FISSIPAROUS SEA CUCUMBER (*Holothuria atra*) POPULATION IN NORTH LOMBOK, INDONESIA

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Received: October 2017 Accepted: January 2018

ABSTRACT

Sea cucumbers belonged to the species of *Holothuria atra* are one of the holothurians that could reproduce asexually through fission particularly when their ambient environments are not optimum to reproduce sexually. The reproductive pattern of *H. atra* has been shown to correlate with the condition of *H. atra* population as well as their habitats and environments. This study aims to investigate the asexual reproduction and fission rate of *H. atra* in Tanjung, North Lombok, Indonesia. We conducted surveys in February, May, July, August, September and December 2016 by applying the free collection method. A total of 1,383 individual *H. atra* were collected consisting of 931 intact or normal individuals, 174 anterior individuals, 261 posterior individuals and 17 uncategorized individuals. Fissioned *H. atra* were found in every sampling conducted in the six different months with fission rates range from 5.53 to 30.40%. This research shows that the population of *H. atra* in Tanjung, North Lombok, are susceptible to asexual reproduction (fission). Fission rate value of $> 5\%$ is associated with an adaptation mechanism of *H. atra* to maintain their population in the wild. Further investigation on the environmental conditions stimulating *H. atra* fission is needed to understand the reproduction and population dynamic of this species in Tanjung waters.

Keywords: *Holothuria atra,* asexual, fission rate, population, reproduction.

INTRODUCTION

Holothuria atra is a low economic value species of sea cucumber (holothurians) with promising mariculture benefits. The species is widely distributed in the Indo-Pacific region (Harriot, 1982; Boyer *et al*., 1995) and commonly exported as dried sea cucumbers (Choo, 2008; Kinch *et al*., 2008). Pharmacological studies have revealed that the extract of *H. atra* contains potential bioactive compounds for the pharmaceutical industry. These include 59 compounds (*i.e.*, flavonoids, phenolic components, terpenoids, saponins, alkaloids) that have been extracted from *H. atra* that could proliferative cervical cancer and inhibit herpes simplex viruses (Dhinakaran and Lipton, 2014). The exploitation of *H. atra* has been increasing in Indonesia, Papua New Guinea, Solomon Islands, India and Ecuador (Conand, 2006; Friedman *et al*., 2006). In Indonesia, low economic value sea cucumbers such as *H. atra* cater to local market demands (Natan *et al*., 2016).

With the increased exploitation of wild *H. atra*, maintaining its sustainable population dynamics by the understanding of its reproductive biology becomes key. Among the identified sea cucumbers in Indonesia, 16 species including *H. atra* can reproduce asexually (Dolmatov, 2014). *H. atra* has been widely reported to be a fissiparous species that reproduce asexually by fission producing anterior and posterior (Harriot, 1982; Chao *et al*., 1993; Conand, 1996; Uthicke, 1997; Lee *et al*., 2008; Purwati *et al*., 2009). The body parts resulted from fission can develop as an individual (Purwati *et al*., 2009), therefore highlighting its potential for natural population restocking. Indeed, previous studies have reported that fission is one of the crucial mechanisms in maintaining the population of this species (Uthicke, 2001; Thorne *et al*., 2013; Thorne and Byrne, 2013). Previous works in Indonesia have evident the role of asexual reproduction for the population of wild *H. atra* (Dwiono *et al*., 2008; Indriana and Supono, 2012).

In this study, we investigate the asexual reproduction rate of *H. atra* in Tanjung (North Lombok, Indonesia; Figure 1) with relation to their ambient environmental conditions. Tanjung and its adjacent waters are known to have a suitable ecological condition for *H. atra* habitat. Some anterior and posterior bodies of *H. atra* have been found in the area that could be generated from asexual reproduction. It has been reported that asexual reproduction on sea cucumber is one of the defense mechanisms in response to environmental stressors (Chao *et al*., 1993; Chao *et al*., 1994; Purwati *et al*., 2009; Asha *et al*., 2015). Natural asexual reproduction can be stimulated by environmental parameters (*e.g.*, temperature, salinity) that are outside their optimum range for *H. atra*, as well as population density and other ecological factors (Uthicke, 1997).

