

A Tale of Two Urchins - Implications for In-Situ Breeding of the Endangered Banggai Cardinalfish (*Pterapogon kauderni*)

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Abstract

Samliok Ndobe, Jamaluddin Jompa and Abigail Moore. 2018. A Tale of Two Urchins - Implications for In-Situ Breeding of the Endangered Banggai Cardinalfish (*Pterapogon kauderni*). *Aquacultura Indonesiana*, 19 (2): 65-75. The endangered Banggai cardinalfish *Pterapogon kauderni*, endemic to the Banggai Archipelago in Central Sulawesi, Indonesia, is a national and global priority conservation species. To support stock recovery based on *in-situ* breeding, using the symbiosis between the Banggai cardinalfish and its microhabitat (especially urchins of Genus *Diadema*), specific research objectives were: (i) identify the *Diadema* species associated with Banggai cardinalfish in the wild; (ii) investigate Banggai cardinalfish preference between these *Diadema* species. Belt transect data (5 sites) found wild Banggai cardinalfish of all size classes associated with *Diadema setosum* and *Diadema savignyi*. Preference trials were conducted in a controlled environment (concrete tanks) with three replicates. Nine sub-adult Banggai cardinalfish (35-42 mm SL), 12 *D. setosum* and 12 *D. savignyi* were placed in each tank. Banggai cardinalfish association (*D. savignyi*, *D. setosum*, none) was recorded hourly (06:00-18:00) for three days and results analysed in RStudio-1.0.143. Banggai cardinalfish did not show significant preference for either *D. savignyi* or *D. setosum*. These results indicate that *D. savignyi* and *D. setosum* can be used impartially in further research on *in-situ* breeding to facilitate recovery of Banggai cardinalfish stocks. However stock recovery measures should consider genetic connectivity and the natural balance between the two urchin species.

Keywords: Banggai cardinalfish; *Diadema*; In-situ breeding; Microhabitat preference; Stock recovery; Symbiosis

Introduction

There is no longer any reasonable doubt that humanity is radically changing the biosphere in which we all live, to the extent that many scientists believe we are now in a new geological era, the Anthropocene or Age of Mankind (Crutzen, 2002; Steffen *et al.*, 2011; Zalasiewicz *et al.*, 2011; Barnosky, 2014), an age where "humans are becoming the dominant force of change on earth" (Schwägerl, 2014). As a species, we are altering the chemical composition of both our atmosphere and oceans, bringing changes in climate and threats to ecosystem integrity, including key marine and coastal ecosystems (Ellis, 2011; IPCC, 2014; Hoegh-Guldberg *et al.*, 2017). In particular, there is strong scientific consensus that anthropogenic activities are responsible for a growing number of species extinctions, to the point where many consider that we are both causing and witnessing the 6th major extinction event in the history of life on earth (Dirzo *et al.*, 2014), especially life in the seas (Jackson, 2008). Major shifts in marine fauna species ranges and community composition have begun, and especially

high extirpation (local/regional extinction) rates are predicted in the Indo-Pacific (Cheung *et al.*, 2009; Jones and Cheung, 2014; Molinos *et al.*, 2016).

Initiatives at all levels (genetic, species, ecosystem, ecoregion, etc.) based on sound science are vital to reduce the rate of biodiversity loss and the negative effects upon human beings and the environment (Hoegh-Guldberg *et al.*, 2009; Pendleton *et al.*, 2016). In this context, aquaculture, as "the farming of aquatic organisms [with] some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc." (FAO, 1988), can and should play a significant role in supporting biodiversity conservation efforts. In particular, through both *ex-situ* and *in-situ* research and interventions designed to maintain or assist in the recovery of threatened species, including the integrity of the biotic communities and ecosystems on which they depend.

