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SEASONAL GROWTH, DEVELOPMENT AND MORPHOLOGY OF TWO SPECIES OF *PADINA* ADANSON: *PADINA TETRASTROMATICA* AND *PADINA PAVONICA* FROM THE MANORA COAST, KARACHI, PAKISTAN

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Abstract

The study was an attempt to investigate some biological aspects dealing with seasonal growth and developmental morphology of two dominant species of brown algae *Padina* Adanson (Pheaophycota, Dictyotales). Manora Island near Karachi was selected as the area for collection of plants known to have very rich algal flora and *Padina* is one of the prominent and dominant algae at Manora and found to grow on all types of habitats and on each tide level. It is a perennial alga and shows its presence throughout the year. In order to investigate the seasonal growth and developmental morphology, changes in the plant length, breath, presence of vegetative or reproductive plants, percentage maturity of thallus during the different seasons of a year was observed of the two most common species of genus *Padina* namely *P. tetrastromatica* Hauck and *P. pavonica* (L.) Thivy. The peak season for growth and reproduction is winter, whereas plants found in summer were in small size and mostly in the form of juvenile or early mature stage. There was a slight difference in thallus size and reproductive structures (tetrasporophytes or gametophytes) among the two species and between different areas of the shore, but the pattern of growth and reproduction is same. Environmental factors (Mean air temperature and sea water temperature) show significant negative correlation that influence on the growth and development of *Padina* species.

Key words: Seasonal growth and development, *Padina tetrastromatica*, *Padina Pavonica*, Manora coast, Karachi.

Introduction

A typical life history of seaweeds involves a seasonal growth cycle caused by climatic changes occurring throughout the year. Almost all algae show to exhibit seasonal cycles of growth, development, reproduction, senescence and die back. The present work is an attempt to investigate the biological behavior in terms of seasonal life in growth, development and morphological changes. Holmes & Brodie (2004) worked on the morphology and seasonal phenology and observed some aspects of the life history in culture of *Porphyra dioica*. Paul *et al.* (2005) studied the morphology of brown algal genus *Padina* from Southern Philippines, including the three new species of the genus *Padina* to Philippines. Distinctive morphological features, life cycle phases and seasonal variation of *Dictyota dichotoma* from sub-tropical populations was mentioned by Ana *et al.* (2008). Population structure and recruitment of two populations of *Padina boryana* in Thailand was investigated by Bongkot *et al.* (2010). Morphology, vegetative and reproductive development of red alga *Portieria hornemannii* was investigated by Dioli *et al.* (2011). Spatial and temporal patterns of growth, erosion, productivity, and morphology of *Ecklonia radiata* was studied by Sheryl *et al.* (2011). Sfrizo & Facca (2013) studied the growth and autoecology of two alien invasive species: *Sargassum muticum* and *Undaria pinnatifida*. Lucía *et al.* (2015) explains the evolution and maintenance of haploid-diploid life cycles of brown alga *Ectocarpus* in natural populations.

Materials and Methods

Field observations and collection of material: Collection of plants and observation of study area was made on monthly basis from February, 2007 to January, 2008. All the material collected and brought to the laboratory for further observations.

On every visit physical parameters of the study sites were noted including date and time of collection, tide level, air and water temperature, pH and salinity. Atmospheric and water temperature were recorded using a simple centigrade thermometer. For pH and salinity, water samples were taken in sample bottles and brought to laboratory for analysis. pH was determined by pH Digital Meter (WPA) while water salinity and OSKH by Refractometer.

Morpho-anatomical observations: After returning to laboratory, growth, developmental and reproductive stages were studied. Morphological observations like length of plant, breath of plant, reproductive organ in a zone and number of zones on thallus were noted from the collected plants. 05 plants of each species were taken randomly from every spot (05 spots) of each area (Area-I to Area-IV). A total of 50 plants from each area (25 each species) were selected for sampling. Hence, a grand total of 200 *Padina* plants (100 each species) were taken as sample for each month.