MATERIALS AND METHODS

Data collection was carried out six times between February and December 2016 during low tidal conditions (February 9, May 9, July 21, August 22, September 15 and December 7) by applying the free collection method. The individual *H. atra* was defined and recorded based on characteristics described by Boyer *et al*. (1995) with modifications as the following:

Normal
: Specimens with complete organ demonstrating mouth and anus, absent of fission sign or narrowing integument.

Anterior
: Specimens demonstrating mouth (anterior part) but absent of anus.

Posterior
: Specimens demonstrating anus (posterior part) but absent of mouth.

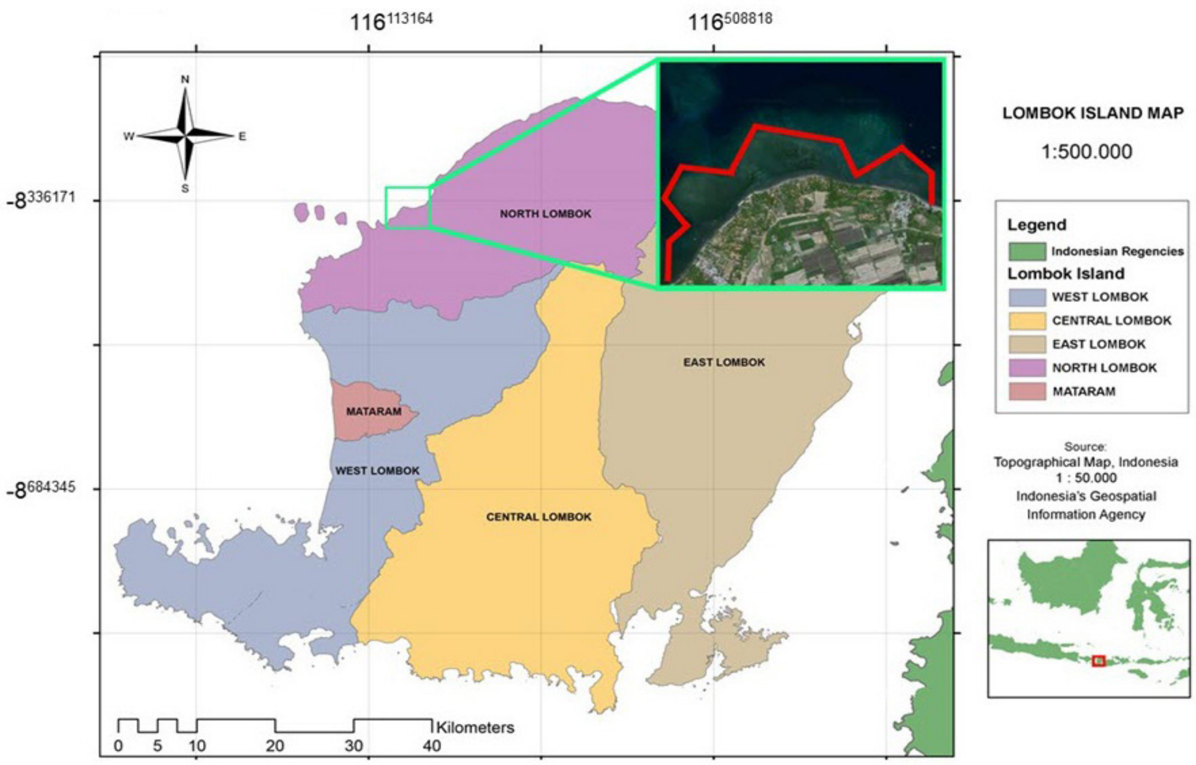

Figure 1. Study location in Tanjung, North Lombok, Indonesia.

