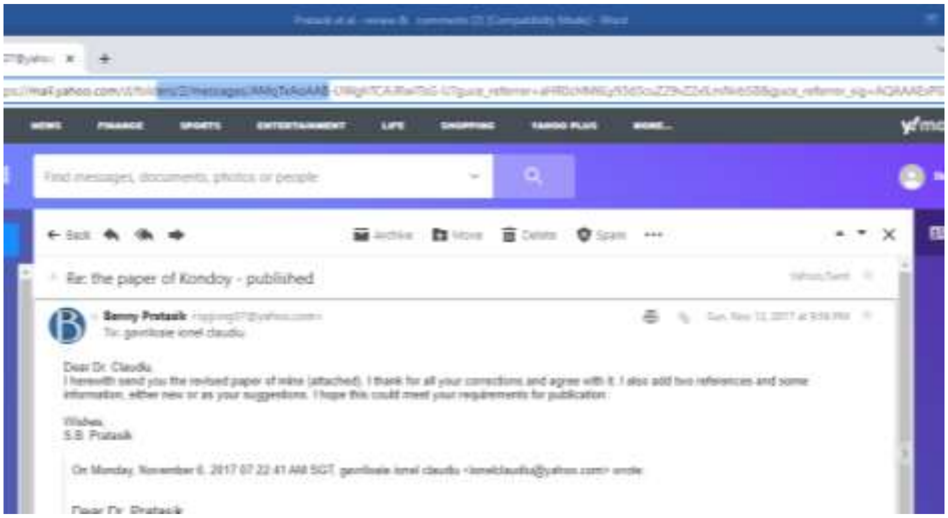

Korespondensi Revisi 1.

Egg placement habitat selection of cuttlefish, *Sepia latimanus* (Sepiidae, Cephalopoda, Mollusca) in North Sulawesi waters, Indonesia

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Abstract. The availability of suitable egg-laying site will support animal's reproductive success. This study was intended to gather information on habitat selection of *S. latimanus* to lay their eggs and to describe possible factors influencing this behavior. This study was carried out in Manado Bay and Lembeh Strait and dive surveys were employed to collect the data. Results showed that there were 8 species of branching corals selected, *Acropora microphthalma, A. brugemanni, Porites cylindrica, P. nigrescens, Hydnophora rigida, Echinopora horrida, Merulina scabricula, and Millepora* sp. Bioactive compounds found in all selected coralswere also possible factors driving this selection.
Key Words: behavior, reproduction, coral, bioactive compound.

Introduction. Habitat availability is crucial for animals to complete their life cycle and to have good population development. It is related with degree of protection, food availability, and survival of youngsters (Litvaitis et al 1994). Habitat use is very important to understand the abundance and the distribution of organisms (Henkel & Pawlik 2005). It is an adaptive behavioral process shaped by multiple cost-benefit tradeoffs, such as food acquisition, risk of predation, reproductive success, etc. (Bastille-Rousseau et al 2010).

Habitat use of a species can reflect the habitat distribution, and it can vary among populations. Therefore, animal populations that occur in the same environment can utilize different habitats or populations in different environments can show the same habitat utilization (Johnson et al 2006). Moreover, different habitat utilization will also reflect geographic variations where the animal population occurs. Habitat distribution can also be an output of population differentiation as a response to the presence of predators or different competitors or the presence of genetic shift among populations (Kie et al 2002) that is possible factor regulating the habitat occupancy (Block & Brennan 1993). High competition and presence of predators cause an individual be able to select different locations of less optimal resources. Once predators are removed, areas of needed resources could be occupied (Rosenzweig 1991). Therefore, habitat selection is an active behavior of the animal, each of which finds environmental performances directly of indirectly related with the resources needed by the animal to reproduce, live and exist. It is also a collection of natural behavior learned from a sustainability of genetic programs (Wecker 1964) – a program that gives initial to behave in a certain way. Hence, initial adaptation to certain environmental signals plays important roles in habitat selection (Morrison et al 1985).

Coral reef ecosystem possesses numerous types of habitats providing food and protection for fish and various marine biota, such as butterflyfish (Chaetodontidae), cardinalfish (Apogonidae) and gobie (Gobiidae) that are more dependent upon coral occurrence than other families and coud directly be affected by loss of corals (Pratchett et al 2006; Wilson et al 2006). In average, 62% of fish species studied have shown abundance decline after 10% of coral cover decreased, and it mostly occurred in coral residents, coral feeders, invertebrate feeders, and planktivores (Wilson et al 2006), and it could be highly correlated with their proportional use of live corals. Therefore, many coral fishes prefer to live near the live corals even though the adults are not dependent upon corals (Jones et al 2004; Feary et al 2007a, b), since they are close to their food, corals or epibiont (Rotjan & Lewis 2008). Many reef fish also utilize coral colonies as shelter from predators. It could explain why many species reflect a response to large-scaled coral loss than predicted based on merely habitat association (Booth & Beretta 2002). Thus, loss of suitable habitats and pressures on factors supporting the inhabiting level of the organisms may be responsible for the decline of coral fish abundance after high loss of corals.

