Recruitment Pattern of Tropical Limpet *Cellana testudinaria* (Class: Gastropoda, Family: Patellidae) Living on the Rocky Shore of Ohoiwait, Southeast Moluccas, Indonesia

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Abstrak

Pola rekrutmen dari limpet daerah tropik C. testudinaria diestimasi dan digambarkan secara spasial untuk ketiga zona dari daerah intertidal dengan mempergunakan dua metode yang berbeda: menghitung langsung juvenil yang berukuran kurang dari 4 mm dan settlemen dari individu berukuran kurang dari 8.5 mm. Sebanyak 2402 ekor limpet diperoleh dari 12 bulan pengambilan sampel yakni dari Oktober 2001 sampai September 2002. Kedua metode tersebut memberikan hasil yang sama yang mengindikasikan bahwa rekrutmen tertinggi terjadi pada musim peralihan yakni pada bulan Maret dan April.

Kata kunci *: Limpet daerah tropik, Cellana testudinaria, rekruitmen, level pantai, juvenil*

Abstract

The recruitment pattern of the tropical limpet C. testudinaria are estimated and described spatially for the three shore levels of the intertidal, using two different methods: direct counting of juveniles of the size less than 4 mm in length and the settlement of the first < 8.5 mm mid-class juveniles. A total of 2402 limpets were obtained in 12 monthly collections (from October 2001 to September 2002). Both methods yielded similar results, which indicated that recruitment was highest during the transition season (March and April).

Key words *: Tropical limpet, Cellana testudinaria, recruitment, shore levels, juvenile*

Introduction

Recruitment often plays a critical role in controlling the distribution and abundance of marine invertebrate species (Butman, 1987), and it has been identified as a major factor (Meadows & Campbell, 1972; Delany *et al.*, 1998), organizing the general character of rocky shore communities (Bowman & Lewis, 1977). The spatial and temporal arrival and subsequent survival of new individuals is a fundamental process shaping community structure in most habitats (Connell, 1985) and is responsible for the supply of individuals to existing assemblages as well as the re-colonisation of denuded areas or newly created space (Sousa, 1984). In practice, recruitment is defined as the abundance of juveniles, which have survived for a certain period of time (Keough & Downes, 1982).

Recruitment fluctuations are not simply "noise" but can rather be viewed as an adaptive response to an inherently variable environment. Numerous physical and biological factors play important roles in the arrival and subsequent survival of recruits in intertidal assemblages (Hutchinson & Williams, 2001). These include limited local scale processes such as the impact of predators and benthic grazers (Menge & Sutherland, 1987), substrate type and their complexity (Raimondi, 1988), the availability of free space to recruit (Paine & Levin, 1981), non-local processes including oceanic currents (Roughgarden *et al.*, 1987) and change in climate conditions (Barry *et al.*, 1995).

Spatial variation in the recruitment of sessile marine invertebrates with planktonic larvae generally involves four phases: (1) development; including dispersal as a planktonic form, (2) testing of a habitat for suitability, (3) settlement; including attachment to the substratum and metamorphosis, (4) recruitment of juveniles into the population (survival until the organism is counted by an observer). The last phase may last from hours to months (Scheltema, 1974), but it is not a true life-history stage, merely a reflection of the limitation of the observer (Keough & Downes, 1982). The number of organisms passing to the third

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phase is termed *settlement*, while the number passing through the fourth phase, which is a composite of larval and juvenile stages, is termed *recruitment*.

According to Keough & Downes (1982) that it is important to distinguish between settlement and recruitment. However, although these phases are differentially susceptible to biotic and abiotic influences, many studies of recruitment have not discriminated between phases leading to an over- or under-estimation of the influence of certain factors in limiting the subsequent distribution and abundance of adults (Pulfrich, 1995).

Smith (1935) found that newly-settled juveniles of the common patellid limpets are 0.2 mm in size. In this study, it was not possible to sample this size class quantitatively, hence the specific aim of this research is only to study the recruitment pattern of tropical limpet *Cellana testudinaria* with particularly concerned to the limpet of < 4 mm in size.