Figure 2. Habitat characteristics in Tanjung, North Lombok, Indonesia.

Uncategorized

Specimens with absent or small mouth and anus with fission sign or narrowing integument that cannot be categorized as an anterior, posterior or normal individual.

Fission rate of *H. atra* is determined following Boyer *et al*. (1995) with modifications as follows:

$$F\ (\%) = 100 \times (A+P+U)/2T \qquad \text{(Eq. 1)}$$

where:

- F = Fission rate
- A = Number of anterior individual
- P = Number of posterior individual
- U = Number of uncategorized individual
- T = Total individual

To determine the population distribution, individual *H. atra* is recorded, measured for its length and weight. Each animal is categorized into different groups of length (0-10 cm, 11-20 cm and 21-30 cm) and weight (0-50 g, 51-100 g, 101-150 g and >150 g). Correlation analysis between fission and environmental parameters (*i.e.*, temperature, rainfall) is carried out using the SPSS software.

RESULTS

Habitat Characteristics

Our study site is an intertidal area whose bottom substrates are dominated by sands, gravels and coral rubbles (Figure 2). Seagrass vegetation is dominated by the species of *Cymodocea sp.* Macroalgae are also present in the sampling areas.

Environmental Conditions

Tanjung has sea-surface temperatures ranging between 27-30°C (mean 28.7°C) and a clear difference between wet and dry seasons (Figure 3). In 2016, there were 190 rainy days, consisting of 135 rainy days during the wet season (October-April) and 55 rainy days in the dry season (May-September). In the wet season, the rain was very heavy with an average rainfall of 195 mm per month. In the dry season, the rainfall was lighter with intensities around 58 mm per month.

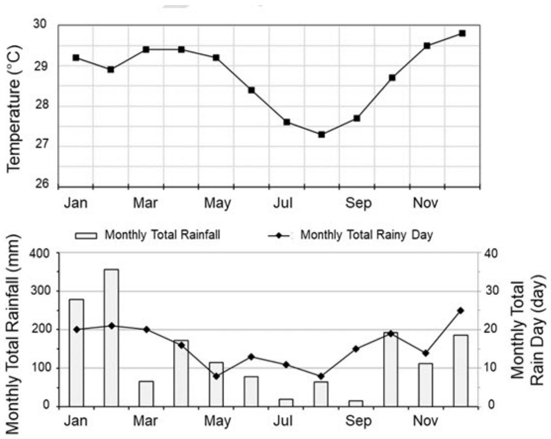

Figure 3. (Top) Monthly sea-surface temperature in Tanjung in 2016. Source: seatemperature.org. **(Bottom)** Monthly total rainfall and rainy day in North Lombok in 2016. Source: Meteorological, Climatological, and Geophysical Agency.

Fission Rate

All individual categories (*i.e.*, normal, anterior and posterior) were found during all of our sampling times with some individuals fall under uncategorized individual. We found 17 individuals that cannot be categorized as a

Table 1. Frequency and fission rate of *H. atra* by asexual reproduction in Tanjung, North Lombok, Indonesia.

Sampling Date	Number of Individual (%)				Total Individual	Fission rate (%)
	N	A	P	U		
9 February 2016	185 ± 88.94	10 ± 4.81	13 ± 6.2	-	208	5.53
9 May 2016	129 ± 38.05	71 ± 20.94	129 ± 38.05	10 ± 2.95	339	30.40
21 July 2016	148 ± 69.48	33 ± 15.50	32 ± 15.02	-	213	15.26
22 August 2016	98 ± 86.73	7 ± 6.19	7 ± 6.19	1 ± 0.88	113	6.25
15 September 2016	290 ± 77.13	37 ± 9.84	47 ± 12.50	2 ± 0.53	376	11.17
7 December 2016	81 ± 60.45	16 ± 11.94	33 ± 24.63	4 ± 2.99	134	18.28
Mean ± SD	155.17 ± 75.60	29.00 ± 23.91	43.50 ± 44.33	2.83 ± 3.82	230.50 ± 106.65	14.48 ± 9.24