One species considered at risk of extinction is the Banggai cardinalfish (*Pterapogon kauderni*), listed as Endangered in the IUCN Red List (Allen and Donaldson, 2007) and a conservation priority

species at national (MMAF, 2015) and international (Conant, 2015; CITES, 2016a; CITES, 2016b) levels. This small shallow-water paternal mouth-brooding fish with direct development has an endemic distribution of around 5000 km² and habitat of around 30-34 km² (Vagelli, 2008; Vagelli, 2011). The Banggai Archipelago, Central Sulawesi Province, Indonesia, comprises over 90% of the Banggai cardinalfish endemic range and populations, including 18 of 21 reproductively isolated stocks inferred based on DNA analyses (Bernardi and Vagelli, 2004; Hoffman *et al.*, 2005; Vagelli *et al.*, 2009; Ndobe, 2013; Moore *et al.*, 2017a). Banggai cardinalfish associate with benthic organisms which serve as microhabitat; despite strong empirical evidence for an ontogenetic shift in microhabitat (Vagelli, 2004; Ndobe *et al.*, 2008), they remain sedentary and strongly site-attached (Kolm *et al.*, 2005). Banggai cardinalfish population abundance and age structure (parameters indicative of reproductive success) are strongly correlated with the abundance of key microhabitat, in particular sea anemones and *Diadema* sp. urchins (Ndobe *et al.*, 2005; Ndobe *et al.*, 2013a; Ndobe *et al.*, 2013b; Ndobe *et al.*, 2013c; Ndobe *et al.*, 2013d; Moore *et al.*, 2011; Moore *et al.*, 2012).

Urchins of the Genus *Diadema* are not only important as microhabitat for other organisms, including the Banggai cardinalfish, but also for their wider ecological role in coastal ecosystems (Macia *et al.*, 2007; Muthiga and McClanahan, 2013; Precht and Precht, 2015). In particular, these urchins are considered as key herbivores which can promote or help maintain coral reef resilience under stresses such as eutrophication, overfishing and climate change related coral bleaching (Carpenter and Edmunds, 2006; Mumby *et al.*, 2006; Mumby *et al.*, 2007). However the past decade has seen a sharp decline in the populations of *Diadema* urchins across much of the Banggai Archipelago, apparently due to increased exploitation for human consumption and as feed for carnivorous reef fish, with a concurrent decline in (both fished and unfished) Banggai cardinalfish stocks (Moore *et al.*, 2012; Moore *et al.*, 2017a; Moore *et al.*, 2017b; Ndobe *et al.*, 2017). This situation calls for an integrated approach to the conservation of the Banggai cardinalfish and its microhabitat (symbionts). One such is the "BCF Gardens" concept (Ndobe *et al.*, 2013d), based on *in-situ* breeding (Ndobe and Moore, 2005), using the symbiosis between the Banggai cardinalfish and its microhabitat

(especially *Diadema* sp. urchins and sea anemones) to increase reproductive success through reduced predation, including a reduction in cannibalism of recruits (Ndobe *et al.*, 2013c).

In this context, it is important to identify microhabitat (symbiont) taxa to species level. Studies on the Banggai cardinalfish and its microhabitat highlight the importance of the symbiosis between Banggai cardinalfish and *Diadema* sp., however which species is not clear. Most studies on Banggai cardinalfish association with urchin microhabitat have recorded data at the genus level (e.g. Lunn and Moreau, 2004; Ndobe *et al.*, 2005; Ndobe *et al.*, 2008; Ndobe *et al.*, 2013a; Ndobe *et al.*, 2013b; Ndobe *et al.*, 2013c; Ndobe *et al.*, 2013d; Moore *et al.*, 2011; Moore *et al.*, 2012). While some publications mention *Diadema setosum* (e.g. Vagelli and Erdmann, 2002; Vagelli, 2004, Kolm and Berglund, 2003), studies on *Diadema* sp. phylogeography and species distribution indicate that at least two species, *Diadema setosum* and *Diadema savignyi*, could be present in the Banggai Archipelago (Pearse, 1998; Lessios *et al.*, 2001). There was thus a gap in knowledge regarding the identity and number of *Diadema* urchin species present, as well as their relative abundance and respective level of use as microhabitat by endemic Banggai cardinalfish populations.

