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## Microhabitat preference of the Banggai Cardinalfish (*Pterapogon kauderni*): a behavioural experimental approach

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## Microhabitat preference of the Banggai Cardinalfish (*Pterapogon kauderni*): a behavioural experimental approach

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**Abstract.** The Banggai cardinalfish *Pterapogon kauderni* is an endangered endemic species with an exceptionally small native distribution and an unusual life history. A paternal mouthbrooder with direct development, symbiosis with benthic organisms (referred to as microhabitat), is crucial to its survival. This is especially so for postflexion larvae (recruits), after their release from the male parent's buccal cavity. Microhabitat preference in *P. kauderni* has been studied empirically based on a survey of wild populations. This study adopted an *ex-situ* experimental approach to *P. kauderni* microhabitat preference using two well established behavioural trial methods: choice flume and choice tank. The experimental animals were sourced from the introduced *P. kauderni* population in Palu Bay, Central Sulawesi, Indonesia. The choice flume method was applied at the Central Sulawesi Marine and Fisheries Service Hatchery in Mambo, Palu, with microhabitats *Diadema setosum* and *D. savignyi*. The choice box method was applied at the Universitas Hasanuddin Centre for Research and Development in Makassar, South Sulawesi, Indonesia, with microhabitats *Heteractis crista*, *Entacmaea quadricolor*, *D. savignyi* and *D. setosum*. The results indicate the most to least preferred preference hierarchy of *D. savignyi*, *D. setosum*, *H. crista*, *E. quadricolor*. While the preference for the sea anemone *H. crista* compared to *E. quadricolor* is consonant with empirical *in situ* studies on *P. kauderni*, the observed preference for *D. savignyi* over *D. setosum* was unexpected, and points to the need for further research. Observations made during the trials also raise the possibility that imprinting may occur and influence subsequent microhabitat preference.

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### 1. Introduction

The Banggai cardinalfish *Pterapogon kauderni* is a small marine fish popular in the marine aquarium trade and considered at risk of extinction across its exceptionally restricted endemic range [1–3]. A paternal mouthbrooder with direct development, *P. kauderni* is an obligate commensalism species [4] associated with protective microhabitat (mostly benthic invertebrates) in shallow (0–5m, mostly less than 3m depth) coastal waters including coral reefs and reef flats, seagrass meadows, and lagoons [4–13], in particular diadematid sea urchins and sea anemones [4,10,14,15]. All *P. kauderni* size classes are commonly observed associated with sea urchins. However, empirical data indicate an ontogenetic shift in *P. kauderni* microhabitat use [4,7,10,12,16]. While *P. kauderni* observed in sea anemones are strongly dominated by recruits (standard length SL < 18mm) and smaller juveniles (SL 18–25 mm), most *P. kauderni* in hard coral microhabitat are sub-adults or adults (SL > 35 mm). Association of

recruits with hard (scleractinian) corals other than the anemone-like *Heliofungia actiniformis* is extremely rare. It is not known whether the observed association patterns are due to innate preference, availability, ontogenetic changes (e.g. differential levels of immunity to sea anemone predation during ontogeny), stochastic, or other factors.

Previous studies have quantified the relative prevalence of *P. kauderni* associations with sea urchin and sea anemone species in the wild [14,15,17,18]. The two most common sea urchin *P. kauderni* microhabitat species are *Diadema setosum* and *D. savignyi* [14,17], while in recent studies the most frequently observed sea anemone microhabitats were (in descending order) *Actinodendron* sp., *Heteractis crispa*, *Entacmaea quadricolor*, *Stichodactyla gigantea*, *S. haddoni* and *H. aurora* [18]. Although one experimental study found no apparent preference between *D. savignyi* and *D. setosum* in sub-adult and adult *P. kauderni* [14], there is a lack of experimental studies on microhabitat preference in *P. kauderni*. One major threat to the endemic *P. kauderni* populations is the widespread decline in *Diadema* and sea anemone abundance, due mainly to heavy exploitation, predominantly for human consumption [3,18–21].