N: Normal individual. A: Anterior individual. P: Posterior individual. U: Uncategorized individual.

normal, anterior or posterior individual. This group has an absent or small mouth and anus with fission sign or narrowing integument, therefore labeled as uncategorized individuals. A total of 1,383 individual was recorded during the six sampling times consisting of 931 normal, 174 anterior, 261 posterior and 17 uncategorized *H. atra* individuals. The percentage of normal individuals in the population range between 38.05-88.94%, anterior individuals between 4.81-20.94%, posterior individuals between 6.19-38.05% and uncategorized individuals between 0.53-2.99%. The fission rates in the present study range between 5.53-30.40% (see Table 1).

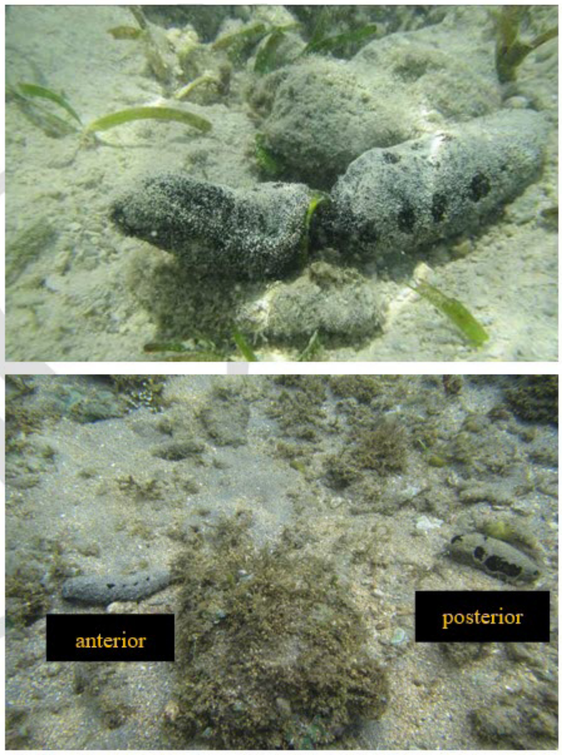

Figure 4. Natural fission process (shown in the top figure) compared to anterior and posterior individuals of *H. atra* produced from natural fission as shown in the bottom figure.

Size Distribution

The population distribution of *H. atra,* intact and fissioned animals vary among different length and weight categories. In general, individual *H. atra* produced from fission in the present study demonstrated less than 20 cm in length and less than 150 g in weight (small fissioned *H. atra*). The medium size of fissioned individuals range from 11 to 20 cm in length and 51 to 150 g in weight. Meanwhile, natural fission is not demonstrated by individuals with more than 20 cm body length and 150 g body weight.

Holothuria atra with body lengths between 0 - 10cm are dominated by anterior and posterior individuals (86 and 97 animals, respectively), suggesting that the fission rate is evident at our study site. The number of fissioned *H. atra* decreases gradually with increased body length. The population of 11 to 20 cm *H. atra* is dominated by the normal individual (811 animals) followed by 45 anterior and 129 posterior individuals. Meanwhile, the population of 21 to 30 cm *H. atra* is dominated by the normal individual where fission is not evident (Figure 4).

