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Original Article

Wave exposure changes reproductive output and timing on *Padina boryana* Thivy (Dictyotales, Phaeophyceae)

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Abstract

Different environments can affect the reproduction on marine organisms. Along the coastline, wave action is seemed to be the strongest stress factor that influences seaweed populations. Seaweed can grow under two different conditions of wave action. The life phases, reproduction and quantity of reproductive cells were recorded for each individual in *Padina boryana*. Both populations had the highest percentage of reproduction in May and dominance of sporophytes. The number of spore and time of production were different at the two sites. Increasing temperature, day length and emersion time enhanced desiccation and high temperature influencing the spore production and maturation of life phases at both locations. More wave exposure decreased the number of reductive cell and changed the pattern of production time in difference scenarios. Energy allocations can play an important role for explaining the cycle of reproduction time in a year on different populations under different wave exposure.

Keywords: *Padina boryana*, Phaeophyceae, population dynamics, reproduction pattern, wave exposure

1. Introduction

Physical environments govern the structure of tropical coastlines. Especially, the intertidal zone of shoreline is exposed directly to wave action, enhanced by high temperatures and desiccation as co-factors of the stressor.

Reproduction is the key parameter of population structure for survival measuring. There are many reports on the environment factors influencing on the population. The relative proportion of phases is one of the obvious biological parameters reflecting environment factors. In *Macrocystis pyrifera* (Linnaeus) C. Agardh, for example, gametophyte production was enhanced by decreased temperature. Also, the changes of sex of gametophytes was determined by the temperature in *Laminaria ochroleuca* Bachelot de la Pylaie and the cell development of gametophytes. (Izquierdo *et al.*, 2002)

Seaweeds have wider distribution in many habitat areas. They have been influenced by many different factors. Therefore, the adaptation would shape the population in each particular area making it fit for survival. Seaweeds have haploid-diploid alternation life cycles with differences in dominance of haploid and diploid phases. The differences of proportion of sporophyte and gametophyte phases may reflect their responses to habitat conditions (Perrot *et al.*, 1991); for example, gametophytes dominated in *Mazzaella flaccida* (Setchell & N.L. Gardner) Fredericq (Thornber and Gaines, 2004) and sporophytes in *Gelidium pusillum*

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(Stackhouse) Le Jolis (Prathep *et al.*, 2009) and in the brown algae, *Dilophus okamurae* E.Y. Dawson (Hwang *et al.*, 2009) and *Padina sanctae-crucis* Børgesen (Liddle, 1975).

The genus *Padina* has a worldwide distribution in tropical and subtropical climate zones. *Padina boryana* Thivy is one of the most common species along the coasts of Thailand and other sites in South East Asia (Teo and Wee, 1983; Verheij and Prud' homme van Reine, 1993; Lewmanomont and Ogawa, 1995). It forms extensive communities in intertidal and shallow subtidal zones in many provinces in Thailand (Wichachucherd and Prathep, 2013). *Padina* is classified to be the member group under the order Dictyotales showing the general character of apical growth, flattened parenchymatous thalli and hairs on the thallus surface (De Clerck *et al.*, 2006). *Padina*, like all other genera in the order Dictyotales, exhibits an alternation of generations life history with three possible isomorphic thalli: a sporophyte and two gametophytes (male and female) which can only be distinguished after reproductive cell formation. However, sporophyte phases are more common (Liddle, 1975; Ni-Ni-Win *et al.,* 2008). The reproductive patterns and life history of tropical *Padina* have not been studied. Therefore the physical and biological factors that regulate the development of *P. boryana* are not well understood. Since it grows rapidly and forms reproductive cells throughout the year (Wichachucherd *et al.*, 2010), *P. boryana* is a good model subject to study year round in order to assess its reproductive potential and the factors that influence this potential. In our study, we hypothesized that wave exposure could influence the reproductive pattern of *P. boryana*.

