# **ASHMORE REEF NATIONAL NATURE RESERVE**

# MARINE SURVEY 2013 - METHODS FIELD TEST

## Daniela Ceccarelli, David Williamson, Tony Ayling, Avril Ayling and Morgan Pratchett



June 6<sup>th</sup>, 2013

Produced for Department of Sustainability, Environment, Water, Population & Communities





Corresponding author – Professor Morgan Pratchett, ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville QLD 4811. E-mail: <u>Morgan.Pratchett@jcu.edu.au</u>, Telephone/ Fax: (07) 47815747/ 47816722

## **Executive Summary**

Monitoring wild populations is important to assess long-term trends in abundance, especially in remote exploited species. However, the accurate detection of population trends requires standardised or comparable monitoring protocols to be adopted during successive surveys. Exploited species of holothurians (e.g. Holothuria nobilis, Holothuria fuscogilva) and topshell (Tectus niloticus) have been surveyed on six separate occasions at Ashmore Reef National Nature Reserve from 1999 and 2009, but survey locations, sampling effort and methods have varied greatly. In March 2013, a team of surveyors aboard the Australian Customs Vessel Ashmore Guardian tested a new method, designed by the CSIRO, to determine its suitability for ongoing monitoring of holothurian and *T. niloticus* abundances. The team was provided with a list of 384 waypoints (sites), classified as 'flat', 'north edge' and 'south edge' sites. The team was requested to survey as many of these as possible given time and logistical constraints, with 12.5% of overall sampling effort on edge sites. At each site, a single 40m long and 2m wide transect was surveyed and all holothurians, T. niloticus, tridacnid clams, Linckia and crown-of-thorns sea stars and pearl oysters were counted. The maximum basal shall diameter of each T. niloticus was also measured and recorded. A guadrat of approximately 250cm<sup>2</sup> (50cm x 50cm) was photographed at the beginning and end of each transect, and the benthic cover was estimated from these photos using the software program CPCe (Kohler and Gill 2006).

Surveys were carried out over a total of 6 days (5 full days and 2 afternoons), with approximately 6-7 hours available for surveys on each full day. A total of 95 sites were surveyed, 75 in areas classified as "reef flat", 11 along the "northern edge" and 9 along the "southern edge". The most important issues encountered with this sampling design were:

- The number of sites surveyed was severely restricted by constraints on operation times for Customs staff that were critical to the conduct of these surveys.
- At low tide, which limited access to the reef flat, the only areas that could be surveyed along exposed reef margins were edge sites. Whilst waiting for access to flat sites an increased number of edge sites were surveyed, leading to >12.5% representation of this habitat type.
- Pre-determined sites on a map did not capture the relevant habitats for the key target organisms, and did not allow for an even spread of effort across the specific reef and non-reef habitats that are relevant for the distribution and habitat preferences of the focal species.
- It is not possible to survey all (384) sites within 10-14 days, especially given constraints imposed by weather, tides and logistics, and safe SCUBA diving regulations.
- The small size of the sampling units (transects), and the use of a single transect at each site, makes it less likely to effectively survey the focal species present at each site.
- Given the error associated with using GPS waypoints, future transects are highly unlikely to cover exactly the same ground. These transects should not be viewed as permanent transects.
- Estimating percent bottom cover from two digital photographs per transect does not provide a representative sample of the benthic cover at each site.

We highly recommend the use of a dedicated research vessel for these surveys; while use of the Australian Customs Vessel Ashmore Guardian limits direct costs for environmental surveys at Ashmore Reef, there is an increased risk that these surveys will be interrupted or prevented due to conflicting demands. The number of berths available for scientists (4) and the single tender available when using the Ashmore Guardian also significantly constrain the surveys that can be completed within a two week sample window. We also recommend that the survey design be re-structured according to a habitat stratification that reflects the extent of different habitats at Ashmore Reef, and the habitat preferences of the species of interest (exposed reef slope, exposed reef crest, sheltered reef slope, sheltered reef crest, reef flat, sand flat, eastern lagoon, western lagoon). Given the difficulties associated with surveying along pre-defined transect paths, we recommend replicate transects (e.g. three replicate 50 x 2m transects) per site. This would not add greatly to the total survey time and provide opportunities to test for variation in abundance of target species at specific sites. Benthic habitats cannot be effectively represented based on just two photographs. We strongly recommend using standard point or line intersect techniques to document benthic cover. Significant value could also be added by combing surveys of holothurians and T. niloticus with surveys of other important reef organisms (e.g. sharks).

This exercise has clearly shown that the development of an effective survey methodology should combine theoretical and analytical considerations with an understanding of the practical and logistical constraints by experienced field ecologists. A Standard Operating Procedure is important to maximise the benefit of ongoing ecological monitoring at Ashmore Reef, and other offshore Commonwealth Reserves, but this needs to be both technically sound and practical.

## Contents

Executive Summary	2
List of Figures	5
List of Tables	5
Introduction	6
Methods	7
Results	12
Number of sites and survey effort Travel and survey time Holothurians, <i>Tectus</i> and other invertebrates Benthos and habitat	12 14 15 17
Discussion	18
Recommendations	21
Acknowledgements	28
References	29
Appendix 1	31

## List of Figures

Figure 1. Location of Ashmore Reef National Nature Reserve
Figure 2. Example of an afternoon's trajectory of travel between sites
Figure 3. Surveyor counting invertebrates along a southern edge transect
Figure 4. Limits of tender operations from a single tender of the Ashmore Guardian
Figure 5. All sites across Ashmore Reef13
Figure 6. The exposed SW edge of Ashmore Reef on the low tide of March 18th
Figure 7. Counts of all holothurians by habitat across Ashmore Reef15
Figure 8. Species composition of holothurians counted at Ashmore Reef
Figure 9. Average counts per transect of <i>T. niloticus</i> in different habitats of Ashmore Reef16
Figure 10. Average counts per transect of all tridacnid clams in different habitats of Ashmore Reef .17
Figure 11. Species composition of other invertebrates counted at Ashmore Reef
Figure 12. Benthic percent cover, estimated from the two photo quadrats taken at each site
List of Tables
Table 1. Field names to establish consistent metadata among surveys
Table 2. Time spent travelling, at the site, and conducting the survey at each site. 14
Table 3. Broadly typical habitats of Ashmore Reef, and number of sites surveyed
Table 4. List of tasks to be undertaken during this survey

## Introduction

Fishing for coral reef invertebrates such as holothurians, bivalves and gastropods has depleted stocks in many regions of the world (Kinch *et al.* 2008; Toral-Granda *et al.* 2008). The life history of a number of commercially valuable species means that once depleted, population recovery occurs very slowly or not at all (Bell *et al.* 2005). The most popular way of avoiding overexploitation or assisting the recovery of target species is to establish marine protected areas with a no-take policy within its boundaries. This strategy has led to increased abundance and / or biomass of exploited finfish (Russ *et al.* 2008; Babcock *et al.* 2010), sharks (Robbins *et al.* 2006; Heupel *et al.* 2009) and commercially important invertebrates (Lincoln-Smith *et al.* 2006; Price *et al.* 2009).

Ashmore Reef National Nature Reserve (the Reserve, 12°17'S, 123°02'E), established in 1983, encompasses an area of approximately 583 square kilometres and comprises three small vegetated islands, a number of sand cays, two lagoons and extensive reef and sand flat habitats. Prior to the establishment of the Reserve, Indonesian fishers regularly harvested holothurians, topshell (Tectus niloticus) and tridacnid clams on Ashmore Reef and other reefs on the North West Shelf. In recognition of these traditional fishing grounds, a Memorandum of Understanding (MOU) between the Australian and Indonesian governments (established in 1974 and reviewed in 1989), sets out arrangements by which traditional fishers may access marine resources in the region (Figure 1). Traditional Indonesian fishermen are permitted to visit the MOU Box area, including a small area known as West Island Lagoon in the Reserve. Access to the remaining area of the Reserve is prohibited unless under authorisation from the Director of National Parks. Despite the MOU, illegal harvesting and fishing of holothurians, T. niloticus, clams, turtles, sharks and other resources remain a constant threat in the Reserve (Ceccarelli et al. 2007). The Australian Customs Service (Customs) has provided a regular compliance and enforcement presence at Ashmore for many years, however it became clear that a dedicated vessel was required to protect the Reserve's unique environment. Since April 2008, the Australian Customs Vessel Ashmore Guardian has provided a near permanent presence at Ashmore, offering an unparalleled level of protection.

Regular monitoring of target populations over time can provide crucial information to management agencies about the success of current management arrangements. Ideally, adaptive management would provide for prescribed reactive changes to management arrangements in response to information provided by monitoring (Gerber *et al.* 2005). Between 1998 and 2009, there were six surveys of Ashmore Reef focusing on a range of ecological variables, including holothurians and *T. niloticus* (Skewes *et al.* 1999; Smith *et al.* 2001; Rees *et al.* 2003; Kospartov *et al.* 2006; Ceccarelli *et al.* 2007; Richards *et al.* 2009). Differences in sampling protocols have made it difficult to compare results between years, and therefore the temporal dynamics of populations remain uncertain (Hosack and Lawrence 2013). An attempt to standardise abundance estimates among surveys found that densities of some holothurians and *T. niloticus* increased over time, but for many holothurian species, especially the most valuable ones, overall densities were too low for significant changes to be detected (Ceccarelli *et al.* 2011). To address these difficulties, DSEWPaC commissioned the development and field testing of a standardised method for monitoring holothurian and *T. niloticus* populations based on data and methods from all previous surveys combined (Hosack and Lawrence 2013).

The objectives of this survey were to field test the methods developed by Hosack and Lawrence (2013) for obtaining estimates of reef-wide abundance of holothurians and *T. niloticus* at Ashmore Reef.

