Dietary preference of key microhabitat *Diadema setosum*: a step towards holistic Banggai cardinalfish conservation

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Abstract. The sea urchins *Diadema setosum* and *D. savignyi* are key symbionts of the Banggai cardinalfish *Pterapogon kauderni*, a species of global and national conservation concern. There is growing interest in *Diadema* propagation for conservation and human consumption; however little is known about the dietary preferences of Indo Pacific *Diadema* species. This study explored the feeding preference of *Diadema setosum* offered a choice of two macroalgae (*Ulva reticulata*, *Gracilaria* sp.) and two seagrasses (*Thalassia hemprichii*, *Enhalus acoroides*). Portions (5g) of the four feeds were attached to the corners of rectangular plastic cages and one *D. setosum* released in the centre of each cage. Uneaten feed was weighed after 24 hours. This procedure was repeated for 5 days with 8 replicates. Mean daily feed consumption/urchin was 4.31g. All urchins ate a varied diet comprising all four feed types, with a significant preference (p < 0.01) for macroalgae (on average 80%). Our results point to an important role of *D. setosum* in macroalgal control; however this urchin might graze on cultivated seaweeds. Further research avenues include the use of macroalgae in prepared feeds for *Diadema* culture, including in the context of *P. kauderni* microhabitat rehabilitation.

1. Introduction

Sea urchins of the genus *Diadema* play important ecological roles as herbivores [1,2,3] and as symbionts of other organisms [4]. In particular, Diadematisid urchins are key symbionts of the Banggai cardinalfish *Pterapogon kauderni* [5,6,7,8], in particular *Diadema setosum* and *D. savignyi* [9]. A species of global and national conservation concern, *P. kauderni* is threatened by overexploitation and habitat degradation [8,9,10,11,12,13,14]. The Banggai cardinalfish is one of four conservation management priorities of the recently declared Banggai Dalaka MPA [15], which comprises over 90% of endemic *P. kauderni* populations and habitat [7,15]. While sea anemones are considered important as microhabitat for juvenile *P. kauderni* and hard corals provide habitat for many adult *P. kauderni*, all over half of all *P.
that (including all age/size classes) associate with Diadema urchins as microhabitat
[6,7,8,9,12,13,16,17]. However, at many P. kauderni population sites within the MPA, the abundance
of Diadema urchins has declined drastically (by an order of magnitude) over the past decade, largely
due to overharvesting for human consumption [12,13,14,15].

Restoring Diadema populations is a key factor in rebuilding P. kauderni populations
[6,9,12,13,14,15]. However, although D. setosum is known to eat a variety of seagrasses and algae
[18,19], there are still many gaps in our knowledge regarding the ecology, and in particular the dietary
preference, of this Indo Pacific Diadema species.

Flocks of D. setosum feeding on a seasonal bloom of Ulva reticulata were observed on the reef flat
of Barrangcad Island, Spermonde Archipelago, while collecting seagrasses as feed for D. setosum kept
in the laboratory during a study on the symbiosis between P. kauderni and Diadema urchins and the
possible effects of climate change on these organisms. This observation prompted opportunistic
harvesting of Ulva, which proved palatable as an alternative feed for D. setosum, as did several other
macroalgae entangled in the Ulva thalli collected. However these qualitative (unquantified) field and
laboratory observations of D. setosum feeding on macroalgae (seaweeds) and seagrasses did not provide
information on feeding rates or preference. To further elucidate D. setosum feeding patterns and
preferences, this study used a quantitative experimental approach to explore the dietary preferences of
Diadema setosum offered a choice of four feeds, all of which had been observed to be palatable to
(consumed by) D. setosum, comprising two macroalgae (Ulva reticulata and Gracilaria sp.) and two
seagrasses (Thalassia hemprichii and Enhalus acoroides).

