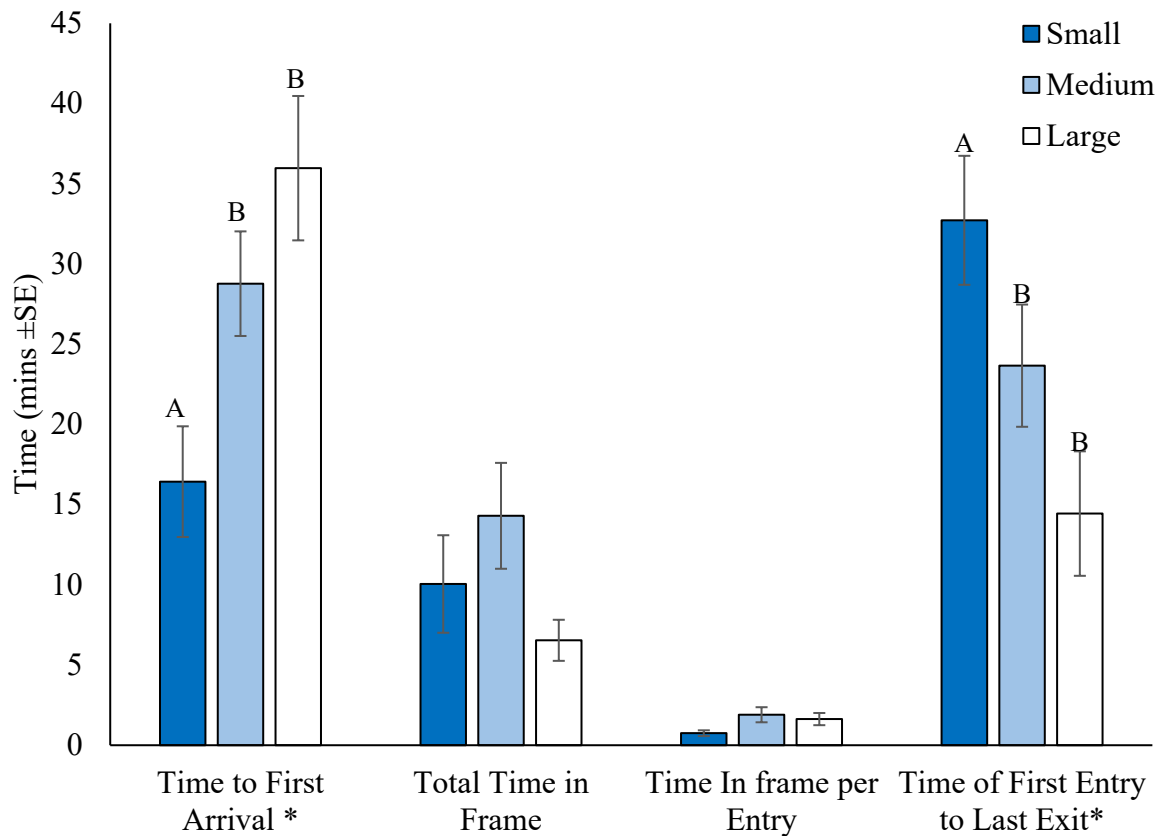
Figure 9. Mean timing of each timing metric investigated for all three size classes of giant morays, where * indicates a significant difference across the size classes and lettering indicates where differences occur within significant metrics.

4. DISCUSSION

Both the focal species, the giant moray and the yellow-edged moray, are tropical species observed across the Indian and Pacific oceans and around Australia (Bohlke & Randall 2000, Bohlke & McCosker 2001, Reece et al. 2010). However, there were no observations of the yellow-edged moray within the Australian Exclusive Economic Zone despite Australia being located at the centre of its distribution (Aquamaps 2016a). It is possible that this method under sampled the yellow-edged moray as our sampling focused on the 10 - 50 m depth range and the yellow-edged morays depth range is reported as 1 - 150 meters (Froese & Pauly 2019). Comparatively, we sampled the entirety of the giant morays depth range, 0 – 50 meters (Myers 1991, Sommer et al. 1996). As such, it is not clear whether the yellow-edged moray is less common than the giant moray or the sampling regime in these surveys did not adequately capture its depth range. The latitudinal distribution of the giant moray was also relatively narrow compared to that reported (Froese & Pauly 2019), with no observations at locations such as Shark Bay on the western coast of Australia (-25.8°, 113.3°) despite it likely to be

present here (AquaMaps 2016b). The lack of sightings in Princess Charlotte Bay for both species likely reflects the nature of the seabed which is largely soft sediments, confirming that both species are reef associated. The capacity to use stereo BRUVS to gather distributional data on a wide range of species, including morays can be used to help update and refine distributional maps.