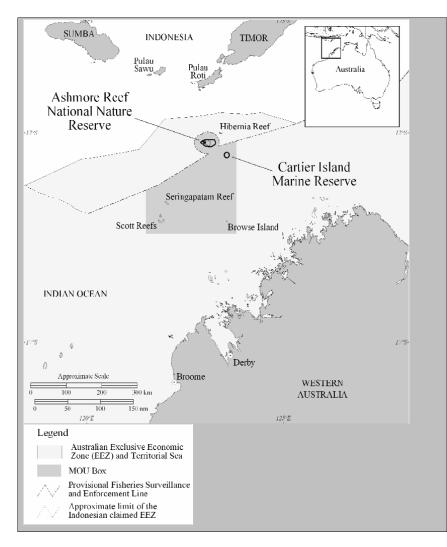


Figure 1. Location of Ashmore Reef National Nature Reserve within the area covered under the Memorandum of Understanding between Australian and Indonesia (the MOU Box) in the Indian Ocean (image from DEH 2005).

### **Methods**

Field testing of the monitoring method for holothurians and *T. niloticus* at Ashmore Reef took place between the 13<sup>th</sup> and 22<sup>nd</sup> of March, 2013. To comply with Customs schedules, only 6-7 hours were available during each full day of surveys, and between three and four hours on half-days (13<sup>th</sup> and 21<sup>st</sup> of March). During this period, the survey team was stood down for 2.5 days (19<sup>th</sup>, 20<sup>th</sup> and morning of the 21<sup>st</sup>) due to Customs duties. A total of six full days were therefore available for survey work. Surveys were carried out during daylight hours only. All four surveyors on the team are highly experienced, and all tasks were rotated during the field trial to account for observer differences.

On each sampling occasion (morning or afternoon), a sector of Ashmore Reef was chosen based on weather, tides and accessibility. Within that sector, a trajectory was planned to link between six and ten waypoints (Appendix 1)<sup>1</sup> with the highest priority (lowest) numbers and the shortest travel distance. We initially aimed for numbers under 100, on the assumption that 100 might be a realistic maximum number of sites surveyed during the trip. Short-listed sites were subsequently added or dropped based on a re-evaluation of time, tide and safety constraints (Figure 2). In situations where there was time for one extra site, the closest available site was chosen with the lowest number, even if this was a number above 100.

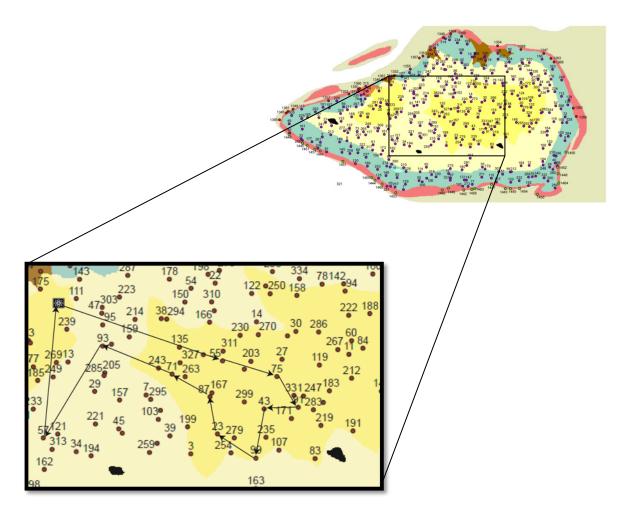


Figure 2. Example of an afternoon's trajectory of travel between sites in the eastern lagoon of Ashmore Reef. After Site 93, time allowed for the survey of Site 57, so it was added as the last site for the day.

At each site, the pre-defined waypoint was approached and a buoy dropped as exactly as possible on the waypoint. Two divers on SCUBA or snorkel (depending on depth and visibility at the site) entered the water, and the first diver laid a 40m transect along a roughly linear path from the buoy, either following the depth contour, or following the habitat type, or parallel to the habitat edge. In instances where there was strong current, there was no option but to swim directly with or against

<sup>&</sup>lt;sup>1</sup> The table of waypoints used in the survey (Appendix 1) is the same table that was provided as an attachment to the Service Agreement. Note that the waypoints for the sites differ from those in Table A.1. of Hosack and Lawrence (2013).

the current (cross-current transects tend to balloon the tape sideways, preventing the roughly linear format required for the transect). The first diver also took a photograph of the slate with the site name, and the benthic photo quadrat at the beginning and end of each transect. The diver aimed to capture a quadrat approximately 0.25m<sup>2</sup>, using the tape for guidance. A number of additional photographs were also taken at most sites to document habitat structure and attributes.

The second diver carried a 1m length of measuring tape and surveyed a 2m belt along the transect. Where the habitat allowed, both sides of the tape were surveyed at once (e.g. over bare sand), whilst in highly complex habitats (e.g. reef crest and slope), or where visibility was poor (e.g. parts of the eastern lagoon), one side was surveyed on the first pass along the transect, and the other side on the return swim. The second diver recorded numbers of holothurians (identified to species level), tridacnid clams, crown-of-thorns (COTS), *Linkia* and *T. niloticus*. The basal shell width of all *T. niloticus*, dead and alive, was measured to the nearest millimetre using a standard ruler mounted on the clipboard (Figure 3). Before winding in the tape, a second buoy was placed at the end of the transect. Before leaving the site, a GPS endpoint was marked and the buoys retrieved. The time was recorded on arrival at the site, on commencing and completing the transect, and on departure from the site. There was only one day when two tenders were available, with one survey team (two surveyors) per tender. On all other days the team of four worked from one tender, with pairs of surveyors alternating sites.

Restrictions included the requirement to follow Customs daily schedules (08:30 – 12:00, 13:00 – 16:30)<sup>2</sup>, the assignment of Customs crews to their priority tasks upon the arrival of two Suspected Illegal Entry Vessels (SIEVs), the availability of only one tender and coxswain, and the limits of permitted travel distance imposed on a single tender (Figure 4). We attempted to overcome these limitations by using a second tender, provided by the ACV Roebuck Bay, on one day, and by requesting the repositioning of the Ashmore Guardian to the eastern edge of Ashmore Reef for two days (overnight).



Figure 3. Surveyor counting invertebrates along a southern edge transect (left) and measuring *T. niloticus* (right).

<sup>&</sup>lt;sup>2</sup> Note: Customs staff at Ashmore Reef operate on Central Standard Time (CST). Time in this report and associated data will also be in CST, unless otherwise stated.

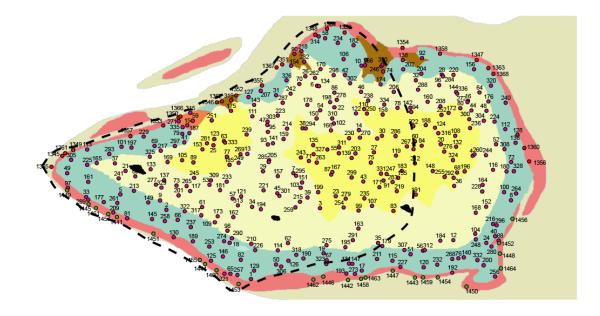


Figure 4. Limits of tender operations for a single tender of the Ashmore Guardian. The dashed line bounds the area accessible to the tender operating alone.

Where a pre-determined waypoint could not be surveyed, the following protocol was followed (as prescribed by DSEWPaC):

1. Proceed to GPS checkpoint. Start transect at the minimum distance to the waypoint that can be safely reached and record the new starting coordinates.

2. If no such point can safely be found within 100 m of the waypoint, then drop this site and replace from the GRTS oversample.

3. Please note why a site is difficult or impossible to sample.

Photographs were downloaded each day and prepared for benthic percent cover analysis. The program Coral Point Count with Excel extensions (CPCe, Kohler and Gill 2006) was used to analyse the percentage cover of the major benthic categories from the digital photos taken at each end of each survey transect. Twenty random points were generated on each habitat photo and the benthos beneath each point recorded in the following categories: sand, rubble, consolidated rubble, boulders, pavement, live hard coral, dead standing hard coral, soft coral, algae, seagrass, ascidians, zoanthids, hydroids, sponges and *Heliopora*. The points assigned to each benthic category were totalled for each photo and converted to a percentage cover estimate. All data were entered into a spreadsheet with rows corresponding to sites and fields to the measured variables (Table 1).

Table 1. Field names to establish consistent metadata among surveys of holothurians, *T. niloticus* and additional ecological variables (modified from Skewes et al., 1999a,b). Variables in bold were those added in the field.