2. Methods

The experimental research (feed preference trials) took place at the Multitrophic Research Group
(MTRG) Laboratory of the Center of Excellence for Development and Utilization of Seaweed (CEDUS),
Universitas Hasanuddin, Makassar, Indonesia. With the exception of Gracilaria sp., which was sourced
from Ujung Baji village in the Takalar District of South Sulawesi, experimental organisms were
collected from the Spermonde Archipelago, South Sulawesi, Indonesia. Diadema setosum sea urchins
(test diameter close to 5 cm) were obtained from Barranglompo Island. The two seagrass species
Thalassia hemprichii and Enhalus acoroides were collected from seagrass meadows on the east side of
Barranglompo Island, Spermonde Archipelago. The seaweed Ulva reticulata was collected from the reef
flat of Barangcad Island, Spermonde Archipelago. Eight D. setosum urchins were acclimated to
conditions similar to the feed preference trial tank for 5 days. Seaweeds and seagrasses were kept in
aerated seawater, confined to floating cages in a tank similar to the trial tank.

The trial comprised eight experimental units (replicates), each consisting of a rectangular plastic cage
(50 x 30cm², depth 15cm), floated in a 100cm x 200cm tank filled with clean, filtered seawater (Figure
1). To prevent the egress of feed, the exterior of each cage was covered with fine mesh netting. An aliquot of 5g of each feed (digital scales, precision 0.01g) was attached to each unit using plastic coated
ties. The position of the feeds within each unit on each day was random. One Diadema setosum was
carefully placed in the centre of each replicate (cage). Initial action was observed and recorded
(photographed). After 24 hours the feed remaining in each unit was removed and weighed using the
same scales. The process was repeated for 5 x 24 hour cycles, providing 5 observations for each of the
8 replicates (cage and individual D. setosum).

The difference between initial (5g) and final weight of each feed was calculated for each trial and
considered to have been consumed by the urchin. For each replicate (urchin) the mean weight of each
feed consumed was calculated by adding the total weight consumed over 5 days and dividing by 5. The
mean, standard deviation, and standard error were calculated using the mean daily values for each of the
8 replicates (cage/urchin).

The linear model (Lm) function in R version 3.4.2 (implemented in the RStudio Version 1.1.456
environment) was applied to evaluate the effect of day and urchin on diet composition. Analysis of
variance (ANOVA) was implemented to evaluate the significance level of the difference in feed weight
consumed between the feed types at 95% and 99% confidence levels ($\alpha = 0.05$ and $\alpha = 0.01$).
Figure 1. Experimental set-up on Day 1: one D. setosum was released in the centre of each cage (experimental unit) containing four feeds (Thalassia hemprichii, Enhalus acoroides, Ulva reticulata, Gracilaria sp.) attached to the corners of each cage in a random sequence.

3. Results and Discussion

The mean daily feed consumption (N = 8) was 4.45 g/urchin/day (Table 1). There was, however, considerable variation in mean feed volume (wet weight) and composition between replicates (urchins) and between days, as reflected in the standard deviation (SD) values.

Table 1. Mean daily diet composition and volume of 8 Diadema setosum over 5 days

| Replicate (urchin) | Gracilaria | Ulva  | Thalassia | Enhalus | Total |
|--------------------|------------|-------|-----------|---------|-------|
| 1                  | 1.45       | 0.44  | 0.32      | 0.32    | 2.54  |
| 2                  | 3.53       | 0.90  | 0.76      | 0.68    | 5.87  |
| 3                  | 1.30       | 0.98  | 0.19      | 0.58    | 3.06  |
| 4                  | 4.56       | 1.43  | 0.53      | 0.85    | 7.37  |
| 5                  | 3.25       | 0.78  | 0.38      | 0.65    | 5.05  |
| 6                  | 3.21       | 1.32  | 0.56      | 0.42    | 5.51  |
| 7                  | 1.28       | 0.71  | 0.42      | 0.21    | 2.61  |
| 8                  | 2.20       | 1.15  | 0.08      | 0.15    | 3.58  |
| Average            | 2.60       | 0.96  | 0.41      | 0.48    | 4.45  |
| SD                 | 1.22       | 0.33  | 0.22      | 0.25    | 1.76  |