Behavioural experiments can be used to test hypotheses developed based on empirical (e.g. survey) data; choice flumes and choice tanks are well-established methods in behavioural studies on preference in aquatic animals, including marine fishes [22–29]. The choice tank method (e.g. [27]) and choice flume method (e.g. [28,29]) both present trialled organisms with a choice of attractants within a confined space. Behavioural experiments on *P. kauderni* microhabitat type preference could provide important information to guide conservation, including habitat and microhabitat rehabilitation. This is particularly relevant within the context of the BCF Gardens concept [30], in which microhabitat protection and, where necessary rehabilitation, are advocated as crucial to the recovery and sustainable management of *P. kauderni* populations.

This research studied the Banggai cardinalfish - microhabitat symbiosis, in particular the between-species preference of *P. kauderni* for particular sea anemones and *Diadema* species, as well as the preference of *P. kauderni* for sea anemones compared to *Diadema* urchins. The aim of the experimental behavioural trials was to test for significant preference between microhabitat types in *P. kauderni* of different age/size classes.

## 2. Materials and methods

Experimental animals obtained for the experimental research were *Pterapogon kauderni*, sea anemones, and *Diadema* sea urchins (Table 1). All animals were collected, transported, and handled in compliance with existing regulations and protocols.

**Table 1.** Experimental animals used in *P. kauderni* microhabitat preference trials.

Taxon	Source	Experimental method	
		Choice tank	Choice flume
<i>Pterapogon kauderni</i>	Mamboro <sup>a</sup>		
(various life-stages)	On-site (re-released to wild post-trial)	--	90
	Airfreighted to Makassar	48	--
<i>Diadema setosum</i> and <i>Diadema savignyi</i>	Mamboro (re-released to wild post trial)		6 each
Sea anemones:	Spermonde Archipelago <sup>b</sup>	4 each	
<i>Heteractis crispa</i>	(obtained from ornamental fishermen/	2	--
<i>Entacmaea quadricolor</i>	traders on Barrang Lompo Island)	2	--

<sup>a</sup> Introduced *P. kauderni* population site in Palu Bay, Central Sulawesi [31]; this population appeared to have been extirpated by the 28 September 2018 tsunami in Palu Bay [17]

<sup>b</sup> Barrang Lompo Island (*D. setosum*) and Badi Island (*D. savignyi*)

In addition to the animals listed in Table 1, specimens of *Actinodendron* sp. collected in the Spermonde Archipelago were also obtained from ornamental fishermen/traders. However, these highly venomous anemones [32] proved hard to keep in captivity [18] and could not be used in the trials.

Separate holding tanks were provided for trialled and untrialled organisms to prevent the same individual from being used more than once during the same trial. These tanks were provided with aeration. *Diadema* urchins were given seaweed (*Ulva* sp.) and *Enhalus acoroides* leaves (collected at the same time as the sea urchins) as feed (*ad libitum*). *P. kauderni* were fed on *Artemia* sp. (cultivated on-site) and/or small mysid shrimp (purchased frozen from an aquarium shop) both before and after the trials and, for the choice tank experiments, during the trials. The anemones were also fed frozen mysid shrimp, delivered close to the oral disc with a pipette. It was noted that the *Diadema* urchins also consumed mysid shrimp, which settled on the test or accessible areas of the tank floor.

The *P. kauderni* microhabitat preference trials were carried out from June to August 2018, using two experimental approaches, a choice tank, and a choice flume. *Pterapogon kauderni* at different life-stages (based on the standard length – SL) were used in the experiments. Based on biological characters, the main life stage classes are recruits (SL ≤ 18 mm, skeleton lacking some elements [33]), juveniles (18 mm < SL < 35 mm), and adults (SL > 40 mm, largest recorded 61 mm) [13]. Fish in the 35-40 mm range may be juveniles, sub-adults with developing gonads, or fully matured adults [13], but in this study were categorized as sub-adults. The microhabitat pairings and number/size classes of *P. kauderni* trialled using both methods are shown in Table 2.

