Department of Environment and Agriculture
School of Science

Seasonality and nutrient-uptake capacity of *Sargassum* spp. in Western Australia

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Doctor of Philosophy
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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature:……………………

Date: …………………………

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PREAMBLE

The thesis consists of nine chapters. Chapter 1 presents a comprehensive literature review on satellite remote-sensing of marine habitats mapping and the importance of *Sargassum* spp., taxonomy, distribution, reproduction, phenology effects of environment factors and nutrient-uptake capacity, all under the influence of the seasonality of Western Australia (WA).

Chapter 2 introduces and provides an overview of the thesis with detail of the site selection criteria and the description of the study sites. This chapter also presents justification for the study and states the research questions, followed by the aims and objectives of the study.

Chapter 3 to 8 address the main objectives of the thesis. These chapters have been either presented at national or international conferences, seminars or independently submitted to peer-review scholarly journals for publication. The full list of attended conferences, seminars, and published journals is provided on pages *xxvii–xxix*.

Chapter 3 presents the spectral response of marine submerged aquatic vegetation (SAV) habitat along the WA coast. This chapter provides a comprehensive study on spectral reflectance profiles of SAV species and is an important pre-requisite study to meet the objectives in Chapters 4 and 5. Chapter 4 identifies and maps the marine SAV in the shallow coastal water using WorldView-2 (WV-2) satellite data. This chapter also presents methods and interprets the results when using WV-2 on classification and building SAV distribution maps including *Sargassum* spp. Chapter 5 presents the remoted-sensing mapping of *Sargassum* sp. distribution around Rottnest Island, WA using high spatial resolution WV-2 satellite data.

Chapter 6 describes the life cycle and ecology of *Sargassum* species in WA by presenting the primary data on distribution, growth rate, and reproduction of the species. This chapter documents the life cycle of *Sargassum spinuligerum* along the WA coast based on field observations and laboratory works. This base line knowledge of the biology and ecology of the local species gives vital information on the seasonality of *Sargassum* that is described in Chapter 7.

Chapter 7 illustrates the seasonal variations in water quality as a driver of seasonal changes in *Sargassum* at the study site. Based on the findings of the seasonality study in chapter 7, a laboratory experiment was conducted which is described in chapter 8. The chapter 8 explores the effects of nutrient and initial biomass on the growth rate and nutrient-uptake of *S. spinuligerum* thus providing essential information on the aquaculture potential of *Sargassum*.

The thesis is completed with a general discussion of key findings and conclusions in Chapter 9. This chapter also summarizes all of the main results of this study, and discusses the findings in a broader context. The chapter ends with several recommendations for potential studies on the topic.

ABSTRACT

Sargassum is one of the most diverse genera of the marine macroalgae, is distributed worldwide, and is mainly dominant in tropical and sub-tropical shallow waters. The *Sargassum* spp. play a major role in forming the SAV (submerged aquatic vegeteation) habitats with high productivity and biomass to maintain healthy coastal ecosystems. To date, there have been a limited number of studies on seasonal variation on *Sargassum* communities and no studies on the ecological characteristics of growth, reproduction, and the life cycle of *Sargassum* spp. undertaken along the subtropical zone of the WA coast. Moreover, there have been no studies using multi-spectral satellite remote-sensing to map *Sargassum*, canopy macroalgae, seagrasses, and its associated habitats along the WA coast. Recently, *Sargassum* species have also been proposed as potential candidates for marine culture in the large scale macroalgae farms. However, there are limited reported studies on the cultivation conditions and ecology of *Sargassum* spp.

The main aim of the present study was to investigate the influence of seasonality of water qualities, canopy cover, thallus length, and distribution of *Sargassum* beds around Point Peron and Rottnest Island, WA. The study presented primary data on growth rate and, reproduction, and documented the life cycle of *Sargassum spinuligerum* based on field surveys and laboratory works. The spectral reflectance optical data of macroalgae (red, green, and brown), seagrasses, and sediment characteristics in coastal waters were measured in order to support the selection of suitable satellite sensors for different benthos. The feasibility of the WV-2 satellite data for identifying and evaluating classification methods has been validated for mapping the diversity of SAV in coastal habitats. Therefore, assessing the distribution of *Sargassum* beds using high-spatial resolution WV-2 satellite images from this study could be considered the first such approach.

The data on canopy cover, thallus length, biomass, reproduction, and distribution patterns of *Sargassum* spp. were collected every three months during the period from 2012 to 2014 from four different reef zones along monitored transects. *In air* spectral reflectance profiles of *Sargassum* spp. and other SAV species were acquired using the FieldSpec® 4 Hi-Res portable spectroradiometer. High spatial resolution satellite images WV-2 with 2-m spatial resolution was employed to estimate the spatial

distribution pattern of *Sargassum* and SAV using different classifiers. A series of experiments were also conducted to test the effects of different initial biomass and nutrient loads on the growth rate and nutrient-uptake capacity of *Sargassum* spp.

The *Sargassum* beds in Point Peron indicated significant seasonal changes in canopy cover and thallus length. However, there were no significant differences between the reef zones. The results also showed that the *Sargassum* spp. coverage and mean thallus length are significantly influenced by the concentrations of PO_4^{3-}. *Sargassum* spp. in the WA coast have several distinct growth phases, varied growth rates, seasonal reproduction and an annual life cycle. The growth rate of *Sargassum* was highest in the cooler months, and canopy cover and biomass were also highest toward the end of the growing period in spring to summer.

The eight-band high-resolution multi-spectral WV-2 satellite imagery has great potential for mapping and monitoring *Sargassum* spp. beds as well as other associated coastal marine habitats. Both Mahalanobis distance (MDiP), supervised minimum distance (MiD) classification methods showed greater classification accuracy than the spectral angle mapper (SAM) classifier. *S. spinuligerum* could be cultivated under the outdoor conditions with the optimum initial stocking biomass at 15.35 ± 1.05 g per 113-L of seawater enriched with Aquasol®, which contributed to a relatively higher SGR than other tested commercial fertilizers.

The research contributed to a better understanding of the seasonal abundance of *Sargassum* spp. in WA waters which in turn will assist in the farming of *Sargassum* spp. These data provide the necessary information for coastal marine management and conservation as well as sustainable utilization of this renewable marine resource. The methods can also be extended and applied in a bio-monitoring program for *Sargassum* beds along the Australian coast and in other similar regions.

TABLE OF CONTENTS

Chapter 2. INTRODUCTION 65

Chapter 3. SPECTRAL RESPONSE OF MARINE SUBMERGED AQUATIC VEGETATION 73

Chapter 7. SEASON CHANGES IN WATER QUALITY AND *SARGASSUM* BIOMASS IN WESTERN AUSTRALIA 129

LIST OF FIGURES

LIST OF TABLE

LIST OF ABBREVIATIONS

Acronym	Description and typical units
AHC	Agglomerative hierarchical clustering
ALOS	The Advanced Land Observing Satellite
ANOVA	Analysis of variance
APHA	American Public Health Association
AVNIR-2	The Advanced Visible and Near Infrared Radiometer Type-2
BoM	Bureau of Meteorology, Australian Government
BR	Back reef
CARL	Curtin Aquatic Research Laboratory
CC	Canopy cover (%)
CDOM	Colored dissolved organic matter
CHRIS-PROBA	Compact High Resolution Imaging Spectrometer-Project for On-Board Autonomy
DO	Dissolved oxygen (mg L^{-1})
DPW	Department of Parks and Wildlife, Government of Western Australia formerly named the Department of Environment and Conservation (DEC)
ED	Euphotic depth (m)
EEZ	Exclusive economic zone
ENVI	Environment for visualizing image
FB	Fresh biomass (g 0.25 m^{-2})
FR	Fore reef zone
GHG	Greenhouse gases
GPS	Global Positioning System
HAI	Hyperspectral airborne imagery
HICO	The Hyperspectral Imager for the Coastal Ocean
IOPs	The inherent optical properties
IPCC	The Intergovernmental Panel on Climate Change
ISB	Initial stocking biomass
KM	K-means
LZ	Lagoon zone
MaxAT	Maximum air temperature (^{o}C)
MDiP	Mahalanobis distance
MiD	Supervised minimum distance

MiL	Minimum likelihood method
MinAT	Minimum air temperature (^{o}C)
MODIS	Moderate Resolution Imaging Spectroradiometer
MHSR	Multispectral high-spatial resolution
MTL	Mean thallus length (cm)
NASA	The National Aeronautics and Space Administration
NIR	Near-infrared
NW	Northward wind (m s^{-1})
PAR	Photosynthetically active radiation (Einstein m^{-2} d^{-1})
PCA	Principle Component Analysis
RC	Reef crest
SAM	Spectral angle mapper
SAV	Submerged aquatic vegetation
SE	Solar exposure (MJ m^{-2})
SLP	Sea level pressure (hPa)
SPOT-4	Satellite Pour l'Observation de la Terre-4
SRSI	Satellite remote-sensing imagery
SSTs	Sea surface temperatures (^{o}C)
WA	Western Australia
WV-2	World View-2

LIST OF PUBLICATIONS

Articles

The following Ph.D. outcomes that have been published or are in the review process at the time of submission this dissertation:

1. Hoang C. Tin, Mick O'Leary, Ravi Fotedar, Rodrigo Garcia, 2015. Spectral response of marine submerged aquatic vegetation: A case study in Western Australia coast. **IEEE/MTS *OCEAN'S 15*** Proceedings, Washington, DC, USA. pp. 1–5. [*Chapter 3*]

2. Hoang C. Tin, Garcia, R., O'Leary, M., and Fotedar, R., 2016. Identification and mapping of marine submerged aquatic vegetation in shallow coastal waters with WorldView-2 satellite data. ***Journal of Coastal Research***. SI 75(2), 1287−1291. doi:10.2112/SI75-258. 1. [*Chapter 4*]

3. Hoang C. Tin, Michael J. O'Leary, Ravi Fotedar, (in press) Remote-sensed mapping of *Sargassum* spp. distribution around Rottnest Island, Western Australia using high spatial resolution WorldView-2 satellite data. ***Journal of Coastal Research***. doi:10.2112/jcoastres-d-15-00077.1. [*Chapter 5*]

4. Hoang C. Tin, Anthony J. Cole, Fotedar Ravi, O' Leary J. Michael, Lomas Michael, Roy Shovonlol, (in press) Season changes in water quality and *Sargassum* biomass in Southwest Australia, ***Marine Ecology Progress Series***. 551, 63−79. doi:10.3354/meps11735. [*Chapter 7*]

5. Hoang C. Tin, Cole J. Anthony, Fotedar Ravi, O' Leary J. Michael, Effects of nutrient media and initial biomass on growth rate and nutrient-uptake of *Sargassum spinuligerum* (Sargassaceae, Phaeophyta). ***Springer Plus*** [*Under revision - Chapter 8*]

6. Hoang C. Tin, Ravi Fotedar, Anthony J. Cole, Michael J. O' Leary, Chapter 6. The spatial distribution, life cycle and seasonal growth rate of *Sargassum spinuligerum* in the Western Australian coast. ***Journal of Experimental Marine Biology and Ecology*** [*Under preparation - Chapter 6*]

Abstracts

The following scientific conferences and seminars that I have been presented results with either oral or poster presentations from Ph.D. project outcomes:

1. Hoang C. Tin, Ravi K. Fotedar, Michael J. O'Leary, 2016. Identification and mapping of marine submerged aquatic vegetation in shallow coastal waters with WorldView-2 satellite data. *The 14th International Coastal Symposium*, Sydney, NSW, Australia [*Oral Presentation*].

2. Hoang C. Tin, Anthony J. Cole, Ravi Fotedar, Mick O'Leary, 2015. Seasonality and distributions of macroalgae *Sargassum* beds at Point Peron, Shoalwater Islands Marine Park, Western Australia. *Journal of Royal Society of Western Australia*. 98: 97−98 [*Extended Abstract*].

3. Hoang C. Tin, Rodrigo Garcia, Michael J. O'Leary, Ravi Fotedar, 2015. Spectral response of marine submerged aquatic vegetation: a case study in Western Australia coast. *IEEE/MTS OCEAN'S 15 Conference,* Washington, DC, USA [*Oral Presentation*].

4. Hoang C. Tin, Ravi K. Fotedar, Michael J. O'Leary, 2015. The ecology of *Sargassum* spp. in Southwest Australia: seasonality, abundance, and habitat mapping. *NOAA Science Seminar Series*, NOAA Headquarter, Washington, DC, USA [*Oral Presentation*].

5. Tin C. Hoang, Michael J. O'Leary, Ravi Fotedar, 2015. Mapping of the Brown macroalgae beds around Rottnest Island using 2-meter resolution WorldView-2 satellite data. *Oceans and Society: the Blue Planet Symposium*, Cairns, QL, Australia [*Poster*].

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7. Rodrigo Garcia, John Hedley, Tin C. Hoang, Peter Fearns, 2014. Optimal choice of spectral classes for higher precision benthic classification, *Ocean Optics XXII*, Portland, Maine, USA [*Poster*].

8. Tin C. Hoang, Ravi Fotedar, Mick O'Leary, 2012. Seasonality and nutrient-uptake capacity of *Sargassum* spp. in Western Australia. *Tropical Research Network Conference*, Cairns, QL, Australia [*Oral presentation*].

Chapter 1. LITERATURE REVIEW

1.1. TAXONOMY OF *SARGASSUM*

Sargassum genus is known as marine brown macroalgae. Its name is derived from the word sargazo, meaning gulfweed or macroalgae, which was used by Portuguese navigators when it was first found in tropical Atlantic waters in the 15th century (Thabard, 2012). *Sargassum*, described over 190 years ago (Agardh, 1820 '1821'), belongs to the Sargassaceae family, the order Fucales, one of 16 orders of Phaeophyta (Brown algae), and the class Phaeophyceae (Luning, 1990). Since then, according to Algae Base, there have been 878 *Sargassum* species identified, but only 340 species have been taxonomically accepted (Guiry and Guiry, 2014).

The *Sargassum* genus has been identified around the globe (Guiry and Guiry, 2014). Agardh was a well-known scientist who conducted numerous studies on *Sargassum* taxonomy (Figure 1.1). In 1820, Agardh had established a classification system for the *Sargassum* genus and described 62 species. He divided the genus into seven groups and arranged them into the Fucoideae order.

The classification of *Sargassum* genera and number of accepted taxonomically:

Doman: -Eukaryota (27519 species)

Kingdom: -Chromista (11066 spe.)

Sub-kingdom: -Chromobiota (10927 spe.)

Phyllum: -Heterokontophyta (3101 spe.)

Division: -Phaeophyta – brown algae

Class: -Phaeophyceae (1760 spe.)

Order: -Fucales (521 spe.)

Family: -Sargassaceae (480 spe.)

Genus: -*Sargassum* (340 spe.)

In 1824, Agardh increased the number of *Sargassum* to 67 identified species. Based on morphogenic relations between stem and blade, Agardh divided the *Sargassum* genus classification system into five subgenera including *Phyllotricha, Schizophycus, Bactrophycus, Arthrophycus,* and *Eusargassum* (~ *Sargassum*) (Agardh, 1820 '1821').

In 1983, Yoshida divided the *Sargassum* genus into two subgenera including *Arthrophycus* and *Bactrophycus*, and the three remain genera. However, the classification method was not followed by the International Botanical code and created confusion in classifying this genus into species level (Phillips, 1994b). In addition, the classification at species level also found difficulty to separating the *Arthrophycus* and *Bactrophycus* subgenera (Yoshida, 1989).

The overview of classification works and the number of species that have been taxonomically accepted in a number of countries and territories in the East, West Asian, Pacific and Oceania regions will be discussed.

1.2. MORPHOLOGY

Sargassum is one of the genera that has the most complex morphology of the Phaeophyta division. They have two main life forms, pelagic and submerged. In the submerged group, *Sargassum* have a rather extensive holdfast that is securely attached onto substrates and the main branch (thallus) carries leaf-like structures, and vesicles that assist the thalli staying upward in the water column (Figure 1.1). The average length of the main branches and leaves are different from species to species and with different geographic distributions. In the pelagic group, *Sargassum* is a floating and drifting type based on currents and the waves (Butler and Stoner, 1984; Hanson, 1977; Hu *et al.*, 2015).