The overall mean feed consumed (Figure 2) reflects the predominance of macroalgae in the diet of most urchins on most days. The preference for macroalgae, which comprised on average 80.1% of diet by weight, was significant at the 99% confidence level (p < 0.01). In terms of percentage, Gracilaria sp. accounted for 58.4% of all feed consumed, followed by Ulva reticulata (21.7%), while the two seagrass species each contributed around 10% (E. acoroides 10.8% and T. hemprichii 9.1%) by weight.
Figure 2. Feed consumption of *Diadema setosum* during the 5 day trial (g/urchin/day)

There was some variation in mean feed volume (wet weight) and composition between days, as reflected in the standard error (SE) values in Figure 2. Each urchin tended to vary its diet between days, as can be seen in the boxplots for consumption of the four feed types (Figure 3). Figure 4 shows the initial choices of two out of the 8 urchins on day 2, one immediately feeding on *Gracilaria* sp. the other on *T. hemprichii*.

![Figure 2. Feed consumption of *Diadema setosum* during the 5 day trial (g/urchin/day)](image)

**Average daily consumption per urchin**

\[(N = 8, \text{mean} \pm \text{SE})\]

| Feed Type       | Wet weight consumed (g) |
|-----------------|-------------------------|
| Gracilaria sp.  | 2.60                    |
| Ulva sp.        | 0.96                    |
| Thalassia sp.   | 0.41                    |
| Enhalus sp.     | 0.48                    |
| Total           | 4.45                    |

**Figure 3.** Box plots of four feed types consumed by each of the 8 urchins over 5 days (in grams/urchin/day). Vertical axes: weight consumed (g); horizontal axes: urchin (replicate) number; box: interquartile range; bold lines: median values, whiskers: most extreme values not more than 1.5 x interquartile range outside the box; circles: extreme outlier values.
3.1. Feeding rate

The comparison between our data and that from other studies in which feeding rates of *D. setosum* and/or related species are reported (Table 2) indicates that the urchins in our trials had a highly variable (0.45-8.74 g/day) but on average relatively high daily feed consumption compared to the only published study found from Indonesia [19] and a study in Kenya [20], but much lower than the rates reported from Fiji [18]. It is interesting to note that [19] found that *D. setosum* which had been fasted before trials had considerably higher feeding rates than non-fasted individuals in the laboratory but that under field conditions (in the wild) there was no significant difference.

![Figure 4. Initial feed choices of two urchins on day 2; A. Gracilaria sp; B. T. hemprichii](image)

### Table 2. Comparison of some published feeding rates of Indo-Pacific *Diadema* urchins

| Species       | Mean rate (g/day) | Feed type            | Remarks                                      | Reference |
|---------------|-------------------|----------------------|----------------------------------------------|-----------|
| *D. setosum*  | 4.45              | algae and seagrass   | laboratory (range 0.45-8.74 g/day)           | This study|
| *D. setosum*  | 2.32              | unknown (multispecies seagrass bed) | Barranglompo Island, field conditions (fasted/non-fastened) | [19]      |
|               | ≈ 3               | 7 species of seagrass | laboratory; fasted                          |           |
|               | < 0.5             |                      | laboratory; non-fasted                       |           |
|               | 1.52              |                      | laboratory (mean, all trials)                |           |
| *D. setosum*  | 19.6              | algae and seagrasses | Fiji, field conditions                       | [18]      |
| *D. savignyi* | 24.8              | algae and seagrasses |                                               |           |
| *D. setosum*  | 1.1               | herbivory            | Kenya, field conditions                       | [20]      |
| *D. savignyi* | 0.4               | herbivory            |                                               |           |
3.2. Diet composition and feeding preference
As in this study, Diadema urchins in Fiji [18] were found to favour macroalgae rather than seagrasses, and to select a varied diet rather than concentrating their efforts on one favoured food species. The macroalgae and one seagrass species, in order of preference, were: Codium geppiorum; Hydroclathrus clathratus; green filamentous algae; Padina pavonica; Halophila ovalis; brown filamentous algae; Amphiroa foliacea; Caulerpa racemosa; and Galaxaura marginata.