This research was performed under a multi-year study on the symbiosis between the Banggai cardinalfish and *Diadema* sp. urchins. The specific objectives were: (i) to identify the *Diadema* species associated with endemic Banggai cardinalfish populations in the wild; (ii) to evaluate Banggai cardinalfish preference between the *Diadema* species found to be present.

Materials and Methods

The research was conducted during May-July 2017. A field study (survey) was conducted within the endemic range of the Banggai cardinalfish in the Banggai Archipelago, Central Sulawesi Province, Indonesia. At each of 5 sites in the Banggai Archipelago (Figure 1), 10 belt transects (20 m x 5 m, following Ndobe *et al.*, 2008) were randomly placed in known Banggai cardinalfish habitat, comprising a cumulative area of 1000 m²/site. Experimental research was conducted at the Hasanuddin University Marine Station Hatchery on Barranglompo Island, South Sulawesi Province, Indonesia.

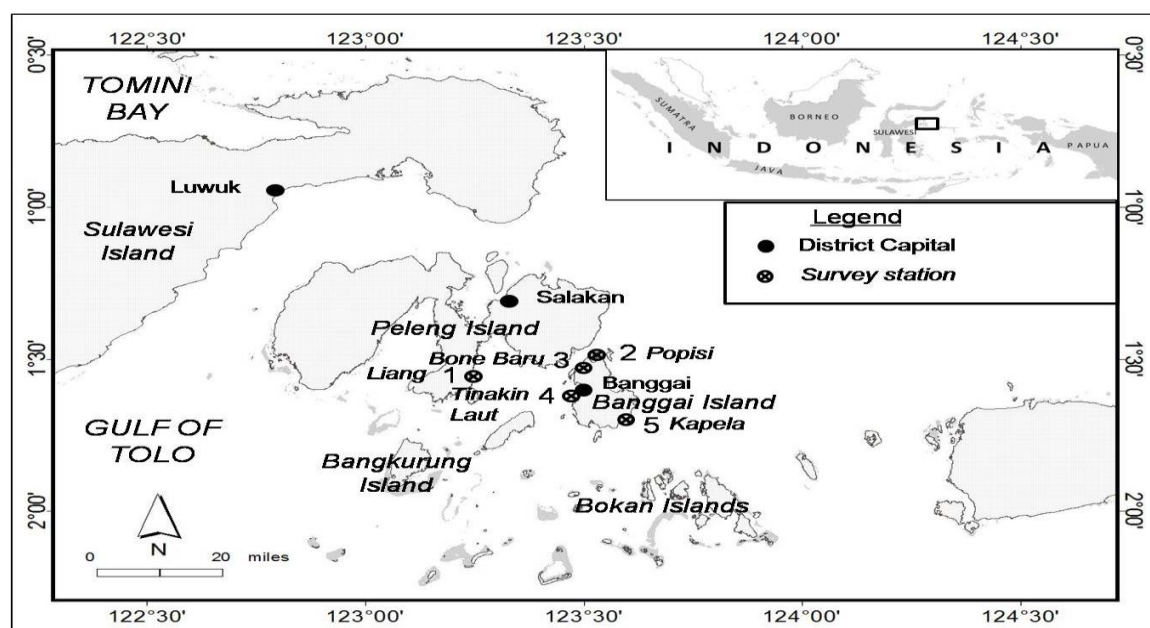


Figure 1. Map of the Banggai Archipelago showing the 5 survey sites (1 Liang, 2 Popisi, 3 Bone Baru, 4 Tinakin Laut, 5 Kapela)