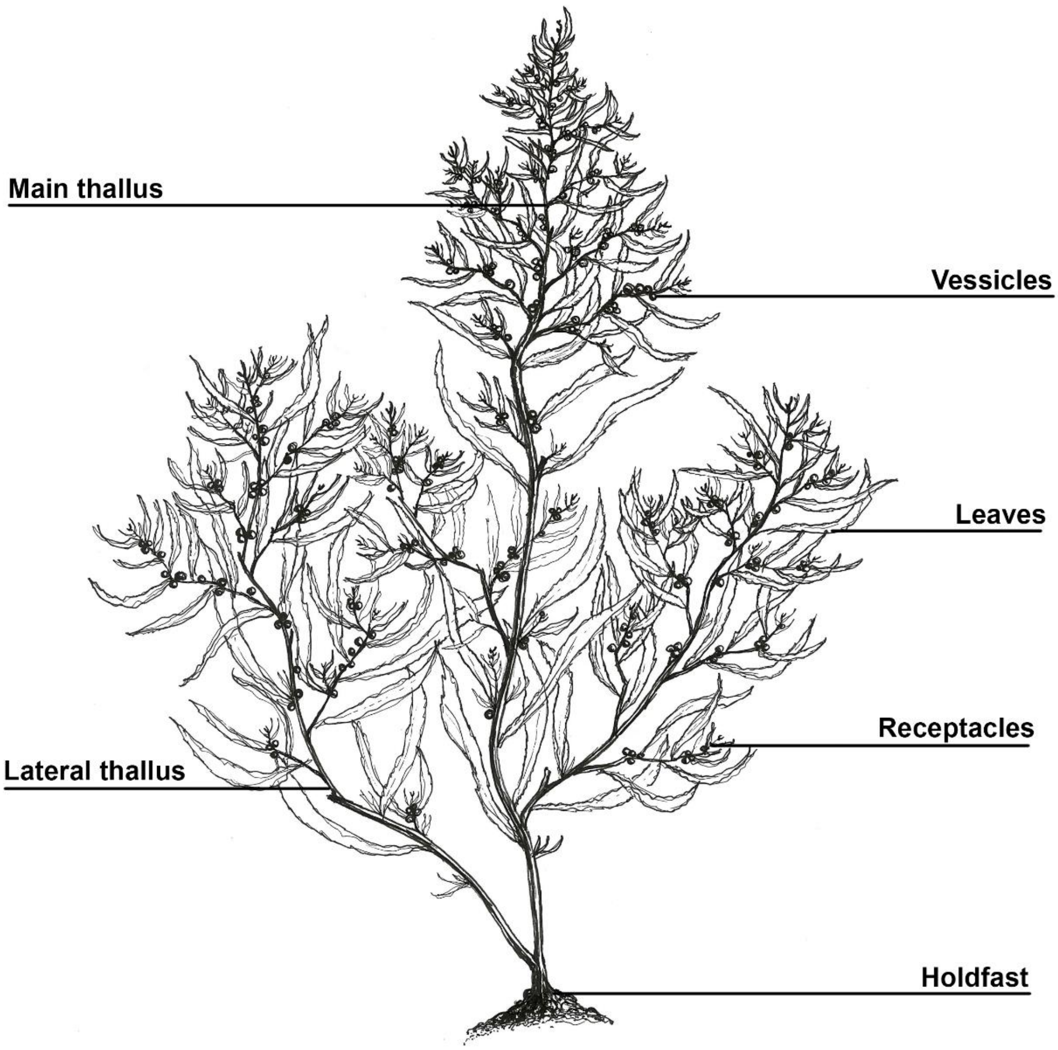

Figure 1.1 The synthetic morphological of *Sargassum* spp. (The line drawing from the collected fresh samples by author).

The morphology of *Sargassum* varies from species to species. The traditional *Sargassum* taxonomy is based on the morphology of holdfast, stem (stipe/blade), branches, leaves, vesicles, and receptacles (reproductive structure) (Figure 1.2) (Yoshida, 1985; 1989). Of these, air blades or vesicles play an important role in keeping the thalli upright in the water column. Moreover, the vegetation structure of *Sargassum* is polymorphic. For instance, Kilar and Hanisak (1988) have found 47 different morphology structures of *S. polyceratium* species in the *Sargassum* community in Florida, USA (Kilar and Hanisak, 1988).

Even at the species level, there is a great variation in vegetation morphology throughout the life cycle of *Sargassum*. There are three main factors that change their morphology: *i*) the effects of environment such as wave, depth and tidal flat; *ii*) the changing morphological structure throughout their life cycle; and *iii*) the different geographical distribution (Yoshida, 1989). Another study on receptacle morphology of *Sargassum* spp. at Reunion Rocks, South Africa, showed that there are three types of receptacles that have been found in *S. elegans* and *S. incisifolium* in the wild. These are *i)* terete, *ii)* three-cornered, and *iii)* twisted receptacles (Gillespie and Critchley, 1997).

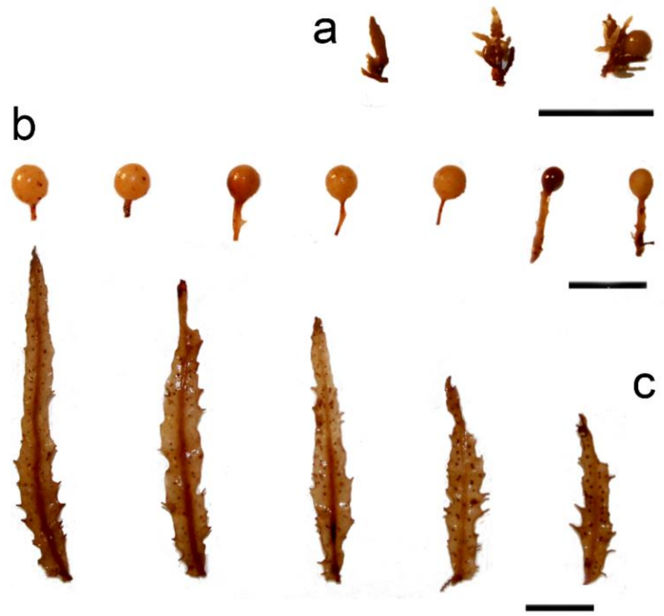

Figure 1.2 The variability of *Sargassum* morphology characters a) receptacles, b) vesicles, and c) blades (leaf-like).

In summary, *Sargassum* is a polymorphic species, but the majority of *Sargassum* taxonomy studies have employed morphological methods. In the recent years, therefore, the phylogenetic and molecular classification techniques have been introduced to retrieve and revise the results of traditional morphology methods (Kantachumpoo *et al.*, 2015; Mattio *et al.*, 2015a, b).

1.3. IMPORTANCE OF *SARGASSUM* SPP.

Sargassum, like many other macroalgal species, has a significant contribution to both marine ecosystems and human needs. In coastal areas and surrounding off-shore islands, well-developed *Sargassum* beds play vital ecological roles as a food resource and nursery areas for numerous organisms and for fish breeding. They also provide feeding grounds for several types of sea birds and sea lions that live on and around these islands (Tyler, 2010). *Sargassum* also contribute to CO_2 absorption from the atmosphere and distribute it to the deep layers of the ocean (Figure 1.3) (Aresta *et al.*, 2005a, b; Feely *et al.*, 2001; Gao and McKinley, 1994; Ito *et al.*, 2009; Muraoka, 2004; Sahoo *et al.*, 2012; Tang *et al.*, 2011).

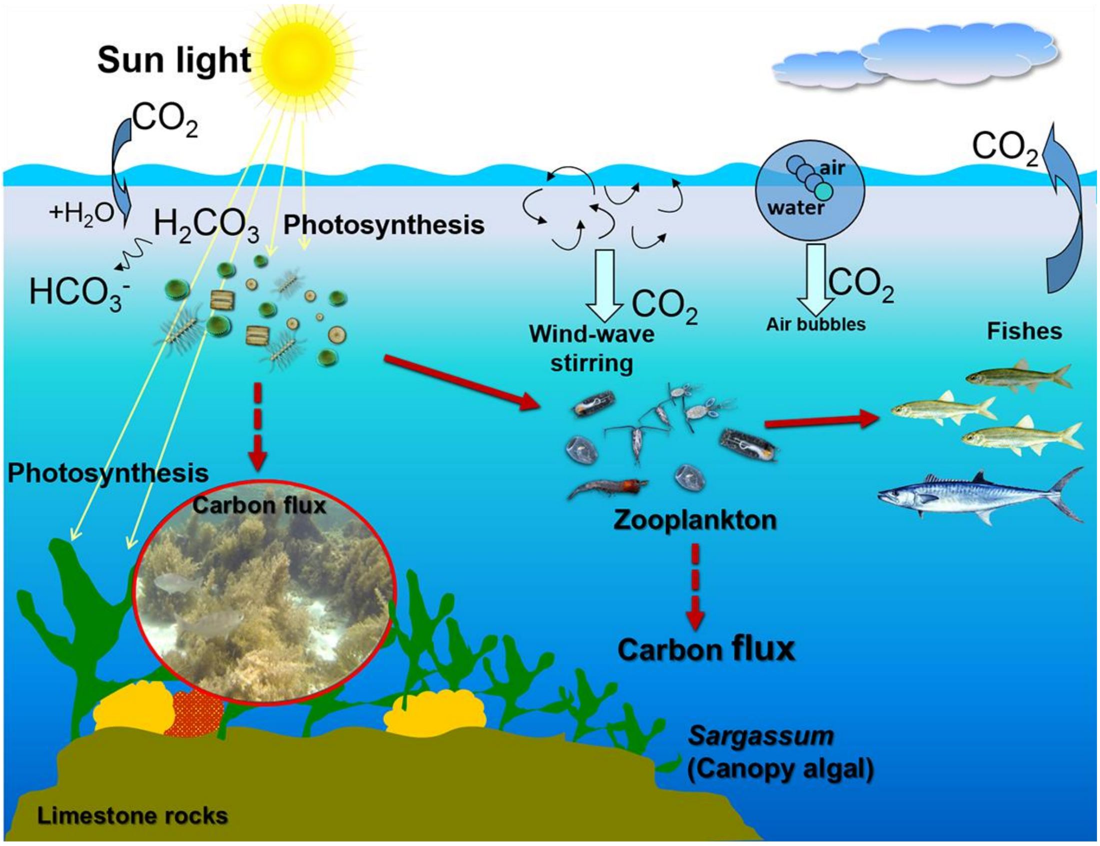

Figure 1.3 A typical coastal marine ecosystem, where macroalgae (e.g. *Sargassum* spp.) plays as primary producer after phytoplankton. Macroalgae are significant contributors to carbon flux in the coastal ocean. In the oceans' carbon cycle, macroalgae are responsible for the transfer of carbon dioxide from the atmosphere to the deep ocean (©The author).

Macroalgae are rich in nutrients for human needs (Gellenbeck and Chapman, 1983), as medicines (Chengkui *et al.*, 1984; Kumar *et al.*, 2011) and fertilizers (Hong *et al.*, 2007), and can be used as living renewable resources in the form of bio-fuels (Aresta *et al.*, 2005a; Borines *et al.*, 2011; Langlois *et al.*, 2012; Sahoo *et al.*, 2012). Of those, *Sargassum* have been used for medicinal purposes for several thousand years. The Chinese literature has recorded that they used macroalgae for herbal uses and decoction as drugs about two thousand years ago (Figure 1.4). According to ancient Chinese literature, several macroalgal species such as *Sargassum fusiforme*, *Laminaria japonica, Porphyra* spp. and *Ulva* spp. have been used in herbal medicine for over 20 centuries (Chengkui *et al.*, 1984).

Figure 1.4 Dried *Sargassum* sp. is sold in a local market at Phu Quoc Island, Vietnam as (a) herbal plants and (b) processed as sweet soup. Photos taken on January 18, 2013 (©The author).

At the present, 95% of the world's bio-fuel products originates from edible oils. The input materials for this industry are primarily based on the seasonality of crops and land-based agriculture area. Therefore, microalgae and macroalgae have great potential to partly replace the land-based agriculture products and provide sustainable bio-fuel and bio-materials (Bharathiraja *et al.*, 2015). Macroalgae biomass is a product that synthesizes atmospheric CO_2 via the photosynthesis processes. This product is known as a clean, non-toxic, and biodegradable energy and does not affect the greenhouse gases (GHG); also, it is a renewable source (Aresta *et al.*, 2005b). The bioenergy farms also do not affect the traditional agriculture land and fresh water bodies (Aitken *et al.*, 2014; Alvarado-Morales *et al.*, 2013; Aresta *et al.*, 2005a; Fargione *et al.*, 2008; Kerrison *et al.*, 2015; Nassar *et al.*, 2011; Romijn, 2011). Therefore, the research and development into bioenergy projects from macroalgae has received more attention and investment (Kerrison *et al.*, 2015). Table 1.1 presents the ethanol production from the major land crops and macroalgae. Consequently, bio-oil has recently been widely produced and used in Europe, the United States, and Asia (Aresta *et al.*, 2005b).

Table 1.1 Ethanol production from the major land crops and macroalgal*

Raw material	Moisture in raw material (%)	Carbohydrates etc. (%) **	Ethanol production per 1 ton of raw material	
			Kg/ton	L/ton
Corn	14.5	70.6	360.8	462.6
Barley	14.0	76.2	389.5	499.3
Wheat	10.0	75.2	384.4	492.8
Rice	15.5	73.8	377.2	483.6
Sweet potato	66.1	31.5	161.0	206.4
Potato	79.8	17.6	90.0	115.3
Sugarcane	60.0	15.0	76.7	98.3
Macroalgae *(S. horneri)*	90.0	5.8	29.6	38.0

**. Based on Aizawa et al. (2007)*

***. Macroalgae contains different component subject to fermentation (alginates, etc.) than that of land crops (starches, glucose).*

1.4. SPECIES DISTRIBUTION

The distribution of marine benthic communities are controlled by substrate availability, depths, and light intensity of the study area, and salinity of marine provinces (Nord, 1996). In addition, previous studies have shown that the growth, development, and distribution of *Sargassum* beds are strongly influenced by physico-chemical water parameters (Mattio *et al.*, 2008; Payri, 1987; Ragaza and Hurtado, 1999), as they play an important role in influencing nutrient-uptake through photosynthesis (Nishihara and Terada, 2010). The seasonal variations in physico-chemical parameters of seawater strongly influence changes in *Sargassum* canopy structure which in turn affects the density of the local populations (Ang and De Wreede, 1992; Arenas and Fernández, 2000; Ateweberhan *et al.*, 2009; Rivera and Scrosati, 2006).

Sargassum is usually found along the sheltered shores where they form abundant meadows on common substrates such as limestone rocks, dead coral, and rubble (Arenas *et al.*, 2002). A study on the distribution of *S. muticum* found that the hard benthos are the favored substrates for this species for growth and development (Staehr and Wernberg, 2009). Meanwhile, other studies showed that the distribution of *S.*

muticum had a strong relationship with rock substrates where the diameter of rocks are larger than 10 cm, but those areas structured with little stones, gravels and sand are unsuitable for the growth of *Sargassum* (Arenas *et al.*, 1995; Norton, 1977; Uchida *et al.*, 1991). In general, *Sargassum* species are usually found in sheltered and hard substrates in the coastal areas to settle and develop (Arenas *et al.*, 2002; Staehr and Wernberg, 2009).

1.4.1. Spatial distribution and diversity

The *Sargassum* genus is distributed worldwide, and is especially dominant in tropical and shallow sub-tropical waters (Hanisak and Samuel, 1987; Mattio *et al.*, 2008; Mattio and Payri, 2011; Yoshida, 1985). About half of *Sargassum* species are found (> 360 species) in tropical and subtropical regions. *Sargassum* are mostly attached to rocks but also have floating forms such as pelagic species such as *S. fluitans* and *S. natans* in the Sargasso Sea, the Gulf Stream (Hanisak and Samuel, 1987; Hwang *et al.*, 2007a; Komatsu *et al.*, 2003; Yatsuya, 2007).

The majority of *Sargassum* species are distributed in the northern and southern Pacific Ocean at the biodiversity hotspots including the Indian Ocean, Southeast Asia (SEA) and Australia. Shimabukuro *et al.* (2008) estimated that there are approximately 140 tropical and subtropical *Sargassum* species in the eastern Asian countries such as Japan, China and the Philippines (Shimabukuro *et al.*, 2008).

S. muticum originated from Southeast Asia and the Japan Sea (Arenas *et al.*, 1995; Kerrison and Le, 2016; Tsukidate, 1984; Uchida *et al.*, 1991; Wright and Heyman, 2008), but this species is also known as a non-native or invasive species in the West Atlantic Ocean including United States, Mexico (Aguilar-Rosas and Machado, 1990), Irish West Coast, Ireland (Baer and Stengel, 2010; Kraan, 2009), Danish (Thomsen *et al.*, 2006), and European coasts (Rueness, 1989). This species is considered one of the greatest threats to marine biodiversity and valuable natural resources of the global oceans (Kraan, 2009; Lawrence, 1984; Rueness, 1989).

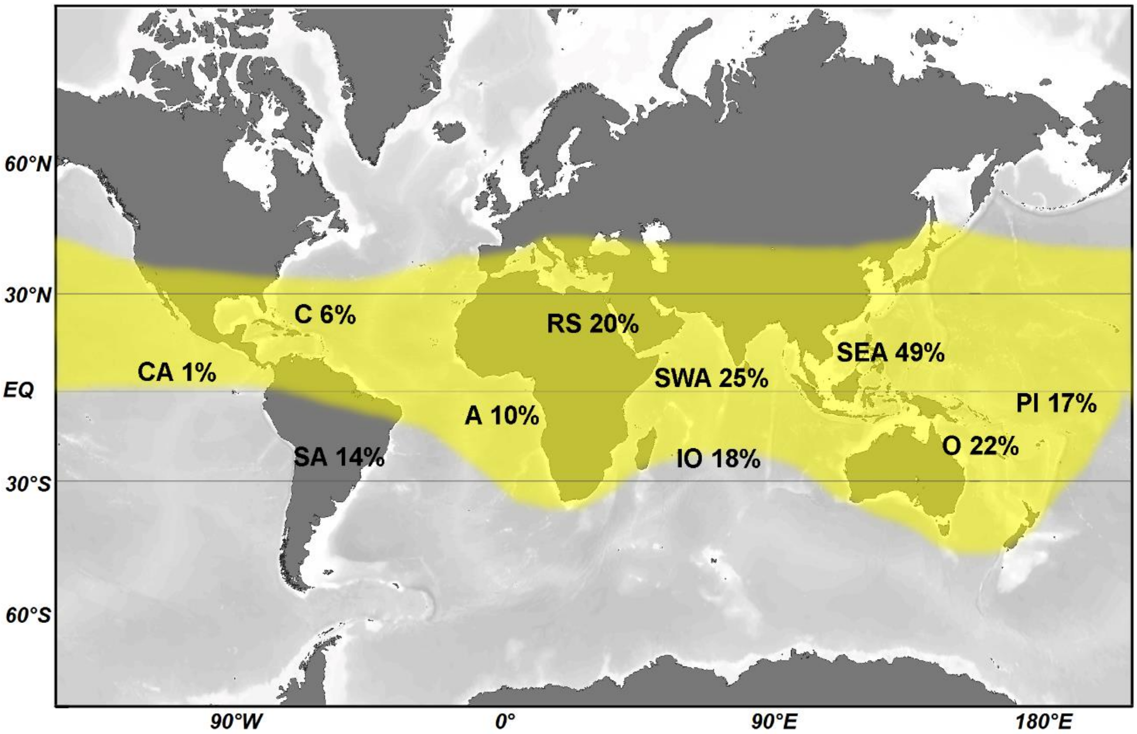

Figure 1.5 The distribution of tropical and sub-tropical *Sargassum* species (yellow area). The total number of species in each region was compared with the total number of the world wide *Sargassum* species. CA: Central America, C: Caribbean, SA: South America, A: Africa, RS: Red Sea, IO: Indian Ocean, SWA: South West Asia, SEA: South East Asia, O: Oceania, PI: Pacific Islands. Data was adopted from Thabard (2012) and (Yoshida, 1989) and the base map was derived from Ocean Data View.