Developmental observations: Each plant was observed under the microscope to identify and categorize it according to the below mentioned four phases and also as to whether it was a tetrasporophyte, female gametophyte or male gametophyte. The percentages of maturity phase were calculated and phases of life cycle were also noted. Percentage of maturity was calculated by the number of mature thalli multiplied by 100 and then divided with the total number of thalli as the following:

$$\text{Maturity (\%)} = \frac{\text{Number of mature thalli (Phase 3-4) x 100}}{\text{Total number of thalli}}$$

Field and laboratory observations indicated that the thalli of *Padina* plants may be conveniently divided into 04 different phases of their morphological and reproductive stages to complete the life cycle of *Padina*.

Phase 1: Juvenile: Thalli with small blades, no lobes, no reproductive sorus.
Phase 2: Early mature adult: Thalli with a single reproductive sorus.
Phase 3: Mature adult: Thalli with more than one reproductive sori.
Phase 4: Late mature Thalli: the tetraspores already released from the reproductive sori.
Vaughaniella stage: A thin, flattened, prostrate stage where tetraspores start to germinate on tetrasporophytic plant, which may be a young gametophyte or tetrasporophyte.

Statistical analysis: All data collected in this study were keyed into Microsoft Office Excel 2010 while all statistical analysis was conducted using IBM Statistical Package for Social Sciences (SPSS ver 20.0) software. One way ANOVA was applied to test for significance changes in sample measurements i.e. Mean Thallus Length (MTL) and Breadth along the areas for each month and overall average MTL and breadth of all areas among months.

Spearman Rank Correlation Coefficient was employed to test the effects of physical factors such as air temperature, water temperature, pH, day length and salinity on mean thallus length, average percentage of maturity, average percentage of tetrasporophyte and gametophyte and average percentage of fertility.

Results and Discussion

Padina is a cosmopolitan genus, predominantly found at the coast of Pakistan and permanent part of the flora and found to grow on all types of habitats and on each tidal level. It is a fan-shaped perennial alga and shows its presence during un-favorable seasons in the form of rhizoid, filamentous thallus or sporlings. The present study gives a report about the developmental morphology and reproductive phenology dealing with in detail the aspects of seasonal growth pattern, fruiting behavior and different life phases of two common species *P. tetrastromatica* and *P. pavonica* of genus *Padina* occurring on Karachi coast.

Overall observations by months: Total number of plants collected during the study amounted to 2000 plants (1000 *P. pavonica*; 1000 *P. tetrastromatica*). 250 plants of each species from each area are selected for sampling. Both the species found in every area and almost every season of the year, but *P. pavonica* plants are small in size and were found more common as compared to *P. tetrastromatica*. However, *P. tetrastromatica* found larger in size.

To investigate the growth and developmental morphology of any macroalgae, mean thallus length is considered as an important factor, but breadth and percentage maturity were also considered to determine the developmental stages and growth pattern of *Padina* species. Number of zones (consist of reproductive and sterile hair zone) present on the thallus did not show any significant changes during the study period so this parameter was not taken as a critical factor to determine developmental morphology in this study. The maturity of plant depends upon the presence or absence of reproductive sori on the surface of thallus. The absence indicate that the plant is young and at juvenile stage. The early mature plant has only one reproductive sorus, while adult plants have more than one reproductive sori.

Marine algae show seasonal changes both in the thallus form and floristic composition of plant community. Seasonal fluctuations in the environment typically induce growth and reproduction in perennial organisms that live long enough to experience repeated seasonal changes (Boney, 1965). Working on the brown algae of the Karachi coast, Khatoon (1990) has reported that few species were present in more or less same morphological state throughout the season (except during un-favorable seasons) many other are well developed only during a certain period of the season.

