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Asexual propagation of two sea anemone taxa for Banggai cardinalfish microhabitat enhancement

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Abstract. The iconic symbiosis with clownfish is not the only sea anemone-fish association. Several tropical sea anemones provide microhabitat for the Endangered (IUCN Red List) Banggai cardinalfish *Pterapogon kauderni*. Microhabitat loss from declining sea anemone populations is a serious threat to native *P. kauderni* populations or evolutionarily significant units (ESUs). One measure advocated to maintain and rehabilitate these *P. kauderni* ESUs is to restore microhabitat abundance. The objective of this study was to evaluate the feasibility of the asexual propagation of wild anemone broodstock with the subsequent release. Two species with which *P. kauderni* commonly associates (*Heteractis crispa* and *Entacmaea quadricolor*) were identified through field surveys. Parent anemones were bisected, cutting through the center of the oral disc. Propagules (half anemones) were placed in empty clam shells as hard substrate. Treatments provided different levels of protection from potential predators, in aquaria (*P. kauderni* present/absent), concrete tanks (*P. kauderni* and *Diadema* urchins excluded/not excluded) or in a net cage on the natural substrate (fish and invertebrate fauna including *P. kauderni* and *Diadema setosum*). The cut edges of sectioned anemones joined together within seconds, with wounds typically healing in around 7 days and joins hard to see after 3-4 weeks. Growth was faster and long-term survival higher in the net cage compared to tanks for uncovered and covered anemones. The results indicate the potential for this propagation method in the context of *P. kauderni* conservation and suggest sectioned anemones should be moved to the natural rehabilitation areas once capable of adhering firmly to a hard substrate.

1. Introduction

Sea anemones are widespread in tropical shallow-water communities, and serve as hosts for many other marine animals, including fish and invertebrates [1]. While the iconic clownfish-anemone symbiosis [2] is arguably the best known, many other fish-anemone associations are reported [3,4]. One of these is the association of the Banggai cardinalfish (*Pterapogon kauderni* Koumans, 1933) with several species of sea anemone [5,6]. The Banggai cardinalfish is, in the words of Vagelli [7], an "obligate commensalism species [which] must remain associated with other organisms to survive". Along with sea urchins and hard corals, sea anemones are among the most commonly observed *P. kauderni* microhabitats, especially for recruit and small juvenile size classes [8,9].

Like clownfishes of the genera *Amphiprion* and *Premnas* [2], *P. kauderni* recruits and juveniles are often observed entering and hiding among sea anemone tentacles with impunity [5,9], while many other fish species become prey for their predominantly carnivorous sea anemone host [3]. There is strong empirical evidence for the importance of these host anemones in *P. kauderni* reproductive success, specifically with regard to recruiting survival [6].

The sea anemones (and other microhabitats) with which *P. kauderni* associates are under pressure from human activities across much of the native range of *P. kauderni*, due to exploitation and habitat degradation [7,9,11], with declines in sea anemone abundance recorded at several sites [12]. Despite species listings, previous studies have not quantified the relative frequency or prevalence of *P. kauderni*-sea anemone associations at the anemone species level. Microhabitat rehabilitation, including measures to increase sea anemone abundance, has been proposed as a tool to promote *P. kauderni* population recovery [5,8,12–14]. Previous studies have shown the feasibility of asexual propagation of several species of sea anemone [15,16], mostly within the context of the marine aquarium trade, although the possible application for conservation purposes has been suggested [15].

A previous study at the Universitas Hasanuddin Barranglompo Marine Station successfully used a sectioning method for the asexual propagation of *Stichodactyla gigantea* (Sven Blankenhorn, pers. com. to Inayah Yasir). The objectives of this study were to identify the sea anemones with which *P. kauderni* most often associates in the wild, to apply a simple asexual propagation (sectioning) method to these sea anemone species, and to perform an initial evaluation of the potential use of the method for *P. kauderni* microhabitat recovery or enhancement.

2. Methods

This study comprised field surveys in the Banggai Archipelago, Central Sulawesi (October 2017), and an experimental component at the Universitas Hasanuddin (Unhas) Marine Station on Barranglompo Island (2017-2018). Quantitative data were tabulated in Microsoft Excel (Microsoft Office 2010) and analyzed in R v.3.4.4 (implemented in the RStudio v.1.1.456 environment). Qualitative data were analyzed descriptively.