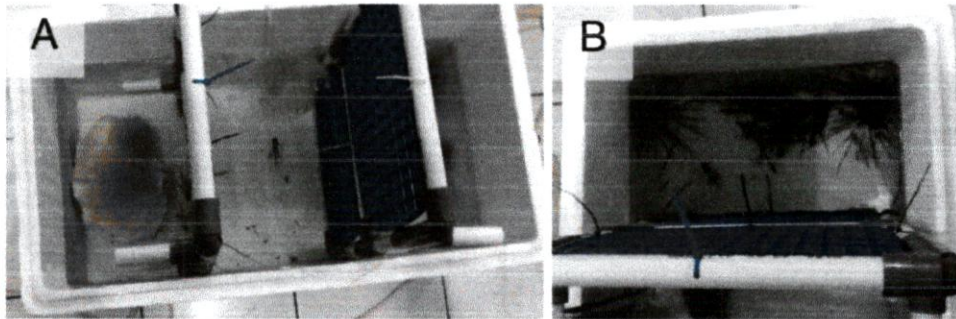
**Table 2.** Choice box and choice flume trial data.

Trial set	Microhabitat Organisms	No. of trials	<i>P. kauderni</i> size (SL)		
			Mean	SD <sup>a</sup>	Class <sup>b</sup>
Choice Box			3 fish/trial = 12/microhabitat pair		
1	<i>Heteractis crispa</i> vs. <i>Entacmaea quadricolor</i>	4	53.6	4.2	A + SA
2	<i>Diadema setosum</i> vs. <i>D. savignyi</i>	4	34.8	2.2	J + SA
3	<i>H. crispa</i> vs. <i>D. setosum</i>	4	49.8	5.6	A + SA
4	<i>H. crispa</i> vs. <i>D. savignyi</i>	4	34.5	2.7	J + SA
Choice Flume			1 fish per trial		
5	<i>Diadema setosum</i> vs. <i>D. savignyi</i>	30	11.49	4.48	R
6	<i>Diadema setosum</i> vs. <i>D. savignyi</i>	29	36.18	4.09	J + SA
7	<i>Diadema setosum</i> vs. <i>D. savignyi</i>	30	49.18	3.10	A
8	<i>Diadema setosum</i> vs. none	10	46.8	2.6	A
9	<i>Diadema savignyi</i> vs. none	10	47.6	4.8	A

<sup>a</sup>SD = standard deviation; <sup>b</sup>R = recruits; J = juveniles; SA = sub-adults; A = adults

The choice tank design was adapted from [27], and was constructed and deployed in the Medaka Centre and Water Quality Laboratory, Centre for Research and Development, Hasanuddin University, Makassar, South Sulawesi. The choice tank (Figure 1) was constructed by dividing a polystyrene tank (approximately 45 x 80 cm, internal width around 36 cm) into three sections using movable grids. Microhabitat organisms were placed at either end, with no microhabitat in the central section into which the trialled fish were released.

The placement of the two microhabitat types was exchanged after each replicate to avoid possible end bias, with four replicates per microhabitat pair. Although the microhabitat organisms were confined to their ends by the grids, the grid mesh size was large enough to enable *P. kauderni* (3 fish per trial replicate) to move freely to all sections of the tank. Observations of *P. kauderni* microhabitat association were made at intervals over 24 hours (minimum 30 observations per replicate). Water condition and aeration were maintained through intermittent use of a protein skimmer in the centre compartment.