Materials and Methods

Study site

The study was carried out on the intertidal rocky shore of Ohoiwait (latitude 5°45'15" S, longitude 132°57'20" E), Big Kai Island, Southeast Mollucas, Indonesia (Fig. 1) from October 2001 to September 2002. The intertidal region investigated is about 0.25 km^2 (1 km long and 0.25 km wide). Thus, it is small enough that atmospheric conditions may be assumed, for most purposes, to be uniform over the whole area. The shore consisted of shingles, pebbles, medium and big boulders. The physical conditions in the tidal zones are quite different; the higher shore is wetted almost exclusively by tidal sea level rise, but the lower shore receives considerable wave action.

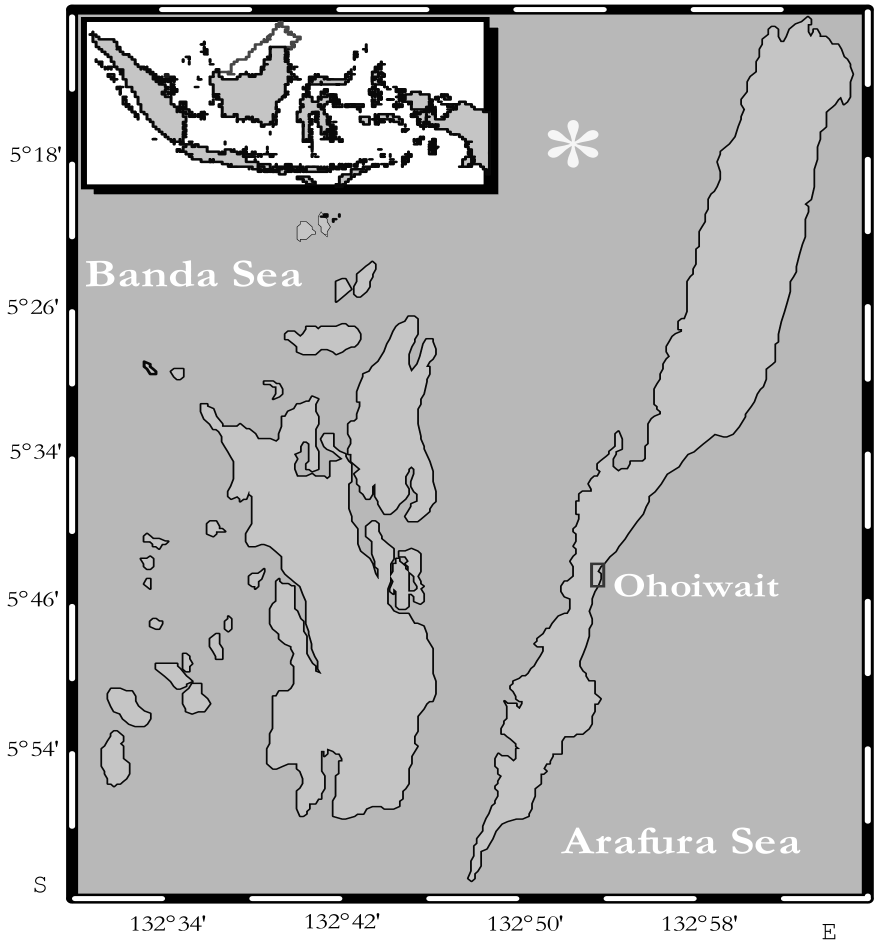

Figure 1. Map of study site of Ohoiwait located at the Big Kai Island

Sampling

Standard quadrate sampling technique was applied in order to obtain a rough estimate of the monthly patterns of juvenile dispersal and recruitment. A sequence of 9 quadrates (1x1 m) was exposed randomly to the three different shore levels in which 3 quadrates were fixed per shore level. All individuals *C. testudinaria* of < 4 mm length in each frame were counted. Allocation of quadrates was done haphazardly by tossing the sample devices to the shore, since true randomisation by using the random number tables or grids is often difficult to realise in the field.