2.1. Species-level prevalence of P. kauderni-sea anemone symbiosis

The prevalence of *P. kauderni* associations with sea anemones at species level was evaluated through field surveys in native *P. kauderni* habitat at 15 sites (Figure 1). A minimum of six belt transects (5m x 20m, 100m^2 area/transect) was placed randomly in *P. kauderni* habitat and microhabitat associations noted at species or genus level.

2.2. Sea anemone asexual reproduction

Sea anemones of three species (*Heteractis crispa*, *Entacmaea quadricolor*, and *Actinodendron* sp.) were obtained from fishermen or marine ornamental traders on Barranglompo. The *Actinodendron* sp. anemones died during the 2 week acclimation period. Asexual reproduction of *Heteractis crispa* and *Entacmaea quadricolor* was carried out through sectioning (Figure 2). Each parent sea anemone was bisected using a clean, sharp stainless steel knife, taking great care to ensure that the knife passed through the center of the oral disc. Each half (propagule) then typically folded itself round to form a smaller anemone, a process completed in seconds. Before placement in one of the treatment units, each propagule was placed in an empty clamshell (with the inner face upwards), in order to provide the hard substrate to which the anemone could attach.

2.3. Evaluation of P. kauderni-sea anemone symbiosis

The post-section treatments are shown in Table 2. The anemones were monitored daily for 3 days then distributed to the treatments and observed weekly for 6 weeks and at (irregular) intervals for 6 months. Mortality was recorded; general condition and behavior were observed.

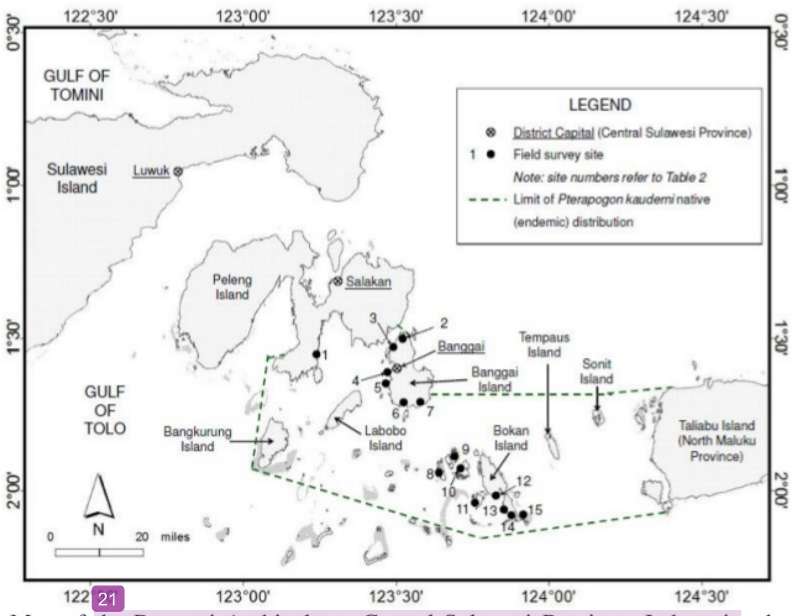

Figure 1. Map of the Banggai Archipelago, Central Sulawesi Province, Indonesia, showing the 15 survey sites within the native (endemic) range of *Pterapogon kauderni*.

Note: the section was made through the center of the oral disc, to ensure the disc and other vital organs were divided as equally as possible.

Figure 2. Asexual propagation: anemone specimen shown is *Entacmaea quadricolor*.

Table 1. Experimental treatments.