One-way ANOVA on data tested significant ($p<0.05$) in the mean thallus length MTL changes of both species (*P. tetrastromatica*: F = 194.222; *P. pavonica*: F = 72.621) and Breadth (*P. tetrastromatica*: F = 56.530: *P. pavonica*: F = 61.426). There is a significant difference in MTL of *P. tetrastromatica* and *P. pavonica* species along months. Highest mean thallus length was observed in March, 2007, while the smallest was found in May, 2007 among sampled *P. tetrastromatica* plants. Highest mean thallus length of *P. pavonica* plants was found in January, 2008 and the smallest was found in May, 2007 (Fig. 1). Highest mean thallus breadth was observed in February, March 2007 and January 2008 while the smallest breadth was found in May, 2007 among the sampled *P. tetrastromatica*. Highest mean thallus breadth of *P. pavonica* was recorded in January, 2008 and the smallest was in May, 2007 (Fig. 2).

Life phase of *Padina* during the study period is fluctuating in a unique pattern. High percentage of mature plants was observed in winter, while this ratio remains low during summer. No mature plant was found in the month of May, 2007. Overall life cycle of *Padina* clearly showed the dominance of mature stages. Vaughaniella stage in both the species of *Padina* was found in the months of Feb-07, Mar-07, Apr-07, Nov-07, Jan-08 and Mar-08 (Fig. 3a, 3b & 3c).

Both the species of *Padina* showed similar growth pattern with only minor variations. The growth of plants of *P. tetrastromatica* and *P. pavonica* was highest in February and March, 2007 and data from these results confirmed by comparing data from the months of January and March, 2008, whereas lowest in the month of May, 2007. Highest decrease in MTL and breadth observed in May, 2007 which may be continued up-to the months of June and July, 2007. We observed gradual increase from August to October, 2007 followed by decrease in the month of November, 2007. The growth was started to increase and peak in growth was observed in January, 2008. This clearly shows that there may be two degenerative phases occurred during the study period. The first was in May, 2007 and the other was in November, 2007 and two peak growth periods i.e. first in October, 2007 and the second were from January to March, 2008. The percentage maturity exhibited the higher ratio throughout the year, especially in the months of February–April, 2007 and October, 2007–March, 2008. However, there were no mature plants observed in the month of May, 2007. There was a definite difference in the mean thallus length and breadth among *P. tetrastromatica* and *P. pavonica* plants. *P. tetrastromatica* plants were larger in size, while *P. pavonica* were smaller in size. *P. tetrastromatica* plants have relatively higher maturity percentage throughout the year, whereas this ratio is relatively lower in *P. pavonica*.

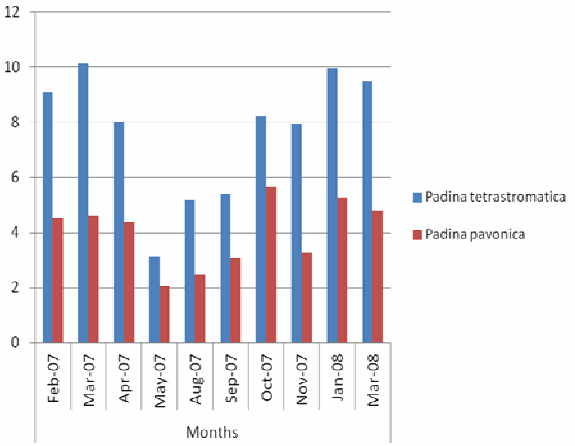

Fig. 1. Mean Thallus Length (MTL) of both *Padina* species in different months.

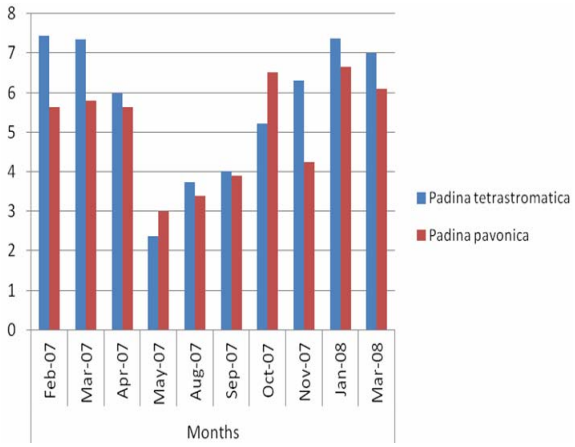

Fig. 2. Mean Thallus Breadth of both *Padina* species in different months.