A similar pattern is shown for population distribution based on body weight of *H. atra.* The population of 0-50 g *H. atra* is dominated by individuals resulted from fission (179 posterior and 141 anterior animals). The number of fissioned individuals decreases with increased body weight (32 anterior and 78 posterior individuals for the 51-100 g group, 1 anterior and 4 posterior individuals for the 101-150 g group

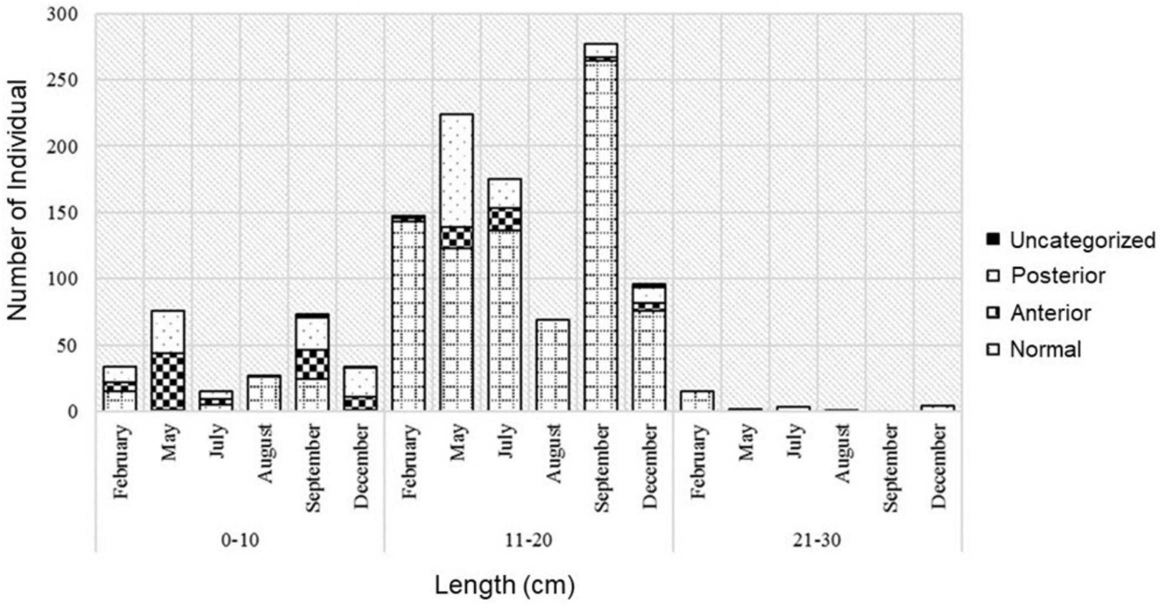

Figure 4. Population distribution based on body length of *H. atra* over different sampling periods.

