New host record of microhabitat preferences of the Banggai cardinalfish (*Pterapogon kauderni*) in the introduced habitat in Luwuk waters, Sulawesi

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Abstract. Banggai cardinalfish *Pterapogon kauderni* is an endemic apogonid fish from Banggai Islands, Central Sulawesi, which is discovered in strong association with black sea urchin (*Diadema* spp.). These fish also protected themselves from predators associated with sea anemones, hard coral branching, mushroom coral, soft coral, seagrass, and mangrove roots. The Banggai cardinalfish juveniles generally prefer to associate with sea anemones or mushroom corals as microhabitats. However, they are looking for similar microhabitats if they cannot find sea anemones or mushroom coral *Heliofungia actiniformis*. Microhabitat observation of Banggai cardinalfish was carried out in Luwuk waters, Banggai Regency. The result showed a new host record for juvenile phases of Banggai cardinalfish associated with upside-down jellyfish *Cassiopea andromeda* as a microhabitat. Further study is needed to represent areas and seasons to get more evidence between the Banggai cardinalfish and jellyfish association concepts.

Keywords: Banggai cardinalfish, endemic, microhabitat selection, symbiosis

1. Introduction

The Banggai cardinalfish *Pterapogon kauderni* is a paternal mouthbrooder with tiny clutch size and no juvenile pelagic larval phase [1-3]. *Pterapogon kauderni* has limited distribution in less than 10,000 km² and has been found naturally only in the Banggai Archipelago off the east coast of Sulawesi, Indonesia because a pelagic larval phase is the main dispersal method in most reef fish [4-6]. Population in small portions inhabit Luwuk Harbor in Central Sulawesi, which enclosure separated from the Banggai Islands group by the Peleng Strait [3, 6, 7]. Some introduce populations were established along the trade routes, such as Lembeh Strait – North Sulawesi [6, 8-10], Tumbak – North Sulawesi [6, 11, 12], Palu – Central Sulawesi [13-15], Luwuk – Central Sulawesi [3, 13], Kendari – South East Sulawesi [16-18], Bali [6, 19, 20, 21], Ambon – Molluca [22, 23], and Ternate – North Molluca [24].

The Banggai cardinalfish lives in shallow waters in a varied habitat, including coral reefs, seagrass beds. However, they are less frequently in open habitats such as low branching coral coverage and rubble [16, 25] and are commonly found in calm and protection bays [5, 11]. *Pterapogon kauderni* is a site-attached species that stays associated with various benthic living substrates. The Banggai cardinalfish lives in groups mainly hovering within and above groups of long-spined sea urchins (*Diadema setosum*) [1, 3, 26-28]. They are also closely associated with other sea urchins (*Diadema setosum* and *Tripneustes gratilla*) [25, 29], sea anemones (*Actinodendron* spp., *Entacmaea quadricolor*, *Heteractis crispa*, *Macroductyla doreensis*, *Stichodactyla haddoni*) [2, 3, 5, 25, 27, 28], soft corals (*Nepthia* spp.) [3, 5, 25, 27], branching stone corals (*Acropora* spp., *Anacropora* spp., *Porites* spp., *Goniopora* spp., etc.) [2,
3, 5, 25, 30], mushroom stone coral, *Heliofungia actiniformis* [27], hydrozoans (*Millepora* spp.) [25, 27], sponges [27], and mangrove roots (*Rhizophora* spp.) [5, 11, 25]. Microhabitat is important as a refuge from predation, wherein anemones to be particularly important microhabitats for newly released and juveniles of Banggai cardinalfish. Almost all predators of juveniles, including adult and sub-adult of *Pterapogon kauderni*, seem to avoid the tentacles among which juveniles often hide and are also protected by anemone resident clownfish when present [16].

Banggai cardinalfish will choose other biotas similar to anemone as their microhabitat if sea anemones are not present. For example, mushroom coral *Heliofungia* spp., soft coral, or another biota with many tentacles, such as jellyfish *Cassiopea andromeda*, which physically resemble anemones. *Cassiopea andromeda* (Forsskål, 1775), or Upside-down jellyfish, belongs to the family Cassiopeidae. These usually live in intertidal sand or mudflats, shallow lagoons, or around mangroves and are commonly mistaken for sea anemones. The distribution is widely around the world, especially in the Indo-Pacific [31], but the population in Eastern Mediterranean waters is recorded to increase [32-38], and also in the Caribbean Sea and the southern tip of Florida [39].