2. Materials and Methods

This study was carried out at two field sites in Phuket Province in the southern part of Thailand. The exposed area was part of the Sirinart National Park (SNP; 8°5'11.98" N, 98°17'3.10" E) on the northwest side of Phuket; the sheltered area was at Tang Khen Bay (TKB; 7°48'37.56" N, 98°24'15.92" E) on the southeast side. There was plentiful dead coral and rock substrate. Both are rich intertidal areas supporting sea grasses, sea cucumbers, mollusks, crustacean and various macroalgae (Wichachucherd *et al*., 2010).

All the possible physical factors that could influence the *Padina* phenology at the two sites were documented. Salinity was measured by a salinity refractometer (model XHO RHS-10ATC, Huake Instrument Co. Ltd., Zhejiang, China) and air and seawater temperature was measured by thermometer in the field at both sites on the collecting day in each month of the research. The time of direct light that *Padina* thalli were exposed to was determined each month. Rainfall, day length, the average temperature and the hours exposed to the air were obtained from the Hydrographic Service Department of Thailand (www.tmd.go.th). The study began in September 2005 and was completed in August 2006.

To compare the reproductive potential and life phases of *P. boryana*, each month approximately one hundred and eighty samples were collected at random over 120 m in the littoral zone. Samples were collected and sorted into sporophytes, male or female gametophytes in the laboratory. The percentage of fertile specimens, the percentage of sporophytes and gametophytes were calculated. The numbers of spores on the sporophytes were estimated.

2.1 Identification of life phases and reproduction measurement

Padina boryana has a haplo-diplontic isomorphic life cycle; the sporophytes and gametophyte are identical when they are infertile. The fertile sporophytes and gametophytes can be distinguished by sori made up of aggregations of minute dark spots in concentric rows mostly on the lower surface of the incurved blade. Preliminary investigations showed that the size of tetrasporangial, oogonial and antheridial sori was 0.01-0.02 mm, 0.008-0.013 mm, and 0.008-0.01 mm wide, respectively. Sporangia are elongate and larger than the spherical oogonia which are packed more tightly. Antheridia were difficult to detect by the naked eye. They are colorless and must be identified by a dissecting microscope.

To compare the potential of reproduction and the difference of life phases of *Padina*, the calculation was presented as percentage value due to the different number of sample collection in each time. All specimens were checked through the stage of mature by presence of reproductive line. A number of mature specimen was calculated as reproduction percentage with all samples and then a mature plant would be examined either of tetrasporangia, female gametangia or male gametangia. The number of sporophytes and female and male gametophytes were counted separately and calculated for percentage of each life phases comparing to the total of mature specimens. The percentages of life phases in each month and the relative amounts of each phase and the number of spores were calculated for each habitat.

2.2 Calculation of number of spores

Preliminary observations showed that there were different distribution patterns of tetrasporangia sori on a blade surface depending on the age of sporangial sori and also thallus. The sori are basically uniform in the number of sporangia per area. Therefore, the type of sporangia arrangement was categorized and the constant number of tetrasporagia of each type of reproductive row was used to calculate for the entire length of reproductive row on blade surface. To find those constant numbers, the average number of sporangia for each reproductive row in different patterns of reproductive sori distribution was calculated based on a 1-cm^2 area for each type of the 30 thalli.

The tetrasporangia sori arrangements can be categorized as five types below (Figure 1 to 5) with the different mean of number of sporangia sori. The number of sorus varied as expected: *Type 1*: a loose arrangement of small