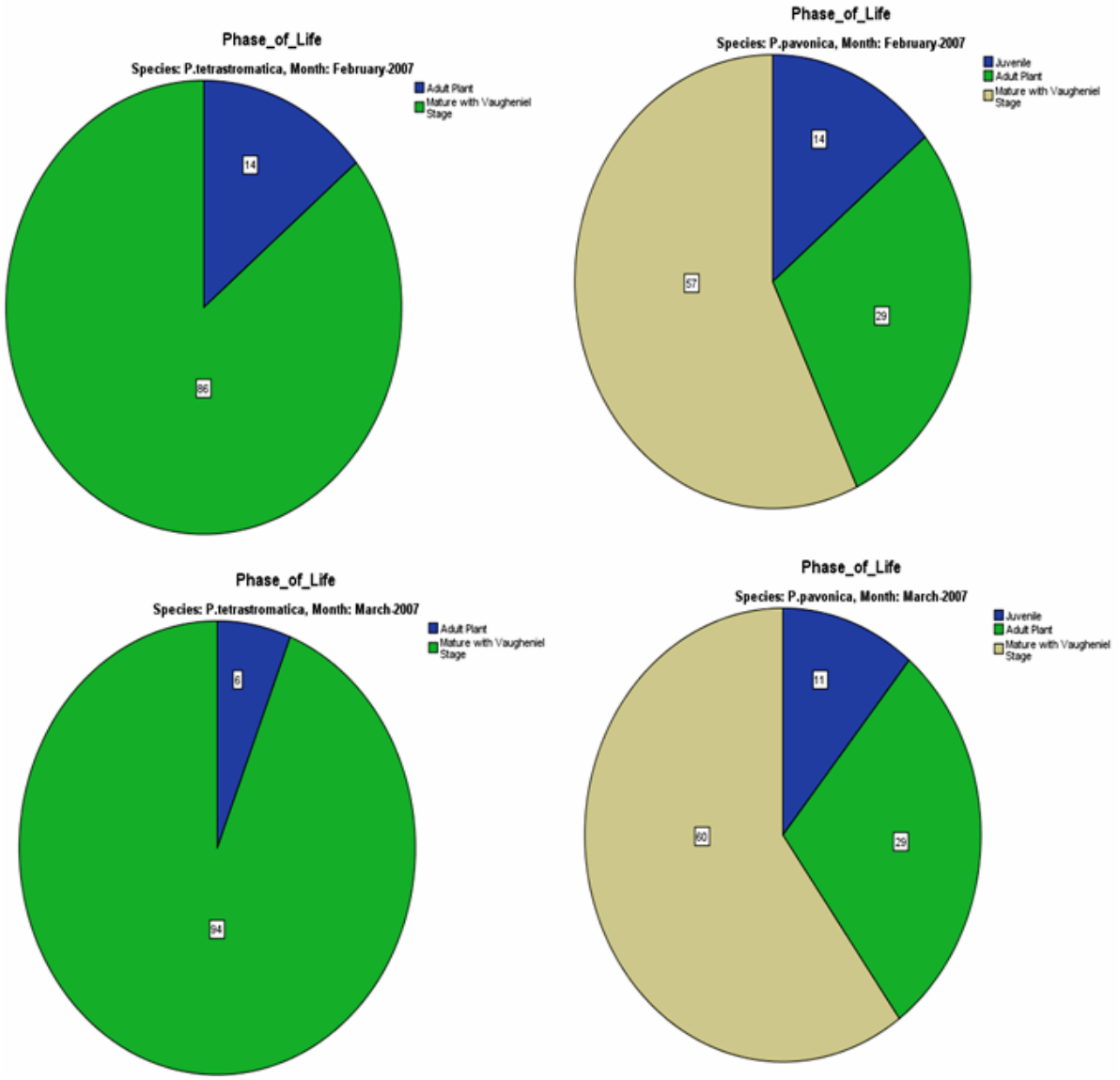

Fig. 3a. Overall Percentage of different Life Phases of both *Padina* species (Feb-07 and Mar-07).