Numerous Upside-down jellyfish were found as a host for juveniles of Banggai cardinalfish during exploratory dives at the Luwuk harbor, Banggai District, Central Sulawesi. This phenomenon offered the opportunity to investigate the possibility of jellyfish as a new host preference for Banggai cardinalfish.

2. Materials and Methods

This study was carried out between April to May 2019 around Luwuk waters, Banggai District, Central Sulawesi. Our work focused on introducing Banggai cardinalfish at five locations (Figure 1), which were reported as introducing areas by trading impact, restocking, and another purpose.

![Figure 1](image_url)

*Figure 1.* Map of the survey location is the location of Banggai cardinalfish introduction in Luwuk waters, Sulawesi.

The modified belt transect method was used to observe the density of the Banggai cardinalfish. A total of 20 m long within 5 m on either side of transect along the coastlines, and six times were conducted at each sampling site (Figure 2). The density was carried out based on the number of individual and microhabitat preferences of each size category using the Underwater Visual Survey (UVS) method along the transect [40]. The size categories were estimated based on standard length (SL) in 3 classes, such
the larvae (less than 1.8 cm), juveniles (1.8 – 3.5 cm), and adults (more than 3.5 cm). To avoid bias records, observers should swim at a constant speed and not count the same fish twice [41]. The density was calculated using the formula:

\[ d = \frac{c}{A} \]

Where:
- \( d \) = Density (ind/m\(^2\))
- \( c \) = Number of individuals
- \( A \) = Extent of observation area (m\(^2\))

**Figure 2.** The modified belt transect method was used to observe the density of the Banggai cardinalfish.

Quantitative analysis using the Chi-square test (\( \chi^2 \)) determines trends in habitat selection and Neu’s Index analysis (Preference Index) to determine the habitat preferences based on differences in fish density in each habitat. Friedman Test was used to test three or more paired populations. A group of subjects was subjected to three or more different treatments. In this study, the comparison of the density of three stages of fish development at each station was analyzed using Friedman Test by SPSS 25 software with a 95% confidence level (\( p = 0.05 \)). Neu Index was used to determine the habitat preference index by animals and to find out the most preferred habitat for these animals that carried out with the following equation:

\[ w = \frac{ui}{pi}, \text{and} \quad b = \frac{wi}{\sum wi} \]

Where:
- \( wi \) = Selection / Preference Index of each station
- \( pi \) = Proportion of coverage area (m\(^2\))
- \( ui \) = Proportion of the number of individuals (individu)
- \( b \) = Standardized index

The order of the level of preference or preferential habitat is based on the value of the variable \( b \) (standardized index), where the standardized index provides comparisons between studies because the number is always one. The largest \( b \) value indicates the primary habitat preference, the second-largest \( b \) value indicates the second habitat preference, and so on.
We observed the upside-down jellyfish whether the presence or absence of Banggai cardinalfish in each jellyfish was found. The number of individuals and the size of the Banggai cardinalfish was calculated on each jellyfish. Furthermore, the behavior and position of the Banggai cardinalfish against their host jellyfish were also recorded.

3. Results and Discussion
Banggai cardinalfish was found in a small population in several locations in Luwuk waters, especially around the Luwuk Harbor. The harbor has around 1.2 km length, 0.5 widths, and 150 m open to the ocean through a passage exposed to high levels of pollution, including regular fuel spills, freshwater, and sewage discharges [42]. The nearest introduction area of Banggai cardinalfish is in Patikaman (southcentral Paleng), about 120 km, which is separated by Paleng Strait with a strong current and 920 m depth [5]. Furthermore, the Banggai cardinalfish populations in Luwuk are also suggested as an introduced population [7, 43].

3.1. Density
Banggai cardinalfish in Luwuk waters was found at five locations with several phases, e.g., recruits (<25 mmTL), juveniles (25-60 mmTL), and adults (> 60 mmTL). The phase compositions in each location have a different encounter pattern. Phase recruit and juvenile in Station I were not found and a juvenile in Station IV. However, the Banggai cardinalfish phase composition in each location also has different densities (Figure 3). According to [43], these results are consistent, wherein most Banggai cardinalfish population found in several phases composition in a location while the juvenile is a generally dominated. The population formed by an adult is rarely found and usually consists of several phases. The recruit phases are often found in a habitat that their parents inhabit [45].