Even though, investigations of the recruitment of juvenile limpet should consider the relative roles of initial settlement patterns and differential post-settlement, the organisms are unlikely to be detected immediately, hence the total numbers of settling could not be determined accurately. Consequently, more precise information on the intensity of recruitment of juveniles was gained from the number of recruits of < 4 mm in length found at any time during the following year. These were done by searching regularly and intensively for the presence of juveniles and these specimens were taken as the measure for estimating the actual "recruitment". This method allows only minimum estimates of the numbers of recruitment because a sampling problem arose from considerable variation in limpet size. Here, the larger initial size of 4 mm in length of recruits and conspicuous animals was considered, which may be attached or stationary on the substratum.

Analyses

The recruitment pattern of *C. testudinaria* populations was estimated by 2 methods: (1) direct counting of juveniles of the size at recruitment (< 4 mm in length), where recruitment means entering the population that can be sampled or caught and not necessarily the size at settling. Spatial and temporal variation in recruitment densities on the three shore levels were analysed using a mixed model ANOVA with the differences in shore level and sampling date as experimental factors (Morrisey *et al.*, 1992; Underwood, 1997). (2) The settlement of the first < 8.5 mm mid-class juveniles perceiving data for the growth study was facilitated by a recruitment-based approach method with the aid of computer software FiSAT (Sparre & Venema, 1998). This routine reconstructs the recruitment pulses from a time series of length-frequency data along a trajectory defined by the von Bertalanffy growth formula (VBGF) to determine the number of pulses per year and the relative strength of each pulse. The pattern would be decomposed automatically by the integrated program NORMSEP and the peak recruitment fitted with up to two Gaussian distributions (Moreau and Cuende, 1991).

Statistical analyses

A *t*-test of variance was used to test whether the number of recruited limpets estimated by both methods are not different during the whole experimental period of 12 months. Therefore, each pair of data from the monthly sampling was treated as an independent variable. This test has the advantage of having a greater power of detecting a real difference and is useful for small samples where the true variances of the two populations are assumed equal (Bakus, 1990).

As the shore is not a uniform environment, the time when conditions favour spat survival must vary with habitat as well as season (Bowman & Lewis, 1986). For hypothesis testing that the recruitment rates may be different between the dry and rainy seasons, the post hoc *R X C G*-test of independence (Sokal and Rohlf, 1995) was chosen. Such a test would examine whether the recruit densities exhibited by the margin totals are independent of the seasons at which the individuals were sampled. Of interest was whether the percentage of recruits changed significantly during the time of observation. I therefore tested whether the proportion of recruitment is independent of the time of collection. Recruit densities of both methods for the three shore levels were summed according to the season; June to August represent the dry season, September to November represent the second transition period, December to February represent rainy season, and March to May represent the first transition period. In this analysis, however, it should be noted that I did not consider the kind of factors affecting the recruit densities itself but only focused on whether the number of recruitment was different among the seasons. Even though this test only tested the differences among the season, it was useful to differentiate the result in which interpretation could be made (Sokal and Rohlf, 1995). Subjecting the result from *R X C G*-test to whether the differences between successive points are significant, the Student-Newman-Keuls (SNK) test was used additionally with the aid of computer program SPSS-10 to give the status of variance according to which analysis is used (Sokal and Rohlf, 1995).

Results and Discussion

Estimation by direct counting of recruits

The numbers and timing of juvenile *C. testudinaria* recruited at the three shore levels are summarised in Table 1 and are shown in Figure 2. Judging from the figure, it is suggested that main recruitment events could be observed throughout the year by following curve trends. New juveniles were observed abundantly on the three shore levels during the periods of rainy season (January – February) following higher settlement of juveniles occurring in April (± 4.78 ind.m^{-2}). However, in the beginning of March, juvenile limpets were almost totally absent. From May to August (at the end of transition to the dry season), the recruits decreased constantly to around 1 ind.m^{-2} following the spawning time. During the period of September to December, the settled population remained relatively stable. A marked rejuvenation of the limpets at the three shore levels occurred in late February, followed by a second, less pronounced, recruitment in June (Fig.2).