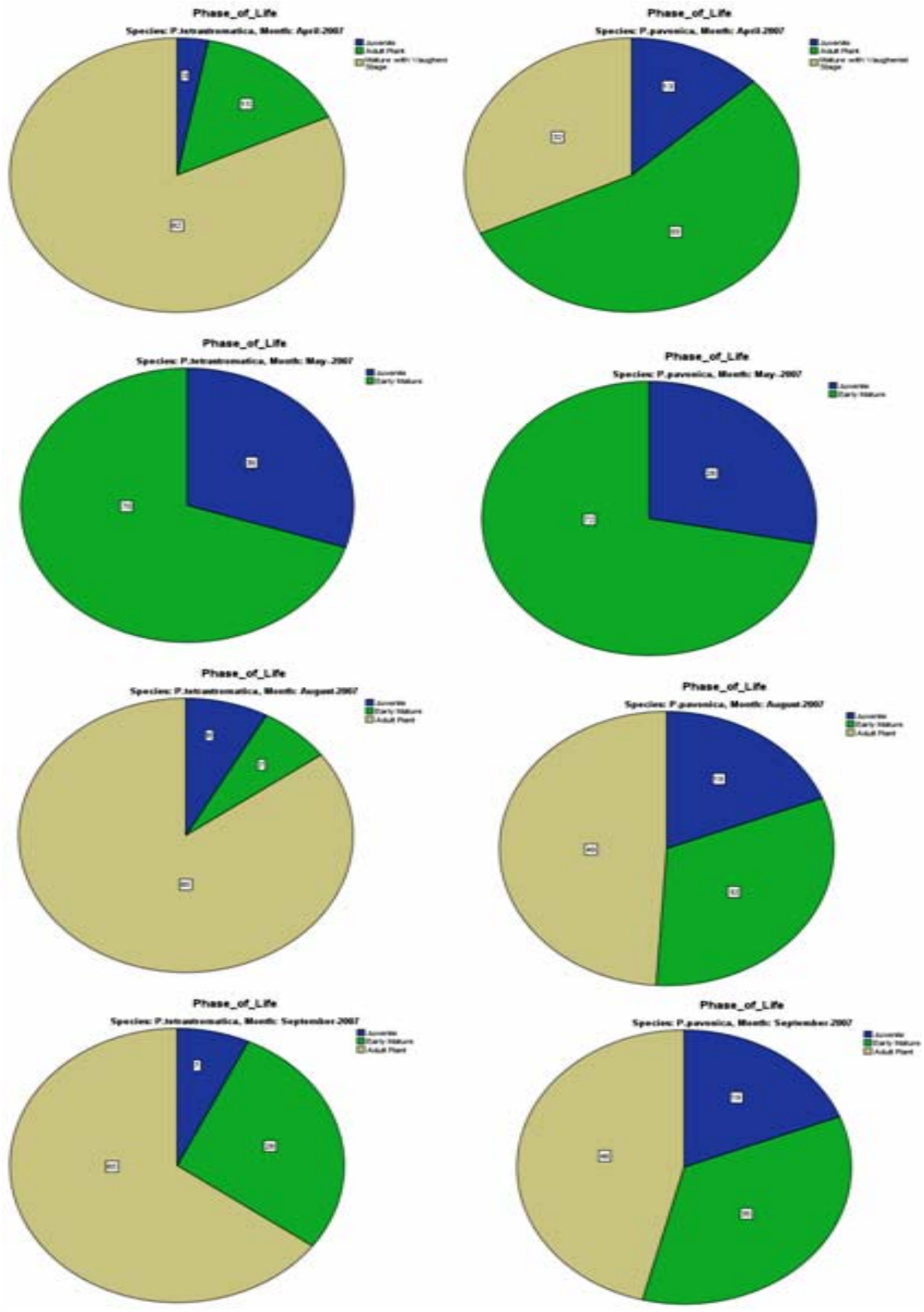

Fig. 3b. Overall Percentage of different Life Phases (Apr-07, May-07, Aug-07, Sep-07).