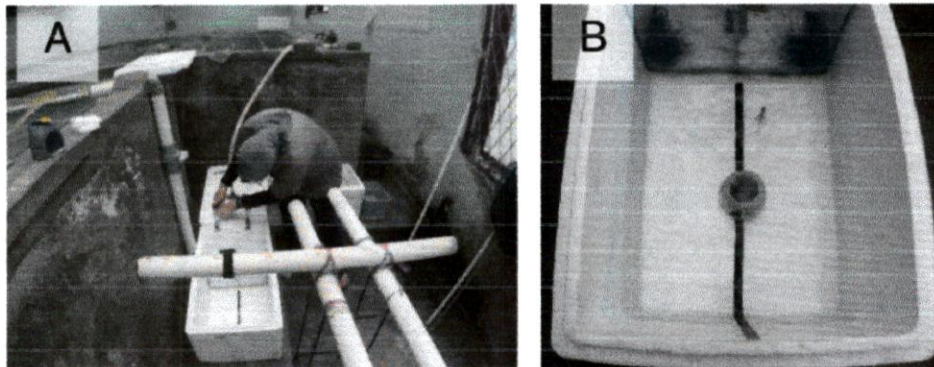
**Figure 1.** Choice tank: microhabitat organisms (A: anemones; B: sea urchins) confined to end compartments; *P. kauderni* (3 individuals per trial) released into the centre compartment.

In essence, the choice flume method allows large numbers (sufficient to be considered statistically valid, typically around 30) of the organism being tested for preference to be exposed to water flowing past two alternative attractors or repellents to which the tested individual might be expected to respond. While vision may be a factor (depending on flume design), olfaction is usually the main clue [23,34]. Examples of attractors/repellents could be predators [24,35,36], conspecifics [23,26,28], habitat cues [23,28,29,34,37], or microhabitat (e.g. type or condition) [22,24,25,38]. Methods of "infusing" the two water sources with the desired attractant/repellent vary, as do the ways in which water is supplied to and flows out of the flume. In the test chamber, where individuals will be tested for preference, there is no physical barrier to movement; however, the water flow must be laminar (as turbulence would promote mixing) and carry each of the desired individuals on just one side of the tank.

The choice flume design was adapted from [23] and trialled at the Hasanuddin University Marine Station Hatchery on Barrang Lompo Island. The flume was deployed at the Mambo Marine and Fisheries Service Hatchery in Palu, Central Sulawesi, in June 2018. The choice flume (Figure 2) was constructed using simple and inexpensive materials available locally. A polystyrene box (approximately 45 x 80 cm, internal width around 36 cm) formed the body of the flume. A two-chamber cage was constructed from semi-transparent laminated plastic and polystyrene, purpose-built to fit snugly at one end of the flume (dimensions approximately 25 cm high and 24 cm fore-aft, with two 18 cm wide compartments). Plastic mesh was fitted over the open front of the compartments, enabling the flow of water but preventing the passage of experimental animals, either fish or microhabitat. The mesh also helped to maintain laminar flow conditions. A series of holes were made along a horizontal line at the other end of the flume, enabling through flow and maintaining water height at around 15cm. A black line denoted the centre of the flume.

A clay pot with a hole about halfway up one side was used for transferring the fish from the holding tanks and releasing them into the choice flume and was fitted with a removable mesh cover. The cover was removed to release the fish after an acclimation period of 1 minute. A stand was constructed of polypropylene piping and fitted with a holder for attaching the GoPro (Hero4) camera. The rate of flow was adjusted to maintain laminar flow without posing a significant challenge or stress to the fish being tested (e.g. well within the range of local tidal currents). Prior to operating the choice flume, the laminar flow was tested using coloured non-toxic (food) dyes, which were fully flushed from the system before trials were commenced.

After acclimation and release, each fish was filmed for 2 minutes (120 seconds). The observer viewed the resulting videos and for each trial noted the time spent by the fish in each of four behaviour categories: POT = fish remaining in or close to the pot; MH1 = fish approaching or remaining close to microhabitat 1 (left of the centreline); MH2 = fish approaching or remaining close to microhabitat 2 (right of the centreline); OT = other (e.g. fish swimming around at the outflow end of the flume). MH1 and MH2 could be *D. setosum*, *D. savignyi*, or none, depending on the trial.