The distribution area and percentage of *Sargassum* species composition that have been identified at different geographical areas is given in Figure 1.5. Of these, the number of species in SEA is the highest all over the world, followed by Southwest Asia and Australia (Oceanic). The lowest number of species was found in Central America (CA) (1%).

In Japan, Murase *et al.* (2000) reported that *S. macrocarpum* is extensively distributed from Aomori Prefecture to Kyushu in the Sea of Japan and from Chiba Prefecture to Kyushu in the Pacific Ocean. In Korea, *S. fulvellum* has a wide spatial distribution that ranges from the southern to the eastern coast of Korea (Hwang *et al.*, 2007a). In the Pacific Ocean, Mattio *et al.* (2008) revealed that there were significant differences in the spatial distribution of similar species in the same tidal flat areas.

In Japan, one of the earliest and most comprehensive *Sargassum* studies was described by Yoshida (1985). The author reported that there were around 50

Sargassum species found in the Japan Sea (Yoshida, 1985; 1989). Of these, there were 19 *Sargassum* species and their relative genera (*Hizikia*, *Coccophora*, and *Myagropsis*) which are dominant and formed large pelagic macroalgal beds around the Japan Sea and Pacific Ocean (Yoshida, 1985). In addition, a study by Shimabukuro *et al.* (2008) reported that there are 60 *Sargassum* species distributed around the Japan Sea, most of which are of subtropical origin (Shimabukuro *et al.*, 2008).

In India, according to a recent study on species composition of macroalgae in the Goa coastal area, 145 macroalgae species have been found belonging to 64 species of red algae, 41 species of green algae, and 40 species of brown algae. Of these, 12 *Sargassum* species have been identified at the study area (Pereira and Almeida, 2014).

In Korea, according to Lee and Kang (2007), 28 *Sargassum* species have been identified in Korea. Of these, *S. fulvellum* is one of the most abundant in the Korean Sea and has been widely used for salad and soups (Hwang *et al.*, 2007a; Sohn, 1993). The effect of environment parameters on *S. horneri* has also been studied (Choi *et al.*, 2009).

In Vietnam, the latest review of macroalgae studies has been published; in total 827 macroalgae species have been reported belonging to red macroalgae (412 species), green macroalgae (180 species), brown macroalgae (147 species), and cyanobacteria (88 species). The review also showed that Vietnam and the Philippines have a similar number of identified *Sargassum* species and subspecies (~70), but this was higher than other neighbouring countries such as Taiwan, Thailand, and Malaysia (Nguyen *et al.*, 2013). In particular, 14 *Sargassum* species have been found in the coastal areas of Quang Ngai province, Central Vietnam (Le *et al.*, 2015). Of these, *S. mcclurei, S. polycystum, S. ilicifolium, S. oligocystum, S. siliquosum*, *S. berberifolium, S. herklotsii* and *S. heslowianum* are the most abundant species in the region and can produce an annual biomass of approximately 1,680 tons per year.

In the Philippines, eight *Sargassum* species have been found at Balibago, Calatangan, Batangas, Philippines. Of these, *S. siliquosum* and *S. paniculatum* are the most abundant species at the reef-flat edge (Ang, 1986). In 1992, Trono reported 28 taxa belonging to the *Sargassum* genus in the Philippines waters (Trono, 1992). Of these, there were 13 taxa that have not been identified to the species level and could be considered new species for science. *S. crassifolium, S. feldmannii, S. gracillimum, S. kushimotense,* and *S. turbinarioides* species have been identified for the first time in the Philippines' flora (Trono, 1992). Noteworthy, a recent review of Philippines' macroalgae diversity showed that 73 *Sargassum* species were described in the period from 1980−1999 (Ganzon-Fortes, 2012).

In Malaysia, according to Wong and Pang (2004), 32 *Sargassum* species have been identified and only one subgenus of *Sargassum* has been found in Malaysian waters (May-Lin, 2011; Wong and Phang, 2004). Of these, *S. polycystum*, *S. binderi,* and *S. siliquosum* have been identified as the most abundant species in the Teluk Kemang area (May-Lin and Ching-Lee, 2013).

In Thailand, *Sargassum* taxonomy studies by Noiraksar and Ajisaka (2009) reported ten species along the Gulf of Thailand. Of these, *S. polycystum* was widely distributed at all of the sampled sites. *S. cinereum, S. longifructum* and *S. swartzii* were found for the first time in the Thai macroalgae community (Noiraksar and Ajisaka, 2009). A recently published study on *Sargassum* taxonomy employed the Nuclear Ribisomal Spacer 2 (ITS2) sequences and morphological data for reclassification of the *Sargassum* genus in Thailand (Kantachumpoo *et al.*, 2015). The results showed that eight popular *Sargassum* species have been identified. However, there were only six distinct clades obtained when the ITS2 method was applied. Therefore, it is required to have further/in-depth evaluations on phylogenetic relationships and species boundaries of *Sargassum* species from Thailand using morphological and other DNA markers (Kantachumpoo *et al.*, 2015).

In New Caledonia, eight macroalgal species belonging to Sargassaceae have been found. Of these, 6 species belonging to *Sargassum* genus and two species of *Hormophysa* and *Cystoseira* genus (Mattio *et al.*, 2008).

In brief, to date, the largest numbers of *Sargassum* species have been identified in the Philippines (73) and Vietnamese (72) waters belonging to the Southeast Asian Sea, followed by Japan (60 species), Australia (43), Korea (28), Malaysia (21), India (12), Thailand (10), and New Caledonia (8).

There are about 46 *Sargassum* species along the WA coast (DEC, 2011), whereas eight *Sargassum* species were recorded at Rottnest Island (Kendrick, 1993). The majority of them have been studied to determine their taxonomic affiliation, including the molecular basis of identification (e.g. Dixon and Huisman, 2010; Goldberg and Huisman, 2004; Kendrick, 1993; Kendrick and Walker, 1994), and physiology (De Clerck *et al.*, 2008; Huisman *et al.*, 2009; Kumar *et al.*, 2011; Muñoz and Fotedar, 2010; Staehr and Wernberg, 2009). However, there are a limited number of studies on the impacts of seasonality on *Sargassum* along the subtropical/temperate coastal zone of WA (Kendrick, 1993; Kendrick and Walker, 1994).

For instance, *S. howeanum* has the highest biomass in autumn and the lowest biomass in the summer. However, this was in contrast to a nearby region where the greatest biomass was found in the summer and lowest in the autumn.

On the shallow subtidal and intertidal reefs around the WA coast, *Sargassum* spp. form a dominant brown macroalgal group that shows strong seasonal variation (Kendrick and Walker, 1991). The highest biomass and density of reproductive thalli are recorded in spring (September to November) (Kendrick, 1993; Kendrick and Walker, 1994). According to Kendrick (1993), *S. spinuligerum* in the subtidal zone has an increased density of vegetative thalli from autumn (March to May) to winter (June to August). *S. spinuligerum* reproduces in spring (September to November) and summer (December to February), when it reaches the greatest biomass. To date, there has been little research on the distribution of *Sargassum* around Australian waters in general and WA coastal waters in particular.

1.4.2. Temporal distribution of *Sargassum*

The growth of macroalgae varies among seasons and life cycle stages. Seasonal patterns in growth of *Gracilaria foliifera, Ulva* species (Rosenberg and Ramus, 1982), *Laminaria longicruris* (Gagné et al., 1982), *Callophyllis variegata* (Ávila *et al.*, 2014), and *Sargassum* from New Caledonia, South Pacific (Mattio *et al.*, 2008) and from Florida, USA have been studied. The growth of macroalgae is dependent on the bioavailability of nutrients, irradiance, and photosynthetic quantum yield (Hwang *et al.*, 2007b), water temperature, salinity (Hanisak and Samuel, 1987) and wave exposures (Andrew and Viejo, 1998; Engelen *et al.*, 2005). In addition, different

Sargassum species are temperature, depth and cultivation stage dependent for growth (Hwang *et al.*, 2004). The variation of *Sargassum* biomass is based on seasonal changes and regional climate in different regions. For instance, in Malaysia, the seasonal variation of two *Sargassum* biomass of *S. baccularia* and *S. binderi* has been observed and the variation of reproduction is also examined (Wong and Phang, 2004).

In Japan, the annual variation of five common *Sargassum* species: *Myagropsis myagroides, S. paten, S. macrocarpum, S. siliquastram,* and *S. piluliferum* was studied in Yoro, western Wakasa Bay, Japan sea (Yatsuya *et al.*, 2005). Another study by Haraguchi (2008) revealed that *S. horneri* usually develops slowly in the summer when the temperature warms up and increase their growth rate in autumn as the temperature decreases (Haraguchi and Sekida, 2008). This study showed that a decrease in sea temperatures in the winter promoted the growth of *Sargassum* germlings of the subtropical species. Meanwhile, in temperate species, the development has a broader temperature and it has also been shown that several *Sargassum* species develop easily in the high temperature of the summer (~25^{o}C) (Haraguchi *et al.*, 2005; Haraguchi and Sekida, 2008).

In another study of *Sargassum* communities in Hiroshima coast, Japan Sea, it was revealed that the majority of *Sargassum* species are well developed in the autumn when the water temperature is decreasing. The *Sargassum* community began to mature in May and June when the sea temperature rose to around 18°C (Tsukidate, 1992).

An ecological study of *S. thunbergii* was completed early in the Gulf of Maizuru, Japan and showed that holdfast and stem remains after the main branch (primary laterals) had decayed (Umezaki, 1974). In the winter, *Sargassum* thalli are relatively short and grow slowly. In the summer, the water temperature is increasing, and *Sargassum* thalli show rapid growth in both length and productivity. The optimum temperature for *Sargassum* to achieve the highest length ranges from 27 ^{o}C to 29^{o}C. Yoshida (1985) reported that the floating *Sargassum* around the Japanese coast reached its peak biomass in May and can obtain up to 1.6 tons per km^{-2} or 5.6 tons per square nautical mil (Yoshida, 1985). However, to date, the seasonality of *Sargassum* from WA has not yet been fully documented.

1.5. REPRODUCTION IN *SARGASSUM*

Several *Sargassum* species can have both sexual and vegetative reproduction (Singh *et al.*, 2010). In vegetative reproduction, some thalli fragments detach from the mother thalli and then grow as new plants. The vegetative reproduction is relatively common in pelagic *Sargassum* species such as *S. natans, S. muticum* and *S. hystrix* (Trainor, 1978).

In sexual reproduction, fertilization occurs between male and female gametes into a new generation. The receptacles have abundant male and female reproductive structures. Male reproductive structures are called antheridia while female reproductive structure are called oogonia. Male and female conceptacles can be placed on the same plants or different plants, which are called monoecious or androgynous (dioecious), respectively.

In general, most *Sargassum* species reproduce annually. *S. thunbergia* in China has a peak biomass in the spring until early summer (Zhang, *et al.*, 2009). However, the reproduction is dependent on the environment conditions; several *Sargassum* species can have two reproduction times per year such as *S. thunbergia* in Chiba and Nagasaki (Akira and Masafumi, 1999).

Gabriela and Rafael (2011) studied the vegetation morphology and reproduction of *S. lapazeanum* in the south-western Gulf of California, Mexico and showed this species to be dioic, as female and male conceptacles are located on the same receptacle. However, male and female conceptacles have a different maturation time. This study also reported that the presence of cauline structures could be in response to the development of the main thallus as well as in response to reproduce new generations (Gabriela and Rafael, 2011). The cauline structure was also described in previous studies for *S. horridum* and other species in Japan (Noro *et al.*, 1994; Yoshida *et al.*, 1999).

Yoshida *et al.* (1999) studied the development of embryos in cauline leaves of *S. macrocarpum* (Fucales, Phaeophyta) under laboratory conditions. The authors revealed that it was not clear whether adventive embryos were produced in wild conditions, and what their role in the establishment of wild populations of *S. macrocarpum* is. The vegetative propagation from remaining holdfasts plays an important role for *S. thunbergia* and *Hizikia fusiformis* (Sargassaceae) species in the wild population (Yoshida *et al.*, 1999).

Recently, Yan and Zhang (2014) conducted studies on the development of zygote embryos and on the development of *S. vachellianum* germlings in the East China Sea. The results showed that temperature has a significant effect on germling development during the first 20 days of cultivation. The optimum temperature to cultivate this species is 21^{o}C and 40 μmol photon m^{-2} s^{-1} of irradiance (Yan and Zhang, 2014).

Male gametangia release a large amount of sperms, while most female conceptacles produce from four to six oogonia with a diameter of 40 × 32 μm, which increases to 157 × 105 μm when they are young and released, respectively (Critchley *et al.*, 1991) (Figure 1.6). However, Trainor (1987) reported that only *Ascophyllum* genus released four oogonium, the remaining genera produced two (e.g. *Pelvetia*) or one egg (e.g. *Sargassum*) (Trainor, 1978).

In *S. heterophyllum*, gametes are released in the winter with semi-lunar cycles. The release of gametes occur during the winter prevent the germling stages from hot temperatures in the summer (~30−33^{o}C) (Critchley *et al.*, 1991). In addition, the release of gametes is also dependent on several environment factors such as tide/lunal phase, day length, temperature, and hormones (Critchley *et al.*, 1991). Likewise, Yoshikawa *et al.* (2014) stated that the development of receptacles at *S. horneri* were affected by the irradiance and day length.

The studies of *Sargassum* communities in the Japan Sea showed that while most of the thalli are detached from the substrates after maturation, only a few species detach before maturation (Yatsuya, 2007). An evaluation study on the floating *Sargassum* in the Japan Sea also revealed that fragments can float for less than two weeks in nature. Only a few species can survive prolonged floating of over eight weeks (Yatsuya, 2007).

Figure 1.6 The development and releasing of Oogonial in *Sargassum heterophyllum*. Adopted from Critchley *et al.* (1991).

In general, *Sargassum* usually has an annual life cycle. However, their life cycles is affected by the local environment, climate, and local geographic conditions so that several species have life cycles with biannual reproduction.

1.6. LIFE CYCLE AND PRODUCTIVITY OF *SARGASSUM* SPECIES

The growth and mortality rate of *Sargassum* species varies among seasons and life cycle stages and is dependent on the bioavailability of nutrients, salinity, irradiance, water depth, pH, photosynthetic quantum yield, water temperature, and salinity (Hanisak and Samuel, 1987; Hwang *et al.*, 2004; 2007a; Liu *et al.*, 2007; Pustizzi *et al.*, 2004; Schaffelke and Klumpp, 1998).

Sargassum has either an annual or fairly long life-time of 3−4 years. A typical life cycle of *Sargassum* species includes growth phase, reproduction, maturation, and die-off (decay) (Ang, 2007). *Sargassum*'s life cycle includes diploid and haplobiotic phases (Critchley *et al.*, 1991). The receptacles' structure holds conceptacles. In *S. heterophyllum* both male and female conceptacles can be found on the same receptacle. Of these, oogonial conceptacles are the most predominant (Critchley *et al.*, 1991).

Sexual reproduction is marked by the production of eggs on the oogonial conceptacles and antheridia developed from the male conceptacles. The fertilization occurs in the water column and become a diploid spore. Then, the spores develop inside the sporangia to produce gametes (De Wreede, 1976). There are several studies that have succeeded in documenting the sexual and vegetative reproduction of several *Sargassum* species at different geographical areas, such as the trade-off between sexual and vegetative reproduction of *S. thunbergia* along the Shandong Peninsula coast, China (Chu *et al.*, 2011), reproductive phenology of *S. muticum* at Friday Harbor, Washington USA (Aguilar-Rosas and Machado, 1990; Baer and Stengel, 2010; Lawrence, 1984; Norton, 1977), *S. muticum* at Kurosima, Japan (Tsukidate, 1984), *S. horneri* at Maizuru Bay and Obama Bay, Japan (Choi *et al.*, 2009; Pang *et al.*, 2009; Uchida, 1993; Umezaki, 1984), *S. yezoense* at the coastal area of Oshika Peninsula, Japan (Yukio et al., 2002), *S. vachellianum* at Zhejiang province, China (Yan and Zhang, 2014), and *S. polyceratium* in the coastal area of Venezuela (Engelen *et al.*, 2005). In Malaysia, the reproduction of *S. baccularia* and *S. binderi* was studied by Wong & Phang (2004).

Productivity of *Sargassum* is species dependent. A study of brown macroalgae productivity in the Sargassaceae family was conducted by Yatsuya *et al*. (2005), where they were monitored over a period of 13 months at the west of Wakasa bay, Japan Sea. The annual net production of five macroalgae species was estimated to be approximately 2407, 2132, 1471, 1458, and 1197 g dry wt m^{-2} for *S. patens, S. macrocarpum, S. piluliferum, S. siliquastrum,* and *Myagropsis myagroides,* respectively (Yatsuya *et al.*, 2005).