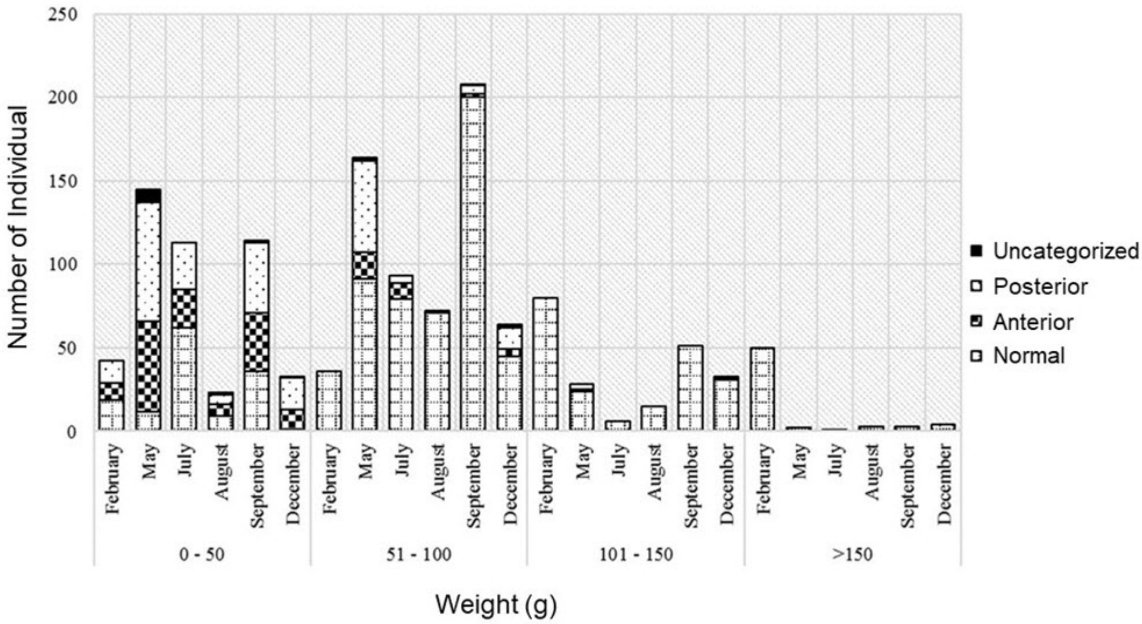

Figure 5. Population distribution based on body weight of *H. atra* over different sampling periods.

and absent of fissioned individual for the >150 g group) (Figure 5).

Effects of Temperature and Rainfall Variation on Fission Rate

Fission rate of *H. atra* in Tanjung, North Lombok is not significantly influenced by either temperature or rainfall variation during our sampling periods. The correlation analysis shows that temperature (r= 0.51, p>0.05) and rainfall variation (r= -0.20, p>0.05) does not demonstrate a strong influence on the fission rate of *H. atra* in our study site (Figure 6).

DISCUSSION

Our study discovers abundant fissioned *H. atra* in Tanjung, North Lombok (14.48 ± 9.24) that varies with seasons. Ecological conditions in Tanjung whose bottom substrate is dominated by sands, gravels and coral rubbles, are suitable for living habitat of *H. atra.* Studies have reported high density of *H. atra* is commonly

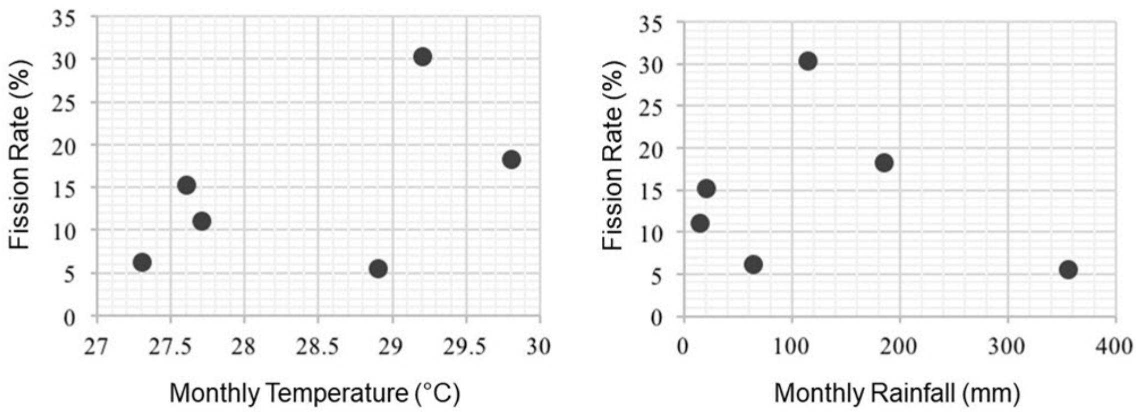

Figure 6. Correlation between fission rate of *H. atra* in Tanjung waters with monthly temperature (left) and rainfall (right).

found in areas with bottom substrate dominated by a mixture of gravel, coral rubber, sand and are covered with macroalgae (Dissanayake and Stefansson, 2012; Asha *et al*., 2015). Similar works on fissiparous holothurians in Pototano (West Sumbawa) and Medana Bay (North Lombok) found fission rates of 18.75% and 1.79-23.68%, respectively (Dwiono *et al*., 2008; Indriana and Supono, 2012). Our data taken between February- December 2016 shows high variations between months. The fission rate of *H. atra* is highest in May and lowest in February. The difference in fission rates from different seasons was also observed by Purwati *et al*. (2009).