**Figure 2.** Choice flume deployed at the Mamboro Hatchery, Palu City. A. Overview of the setup.

B. Screenshot taken from the GoPro camera used to film each trial when fixed in place with microhabitat organisms (in this case, *Diadema setosum* left and *D. savignyi* right) confined to compartments at the upstream end. Trialled fish were placed in the pot (initially covered for 1-minute acclimation) and filmed for 2 minutes (120 seconds) once the cover was removed.

The choice tank and choice flume data were tabulated in Microsoft Excel 2010. For each set of trials, Pearson's chi-square test (chisq.test) in R version 3.4.2 [39], implemented in RStudio version 1.1.456 [40], was applied to test the null hypothesis of no preference between microhabitat types.

### 3. Results and discussion

#### 3.1. Choice tank trials

The preferences recorded in the choice tank experiments (Table 3) were mostly conducted with sub-adult/adult *P. kauderni*. The results indicate a significant preference for microhabitats compared to no microhabitat, and most fish moved several times between microhabitats. *Diadema savignyi* was significantly preferred to *Diadema setosum*, in contrast to [14], who found no significant preference. With regards to *H. crispa* versus either of the two *Diadema* species, despite an overall preference for *Diadema*, *H. crispa* was preferred to *D. savignyi* in a trial replicate with juvenile fish (SL < 35 mm). Of the four trials, preference between the two anemone species had the lowest *p*-value ( $p < 0.05$ ); however, some preference for *H. crispa* to *E. quadricolor* is consonant with field survey results [18].

**Table 3.** *P. kauderni* microhabitat preference choice tank trial results.

Mean fish in microhabitat			Significant differences	
<i>D. savignyi</i>	none	<i>D. setosum</i>	** significantly different from both,	$p < 0.001$
1.569**	0.634	0.805		
<i>H. crispa</i>	none	<i>D. setosum</i>	** significantly different from both,	$p < 0.001$
0.721*	0.555	1.724**	* significantly different from none,	$p < 0.05$
<i>D. savignyi</i>	none	<i>H. crispa</i>	** significantly different from both,	$p < 0.001$
1.938**	0.436	0.625*	* significantly different from none,	$p < 0.01$
<i>H. crispa</i>	none	<i>E. quadricolor</i>	* significantly different from both,	$p < 0.05$
1.105*	1.003	0.9		

#### 3.2. Choice flume trials

The results of the choice flume experiments carried out at Mamboro (Table 4, Figure 3) indicate a significant preference in *P. kauderni* of all size classes for sea urchins over no microhabitat and for *D. savignyi* over *D. setosum*. However, it was remarkable that the seven *P. kauderni* recruits collected

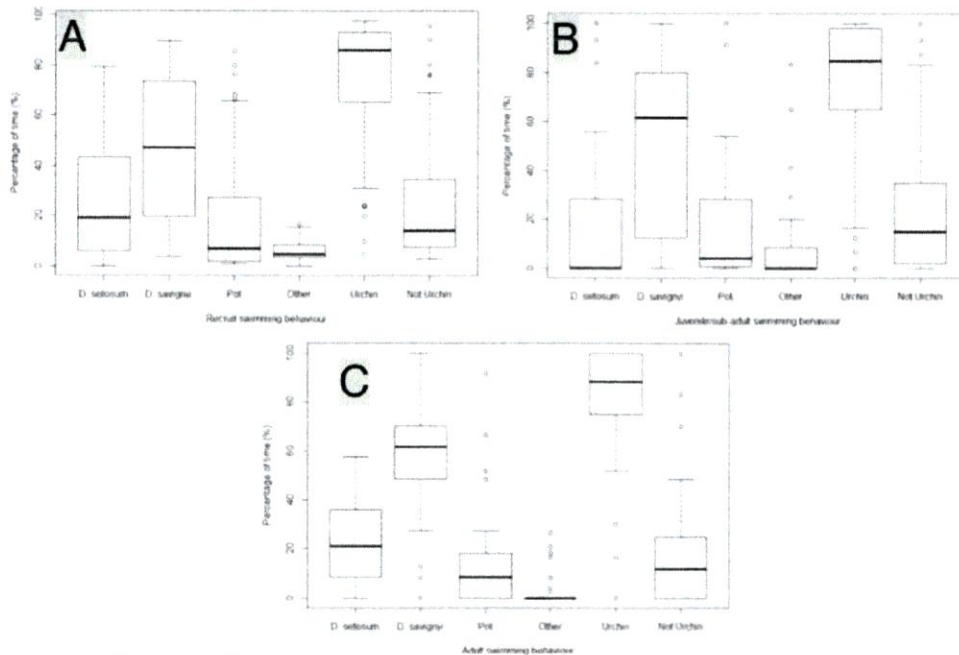
from sea anemones (*Heteractis crispa* and *Stichodactyla gigantea*) showed little or no interest in the sea urchins, and seemed to prefer the pot, remaining in or very close to the pot for most (mean 70%) of the trial. Conversely, when the net cap was lifted, almost all recruits collected from *Diadema* microhabitat swam towards one or other of the urchins immediately or after a very short time interval. These very strong demarcations indicate the possibility of imprinting in *P. kauderni*, as has been reported for clownfishes (*Amphiprion* species) [38,41–43].