![Figure 3](image-url)

**Figure 3.** The density of Banggai cardinalfish based on each phase at each station in Luwuk waters.

Banggai cardinalfish in Luwuk waters had a different average density on all phases in each station, which was statistically significant based on the nonparametric Friedman test with a value of 0.019 (Asymp value Sig <0.05). The lowest density of recruit phases was reported in Station I (not found), while juvenile phases were not recorded in Station I and IV, and the adult phases also showed less density in Station I (1 ind./100m²). However, Station II was reported to have a higher density for recruit, juvenile, and adult phases with a number of average 3 ind./100m², 6 ind./100m², and 11 ind./100m², respectively.

Station I’s low density is related to the coral reef ecosystem, a suitable zone for fish spawning. Population structure and size distribution in Banggai cardinalfish are influenced by predation after initial settlement [4]. Although mating partners and incubating males can be found in all habitats inhabited by fish, this suggests that fish reproduction and recruitment can occur in all zones occupied by species.
However, the number of newly released recruits can be higher in seagrass beds than on coral reefs. Where in this zone, most of the male parents who are incubating eggs are found. During the first two to three weeks after birth, recruits experience high predation rates (including cannibalism of adults of the same breed). After being released by the male, recruits will seek protection on a substrate that is isolated and separated from the parent's microhabitat.

### 3.2. Host Preferences

Similar to other locations, Banggai cardinalfish in Luwuk is associated with microhabitats such as black sea urchin (*Diadema setosum* and *Diadema savignyi*), sea anemones, various hard corals, mushroom corals *Heliofungia actiniformis*, and soft corals. The life cycle of Banggai cardinalfish in each phase has different habitat requirements even in the same waters, causing their habitat preference to change [45]. The preference index for each developmental phase of Banggai cardinalfish is presented in Tables 1 to 3, which show different habitat tendencies.

#### Table 1. Preference Index for recruits of Banggai cardinalfish in Luwuk waters.

| Station | a  | P  | n  | u  | E  | w  | b  | l  |
|---------|----|----|----|----|----|----|----|----|
| I       | 6.04 | 0.08 | 0  | 0  | 23 | 0  | 0  | 5  |
| II      | 36.17 | 0.45 | 278 | 0.917 | 136 | 2.04 | 0.388 | 2  |
| III     | 1.92 | 0.02 | 23 | 0.076 | 7  | 3.19 | 0.606 | 1  |
| IV      | 23.78 | 0.3  | 1  | 0.003 | 90 | 0.01 | 0.002 | 4  |
| V       | 12.59 | 0.16 | 1  | 0.003 | 47 | 0.02 | 0.004 | 3  |
| Amount  | 80.49 | 1   | 303 | 1  | 303 | 5.26 | 1   |

#### Table 2. Preference Index for juveniles of Banggai cardinalfish in Luwuk waters.

| Station | a  | P  | n  | u  | E  | w  | b  | l  |
|---------|----|----|----|----|----|----|----|----|
| I       | 6.04 | 0.08 | 0  | 0  | 45 | 0  | 0  | 4  |
| II      | 36.17 | 0.45 | 593 | 0.98 | 272 | 2.18 | 0.82 | 1  |
| III     | 1.92 | 0.02 | 6  | 0.01 | 14 | 0.42 | 0.16 | 2  |
| IV      | 23.78 | 0.3  | 0  | 0  | 179 | 0  | 0  | 4  |
| V       | 12.59 | 0.16 | 7  | 0.01 | 95 | 0.07 | 0.03 | 3  |
| Amount  | 80.49 | 1   | 606 | 1  | 606 | 2.67 | 1   |

#### Table 3. Preference Index for adults of Banggai cardinalfish in Luwuk waters.

| Station | a  | P  | n  | u  | E  | w  | b  | L  |
|---------|----|----|----|----|----|----|----|----|
| I       | 6.04 | 0.08 | 33 | 0.02 | 132 | 0.25 | 0.04 | 5  |
| II      | 36.17 | 0.45 | 1103 | 0.63 | 793 | 1.39 | 0.22 | 2  |
| III     | 1.92 | 0.02 | 147 | 0.08 | 42  | 3.5  | 0.55 | 1  |
| IV      | 23.78 | 0.3  | 312 | 0.18 | 521 | 0.6  | 0.09 | 4  |
| V       | 12.59 | 0.16 | 169 | 0.1  | 276 | 0.61 | 0.1  | 3  |
| Amount  | 80.49 | 1   | 1764 | 1  | 1764 | 635  | 1   |

Where:

- **a** = coverage of microhabitat inhabited by Banggai cardinalfish (m²)
- **P** = coverage proportion of microhabitat inhabited by Banggai cardinalfish
- **w** = Preference Index / Selection Index
- **n** = number of fishes were counted (individual)
- **u** = proportion of number of fishes were counted
- **e** = expected value of number of fishes were counted (individual)
According to the value of preference index, it showed that the recruits phase prefers in Station II and III ($w\geq1$), with the highest preference index (3.19). The recruits phase was also founded in Station V and IV. However, it has tended not to be their habitat preference ($w\leq1$), while no recruit phases inhabiting Station I ($w=0$). Five stations show that Station III is a preferred habitat for recruit phases which is habitat use by fish is greater than the habitat available. Station III is located in the transitional ecosystem between seagrass and coral reef, with sand as substrate dominated and microhabitat presented by sea urchins. Moreover, the recruit phases basically can be found in all adult phase microhabitats, e.g., sea urchins. In conditions presenting more microhabitat types, the recruit phases will prefer to inhabit anemones and mushroom coral *Heliofungia actiniformis* [5]. The number of sea urchin colonies changes will affect individuals of recruit phases [5]. If recruits remain associated with sea urchins for a long time, fish will passively migrate along with sea urchins to find more suitable microhabitats and switch to different types of microhabitats. For example, if a colony of sea urchins passes through anemones, recruits will move from sea urchin colonies to anemones [46].

Juvenile phases of Banggai cardinalfish in Luwuk waters prefer to inhabit Station II ($w=2.18$). Although found in Station III and V, the juvenile tends to be disliked inhabit ($w\leq1$) and not prefer to inhabit in Station I and IV ($w=0$), for the adult phase was reported present in all stations. However, the highest preference index is at Station III ($w=3.5$) means that Station III is a preferred habitat for Banggai cardinalfish. At the same time, other stations seem to tend not to be the preferred habitat ($w\leq1$). Station II is similar to Station III, which reports as the preferred habitat for recruit phases. Dominated microhabitat by sea urchin at Station II and III as an associate throughout sheltering among the sea urchin spines which have a color similar with the black pattern of Banggai cardinalfish [5], and also sea urchin spines have a stinging cell that protects from predators when Banggai cardinalfish threatened [47].

The previous study has reported microhabitat ontogenetic shifting in Luwuk waters. It is supported by the hypothesis ontogenetic, which tends to shift between microhabitat or host-symbiont and age change [5]. After settlement, Banggai cardinalfish exhibits high site fidelity [4] despite an ontogenetic shift in microhabitat within a given site [42]. Habitat with a relatively high population of black sea urchin, Banggai cardinalfish in various sizes is observed. Conversely, the Banggai cardinalfish will choose another microhabitat if their habitat has a low sea urchin population. Relation between microhabitat types and size composition of Banggai cardinalfish is visually prominent, which is more than 5% and considered significant. The study of Banggai cardinalfish in the Banggai Islands shows the microhabitat preferences among the size-types of Banggai cardinalfish in a habitat with a high sea urchin population. At the same time, the juvenile prefers sea anemone as their microhabitat (80%) and adult at hard coral (60%) [5]. Microhabitat shifts tend to occur slowly from sea anemones to hard corals. The difference in the percentage of juveniles that choose sea anemones is higher than in another microhabitat. However, hard coral is only chosen by adults [27].

Sea anemones are a particularly important habitat for recruit and small juvenile phases of Banggai cardinalfish survive, whereas often to share a host with clownfish. The adult phase dominant inhabits hard coral as their microhabitat, though limited to coral life forms e.g., branching and foliose [27]. There is an interesting relationship between clownfish *Amphiprion* spp. and juvenile Banggai cardinalfish, symbiotic in sea anemones. Clownfish usually throw other fishes from approaching sea anemones they live in, including the adult of Banggai cardinalfish; however, they tolerate the presence of juvenile [13]. There is much evidence that microhabitats, sea urchin, sea anemone, and hard coral life forms are important to support the Banggai cardinalfish populations [40, 48, 49]. The main factor in decreasing the Banggai cardinalfish population is the abundance of microhabitat populations, especially sea urchin and sea anemone [50].
3.3. New Host Preference
As previously explained, the Banggai cardinalfish is a site-attached species that stays associated with various benthic living substrates (Figure 4), lives in groups mainly hovering within and above long-spined sea urchins, hard corals, soft corals, hydrozoans, sponges, sea anemones, seagrass and mangrove roots. The absence of sea anemones allows Banggai cardinalfish to look for a similar one with many tentacles as juveniles inhabit commonly, such as mushroom coral *Heliofungia actiniformis*. Observation in Luwuk Harbor shows a new fact that Banggai cardinalfish has an associate with upside-down jellyfish *Cassiopea andromeda* (Figure 4 photo number 13 to 15). This microhabitat associate has less information before. Little information about this association has been mentioned before, but a more in-depth study has not been carried out [48]. The association between a small number of individual Banggai cardinalfish and jellyfish was recorded in Tinakin Laut, one of the research sites in the Banggai Islands. However, the results of observations at Luwuk Harbor were discovered, and the data will be useful as input for further research to consider the existence of this jellyfish about the selection of microhabitat for Banggai cardinalfish.