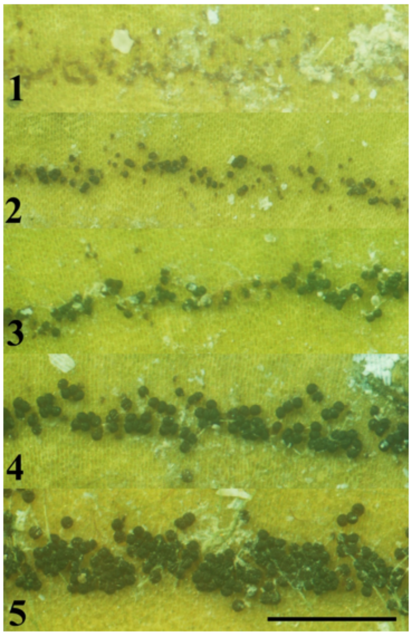

Figure 1 to 5. Sporangium arrangement on the surface of the blade. Scale bar = 0.1 mm:

1) Type 1: a loose arrangement of small sporangium size (0.004-0.007 mm.); 88±7 sporangia.

2) Type 2: a dense arrangement of small sporangium size (0.004-0.007 mm.); 124±7 sporangia.

3) Type 3: a dense arrangement of mixed sporangium size (0.004-0.01 mm.); 93±5 sporangia.

4) Type 4: a loose arrangement of big sporangium size (0.008-0.02 mm.); 101±4 sporangia.

5) Type 5: a dense arrangement of big sporangium size (0.008-0.02 mm.); 152±9 sporangia.

World-Wide Electronic Publication, Thai Meteorological Department, Thailand. Available from: http://www.tmd.go.th. [October 29, 2006].

sporangium size (0.004-0.007 mm.); 88±7 sporangia, (mean± SE). *Type 2*: a dense arrangement of small sporangia size (0.004-0.007 mm.); 124±7 sporangia. *Type 3*: a dense arrangement of mixed sporangia size (0.004-0.01 mm.); 93±5 sporangia. *Type 4*: a loose arrangement of big sporangia size (0.008-0.02 mm.); 101±4 sporangia. *Type 5*: a dense arrangement of big sporangia size (0.008-0.02 mm.); 152±9 sporangia.

To calculate the number of spores using constant numbers, each reproductive row of a specimen was identified a type of tetrasporangia arrangement for matching with the constant number. A reproductive line was measure the entire length in centimeter and then multiplied by which constant number and after multiplied with four for the relative number of spores that thallus could produce.

2.3 Statistical analyses

Two-way ANOVA was applied to compare the percentage of reproductive plants, the percentage of each life phase (sporophytes and male and female gametophytes) and to compare the differences in the numbers of spores produced, among months and between the two sites. The data were tested and transformed (if necessary) to meet the normal distribution assumption (Zar, 1984). Stepwise multiple regressions was employed to test the effects of physical factors such as seawater temperature, air temperature, salinity, rainfall, time exposed to air and day length on the biological variable data (Dytham, 1999). All data were analyzed using SPSS for Windows version 11.0.

3. Results

3.1 Proportion of haploid-diploid phases

Difference of wave exposure did not change the proportion of phases and the peak of reproduction in a year. The two populations of *P. boryana* of SNP and TKB had similar trends in reproduction pattern (p>0.05) with slight differences in the percentage of reproduction (SNP; 35.88±4.20% and TKB; 28.52±3.37%). Mature plants were found throughout the year at both sites with more than 50% reproduction. The peak percentage of reproduction at both sites started in May (SNP; 76±9% and TKB; 66±10%). There were significant differences (p<0.05) between months with both high reproduction during a time of year and low reproduction in other months at both sites. The period of high reproductive thalli was May-August 2006 for SNP and May-June 2006 for TKB. The other months showed low (10-45%) the reproduction (Figure 6).