No	Treatment: Environment	Treatment: Description	Replicates per species: *H. crispa*	Replicates per species: *E. quadricolor*
1	Aquarium, aerated, 40x40x60cm	a. With 3 *P. kauderni* juveniles	0	3
		b. No *P. kauderni*	0	3
2	Concrete tank 150x250 cm, depth 50-70cm, filtered seawater flow	a. *P. kauderni* and *Diadema* sp.[a] present in the tank	2	10
		b. As above, in net-covered basket	2	0

3	Net cage on reef flat, 3mx9m, depth varying with tide (≈1.2-2.5 m)	a. *P. kauderni, Diadema setosum*, reef fish and invertebrates present	2	4
		b. As above, in net-covered basket	2	0

[a] *Diadema setosum* and *D. savignyi*

3. Results

3.1. Field data on *P. kauderni*-sea anemone associations

Over the 15 survey sites, six species of anemone were recorded as hosting *Pterapogon kauderni*. In order of descending frequency these were: *Actinodendron* sp., *Heteractis crispa*, *Entacmaea quadricolor*, *Stichodactyla gigantea*, *S. haddoni* and *H. aurora* (Table 2). The associated *P. kauderni* were predominantly recent recruits and small juveniles; however, a few adults were observed close to the anemones, including brooding males. Collectively, five species of clownfishes shared their anemone hosts with *P. kauderni*. Clownfishes were never observed in the most common *P. kauderni* host anemone, *Actinodendron* sp., but shared 39% of anemones belonging to four species: *E. quadricolor*, *H. crispa*, *S. gigantea*, and *S. haddoni*.

Table 2. *Pterapogon kauderni* host anemones observed at 15 sites in October 2017

No	Name	*Actinodendron* sp.	*Entacmaea quadricolor*	*Heteractis crispa*	*H. aurora*	*Stichodactyla gigantea*	*S. haddoni*
1	Liang	0	2	0	0	0	0
2	Popisi	2	1	0	0	1	0
3	Bone Baru	9	2	5	0	2	2
4	Tinakin Laut	1	5	0	0	0	0
5	Monsongan	4	3	15	0	1	1
6	Tolokibit	0	1	3	0	2	0
7	Kapela	0	5	1	0	0	0
8	Toropot	0	1	1	0	0	0
9	Kombongan	0	3	6	0	0	0
10	Minangga	9	4	4	0	5	2
11	Tj Nggasuang	1	0	1	0	0	0
12	Mandel	0	1	2	0	0	0
13	Mbuang-Mbuang	0	0	0	0	1	0
14	Melilis	1	0	3	0	0	0
15	Toado	21	0	1	2	1	1
	% of total	34.53	20.14	30.22	1.44	9.35	4.32
	% also hosting clownfishes	0	14.29[a] 21.43[b]	23.81[c] 19.05[d]	0	15.38[c] 23.08[e]	33.33[c]

[a]*Premnas biaculeatus*; [b]*Amphiprion frenatus;* [c]*A. clarkii*; [d]*A. akallopisos*; [e]*A. ocellaris*

3.2. Asexual reproduction techniques

The cut edges of sectioned anemones joined together within seconds, with wounds typically healing in around 7 days and joins began to be hard to see after 3-4 weeks (Figure 3). However, some aspects required special care and attention, and several unexpected complications were encountered. During and immediately after bisection, it became clear that it was important to ensure tentacles did not become trapped in the join; thereafter, special care was required during all post-section handling, to avoid re-opening the wound. When either of these occurred, the sea anemone propagules survived under the study conditions, but healing was substantially delayed.

Attempts to measure (pedal disc diameter) and weight (total body weight) the propagated sea anemones during the first trial (treatment 1) were impeded or rendered meaningless by the ability of the sea anemones to change size and weight (up to over 300%) through uptake and release of water. Thus, growth was not measured quantitatively.

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Propagules of both species exhibited a greater capacity for mobility than expected, some moving up to several meters overnight, mainly in treatment 2a. Furthermore, recently bisected *Entacmaea quadricolor* in treatment 2a exhibited a tendency to aggregate, and proved capable of joining together, with two, three or four propagules (half anemones) matching up their sectioned edges to form a larger anemone. To prevent this, care was necessary to ensure newly anemones were kept apart for the first week. In treatment 3b, a storm resulted in the release of the anemone propagules in covered baskets. These anemones found suitable substrate and settled within the caged area, and thereafter experienced the same conditions as those in treatment 3a.