Required	Name	Description				
fields	SITE	Site name				
	START EAST	Easting (UTM)				
	START	Northing (UTM)				
	NORTH					
	END EAST	Easting (UTM)				
	END NORTH	Northing (UTM)				
	OBSERV	Observer ID				
	T.TRAVEL	Travel time to site (minutes)				
	T.SAMPLE	Duration of time required to sample site (minutes)				
	T.DAY	Time of completion (24 hour clock, hh:mm, AWT)				
	AREA	Area sampled at site (square metres)				
	DEPTH	Station depth (metres)				
	T.NILO	<i>Tectus niloticus</i> shell (Count per transect)				
	H.ATRA	Lollyfish, <i>Holothuria atra</i> (Count per transect)				
	H.LEAU	Holothurian, Holothuria leucospilota (Count per	transect)			
	H.NOB	Black teatfish, Holothuria nobilis (Count per tra	nsect)			
	H.FUSC	White teatfish, Holothuria fuscogilva (Count per	transect)			
	H.EDU	Pinkfish, Holothuria edulis (Count per transect)				
	H.GRAE	Holothurian, Pearsonothuria graeffei (Count per	r transect)			
	H.ARGU	Holothurian, <i>Bohadschia argus</i> (Count per transect)				
	H.FUSP	Elephant trunk fish, <i>Holothuria fuscopunctata</i> (Count per transect)				
	H.CHOR	Greenfish, <i>Stichopus chloronotus</i> (Count per transect)				
	H.ANAN	Prickly redfish, <i>Thelenota ananas</i> (Count per transect)				
	H.ANAX	Amberfish, <i>Thelenota anax</i> (Count per transect)				
	H.VARI	Curryfish, <i>Stichopus variegatus</i> (Count per transect)				
	H.SYNA	Holothurian, <i>Synapta</i> spp. and <i>Euapta</i> spp. (Count per transect)				
	H.ACTI	Holothurian, <i>Actinopyga</i> spp. (Count per transect)				
	H. ATRAL	Holothurian, larger subspecies of <i>H. atra</i> (Con				
	H.FUSR	Holothurian, Holothuria fuscorubra (Count p				
Additional	COT	Crown of thorns starfish, <i>Acanthaster planci</i> (Court p				
Fields	T.GIGAS	Giant clam, <i>Tridacna gigas</i> (Count per transect)	built per transcet)			
ricius	PEARL	Pearlshell, <i>Pinctada</i> spp. (Count per transect)				
	LINK					
	CL.CROC	Blue starfish, <i>Linckia laevigata</i> (Count per transect) Clams, <i>Tridacna crocea</i> (Count per transect)				
	CL.SQUA	Clams, <i>Tridacna squamosa</i> (Count per transect)				
	CL.MAXI	Clams, <i>Tridacna maxima</i> (Count per transect)				
	CL.DERA	Clams, <i>Tridacna derasa</i> (Count per transect)				
	CL.HIPP	Clams, <i>Hippopus hippopus</i> (Count per transect)				
	SAND	Percent of bottom: sand				
	RUBBLE	Percent of bottom: rubble	A digital photo quadrat of			
	CONS.RUB	Percent of bottom: consolidated rubble	benthic habitat at the start			
	BOULDERS	Percent of bottom: boulders	and end of each site			
	PAVEMENT	Percent of bottom: pavement	transect will be taken and			
	L.CORAL	Percent of bottom: live coral	used to determine per cent cover of these variables.			
	DS.CORAL	Percent of bottom: dead hard standing coral	cover of these variables.			
	S.CORAL	Percent of bottom: soft coral				
	ALGAE	Percent of bottom: algae				
	SEAGRASS	Percent of bottom: seagrass				
	SPONGES	Percent of bottom: sponges				
	ASCIDIANS	Percent of bottom: ascidians				
	ZOANTHID	Percent of bottom: zoanthids				
	HYDROIDS	Percent of bottom: hydroids				
	SPONGE	Percent of bottom: sponges	1			

### **Results**

#### Number of sites and survey effort

In the six days that were available, 95 sites were surveyed across the Reserve (Figure 5). Efforts were made to survey an even spread of sites around the reef, and to keep the proportional effort in the edge strata low. Overall, 95 sites were sampled, 75 in flat strata and 20 (21%) in edge strata, with 11 on the north edge and 9 on the south edge. The reason for proportional effort in edge strata being higher than the prescribed 12.5% was due to very low tides in the mornings of the first four days of surveys, making access to the flats impossible (Figure 6). Continuing to sample edge strata was deemed preferable to ceasing operations and waiting for high tide.

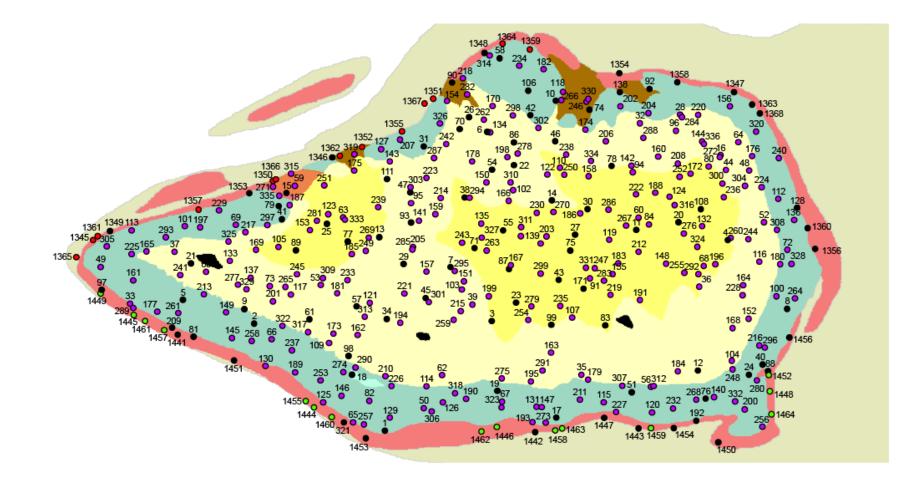


Figure 5. All sites across Ashmore Reef. Purple: flat sites; green: south edge; red: north edge; black: sites completed during this survey.

The waypoint was changed for 12 sites, either because the depth was greater than 12m (8 sites) or because they were exposed and inaccessible at the time of sampling (4 sites) and it was unlikely that that area could be accessed again during the survey trip (Appendix 1).



Figure 6. The exposed SW edge of Ashmore Reef on the low tide of March 18<sup>th</sup> (~1m tidal height), during which the flat stratum was inaccessible.

#### Travel and survey time

The total accumulated travel time between sites, and between the Ashmore Guardian and the first and last site of the day, amounted to 21 hours and 13 minutes (Table 2). The total time spent conducting surveys was just under 10 hours. On average, travel between sites took approximately 11 minutes. An average of 15 minutes was spent at each site, which included the arrival at the waypoint, preparation of divers, and follow-up activities after the dive before departing from the site. Site surveys took between 4 and 14 minutes, with an average survey time of 6.3 minutes per site (Table 2).

Task	Minutes	Hours
Total travel time	1273	21h13m
Total survey time	599	9h59m
Total site time	1499	24h59m
Average travel time	11.23158	
Average survey time	6.305263	
Minimum travel time	2	
Maximum travel time	58	
Minimum survey time	4	
Maximum survey time	14	

Table 2. Time spent travelling, at the site, and conducting the survey at each site.

#### Holothurians, T. niloticus and other invertebrates

Across all sites, a total of 86 individual holothurians, and 32 live and one dead *T. niloticus* were recorded. On the flat stratum<sup>3</sup>, 72 holothurians and 17 live and one dead *T. niloticus* were counted, whilst edge strata yielded 14 holothurians and 15 *T. niloticus*; 7 and 1 (respectively) on the north edge and 7 and 14 (respectively) on the south edge. No holothurians or *T. niloticus* were found at 54 (56.8%) of the 95 surveyed sites. If other organisms (tridacnid clams, COTs, *Linckia* spp. and *Pinctada* spp.) were included, 42 (44.2%) sites returned a zero count.

Differences in the average counts between habitat strata were negligible (Figure 7). Reef-wide and flat counts trended to be slightly higher than edge counts (including north edge, south edge and all edge habitats combined). Counts of holothurians were dominated by few species, and even the most abundant species returned low average counts per transect. The most abundant species were *Holothuria atra, Holothuria leucospilota, Stichopus chloronotus, Stichopus hermanni* and *Holothuria edulis* (Figure 8). The highest-value species, *Holothuria nobilis* and *Holothuria fuscogilva*, were found in very low abundance.

*Tectus niloticus* were distributed much less evenly across the different habitat strata, with the highest counts recorded along the southern edge (Figure 9). Flat strata also hosted low numbers of *T. niloticus*, however, the waypoints for the sites where *T. niloticus* were recorded fell into habitat that would not be classified as flat (either reef or sand flat) in the field. Sites classified as flat strata with present *T. niloticus* included sites 0081, 0192 and 0097; all three sites were on the reef crest in "typical" *T. niloticus* habitat (Smith 1987; Skewes *et al.* 1999).

The average basal shell width of live *T. niloticus* was 85.6mm (+/- 1.1 S.E.). Only one dead *T. niloticus* was recorded, at 85mm.

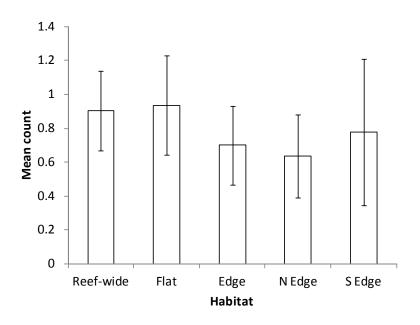


Figure 7. Counts of all holothurians by habitat across Ashmore Reef.

<sup>&</sup>lt;sup>3</sup> Please see section on *T. niloticus* counts by habitat and size structure with regards to concerns with labeling sites as 'edge' or 'flat' strata.

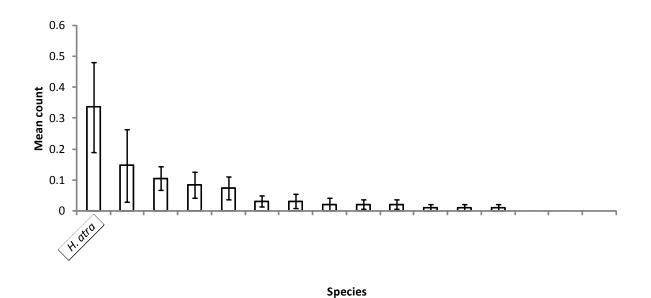


Figure 8. Species composition of holothurians counted at Ashmore Reef, in order of declining abundance.

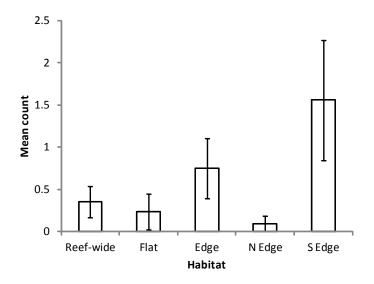


Figure 9. Average counts per transect of T. niloticus in different habitats of Ashmore Reef.

Tridacnid clams were found in greater numbers on both the northern and southern edges of Ashmore Reef than on the flat (Figure 10). As with holothurians and *T. niloticus*, however, inter-site variability was extremely high. Of the other invertebrates counted during the survey, the sea stars *Linckia* spp. were the most abundant, followed by the clams *Tridacna maxima* and *Tridacna crocea*. No crown-of- thorns starfish were recorded during the survey (Figure 11).