Table 1. *Cellana testudinaria*. Number of recruits in 9 quadrates on the three shore levels

Months	Number of recruits			Density
	High	Middle	Low	(ind.m^{-2})
Oct'01	8	7	3	2.00
Nov'01	7	6	0	1.44
Dec'01	6	4	2	1.33
Jan'02	7	6	4	1.89
Feb'02	8	8	4	2.22
Mar'02	3	1	0	0.44
Apr'02	20	15	8	4.78
May'02	8	5	1	1.56
Jun'02	11	5	0	1.78
Jul'02	4	1	1	0.67
Aug'02	1	1	0	0.22
Sep'02	6	5	1	1.33

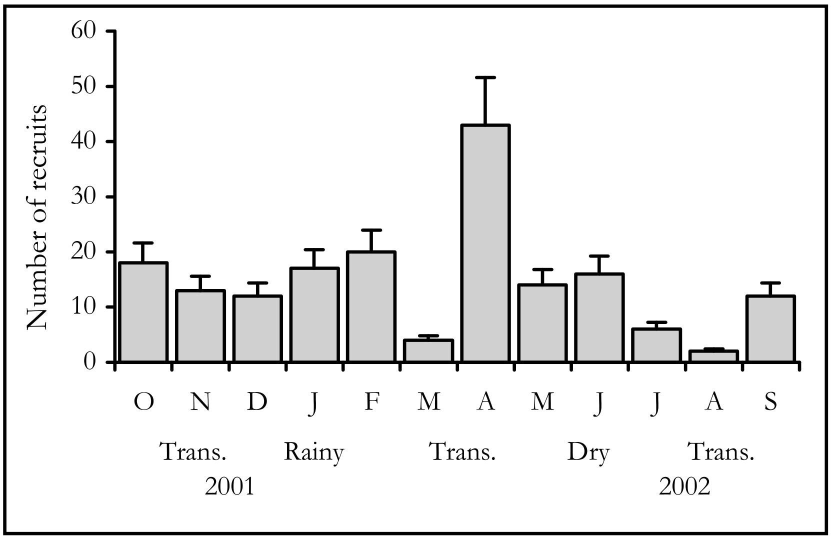

Figure 2. *Cellana testudinaria*. Abundance of recruits on the three shore levels. Nine quadrates of 1 m^2 in size were sampled approximately monthly. Error bar is ± 1 SD.

From regular monitoring of the numbers of juveniles settling at the three shore levels, it was determined that average recruitment observed during the dry season was lower than during the rainy season. Heavy settlements of juvenile limpets were found at the high shore level, those were concentrated on top and below big boulders as well as medium boulders in and around the tide pool.

Estimation of recruitment by FiSAT

The monthly percentages of *C. testudinaria* recruits estimated by FiSAT based on the individual lengths of < 8.5 mm are shown in Figure 3. A decrease in the recruitment occurred in the period between January and September. Although some rejuvenation of limpets were observed during this period, recruitment levels remained weak throughout the year. The expected settlement peak of 17 % occurred in March rather than in April as estimated by the first method. This reflects the fact that, when considering the parameters L_{∞}, *K*, and t_0 on the von Bertalanffy equation, then it can be concluded that the population has already 2 – 3 months old on the shore. Hence, if the time is set arbitrarily to January 1st and the recruitment time is counted down, then the estimated time of real recruitment occurs in March. It also can be seen from the figure that the trends of the peak recruitment did not follow any seasonal pattern. However, the curves represent the period of heavy recruitment, in turn, proposed that the average recruitment on the periods of dry season was lower than on rainy season.

A consideration of the analyses of regular monthly recruitments indicates how synchronous recruitment pattern is between both techniques (compare Fig. 2 and Fig. 3). A *t*-test of variance of the proportion of juveniles in samples both methods showed that there was no significant difference ($t = 0.43$, $P > 0.05$) in the numbers of recruitment per month (Table 2). This confirms that either direct-counting method or FiSAT would give the same result in estimating recruitment rates.