**Table 4.** The proportion of time (%) spent near each microhabitat by trialled *Pterapogon kauderni* during choice flume trials.

No	Microhabitat 1		Microhabitat 2		Pot	Other	<i>P. kauderni</i> life stage	<i>p</i> <sup>b</sup>		
	Type	%	Type	%				All	MH 1-2	DD-N
1	<i>D. setosum</i>	25.26	<i>D. savignyi</i>	46.14	21.88	6.72	R	***	*	***
2	<i>D. setosum</i>	19.78	<i>D. savignyi</i>	51.83	18.99	9.40	J + SA	***	***	***
3	<i>D. setosum</i>	22.36	<i>D. savignyi</i>	58.86	15.53	3.25	A	***	***	***
4	<i>D. setosum</i>	63.67	none	10.58	16.42	9.33	A	***	***	NA
5	<i>D. savignyi</i>	73.25	none	7.33	13.50	5.92	A	***	***	NA

<sup>a</sup> R = recruits; J = juveniles; SA = sub-adults; A = adults

<sup>b</sup> Pearson's chi-square test on the difference between means; All = all behaviours; MH 1-2 = microhabitat 1 and microhabitat 2; DD-N = (*Diadema setosum* + *D. savignyi*) and (pot + other); \*\*\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \* =  $p < 0.05$ ; ns = not significant at 95% confidence level ( $p \geq 0.05$ )



**Figure 3.** Box plot of behaviour indicating preference of *P. kauderni* recruits (A), juveniles/sub-adults (B), and adults (C) based on time spent close to sea urchin microhabitat.

D. setosum = close to *Diadema setosum*  
D. savignyi = close to *Diadema savignyi*  
Pot = remaining in/around the release pot  
Other = exhibiting other behaviour  
Urchin = time close to either urchin species  
Not Urchin = time close to pot + other

### 3.3. Implications for conservation management

In contrast with the results in Tables 3 and 4, an earlier behavioural experiment using different methods [14] found no significant difference in sub-adult/adult *P. kauderni* association with (equal numbers) of *D. savignyi* and *D. setosum*. These contradictory results call for further investigation. With respect to the *P. kauderni* size class, the fish in [14] were sub-adult to adult *P. kauderni* (35-42 mm TL). Collectively, the choice flume and choice box trials cover a full range of *P. kauderni* classes, with recruits, small juveniles, and adults in the choice flume trials, and larger juvenile and sub-adult *P. kauderni* in the choice tank trial of *D. setosum* vs. *D. savignyi*. One possible explanation is that in the large concrete tanks used by [14], any preference was confounded by the tendency of the sea urchins to congregate in mixed-species groups, while in this study, the two *Diadema* species were kept separate. Conversely, despite the high statistical significance of the observed preference for *D. savignyi* in this study, it is still possible that our results could be due to chance.