***Diadema* sp. Identification, Distribution and Relative Abundance**

For each group of *Diadema* sp. within each transect, the number of individual urchins was recorded by species and size. The two size classes used were: (i) large, possibly mature/adult individuals (test diameter > 30 mm); and (ii) small, clearly immature juvenile urchins (test diameter < 30 mm). The 30 mm limit was chosen based on the (minimal) data available on *Diadema* size, growth and maturity reviewed in Muthiga and McClanahan (2007). Identification of *Diadema* species was based mainly on Chow et al. (2014) and Chow et al. (2016). Individuals were classified using the following external characters: (i) *Diadema setosum*: orange ring on the anal cone, with five white spots and dotted blue iridophore lines in the naked space of the interambulacral areas; (ii) *Diadema savignyi*: Y-shaped continuous (at least partially double) blue iridophore lines running along the naked space of the interambulacral areas and no orange ring on the anal cone; (iii) undetermined/possible hybrid: any combination other than (i) or (ii).

***Banggai* Cardinalfish Association with *Diadema* sp. in The Wild**

The number of Banggai cardinalfish within each transect was recorded by microhabitat association and by size class (recruits/small juveniles < 25 mm, large juveniles/sub-adults 25-

42 mm, adults > 42 mm) based on standard length (SL). These size classes were based on previous research (Ndobe et al., 2013a; Ndobe et al., 2013b; Ndobe et al., 2013c). For *Diadema* sp., Banggai cardinalfish associated with each group of urchins were recorded separately. For all biotic microhabitats, the genus (and, where known, the species) was recorded, while for abiotic microhabitat a brief description was recorded.

***Banggai* Cardinalfish Preference Trials**

Wild-caught Banggai cardinalfish from the Palu Bay introduced population (Moore and Ndobe, 2007) were air-freighted to Makassar, transported to the Field Station, and acclimated (without *Diadema* microhabitat) before the trials were conducted. Live feed (*Artemia salina*) was provided twice daily (07:00-0:800 and 16:00-17:00 WITA, GMT+8) during the acclimation and trial periods. The trials were conducted in concrete tanks (area ≈ 1.5 x 1.7 m, water depth ≈ 0.5m) supplied with filtered seawater using a continuous throughflow system. Sub-adult (35-42 mm SL) Banggai cardinalfish (9 individuals) and equal numbers (12 each) of the two *Diadema* species identified in the field (*D. setosum* and *D. savignyi*) were placed in each of three replicate trial tanks. The association of each fish (*D. savignyi*, *D. setosum*, none) was recorded at hourly intervals (from 06:00 to 18:00 WITA, GMT+8) for three days.

Data Analysis

The data were tabulated in Microsoft Excel 2007, and all statistical analyses were conducted in RStudio-1.0.143. Qualitative observations were analysed descriptively. The results were compared with other studies and evaluated in the context of *P. kauderni* conservation and implications for aquaculture, in particular for *in-situ* breeding of the Banggai cardinalfish (Ndobe & Moore, 2005) and the "BCF Gardens" concept (Ndobe & Moore, 2013d; Moore and Ndobe, 2017a).

Results

The field survey results in Table 1 show that urchins exhibiting the characteristics of two species, *Diadema setosum* and *Diadema savignyi*, were observed at all sites. In addition, 36 undetermined individuals exhibiting some characteristics of both species (possible hybrids) were observed. Overall, *D. setosum* was more abundant than *D. savignyi*, however the majority (64%) of groups observed comprised both species, while only 8 out of 167 groups contained *D. savignyi* alone.

Table 1. Species composition, abundance and size class of *Diadema* sp. (N = 2964)

Parameter	Unit	Survey Site					
		Liang	Popisi	Bone Baru	Tinakin Laut	Kapela	Total
Transects with <i>Diadema</i>	%	50	70	70	100	70	72
Total <i>Diadema</i> density	ind/m ²	0.217	0.503	0.299	0.912	1.033	0.593
Juvenile <i>Diadema</i> (diameter < 30 mm)	%	24.0	9.3	12.0	38.7	17.4	22.5
Potentially adult <i>Diadema</i> (diameter ≥ 30 mm)	%	76.0	90.7	88.0	61.3	82.6	77.5
<i>Diadema savignyi</i>	n	12	118	63	135	95	423
	%	5.53	23.46	21.07	14.80	9.20	14.27
<i>Diadema setosum</i>	n	205	385	236	777	938	2541
	%	94.47	76.54	78.93	85.20	90.80	85.73
Suspected hybrids	n	5	5	5	9	12	36
	%	2.25	0.98	1.64	0.98	1.15	1.20