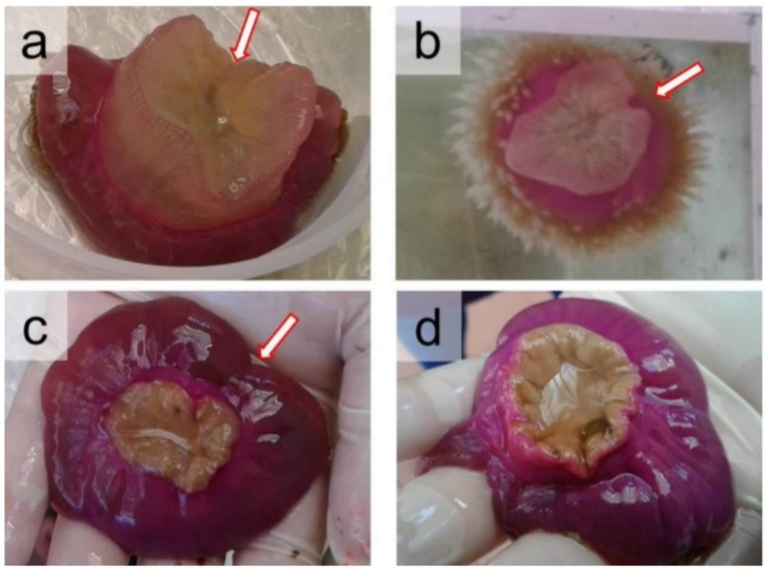

Figure 3. Sectioned sea anemone healing process (*Entacmaea quadricolor*) (a) Cut edges closed together seconds after sectioning (b) Join still clearly visible after 1 week (seen in the aquarium, treatment 1) (c) Join scar fading after 3 weeks and (d) Join scar not discernable after 6 weeks.

3.3. Sea anemone condition, survival, and growth under experimental treatments

Although the numbers in this initial trial were too small for statistical analysis, certain trends were visually noticeable in each treatment. In all treatments, survival to 6 weeks was 100%. No visible predation by fish or invertebrates was observed during this period in any treatment.

In treatment 1, designed to test the effect of introducing or not introducing fish (groups of three *P. kauderni* juveniles) to newly sectioned anemones (*E. quadricolor*) held in aquaria for 6 weeks, there was no detectable difference in anemone growth, survival (100%) or behaviour (e.g. movement, choice of substrate) between treatments. Based on this result, *E. quadricolor* was not given net covering as protection from potential predators in treatments 2 and 3.

After approximately 6 months, all *E. quadricolor* and *H. crispa* in treatment 3a had survived and grown. They each attracted resident clownfish as well as attracting *P. kauderni* recruits from at least one of the several spawning events over this period which were followed by successful brooding (eggs and larvae) and release of recruits. Brooding male *P. kauderni* with hatched larvae still in the yolk-sac stage but close to release as recruits were seen hovering over or near anemones on several occasions, followed a few days later (around full moon) by the appearance of new recruits (SL 6-10 mm) in the anemones (Figure 4a).

While juvenile clownfishes probably entered the cage through the netting as competent post-larvae

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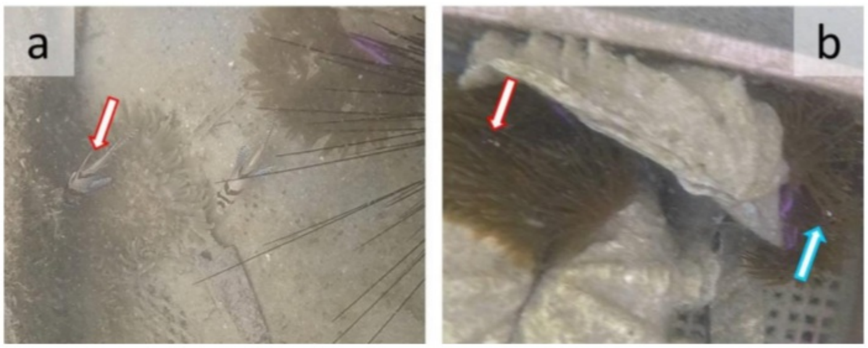
ready to settle, several large individuals were released into the cage by ornamental fishermen who had not realized the purpose of the experimental cage and were later removed. The juvenile clownfish which recruited and shared these anemones with *P. kauderni* were *A. frenatus* (4 individuals), *A. ocellaris* (one individual, Figure 4b) and *A. clarkii* (3 individuals). While all three clownfish species settled on at least one occasion in *E. quadricolor*, only *A. ocellaris* (3 individuals) and *A. clarkii* (2 individuals) settled in *H. crispa* during the study period. The numbers given refer to occurrences observed during monitoring. When recruited to adjacent sea anemones (Figure 4b), all species moved freely between these anemones, as if both were considered as part of their microhabitat or territory.