Baer and Stengel (2010) have carried out comparative studies on the production, development, and reproduction of an invasive *S. muticum*, in different levels of wave exposure in the Irish Sea. The results showed that the development of receptacles have a significant difference between wave exposure conditions. A study on ecology and the life cycle of a green macroalgae species, *Nitella* sp., was carried out at Capel wetlands, WA (Annan and John, 2012). This study analyzed the morphological, ecological and life cycle as well as classification of the *Nitella* sp. The results showed charophyte species commence their life cycle in the autumn when the water temperature starts to fill up the ponds and continue their reproduction phases until the winter. To avoid the increasing temperature, reproduction always takes place in the spring and mature oospores are produced before the approach of summer when the water levels of the ponds are lowest until the ponds dry up (Annan and John, 2012).

1.7. EFFECTS OF ENVIRONMENT PARAMETERS ON *SARGASSUM*

The growth and development of *Sargassum* species depends directly on important environmental parameters such as nutrient concentrations, temperature, light, salinity, rainfall, pH, and wave action (Liu *et al.*, 2007; Newsted, 2004; Pustizzi *et al.*, 2004; Singh and Singh, 2015).

1.7.1. Nutrient requirements

Similar to other terrestrial plants, macroalgae require inorganic carbon, light, water and essential trace elements for photosynthesis and hence for growth and development. There are 21 elements required for the metabolism of plants; more than half of these are present in the macroalgae (Lobban and Harrison, 1994). Besides macro-nutrients, macroalgae also require several essential mineral ions and vitamins for growth. During cultivation, three vitamin groups namely, vitamin B_{12} (cyanocobalamin), thiamine and biotin are usually added to the culture environment (Lobban and Harrison, 1994).

In the marine environment, nitrogen is one of the common factors that frequently affects the growth of macroalgae (Lobban and Harrison, 1994). Following nitrogen, phosphorus is the second element that affects the growth and development of macroalgae. However, the nutrient requirements are different for each macroalgae species. Nitrogen concentration in sea water could be a limiting element for a macroalgal species, while phosphorus can be limiting for other species. A study on *Enteromorpha intestinalis* showed that the addition of nitrogen in sea water increases its growth rate, while the decrease of salinity decreases the growth-rate (Kamer and Fong, 2001).

Nitrate, ammonia and urea are used as nitrogen sources to support macroalgae growth, where ammonium is the basic nitrogen unit that macroalgae need to synthesize amino acids. Macroalgae requires and uses an amount of nitrogen that is approximately 55 to 111 times higher than terrestrial plants, aiming to meet the demands of increasing biomass quickly (Eustance *et al.*, 2013). Rosenberg and Probyn (1984) studied the absorption of nutrients and the growth rate of several brown macroalgae species including *Fucus distichus* subsp. *edentates* and *Chordaria flagelliformis* (Rosenberg *et al.*, 1984).

Zhang *et al.* (2009) observed the seasonal variation of kelp community at Goupi Island, China, and showed that nitrogen is one of the main factors affecting the growth and development of *S. horneri* and also controls nitrate changes in the aquatic ecosystems (Zhang *et al.*, 2009).

1.7.2. Temperature

Temperature is considered the main factor for the growth and maturation of *Sargassum,* with the majority based on the water depth levels of *Sargassum* beds (Plouguerné *et al.*, 2006). According to Haraguchi *et al*. (2005) *S. horneri, S. hemiphyllum,* and *S. micracathum* can grow in the maximum critical temperature of 27^{o}C. Meanwhile, *S. piluliferum, S. fulvellum,* and *Myagropsis myagroides* can grow in the maximum temperature 30^{o}C and this increases to 31^{o}C for *S. patens, S. macrocarpum,* and *S. thumbergii* species (Haraguchi *et al.*, 2005).

Several studies on variation of the macroalgae community in the east of the Cantabrian Sea in relation to the increasing sea surface temperature revealed that there was a strong variation of macroalgae community structure at the study area in the period from 1991 to 2008. The changes include: *i)* the benthos community increased in coralline algal; *ii)* a change in the distribution characteristic of canopy macroalgae; *iii)* the disappearance of kelps (*Ecklonia* sp.); *iv)* the increase of warm/tropical water species; *v)* the introduction of invasive species; and *vi)* a higher biodiversity index (Díez *et al.*, 2012).

A study on the effect of temperature on seasonality of *Sargassum* in Japan showed that sub-tropical *Sargassum* grow well at temperatures in the range of 16–21^{o}C. This temperature range also coincides with seawater temperature in the winter in the southern Japan Sea (Nagai *et al.*, 2011). Choi *et al.* (2009) studied the effects of temperature, irradiance, and day length on the growth pattern in the period from germling to mature *S. horneri* in Korea. The results showed that the germling and blade stages of *S. horneri* have optimum temperature ranges of 10–25°C, irradiances

(20–80 μmol photons m^{-2} s^{-1}), and day lengths (8–24 h). Of these, the optimal conditions to get the highest relative growth rates (RGR) for germlings was found to be 21% day^{-1} (25°C, 20 μmol photons m^{-2} s^{-1}) and 13% day^{-1} (8 h day length). The authors also stated that RGR was similar to the RGR of the wild *Sargassum* community (Choi *et al.*, 2009).

In laboratory conditions, Hamza *et al*. (2015) studied the effect of temperature and substrates on the growth of *S. echinocarpum* spores in Abu Dhabi coast. The results showed that germlings acquired the greatest length at 35^{o}C and limited their growth at temperatures below 25^{o}C. In addition, the rate of germling development was the highest with paper filter substrate (P = 0.02) compared to other substrates including clear base and cotton base (Hamza *et al.*, 2015).

1.7.3. Irradiance

Zou and Gao (2005) reported the effect of irradiance and temperature on photosynthesis of *Hizikia fusiforme* (Sargassaceae) leaves and receptacle structures. The results showed leaves and receptacles are independent of temperature and reach the greatest value of 200 μmol photons m^{-2} s^{-1} at 30^{o}C (Zou and Gao, 2005).

1.7.4. Salinity

Similar to light and temperature, salinity is also one of the most important environmental factors that affects the growth and development of *Sargassum* (Chu *et al.*, 2012; Henning, 2004; Hwang *et al.*, 2006; Qingman *et al.*, 2014; Wong, 1996; Yu *et al.*, 2012). Coastal marine macroalgae communities are usually impacted by the variation of salinity as a result of rainfall and runoff from the inland area (Chu *et al.*, 2012). Thus, most research into the effects of salinity on *Sargassum* growth revealed that this is a genus that can grow in a wide range of salinities and has a broad tolerance. For instance, *S. duplicatum* is a euryhaline species and can grow well in salinity from 15 to 35 psu. Of these, the greatest growth rate was found at 34 psu (Hales and Fletcher, 1989).

1.7.5. Water motion/ current

Wave action is one of the physical limiting factors for the growth and distribution of marine macroalgae, including *Sargassum* in the intertidal zone (Andrew and Viejo, 1998; Engelen *et al.*, 2005; Wichachucherd *et al.*, 2010). A study on the effect of wave exposure and intraspecific density on the survival and growth rate of *S. muticum* in northern Spain revealed that young thalli grow faster in areas that are affected by the waves. However, at the end of the growth phase, the length of macroalgae is similar to those without wave exposure, because the top of the main primary thalli were broken down by waves (Andrew and Viejo, 1998). The results of this study show that waves affect the distribution and development of juvenile stages in the *Sargassum* life cycle in the inter-tidal zone.

Another study on *S. muticum* in the Irish west coast showed that the growth rate and development of receptacle were significantly different between different two wave expose conditions. There were significant differences in the growth rate and development of receptacles at different wave-exposed studied sites. At the sheltered site, just 10% of plants were found to be fertile, while 100% plant fertility was found at the exposed sites and the tidal pools (Baer and Stengel, 2010).

1.7.6. Rainfall and sediment

Rainfall is another physical factor that plays an important role in controlling production biomass of *Sargassum* species (Wong and Phang, 2004). Wong and Phang (2004) studied biomass variation of two *Sargassum* species at Cape Richado, Malaysia and showed that rainfall is a key factor affecting *Sargassum* biomass. Hamedd and Ahmed (1999) showed that the highest *Sargassum* biomass season usually occurs in the pre-monsoon period coinciding with high rainfall (Hameed and Ahmed, 1999). In particular, when an El Niño occurs, on average every 3−7 years, this will cause heavy flooding in the central and eastern Pacific and cause drought in the western Pacific regions.

Sediment is one of limitation factors for *Sargassum* growth and development. A recent study showed that sediment effected the early stages of the *S. horneri* life cycle in the subtidal reefs (Bi *et al.*, 2016). The experimental results on effect of sediment coverage on attachment and development of germlings revealed that attachment rate of zygotes reached 81.5% of the control group, while medium dusting sediment (~0.5 mm thickness) only had 3.6% and 0% for heavy dusting sediment (~0.7 mm thickness). For the germlings, only 1.0% survived and there was 100% mortality when covered by medium sediment and high coverage, respectively (Bi *et al.*, 2016).

1.7.7. CO_2 and pH

An increase in CO_2 concentration leads to an increased in CO_2 concentration in the atmosphere. Ocean absorption of CO_2 contributes to reduce the effect of atmospheric warming due to greenhouse gas triggers (Doney *et al.*, 2009). However, the absorption in turn contributes to decreased pH levels of the ocean, which leads to ocean acidification. Atmospheric CO_2 has increased from approximately 280 to 395 ppm and has caused the pH of the ocean surface to drop by 0.1 pH units since the Industrial Revolution (Raven *et al.*, 2005). According to the Intergovernmental Panel on Climate Change (IPCC) scenario projections, in 2014, atmospheric CO_2 was 760 ppm and will rise above 1000 ppm by 2100. This increase will cause the pH of the surface of the ocean water to reduce from 0.3 to 0.4 pH units (Caldeira and Wickett, 2003, 2005; Raven *et al.*, 2005). The coupling of global temperatures rising to 2ºC and ocean acidification increasing higher than Pre-Industrial levels can lead to coral ecosystems becoming increasingly dominated by macroalgae, loss of biodiversity of marine ecosystems and less of an appeal for the tourism industry (Aronson *et al.*, 2002; Wernberg, Russell, Moore, *et al.*, 2011).

In WA waters, acidification of the sea surface water has increased by about 30% since 1950 (Doney *et al.*, 2009). Projections show that the pH level of sea surface water will be greatly reduced by 2050 (Orr *et al.*, 2005; Raven *et al.*, 2005). The decrease in pH levels of ocean water affects the growth of corals and calcifying organisms because it reduces the number of available carbonate ions needed to build up their skeleton/shells (Doney *et al.*, 2009). In contrast, the increase in CO_2 levels in seawater leads to an increase in the photosynthesis rate of macroalgae/aquatic plants due to more CO_2 diffusing from the external environment into the plant cells. However, there are long-term effects of the increase in CO_2 concentration on the growth rate of algae, because it affects the availability of nutrients. When using CO_2 for photosynthesis, plants need more nutrition to grow (Giordano *et al.*, 2005; Porzio *et al.*, 2011; Xu Zhiguang *et al.*, 2010). Relatively few studies have been conducted to understand the impact of decreasing ocean pH levels on either marine ecosystems or macroalgae of the WA region (Doney *et al.*, 2009). Therefore, it is clear that more studies are required to provide a better understanding on the biological and ecological response of species to climate change in order to determine their vulnerability (Morgan *et al.*, 2008).

1.8. *SARGASSUM* IN AQUACULTURE/MARICULTURE

Macroalgae have been cultivated and utilized since the 1970s (e.g. CMFRI, 1979; Gellenbeck and Chapman, 1983). They have been cultivated all over the world, in tropical and temperate areas, particularly in tropical areas such as the Indian Ocean and the tropical Pacific Ocean (Table 1.2). Among the three macroalgae groups, red and brown macroalgae are most commonly cultivated (Gellenbeck and Chapman, 1983). Generally, there are two kinds of macroalgae cultivations. Macroalgae are cultivated either in shore-based facilities such as tanks and ponds or in open ocean systems (Gellenbeck and Chapman, 1983). These cultivation techniques can also be integrated; for example, initial growth phase in coastal waters can be followed by transfer to shore-based facilities. This approach can be further integrated with prawn and fish farming with macroalgae. The macroalgae can produce a high concentration of dissolved nutrients in the monoculture ponds (Mai *et al.*, 2008). The study by Mai *et al.* (2008, 2010) has shown that integrating *Sargassum* sp. into Western King Prawn culture is beneficial for prawn farming. *Sargassum* can assist in maintaining optimum water quality and thus reduce the environmental impacts of aquaculture activities (Mai *et al.*, 2008).

Figure 1.7 The global aquaculture production (a) and the global capture production (b) of Phaeophyceae macroalgal, according to FAO Fishery Statistic (FAO, 2012)

Sargassum species are cultivable species as they have a high growth rate, long life cycle, and the reproduction spores can be released in the first year of culture (Kraan, 2009; May-Lin and Ching-Lee, 2013). Therefore, both submerged and floating

Sargassum have been considering as "energy farms or biomass farms" that have full potential for biomass development for green/clean energy and alginate industries since the 1960s (Hanisak and Samuel, 1987; Menzel and Ryther, 1961).

Table 1.2 The overview of macroalgae species cultured in tropical waters (McLachlan *et al.*, 1993; Vu *et al.*, 2003)

Region	**Country**	**Species**	**Purpose**	**Remarks**
Pacific Ocean	Fiji islands	*Eucheuma cottonii*		
		E. spinosum		
		Kappaphycus sp.		
	Maldives islands	*Eucheuma* sp.	Industrial production	
	Kiribati	*Eucheuma* sp.	Mariculture	Fanning Atoll
		Eucheuma striatum	Introduced	Marakei Lagoon
	Tuvalu	*Eucheuma* sp.		Funafuti lagoon
	The Philippines	*Eucheuma* sp.		
		*Kappaphycus alvarezii*var. *tambalang*	Economic	Visayas
	Malaysia	*Gracilaria* sp.		
	Hawaii (US)	*Kappaphycus alvarezii*		
		K. striatum	Imported species	
		E. denticulatum		
	Japan	*E. striatum*		
		E. alvarezii		
		Caulerpa lentillifera	Green algae	
	China	*Eucheuma* sp.	Carrageenan	
	Taiwan	*Eucheuma* sp.	Mariculture	
	Vietnam	*Gracilaria asiatica*	Integrated culture, water treatment	
		G. heteroclada		
		G. tenuistipitata	Cultivation	
		K. alvarezii	Introduced for cultivation	
	Australia	*G. eduli*		Townsville
Indian Ocean	India	*G. edulis*		
	Tanzania	*Eucheuma* sp.		
	Madagascar	*Eucheuma* sp.		
Mediterranean Sea		*Caulerpa taxifolia*		Introduced
Caribbean Sea	St. Lucia	*Gracilaria* sp.		

A pilot study of Tsukidate (1992) on the establishment of *Sargassum* beds with artificial reefs at the Japan Sea, showed that *Sargassum* beds were maintain well in the first two years with mono- or multi-species community structure. However, *Sargassum* beds were starting decay and replaced with other invasive species in the third year. Therefore, the supplementation of seeding is necessary to maintain the healthier and longer *Sargassum* beds after two years (Tsukidate, 1992).

A study on breeding of *S. vachellianum* on artificial reefs was carried out in Gouqi Island, China (Chai *et al.*, 2014). The field trail result showed that the average length of young seedlings reached 31.85 ± 0.62 mm and 41.31 ± 1.39 mm at slow current site and rush current site, respectively. After a one year trial, the *S. vachellianum* reached the average length of 15.51 ± 3.42 cm. However, this research did not present the survival and mortality rates after a certain time (Chai *et al.*, 2014).

1.9. COASTAL HABITATS AND *SARGASSUM* BEDS MAPPING

1.9.1. Satellite imagery remote-sensing on coastal habitat mapping

Satellite remote-sensing imagery (SRSI) has been used as a powerful tool to estimate the biomass of terrestrial and marine plants on large areas in order to limit the field study costs, which can be time-consuming, and can even destroy the study areas in several restricted regions (Andréfouët *et al*., 2004). In recent years, satellite remote-sensing studies have been successfully applied to map coastal marine ecosystems (Andréfouët and Robinson, 2003; Andréfouët *et al.*, 2004; Benfield *et al.*, 2007; Casal *et al.*, 2011a, b; Fearns *et al.*, 2011; Maheswari, 2013; Mumby *et al.*, 2004; Vahtmäe and Kutser, 2007; Vahtmäe *et al.*, 2012). Most of these techniques have focused on seagrass meadows (Ha *et al.*, 2012; Hoang *et al.*, 2011; Komatsu *et al.*, 2003) and coral reefs (Mumby *et al.*, 1998; 2004; Scopélitis *et al.*, 2009; 2010). Several initial studies have validated a methodology for mapping *Laminariales* (Kept forest) in turbid waters by using Satellite Pour l'Observation de la Terre-4 (SPOT-4) satellite images (Casal *et al.*, 2011a).

The very first scheme for mapping macroalgae was mentioned in the 1970s by Norton from the Department of Botany, University of Glasgow, Scotland (Norton, 1970; 1971). He used his scheme to map the macroalgae distribution around the British coast as the first knowledge on geographical distribution of marine algae in the British Isles.

To date, there are three universal sampling methods: direct observations, indirect observations, and integrated methods; these are widely used by coastal scientists for mapping marine SAV.

In the direct observation method, marine habitat mapping is usually implemented using ground surveys by side-scan sonar, hydro-acoustic and direct visual observations using either scuba or free-diving techniques. These techniques have been used in numerous studies to map the distribution of macroalgae and seagrasses (e.g. Fearns *et al.*, 2011; Komatsu *et al.*, 2002; Tecchiato *et al.*, 2011). However, all of these techniques are extremely time-consuming, expensive and often unfeasible for large areas.