The populations at both sites also showed the same trend of percentages of the various life phases. Sporophytes were most abundant throughout the year with only a few female gametophytes in particular months at both sites (Figure 7). Female gametophytes were observed in several months at TKB whereas they were found in fewer months at SNP. SNP and TKB were not significantly different (p>0.05) in percentage of gametophytes. However, SNP had a significantly higher (p<0.05) percentage of sporophytes than those of TKB, 32.13±3.71% and 21.30±2.56% respectively (Figure

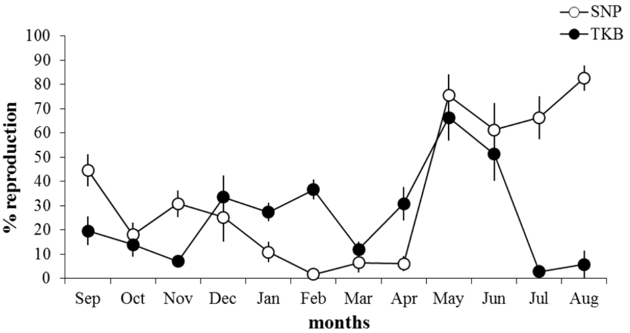

Figure 6. Comparison of the percentage of reproduction from September 2005 to August 2006 at Sirinart National Park and Tang Khen Bay.

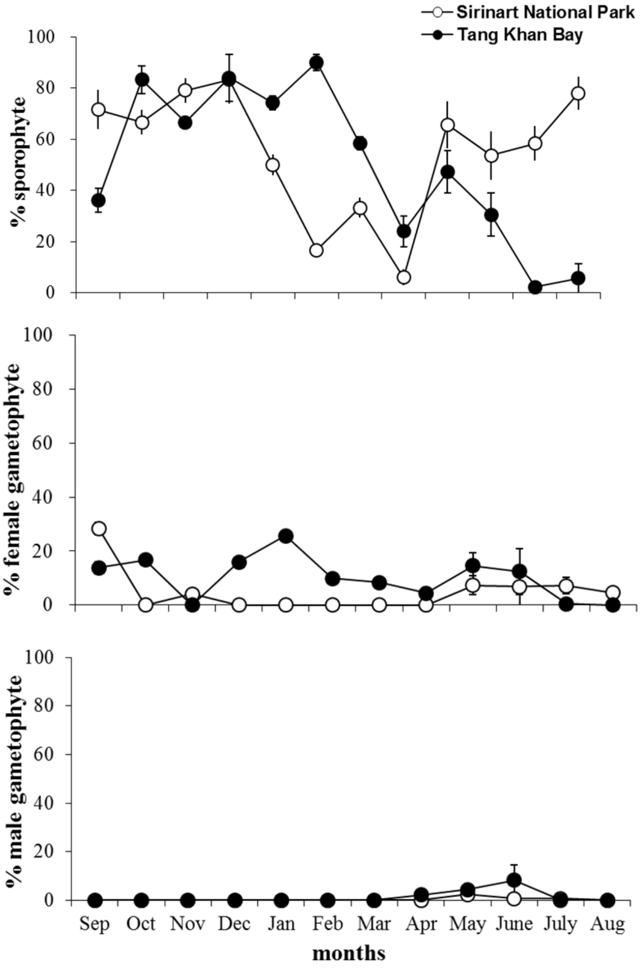

Figure 7. Summary of percentages of each life stage-percentage of sporophyte (top), percentage of female (middle), and male gametophyte (bottom) varied throughout the year from September 2005 to August 2006 at Sirinart National Park and Tang Khen Bay.

7). The percentage of male gametophytes was even lower and were observed only three times during this study (Figure 7): May (2%) to June (1%) to July (1%) 2006 in SNP, and April (2%) to May (4%) to June (8%) 2006 in TKB.

3.2 Reproductive cell production and time of production

The reproductive potential is a function of the number of spores and gametes produced. Since the sporophyte generation was overwhelmingly dominant at both SNP and TKB, the data reflect the reproductive potential based only on spore production. The data of number of spore and time of production thought a year were indicated that wave exposure affected on the differences of number of spore and time of production between two locations.