My research found a strong allometric relationship between head height and total length in giant morays. This relationship has not previously been investigated, although is supported by evidence surrounding strong morphometric relationships in reef fish of similar shapes (Kulbicki et al. 2005). A previous study by Harrison et al. (2017) found a strong positive relationship between head length and total length ($r^2 = 0.97$) in the California moray, *Gymnothorax mordax*. This was not supported by my findings, however the lack of relationship found between head length and total length, could be a result of the small sample size (n = 5) of total lengths in this study compared to the larger sample size (n = 66) in Harrison et al. (2017). It may also be the result of slight methical differences in that this study defined head length as the anterior tip of the snout to the anterior edge of the parabranchial opening compared to the posterior edge of the parabranchial opening defined in Harrison et al. (2017). However, I did find a strong relationship between head height and head length, giving validity to the relationship between head height and total length.

The relationships between head height, head length and total length from this research and Harrison et al. (2017) also provide insights into the trophic roles of morays. Harrison et al. (2017) investigated how developmental changes in jaw and head morphometry corresponded to ontogenetic shifts in diet relating to prey size with a strong allometric relationship between vertical gape distance and head length ($r^2 = 0.83$). Gape is understood to determine the maximum size prey that can be ingested with a relationship of increasing prey size with increasing gape (Goatley & Bellwood 2009, Bachiller & Irigoien 2012, Dunic & K. 2017, Harrison et al. 2017, Mihalitsis & Bellwood 2017). With increasing size, morays can consume larger prey but continued to consume smaller prey. Specifically, as the California moray increased in size, so did the size range of prey suggesting their role as an opportunistic forager. This is consistent with Bachiller and Irigoien (2012) who found similar patterns in small pelagic fishes. As morays increase the size range of prey they consume over ontogeny understanding gape limitation in impacting what they eat allows us to further our understanding of their role as a predator and therefore their role in structuring ecosystems. Given the recognised relationships between head morphometry, gape, and total length, a

significant opportunity exists for future studies to capitalise on the ease of obtaining head measurements from stereo BRUVS to further our understanding of moray trophic ecology in shaping reef community structure and health.

An additional valuable insight from this morphometric analysis relates to the ability to create size distribution and determine the reproductive maturity of the sampled population. This result has three implications. First, it allows documentation of the size structure of moray populations based on a consistently visible attributes: their heads. As such, questions as to how size structure changes through space and time can be answered. Second, it allows inferences to be made about demographics such as proportion of the population that is reproductively mature. Estimates of natural mortality in morays are limited (Froese & Pauly 2019) so the provision of information on size structure and reproductive maturity contributes to improved estimates, or in many cases for the Muranidae, first estimates of these important demographic parametres. The estimate that 25.5% of the observed giant morays were reproductively mature also provides a benchmark against which change through time can be measured. Given the entire depth range of the giant moray was sampled, the large proportion of mature adults is unlikely to be a result of the methodology or ontogenetic partitioning. Instead it may reflect the lack of exploitation of morays given that fished populations of commercially targeted species typically have their adult populations truncated by as much as 90% (Barnett et al. 2017). The relatively large proportion of adults may also be explained by the extended pelagic duration of the leptocephali (moray larvae) and their ability to delay metamorphosis until appropriate conditions are available, therefore resulting in consistently successful 'recruitment'(Reece et al. 2010, Reece et al. 2011).