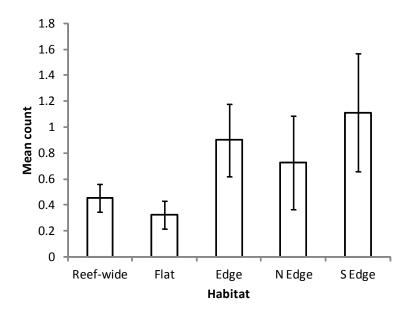


Figure 10. Average counts per transect of all tridacnid clams in different habitats of Ashmore Reef

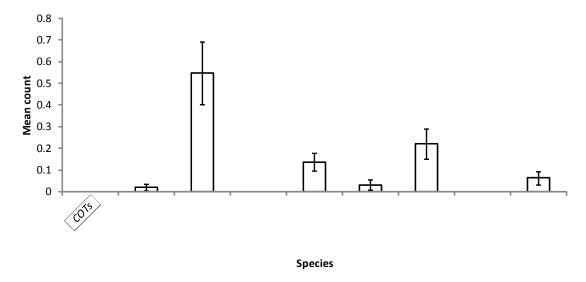


Figure 11. Species composition of other invertebrates counted at Ashmore Reef, including sea stars (COTs or crown-of-thorns and *Linckia* spp.), pearl oysters (*Pinctada* spp.) and tridacnid clams.

#### **Benthos and habitat**

Habitat notes taken during the surveys reveal that 17% of sites were on bare sand, with sparse patches of seagrass or filamentous algae, and 43% of sites fell in sandy lagoonal areas with some rubble and / or patch reefs. A further 16% of sites were on reef flat, 6% on reef crest, 12% on reef slope and 4% were on lagoonal reef slope or large patch reefs (Table 3). Analysis of the photo quadrats revealed that the reef-wide cover of sand was 54%, followed by pavement (22%), rubble (7.6%), live coral (6.5%), algae (2.8%) and soft coral (2.1%). All other benthic categories covered less that 2% of the photographed quadrats (Figure 12). Sand dominated primarily on the flat (68%), whilst pavement covered 65% and 54% of the north and south edge, respectively. Live coral cover

was 12% on the north edge and 21% on the south edge. However, the representativeness of just two photoquadrats (each less than or equal to 0.25m<sup>2</sup>) for each 80m<sup>2</sup> transect is questionable.

Table 3. Broadly typical habitats of Ashmore Reef, and number of sites surveyed within each habitat.

Habitat type	Number of sites
Bare sand	16
Sand and rubble or patch reefs	41
Reef flat	17
Reef crest	6
Reef slope	11
Lagoonal reef	4

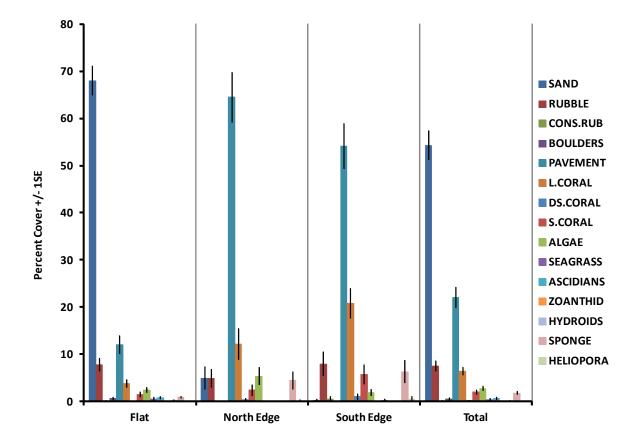


Figure 12. Benthic percent cover, estimated from the two photo quadrats taken at each site.

#### **Discussion**

The number of sites surveyed was severely restricted due to a number of factors, most of them to do with using the Customs vessels as a platform. Travel time to and from Ashmore Reef was longer than planned due to Customs surveillance operations during the transit. While on the Ashmore Guardian, Customs time schedules (08:30-12:00, 13:00-16:30, CST) had to be complied with, shortening the potential time spent on the water by 2-3 hours each day. The arrival of two SIEVs stopped the surveys completely for 2.5 days due to increased demand on the Customs staff. Furthermore, the distance that could be travelled by a single tender from the Ashmore Guardian excluded much of the eastern third of the reef. The distance restriction was overcome by using two tenders on one day,

and through repositioning the Ashmore Guardian to the eastern edge of the reef for two days. However, the northeastern reef flat remains a geographic gap in the sampling effort. Weather conditions were extremely favourable during this survey, but may hamper survey efforts in future, especially as Customs regulations do not permit tender operations, even within the lagoon, in wind above 20 knots.

Low proportional effort in edge sites was achievable only on days when the tide allowed access to flat sites. In the early part of the survey, very low tides (~0.6m) during morning hours meant that very few (and usually low-priority) flat sites could be accessed. Furthermore, following Customs time schedules meant that on at least four days, the survey team was required to be on the Ashmore Guardian during times that would have been favourable for sampling flat sites. Generally, sites were mapped out in groups of eight per half-day sampling session, with one edge site and seven high-priority flat sites. However, at tidal heights when the reef flat is completely inaccessible, the high cost and logistic difficulty of transporting survey teams to Ashmore Reef means that a higher proportion of edge sites should be sampled. If required, the balance of edge to flat sites can be restored during analysis simply by excluding lower-priority edge sites.

The hierarchical prioritisation offered in Table 1 was generally achievable. However, on some occasions sites had to be chosen on the basis of their vicinity, rather than their priority level, in the effort to survey as many sites as possible in the time available. Attempts were made to minimise travel time during each sampling session, but Ashmore Reef is large (measuring 25km between its western and eastern ends) and course trajectories to reach reef flat sites were often slow and not linear, due to sand banks, islands and tracts of shallow reef. Travel time (just over 21 hours) was approximately double the time taken for surveys (just under 10 hours). Non-survey time at the site amounted to 15 hours; this time of travel and pre-and post-dive activities would remain constant if greater effort (i.e. an increase in transect replication or a larger sampling unit) was expended at each site. A further time commitment was the analysis of the photo quadrats to record benthic percent cover. This took approximately 15 hours and would not usually be included in a field survey, but counted as data analysis. Thus, although photo quadrats may shorten the time in the field, this approach adds to data analysis time and presents other problems (see below).

Numbers of holothurians and *T. niloticus* counted on the survey were low, as found in previous surveys; the species composition and relative abundance of different holothurian species roughly matched observations from previous surveys. *Holothuria atra, H. leucospilota* and *S. chloronotus* were the three most abundant species, but others were recorded in lower abundance (e.g. *Pearsonothuria graeffei*) or higher abundance (*S. hermanni*). The most likely cause of these differences is the difference in survey effort between habitat strata. Many species have distinct habitat preferences – for instance, *P. graeffei* is generally most abundant on upper reef slopes (Purcell *et al.* 2012) – and without adequate habitat stratification in the survey design, the recording of such species is likely to be inconsistent. This also applies to *T. niloticus*, where adults tend to be found exclusively in a band below the reef crest, and juveniles tend to occur on outer reef flats (Colquhoun 2001). This survey design, with pre-determined sites on a map, did not capture the relevant habitats for the key target organisms, and did not allow for an even spread of effort across reef habitats that are relevant for the distribution and habitat preferences of the focal species.

The sampling method tested during this survey poses a number of problems in the context of an ongoing monitoring program. Firstly, the number of sites to be sampled will rarely be achievable under standard field trip conditions (taking into account weather, tides and logistics) of 10 days to two weeks. Sampling 'as many sites as possible' during the available survey time, even with a prioritisation scheme of the sites, is unlikely to yield the same number of sites, or the same sites, on each trip. Hosack and Lawrence (2013) state that "designs with fewer than 100 sites have very little chance of capturing the true total abundance within a reasonably narrow confidence interval"; sampling 100 sites or more is unrealistic within the logistic constraints of using the Customs vessels as platforms for the surveys. Sampling more than 100 sites may be possible with a dedicated survey vessel, at least two tenders and at least four surveyors, but this remains a high-risk sampling design in terms of achieving a number of sites that will yield a reasonable estimate of abundance from a few specific locations, rather than attempting to quantify absolute densities and changes through time at the scale of the entire reef.

The small size of the sampling units (transects), and using a single transect at each site, reduces the likelihood of effectively sampling target organisms at individual sites. Furthermore, given the error associated with GPS waypoints, future transects are highly unlikely to cover the same ground, especially with small sampling units. According to Hosack and Lawrence (2013): "In the absence of evidence to the contrary, the proposed survey design assumes that the spatial data obtained by sampling two transects at two separate sites is worth more than sampling two separate transects at the same site." This is untrue in terms of time efficiency, whereby more time spent surveying a site requires the same amount of time travelling to and from the site, and the same amount of pre- and post-dive time at the site. In addition, we have reservations about their premise of using more sites to maximise the chances of surveying at a site where organisms are present, versus using a smaller number of more strategically placed sites, with a greater survey effort at each site, to actually capture organisms that are present. At several sites, the target species were present (in five cases they were photographed or recorded as being off-transect), but not captured by the sampling units. After field testing this method, we therefore still believe that a smaller number of sites, stratified by habitats (see Recommendations below), with a greater sampling effort at each site (three transects or more, longer than 40m), has a greater likelihood of providing a more accurate estimate of abundance than the present survey design.

Estimating benthic structure and composition based on just two digital photographs per transect poses a considerable risk in misrepresenting the transect area. For photo-analysis (e.g. using the program CPCe), photo-quadrats had to be less than or equal to  $0.25m^2$ . As such, benthic composition for each transect was represented by sampling 0.625% of the transect area. Moreover, these to photographs failed to capture changes in habitat structure along transects. Virtually all published papers that quantify benthic composition do so using either point or line intersect surveys along the transect (only marginally more time-consuming for an experienced observer), or to take photographs along the entire transect (very time-consuming to analyse).