The observed associations of Banggai cardinalfish with the two *Diadema* urchin species and other microhabitat types are shown in Table 2. All three Banggai cardinalfish size classes (from recent recruits to adults) were observed associated with mono-species groups of each *Diadema* species as well as (predominantly) mixed species groups (Figure 2a). Banggai cardinalfish association with microhabitat types other than the two *Diadema* species differed between age/size groups. Recruits and (mostly smaller) juveniles were associated with other sea urchins of the Family Diadematidae (*Echinothrix* sp. and *Astropyga radiata*), sea anemones (*Entacmea quadricolor*, *Heteractis crista*, *Actinodendron* sp., *Heteractis magnifica*, *Stichodactyla gigantea*), hard corals of the genus *Heliofungia*, and the upside-down jellyfish (*Cassiopea* sp.). Adult Banggai cardinalfish were commonly associated with corals, mostly branching forms. Recorded microhabitat organisms included 8 hard coral genera (*Acropora*, *Seriatopora*, *Stylophora*, *Goniopora*, *Echinopora*, *Hydnophora*, *Heliofungia*, *Porites*), branching and foliose forms of fire coral (*Millepora* sp.),

soft corals (*Sinularia* sp.), branching sponges, and seagrass (*Enhalus acardoides*). At two sites (Tinakin Laut and under the pier at Kapela), Banggai cardinalfish were observed (possibly temporarily) associated with abiotic microhabitat, including manmade structures and marine debris.

The Banggai cardinalfish preference trial results are summarised in Table 3. An example of a typical observation is shown in Figure 2b. There was no mortality of experimental animals during the trial period. The urchin groupings and Banggai cardinalfish associations varied throughout the observation period, however there was no significant difference between the number of Banggai cardinalfish associated with each urchin species overall, between days, or for any of the 3 replicates ($p > 0.05$). On average (\pm standard deviation), $42.22\% \pm 10.03\%$ were associated with *Diadema savignyi*, $42.03\% \pm 10.19\%$ with *D. setosum* and $15.75\% \pm 13.47\%$ with neither. Banggai cardinalfish not associated with either urchin species were either actively feeding (45%), moving through the water but not obviously feeding (34%), or stationary, usually in a corner of the tank (21%).

Table 2. Observed Banggai cardinalfish (BCF) microhabitat use (N = 4259)

Parameter	Unit	Survey Site					Total
		Liang	Popisi	Bone Baru	Tinakin Laut	Kapela	
Transects with BCF	%	50	70	70	100	70	72
Total BCF density	ind/m ²	0.266	1.174	1.186	0.924	0.709	0.8518
BCF recruits/small juveniles (< 25 mm SL)	%	9.02	11.07	20.40	13.31	10.16	13.88
Juvenile/sub-adult BCF (25-42 mm SL)	%	34.96	25.21	39.88	42.75	20.17	32.87
Adult BCF (> 42 mm SL)	%	56.02	63.71	39.71	43.94	69.68	53.25
BCF in <i>Diadema</i> sp. (DD) microhabitat							
Proportion of all BCF	%	46.24	79.81	23.95	77.81	10.30	50.15
BCF recruits/small juveniles (< 25 mm SL)	%	2.44	8.75	21.13	4.87	47.95	10.07
Juvenile/sub-adult BCF (25-42 mm SL)	%	57.72	19.21	43.66	45.76	15.07	33.47
Adult BCF (> 42 mm SL)	%	39.84	72.04	35.21	49.37	36.99	56.46
Main other microhabitat types (X = observed, - = not observed)							No. sites
<i>Echinothrix</i> sp.		X	X	X	X	X	5
<i>Astropyga radiata</i>		-	-	X	-	-	1
<i>Entacmea quadricolor</i>		X	-	X	X	X	4
<i>Heteractis crispata</i>		-	-	X	-	X	-
<i>Actinodendron</i> sp.		-	X	X	X	-	3
Other sea anemones		-	-	X	-	-	1
<i>Acropora</i> sp. (branching forms)		X	X	X	X	-	4
<i>Seriatopora/Stylophora</i> sp.		-	X	X	-	-	2
<i>Porites</i> sp. (branching forms)		X	X	-	-	-	2
Other branching corals		X	-	-	-	-	1
<i>Goniopora</i> sp. (semi-massive)		X	X	X	-	-	3
<i>Heliofungia</i> sp.		X	-	-	-	X	2
<i>Sinularia</i> sp. soft coral		X	-	X	-	-	1
Branching sponges		-	X	-	-	X	2
<i>Cassiopea</i> sp. jellyfish		-	-	-	X	-	1
<i>Enhalus acaroides</i>		-	X	-	X	-	2
Abiotic substrate/structures		-	-	-	X	X	2