Benthic habitat heterogenity and coral structure complexity affect the composition of fish community and the number of coral fish species occurence (Wilson et al 2007). Increased structural complexity can help mediating competition for space and providing food resources (Munday et al, 2008). Habitat complexity can be considered as variations in habitat topographic structures and measured from relief, crevices, and surface area (Grigg 1994; Beck 2000).

Higher structural complexity of coral reef supports more individuals and fish species than those of lower complexity (Jones 1991; Syms & Jones 2000; Garpe et al 2006; Graham et al 2006). Previous studies found that there is a positive correlation between complexity and abundance (Lawson et al 1999) or biomass (Jennings et al 1996; Grigg 1994) of fish species, and at the community level, habitat complexity is positively correlated with diversity and total abundance (Luckhurst & Luckhurst 1978; Sano et al 1984; Caley & St John 1996; Friedlander & Parrish 1998; Gratwicke & Speight 2005a, b).

Cuttlefish, *Sepia latimanus*, is one of marine organisms utilizing coral reefs as spawning ground and egg placement site. Cuttlefish, as most cephalopods, are a short-lived species and reproduce once in a short period of time at the end of their life (Akyol et al 2011). Their eggs are laid and attached on the seagrass or other objects, and put one by one or in group in different shelter or hard substrates (Arkhipkin 1992). Many species of cephalopods (cuttlefish and squids) come to the coastal waters in group when they are 1-2 years old to spawn and lay their eggs (Hanlon & Messenger 1996). Most molluscs take advantages of chemical cues as social comunication (Boal & Marsh 1998), and chemical attraction to facilitate reproduction (Susswein & Nagle 2004). In coral reef ecosystem, many young and small marine animals benefit the structural complexity of coral life forms for protection from predators.

This study focused on habitat selection for egg placement of cuttlefish, *S. latimanus,* in coral reef habitats, and the information is expected to be able to help promoting conservation effort and population development of the cuttlefish.

Material and Method. This study was mainly concentrated in the coral reefs of Malalayang II, Manado Bay (Fig. 1). Observations on spawning activities of *S. latimanus* were done for about a year, from November 2013 to September 2014, and were conducted using SCUBA dive gear twice a week, so that there were approximately 51 dives, both day and night, done during the study. Occasional observations were also carried out after 2015 up to 2017 through diving activities. The survey applied haphazardous survey technique, and all corals used by the cuttlefish, *S. latimanus*, for egg placement were recorded. Study sites were positioned using a Global Positioning System (GPS).

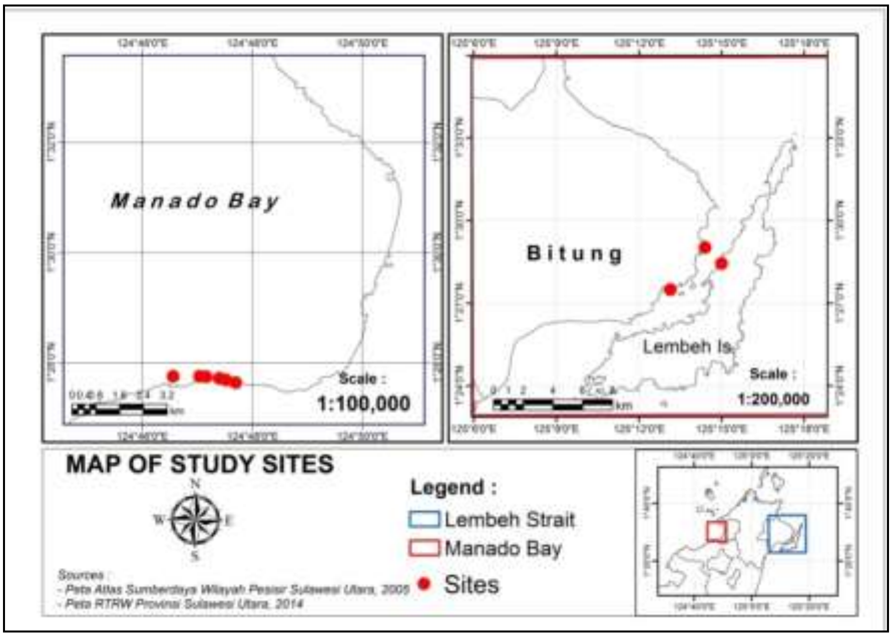

Figure 1. Data collection locations.