Substantial increase and decrease noted in juvenile numbers of limpets following recruitment peaks indicate that recruitment gains are also strongly affected by the conditions on their natural habitat. The juvenile abundance data collected at the three shore levels therefore were found to be different during monthly sampling. The result of *R* X *C* *G*-test confirmed that the number of recruitment was strongly dependent on the seasons (*G*-value is highly significant at $P < 0.05$). Subjecting this result to SNK test, confirmed that either the dry season differs from the rainy season or the period of first transition differs from the second transition (Table 2).

The present study highlights the fact that recruitment of *C. testudinaria* is highly variable within the intertidal rocky shore of Ohoiwait. At any point in time, recruitment to areas of the differencet shore level may differ in the density of new recruits. Both methods of estimating recruitment (direct counting and size-frequency distribution by FiSAT) yielded similar results. Recruitment patterns varied broadly throughout the three shore levels in dry and rainy

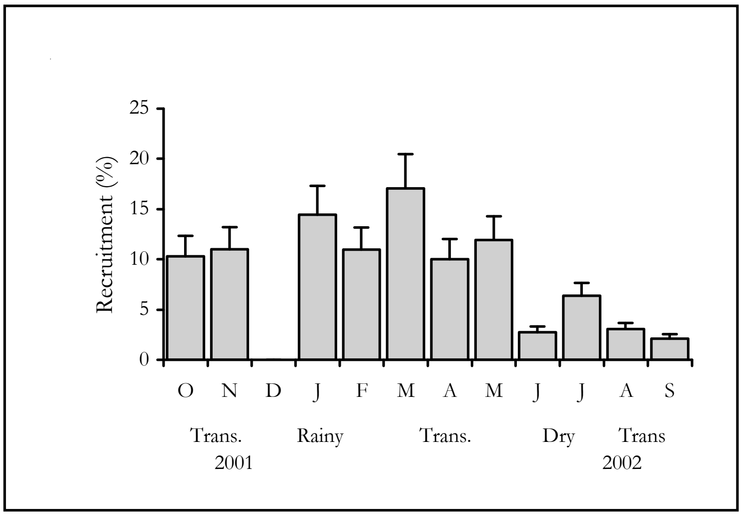

Figure 3. *Cellana testudinaria*. Percentage of recruits estimated by FiSAT. Numbers are based on the individuals of < 8.5 mm mid-class of juveniles. Error bar is ± 1 SD.

Table 2. *Cellana testudinaria*. *G*-test of in-dependence, together with the *SNK*-test. The calculation of these values are presented below.

Computation:

1. Sum of transforms of the frequencies in the body of contingency table
= ΣΣ f ln f = 49 ln 49 + 40 ln 40 + . . . + 62 ln 62
= 1482.6428
2. Sum of transforms of the row totals
= Σ (Σ f) ln (Σ f) = 69 ln 69 + . . . + 105 ln 105
= 1633.2153
3. Sum of transforms of the column totals
= 177 ln 177 + 200 ln 200
= 1975.8420
4. Transform of the grand total
= 377 ln 377
= 2236.4564
5. G = 2 [quantity 1 – quantity 2 – quantity 3 + quantity 4]
= 2 [1482.6428 – 1633.2153 – 1975.8420 + 2236.4564]
= 220.0838
6. William's correction
q = 1 + [(2+1)(4+1)]/6(377)
= 1.0066
G_{adj} *= G/q*
= 218.6408

Since $\chi^2_{.05[3]}$ *= 7.81 , the G – value is significant at P << 0.05, that the recruitment pattern is dependent of season.*

G – test of independence

Season	***by sampling***	***by FiSAT***	***Totals***
Dry	49	40	69
Transition (Sep-Nov)	61	78	139
Rainy	24	20	44
Transition (Mar-May)	43	62	105
Totals	***177***	***200***	***377***

Student-Newman-Keuls (SNK) – test

Season	*N*	***Subset for alpha = .05***
Dry (June – August)	2	44.5
Transition (September – November)	2	69.5
Rainy (December – February)	2	22.0
Transition (March – May)	2	52.5
Sig.		.072

seasons, with the greatest percentage cover of recruits occurring in April (estimated by direct counting, Fig. 2) and in March (estimated by FiSAT, Fig. 3). The substantial difference in the peak of recruitment between both methods was presumably because of the difference in size at recruitment. Initial size at recruitment used by the first method is the overall individual limpet with shell length of < 4 mm whereas by FiSAT method is 8.5 mm in length.