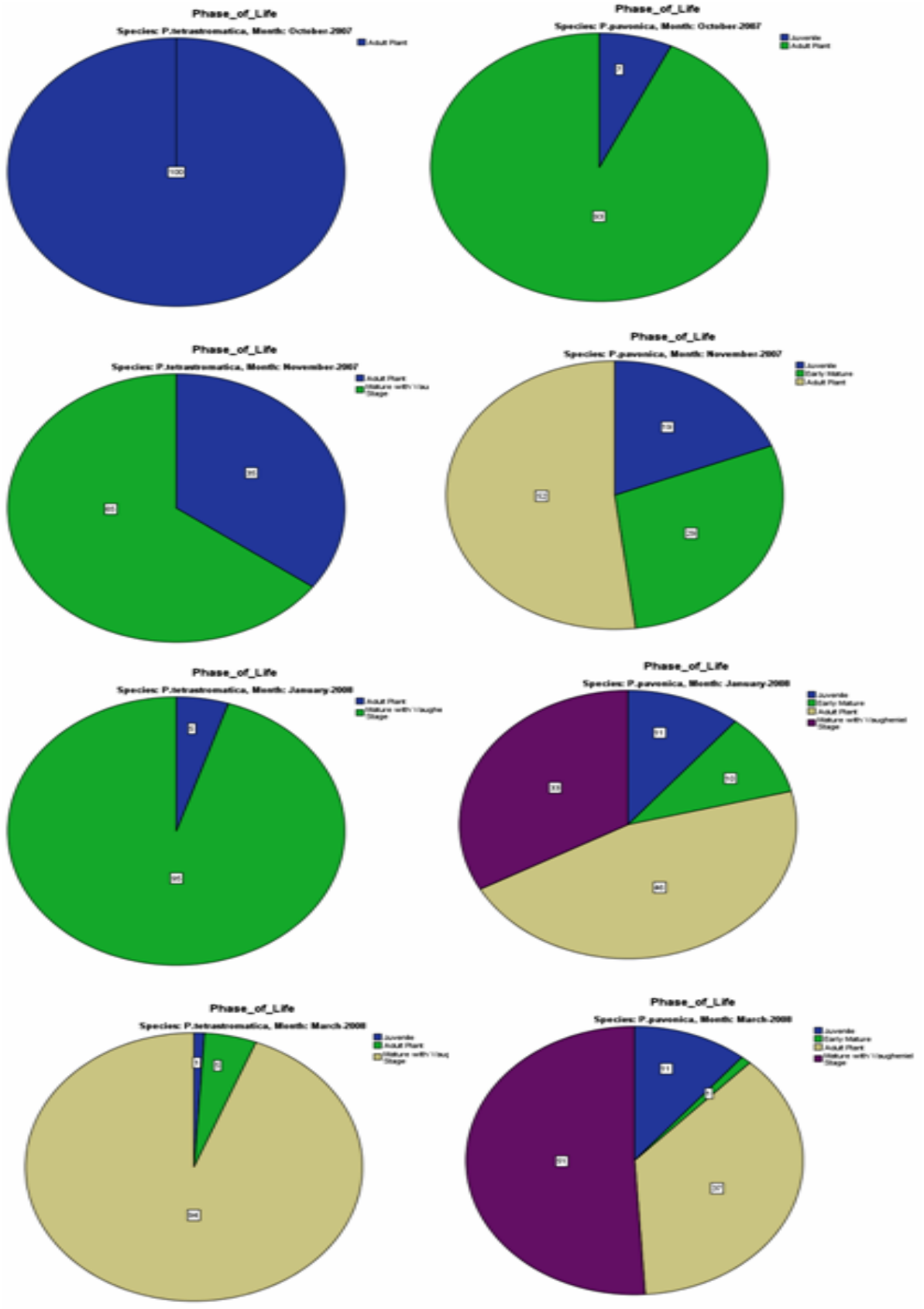

Fig. 3c. Overall Percentage of different Life Phases (Oct-07, Nov-07, Jan-08, Mar-08).