Providing predetermined waypoints has the inherent risk of misrepresenting habitats, especially with the present stratification of habitats into flat and (north and south) edge. Waypoints categorised as edge sites were not in consistent habitats; some were well above the crest on the reef flat, some were around the reef crest, and a number of the southern edge sites were on the

reef slope at depths below 12m and were not viable and repeatable for a remote monitoring program. Similarly, many 'flat' waypoints were in areas of strong currents, such that it will be difficult to survey the same transect path unless currents are running in the same direction at different stages of the tide. The only way to ensure repeatability is for field surveyors to set the starting waypoint of the site, based on knowledge of the area, the availability of the target habitats and common sense.

## Recommendations

Deviations from the prescribed survey methods (Table 4) were necessary due to limitations imposed by either operational constraints imposed by using the Ashmore Guardian, large distances involved in transiting among high priority sites, and inaccessibility of some sites due to tides and weather. The only way to complete the proposed sampling within a two-week period would be to use a dedicated research vessel, with a team comprising at least 8 divers and access to multiple tenders. Given limitations associated with working from the Ashmore Guardian, it may be prudent to focus on sampling within a limited range of specific study sites that capture the full range of habitat types, and then use randomly placed replicate transects within fixed sites. We propose that this stratification include at least the following habitat types:

- o Exposed reef slope (southern and northwestern)
- Exposed reef crest (southern and northwestern)
- Sheltered reef slope (northern and eastern)
- o Sheltered reef crest (northern and eastern)
- o Reef flat (characterised by pavement substrate)
- o Sand flat (characterised by sand substrate)
- o Eastern lagoon (wide, shallow, turbid, dominated by sandy habitats)
- Western lagoon (deep, clear, characterised by coral-dominated slopes and patch reefs)

If reliant on broadly defined sites, then the repeatability of individual transects would be less important than ensuring that sampling is constrained to a specific area and habitat type. By surveying replicate, random (haphazardly-positioned) transects within each site (e.g., three replicate 50 x 2m transects) it will then be possible to test for changes in the abundance of target species between surveys relative to the sample error at a single survey. It is also important to effectively account for benthic structure and composition along each transect, as this has a major bearing on the abundance and composition of holothurians that are likely to be recorded. Standard point or line intersect techniques are well-established and should be adopted both to adequately represent the benthic attributes of each site, and to test for changes in structure and composition over time.

Table 4. List of tasks to be undertaken during this survey, with comments on their execution in the field, benefits and limitations associated with the tasks, and recommendations specifically arising from difficulties faced with the tasks.

Task	Execution	Benefits	Limitations	Recommendations
Survey sites listed in Table 1 of	Sites listed in Table 1 of the	Saves time of deciding	Sites are not based on habitat	The first team of field surveyors are
the contract	contract document were	site locations in the field.	characteristics that influence the	given a list of habitats of Ashmore
	imported into the handheld		distribution and abundance of reef	Reef (we recommend exposed reef
	GPS unit and used to locate		biota. Randomly placing sites on a	slope, exposed reef crest, sheltered
	the starting points of the		map increases the risk of missing	reef slope, sheltered reef crest, reef
	transects.		representative habitats and over-	flat, sand flat, eastern lagoon and
			sampling others.	western lagoon), with a map
				delineating the extent of each and a
				minimum number of sites to survey
				in each habitat. Surveyors then
				locate these habitats and place the
				sites in the correct location. Future
				surveyors then use those waypoints
				for monitoring surveys.
As many sites are to be sampled	As many sites as possible	A large number of sites	The choice of sampling trajectory	See recommendation above.
as possible given the time	were sampled given	can be sampled in a	will always have a subjective	
available, other constraints and	limitations of time, tides, and	relatively short time.	element and therefore will vary	
workplace health and safety	safe diving procedures.		from team to team. Complying	
standards and procedures. The	Sample numbers were		with Customs work schedules	
number of sites completed is not	maximised by planning		effectively shortens the available	
to exceed 384	sampling routes with the		time on the water by 2-3 hours	
	shortest distances between		each day, and the risk of having to	
	highest priority sites within a		cease surveys due to Customs	
	given area of Ashmore Reef.		taskings is high.	
	The number of sites did not			
	exceed 384. It is unlikely that			
	this figure could be			
	approached even if the full			
	ten days of sampling			
	originally intended for this			
The notice of sites are suited in	survey had been available.		To maintain balance and law	
The ratio of sites prescribed in	Keeping the prescribed site	According to the models	To maintain balance and low	See recommendation above.

Task	Execution	Benefits	Limitations	Recommendations
Table 1 is to be followed, i.e.	ratio (12.5% edge sites, equal	in Hosack and Lawrence	proportional effort in edge sites	
12.5% of sites are to be allocated	number of north and south	(2013), maintaining a low	within a short time period, all	
to edge strata, and an equal	sites) was not possible during	proportional effort	strata must be equally accessible	
number of sites in each edge	this survey.	(12.5%) in edge sites	at all times. It is not possible to	
stratum is required		provides for the greatest	maintain this proportion while still	
		utility for the most	completing as many sites as	
		abundant species or	possible overall (see Task above),	
		invertebrates at	and while accounting for the tidal	
		Ashmore Reef	limitations of access to flat strata,	
			distance limitations of a single	
			Customs tender, the large	
			distances to be covered across	
			Ashmore Reef, weather limitations	
			(not experienced on this survey,	
			but certainly a factor to consider),	
			and the short-notice changes to	
			work schedules. Furthermore,	
			waypoints for sites classified as	
			'edge' fell into a diverse range of	
			habitats, from reef slope, to crest	
			to shallow flat.	
Sites sampled should follow in	The sequential order of sites	Prioritisation was the	The distances between high-	See recommendation above. A
sequential order as in Table 1	listed in Table 1 was followed	only way to maintain a	priority sites on a reef the size of	dedicated research vessel should be
	by prioritising the lowest-	geographically balanced	Ashmore made it impossible to	chartered for these surveys.
	numbered sites in the areas	sampling program with a	follow the actual sequence of	
	to be accessed at each	very high number of	sites; the reef had to be broken	
	sampling occasion. All but	potential sites.	down into areas that could be	
	two of the 30 highest priority		accessed within one morning or	
	sites were surveyed.		afternoon, and the prioritisation	
			scheme used to choose sites	
			within each area. Because the first	
			<i>m</i> sites in the table are spatially	
			balanced, they are required to be	
			a long way apart, especially if	
			beginning with Site 1.	

Task	Execution	Benefits	Limitations	Recommendations
Where fewer sites than those	Keeping the prescribed site	According to the models	To maintain balance and low	See recommendation above. A
identified in Table 1 can be	ratio (12.5% edge sites, equal	in Hosack and Lawrence	proportional effort in edge sites	dedicated research vessel should be
completed, site numbers should	number of north and south	(2013), maintaining a low	within a short time period, all	chartered for these surveys.
be completed in multiples of four	sites, multiples of four) was	proportional effort	strata must be equally accessible	
and always with a 12.5% ratio of	not possible during this	(12.5%) in edge sites	at all times. It is not possible to	
edge stratum	survey.	provides for the greatest	maintain this proportion while still	
		utility for the most	completing as many sites as	
		abundant species or	possible overall (see Task above),	
		invertebrates at	while accounting for the tidal	
		Ashmore Reef.	limitations of access to flat strata,	
			distance limitations of a single	
			Customs tender, the large	
			distances to be covered across	
			Ashmore Reef, weather limitations	
			(not experienced on this survey,	
			but certainly a factor to consider),	
			and the short-notice changes to	
			work schedules. Furthermore,	
			waypoints for sites classified as	
			'edge' fell into a diverse range of	
			habitats, from reef slope, to crest	
			to shallow flat.	
Prior to commencing the survey	The sequential order of sites	Maintaining spatial	The eastern quarter of Ashmore	A dedicated research vessel should
site coordinates should be	listed in Table 1 was followed	balance between the	Reef could only be accessed with	be chartered for these surveys.
plotted and then surveyed in a	by prioritising the lowest-	four quadrants of the	two tenders, or by moving the	
logical sequence for efficiency,	numbered sites in the areas	reef ensures a wide	Ashmore Guardian. Sites in this	
e.g. the closest site to Site 1 may	to be accessed at each	spread of geographic	area are at risk of being under-	
be Site 163. However, the spatial	sampling occasion. The area	locations are surveyed,	sampled without the use of a	
balance needs to be maintained	to be accessed was changed	reef areas subjected to	dedicated research vessel.	
and time needs to be allocated	at each sampling occasion to	different exposure		
equally to each quandrant of the	ensure all quadrants would	regimes are sampled.		
Reef, i.e. do not complete all	be surveyed equally. All but			
sites in quadrants 1, 2 and 3 and	two of the 30 highest priority			
no or few sites in quadrant 4.	sites were surveyed.			
Where a site is inaccessible, that	The sequential order of sites	Taking replacement sites	This method only works for sites	The sites used to replace inaccessible

Task	Execution	Benefits	Limitations	Recommendations
site can be replaced by another site taken sequentially from the GRTS reverse hierarchical order (Table 1) so as to retain spatial balance in the design. For example, for the agreed minimum number of sites to be surveyed, plot those sites and survey them; if, say, three of those sites were inaccessible, the next 3 sites in numerical sequence would be chosen as replacement sites.	listed in Table 1 was followed by prioritising the lowest- numbered sites in the areas to be accessed at each sampling occasion. The area to be accessed was changed at each sampling occasion to ensure all quadrants would be surveyed equally. All but two of the 30 highest priority sites were surveyed.	sequentially from a pre- existing list saves time in the field and returns a similarly balanced spatial survey design.	within the same rough area of the reef. If the next three sites in the sequential order are on the other side of the reef, they may be no more accessible than the original sites.	sites should simply be the highest priority sites in the general vicinity, guided more by their geographic proximity than their priority listing.
Recording of site information is set out in Table 2 and Table 3 and is to be followed, and all fields completed	All site information fields set out in Table 2 and Table 3 were completed.	Site information in Tables 2 and 3 allows for the identification of the exact location of the survey and the time taken to travel to the site and to complete the survey.	None.	Use these same categories in future surveys.
Transects 1 x 40m long and 2m wide to be undertaken per site, commencing at the geographic coordinates provided in Table 1	One transect, 40 x 2m, was surveyed at each site, starting at the waypoint provided in Table 1.	One short transect at each site allows for a short survey time and for a greater number of sites to be surveyed.	These sampling units are too small and too few to adequately capture the abundance of target organisms that have a patchy, sparse or clumped distribution. There were numerous sites where organisms were sighted, but not captured by the transect, and therefore not recorded. One transect per site does not allow for the identification of spatial distribution patterns across Ashmore Reef. Some organisms (eg <i>T. niloticus</i> )	See recommendations above. Sample less sites more thoroughly. This will reduce the proportion of travel time and pre- and post-dive at the site to actual survey time, increasing efficiency and increasing the quality of data collected at each site.