In the indirect methods, a range of earth observation satellite imageries such as IKONOS, SPOT, Landsat, the Compact High Resolution Imaging Spectrometer–Project for On-Board Autonomy (CHRIS-PROBA), and Medium Resolution Imaging Spectrometer (MERIS) can be used for mapping spatial and temporal distribution of marine habitats (e.g. Andréfouët *et al.*, 2004; Casal *et al.*, 2011a, b; Gower *et al.*, 2006; Hoang *et al.*, 2011). For example, Gower *et al.* (2006) combined the MERIS and Moderate Resolution Imaging Spectroradiometer (MODIS) to interpret extensive lines of floating *Sargassum* in the Gulf of Mexico. The satellite observation results showed that *Sargassum* biomass was higher than previously estimated. Fearns *et al.* (2011) reported for the first time the application of airborne hyperspectral, HyMap, remote-sensing model for coastal habitat mapping in WA’s waters. Thus, these indirect methods have been used as the most cost-effective method in order to collect data quickly on a large scale. However, the ground truth data from the field trips are also required to improve the accuracy and validate the interpretation results. Satellite remote-sensing imagery requires fewer field surveys, and with the cost of imagery decreasing with concurrent improvements in spatial and temporal resolution, there has been a rapid increase in the use of SRSI for various marine-mapping applications.

Thus, recent studies have shown the advantages of several integrated methods in mapping and detecting the most abundant of macroalgae along the coast. The clearest and most direct method for marine habitat mapping is integrated visual observations ground-truth data using either scuba or snorkeling techniques which provides an essential input to the remote-sensing interpretations (Komatsu *et al.*, 2002). For instance, a methodology for mapping *Laminariales* (Kelp) in turbid waters of the Seno de Corcubión (Northwest Spain), using SPOT-4 satellite images was developed, which showed that the mapping of *Sargassum* beds could be improved through the application of higher spectral images, increasing spatial and radiometric resolution as well as by performing new field calibrations simultaneously to the acquisition of images (Casal *et al.*, 2011a). Lower resolution Landsat (30 m) and higher resolution QuickBird (2.4 m) satellite images have been used to estimate the spatial distribution of 11 *Sargassum* beds in South West Lagoon, New Caledonia (Mattio *et al.*, 2008). Le *et al.* (2009) applied remote-sensing techniques to detect the distribution of *Sargassum* meadows in coastal waters of Khanh Hoa province, Vietnam. The Depth Invariance Index (*DII*) algorithm was mainly employed to detect *Sargassum* meadows. This was also the first distribution map of *Sargassum* meadows in coastal waters of Khanh Hoa province, Vietnam, to have been established. In 2010, by using satellite and field images, Liu and co-workers also demonstrated that green-algal tide re-occurred in June 2009 from the Jiangsu coast (Liu *et al.*, 2009; 2010). Another study by John *et al.* (2011) reported on the inter- and intra-annual patterns of *Ulva prolifera* green tides in Yellow Sea during 2007-2009 periods. The authors concluded that *Ulva prolifera* waste from *Porphyra* aquaculture rafts caused the blooms during these periods (John *et al.*, 2011). Another study by Zavalas *et al.* (2014) on macroalgae community classification shows that the application of bathymetric Light Detection and Ranging (LiDAR) yielded high accuracy rates of classification of mixed brown algae and sediment substrata, which were 74% and 93%, respectively (Zavalas *et al.*, 2014).

1.9.2. Mapping *Sargassum* and other associated habitats

Satellite remote-sensing imagery has been successfully applied for mapping marine habitats in shallow coastal waters, especially in clear water with good light penetration, where it is easy to carry out field observations (Green *et al.*, 2000). A range of different satellite imagery has been used for mapping the spatial and temporal distribution of macroalgae and their associated habitats, including MERIS (Gower *et al.*, 2005), SPOT-2/4 (Casal *et al.*, 2011a), Landsat (Vahtmäe and Kutser, 2007), IKONOS (Andréfouët *et al.*, 2004; Sagawa *et al.*, 2008; 2012; Stumpf *et al.*, 2003), CHRIS-PROBA (Casal *et al.*, 2011b), The Advanced Land Observing Satellite

(ALOS) – The Advanced Visible and Near Infrared Radiometer Type-2 (AVNIR-2) (Hoang *et al.*, 2009; Phauk *et al.*, 2012; Sagawa *et al.*, 2012) (Figure 1.8).

The recent launch of the commercial WV-2 satellite has further increased the spatial and spectral resolution of SRSI, with images having a 0.5-m spatial resolution for the single panchromatic band (450–800 nm) and a 2-m resolution for the eight multispectral bands. In addition to the four standard colors: blue, green, red and near-infrared 1 (NIR-1), WV-2 includes four new colors *i.e.*, coastal band (400–500 nm), yellow band (585–625 nm), red edge (705–745 nm) and NIR2 (860–1,040 nm), which are particularly useful for coastal ecosystem studies (DigitalGlobe, 2013; Updike and Comp, 2010). Thus, to date, WV-2 satellite images provide the highest available spatial and spectral resolutions (eight spectral sensors ranging from 400–1040 nm) (DigitalGlobe, 2013; Lee *et al.*, 2011).

Figure 1.8 The concept diagram presents the spatial and temporal resolution of available satellite remote-sensing imagery for various earth science applications (Adopted from Hedley *et al.*, 2016).

However, there have been no studies using these high-resolution satellite images for the detailed mapping of SAV and *Sargassum* beds in coastal waters. In addition, a direct visual approach integrated with high spatial resolution remote-sensing observations could be a robust approach to minimize the costs while increasing the accuracy of detection and distribution patterns of *Sargassum* shallow coastal waters.

Table 1.3 Studies that have used the WV-2 and other high spatial resolution satellite imagery to map coastal ecosystem/habitat vegetation since October 2010.

Location	Depth (m)	Satellite imagery	Employed techniques/methods	Identified habitats	Overall habitat map accuracy	Cited
Offshore island of Singapore	0–0.8	WV-2 (eight bands)	Unsupervised classification and contextual editing.	Seagrass meadow, mangroves and coral reefs.	n.a.	(1)
Singapore coastal waters	0.1–20	WV-2 (eight bands)	Computed reflectance spectra of SAV.	SAV, seagrasses.	Possible to detect SAV in low-turbidity water (up to 25 NTU).	(2)
Recife de Fora, Brazil	n.a.	WV-2 including "Coastal Blue" band	A back-and-forth shifting by selecting RGB triplets generates polygons; Inverse Distance Weighted model.	Coral reef system, seagrass & macroalgae, coralline algae, coral & octocoral, zooanthidean.	A map of coral reef and benthic habitat at the scale of 1:5,000.	(3)
Gulf of Mannar, India	n.a.	WV-2 including "Coastal Blue" band	Atmospheric correction, Radiance conversion, ToA reflectance.	Water, seagrass, island, coral reef	n.a.	(4)
Almería coast (Southern Spain)	n.a.	GeoEye-1 and WV-2	Sensor models and geometric quality assessment tests	Assessing geometric accuracy	n.a.	(5)
The Wax Lake delta, USA	n.a.	WV-2, IKONOS, OrbView-3	MLC and SVM classifiers.	Fresh water marsh species	75% for MLC from WV-2 imageries	(6)
Dukuduku, South Africa	n.a.	WV-2 (eight bands)	The object-based and pixel-based classification	Subtropical coastal forest.	86.9% (k = 0.82)	(7)
Darwin, Australia	n.a.	WV-2 and aerial photographs	The object-based image analysis and SVM classifiers	Mangrove Species Classification	89% (k = 0.86)	(8)

Abbreviations: n.a.: not available, ToA: Top-of-atmosphere, MLC: Maximum likelihood classification, SVM: Support vector machine. Literature cited: (1) Chen *et al.*, 2011; (2) Soo-Chin and Chew-Wai, 2012; (3) Seoane *et al.*, 2012; (4) Maheswari, 2013; (5) Aguilar *et al.*, 2013; (6) Carle *et al.*, 2014; (7) Malahlela *et al.*, 2014; (8) Heenkenda *et al.*, 2014.

The WV-2 satellite has been effectively used to map seagrass, and macroalgae since 2010 (Aguilar *et al.*, 2013; Cerdeira-Estrada *et al.*, 2012; Chen *et al.*, 2011; Heenkenda *et al.*, 2014; Maheswari, 2013; Midwood and Chow-Fraser, 2010; Seoane *et al.*, 2012; Soo Chin and Chew Wai, 2012). However, no systematic study into the utility of WV-2 imagery for the remote-sensing of macroalgae has been performed, particularly of *Sargassum* spp. No studies have used multispectral satellite remote-sensing to map marine habitats in general or to map canopy macroalgae along the WA Southwest coast. Therefore, assessing the distribution of *Sargassum* beds using high spatial resolution satellite images WV-2 from this study could be considered the first such approach.

Noiraksar *et al*. (2014) has shown the potential of using the objective-based classification methods to interpret the distribution of macroalgal canopy and *Sargassum* spp. by ALOS AVNIR-2 satellite images (Noiraksar *et al.*, 2014). Within four years from the date of successful operation (October 2010) there has been a number of research studies using the WV-2 images data in assessing coastal ecosystems. So far, we have found that the number of published applications of remote-sensing image by WV-2 data in coastal ecosystems studies is still minimal. For instance, the WV-2 imageries were used to map seagrass, mangroves, and coastal reef ecosystems in Singapore (Chen *et al.*, 2011). In Brazil, Seoane *et al*. (2012) used WV-2 and Quickbird images to map benthic habitats such as coral reefs, macroalgae, and coralline algae ecosystems (Seoane *et al.*, 2012). Another study in the Gulf of Mannar, India, used WV-2 satellite data and GIS to evaluate the water quality parameters, seagrass and coral reef in marine protected areas (Maheswari, 2013). Therefore, assessing the distribution of *Sargassum* beds by using high spatial resolution satellite images WV-2 from this study could be also considered the first approach. This approach allows a sufficiently accurate classification of results that showed the advantages of its satellite image source and practicality.

1.9.3. Methods on the identification and mapping of marine benthic habitats

1.9.3.1 Reflectance response library of marine submerged aquatic vegetation

Satellite remote-sensing imagery is one of the main techniques in mapping the distribution and coverage of macroalgae based on the difference of optical signatures and spectral reflectance profiles (Kutser *et al.*, 2006). Spectral reflectance profiles that allow the differences between benthic substrates to be determined are usually narrow. Therefore, the hyperspectral instruments are encouraged in mapping the distribution of benthic habitats (Kutser *et al.*, 2006). The different between benthic habitats and substrate types at different water depths were calculated by subtracting the reflectance of a substrate type with another substrate at the same depth (Kutser *et al.*, 2006).

In order to provide the input data for SRSI classification, it is necessary to collect and analyze the spectral reflectance profiles of various macroalgae species and their associated substrates. For instance, to classify and identify invasive macroalgae species including *Caulerpa racemose* var. *cylindracea* and *C. taxifolia* in the Adriatic Sea, Kisevic *et al.* (2011) collected and analyzed the spectral reflectance profiles of these species to initially separate the invasive species with other native species in the same genus (e.g. *Caulerpa* cf. *laetevirens*) (Kisevic *et al.*, 2011).

According to Kutser *et al.* (2006) the reflectance profiles of optically dark bottom macroalgae such as brown and red macroalgae are related to their depth in the water column when using the multispectral sensors. Landsat 7 ETM+ and IKONOS imagery are not suitable to determine these differences. Both brown and red macroalgae could be identified from the water column if the depth is more than 1 m. Landsat 7 ETM+ and IKONOS cannot be used for mapping the *Fucus* meadows in the coastal area of Estonia, as these images cannot detect *Fucus* sp. in the deep waters. While the Advanced Land Imager (ALI) can be detected the differences of benthic habitats at the depth up to 1.5 m (using band 2), MERIS can identify *Furcellaria* at a depth of around 4 m. Moreover, sand and *Furcellaria* can be separated by MERIS sensors at a water depth of up to 6 m. Therefore, the majority of *Furcellaria lumbricalis* can be mapped with MERIS data as the mentioned conditions (Kutser *et al.*, 2006). According to Andréfouet *et al.* (2004), the integrated results of ground truth and IKONOS 4-m resolution imagery gave the overall accuracy up to 70%.

1.9.3.2 Maximum Chlorophyll Index techniques

The Maximum Chlorophyll Index (MCI) method has been used as a helpful tool to detect algal bloom. MCI employs spectral band 709 nm of MERIS data. The MERIS can be analyzed and the real-time data used at the global scale for an early warning system of algae bloom (Gower *et al.*, 2005).

The MCI methods of MERIS imagery was also used to detect the massive pelagic *Sargassum* at the Gulf of Mexico in July, in which the highest biomass was found at the north-west of the Gulf (Gower *et al.*, 2006). It is also considered the first scientific report on the application of satellite remote-sensing detect *Sargassum* which opens up a novel theme in the study of distribution, spatial and temporal variation of *Sargassum*.

The integration between satellite remote-sensing and the ground truth that is usually collected by the research vessels provide an advantageous data for *Sargassum* studies, but still encounter some limitations on spatial resolution, cloud cover, and sunlight glints (Grower and King, 2008). However, knowledge of the annual life cycle of *Sargassum* is not sufficient to meet the requirements of recent studies. For instance, the original locations of *Sargassum* in the Gulf of Mexico and Sargasso Sea are not clear.

1.9.3.3 Supervised and Unsupervised Classification methods

Interpreting the acquired data and classifying land cover features are the main objectives of satellite remote-sensing studies. There are two major classification approaches: supervised and unsupervised classification methods (Xie *et al.,* 2008).

Of these, supervised classification methods are essential tools for extracting information from SRSI (Richards, 2013). The classification processes are based on a training dataset which is related to each class of interest. Maximum likelihood, Mahalanobis, Minimum distance, Parallelepiped classification, and Spectral angle mapper are commonly used to classify vegetation and land use land cover applications (Movia *et al.*, 2015; Richards, 2013). As such, the supervised classification methods usually go through two processes including training and classification. The accuracy of supervised classification is mostly dependent on the quantity and quality of the training dataset (Lang *et al.*, 2008; McCaffrey and Franklin, 1993; Xie *et al.*, 2008). For instance, a study on mapping kelp forests in turbid waters by SPOT-4 satellite imagery showed that the interpreter, analysis, and supervised classification methods were given an accuracy over 70% for each method (Casal *et al.*, 2011a).

By contrast, unsupervised classification methods are based on a clustering algorithm to classify satellite remote sensing data, without the support of a training dataset in the study areas (Richards, 2013). The unsupervised classification is mainly based on the spectral values of land cover features. The similar spectral values are classified into the same group of the SRSI (Lang *et al.*, 2008; Lillesand *et al.*, 2004). In unsupervised classification groups, there are numerous classification algorithms that have been widely used in vegetation classification such as K-means and Isodata Clustering (Richards, 2013). For instance, Movia *et al.* (2015) employed three unsupervised classification methods to classify very high resolution (VHR) aerial images to provide information in agricultural and urban features. This study used K-means, Self-organizing map and Maximum likelihood techniques to interpret five cover classes of the study area (Movia *et al.*, 2015).

1.9.4. The limitation of mapping benthic habitat methods

The high density of phytoplankton and particulate organic matter could limit the detection and mapping of macroalgal habitats in the water column, especially during a phytoplankton bloom. Kutser *et al.* (2006) showed that chlorophyll concentration ranged from 1−4 mg m^{-3} from winter to summer at the Bantic Sea. However, the chlorophyll concentration increased up to 30−100 mg m^{-3} in the spring (Kutser *et al.*, 2006).

In addition, the distribution of macroalgal communities are significantly controlled by substrate availability, water depths, salinity, and photoperiod of the coastal oceans (Nord, 1996). In the Bantic Sea, green macroalgae are usually found in hard substrates and the shallowest of the subtidal areas (Nord, 1996). Green macroalgae present a high diversity of species, polymorphic including ephemeral filamentous to perennial species with large thalli (Kutser *et al.*, 2006). However, according to Martin and Torn (2004), the brown macroalgae *Fucus vesiculosus* develops in belts at the depth of about 6 m (Georg and Kaire, 2004).

1.10. *SARGASSUM* AND GLOBALLY CHANGING ENVIRONMENTS

Through photosynthesis, phytoplankton, macroalgae and shellfish can directly and indirectly absorb a significant volume of CO_2 in the coastal oceans (Tang *et al.*, 2011). Marine phytoplankton and macroalgae contribute approximately 71% of carbon storage in oceanic sediments during their life cycles, where macroalgae contribute at least 50% of the world's carbon fixation (Chung *et al.*, 2011). Therefore, macroalgae play a significant role in carbon sequestration in oceans and in coastal waters (Chung *et al.*, 2011).

Climate change, especially the increase in sea surface temperature (SST) and ocean acidification, is causing long-term effects to coral reefs and marine ecosystems' health around the world oceans. *Sargassum* macroalgae also play vital roles in climate change responses such as carbon fixation and sequestration of coastal ecosystems (Xu Zhiguang *et al.*, 2010). They absorb CO_2 from the atmosphere and distribute it in the deep layers of the ocean as one important part of the ocean carbon sink (Aresta *et al.*, 2005a; CMFRI, 1979; Gellenbeck and Chapman, 1983; Hong *et al.*, 2007).

In WA, the annual average overnight minimum temperature has been increasing by about 1.1^{o}C and SST has been increasing by around 0.84^{o}C since 1910 (Bidwell and McLachlan, 1985; Cresswell and Golding, 1980; IOCI, 2009; Pearce and Feng, 2007). Warming around the whole of the WA state is occurring in the winter and spring (BoM-Australia, 2013). This increasing SST may affect the Leeuwin Current that has a major influence on marine environments and ecosystems along the southwest WA coast (Morgan *et al.*, 2008). A number of coral species in coastal WA have moved south with cooler waters by about 100−200 km due to their reaction to the warming of ocean water in the coastal region of WA (Greenstein and Pandolfi, 2008)

Temperature is a common factor that has a major effect on an aquatic organisms' health (Petton *et al.*, 2013). The coupling of increased global temperatures rising to 2ºC and ocean acidification going higher than the Pre-Industrial Revolution can lead to coral ecosystems becoming increasingly dominated by macroalgae, loss of biodiversity of coral reef ecosystems and the attractiveness for the tourism industry (Aronson *et al.*, 2002). Western Australia is one of the 25 biodiversity hotspots of the world and holds up to five of Australia's 15 biodiversity hotspots (Morgan *et al.*, 2008). However, relatively few studies have been conducted to better understand the impact of increasing SST and ocean acidification on either marine ecosystems or macroalgae of the WA region (Doney *et al.*, 2009).