In behavioural analysis between the two focal studies there were three common behaviours relating to entries and exits to and from the reef matrix, breathing (pumping) and feeding (nudging). Despite morays having a reputation for being aggressive (Reece et al. 2010), aggressive behaviours were rarely observed in this study. This suggests that, for the most part, morays were able to coexist with other organisms even when a food source is present. Such coexistence among predators with few displays of aggression may be the result of existing hierarchical dominance negating the need for such displays (Myrberg & Gruber 1974, Sperone et al. 2010). Established dominance hierarchies are well documented as being evolutionarily favourable as a means of settling disputes with low energetic cost (Neat et al. 1998, Lopez & Martin 2001, Horova et al. 2015).

The two species showed the same relative proportion of behaviours but the yellow-edged moray was more active in absolute terms. Yellow-edged morays are reported to be particularly sensitive to injured fish, although it is unclear whether this is in response to the release of blood or to chemicals related to stress (Hobson 1974). If yellow-edged morays are sensitive to blood, the higher frequency of activity around the bait bag is unsurprising. There were no differences between the two species with respect to any of the behavioural timing metrics. The short mean time of first arrival for both species may reflect limited movements by morays although Pereira et al. (2017) and Bassett and Montgomery (2011) both document relatively large home ranges for other Muranidae. However, the nocturnal behaviour of morays may have resulted in a lack of movement during the day. The lack of differences in timing was unexpected given the yellow-edged moray otherwise was more active and suggests that sensitivity to stimuli (Hobson 1974) was relatively unrelated to timings of behaviours.

Frequency of dominant behaviours varied by size classes within the giant morays. The difference was driven by medium sized morays having a higher frequency of common behaviours compared to small and large morays. This result is inconsistent with other studies that show smaller individuals to be more active and aggressive than larger animals, including reef sharks (Thompson 2014). It may be that smaller morays are more vulnerable to predation given their slender forms as they are known to be preyed upon by sharks and barracudas. Medium size morays might have escaped the gape size of their predators (Barley et al. 2019) but still show elements of increased behaviour due to higher energetic costs relative to large individuals (Peters 1983) .Unlike species, there was variation in the timing metrics by size class. Differences were detected in two timing metrics, time to first arrival and time from first entry to last exit. The difference in time to first arrival was driven by small morays arriving earlier. Whilst literature does not exist on the relationship of size and this timing metrics for morays specifically, it does exist for a similar metric relating to the freshwater killifish *Brachyraphis episcopi* (Brown & Braithwaite 2004). Brown and Braithwaite (2004) investigate the relationship between body size and time taken to emerge from a shelter, finding that there was a positive relationship with smaller fish taking a shorter time to emerge compared to larger fish. The authors argue that the positive relationship is related to metabolic theory, such that smaller individuals and juveniles have lower body fat reserves and faster metabolic rates hence they are compelled to emerge from shelters and begin foraging sooner than larger fish, consistent with (Peters 1983).

This study demonstrates that stereo BRUVS represent an effective way of sampling size and behaviour of morays and can be utilised as an alternative to extractive lab-based surveys and diver-based surveys. High volumes of information can be obtained from the videos such as distributional evidence, behavioural data and size data. Stereo BRUVS also overcome issues associated with other underwater sampling methods such as depth, safely observing aggressive behaviours, reproducibility and seafloor rugosities (Cappo et al. 2006, Harvey et al. 2007). Whilst sampling for this study occurred solely during daylight hours there is also documentation of adapting the technology for nocturnal studies with the use of blue light (Harvey et al. 2012). Finally, the potential of the bait to influence behaviour must be considered and thus the generality of the results to behaviour in the absence of bait treated cautiously. However, as the giant moray is believed to be an opportunistic forager (Harrison et al. 2017) and hence differences in foraging relating to the bait bag seem unlikely.

Future research on morays should utilise stereo BRUVS. Additional samples are needed, both to expand the size range of sampled morays and to extend this approach to species not included here due to inadequate samples. Size analysis should continue to focus on total lengths as explained by head morphometry given the relationships found in this study and by Harrison et al. (2017), however should also continue to collect total length data, where possible, to consolidate these relationships further. The importance of understanding size and its ecological implications is highlighted in this study given the variation in behavioural frequency and timing seen. Additionally, behavioural sampling should continue for these species whilst adding in more species allowing for more tangible conclusions regarding the role of morays on reef systems as a whole.