a. *Heteractis crispa* with brooding *P. kauderni* male (red/white arrow); a week later *P. kauderni* recruits were seen in this anemone and remained there, growing to the juvenile stage (SL > 18mm) by the end of the study period

b. *Entacmaea quadricolor* anemones with new (SL < 10 mm) *P. kauderni* recruits (one visible, red/white arrow) and an *A. ocellaris* juvenile which recruited naturally from seawater flowing through the cage (turquoise/white arrow)

Figure 4. Propagated sea anemones in the net cage with captive *P. kauderni* broodstock, four (a) and five (b) months post sectioning.

In treatments 2 and 3 there was no visible difference in growth between covered and uncovered propagules until the covers were removed at the end of the six week period (treatment 2) or due to storm damage (treatment 3). Over the initial 6 weeks, anemones released into the net cage (similar to wild conditions) initially showed similar growth compared to those kept in a controlled environment (concrete tanks or aquaria).

Over the remainder of the study period, *H. crispa* exhibited slightly faster growth in the cage (treatment 3), while *E. quadricolor* growth and survival were higher in the cage. The difference in growth rate became noticeable about 2 months after sectioning. There were no anemone fatalities in the cage, despite several severe storms. Some anemones were buried in the soft substrate or had their hard substrate turned over, but proved capable of re-surfacing and/or relocating themselves. In the tanks, although initial growth (similar to cage) and survival (100%) of *E. quadricolor* were satisfactory, after 2-3 months growth slowed down, and in some cases became negative. By the end of the study period, *E. quadricolor* survival rate was down to 50% in treatment 2.

4. Discussion

4.1. P. kauderni-sea anemone symbiosis at the species level

The field and experimental results add to the body of knowledge on the *P. kauderni*-sea anemone symbiosis. With regard to the preference for certain sea anemones, it is likely that *P. kauderni* associations were influenced by the limited choice available. Behavioral experiments should help to distinguish between possible choice mechanisms (e.g. innate preference, imprinting, and propinquity).

The host sea anemone *Stichodactyla gigantea*, the fourth most common host in this study, was rare or absent from most sites during the 2017 survey. Visual records from 2004 and 2006 (Moore and Ndobe, unpublished data) indicate that this anemone was once more prevalent, both in terms of absolute abundance and as a *P. kauderni* microhabitat (host). 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. This species is also collected for the marine ornamental trade, for example in the Spermonde Archipelago, Indonesia [17] and the Philippines [18], and collection may well extend to the Banggai Archipelago.

Anemones of the genus *Actinodendron* are not exploited and their abundance does not seem to have changed noticeably over time, even at sites with noticeable environmental degradation. Thus relative abundance (availability) could well be one reason for the high frequency of this species as *P. kauderni* host. Furthermore, data on *P. kauderni* abundance indicate that on average *Actinodendron* hosted more *P. kauderni* per anemone than other species. *Actinodendron* anemones have a particularly strong venom [19] and are known as “fire anemones” due to the severe burn-like skin lesions (blistering and swelling, with pain and itching) which they can cause in humans. Compared to the other *P. kauderni* host anemones, they appear to host a limited number of fish and invertebrate species. The ability of *P. kauderni* to take refuge within a wide range of sea anemone species, especially the close-knit and intricately branching tentacles of the highly venomous *Actinodendron* raises questions regarding the mechanisms of venom immunity (or avoidance) in *P. kauderni*.