The average number of spores varied throughout the year and showed significant differences at both sites (Figure 8, $p<0.05$). TKB produced an average number of 496,296 spores and SNP 258,657 spores. Throughout the year, there was a significant difference in spore production among months at both sites ($p<0.05$). TKB had peak of spore production in October (83,140±47,659 spores), February (93,041 ±17,768 spores) and July (100,725±24,117 spores). At SNP, maximum spores were estimated in May (45,827±5,589 spores), June (44,649±7,283 spores) and July (37,811±7,495 spores). Many plants died at both sites after July.

Field physical parameter measurement

All physical parameters showed seasonal fluctuations at both sites (Figure 9) but with a few differences in some months. High temperatures, hours of exposure and day length during December to May correlated with the lower average rainfall at both sites. Seawater temperature showed the same trend as the air temperature which peaked in April at 32°C. Seawater temperature was slightly different between the sites with a maximal value of 35°C in April at TKB and 33°C in February and June at SNP. The exposure times to the air and day length were approximately 7-8 hours and 7-9 hours, respectively. The rainy season, June to December, brought lower temperatures and fewer hours of exposure at low tide. The range of low temperature between air and seawater temperature was not different at the two sites. The exposure time was not much different in the dry season. The average day length increased approximately four hours during in the dry season. Salinity was not much different throughout the year between the sites. It fluctuated from September to December from 20 to 33 ppt. although the average salinity was 30 ppt for both sites from January to August.

Most physical parameters influenced the biological variables more at SNP than at TKB (Table 1). Over all, temperature, exposure time and day length were correlated strongly with life phases ($p<0.05$) at SNP. Temperature showed high correlation with the spore production and female gametophyte. Also, the temperature factor stated a high correlation with spore product in TKB and the exposure time correlated with percentage of sporophyte.

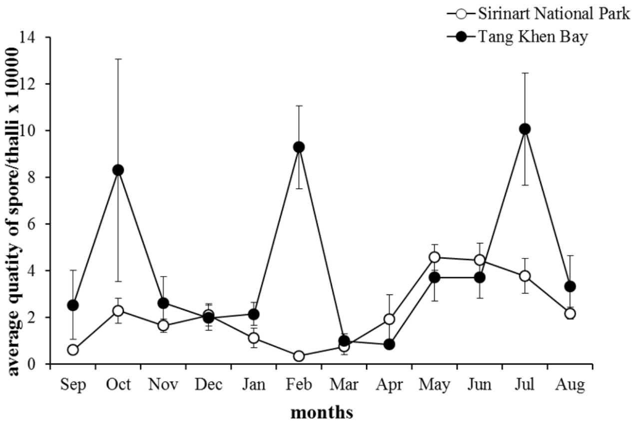

Figure 8. Comparison of the average number of produced spores/ thalli from September 2005 to August 2006 at Sirinart National Park (N=596) and Tang Khen Bay (N=341).