Through my findings I have shed some light on the role of morays in the structuring and function of reef ecosystems. Through demonstrating a relationship between head height and total length, I have furthered our understanding of moray demography and also provided insights into how prey size may vary ontogenetically. By building a size distribution for the sample population and through the back-calculation of length at maturity, I have provided a tentative indicator of fishing mortality. The potential presence of hierarchical dominance as interpreted from the behavioural data suggest that morays may play a role as a high level predator and thus contribute to coral reef health.

5. REFERENCES

(2016a) Reviewed distribution maps for Gymnothorax flavimarginatus (Yellow-edged moray), with modelled year 2100 native range map based on IPCC A2 emmissions scenario. www.aquamaps.org

(2016b) Reviewed distribution maps for Gymnothorax javanicus (Giant moray), with modelled year 2100 native range map based on IPCC A2 emmissions scenario. www.aquamaps.org

Abdo D, Bellchambers L, Evans S (2012) Turning up the heat: increasing temperature and coral bleaching at the high latitude coral reefs of the Houtman Abrolhos Islands. Plos One 7

Bachiller E, Irigoien X (2012) Allometric relations and consequences for feeding in small pelagic fish in the Bay of Biscay. ICES Journal of Marine Science 70:232-243

Barley S, Meekan M, Meeuwig J (2017a) Diet and condition of mesopredators on coral reefs in relation to shark abundance. Plos One 12

Barley S, Meekan M, Meeuwig J (2017b) Species diversity, abundance, biomass, size and trophic structure of fish on coral reefs in relation to shark abundance. Marine Ecology Progress Series 565:163-179

Barley S, Mehta R, Meeuwig J, Meekan M (2016) To knot or not? Novel feeding behaviours in moray eels. Mar Biodivers 46:703-705

Barley SC, Clark TD, Meeuwig JJ (2019) Functional redundancy between coral reef sharks and predatory teleosts. . Submitted to Reviews in Fish Biology and Fisheries

Barnett LAK, Branch TA, Ranasinghe RA, Essington TE (2017) Old-Growth Fishes Become Scarce under fishing. Current Biology 27:2843-2848

Bohlke E, McCosker J (2001) The Moray Eels of Australia and New Zealand, with the Description of Two New Species (Anguilliformes- Muraenidae). Records of the Australian Museum 53:71-102

Bohlke EB, Randall J (2000) A Review of the Moray eels (Angulliformes- Muraenidae) of the Hawaiian Islands, with Descriptions of Two New Species. Proceedings of the Academy of Natural Sciences of Philidelphia 150:203-278

Brown C, Braithwaite VA (2004) Size matters: a test of boldness in eight populations of the poeciliid Brachyraphis episcopi. Animal Behaviour 68:1325-1329

Cappo M, Harvey E, Shortis M (2006) Counting and measuring fish with baited video techniques -an overview. Australian Society for Fish Biology Workshop Proceedings:101-114

Chave N, Randall H (1971) Feeding Behavior of the Moray Eel, Gymnothorax pictus. Copeia 1971:570-574

D'Aout K, Aerts P (1999) A kinematic comparision of forward and backward swimming in the eel, Anguilla anguilla. The Journal of Experimental Biology 202:1511-1521

Dunic JC, Baum JK (2017) SIze structuring and allometric scaling relationships in coral reef fishes. Journal of Animal Ecology 86:577-589

Dunic JC, K. BJ (2017) Size structuring and allometric scaling relationship in coral reef fishes. Journal of Animal Ecology 86

Frederiksen M, Edwards M, Richardson AJ, Halliday NC, Wanless S (2006) From plankton to top predators: bottom-up control of a marine food web across four trophic levels. Journal of Animal Ecology 75:1259-1268

Froese R, Pauly D (2019) FishBase. www.fishbase.org

Goatley CHR, Bellwood DR (2009) Morphological structure in reef fish assemblage. Coral Reefs 28

Halpern B, Walbridge S, Selkoe K, Kappel C, Micheli F, D'Agrosa C, Bruno J, Casey K, Ebert C, Fox H, Fujita R, Heinemann D, Lenihan H, Madin E, Perry M, Selig E, Spalding M, Steneck R, Watson R (2008) A global map of human impact on marine ecosystems. Science 319:948-952