Task	Execution	Benefits	Limitations	Recommendations
			occur only in very narrow habitat types and it is not appropriate to estimate abundance reef-wide, but only in relation to that particular habitat type. One short transect per site makes it difficult to relate the abundance of these organisms to the habitat types they typically occupy.	
Follow a linear path along a contour of more or less constant depth	A linear path was followed along a depth contour. On the reef and sand flats and on the lagoon floor, a roughly linear path was followed that remained within a consistent benthic habitat type. The direction of the transect was generally into the current, or with the current when it was too strong to swim against.	Following a depth contour, when in an environment of variable depth, ensures that the sample stays within the same habitat.	None.	Follow a linear path along a contour of more or less constant depth.
The species listed in Table 2 and Table 3 are to be counted	All species listed in Table 2 and Table 3 were completed, and four extra categories added.	Information in Tables 2 and 3 is more or less consistent with information gathered during previous surveys, potentially allowing for temporal comparisons.	None.	Use these same categories in future surveys.
Two digital photo quadrats are to be taken for each transect, one at the start and one at end, % cover of benthos is to be determined from these photographs.	Two digital photo quadrats were taken for each transect, one at the start and one at end, % cover of benthos was determined from these photographs using CPCe.	Allows for a permanent record of the benthos at the start and end of the transect.	Two digital photo quadrats, at the start and the end of each transect, do not adequately represent the benthic community at the site. To have a resolution high enough for photo-analysis, a quadrat no larger than 0.25m <sup>2</sup> can be	Either conduct point intercept or line intersect benthic surveys on site (any experienced surveyor would be able to complete this in a time-efficient manner) or take digital photo quadrats all along the transect, and allow for photo-analysis time after

Task	Execution	Benefits	Limitations	Recommendations
			photographed. This is much too small an area to use for an accurate estimate of benthic percent cover.	the field survey.
Live and dead <i>T. niloticus</i> should be counted and their basal width measured and recorded at each site.	Live and dead <i>T. niloticus</i> were counted and their basal width measured and recorded at each site.	Allows for both abundance estimates and size frequency distribution, providing information about population structure.	Measuring basal shell width is time-consuming.	Live and dead <i>T. niloticus</i> should be counted and their basal width measured and recorded at each site.
<i>Tectus pyramis</i> should be excluded from the count of <i>T.niloticus</i> where possible	<i>Tectus pyramis</i> were excluded from the count of <i>T.niloticus</i> .	Avoids time and effort spent on non- commercial species.	The two species can be difficult to tell apart by untrained observers.	<i>Tectus pyramis</i> should be excluded from the count of <i>T.niloticus</i> where possible
The measured variables should be stored in a traditional spreadsheet format: rows should correspond to sites and fields to the measured variables above; fields should contain both the site name and the GPS coordinates taken at each site in the UTM projection.	The measured variables have been stored in a traditional spreadsheet format: rows correspond to sites and fields to the measured variables above; fields contain both the site name and the GPS coordinates taken at each site in the UTM projection.	Establishes standards for a database that can be stored and updated with successive surveys in the same format.	The primary limitation with this task is the use of the UTM coordinate system in the Map Grid of Australia projection, which is uncommon in the field. It is not generally used in handheld GPS units or in mounted GPS units on vessels. Using this coordinate system and projection increases the risk of errors in the field.	We recommend using degrees- decimal-minutes in the WGS 84 projection commonly found in all GPS units and most widely utilised in the marine industry.
Species counts are to be recorded in count per transect.	Species counts were recorded in count per transect.	This is the standard method for recording species abundances in the field.	None.	Species counts are to be recorded in count per transect.
Quadrat photos of the benthic habitat at each site are to be clearly labelled with Site number and the GPS coordinates of each photo recorded	Quadrat photos of the benthic habitat at each site were clearly labelled with Site number and the GPS coordinates of each photo recorded	Provides a permanent record of benthic structure at the site, with a clear label for easy recalling of the location of the photo.	Time-consuming.	Labelling photographs with site numbers should be enough. If the exact location of the photograph is required, the database can be consulted.

## Acknowledgements

This study was funded by the Department of Sustainability, Environment, Water, Population and Communities. The field team would like to thank James Cook University administration staff, the Customs crew aboard the ACV Roebuck Bay, and the Customs staff and crew aboard the Ashmore Guardian for their assistance.

#### **References**

Babcock RC, Shears NT, Alcala AC, Barrett NS, Edgar GJ, Lafferty KD, McClanahan TR, Russ GR (2010) Decadal trends in marine reserves reveal differential rates of change in direct and indirect effects. PNAS 107:18256-18261

Bell JD, Rothlisberg PC, Munro JL, Loneragan NR, Nash WJ, Ward RD, Andrew NL (2005) Restocking and stock enhancement of marine invertebrate fisheries. Advances in Marine Biology 49:1-370

Ceccarelli DM, Beger M, Kospartov MC, Richards ZT, Birrell CL (2011) Population trends of a remote invertebrate resource in a marine reserve: the case of trochus at Ashmore Reef. Pacific Conservation Biology 17:132-140

Ceccarelli DM, Kospartov MC, Beger M, Richards ZT, Birrell CL (2007) Survey of the distribution and abundance of trochus, holothurians and tridacnid clams at Ashmore Reef in 2006, to assess the impact of recent illegal fishing on target invertebrate stocks. Report to the Department of Environment and Water by C&R Consulting, Townsville

Colquhoun JR (2001) Habitat preferences of juvenile trochus in Western Australia: implications for stock enhancement and assessment. SPC Trochus Information Bulletin 7:14-20

Gerber LR, Beger M, McCarthy MA, Possingham HP (2005) A theory for optimal monitoring of marine reserves. Ecology Letters 8:829-837

Heupel MR, Williams AJ, Welch DJ, Ballagh A, Mapstone BD, Carlos G, Davies C, Simpfendorfer CA (2009) Effects of fishing on tropical reef associated shark populations on the Great Barrier Reef. Fisheries Research 95:350-361

Hosack GR, Lawrence E (2013) Survey design for holothurians and *Tectus* at Ashmore Reef. Interim report. CSIRO, Hobart

Kinch J, Purcell S, Uthicke S, Friedman K (2008) Population status, fisheries and trade of sea cucumbers in the Western Central Pacific. In: Toral-Granda V, Lovatelli A, Vasconcellos M (eds) Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper. No. 516., Rome, pp 7–55

Kohler KE, Gill SM (2006) Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. Computers and Geosciences 32:1259-1269

Kospartov M, Beger M, Ceccarelli D, Richards Z (2006) An assessment of the distribution and abundance of sea cucumbers, trochus, giant clams, coral, fish and invasive marine species at Ashmore Reef National Nature Reserve and Cartier Island Marine Reserve: 2005. Report prepared by UniQuest Pty Ltd for the Department of the Environment and Heritage, Canberra

Lincoln-Smith MP, Pitt KA, Bell JD, Mapstone BD (2006) Using impact assessment methods to determine the effects of a marine reserve on abundances and sizes of valuable tropical invertebrates. Canadian Journal of Fisheries and Aquatic Sciences 63:1251-1266

Price ARG, Harris A, McGowan A, Venkatachalam AJ, Sheppard CRC (2009) Chagos feels the pinch: assessment of holothurian (sea cucumber) abundance, illegal harvesting and conservation prospects

in British Indian Ocean Territory. Aquatic Conservation: Marine and Freshwater Ecosystems 20:117-126

Purcell SW, Samyn Y, Conand C (2012) Commercially important sea cucumbers of the world. FAO, Rome

Rees M, Colquhoun J, Smith L, Heyward A (2003) Surveys of Trochus, Holothuria, giant clams and the coral communities at Ashmore Reef, Cartier Reef and Mermaid Reef, Northwestern Australia: 2003. AIMS Report Produced for the Department of Environment and Heritage, Townsville

Richards Z, Beger M, Hobbs JP, Bowling T, Chong-Seng K, Pratchett M (2009) Ashmore Reef National Nature Reserve and Cartier Island Marine Reserve - Marine survey 2009. Report to the Department of the Environment, Water, Heritage and the Arts by the Australian Research Council Centre of Excellence for Coral Reef Studies, Townsville

Robbins WD, Hisano M, Connolly SR, Choat JH (2006) Ongoing collapse of coral-reef shark populations. Current Biology 16:2314-2319

Russ GR, Cheal AJ, Dolman AM, Emslie MJ, Evans RD, Miller I, Sweatman H, Williamson DH (2008) Rapid increase in fish numbers following creation of world's largest marine reserve network. Current Biology 18:514-515

Skewes TD, Dennis DM, Jacobs DR, Gordon SR, Taranto TJ, Haywood M, Pitcher CR, Smith GP, Milton D, Poiner IR (1999) Survey and stock size estimates of the shallow reef (0-15 m deep) and shoal area (15-50 m deep) marine resources and habitat mapping within the Timor Sea MOU74 Box. CSIRO Marine Research

Smith BD (1987) Growth rate, distribution and abundance of the introduced topshell *Trochus niloticus* Linnaeus on Guam, Mariana Islands. Bulletin of Marine Science 41:466-474

Smith L, Rees M, Heyward A, Culquhoun J (2001) Survey 2000: bêche-de-mer and trochus populations at Ashmore Reef. Report to the Department of Environment and Heritage, AIMS, Townsville

Toral-Granda V, Lovatelli A, Vasconcellos M (2008) Sea cucumbers: A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical Paper 516, Food and Agriculture Organization of the United Nations, Rome

## **Appendix 1**

Geographic coordinates (Universal Transverse Mercator coordinate system, zone 51L) and strata for the GRTS survey that allocates 12.5% to edge strata and 24 sites to each edge stratum (384 sites in total). The estimated areas of the strata are: flat, 19660 ha; north edge, 326 ha; south edge, 346 ha. Sites are presented in reverse hierarchical order. Sites completed during this survey are shown in bold.