As a consequence of increasing SST, new macroalgae species have appeared or have been introduced by scientists and cultivators (Boudouresque and Verlaque, 2010; Philippart *et al.*, 2011; Wells *et al.,* 2009). For example, 106 non-native macroalgae species have been introduced into the Mediterranean waters (Boudouresque and Verlaque, 2010) and five introduced macroalgae species were discovered in WA's coastal waters (Wells *et al.,* 2009) (Table 1.4). Therefore, it is clear that more studies are required to provide a better understanding of the biological and ecological response of species to climate change in order to determine their vulnerability (Morgan *et al.*, 2008).

Table 1.4 Introduced macroalgae species in the coastal areas of Western Australia (Wells *et al.*, 2009).

No.	**Common name**	**Scientific name**	**Classification**	**Remarks**
1	Dead man's fingers, oyster thief	*Codium fragile* ssp. *fragile*	Green algae *(Chlorophyta)*	Introduced via hull fouling, vessels and their equipment
2	Forked grateloup's weed	*Grateloupia imbricata*	Red algae *(Rhodophyta)*	Originally from Japan, introduced via hull fouling
3	False codium	*Pseudocodium devriesii*	Green algae *(Chlorophyta)*	Original described from South Africa, introduced via hull fouling
4		*Elachista orbicularis*	*Ochrophyta*	
5		*Stictyosiphon soriferus*	*Ochrophyta*	

Chapter 2. INTRODUCTION

2.1. RATIONALE OF THE STUDY

Sargassum belongs to the marine SAV groups and is one of the most important genera of aquatic ecosystems in providing food, shelter, habitats for reproduction and a feeding ground for aquatic animals. Similarly to seagrasses and coral reefs, *Sargassum* also plays an important role in forming coastal marine ecosystems as primary producers and ecosystem engineers (Harley *et al.*, 2012; Tyler, 2010). As ecosystem engineers, the canopy of *Sargassum* species contributes to form a benthic ecosystem where coastal marine aquatic species can find refuge and reproduce (Mattio *et al.*, 2013; Tanaka and Leite, 2003). Like other coastal ecosystems, *Sargassum* beds have high biodiversity, but they are under growing pressures from human activities in terms of water quality, and natural pressure from seasonal variation, as well as the changing climate (Andrew and Viejo, 1998; Ang, 2007; Ateweberhan *et al.*, 2009; Cortés *et al.*, 2014; Engelen *et al.*, 2005; Espinoza and Rodriguez, 1987; Wernberg *et al.*, 2011; Zhang *et al.*, 2009). It is predicted that by the middle of the 21st century, commercial fish and marine stocks will collapse beyond the recovery point if the human impacts are not mitigated effectively (Worm *et al.*, 2006).

Understanding the biology and ecology of *Sargassum* species is one of the important tasks that needs to be addressed on both regional and global scales, and is of worldwide concern. For example, *Sargassum* biomass has been steadily declining in both biomass and coverage area in Asia (Zhang *et al.*, 2009) and there is limited information on the ecology and distribution of *Sargassum* populations in the Asia Pacific region (Cortés *et al.*, 2014; Mattio *et al.*, 2008; McLachlan *et al.*, 1993; Phillips, 1994a). Furthermore, there has been an increase in the distribution area of invasive *S. muticum* in European waters (Cacabelos *et al.*, 2013; Hales and Fletcher, 1989; Norton, 1977; Rueness, 1989; Thomsen *et al.*, 2006) and a massive bloom of pelagic *Sargassum* in North Atlantic (Maria *et al.*, 2012), Caribbean Sea (Camacho and Hernandez-Carmona, 2012; Camacho *et al.*, 2015; Gower *et al.*, 2006), and in the Sargasso Sea due to effect of environmental stressors (Gower *et al.*, 2006; Hu *et al.*, 2015).

In addition, knowledge of the spatial and temporal distribution characteristics of *Sargassum* beds in coastal waters is essential information in understanding the past and current status of spatial distribution which can be compared with changes in the future. However, our knowledge of the current distribution, geographical locations, and ecological functioning of *Sargassum* and other SAV is still poor due to the limitations of expensive traditional survey methods (Brown *et al.*, 2011). Moreover, it is estimated that only 5–10% of the seafloor area has been mapped with the same resolution as in terrestrial studies (Wright and Heyman, 2008). Therefore, the effective management and sustainable exploitation of *Sargassum* resources in coastal waters is faced with several limitations.

To the best of our knowledge, to date, there are only three publications on *Sargassum* mapping using satellite remote-sensing (Andréfouët *et al.*, 2004; Mattio *et al.*, 2008; Noiraksar *et al.*, 2012). Of these, there were two studies that applied SPOT imagery (Andréfouët *et al.*, 2004; Mattio *et al.*, 2008) and a study with ALOS AVNIR-2 imagery (Noiraksar *et al.*, 2014) with the spatial resolution of 5 m and 10 m, respectively. The previous studies merely reported the distribution and location of *Sargassum* beds rather than assessing adjacent SAV habitats and their classification accuracy was limited.

The coastal areas of WA have received little scientific attention on the ecology and biology characteristics of macroalgae in general and *Sargassum* in particular. Several publications of *Sargassum* studies were carried out in WA waters including on taxonomy (Dixon and Huisman, 2010; Goldberg and Huisman, 2004) and recruitment in structuring beds (Kendrick and Walker, 1994). As such, there are no published data on: 1) the evaluation of seasonal changes in biomass, and growth rate of *Sargassum* in WA waters; 2) the use of high spatial and spectral resolution to assess the spatial distribution characteristic of *Sargassum* and other associated habitats in the coastal shallow waters; 3) the ecology and life cycle of most *Sargassum* species, which has not yet been documented; and 4) the baseline information on the requirements of initial *Sargassum* biomass for successful cultivation.

Therefore, studies on distribution, reproduction and life cycle of the most abundant *Sargassum* species in WA waters would provide baseline information for management. The present study approaches the interdisciplinary methods including satellite remote-sensing techniques, ecological studies, and aquatic plant cultivation that aim to fully understand the knowledge gaps (1–4). The aim of this study is to comprehensively understand the biology, ecology, life cycle, distribution, and seasonal changes of *Sargassum* and determine how this impacts the broader spatial distribution of *Sargassum* beds using *in situ* observations and satellite remote-sensing methods.

2.2. AIM OF THE STUDY

The main aim of the research was to study the seasonal abundance and distribution of *Sargassum* spp. and their associated habitats around Point Peron and Rottnest Island in WA, and nutrient-uptake capacities of the native species of *Sargassum spinuligerum.*

2.3. OBJECTIVES

This aim of the research was achieved by meeting the following objectives:

1. To identify and select sampling sites to study the abundance, productivity and distribution of *Sargassum* spp. based on the secondary data and field surveys.

2. To contribute as the primary spectral reflectance profile library for a comprehensive data on SAV species and their associated substrates.

3. To validate the feasibility of the WV-2 satellite data for identifying and mapping SAV in coastal habitats.

4. To evaluate the supervised and unsupervised classification methods for classifying SAV and other associated benthic habitats.

5. To identify and map the distribution of *Sargassum* and other associated benthic habitats in shallow coastal waters with WV-2 satellite data.

6. To document the reproduction and life stages of *Sargassum spinuligerum* in the WA coast.

7. To determine the seasonal variation in water quality and biomass changes of *Sargassum* spp. around Point Peron, WA.

8. To estimate the growth rates and productivity of the most abundant *Sargassum* species in WA waters.

9. To investigate the effect of different commercially available fertilizers and different quantities of initial stocking biomass on the growth rate and nutrient uptake of *S. spinuligerum* in outdoor cultivation conditions.

2.4. SIGNIFICANCES

The outcomes of this research project aim to contribute to improving knowledge of the *Sargassum* community along the WA coast by

1. Providing an understanding of the seasonal abundance and variety of the *Sargassum* community around Point Peron and Rottnest Island, WA.

2. Providing understanding of the effects of environmental parameters on the abundance and biomass of *Sargassum* species.

3. Contributing to the collection of data on the growth and productivity of *Sargassum* both under outdoor and laboratory conditions.

4. Documenting the life cycle of *Sargassum spinuligerum* in WA waters.

5. Assessing the distribution of *Sargassum* beds and other coastal marine SAV ecosystems using remote-sensing studies.

6. Providing an estimate of the nutrient-uptake capacity of *Sargassum* species in outdoor cultivation conditions.

2.5. OVERVIEW OF THE STUDY SITES

2.5.1. Marine environments of Western Australia

Western Australia is the largest state in Australia and has the longest coastal line, with more than 13,500 km, excluding islands (Searle and Woods, 1986; Wells *et al.*, 2009). WA is surrounded by the Indian Ocean to the west and the Great Australian

Bight to the southeast of the state. WA has unique geology topography and geological history has formed an area that has high biodiversity and unique marine organisms. Environment and natural resources in WA are recognized as the distinctive habitats of several corals, mangroves, rocky shores, sandy beaches, salt marshes, macroalgal, and seagrass species (Fox and Beckley, 2005). The WA waters have also been identified as the home of several marine habitats and wildlife on the world such as whale sharks, humpback whales, and several threatened sea turtles species.

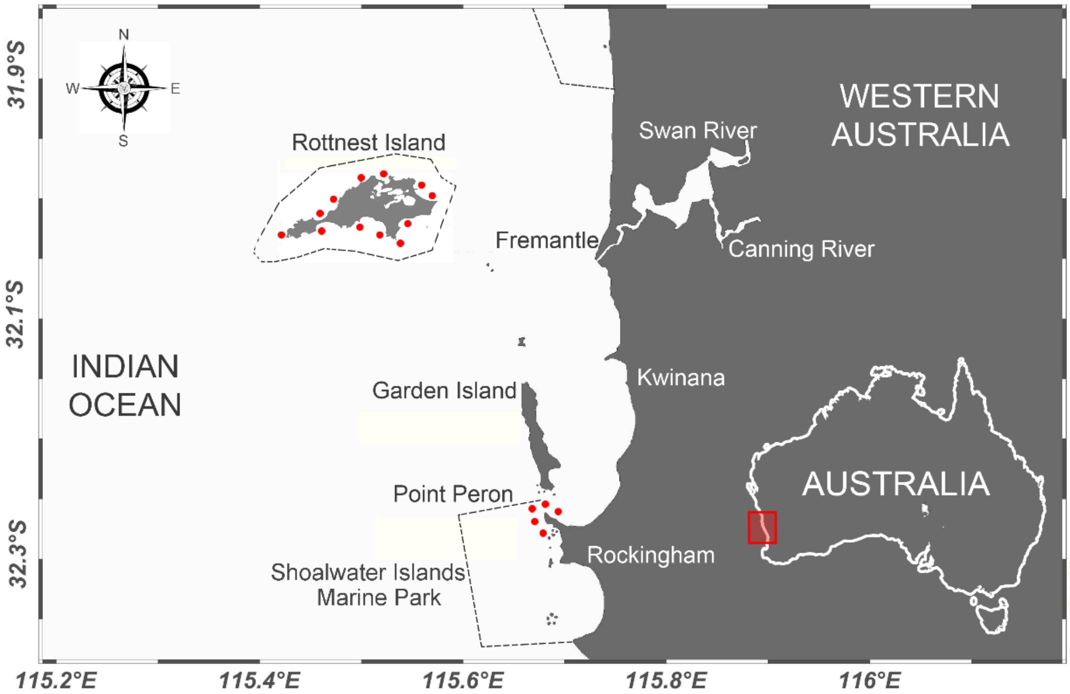

Figure 2.1 Map showing the selected study locations along the Western Australian coast, Australia. The dash lines symbolize marine protected areas' boundaries.

The poleward Leeuwin Current is a unique current in the WA waters. The current moves in a southerly direction and related to increasing SST. The Leeuwin Current brings low salinity and warm tropical water along the continental shelf break of WA. Several previous studies have shown that the Leeuwin Current is stronger in the austral winter as the current has a major thermosteric effect and brings warm water from the Pacific to Indian Oceans (Feng *et al.*, 2003; Schwartz, 2005).

There are all three tidal environments along the coast of WA including micro-tidal (less than 2 m), meso-tidal (range 2−4 m), and macro-tidal (range greater than 4 m). Of these, the tides belongs of the coastal line of Perth, WA, is in a micro-tidal environment (< 2 m). Point Peron belongings to Cockburn Sound, Southwest Australia where there is a low-energy, diurnal, and micro-tidal environment with a maximum of 0.6 m in the spring tide (Masselink and Pattiaratchi, 2001; Travers, 2007).

Rottnest Island and Point Peron, Rockingham are recognized as biodiversity hotspots of the WA coast and have been selected as pilot study sites for the region. Ten and four survey transects have been carried out at Rottnest Island and Point Peron, respectively (Figure 2.1). Both study sites are dominated with canopy forming macroalgae (i.e. *Sargassum* sp., *Ecklonia* sp.), seagrass (i.e. *Amphibolis* sp., *Posidonia* sp.), and other associated SAV species such as *Ballia* sp., *Metagoniolithon* sp., *Asparogopsis* sp., *Gracilaria* sp., and *Ulva* sp.

2.5.2. Marine environments around Point Peron, Shoalwater Islands Marine Park

Point Peron, a small cape west of Rockingham City, is located 50 kilometers south of Perth, WA (Figure 2.2). The study site was selected to be Point Peron, WA, which is a small peninsula located within the Shoalwater Islands Marine Park, an area of approximately 67 km^2, west of Rockingham city. The point is approximately 930 m long and 1,450 m wide and is surrounded by a chain of limestone reefs and islands, including Garden Island to the north. As part of the Shoalwater Islands Marine Park, Point Peron has a high diversity of marine fauna and flora and is one of the fifteen biodiversity hotspots in Australia (DEC, 2011).

Figure 2.2 Study area, with sampling sites shown by arrows. Point Peron is located approximately 50 kilometers south of Perth City, WA.

2.5.3. Marine environments around Rottnest Island

Rottnest Island is one of the largest A Class Reserves in WA. The island is 11 kilometers long and 4.5 kilometers at the widest part, with a total area of about 1,900 hectares (Figure 2.3). Rottnest Island is ecologically unique because it is located exactly at the boundary between tropical and temperate zones, with both temperate and tropical species co-inhabiting the marine environment (Rottnest, 2014). This is a unique habitat for a range of flora and fauna such as nine seagrass species, around 10 thousand quokkas, 16 species of butterflies and 360 species of fish, some of which cannot be found in WA coastal waters. In addition, coral communities on the south coast of Rottnest Island, Parker Point, are the most southerly tropical coral in the world (Rottnest, 2014).

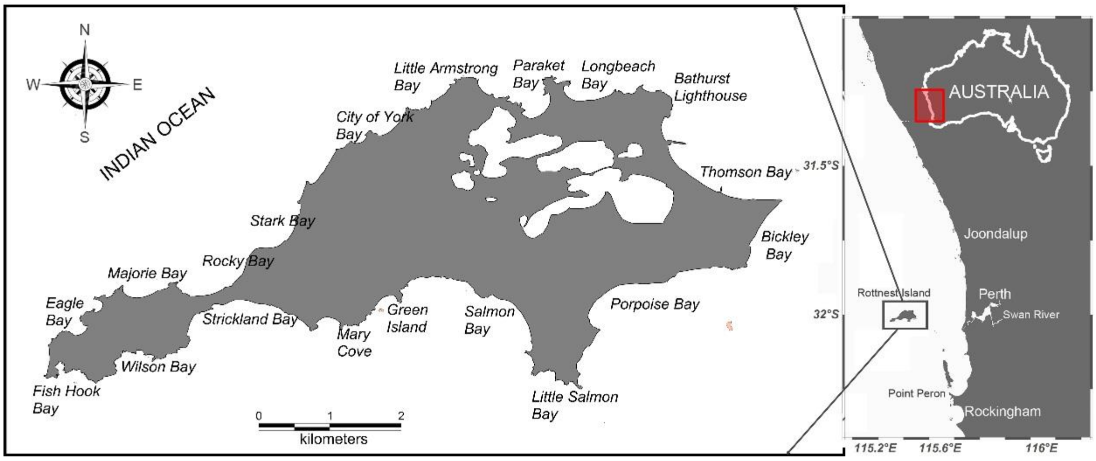

Figure 2.3 The map of study area, Rottnest Island (32°00 – 115°30 E), off the WA coast about 19 kilometres west of Fremantle. One of the largest A Class Reserves in the Indian Ocean.

Rottnest Island is one of the first places to be discovered and named in WA. The name of Rottnest originated from "*Rat's nest*" by a Dutch maritime officer when he found many quokkas on the island (Rigby and Ward, 1969). Tropical marine species are sustained by the Leeuwin Current, which transport warm tropical waters south along the WA coastline. This allows the island to host one of the southernmost coral reefs in Australia and in the world. The island's biodiversity is internationally acknowledged as having a high conservation value, consisting of mollusks, seagrasses, macroalgae, coral, and fish species with both tropical and temperate affinities (Rottnest, 2014).

In the earliest days (1830s), the island was used as a prison for Aboriginal men from WA regions. Then, the island was a key military base and a holiday place for WA governors. In the early 1900s, the Island was employed as a tourist destination when ferries carried tourists from the mainland to Rottnest Island on Sundays. With the Island's Mediterranean climate, scenic natural environment, biodiversity and turquoise waters, with 63 sheltered beaches and 20 bays, Rottnest Island has become one of the most well-known islands for holiday makers and marine conservation in the WA region and across Australia (Phillip, 1988).

