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# The effect of habitats, densities and seasons on morphology, anatomy and pigment content of the seagrass *Halophila ovalis* (R.Br.) Hook.f. at Haad Chao Mai National Park, Southern Thailand

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ABSTRACT

*Halophila ovalis* is a small marine angiosperm that is widely distributed throughout the world. In this study, the morphology, the anatomy and pigment content of *H. ovalis* leaves from two habitat types (sand vs. tide pool) on the upper shore, each from high density and ca. 50% of high density locations, which represents the differences in emersion times, were investigated at Haad Chao Mai National Park, Southern Thailand, during the dry (December–April, 2011) and rainy seasons (May–November, 2011). Although the average light intensity and temperature were significantly higher during the dry season, they did not differ between the sand and tide pool habitats. Leaf length, width and area as well as petiole length, were significantly greater in high density beds of the sand habitat. The rhizome internode length was significantly longer in the tide pool habitat during the rainy season. The size of the air lacunars was significantly smaller during the dry season in the low density sand habitat. The amount of chlorophyll *a* and total chlorophyll were significantly lower in high density tide pool populations during both seasons. Anthocyanin, as indicated by a high amount of red colour in the leaves, was greatest in the rainy season sand habitat. This study is provides information on a range of *H. ovalis* variations and helps to better understand the *H. ovalis* response to changes in exposure conditions in the upper intertidal area.



## 1. Introduction

*Halophila ovalis* is a small dioecious seagrass that is broadly distributed throughout temperate and tropical regions. It is arguably the most common seagrass species worldwide, is especially common in the Tropical Indo-Pacific, and has recently been reported in the Atlantic Ocean (Short et al., 2007, 2010). In Thailand, *H. ovalis* grows along the coasts of the Andaman Sea and the Gulf of Thailand on various types of substrates in the intertidal and subtidal zones. The variety of *H. ovalis* habitats reflects the ability of this species to tolerate and thrive in a broad range of physical environments subjected to diurnal fluctuation. For example, the light intensity and quality at different water depths affects the distribution, productivity and survival of seagrasses (Livingston, 1984; Longstaff et al., 1999). These factors vary based on differences in emersion time and also affect the morphology and physiology of *H. ovalis* (Ralph and Burchett, 1995; Ralph, 1998a,b; Shafer et al., 2007). Spatial morphological variations have been described in many seagrass species. For instance, *Cymodocea rotundata* and *Thalassia hemprichii* reduce their leaf size in response to emergence stress, which is the effect that maintains water content for supporting physiological processes (Tanaka and Nakaoka, 2004). *Thalassia testudinum* reduces their leaf size in exposed sites with more physical stressors than sheltered areas (Krupp et al., 2009). *Halophila stipulacea* showed smaller leaf width and length in shallow shores, which have greater levels of light, temperature and hydrodynamics (Procaccini et al., 1999). Longer internodal distances in *Halodule wrightii* at the periphery compared to the centre of patches are likely related to sediment nutrient differentiation (Jensen and Bell, 2001).

Morphological plasticity of plants which exhibit under different environmental conditions as their traits to cope with the combination of environmental stressors are well studies in terrestrial plants both vegetative and regenerative traits (Cornelissen et al., 2003; Pérez-Haeguindeguy et al., 2013). *H. ovalis* plants from our study sites show distinct morphological variations depending on the habitat. For example, leaves are smaller in the higher intertidal than at greater depths. Differences in leaf size vary greatly within the high intertidal where plants are exposed to different emersion levels due to the heterogeneity of substrate. Such small-leafed *H. ovalis* are sometimes confused with *H. minor* and the large-leafed form of *H. major*. The large variation in leaf size of *H. ovalis* could be an adaptation or traits off of the plant under the extreme stressful conditions on the upper intertidal habitat; such studies are limited.

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The adaptation ability could be a strategy of the plant to maintain their population worldwide.

Leaf anatomy is an important adaptation trait of plants that benefits them under different conditions. Seagrass, as an aquatic plant, has an air lacunar system that plays an important role in gas exchange during photosynthesis (Enríquez, 2005; Kuo and den Hartog, 2006). Seagrass in the genus *Phyllospadix* has smaller lacunae when it grows on exposed rocky substrate (Cooper and McRoy, 1988). The size of lacunae may show the large variation, as well as the morphological changes, that occur in response to the surrounding environment. Studies of seagrass anatomy are still limited.

Physiological processes are very important functions that underlie all plant responses. To better understand the ecophysiology of seagrass, it is important to analyse changes in their photosynthetic and non-photosynthetic pigment content. Previous studies of plant pigments have focused on the roles of chlorophyll *a* and *b* (Touchette and Burkholder, 2000) and carotene and xanthophyll cycle pigments (Demming-Adams and Adams, 1996; Marín-Guirao et al., 2013) in the photo-physiology of seagrass. For example, the reddening of *H. ovalis* leaves due to an increase in anthocyanin is a global phenomenon (Novak and Short, 2011a). These red leaves have also been observed widely in our site. Novak and Short (2011b) suggest that the reddening of *T. testudinum* leaves that were transplanted to the upper intertidal was induced by UV-B radiation, but no experimental evidence has confirmed this explanatory hypothesis. The aim of this study was to better understand the impact of environmental fluctuations in the intertidal habitat on the morphological, anatomical and pigment content characteristics of the leaves of *H. ovalis*; and extent the understanding of ecological traits of *H. ovalis* in extreme conditions.