*Diadema savignyi* and *D. setosum* appear to have overlapping, but significant distribution across habitat types, and thus together may enlarge the potential habitat for *P. kauderni* [17]. While increased exploitation of *Diadema* spp., mostly for human consumption, is the main driver of declining urchin populations across much of the *P. kauderni* endemic range [3,18–21], it is not known whether collection affects both species proportionally or not. Captive breeding has not been developed for either *Diadema* species. In addition, analysis of mitochondrial DNA (mtDNA) has shown that *Diadema clarki* mtDNA is also present in the Banggai Archipelago, possibly as a full species, in hybrids or through introgression [17]. It is clear that there is still much to learn about the *P. kauderni* - *Diadema* symbiosis; however, the study results support the view that *D. savignyi* is important as a *P. kauderni* microhabitat and that efforts to rehabilitate *Diadema* microhabitat should aim to maintain both species rather than concentrating on the generally more abundant *D. setosum*.

To some extent, the frequency of *P. kauderni* association with different sea anemone species recorded during a field survey in 2017 [18] may have been due to availability as much as preference. For example, the host sea anemone *Stichodactyla gigantea* (fourth most common host) was rare or absent from most sites [18]. Visual records (Moore and Ndobe unpublished data, 2004-2006) indicate that this anemone was once more prevalent, both in terms of absolute abundance and as a *P. kauderni* microhabitat. In the Banggai Archipelago, this large and fleshy anemone has been more heavily collected (mostly for human consumption) than any other anemone, especially since 2007 [3,7]. This species is also collected for the marine ornamental trade. For example, in the Spermonde Archipelago, Indonesia [44] and the Philippines [45], and collection may well extend to the Banggai Archipelago.

By comparison, both *H. crispa* and *E. quadricolor* are still relatively abundant in the Banggai Archipelago. *Heteractis crispa* is both easier to propagate asexually than *E. quadricolor* [18] and appears to be preferred as a microhabitat in the wild [18] and under experimental conditions (Table 3). These findings indicate that *H. crispa* could be a primary target species for microhabitat rehabilitation to promote *P. kauderni* population recovery, as proposed in the BCF Garden concept [2,30].

The results indicate that there may be factors other than ontogeny influencing microhabitat preference. For example, as is thought to occur in clownfishes of the genus *Amphiprion* [38,41–43], imprinting may play a significant role in *P. kauderni* microhabitat preference. In addition, it has been postulated that imprinting may affect the choice of egg-laying sites in clownfishes [42]. The ambiguous (and in some cases conflicting) data on microhabitat preference point to a possible role of imprinting in *P. kauderni* microhabitat choice. It is not impossible that imprinting of recruits could affect microhabitat choice at later life stages, in particular the choice of recruit release sites selected by

brooding male *P. kauderni*. This is just one of the questions still unanswered regarding the *P. kauderni* symbiosis with its various microhabitats, including sea anemones.

#### 4. Conclusion

The choice tank results indicate a descending preference hierarchy of *D. savignyi*, *D. setosum*, *H. crispa*, *E. quadricolor*. The preference for the sea anemone *H. crispa* compared to *E. quadricolor* is consonant with empirical *in situ* studies on *P. kauderni*, and supports the use of *H. crispa* in particular in efforts to promote the recovery of *P. kauderni* stocks through microhabitat rehabilitation. The unexpected preference for *D. savignyi* over *D. setosum* in both choice tank and choice flume trials calls for attention to fine-scale *Diadema* species structure in conservation management. The findings point to the need for further research on *P. kauderni* – *Diadema* symbiosis as well as on aspects of *Diadema* biology, ecology, biogeography, and exploitation. Observations made during the trials raise the possibility that imprinting may occur in *P. kauderni* and could influence subsequent microhabitat preference.

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