Table 1. Spearman Rank Correlation Coefficient (Non-parametric, 2-tailed) values of different biological parameters of *P. tetrastromatica* and *P. pavonica*.

Environmental factors	Mean thallus length (MTL)	Mean breadth	Tetrasporophyte (%)	Female gametophyte (%)	Male gametophyte (%)	Maturity (%)	Fertility (%)
	Padina tetrastromatica						
Air temperature	-.805**	-.835**	-.704*	-.112	.003	-.761*	-.761*
Sea water temperature	-.578	-.736*	-.529	.028	-.284	-.746*	-.772**
Day length	-.345	-.491	-.464	.068	-.615	-.588	-.614
Salinity	-.286	-.121	-.171	-.427	.184	-.115	-.068
pH	.305	.267	-.105	.570	-.223	-.163	-.163
	Padina pavonica						
Air temperature	-.713*	-.762*	-.043	-.299	.169	-.433	-.581
Sea water temperature	-.547	-.602	-.223	-.152	.446	-.195	-.330
Day length	-.539	-.564	-.079	-.394	.116	-.224	-.354
Salinity	-.172	-.102	-.157	-.051	-.387	-.648*	-.371
pH	.000	-.230	.197	.317	.305	.130	1.000

Note: ** showed significant at the level of 0.01 and * significant at the level of 0.05

Correlation with environmental factors: Spearman Rank Correlation Coefficient was employed to test the effects of different environmental factors such as mean air temperature, sea water temperature, salinity, day length and pH on biological parameters like mean thallus length, mean breadth, percentages of tetrasporophytes, female gametophytes, male gametophytes, percentage maturity and mean percentage fertility of both *Padina* species (Table 1).

Padina tetrastromatica: There was a significant negative correlation between air temperature and mean thallus length (r = -0.805) and mean breadth (r = -0.835). There is also a negative correlation between mean air temperature and percentage of tetrasporophytes (r = -0.704), percentage maturity (r = -0.761) and percentage fertility (r = -0.761). Significant negative correlation was found between percentage fertility and sea water temperature (r = -0.772), also negative correlation between sea water temperature and mean breadth (r = -0.736), and percentage maturity (r = -0.746) (Table 1). No other factor (Salinity, pH, Day Length) was found to effect significantly on any biological parameter of *P. tetrastromatica*.

Padina pavonica: There was a negative correlation between air temperature and mean thallus length (r = -0.713) and mean breadth (r = -0.762). Another negative correlation was found between salinity and percentage maturity (r = -0.648). No other factor (Sea Water Temperature, pH, Day Length) was observed to effect significantly on any biological parameter of *P. pavonica*. The values of Pearson Rank Correlation Coefficient are mentioned in Table 1.

Temperature is an important factor that not only regulates seasonal changes in growth pattern, but also effects seaweed morphology (Chung *et al.,* 2007). Agarwal (2009) stated that temperature also regulates the survival and reproduction of algae. The observations of present study proved that air temperature played a major role in affecting the growth of *Padina* species. The mean air temperature has a significantly negative correlation with the mean thallus length and breadth of *P. tetrastromatica* and *P. pavonica*, but sea water temperature has a negative correlation with the breadth of *P. tetrastromatica* and air temperature is also negatively correlated with percentage maturity. All these results suggest that the decrease in temperature is suitable for the growth and development of *Padina* species.