Numerous upside-down jellyfish *Cassiopea andromeda* was found in Luwuk Harbor as a host for juvenile Banggai cardinalfish (Table 4). Nine jellyfish individuals encountered during the observation, as many as 99 individuals of Banggai cardinalfish from various phases of development were found. From nine jellyfish individuals were found, the Banggai cardinalfish do not inhabit only one individual. Data shows, the Banggai cardinalfish that is associated with jellyfish is dominated by recruit and juvenile. A total of 55.77% (58 individuals) of Banggai cardinalfish found were recruited, 40.38% (42 individuals) were juvenile, and only 3.85% (4 individuals) were adult. Four adults recorded were only found in two jellyfish individuals; one adult of fish was found in one jellyfish while three individuals were found in other jellyfish. Like anemones, the Banggai cardinalfish that chose this microhabitat were dominated by recruit and juvenile phases. The type of microhabitat with many tentacles is important as a refuge from predation, wherein anemones are particularly important microhabitats for newly released and juveniles of Banggai cardinalfish [3, 27]. Almost all predators of juveniles, including adult and sub-adult of *Pterapogon kauderni*, seem to avoid the tentacles among which juveniles often hide and are also protected by anemone resident clownfish when present [3, 5, 16, 27, 28]. Upside-down jellyfish *Cassiopea andromeda* has a morphology similar to anemones which have tentacles-like shaped organs. The absence of anemones at the observation site was replaced by the presence of these jellyfish, which are used by Banggai cardinalfish for shelter, especially for recruits and juveniles [3, 27, 28].

In general, these observations indicate that upside-down jellyfish *Cassiopea andromeda* is important symbionts for Banggai cardinalfish, especially in the recruit and juvenile phases. The absence of anemones, known to be the main choice for the recruit and juvenile phases of the Banggai cardinalfish, has been replaced by the jellyfish for protection (Figure 5). However, information on the relationship between Banggai cardinalfish and jellyfish as their hosts is still needed. Information on whether the season affects the presence of jellyfish, whether there is a difference between the rainy season and the dry season, and other information related to the jellyfish in a given location. Furthermore, it may be necessary to research on a laboratory scale to determine the microhabitat selection of Banggai cardinalfish if anemone and jellyfish are found in one location.
Figure 4. Microhabitat preferences of Banggai cardinalfish observed in Luwuk waters, namely long-spined sea urchin *Diadema* spp. (1-3), branching hard corals *Acropora* spp. (4-6), various sea anemones (7-9), mushroom coral *Heliofungia actiniformis* (10-12), and upside-down jellyfish *Cassiopea andromeda* as a new microhabitat preference (13-15).
Table 4. Banggai cardinalfish associated with jellyfish, indicating the presence of selection in each phase of development.

| Cassiopea andromeda | Pterapogon kauderni |
|--------------------|---------------------|
| Specimen No.       | recruit | juvenile | adult | Total |
| 1                  | 6       | 5        | 3     | 14    |
| 2                  | 8       | 7        |       | 15    |
| 3                  | 9       | 6        |       | 15    |
| 4                  | 5       | 5        | 1     | 11    |
| 5                  | 13      | 7        |       | 20    |
| 6                  | 10      | 11       |       | 21    |
| 7                  | 3       |          |       | 3     |
| 8                  | 4       | 1        |       | 5     |
| 9                  | 0       |          |       | 0     |
| Total of individual| 58      | 42       | 4     | 104   |

Figure 5. A group of recruits and juveniles of Banggai cardinalfish swim between the jellyfish's tentacles for protection.

4. Conclusion
The absence of anemones, known as the main choice for the recruit and juvenile phases of the Banggai cardinalfish *Pterapogon kauderni*, has been replaced by the upside-down jellyfish *Cassiopea andromeda*. The upside-down jellyfish, which physically resemble anemones was important symbionts for Banggai cardinalfish for protection. From the results, further research is needed to determine the mechanism of selection of the upside-down jellyfish as Banggai cardinalfish microhabitats, both in nature and on a laboratory scale.
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