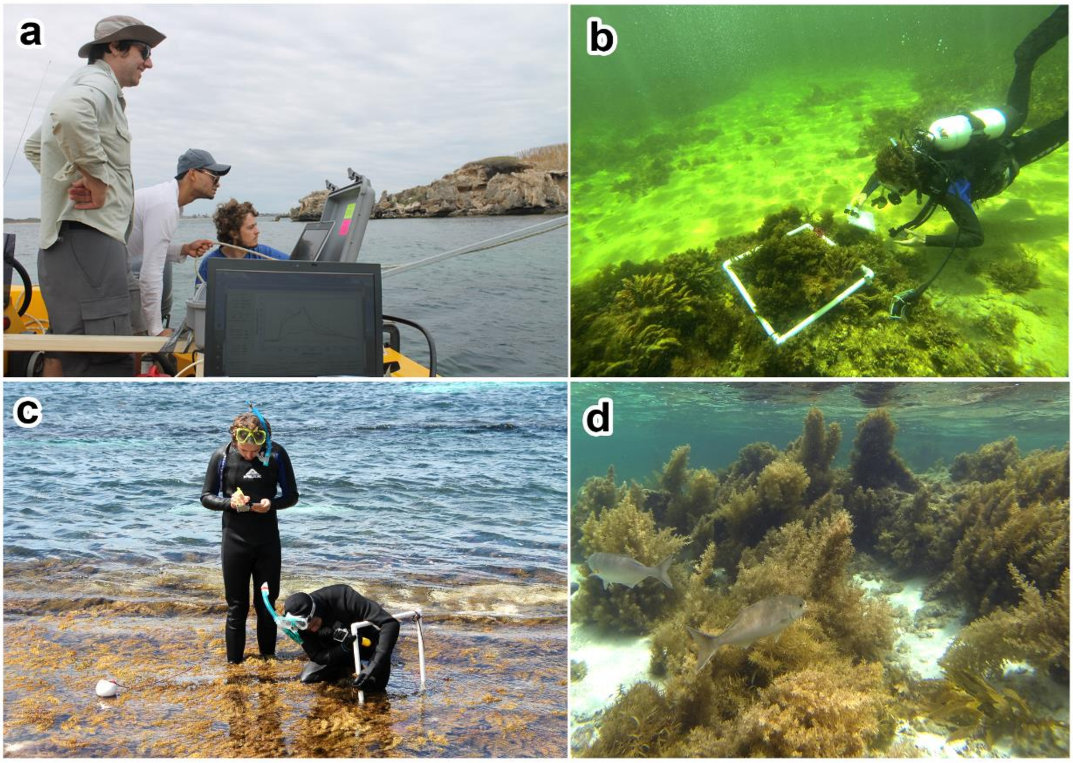

Figure 2.4 Several survey activities were carried out at the study sites (a) sampling water quality and spectral reflectance, (b) monitoring and measuring *Sargassum* by SCUBA technique at Point Peron, (c) free-diving and monitoring the *Sargassum* beds at the intertidal areas, and (d) illustrating a typical subtidal *Sargassum* bed as feeding grounds for marine species around Rottnest Island.

The marine environment of Rottnest Island is mainly affected by the Leeuwin Current. The Leeuwin Current moves southward along the continental shelf and then turns to the east. The regular monitoring program at Rottnest Island shows that the current causes a decrease of salinity, and SST remains around 22^{o}C in the autumn-winter period (Cresswell and Golding, 1980). Thus, all of these unique features make Rottnest Island an ideal place to study the ecological and spatial distribution of macroalgae in general, and *Sargassum* spp. in particular (Figure 2.4).

Chapter 3. SPECTRAL RESPONSE OF MARINE SUBMERGED AQUATIC VEGETATION

Paper published in the MTS/IEEE Proceedings of OCEAN'S 15 Washington DC, USA, 1–5.
(Appendix 3)

3.1. INTRODUCTION

Marine SAV is an important component of the coastal intertidal and subtidal ecosystems due to their ecological and conservational values (Michael *et al.*, 2004; Zavalas *et al.*, 2014). In coastal and estuarine areas, SAV is well-defined as a combination of seagrasses, oligo-haline grass, benthic macroalgae, and floating macroalgae that covers from 10 to 100% substrates. In this study, we focused on the two main abundant SAV groups of seagrasses and benthic macroalgae, which play an important role in coastal marine ecosystems in WA. There are three basic groups of reef macroalgae that consist of turf algae, crustose calcareous algae, and fleshy macroalgae. Of those, turf algae and macroalgae are the main sources of carbon fixation and provide primary productivity for reef organisms (Berner, 1990). Based on the characteristics of pigment composition we have divided the benthic macroalgae groups into three sub-groups consisting of red, green, and brown macroalgae.

However, the evaluation of large scale SAV distribution, seasonal fluctuations, and the effect of environmental factors is quite demanding due to the limited study areas and the combination of interdisciplinary knowledge (Nieder *et al.*, 2004). Therefore, remote-sensing has been used as a useful tool for SAV monitoring in both freshwater and saltwater environments (Visser *et al.*, 2013). A number of techniques have been widely adopted for the classification of marine habitats using *in situ* substrate reflectance (Nurdin *et al.*, 2012; O'Neill *et al.*, 2011). There are two common techniques of remote-sensing now includes access to specific study objectives and spectral reflectance. *i)* Understand the characteristics of spectral reflectance of SAV objects to distinguish them; *ii)* Based on the ground truth data to extract the spectral reflectance from remote-sensing data. The spectral reflectance of SAV has an important role in the analysis and interpretation of habitat classification. However, the collection of SAV specimens and their spectral reflectance, with measurements of the optical properties of water in the field are time and labor-intensive and may also be affected by weather conditions (e.g. Gómez, 2014; Hochberg *et al.*, 2003; Visser *et al.*, 2013). Therefore, the establishment of a spectral reflectance library of SAV spectral is necessary to assess the distribution of coastal marine SAV in larger scale remotely sensing.

There have been a number of studies analysing many spectral reflectance characteristics of marine benthos objects. However, these studies have focused on the spectral reflectance of the sea surface (Chen *et al.*, 2014; Gómez, 2014; Lubac and Loisel, 2007; Vandermeulen *et al.*, 2015), planktonic algae such as pelagic *Sargassum* (Dierssen *et al.*, 2015; Hu *et al.*, 2015; Suwandana *et al.*, 2012), analysing the spectral reflectance of coral reef benthos such as live and dead corals (Hedley and Mumby, 2002; Hochberg and Atkinson, 2003; Mumby *et al.*, 2004; Nurdin *et al.*, 2012), fresh water SAV (O'Neill *et al.*, 2011; Visser *et al.*, 2013; Zou *et al.*, 2013), seagrasses (Barillé *et al.*, 2010; Shuchman *et al.*, 2013; Suwandana *et al.*, 2012; Yuan and Zhang, 2008; Zou *et al.*, 2013), and terrestrial mangroves (Ajithkumar *et al.*, 2008; Fitoka and Keramitsoglou, 2008; Peng *et al.*, 2013; Thackrah *et al.*, 2004). At present, there are a few studies that document the spectral reflectance of the coastal substrate components including seagrass, macroalgae, and their substrates (sandy, coral, and limestone rocks) (Hochberg and Atkinson, 2003; Peñuelas *et al.*, 1993). Therefore, the major objective of the present study aimed to gather optical data of macroalgae (red, green, and brown), seagrasses, and sediment characteristics in coastal waters in order to support the selection of spectral bands and bandwidths for different environmental conditions such as clear water and high turbidity water bodies and to choose suitable satellite sensors for different benthos. This study may contribute as the primary spectral reflectance profile library for marine macroalgae with a comprehensive data on SAV species components and their associated substrates to provide input foundation information for remote-sensing studies that assess the distribution and seasonal variation of coastal marine SAV ecosystems.

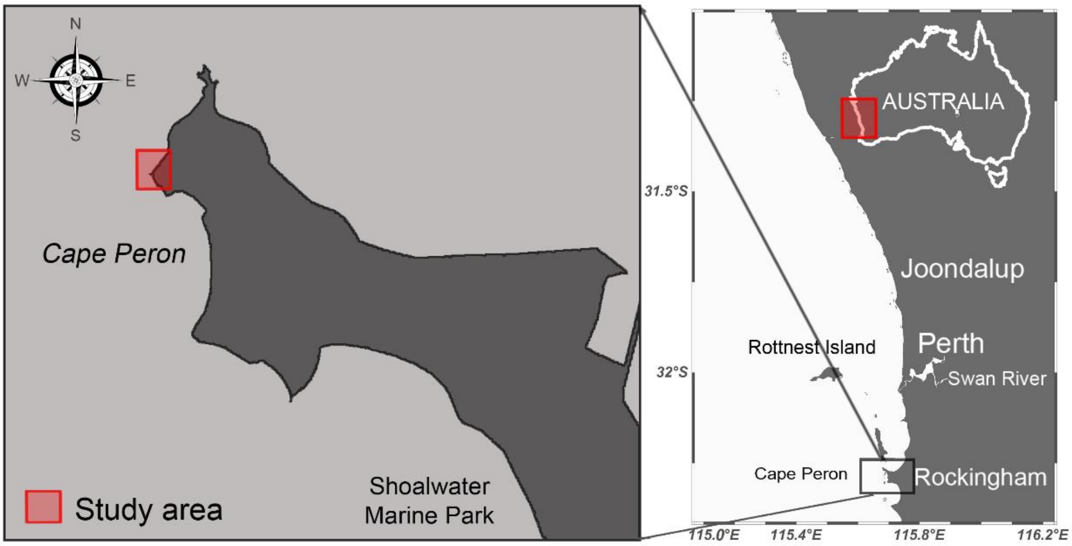

Figure 3.1 Location of the study area, Point Peron in indicated in the red box and is part of the Shoalwater Islands Marine Park, Rockingham, WA.

3.2. DATA AND METHODS

3.2.1. Study area and sample collection

Twenty-two (22) submerged coastal aquatic plant species that included red, green, brown macroalgae, and seagrasses (Table 3.1) were collected from Point Peron, WA (32.2715 °S−115.6865 °E) to measure their *in situ* spectral reflectance (Figure 3.1). Point Peron belongs to the Shoalwater Islands Marine Park, Rockingham, WA (Figure 3.2 a-b) and is one of the fifteen biodiversity hotspots in Australia.

SAV samples were collected by free-diving at intertidal and subtidal areas of Point Peron at the depth range from 0.5 to 2.0 m. The collected samples were stored in chilled containers and transferred to Curtin Aquatic Research Laboratory (CARL) within two hours. The fresh SAV samples were overnight stocked in bulk tanks with the filtered seawater and aeration. The spectral reflectance of the SAV samples was measured the following day under clear sky conditions.

3.2.2. Spectral data collection

The spectral reflectance of SAV has an important role in the analysis and interpretation of habitat classification. However, the collection of SAV specimens and their spectral reflectance, with measurements of the optical properties of water in the field are time and labor-intensive and may also be affected by weather conditions (e.g. Gómez, 2014; Hochberg *et al.*, 2003; Visser *et al.*, 2013). Therefore, the establishment of a spectral reflectance library of SAV spectral is necessary to assess the distribution of coastal marine SAV in larger scale remote-sensing.

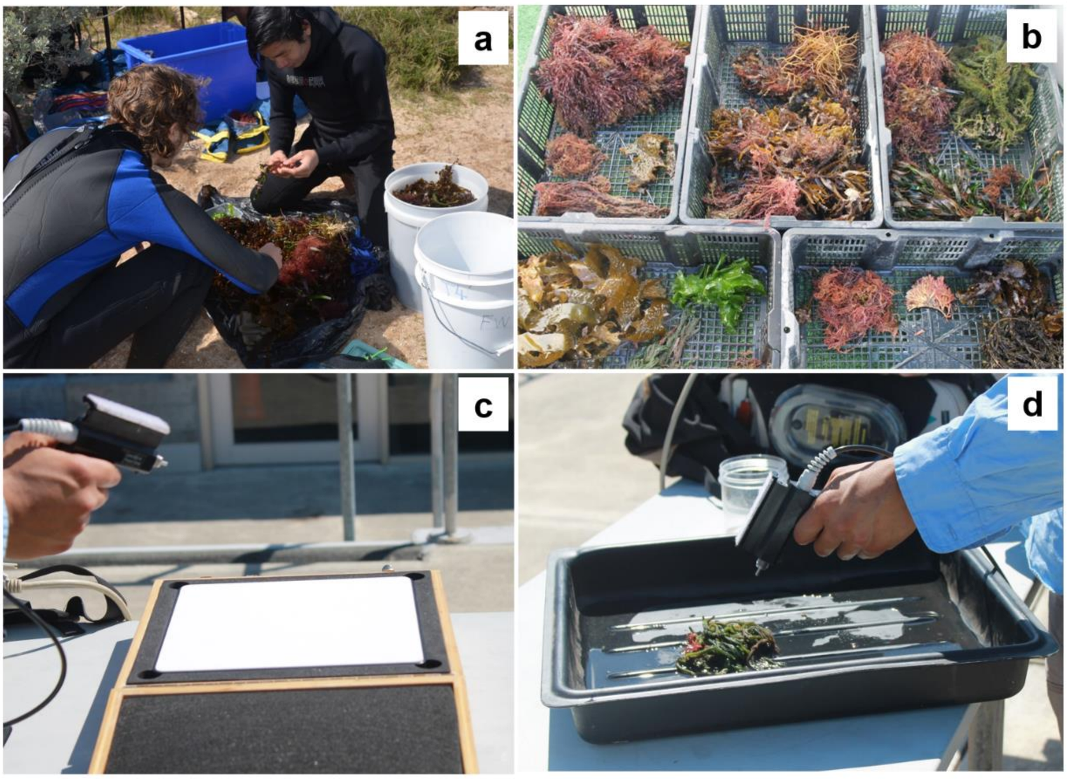

Figure 3.2 (a) Sample collection at Point Peron, the Shoalwater Islands Marine Park, Rockingham, WA, (b) Classification the collected samples into differences SAV groups, (c) A white spectral on plate was used as the reference and optimization, and (d) The FieldSpec® 4 Hi-Res portable spectroradiometer for reflectance collection.

In air spectral reflectance profiles were acquired at wavelengths between 350 to 2151 nm at 1 nm resolution. The FieldSpec® 4 Hi-Res foreoptic via the pistol grip was clamped to a retort stand 30 cm directly above the test sample at a small angle off nadir. At this distance the 25º field of view of the foreoptic completely captured the area occupying the benthic sample. The reflectance measurements were carried out by the FieldSpec® 4 Hi-Res portable spectroradiometer on a cloudless day between 10:00 am and 2:00 pm local hours. A white spectral on plate was used as the reference and optimization was done first before recording the reflectance of each sample. The spectral reflectance profiles were measured at the outdoor experiment facility of the Physics Department, Curtin University, Australia (Figure 3.2 c-d).

3.2.3. Data analysis

The spectral data were transferred into the statistic package to analysis. Correlation and spectral clustering were employed to evaluate the differences between SAV groups. One-way analysis of variance (ANOVA) was used to determine any significant different SAV pair at each wavelength band. Principle Component Analysis (PCA) technique was done with IBM® SPSS® 20 (IBM Corporation, Chicago, USA) factor analysis.

Table 3.1 The check list of collected submerged aquatic vegetation species from Point Peron, the Shoalwater Islands Marine Park

SAV groups	Species	Acronym
Brown macroalgae	*Sargassum longifolium*	*Sal*
	Sargassum spinuligerum	*Sas*
	Ecklonia radiata	*Ecr*
	Colpomenia sinuosa	*Cos*
Red macroalgae	*Asparagopsis armata*	*Asa*
	Hypnea ramentacea	*Hyr*
	Ballia sp.	*Bas*
	Amphiroa anceps	*Amp*
	Euptilota articulata	*Eua*
	Ballia callitrichia	*Bac*
	Metagoniolithon stelliferum	*Mes*
Green macroalgae	*Ulva australis*	*Ula*
	Entermorpha sp.	*Ens*
	Codium duthieae	*Cod*
	Caulerpa germinata	*Cag*
	Caulerpa flexis	*Caf*
	Bryopsis vestita	*Brv*
Seagrass	*Amphibolis antartica*	*Ama*
	Posidonia sp.	*Pos*
Sand/sediment	Sediment	Sed
	Sediment/Rubble	Ser
	Limestone rocks with red coralline algae covering	Lir

3.3. RESULTS AND DISCUSSION

3.3.1. The spectral characteristics of SAV

The comparison of the measured spectral reflectance value of SAV groups and their associated substrate revealed the significant spectral reflectance and absorption regions. There are significant differences in reflectance spectra characterizing red, green, brown macroalgae, and seagrass (Figure 3.3). The red macroalgae contained reflectance peaks in the red spectral regions (580 nm) and strong absorption features in blue and green spectral regions (Figure 3.3a).

The green macroalgae had common absorption features with peaks at blue spectral regions (450 nm) and maximizes spectral reflectance in the green spectral regions (550 nm). Of those, *Ulva australis* and *Bryopsis vestita* have spectral reflectance value of 0.06 and 0.09, respectively, and greater than the four remaining species in the group with the spectral reflectance peaks value of 0.04 (Figure 3.3b).