## 2. Materials and methods

### 2.1. Sampling site, environmental measurement and experimental set-up

Monospecific beds of *H. ovalis*, a common species in the high intertidal zone at Leam Yong Lam, Haad Chao Mai National Park, Trang Province, along the Andaman coast in Southern Thailand (7°23′ N, 99°20′ E), were chosen for this study. This area, which is an important grazing ground of dugong, hosts the largest and richest seagrass communities in Thailand. Single species and mixed strands form continuous beds over 18 $km^2$, and they are exposed to the air for approximately 3–4 h during tidal fluxes from December to April. The emersion time in the sand habitat is almost 5 times larger in the dry season (4.7 h) than in the rainy season (1 h) (Rattanachot and Prathep, 2011). Other patches are in permanent tide pools with an average size of $1.8 \pm 0.4$ m by $2.2 \pm 0.6$ m (mean ± S.E.) that are 5–10 cm in depth. The average densities of plants from the sand and tide pool habitats were $175 \pm 4$ and $191 \pm 11$ (mean ± S.E.) leaf pairs, respectively.

*H. ovalis* plants were collected twice during each season from January to November 2011, covering both the dry (December–April) and rainy (May–November) seasons. Light intensity and temperature were recorded hourly from 7 a.m. to 6 p.m. using an Onset-Hobo® LI light logger (Onset Computer Corporation, USA). Two replications of data logger were placed on the sandy habitat for 7 days during each season, but these two parameters were measured only during the rainy season for the tide pool habitat. Light data were converted from μmol photon $m^{-2}\,s^{-1}$ to photon $m^{-2}\,d^{-1}$. The average total rainfall for each season was calculated using the 2008–2012 monthly rainfall data provided by the Meteorological Department. Plant rhizomes were collected from high density at the centre and ca. 50% high density at the periphery of patches in the sand and tide pool habitats. From each, one hundred rhizomes of were randomly sampled and brought back to the laboratory for further examination. A total of 580 rhizomes were examined.

### 2.2. Morphological and pigment measurements

Leaf length, leaf width and leaf area from a single leaf of the third youngest leaf pair as well as petiole length and internode length between the second and third youngest leaf pair of each rhizome were measured using a digital calliper and the image analysis program Image-Pro Express 6.0 (Fig. 1a). To examine pigment content, the whole plant leaf with known area, was submerged in 1 ml of *N,N′*-dimethylformamide (DMF) and 1% HCl in methanol for chlorophyll-carotenoid and anthocyanin extractions, respectively. Samples were kept in the dark at 4 °C for 10 days for chlorophyll and carotenoid extraction or overnight for anthocyanin extraction before measuring the optical density at specific wavelengths according to standard spectrophotometric methods. All pigments were calculated as follows: chlorophyll $a = 11.65A_{664} - 2.69A_{647}$, chlorophyll $b = 20.81A_{647} - 4.53A_{664}$ (Wellburn, 1994), carotenoid $= (1000A_{480} - 0.89C_a - 52.02C_b)/245$ (Wellburn, 1994; Vicaş et al., 2010) and anthocyanin $= A_{530} - 0.25A_{657}$ (Mancinelli, 1994). The calculated values were μg/ml and divided by leaf area, thus pigments were presented in μg $cm^{-2}$. Because the percentage of anthocyanin coverage was extremely variable, we corrected the anthocyanin concentration determined through the extraction to the real coverage, and we categorised the leaves into three classes depending on the red leaf area observed: low, medium and high. Low referred to less than 20% coverage, medium referred to 20–80% coverage, and high referred to more than 80% coverage (Fig. 1b).

### 2.3. Anatomical measurement

Anatomical variables include the width and length of cells from the leaf margin, superficial cells, mesophyll cells and cells from the epidermis. All were measured using the picture analysis tool Image-Pro Express 6.0. The width and length of the two largest air lacunars were also measured (Fig. 1c and d).

### 2.4. Data analyses

SPSS for Windows 13.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. The Mann–Whitney *U* test was used to compare the differences in temperature and light intensity between the two seasons as well as between the sand and tide pool habitats of the rainy season. Three-way ANOVA was employed to test the differences in morphology, anatomy and pigment content among different habitats, densities and seasons at the significance level of 95%, and the *Z*-test was conducted to compare the differences in percentage coverage of anthocyanin between habitats.

## 3. Results

### 3.1. Environmental factors

The average temperature and daily light exposure during the dry season were $31.97 \pm 0.08$ °C and $79.09 \pm 3.04$ mol photon $m^{-2}\,d^{-1}$, respectively, which were significantly higher than those during the rainy season, $30.63 \pm 0.07$ °C and $57.61 \pm 1.30$ mol photon $m^{-2}\,d^{-1}$, respectively ($p < 0.05$). There were no significant differences in temperature and light intensity between the sand and tide pool habitats. The lowest average of the total rainfall during the dry season was 20 mm in February, and the highest average of the total rainfall during the rainy season was 310 mm in September.