One way ANOVA and Post Hoc Test (Tukey HSD) were applied to test for significance changes in sample measurements (Mean Thallus Length and Breadth) along the four Areas (Area-I, II, III, IV) of each month and overall average MTL and breadth of all areas among months. (Table of One way ANOVA and Post Hoc Test (Tukey HSD) will be provided on request). Area-I and Area-III are mostly consists of rocky pools and exposed rocky bottoms where the plant size of *Padina* (*P. tetrastromatica* and *P. pavonica*) were larger in size (measured in length and breadth). The large sized *Padina* plants for both species were collected from Area-III throughout the year whereas Area-II and Area-IV occupied by long channels of sandy areas and sandy bottom pools. Plants for both species of *Padina* were small in size all year round and more juvenile plants were also observed. The percentage of gametophytic plants is much higher in these two areas (Area-II and Area-IV) as compared to the other two areas of collection (Area-I and Area-III). Lesser growth rate, more gametophytes and recruitment of new plants in Area-II and Area-IV occupied by the sandy areas may support the hypothesis that these plants invest more energy on reproduction than on growth (Han *et al.,* 2003). The probable reason for this is the fact that new recruits from small sized substrates like sand would only survive for a short period of time before strong currents washed out or displaced both new recruits of small size plants and substrate from their origin. On the other hand rocks provide more solid base for the holdfast attachment, so the *Padina* plants on the Area-I and Area-III are larger in size. It may be confirmed by field observations that the *Padina* plants harvested from sandy areas were very easily detached from their substratum.

Observations of present study revealed that the life cycle of *Padina* started from the beginning of summer i.e. in the month of May when small sized plants and new juvenile recruits were observed in a reasonable quantity and growing along with early mature plants. The fertility rate was also lower and no gametophytic plant was observed. However, tetrasporophytes with single reproductive sori

were recorded dominantly. The same condition remained continue during the months of June and July. With the start of August, the gradual increase in thallus size was observed. Plants bearing reproductive structures started to become mature and some gametophytes were also observed. Fertility and maturity rate (adult plants) also increased, but still, there were a few juvenile plants were noted along with some early mature plants. Vaughaniella stage of life cycle was totally absent in the summer season. Month of September complimented the month of August in the life cycle of *Padina*, but in the month of October, relatively larger plants of *Padina* plants in mature stage of life cycle were recorded. Fertility rate was also higher. A few juvenile plants were also observed. Vaughaniella stage was not observed in the autumn. Interestingly, in the month of November, the thallus size of plants was reduced a little and the fertility of *P. pavonica* was also decreased. However, tetrasporophytes were dominant and the ratio of female and male gametophytes was also the same. *P. pavonica* were in mature stage of life cycle but no Vaughaniella stage was observed. Whereas *P. tetrastromatica* plants were also in mature stage of life cycle and there were some tetrasporophytic plants found with Vaughaniella stage.

This study revealed that the months of January, February and March were found most suitable for the growth and development of both species of *Padina*, correlated with the lowest air and sea water temperature during the year. Well-developed and mature plants with peak size in length and breadth were also abundantly collected and highest percentage of mature and fertile plants of *Padina* was found in these months. The number of gametophytic plants was relatively higher than the other seasons. Winter proved to be the most important season in the life cycle of *Padina* species as most of the mature plants were found with Vaughaniella stage. However, there were still some juvenile plants collected from some areas. In the month of April (spring season), the last stages of seasonal life cycle of *Padina* species was observed. The gradual decrease of thallus length and breadth i.e. deterioration of plants was noticed. Maturity and fertility rates were still higher. Most of the plants were at Vaughaniella stage. There were still some juvenile plants of *P. pavonica* were observed.

It can be concluded from above observations that the lowest growth and development found during the long summer season in Karachi. Autumn season is the season when the development of plants starts. Winter season is the most appropriate season for the growth and development of *Padina* plants, while spring season is the time when the life cycle of *Padina* starts declining just after the peak season in winter. Both species of *Padina* show same behavior.

This study presents a clear picture about the seasonal morphological changes and developmental stages of the *Padina* species, and provides thorough knowledge about the growth, development, fertility and reproduction at various seasons of the year. It is the first basic biological research in Pakistan which provides a typical seasonal life cycle of one of the most dominant alga from the coast of Karachi. Present research will also be helpful for future researchers by giving the information about suitable time of collections and harvesting of one of the most dominant and common genus *Padina* on the coast of Karachi.

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