There were also some observations in Lembeh strait through free dive adventures in order to gain similar information as comparison. Some pieces of corals were also collected for species identification following Veron (1993). The physical structures of the corals selected as egg placement media were also described. Number of egg clumbs and number of eggs in the clumb were recorded as well. These observations were conducted twice a week for about a year. It enabled to gain information on egg placement activities and spawning season.

Egg placement habitat selection applied Ivlev's (1961) electivity index as follows:

$$\mathbf{E} = \frac{ri - pi}{ri + pi} \qquad (1)$$

where *E* is electivity index, *ri* is proportion of branching coral selected, and *pi* is proportion of the branching coral occurs in nature. The index ranges between -1 and +1, in which negative value indicates avoidance, zero indicates random selection, and positive indicates preference.

Since the distribution of branching corals is not even and patchy, and the area size and number of branching coral species are not the same, data need to be uniformed. Thus, non-parametric statistics was used to compare the utilization level, in which expected value and observation value were used as reference, under an assumption that number of species presence frequencies were the same. Phytochemical analysis of the selected corals was also done using Cannel (1988) in order to gather information on possible coral's bioactive compounds that could drive the habitat selection. These analyses used Dragendorf, Meyer, and Wagner reagents for alkaloid, H_2SO_4 for flavonoid, Liebermann Burchard for steroid, in which the compound presence was indicated with color change, and shaking for saponin (10 min. foam).

Results and Discussion. Organisms need certain habitats to live and develop, and therefore, the habitat should be able to promote partly or entirely their necessities to live and develop. This study found that cuttlefish, *S. latimanus*, utilized 8 species of branching corals for their egg placement consisting of 2 species of genus *Acropora*, *A. microphthalma* and *A. bruggemanni,* 2 species of genus *Porites*, *P. cylindrica* and *P. nigrescens*, 1 species of *Hydnophora*, *H. rigida*, 1 species of *Echinopora, E. horrida,* 1 species of *Merulina*, *M. scabricula*, and *Millepora* sp.

Based on Line Intercept Transect (LIT) survey, branching corals inhabited only 9.88% of total survey area in Manado Bay, and there were 42 colonies of branching corals eligibly selected for egg placement. It means that the branching corals have only very small portion in relation with habitat selection behavior of the cuttlefish, especially *S. latimanus,* for egg placement, and therefore, this condition reflects its high contribution to the continuity of the cuttlefish population.

This finding supports previous studies (Munday et al 2007; Garcia et al 2008) in other different observations on the ecolological interaction between coral structures and marine organisms, in which the complexity of the coral branches holds up the biodiversity and reduce (Coker et al 2009) and help mediating the biological interactions (Holbrook & Schmitt 2002), such as competition and predation. For gobiid fish, big coral colony is preferred since bigger colony size could provide better shelter (Untersteggaber et al 2014). Similar condition was also shown by the cuttlefish, *S. latimanus*, in which bigger coral colonies enabled the cuttlefish to lay more eggs in several different groups of the same coral. Our field observations revealed that more than 4 egg groups were placed inside *E. horrida* (approximately 54 m^2 area) and *M. scabricula* (about 1 m^2 area).

Based on the complexity of the coral structure, this study categorized the feasibility condition of the branching corals as suitable and unsuitable used habitat for egg placement. This classification was based on the capacity of holding the eggs inside their crevices. The distribution of coral species used for egg placement varied with observation sites (Table 1).

Table 1

Distribution of properly used branching corals

Species	*Station*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*Lembeh*
Acropora microphthalma	1	2						
Acropora brugemanni	6							
Merulina scabricula		6			1			
Porites cylindrica					5			
Porites nigrescens		4						
Echinopora horrida		3						
Hydnophora rigida	1	1	3	1	10	3	5	5
Millepora sp.	1							1

There were also many similar species of branching corals not proper to use as egg placement sites distributed in Manado Bay, particularly Malalayang II waters, due to small colony size or low complexity of branch growth. In station 1, we found that 7 colonies of *M. scabricula,* 10 colonies of *H. rigida,* 5 colonies of *E. horrida*, and 50 colonies of *P. nigrescens*. In station 3, there were recorded 8 colonies of *H. rigida*, 1 colony of *Millepora* sp., and 6 colonies of *Porites* sp. improper to use for egg placement. Only one colony of *H. rigida* was found in station 4 in damaged condition, while station 5 had 11 colonies of *M. scabricula* and 15 colonies of *H. rigida* improper to use as egg placement habitat. Moreover, station 6 held also 4 colonies of *H. rigida* improper to use as egg placement site. Despite the presence of proper *H. rigida* touse as egg placement site, the cuttlefish, *S. latimanus*, did not lay eggs in this corals, because this coral occurred on the reef flat that nearly got many disturbances and drought at the lowest tide. At this time, many people come to collect fish or other organisms trapped between corals.