A study from Zanzibar [21] reports the following order of feed preference for D. setosum: Galaxaura oblongata, Padina gymnospora, Halymenia venusta, Dictyota sp., Eucheuma striatum, Gracilaria crassa, and Cystoseira trinodis. The authors also note a “sustained avoidance” of the macroalgae Sargassum sp. and Turbinaria sp. even after prolonged fasting with no other feed choices. Sargassum sp. and Turbinaria sp. were among the algae presented to D. setosum and D. savignyi held in aquaria prior to this study. Both urchin species appeared to avoid Turbinaria; however, neither Diadema species appeared to exhibit any reluctance to consume Sargassum when provided alone (Figure 5A) or together with E. acoroides and/or T. hemprichii, although Ulva was consumed first when present. However, unlike Ulva and Gracilaria which could be kept for several weeks, Sargassum quickly began to decompose under holding conditions in the laboratory, emitting unpleasant odours after 2-4 days.

It is also of interest that [21] reports D. setosum as feeding on two species from genera widely used in mariculture, Eucheuma and Gracilaria. In our study, Gracilaria sp. was the preferred feed. Neither of the two species which dominate mariculture in Indonesia (Kappaphycus alvarezii and Eucheuma spinosum) was included in the feeding trials under this study. However, the authors have observed that D. setosum and D. savignyi will consume fragments of eucheumatoid algae entangled in (and thus collected together with) U. reticulata blooms. The unusually high density of D. setosum observed feeding on a U. reticulata bloom before and during feed collection for this study (Figure 5B) indicates a possible role for Diadema urchins in the control of such macroalgal blooms.

Figure 5. Diadema setosum feeding on: A. Sargassum sp. (Marine Environmental Quality Laboratory, Universitas Hasanuddin); B. Ulva reticulata bloom on Barrangcadi reef flat, Spermonde Archipelago

Of the seven seagrass species found around Barranglompo Island by [19] (Enhalus acoroides, Thalassia hemprichii, Cymodocea rotundata, Syringodium isoetifolium, Halodule uninervis, Halophila ovalis and Halodule pinifolia), D. setosum is reported as consistently preferring E. acoroides. One reason put forward for this preference is the relatively high protein content of E. acoroides compared to the other seagrass species. In our study, the urchins did consume slightly more E. acoroides than T. hemprichii, but the difference in preference was not significant (p > 0.05). However, the consumption
of both seagrasses did exhibit an increasing trend over the 5-day study period. One reason for this trend could be the reduced toughness of the seagrass leaves over time, due to decomposition processes. Nutrient content (e.g. protein and lipid), as well as texture and palatability, might influence D. setosum dietary choices. Somatic growth and gonad development of sea urchins, including Paracentrotus lividus [22] and Tripneustes gratilla [23], can be promoted through the addition of algae to feeds, especially Ulva sp.

3.3. Potential implications and future directions

Our data point to a potentially important role of D. setosum in macroalgal control in tropical coastal ecosystems. With respect to aquaculture, it has been suggested that D. setosum may have potential in multi-trophic context. However the preference for Gracilaria in this study and the reports of Diadema urchins feeding on Eucheumatoid algae mean that (like Tripneustes sp. [24]) these urchins could potentially become pests in the context of seaweed farming.

There is considerable interest in sea urchin culture [25], including Diadema propagation, mostly for conservation [26], including in the context of P. kauderni population and habitat management [12,13,14,15], and as an alternative to unsustainable harvest practices [9,13,14]. The varied diet of urchins in our study indicates that formulations of feed for captive D. setosum could be flexible. For example, while seagrasses could be used when leaves are abundant and can be harvested without undue damage to seagrass ecosystems, care should be taken not to degrade these highly productive ecosystems, under threat across much of Indonesia [27].

4. Conclusion

Our study contributes to the body of knowledge on the dietary preferences of Diadema setosum, and tends to support the ecological importance of this tropical shallow-water invertebrate which is also of socio-economic importance. Results indicate that feed to support the development of captive D. setosum could be flexible. For example, while seagrasses could be used when leaves are abundant and can be harvested without undue damage to seagrass ecosystems, care should be taken not to degrade these highly productive ecosystems, under threat across much of Indonesia [27].

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