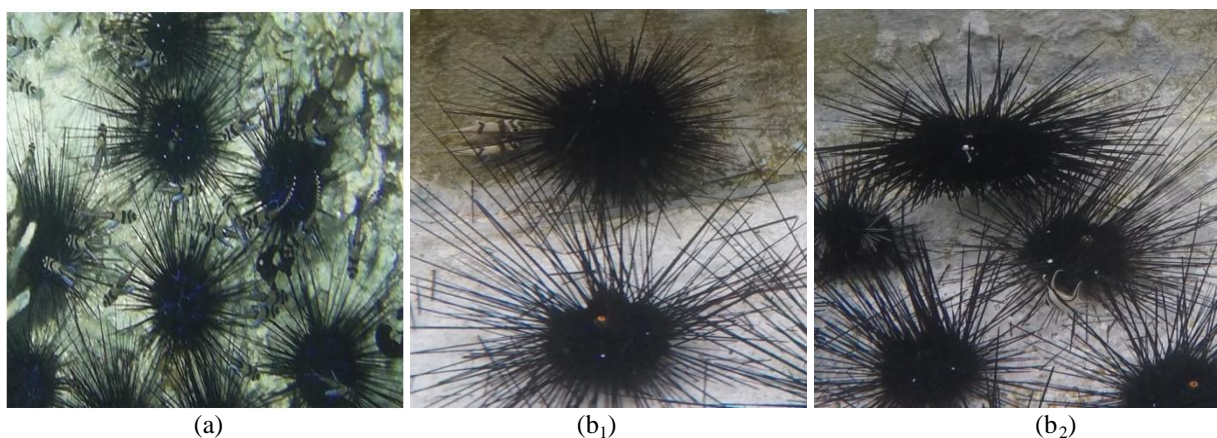


Figure 2. Banggai cardinalfish associated with two *Diadema* species: a. in the wild, at Bone Baru and b. during the preference trials (b₁, 2 fish associated with *Diadema savignyi*; b₂, 1 fish associated with *Diadema setosum*).

Table 3. Banggai cardinalfish *Diadema* sp. preference trial results.

Period	Proportion (% \pm SD) of Banggai cardinalfish associated with		
	<i>Diadema savignyi</i>	<i>Diadema setosum</i>	None
Trial (day 1-3)	3.80 \pm 0.90	3.79 \pm 0.92	1.42 \pm 1.21
08:00	3.22 \pm 0.67	3.11 \pm 0.60	2.67 \pm 1.12

¹ The linear model (lm function) analysis implemented in R did not reveal any significant difference between days or between replicates ($P > 0.05$); however there was a significant difference between 08:00 observations and all other time periods (06:00-0:700 and 09:00-18:00)

Table 4. Some implications of the study results for Banggai cardinalfish (BCF) conservation, including the potential role of aquaculture.