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0001	502336	8640888	Flat	Reef flat	
Site-0002	497924	8644508	Flat	Sand flat	
Site-0003	505945	8644596	Flat	Sand flat	
Site-0004	513926	8647325	Flat	Sand flat	
Site-0005	495518	8645314	Flat	Sand and rubble /	
Site-0006	505799	8650979	Flat	Sand and rubble /	
Site-0007	504563	8646411	Flat	Sand flat	
Site-0008	515930	8645009	Flat	Reef flat	
Site-0009	497596	8644999	Flat	Sand and rubble /	
Site-0010	508112	8652025	Flat	Sand and rubble /	
Site-0011	510832	8647665	Flat	Lagoonal reef	
Site-0012	512919	8642918	Flat	Sand flat	
Site-0013	502153	8647428	Flat	Sand and rubble /	
Site-0014	507977	8648666	Flat	Sand flat	
Site-0015	499017	8648917	Flat	Lagoonal reef	
Site-0016	513683	8650193	Flat		
Site-0017	508129	8641332	Flat	Reef flat	
Site-0018	501227	8642784	Flat	Lagoonal reef	
Site-0019	506134	8642232	Flat	Reef flat	
Site-0020	512259	8647926	Flat	Sand and rubble /	
Site-0021	495787	8646545	Flat	Sand flat	
Site-0022	506699	8649856	Flat	Sand and rubble /	
Site-0023	506771	8645211	Flat	Sand and rubble /	
Site-0024	514642	8642775	Flat	Sand and rubble /	
Site-0025	500377	8647878	Flat	Lagoonal reef	
Site-0026	505181	8651477	Flat	Sand and rubble /	
Site-0027	508764	8647509	Flat	Sand and rubble /	
Site-0028	512311	8651555	Flat		
Site-0029	502975	8646522	Flat	Sand flat	
Site-0030	509192	8648370	Flat	Lagoonal reef	
Site-0031	503661	8650486	Flat	Sand and rubble /	
Site-0032	510954	8651270	Flat		
Site-0033	493730	8645179	Flat		
Site-0034	502405	8644665	Flat	Sand flat	
Site-0035	508982	8642780	Flat		
Site-0036	512957	8645752	Flat		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0037	495242	8646930	Flat		
Site-0038	505034	8648763	Flat	Sand and rubble /	
Site-0039	505298	8645152	Flat	Sand flat	
Site-0040	515094	8643132	Flat	Reef flat	
Site-0041	498861	8648026	Flat	Sand and rubble /	
Site-0042	507261	8651544	Flat	Sand and rubble /	
Site-0043	508211	8645982	Flat	Sand and rubble /	
Site-0044	513863	8649694	Flat		
Site-0045	503718	8645370	Flat	Sand flat	
Site-0046	508095	8650663	Flat	Sand and rubble /	
Site-0047	503196	8648928	Flat	Sand and rubble /	
Site-0048	514643	8649670	Flat		
Site-0049	492736	8646417	Flat		
Site-0050	503656	8641646	Flat		
Site-0051	510673	8642187	Flat	Reef flat	
Site-0052	515145	8647931	Flat		
Site-0053	500177	8645813	Flat	Sand flat	
Site-0054	505954	8649699	Flat	Sand and rubble /	
Site-0055	506928	8647464	Flat	Sand flat	
Site-0056	511324	8642381	Flat		
Site-0057	501387	8645088	Flat	Sand flat	
Site-0058	506218	8653466	Flat	Reef flat	
Site-0059	499296	8649156	Flat		
Site-0060	510913	8648084	Flat		
Site-0061	499798	8644652	Flat	Sand flat	
Site-0062	504273	8642767	Flat		
Site-0063	500931	8648092	Flat		
Site-0064	514275	8650627	Flat		
Site-0065	501265	8641175	Flat		
Site-0066	498513	8643980	Flat		
Site-0067	506298	8641871	Flat		
Site-0068	513109	8646453	Flat		
Site-0069	497304	8647843	Flat		
Site-0070	504866	8651068	Flat	Sand and rubble /	
Site-0071	505367	8647056	Flat	Sand and rubble /	
Site-0072	515934	8646999	Flat		
Site-0073	498453	8645778	Flat		
Site-0074	509251	8651733	Flat	Sand and rubble /	
Site-0075	508605	8646978	Flat	Sand and rubble /	
Site-0076	513177	8641973	Flat	Sand and rubble /	
Site-0077	501081	8647279	Flat		
Site-0078	510001	8649825	Flat	Sand flat	
Site-0079	498757	8648469	Flat	Reef flat	
Site-0080	513278	8649804	Flat		
Site-0081	495875	8644065	Flat	Reef crest	
Site-0082	501815	8641904	Flat		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0083	509784	8644451	Flat	Sand and rubble /	
Site-0084	511262	8647852	Flat		
Site-0085	496302	8646249	Flat		
Site-0086	506702	8650618	Flat	Sand and rubble /	
Site-0087	506537	8646354	Flat	Sand and rubble /	
Site-0088	515275	8642907	Flat	Reef slope	
Site-0089	499332	8646985	Flat	Lagoonal reef	
Site-0090	504617	8652641	Flat	Reef flat	
Site-0091	509268	8646034	Flat	Sand flat	
Site-0092	511279	8652420	Flat	Sand and rubble /	
Site-0093	503227	8647921	Flat	Sand and rubble /	
Site-0094	510713	8649615	Flat		
Site-0095	503226	8648573	Flat		
Site-0096	512173	8651013	Flat		
Site-0097	492777	8645643	Flat	Reef slope	
Site-0098	501110	8643419	Flat	Sand flat	
Site-0099	507957	8644464	Flat	Sand and rubble /	
Site-0100	515569	8645431	Flat		
Site-0101	495580	8647795	Flat		
Site-0102	506672	8649010	Flat		
Site-0103	504942	8645658	Flat		
Site-0104	514064	8643257	Flat		
Site-0105	498748	8647093	Flat		
Site-0106	507186	8652374	Flat	Reef flat	
Site-0107	508668	8644712	Flat		
Site-0108	513016	8648375	Flat	Lagoonal reef	
Site-0109	500452	8643853	Flat		
Site-0110	508260	8649771	Flat		
Site-0111	502407	8649381	Flat	Sand and rubble /	
Site-0112	515628	8648714	Flat		
Site-0113	493787	8647653	Flat		
Site-0114	503750	8642387	Flat		
Site-0115	509729	8641851	Flat		
Site-0116	514987	8646595	Flat		
Site-0117	499162	8645485	Flat		
Site-0118	508355	8652323	Flat		
Site-0119	509919	8647332	Flat		
Site-0120	511360	8641508	Flat		
Site-0121	501836	8645208	Flat		
Site-0122	507827	8649539	Flat		
Site-0123	500428	8648197	Flat		
Site-0124	511957	8648785	Flat		
Site-0125	500256	8641851	Flat		
Site-0126	504297	8641851	Flat		
Site-0127	502213	8650397	Flat		
Site-0128	516267	8648418	Flat	Reef flat	