In station 7, there were recorded 1 colony of *H. rigida* and 3 colonies of *M. scabricula* improper to use as egg placement site as well. This condition reveals that coral reefs in Manado Bay are very potential to support early life stages of *S. latimanus* population due to the presence of high number of young suitable branching coral species for egg placement sites. Two of 3 data sampling points in Lembeh strait showed also the occurrence of coral species used by *S. latimanus* for laying eggs, such as *H. rigida* and *Millepora* sp., indicating the potential of Lembeh strait coral reefs as egg placement habitat.

There were a total of 35 selections recorded based on the distribution of coral selected as egg placement site of *S. latimanus*. The occurrence of branching corals used for egg placement habitat of *S. latimanus* reflected that *H. rigida* occupied the highest occurrence in the coral reef of Manado Bay and Lembeh strait, while the lowest proportion was recorded in *A. brugemanni, P. cylindrica* and *Millepora* sp. (Table 2). However, the selectivity index revealed that *A. brugemani, P. cylindrica,* and *Millepora* sp. had the highest preference, while *H. rigida* had the lowest preference.

Table 2

Branching coral occurrence and selectivity

Species	*Occurrence*	*Occurrence (%)*	*No. utilization*	*Utilization rate (%)*	*Selectivity index*
A. microphthalma	8	13.3	2	25	-0.40
A. brugemanni	1	1.66	1	100	**0.26**
M. scabricula	11	18.3	3	27.3	-0.36
P. cylindrica	2	3.33	2	100	**0.26**
P. nigriscencens	4	6.66	1	25	-0.40
E. horrida	4	6.66	1	25	-0.40
H. rigida	28	46.6	23	82.1	**0.169**
Millepora sp.	2	3.33	2	100	**0.26**
	60		35		

Note: Bold indicates preference.

Field observations also exhibited that *S. latimanus* tend to avoid laying eggs in the branching corals that open up or having large-hollowed branches because this condition cause the eggs be easily swept by the wave and removed from the coral branches. The tight complexity of the coral habitat structure seems to provide better shelter to marine organisms, particularly *S. latimanus*. This result is in agreement with fish assemblage study of Menard et al. (2012) that habitat structure complexity is important, and loss of this structure will reduce shelter availability and will impact on the habitat use. Figure 2 demonstrates egg position in the coral crevices. *S. latimanus* preferred to choose small crevices to lay their eggs. Our measurements revealed that mean size of the crevices selected was 0.5-1.2 cm.

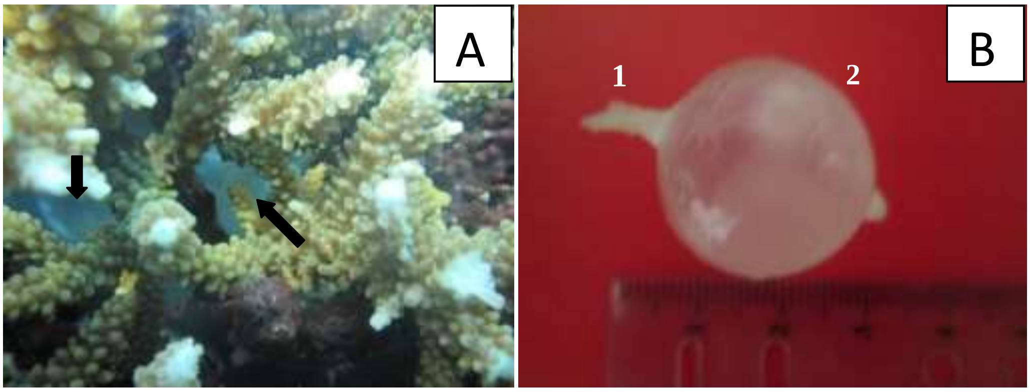

Figure 2. Egg position in branching corals (arrow). A. egg in the coral crevices; B. egg shape: 1) egg part attached on the coral; 2) free moving part.