No.	Aspect	Implications	Recommendation
1	Genetic biodiversity and connectivity	<ul style="list-style-type: none"> <i>Diadema</i> urchins (wild caught or captive breeding) for re-stocking should be from same genetic stock as those at target site 	<ul style="list-style-type: none"> Research on <i>Diadema</i> sp. genetic connectivity/stocks Careful selection of <i>Diadema</i> sp. broodstock
2	Species diversity (<i>Diadema</i>)	<ul style="list-style-type: none"> <i>Diadema</i> population species composition varies between sites and within sites (e.g. between habitat types) Interventions should avoid causing major shifts in species balance 	<ul style="list-style-type: none"> Research on the distribution of each <i>Diadema</i> sp. at multiple scales and across habitat types Need to develop breeding and release protocols for each species (<i>D. savignyi</i> & <i>D. setosum</i>)
3	Ecosystem health, diversity and balance	<ul style="list-style-type: none"> <i>Diadema</i> urchins are known to play important ecological roles which are density dependent; however knowledge on the roles of <i>D. savignyi</i> and <i>D. setosum</i> is limited and contradictory 	<ul style="list-style-type: none"> Research on the ecological roles of each <i>Diadema</i> species and density effects (including carrying capacity) in the context of ecosystem health and resilience, etc.
4	Research on the BCF - <i>Diadema</i> symbiosis	<ul style="list-style-type: none"> BCF do not appear to exhibit preference or different behaviour with the two <i>Diadema</i> species Each <i>Diadema</i> species will have different habitat niches and most likely respond differently to environmental cues and changes, whether natural or experimental 	<ul style="list-style-type: none"> Use of "samples of convenience" (locally abundant urchins) for some research (e.g. many BCF behavioural studies) Species will be important where environmental variables are a factor (e.g. studies on climate change/ocean acidification)

Discussion

The results show that at least two sea urchin species of the Genus *Diadema* are present in the Banggai Archipelago. As reported by De Beer (1990) in the Spermonde Archipelago and Pearse (1998) in North Sulawesi and the Sangihe Islands, the relative abundance of the two species varies between sites. These variations could reflect the influence of intrinsic factors (e.g. reproductive biology and larval dispersal patterns), external factors (e.g. levels of pollution and/or exposure to wave action and currents), and stochastic factors. *Diadema* urchins are heavily exploited in the Banggai Archipelago (Moore *et*

al., 2012; Ndobe *et al.*, 2017). It is not known whether the fishers or consumers recognise or have any preference between *Diadema* species; however it is possible that, in addition to overall abundance, the relative abundance of each species could, at least to some extent, be affected by *Diadema* exploitation patterns.

Both De Beer (1990) and Pearse (1998) found that relative abundance appeared to be related to environmental conditions. *D. setosum* seemed to be more common than *D. savignyi* at sites with higher levels of terrestrial or anthropogenic influence, particularly sedimentation, eutrophication, and other forms of pollution. Conversely, *D. savignyi* has been reported as

more common at sites further from large islands or human settlements, typically with clearer waters, and often more exposed to wave action. The seemingly greater ability of *D. savignyi* to cope with relatively violent water movement is possibly related to the finding by Muthiga (2003) that *D. savignyi* can outcompete *D. setosum* in securing protective microhabitat such as crevices within or between coral colonies and rocks. In addition, Coppard and Campbell (2007) report differences in grazing preferences between Diadematid urchins, including the two species in this study. Thus, it seems likely that environmental factors may be important in determining *Diadema* urchin community composition in the Banggai Archipelago.