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0129	502511	8641332	Flat		
Site-0130	498310	8643072	Flat		
Site-0131	507467	8641701	Flat		
Site-0132	513061	8647792	Flat		
Site-0133	497107	8646619	Flat		
Site-0134	505892	8650959	Flat		
Site-0135	505596	8647884	Flat		
Site-0136	516166	86	Flat		
Site-0137	497805	8646052	Flat		
Site-0138	510276	8652316	Flat	Lagoonal reef	
Site-0139	507599	8647206	Flat		
Site-0140	513459	8642027	Flat		
Site-0141	503497	8647979	Flat		
Site-0142	510668	8649838	Flat		
Site-0143	502507	8649977	Flat		
Site-0144	513060	8650658	Flat		
Site-0145	497179	8644021	Flat		
Site-0146	500875	8642074	Flat		
Site-0147	507638	8641684	Flat		
Site-0148	511840	8646528	Flat		
Site-0149	496976	8644890	Flat		
Site-0150	505765	8649276	Flat		
Site-0151	505008	8645937	Flat		
Site-0152	514630	8644671	Flat		
Site-0153	499817	8647659	Flat		
Site-0154	504443	8652017	Flat		
Site-0155	510039	8646183	Flat		
Site-0156	513998	8651838	Flat		
Site-0157	503746	8646257	Flat		
Site-0158	509223	8649480	Flat		
Site-0159	504044	8648203	Flat		
Site-0160	511568	8650141	Flat		
Site-0161	493842	8645964	Flat		
Site-0162	501418	8644119	Flat		
Site-0163	507952	8643528	Flat		
Site-0164	514444	8645805	Flat		
Site-0165	494290	8646947	Flat		
Site-0166	506528	8648653	Flat		
Site-0167	506598	8646477	Flat		
Site-0168	514089	8644361	Flat		
Site-0169	497988	8647000	Flat		
Site-0170	505979	8651843	Flat		
Site-0171	509053	8645687	Flat		
Site-0172	512659	8649564	Flat		
Site-0173	500607	8644164	Flat		
Site-0174	509119	8651057	Flat		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0175	501297	8649675	Flat		
Site-0176	514745	8650157	Flat		
Site-0177	494649	8644904	Flat		
Site-0178	505286	8649980	Flat		
Site-0179	509206	8642616	Flat		
Site-0180	515696	8646513	Flat		
Site-0181	500735	8645533	Flat		
Site-0182	507700	8653092	Flat		
Site-0183	510228	8646541	Flat		
Site-0184	512235	8642892	Flat		
Site-0185	501216	8646860	Flat		
Site-0186	508932	8648232	Flat		
Site-0187	499127	8648508	Flat		
Site-0188	511474	8648922	Flat		
Site-0189	499307	8642863	Flat		
Site-0190	505082	8641963	Flat		
Site-0191	510955	8645305	Flat		
Site-0192	512862	8641227	Flat	Reef crest	512841, 8641290
Site-0193	507326	8641179	Flat		
Site-0194	502866	8644538	Flat		
Site-0195	507278	8642552	Flat		
Site-0196	513498	8646511	Flat		
Site-0197	496107	8647762	Flat		
Site-0198	506481	8650127	Flat		
Site-0199	505846	8645432	Flat		
Site-0200	514492	8641615	Flat		
Site-0201	498555	8645257	Flat		
Site-0202	510295	8651840	Flat		
Site-0203	507802	8647448	Flat		
Site-0204	511244	8651613	Flat		
Site-0205	503287	8647095	Flat		
Site-0206	509801	8650664	Flat		
Site-0207	502857	8650716	Flat		
Site-0208	512235	8649910	Flat		
Site-0209	495160	8644373	Flat	Reef crest	
Site-0210	502361	8642733	Flat		
Site-0211	508922	8641941	Flat		
Site-0212	510914	8646928	Flat		
Site-0213	496227	8645495	Flat		
Site-0214	504224	8648742	Flat		
Site-0215	504905	8644925	Flat		
Site-0216	514994	8643774	Flat		
Site-0217	497630	8647594	Flat		
Site-0218	504973	8652781	Flat		
Site-0219	509964	8645473	Flat		
Site-0220	512928	8651550	Flat		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0221	503002	8645518	Flat		
Site-0221	510824	8648867	Flat		
Site-0223	503743	8649427	Flat		
Site-0223	515066	8649109	Flat		
Site-0224	493787	8646857	Flat		
Site-0225	502563	8642397	Flat		
Site-0220	510158	8641528	Flat		
Site-0228	514422	8645464	Flat		
Site-0228	496734	8648326	Flat		
Site-0223	507471	8648249	Flat		
Site-0230	506334	8647654	Flat		
Site-0231	512073	8641643	Flat		
Site-0232	501078	8645976	Flat		
Site-0233	506878	8653205	Flat		
Site-0234	508269	8645108	Flat		
Site-0235	513786	8648771	Flat		
Site-0236 Site-0237		8643606	Flat		
	499197		Flat		
Site-0238	508467	8650216			
Site-0239	502115	8648438	Flat		
Site-0240	515646	8650095	Flat		
Site-0241	495426	8646140	Flat		
Site-0242	504412	8650563	Flat		
Site-0243	504949	8647238	Flat		
Site-0244	514619	8647350	Flat		
Site-0245	499371	8646170	Flat		
Site-0246	509148	8651990	Flat		
Site-0247	509429	8646380	Flat		
Site-0248	514087	8642971	Flat		
Site-0249	501697	8646967	Flat		
Site-0250	508399	8649532	Flat		
Site-0251	500291	8649173	Flat		
Site-0252	512296	8649460	Flat		
Site-0253	500165	8642587	Flat		
Site-0254	507188	8644628	Flat		
Site-0255	512457	8646228	Flat		
Site-0256	515087	8641024	Flat		
Site-0257	501633	8641110	Flat		
Site-0258	497849	8643921	Flat		
Site-0259	504670	8644639	Flat		
Site-0260	514022	8647398	Flat		
Site-0261	495368	8644860	Flat		
Site-0262	505688	8651388	Flat		
Site-0263	505773	8646977	Flat		
Site-0264	516194	8645358	Flat		
Site-0265	498980	8645744	Flat		
Site-0266	508300	8652051	Flat		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0267	510489	8647810	Flat		
Site-0267	510489	8641940	Flat		
Site-0208	501795	8647411	Flat		
Site-0209	508033	8648269	Flat		
Site-0270 Site-0271	498561	8649133	Flat		
Site-0271 Site-0272	513659	8650126	Flat		
Site-0272	507767	8641159	Flat		
Site-0273	500939	8642870	Flat		
Site-0274	506293	8642658	Flat		
Site-0275	512441	8647546	Flat		
Site-0276	497377	8645817	Flat		
Site-0277	506828	8650249	Flat		
Site-0279	507290	8645088	Flat Flat		
Site-0280 Site-0281	514905	8642592	Flat		
	500050	8647975			
Site-0282	505115	8652241	Flat		
Site-0283	509735	8645956	Flat		
Site-0284	512377	8651473	Flat		
Site-0285	503266	8647010	Flat		
Site-0286	509896	8648353	Flat		
Site-0287	503999	8650096	Flat		
Site-0288	511073	8650777	Flat		
Site-0289	493843	8645033	Flat		
Site-0290	501343	8643025	Flat		
Site-0291	507667	8642922	Flat		
Site-0292	512935	8646182	Flat		
Site-0293	494943	8647423	Flat		
Site-0294	505200	8648750	Flat		
Site-0295	504706	8646275	Flat		
Site-0296	515175	8643696	Flat		
Site-0297	498368	8647829	Flat		
Site-0298	506659	8651528	Flat		
Site-0299	507601	8646198	Flat		
Site-0300	513743	8649231	Flat		
Site-0301	503830	8645236	Flat		
Site-0302	507531	8651108	Flat		
Site-0303	503207	8649112	Flat		
Site-0304	514505	8649156	Flat		
Site-0305	492945	8647118	Flat		
Site-0306	503917	8641542	Flat		
Site-0307	510362	8642411	Flat		
Site-0308	515586	8647672	Flat		
Site-0309	500243	8646046	Flat		
Site-0310	506589	8649279	Flat		
Site-0311	506942	8647764	Flat		
Site-0312	511418	8642399	Flat		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-0313	501661	8644736	Flat		
Site-0314	505872	8653551	Flat		
Site-0315	499175	8649561	Flat		
Site-0316	512130	8648511	Flat		
Site-0317	499751	8644226	Flat		
Site-0318	504714	8642156	Flat		
Site-0319	501307	8650223	Flat		
Site-0320	514884	8650990	Flat		
Site-0321	500957	8641168	Flat	Reef flat	
Site-0322	498880	8644426	Flat		
Site-0323	506286	8641673	Flat		
Site-0324	512901	8647104	Flat		
Site-0325	497058	8647281	Flat		
Site-0326	504197	8651260	Flat		
Site-0327	505613	8647405	Flat		
Site-0328	516075	8646527	Flat		
Site-0329	497668	8645662	Flat		
Site-0330	509215	8652089	Flat		
Site-0331	509131	8646400	Flat		
Site-0332	514171	8641884	Flat		
Site-0333	500991	8648016	Flat		
Site-0334	509302	8649996	Flat		
Site-0335	498778	8648825	Flat		
Site-0336	513121	8650562	Flat		
Site-1345	492487	8647326	Edge N		
Site-1346	500564	8650111	Edge N	Reef flat	
Site-1347	514136	8652319	Edge N	Reef flat	
Site-1348	505704	8653638	Edge N	Reef flat	
Site-1349	493058	8647631	Edge N	Reef crest	493027, 8647679
Site-1350	498557	8649330	Edge N		,
Site-1351	503968	8652103	Edge N		
Site-1352	501568	8650465	Edge N		
Site-1353	497766	8648908	Edge N	Reef crest	497716, 8649037
Site-1354	510263	8652956	Edge N	Sand and rubble /	510250, 8652884
Site-1355	502920	8650999	Edge N	·····,	
Site-1356	516874	8647029	Edge N	Reef flat	
Site-1357	496050	8648357	Edge N		
Site-1358	512220	8652649	Edge N	Reef crest	511840, 8652649
Site-1359	507243	8653759	Edge N		,
Site-1360	516624	8647733	Edge N	Reef flat	
Site-1361	492641	8647457	Edge N		
Site-1362	500821	8650170	Edge N		
Site-1363	514747	8651897	Edge N	Reef slope	
Site-1364	506315	8653961	Edge N		
			0-11	Į	
Site-1365	491919	8646748	Edge N		

Site ID	x coordinate	y coordinate	Stratum	Habitat type	New coordinates
Site-1367	503682	8651937	Edge N		
Site-1368	515006	8651604	Edge N	Reef flat	
Site-1441	495349	8644132	Edge S	Reef slope	495430, 8644142
Site-1442	507421	8640851	Edge S	Reef slope	507416, 8640959
Site-1443	510913	8640970	Edge S	Reef slope	510950, 8641125
Site-1444	499944	8641681	Edge S		
Site-1445	493854	8644797	Edge S		
Site-1446	506118	8641015	Edge S		
Site-1447	509750	8641327	Edge S	Reef slope	509714, 8641421
Site-1448	515338	8642223	Edge S		
Site-1449	492741	8645525	Edge S		
Site-1450	513598	8640501	Edge S	Reef crest	
Site-1451	497247	8643266	Edge S	Reef crest	497343, 8643408
Site-1452	515310	8642773	Edge S		
Site-1453	501696	8640637	Edge S	Reef crest	501770, 8640712
Site-1454	512101	8640980	Edge S	Reef slope	512084, 8641085
Site-1455	499665	8641893	Edge S		
Site-1456	516000	8644041	Edge S	Reef flat	
Site-1457	494886	8644281	Edge S		
Site-1458	508099	8640897	Edge S		
Site-1459	511326	8640983	Edge S		
Site-1460	500541	8641351	Edge S		
Site-1461	494244	8644595	Edge S		
Site-1462	505610	8640862	Edge S		
Site-1463	508328	8640977	Edge S		
Site-1464	515400	8641447	Edge S		