4.2. Evaluation of asexual reproduction for P. kauderni sea anemone microhabitat enhancement

The propagation technique applied in this study was simple to use and generally successful. The aggregate experimental results support the suggestion of [15] that the release to the wild of asexually propagated (sectioned) sea anemones should have a good chance of success. The lack of observed predation on sectioned anemones, even on newly sectioned anemones with no protective cover exposed to naturally recruiting reef fauna in the cage (treatment 3a), indicates that sea anemone propagation and release could be implemented in the field without the need for extensive *ex-situ* facilities for anemone husbandry.

Although only two species were propagated in this study, it is likely that, with the exception of *Actinodendron* sp., the other P. kauderni hosting anemones could be propagated in a similar manner, in particular, *Stichodactyla gigantea*. Fishermen have reported that *S. gigantea* can be hard to remove from its natural substrate in the wild, and indeed the first author has found to be the case. When collected by fishermen for human consumption, *S. gigantea* appears to resettle with ease when relocated to holding sites. These are generally on the reef flat below stilt houses of Bajo (sea gypsy) fisherfolk, in areas which are often also *P. kauderni* habitat. During this study, at Monsongan (site 5) *P. kauderni* had recruited to anemones in such a “living larder”. Similar cases have been observed in the past at Toado (site 15) in 2012 and Liang (site 1) on several occasions since 2007 (Moore, unpublished data). Together with the record of successful asexual propagation for *S. gigantea*, these observations indicate a high likelihood of success in applying the sectioning method to replenish depleted populations of this *P. kauderni* microhabitat species.

Although the anemones in this study host symbiotic *Symbiodinium* sp. and thus gain much of their energy from photosynthesis [20,21], the higher growth and survival of sea anemones in the cage compared to the tank could be explained by the greater opportunities for heterotrophy in natural (as opposed to filtered) seawater. These considerations indicate that early release of propagated anemones should be doubly beneficial, reducing the resources needed (e.g. time, facilities, and manpower) as well as improving the chances that the propagated (sectioned) anemones will grow and survive.

4.3. Future directions for research and conservation

Sea anemones that host *P. kauderni* (and clownfishes) have a number of life-history characteristics that make them particularly vulnerable to overexploitation, including long life, limited breeding seasons and density-dependent fertilization, which can make natural recruitment slow and unreliable [16]. To prove that asexual propagation is a realistic option in the context of *P. kauderni* anemone microhabitat rehabilitation and enhancement, the next steps should include the identification of potential "parent" anemones for sectioning, and trials at sites with depleted anemone populations within *P. kauderni* native habitat. These should be sites at which management measures are in place to prevent further collection of sea anemones. The trials should be designed inter alia to optimize release protocols including considerations related to the substrate and other environmental factors as well as timing.

Another consideration is the size of the parent anemones. The anemones used in this research were relatively small for their species. The feasibility of sectioning larger individuals into more than two propagules (e.g. quartering rather than bisecting parent anemones) could be tested.

Advances in knowledge regarding the reproductive biology of several species, including the two anemones propagated in this study (*E. quadricolor* and *H. crispa*) open the possibility of sea anemone culture through sexual reproduction. The advantages of sexual propagation include greater genetic diversity (as all sectioned propagules are genetically identical clones), and far higher numbers of propagules. Although the resources needed would be much greater, if applicative techniques were developed this approach might be a viable alternative in the future, potentially offering the chance of livelihoods through sale of the anemones produced to the marine ornamental trade [16] as well as for conservation purposes.

5. Conclusion

The four sea anemone taxa most commonly associated with *P. kauderni* comprise three species (*Heteractis crispa, Entacmaea quadricolor, Stichodactyla gigantea*) and one genus (*Actinodendron*). Of these, only *Actinodendron* is not (at least as yet) amenable to asexual reproduction using simple techniques that could be readily applied at the local level within the *P. kauderni* native range. *H. crispa* and *E quadricolor* propagules were successfully released in close to natural conditions and attracted *P. kauderni* recruits released over several reproductive cycles. While further research is recommended (e.g. on the optimal substrate and release protocols, the potential for sectioning into more than 2 parts), the results of this study indicate that enhancing *P. kauderni* microhabitat through asexual sea anemone reproduction should be a viable proposition.

Acknowledgments

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ORIGINALITY REPORT

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