The presence of apparent hybrids between the two species is consonant with reports from other areas in the Indo-Pacific (Lessios and Pearse, 1996; Lessios 2007). Some studies, based on morphometric (DNA) and genetic analyses, indicate that external morphology is not always a reliable indication of species (Pearse, 1970; Pearse, pers. comm., 2016), and that hybrids can be fertile as backcrosses have been identified (Lessios and Pearse, 1996). Although phylogeographic connectivity of *Diadema* sp. has been studied over the wider Indo-Pacific area, including Malaysia, Philippines, Australia and Papua New Guinea (Lessios et al., 2001), to date there are no data from Indonesia, at the centre of the Coral Triangle marine biodiversity "hotspot". Genetic (DNA) studies to fill this gap could provide valuable information on connectivity and help inform ecosystem-based conservation of the Banggai cardinalfish and its habitat, including efforts to assist the recovery of *Diadema* stocks, in particular through the development of captive breeding of *Diadema* sp. urchins.

The high level (84.25%) of Banggai cardinalfish association with *Diadema* sp. throughout the preference trial, in the absence of any threat of predation, reinforces the importance of this symbiosis. The lack of any consistent preference for either *D. setosum* or *D. savignyi* when equal numbers of both species were present indicates that, at least under current environmental conditions, the species of *Diadema* present is unlikely to significantly affect Banggai cardinalfish conservation outcomes. It also indicates that many behavioural studies to further elucidate the symbiosis between the Banggai cardinalfish and *Diadema* could be carried out using whichever urchin species was more convenient, e.g. through local abundance or

greater adaptability to a given controlled environment. However a note of caution is felt necessary regarding studies in the wild or where environmental variables are a key part of the study (e.g. climate change and ocean acidification impact studies). Based on empirical (field) studies on *Diadema* species (De Beer, 1990; Pearse, 1998) as well as experimental research on sea urchins more generally (e.g. Brennand et al., 2010; Dorey et al., 2013; Sherman, 2015), the responses of the two *Diadema* species to environmental change are likely to have significant (mostly negative) consequences for fitness, and could be very different from one another.

The lack of significant difference between the observations by day or replicate strengthens the validity of the result. The significant difference in association at 08:00 compared to other times of day was probably related to the time of first daily feed, between 07:00 and 08:00. Throughout the experiment, individuals not associated with *Diadema* sp. urchins tended to exhibit typical feeding behaviour, i.e. short bursts of speed accompanied with mouth opening and closing, including 83% of individuals observed during the 08:00 time period. The percentage was lower (typically around 50%) at other times of day, including after the afternoon feed (between 16:00 and 17:00). Non-feeding unassociated fish were either swimming actively (possibly moving between con-specific groups or urchin clumps) or sedentary in a corner of the tank. Reasons for the higher feeding activity after the morning feed could include greater hunger after a night with little or no feeding opportunity, while some feeding on the remaining (live) morning feed probably occurred throughout much of the day. Thus it is logical that the afternoon feed would not elicit as large a response in terms of concentrated active feeding behaviour as the morning feed.

While the survey and experimental results do not indicate any preference by the Banggai cardinalfish for either sea urchin species, the presence of both *Diadema* species at all survey sites has important implications for many aspects of genetic, species and ecosystem conservation, including interventions where aquaculture could or should play a role. Lessons learned from the (still experimental) culture and release to the wild of *D. antillarum* in the Caribbean (Moe, 2010; Moe pers.com. 2015) could inform such processes in the Indo-Pacific, and in particular the Banggai Archipelago. Aspects of particular

concern in this context include biodiversity at inter- and intra-species levels and connectivity. Table 4 presents a brief overview of some implications of this study for biodiversity conservation, including conservation-oriented aquaculture.

In conclusion, this study shows that two species of the Genus *Diadema* (*Diadema savignyi* and *Diadema setosum*) are important as microhabitat (symbionts) of the endangered Banggai cardinalfish (*Pterapogon kauderni*) within its endemic range. The experimental observations showed no significant preference by Banggai cardinalfish for either *D. savignyi* or *D. setosum*. These results indicate that *D. savignyi* and *D. setosum* could be used impartially in further research on *in-situ* breeding to facilitate the recovery of Banggai cardinalfish stocks. However, any *Diadema* sp. stock recovery measures should take genetic connectivity and structure into account and, furthermore, should aim to maintain the balance between the two urchin species.

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