The egg part attached on the coral helps the egg be inside the crevices and protect from current and wave removals in addition to the crevices as physical inhibitor of the branching coral life forms. The cuttlefish, *S. latimanus*, did not select smaller-sized branching corals, because small colonies do not have sufficiently complex branching structure to maintain the eggs inside the crevices.

Number of eggs laid in each coral colony varied from 21 to 100 eggs/cluster, depending upon the colony size and the availability of branching structures. In bigger colonies, egg placement could be done in several spots of the same colony as recorded in *M. scabricula* and *E. horrida*. Nevertheless, not all proper branching structures are selected by the cuttlefish, *S. latimanus* for egg placement. Our findings revealed that large colony of *E. horrida,* about 9 x 6 m^2, with feasible branch structure for egg placement, was only used once in about 2 years or at least 2 spawning cycles, while several decent big colonies of the same species in other site were not selected, at least during this study.

In addition to the complexity of coral structures, certain coral seletion for the cuttlefish egg placement is influenced by bioactive compounds or nematocyst contained in the host corals. According to Sewell (2007), many sedentary organisms, such as soft corals, anemon, and algae, due to strong space competition in coral reef ecosystem, have to possess certai method to defeat their competitors to grow faster than their competitors around, nematocyst or secondary metabolite release into the water column to inhibit the growth of other species near them. Spine, spicule, andanti-predatory agent are also used to attack and survive through biological interactions in the coral reef ecosystem (Dyrynda 1986).

In this study, 2 colonies of fire corals, *Millepora* sp. (Milleporidae), were selected by *S. latimanus*, to lay their eggs. They look like true corals, but not belong to coral group; they are closer to Hydra and hydrozoa, and possess dactylozoid facilitated with nematocyst holding strong stingability (Borneman 2008). This nematocyst may cause the coral be avoided by other marine biota. Nevertheless, under suitable complexity of the coral branches, the cuttlefish, *S. latimanus* lay their eggs inside the crevices as a safe shelter to predators.

Similar situation could also become the reason for *S. latimanus* to select certain coral species as egg placement sites due to their bioactive compound content. Our present study showed that all these coral species contain some bioactive compounds that allegedly act as defense mechanism against negative biological interactions in nature, such as competition and predation. Phytochemical analyses revealed that all colonies of branching corals selected as egg placement sites, but *Millepora* sp. contained saponin. Alkaloid was recorded in *P. cylindrica, H. rigida, E. horrida, A. brugemanni,* and *A. microphthalma*. Flavonoid was only found in *P. nigrescens* and *A. brugemanni*, and steroid was only found in *A. microphthalma, A. brugemanni, H. rigida, E. horrida* and *M. scabricula.* Thus, these results reconfirm the previous findings (Rocha et al 2011; da Rocha 2013; Sankaravadivu et al 2013; Dyrynda 1986) that inactive or slow moving marine animals contain metabolite compounds used for survival. Field evidence showed that coral *P. cylindrica* released mucus causing ichiness when it was cut (*pers. exp.*). This study also reflects that coral structures and bioactive compounds of the corals provide physical and chemical protection to the cuttlefish eggs. The predators will have to spend more energy to obtain food through predation, particularly the cuttlefish eggs, since predators are inhibited by tight and strong coral branches or avoid any contact with the bioactive substance-containing corals. This condition could also give similar inflammatory effect to predators, so that they tend to avoid contact with or even keep a safe distance from this species.

However, further observations on the previously selected coral species for egg placement revealed that none of them were found holding eggs. It means that there are other factors affecting the egg placement of *S. latimanus*. Field observations showed that spear-gunners also targeted *S. latimanus* in their fishing activities, they might have taken the cuttlefish before they laid eggs.

Conclusions. Cuttlefish, *S. latimanus*, utilized 8 species of branching corals, *A. microphthalma, A. brugemanni, P. cylindrica, P. nigrescens, M. scabricula, E. horrida, H. rigida,* and *Millepora* sp., with preference for *A. brugemanni, P. cylindrica, H. rigida,* and *Millepora* sp. This selection could be driven by crevice size of the corals. This study showed that the eggs had much bigger size than the crevices that could hold the egg inside. The coral selected for egg placement contained several bioactive compounds, such as alkaloid, flavonoid, steroid, and saponin. The availability of suitable size of coral crevices and the presence of these bioactive compounds were believed to influencing factors for egg placement site selection of *S. latimanus.* Nevertheless, no utilization of coral species previously selected for egg placement reflects that other factors may influence the site selection.

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