Harasti D, Malcolm H (2013) Distribution, relative abundance and size composition of the threatened serranid Epinephelus daemelii in New South Wales, Australia. Journal of Fish Biology 83:378-395

Harrison JS, Higgins BA, Mehta RS (2017) Scaling of dentition and prey size in the California moray (Gymnothorax mordax). Zoology 122:16-26

Harvey E, Cappo M, Butler J, Hall N, Kendrick G (2007) Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure. Marine Ecology Progress Series 350:245-254

Harvey ES, Dorman S, Fitzpatrick C, Newman S, McClean D (2012) Response of diurnal and nocturnal coral reef fish to protection from fishing: an assessment using baited remote underwater video. Coral Reefs 31:939-950

Harvey ES, Fletcher D, Shortis M (2001) A comparison of the precision and accuracy of estimates of reef-fish lengths determined visually by divers with estimates produced by a stereo-video system. Fishery Bulletin 99:63-71

Harvey ES, Shortis M (1998) Calibration stability of an underwater stereo--video system: Implications for measurement accuracy and precision. Marine Technology Society Journal 32:3-17

Hobson ES (1974) Feeding Relationships of Teleostean Fishes on Coral Reefs in Kona, Hawaii. Fishery Bulletin 72

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral Reefs under Rapid Climate Change and Ocean Acidification. Science 318:1737-1742

Horova E, Brandlova K, Glonekova M (2015) The First Description of Dominance Hierarchy in Captive Giraffe: Not Loose and Egalitarian, but Clear and Linear. Plos One 10

Hourigan T, Timothy T, Reese E (1988) Coral Reef Fishes as Indicators of Environmental Stress in Coral Reefs. In: Soule DF, Kleppel GS (eds) Marine Organisms as Indicators. Springer New York, New York, NY

Jordan R, Burghardt G (1986) Employing an Ethogram to Detect Reactivity of Black Bears (Ursus arnericanus) to the Presence of Humans. Ethology 73:89-115

Knowlton N (2001) Coral Reef Biodiversity-Habitat Size Matters. Science 292:1493-1495

Knowlton N, Jackson JBC (2008) Shifting Baselines, Local Impacts, and Global Change on Coral Reefs. PLoS Biology 6

Kulbicki M, Guillemot N, Amand M (2005) A general approach to length-weight relationships for New Caledonian lagoon fishes. Cybium: international journal of ichtyology 29:235-252

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnology and Oceanography 45:1119-1131

Lopez P, Martin J (2001) Fighting rules and rival recognition reduce costs of aggression in male lizards, Podarcis hispanica. Behavioural Ecology & Sociobiology 49:111-116

MacNulty D, Mech D, Smith D (2007) A Proposed Ethogram of Large-Carnivore Predatory Behavior, Exemplified by the Wolf. Journal of Mammalogy 88:595-605

Malcolm H (2016) A moray's many knots: knot tying behaviour around bait in two species of Gymnothorax moray eel. Environ Biol Fish 99:939-947

Manuel A, Franco-Trecu V, Franco-Fraguas P, Arim M (2015) Gape and energy limitation determine a humped relationship between trophic position and body size. Canadian Journal of Fisheries and Aquatic Sciences 72:198-205

Matić-Skoko S, Tutman P, Bojanić Varezić D, Skaramuca D, Đikić D, Lisičić D, Skaramuca B (2014) Food preferences of the Mediterranean moray eel, Muraena helena (Pisces: Muraenidae), in the southern Adriatic Sea. Marine Biology Research 10:807-815

May RM, McClean AR (2007) Theoretical ecology principles and applications. Oxford University Press, New York

Mehta RS, Wainwright PC (2007) Biting releases constraints on moray eel feeding kinematics. Journal of Experimental Biology 210:495-504

Mihalitsis M, Bellwood DR (2017) A morphological and functional basis for maximum prey size in piscivorous fishes. Plos One 12