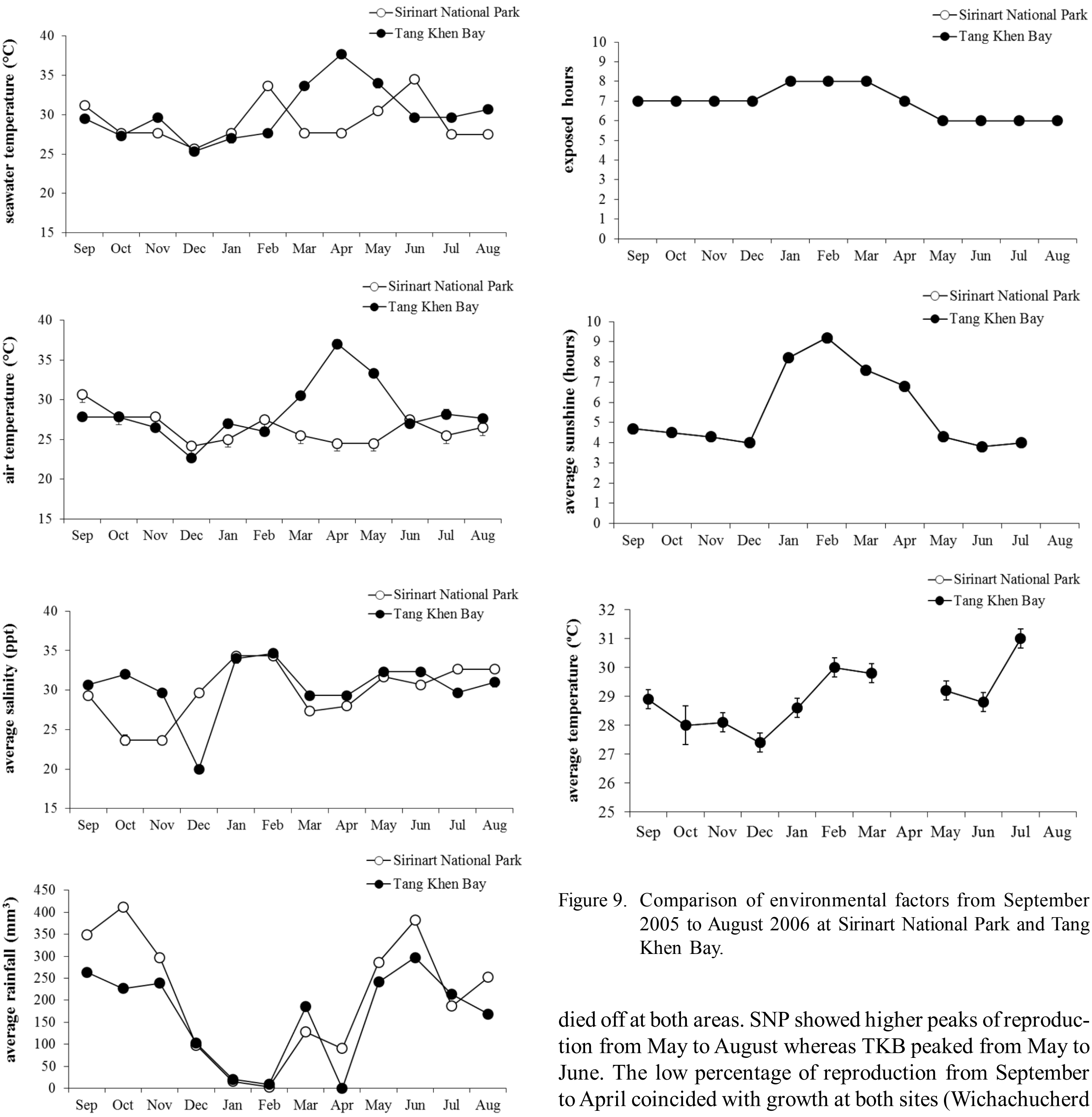

Figure 9. Comparison of environmental factors from September 2005 to August 2006 at Sirinart National Park and Tang Khen Bay.

4. Discussion

Environment can influence on the different aspect of reproduction. Our data indicated that wave exposure effects the amount of reproductive cell and the timing of production that population adapted to the particular habitat as shown in difference between two locations.

In summary, the percentage of reproduction was similar at both SNP and TKB. Mature plants were found throughout the year with peaks in May, when the southwest monsoon begins, with a slight difference in durations at each site. After the monsoon, *P. boryana* thalli were damaged and died off at both areas. SNP showed higher peaks of reproduction from May to August whereas TKB peaked from May to June. The low percentage of reproduction from September to April coincided with growth at both sites (Wichachucherd *et al.*, 2010). Therefore, the difference in wave exposure did not affect the proportion of sporophytes and gametophyte and peak of maturity that started in May.