Size distribution (body length and weight) data of fissioned *H. atra* in the present study show relatively small values. In comparison, previous studies have reported the size of fissioned individuals is commonly higher than 100 g (Chao *et al*., 1994; Conand, 1996; Utchike, 2001; Lee *et al*., 2008).

The high number of fissioned *H. atra* in Tanjung is possible due to population and ecological adaptation. A mean fission rate of > 5% herein suggests that *H. atra* in Tanjung, North Lombok may be of fission population. This is supported by the absence of normal juveniles during all sampling periods. For this type of population, the asexual mode appears to be more successful than sexual reproduction (Chao *et al*., 1993). The absence of juvenile *H. atra* may also be associated with a low density of mature individuals, therefore inhibiting sexual reproduction. Another possible factor is high temperature and salinity fluctuations in Tanjung waters. It has been reported that the early developmental stage of holothurians is susceptible to high mortality rate caused by environmental changes (Chao *et al*., 1994; Lee *et al*., 2008). Indeed, Dwiono *et al*. (2008) suggest that fission may be one of the strategies in maintaining the population of *H. atra* in North Lombok.

The higher number of posterior individuals compared to anterior individuals in the present study is consistent with previous works showing higher mortality rate in anterior individuals (Boyer *et al*., 1995; Conand, 1996). The difference may be due to unequal fission. Naturally, the point of which fission starts is at the anterior part, 45% from the total body length of sea cucumber resulting smaller size of the anterior individual as compared to the posterior individual (Conand and De Rider, 1990; Chao *et al*., 1993). This leads to different survival rates when fissioned parts of sea cucumber develop as single living individuals.

Another plausible reason related to the difference between anterior and posterior populations in the present study could be due to internal organ distribution of *H. atra.* After fission, posterior part features a complete structure of vital organs such as the respiratory tree, gonad and intestine (Boyer *et al*., 1995). The respiratory tree is an essential organ for obtaining a sufficient oxygen supply during recovery and development after fission process. In contrast, anterior part features mouth, partial gonad and intestine; therefore it requires to regenerate intestine, anus and respiratory tree. Anterior part supplies oxygen through diffusion across body wall when the respiratory tree is not fully developed after fission and under regeneration process (Reichenbach *et al*., 1996). Time needed to regenerate respiratory tree by anterior part is thought to be a significant factor causing higher mortality in anterior population (Reichenbach *et al*., 1996; Conand, 1996).

Individuals showing characteristic of multiple fissions also were found in the present study. This group has an absent or small mouth and anus with fission sign or narrowing integument that could not be categorized as the normal, anterior or posterior individual. Emson and Mladenov (1987) reported that in this group, the body is divisible into three areas or more, and that the product could be detected by external morphology or internal organs such as the presence of unusually small respiratory trees and cuvierian organs.

The variation of fission rate recorded in this study does not show strong correlations with monthly temperature and rainfall variations. This finding is contradictory to the previous research by Uthicke (1997) showing seasonality fission in *H. atra*. This discrepancy could be due to the difference between daily temperature during our sampling days (not recorded) and the monthly temperature data. It is also plausible that other environmental factors may be more dominant to variation in fission rates. It has been reported that fission rate of sea cucumber could be affected by tidal cycle, water depth, water dynamic, temperature, salinity and food availability (Harriot, 1982; Emson and Mladenov, 1987; Chao *et al*., 1993; Conand, 1996; Uthicke, 1997).

CONCLUSION

The fission rate of *Holothuria atra* population in Tanjung, North Lombok Indonesia is relatively high compared to other areas with similar ecological conditions. This suggests that the population of adult *H. atra* combined with local environmental factors during our observation periods are not optimum for sexual reproduction. Further research is needed to investigate survival mechanisms of anterior and posterior body parts to enhance sea cucumber restocking program for the natural population of *H. atra*.

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