Miller T, Adlard R, Bray R, Justine J-L, Cribb T (2010) Cryptic species of Euryakaina n. g. (Digenea: Cryptogonimidae) from sympatric lutjanids in the Indo-West Pacific. Systematic Parasitology 77:185-204

Myers R (1991) Micronesian reef fishes. Coral Graphics, Barrigada, Guam

Myrberg AA, Gruber SH (1974) The Behavior of the Bonnethead Shark, Sphyrna tiburo. 1974:358-374

Neat FC, Taylor AC, Huntingford FA (1998) Proximate costs of fighting in male cichlid fish: the role of injuries and energy metabolism. Animal Behaviour 55:875-882

Pelletier F, Clutton-Brock T, Pemberton J, Tuljapurkar S, Coulson T (2007) The Evolutionary Demography of Ecological Change- Linking Trait Variation and Population Growth. Science 315:1571-1574

Peters RH (1983) The ecological implications of body size. Cambridge University Press, Cambridge

Reece JS, Bowen BW, Joshi K, Goz V, Larson A (2010) Phylogeography of two moray eels indicates high dispersal throughout the indo-pacific. J Hered 101:391-402

Reece JS, Bowen BW, Smith DG, Larson A (2011) Comparative phylogeography of four Indo-Pacific moray eel species (Muraenidae) reveals comparable ocean-wide genetic connectivity despite five-fold differences in available adult habitat. Marine Ecology Progress Series 437:269-277

Robinson J, Williams I, Edwards A, McPherson J, Yeager L, Vigliola L, Brainard R, Baum J (2016) Fishing degrades size structure of coral reef fish communities. Global Change Biology 23:1009-1022

Robinson JPW, Baum JK (2015) Trophic roles determine coral reef fish community size structure. Canadian Journal of Fisheries and Aquatic Sciences 73:1-10

RStudio (2015) RStudio: Intergrated Development for R.

Ruppert JLW, Travers MJ, Smith LL, Fortin M, Meekan MG (2013) Caught in the Middle: Combined Impacts of Shark Removal and Coral Loss on the Fish Communities of Coral Reefs. Plos One 8

Sale P (1991) CHAPTER 1 - Introduction. The Ecology of Fishes on Coral Reefs. Academic Press, San Diego

Scheiner SM, Willig MR (2007) A general theory of ecology. Theoretical Ecology 1:21-28

Sommer C, Schneider W, Poutiers JM (1996) The living marine resources of Somalia. FAO, Rome

Sperone E, Micarelli P, Andreotti S, Spinetti S, Andreani A, Serena F, Brunelli E, Tripepi S (2010) Social interactions among bait-attracted white sharks at Dyer Island (South Africa). Marine Biology Research 6:408-414

Thompson C (2014) Predicting shark behaviour: the influence of species, size and brain organisation. University of Western Australia

Tickler D, Letessier T, Koldewey H, Meeuwig J (2017) Drivers of abundance and spatial distribution of reef-associated sharks in an isolated atoll reef system Plos One 12

Verberk W (2011) Explaining General Patterns in Species Abundance and Distributions. Nature Education Knowledge 3

Whitmarsh S, Fairweather P, Huveneers C (2017) What is Big BRUVver up to? Methods and uses of baited underwater video. Rev Fish Biol Fisher 27:53-73

Zar JH (1999) Biostatistical Analysis. Prentice Hall, New Jersey

6. APPENDIX 1: Expedition information for all stereo BRUVS surveys completed between 2015 and 2018.

Location	Year	Month	Total N of drops	N Giant moray	N Yellow-edged moray
Chagos	2015	March	247	10	6
Chagos	2015	November	270	1	0
Chagos	2016	May	332	8	10
Cocos Keeling Islands	2016	November	203	5	2
Princess Charlotte Bay	2017	April	428	0	0
Wandoo	2017	May	100	0	0
Far north Queensland	2017	June	280	8	0
Far north Queensland	2017	November	173	8	0
Far north Queensland	2018	April	385	16	0
North Western Australia	2017	June	359	16	0
Shark Bay	2017	September	40	0	0
Shark Bay	2018	August	75	0	0
Rowley Shoals	2017	November	85	19	0
		Total	2977	91	18