Between the two *Padina* populations, the potential reproductive effort obviously can vary in space and time due to biological and abiotic factors. The shorter period of peak time that they could reproduce at TKB can be explained by the pattern of average number of spores. There was a peak of spore production at SNP at the same time of a year but longer than at TKB. At SNP, the exposed area, the reproduction strategy is to release a large quantity of spores during these months. Otherwise water motion dilutes the quantity of spores and decreases reproductive success (Zacharias and Roff, 2001; Taylor and Schiel, 2003). In addition, energy

Table 1. Correlation coefficients (R^2) between biological factors and physical factors at Sirinart National Park (SNP) and Tang Khen Bay (TKB).

Site	Variables	Physical parameters						
		Seawater temperature	Air temperature	Salinity	Average rainfall	Hours of exposure	Average sunshine	Average temperature
SNP	%reproduction	-	-	-	-	0.939**	-	-
	%sporophyte	-	-	-	-	-	0.678*	0.849*
	%female gametophyte	-	0.542*	-	-	-	-	-
	%male gametophyte	-	-	-	-	0.458*	-	-
	Number of spore production	0.972**	0.927**	-	-	0.822*	-	-
TKB	%reproduction	-	-	-	-	-	-	-
	%sporophyte	-	-	-	-	0.531*	-	-
	%female gametophyte	-	-	-	-	-	-	-
	%male gametophyte	-	-	-	-	-	-	-
	Number of spore production	-	-	-	-	-	-	0.524*

(-) not significant, *$p<0.05$, **$p<0.001$

allocation can be the key role for difference of reproduction phenomena. Energy allocation for reproduction was equally spent throughout the year at TKB; whereas, SNP was more reproductive during the four months before the monsoon. A similar response to exposed and sheltered shores was also reported in *Macrocystis pyrifera* (L.) C. Ag. Protected populations produced spores throughout the year but the exposed population produces only once every few months (Buschmann *et al.,* 2006).

Wave action is the primary factor that defined the exposed and sheltered areas. It affects the intensity of humidity, temperature, and desiccation. Environmental factors had more of an impact on the reproductive biology of *P. boryana* at SNP than at TKB. Temperature was the main correlate with reproduction and life-phase development at both sites for year cycle. At SNP, exposure time and exposure time with day length were strongly correlated with the reproductive phases. Exposure and day length stimulate reproductive cell release. Temperature seems to be the primary trigger for maturation and phase development for *P. boryana* as has been reported in other brown algae such as *Sargassum* (Yoshida *et al.,* 2001), *Dictyota* (Tronholm *et al.,* 2008), *Fucus* (Izquierdo *et al.,* 2002; Muñoz *et al.,* 2004), *Macrocystis* (Reed *et al.,* 1997) and the red algae *Porphyra* (Tala and Chow, 2014) and *Chondrus* (Bellgrove and Aoki, 2006; Bellgrove and Aoki, 2008). There were also correlations between temperature and exposure time with a higher quantity of spores at TKB than at the more stressful site at SNP.

The percentage of reproduction was based on which phases were dominant at both sites throughout the year. Sporophyte is the dominance phase recorded for this research. Likewise, gametophytes were rare as reported by many researchers in both intertidal and subtidal zones (Lewis *et al.,* 1987; Fagerberg and Dawes, 1973; Liddle, 1975). There were possible reasons that can explain the over dominance of sporophyte in nature; however, there are still doubts. Other investigators suggested that sporophytes have the ability of direct germination to new sporophytes with non-meiosis process without going through the gametophyte phase (Allender, 1977; Tronholm *et al.,* 2008) and not exhibit an alternative life cycle. In addition to gametophyte development, a low percentage of female gametophytes were found throughout the year but male gametophytes were found only in May, June, and July at SNP and April, May and June at TKB. The success of fertilization could not be concluded because only a small number of gametophytes were found during this season and the female gametophytes matured in at different times. Sporophytes may be more tolerant than gametophytes under various conditions and also the ability of sporophyte to self-generate may account for the success of *P. boryana* in Southern Thailand.

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