The availability of recruits at the time when suitable conditions exist for their settlement and survival is paramount (Sousa, 1984). The low rates of recruitment among habitats during the periods of sampling may be either the result of different settlement of larvae into areas, or something killing the settlers before they became visible and could be censuses. The last hypothesis seems unlikely because the limpets were observed to recruit at densities of up to 4.78 ind.m^{-2} at the three shore levels. It is more likely that some process during the settlement phase was reducing the numbers recruiting to the areas. The observed spatial variation in density of settled spat may result from differential survival (Butman, 1987), but the comparatively low larval abundances and associated with high mortalities suggest that redistribution and accumulation of settlers is more likely to be responsible for observed recruitment patterns (Pulfrich, 1995). The direct effect of differences in the rates of recruitment within different habitats on the population dynamics of a species has also been reported for *Patella vulgata* (Workman, 1983), *Acmaea scabra* (Sutherland, 1970), *Notoacmaea petterdi* (Creese, 1980), and *Cellana tramoserica* (Fletcher, 1984). As with most other studies of marine invertebrates, the phenomenon of settlement was not observed; therefore, I could not distinguish between these two alternatives. The other consideration that may be applied to the population of limpet *C. testudinaria*; it is possible that this species may be came from other populations from different shores and recruited into the areas. However, in order to confirm this assumption, it is important to consider the magnitude and extent of larval exchange between the populations of other shores, but I did not cover it in the present study.

The success and intensity of recruitment at the shore varied seasonally: fewer recruits became juveniles in dry season than during the cooler rainy season. Even though there have been relatively few studies on settlement and recruitment of sessile species in tropical regions (Walters *et al.*, 1996), previous studies have shown that survival of recruits during summer is low, because some parts have increased levels of physical stress (Kaehler & Williams, 1996). When spat first appeared on the shore in early December, good recruitment usually followed. In poor recruitment periods, spat were not found on the observation shore until the following transition period in March, and were often stunted and atypically coloured. Though there is probably an element of truth in this explanation, the correlation between variability in seawater temperature and salinity and recruitment success was built up. Determination of the effects of seawater temperature and salinity on the recruitment of *C.testudinaria*, using data available, has been hindered by a succession of poor recruitments, so field experimental studies are still lacking. However, analyses in progress with known differences in seawater temperature and salinity regimes during different seasons are indicating that this species is more susceptible to temperature and salinity variation.

Such a widespread recruitment succession during the rainy season, and its being optimal coincided with the temperature and salinity between ca. 31-32 °C and 33-34, respectively. However, heavy recruitment following the period of transition in April, suggests that recruitment intensity is not governed by temperature alone. Bowman and Lewis (1986) studied the effect of geographical differences in breeding cycles on the recruitment variation of the limpets *Patella vulgata* and *Patella aspera* found that the cause of these differences could be correlated with geographical and annual differences in seawater temperature over the potential breeding periods. The spawning period coincides with the proposed temperature "window" optimal for spat settlement and survival, and it appears that the main spawning time for each region occurs during autumn and winter when mean seawater temperature drops below 12 °C.

Although recruitment pattern varied between different seasons and shore levels, there was no evidence to suggest that variation in environmental variables within the shore examined at intertidal of Ohoiwait consistently, or predictably, affected juvenile *C. testudinaria* recruitment patterns. Several factors of the life history during the planktonic phase of the species are not yet quantified, and some aspects of limits to their pre- and post-settlement have not been tested by experiments or observations in the field. It is, therefore, impossible to make coherent general statements about many of the factors influencing the recruitment of this species.

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