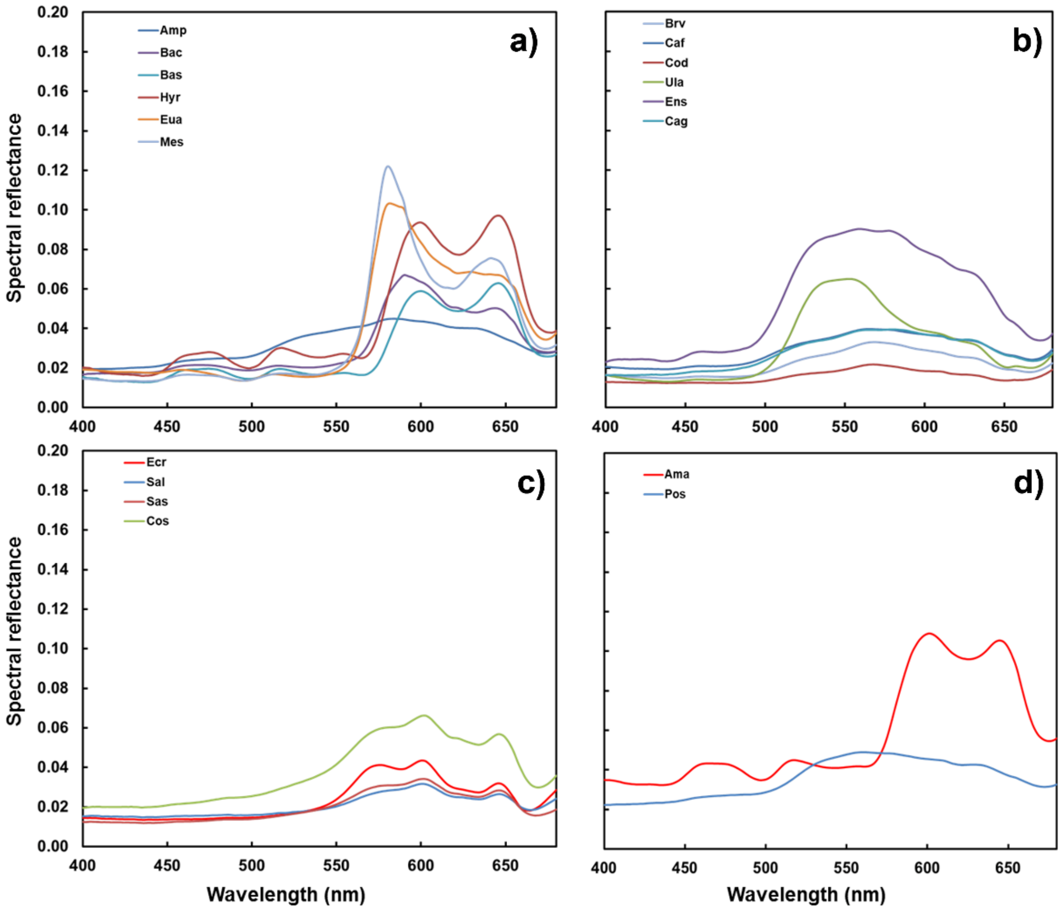

Figure 3.3 The selected mean *in situ* reflectance signature of marine SAV group of (a) red algae (b) green algae, (c) brown algae, and (d) seagrass at visible wavelengths (400−680 nm) (n = 20).

The brown macroalgae had strong spectral absorption peaks in the blue and green spectral regions (400−550 nm). The spectral reflectance peaks were observed between 600 and 650 nm. In particular, *Colpomenia sinuosa* has the highest spectral reflectance with value of following *Ecklonia radiata* the spectral reflectance peak valued at Meanwhile, the lower peak reflectance values of two *Sargassum* species' spectral absorption and reflectance reaches 0.03 (Figure 3.3c).

There was a significant difference in spectral profiles between *Posidonia* sp. and *Amphibolis antartica*. *Posidonia* sp. had maximum spectral absorption in blue and green spectral regions (450−550 nm) and maximum reflectance in the red spectral region (600−650 nm). While, *Amphibolis antartica* had spectral absorption in blue spectral regions (400−500 nm) and spectral reflectance peak in green spectral region (550 nm) (Figure 3.3d).

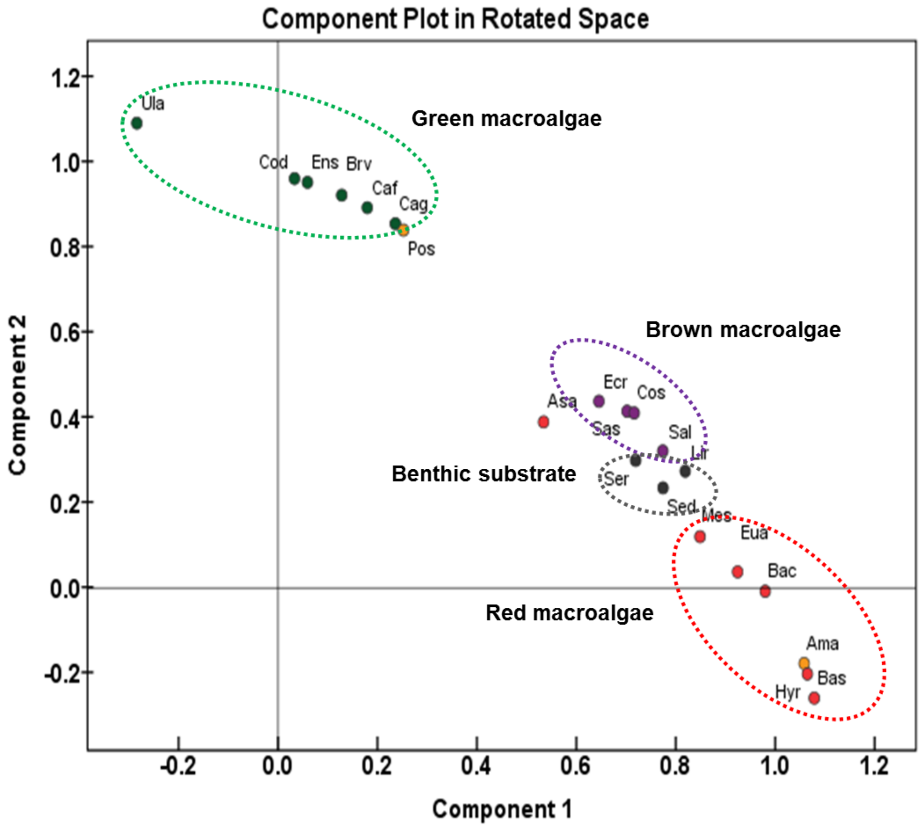

Figure 3.4 PCA component 1 and 2 scatter plot labelled by marine SAV group. Five groups of SAV are indicated by different circles *(i)* red algae in red circles, *(ii)* benthic substrate in black circles, *(iii)* brown algae in violet circles, *(iv)* green algae in green circles.

3.3.2. Factors affecting the SAV spectral characteristics

The score plot (Figure 3.4) represents the first two eigenvectors from the PCA that cumulatively explained 92.3% of the spectral variance. In this figure red macroalgae are predominantly constrained on the lower left hand area of the plot. The first two components of the PCA analysis have high effectiveness in separating the data between macroalgal groups. The classification revealed that there are undoubtedly distinctions between SAV groups based on their spectral characteristic and pigment composition (Peñuelas *et al.*, 1993). There were only a few species of seagrasses and red macroalgae, *Asparagopsis armata* misclassified. For example, the *Posidonia* sp. was classified as near green macroalgae, while *Amphibolis antartica* had spectral characteristics similar to the red macroalgae. In addition, the red macroalgae, *Asparagopsis armata* was hierarchical near the brown macroalgae group (Figure 3.4).

The primary analysis showed that the SAV groups can be accurately classified from remote-sensing data based on their spectral reflectance profiles (Kisevic *et al.*, 2011). PCA results showed the significant differences between SAV groups as well as their associated bottom substrates. The separation of different macroalgae species within the same group is feasible and proven accurate through the t-test. However, the problem with this analysis is based on the hyperspectral measurements at 1 nm. While, multispectral satellite sensors such as WV-2 and Landsat are only few wavebands which will not capture those reflection peaks. The other issue is that there is an impeding water column between the benthos and the sensor. Since the 1970s, it has been known that the water column, especially the depth, greatly reduce the capability of remote-sensing to distinguish between benthic classes at depth (Call *et al.*, 2003).

The collection of SAV samples for establishes the spectral reflectance library should be considered to the seasonality of SAV species. The difference in the spectral profiles of two seagrasses species in this study is a typical example. There were only two seagrass species in this group, but each species had the different absorption characteristic and spectral reflectance. *Amphibolis antartica* had spectral characteristics similar to the green macroalgae, while *Posidonia* sp. had similar spectral characteristics to red macroalgae. This can be explained by biological characteristics of SAV in sample collection time. Notably, at the time of sampling, *Posidonia* sp. species was the end of the growing season (Larkum and West, 1990; West and Larkum, 1979) and the sample of *Posidonia* sp. samples were collected just the above-ground, which could be the remain of plant senescence part. Therefore, there were modified pigment compositions of *Posidonia* sp. itself in different phenology phases of SAV.

The challenge arises when there is a water column which modulates the spectral reflectance of the benthos and where different substrates start to look the same, thereby significantly reducing the capability of remote-sensing. The other issue is that the majority of benthic habitat classifications are done with high spatial resolution satellites that only have a handful of spectral bands in the visible domain, such as WV-2, IKONOS, QuickBird, and Landsat 7, 8. The lower spectral resolution has often led to a lower ability to differentiate between the classes. Hence, combined with the effects of variable water column and we have a very difficult problem to solve.

Chapter 4. IDENTIFICATION AND MAPPING OF MARINE SUBMERGED AQUATIC VEGETATION IN SHALLOW COASTAL WATERS WITH WORLDVIEW-2 SATELLITE DATA

Paper published in the *Journal of Coastal Research*, SI75: 1287−1291.
doi: 10.2112/SI75-258.1. *(Appendix 3)*

4.1. INTRODUCTION

Marine SAV is one of the most important constituents of aquatic ecosystems as a place to provide food, shelter, reproduction and a feeding ground for aquatic animals (Harwell and Sharfstein, 2009; Yuan and Zhang, 2008). SAV habitat is a place to absorb wave energy, nutrients, produce oxygen, and increases the clarity of coastal water. SAV including the submerged macroalgae belong to three different groups, brown macroalgae (phylum Ochrophyta), red macroalgae (phylum Rhodophyta), and green macroalgae (phylum Chlorophyta) and seagrass which growth on sandy, dead coral, and limestone substrates in the shallow waters (Modjeski, 2008).

Therefore, identifying and mapping SAV groups in shallow coastal waters is a vital task and objective for management and conservation of biodiversity and coastal natural resources (Gibbons *et al.*, 2006). However, because of difficulties with the large scale of coastal areas and the limitation of field survey data the understanding of these ecosystems' distribution is still limited. With the large geographical scale of the marine coastal area mapping requires effort and it is time consuming to survey and collect field information (Carle *et al.*, 2014).

High spatial resolution satellite remote-sensing is an effective tool for monitoring, evaluating and mapping biodiversity and natural resources in coastal areas (Gibbons *et al.*, 2006; Green *et al.*, 1996). There are numerous studies that have used high-resolution satellite images for identifying and mapping coastal habitats such as coral reefs (Benfield *et al.*, 2007), seagrass meadows (Guimarães *et al.*, 2011), mangroves (Heenkenda *et al.*, 2014; Ozdemir and Karnieli, 2011), macroalgae (Garcia *et al.*, 2015; Hoang *et al.*, 2015a), and freshwater/ salt marsh (Carle *et al.*, 2014). However, these studies mostly utilized sensors with fewer than four spectral bands in the visible domain, which limited the detailed classification of vegetation (Feilhauer *et al.*, 2013). To overcome the limitations, in October 2010, a WV-2 satellite was successfully launched into orbit and began to acquire high spatial resolution images, 0.5-m for panchromatic and 2-m for multispectral images, and high spectral resolution (eight bands) including four additional spectral bands with additional near-infrared, coastal-blue, yellow, and red-edge bands (Updike and Comp, 2010).

Evaluation and validation of the feasibility of the new spectral bands of WV-2 satellite data on identifying and mapping SAV in coastal habitats are a necessity. This work not only contributes to scientific research but also provides useful information for managers, conservationists, and coastal planners, and is particularly relevant for marine conservation parks' authorities. The main objectives of the present study were: 1) validating the feasibility of the WV-2 satellite data for identifying and mapping SAV in coastal habitats; 2) evaluating three machine learning algorithms/classification methods, Mahalanobis distance (MDiP), supervised minimum distance (MiD), and spectral angle mapper (SAM), for mapping the diversity of SAV.

4.2. METHODS

In this section, we present a description of the study area, spectral reflectance measurements, WV-2 images acquired, processing methods, and the accuracy assessment used in this study.

4.2.1. Study area

Rottnest Island and Point Peron, Rockingham are recognized as biodiversity hotspots of the WA coast and have been selected as pilot study sites for the region. The tidal range of the WA coast is relatively low (± 1 m). Rottnest Island is located off the WA coast approximately 19 kilometers from the port of Fremantle, while Point Peron is a large limestone region at Shoalwater Islands Marine Park on the Rockingham coast (Figure 2.1). The study sites are dominated by canopy forming macroalgae such as *Sargassum* sp., *Ecklonia* sp., seagrasses (*Amphibolis* sp., *Posidonia* sp.), and other associated SAV species such as *Ballia* sp., *Metagoniolithon* sp., *Asparogopsis* sp., *Gracilaria* sp., and *Ulva* sp.

4.2.2. Field surveys and vegetation classes

The purpose of mapping mainly focuses on the abundance of habitats. Consequently, broad-scale field surveys were conducted as much as possible. We used a combination of both SCUBA and free-diving methods to collect ground truth data of the dominant habitat types. Ten and three survey transects were carried out at Rottnest Island and Point Peron, respectively (Figure 2.1). Along the transects, SAV samples, in the depth range between 0.2 and 3.5 m, were collected and underwater photographs taken.

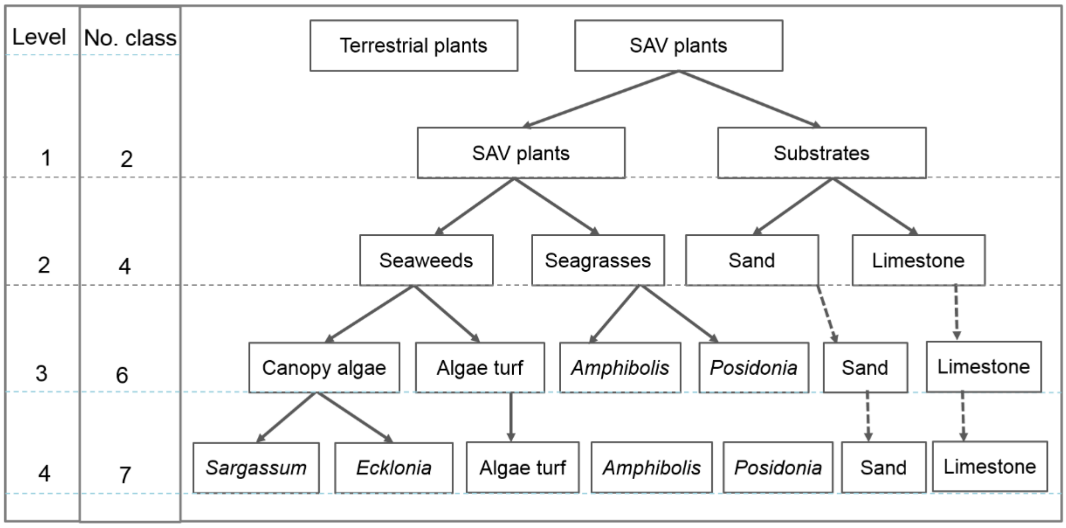

Figure 4.1 The conceptual diagram used to classify marine SAV habitats in clear shallow coastal waters.

The classification of benthic habitats at the two study sites was based on a hierarchal classification scheme that included four levels and five classes (Figure 4.1). Classification at level 1 was based on the pixel reflectance value that best separated vegetated and non-vegetated benthos. The non-vegetated level 1 substrates were subdivided into sandy and limestone substrates by means of reflectance characteristics. Likewise the vegetated level 1 substrates were classed as either macroalgae and seagrass by means of spectral reflectance and the ground truth data from the field surveys. Level 3 classification, including five different habitat classes, largely depended on the characteristics of spectral reflectance to divide them into two groups of canopy algae and algae turf. The group of seagrass, sand, and limestone was also similar to level 2.

4.2.3. Spectral reflectance measurement and processing

Twenty-two SAV samples that included macroalgae, seagrass, sand and limestone substrates were collected at Point Peron (32.2715 ^{o}S−115.6865 ^{o}E), WA on August 22, 2014. The SAV samples were preserved in cold containers and transported to the CARL within four hours of collection. The samples were then identified to species level and their spectral reflectance was measured by an ASD FieldSpec® 4 Hi-Res portable spectroradiometer (1-nm resolution, 350−2500 nm coverage). All samples were placed on a non-reflective black tray and measured from nadir position (~10^{o}). Twenty replicates were measured for each sample.

4.2.4. WorldView-2 image acquistion

Recently, WV-2 satellite images were one of the highest spatial resolutions with 0.5-m and 2-m for panchromatic and multispectral bands, respectively. Moreover, WV-2 satellite images also have a great number of spectral bands (eight multispectral bands) and especially the strengthening of unique bands including coastal band (396–458 nm), yellow (584–632), red-edge (699–749), NIR2 (856–1043) that have not found in the previous remote-sensing satellite data (Updike and Comp, 2010).

Two WV-2 scenes covering the Rottnest Island and Point Peron, Rockingham regions were acquired for study areas in the WA coastal waters. The selected WV-2 were captured on February 7 and October 28, 2013 for Point Peron, Rockingham and Rottnest Island, respectively. The captured time of the satellite corresponds to Australian mid-spring and late summer, coinciding with the most dominant SAV habitat development, particularly the canopy macroalgae such as *Sargassum* sp. and *Ecklonia* sp. (Hoang *et al.*, 2015b; Kendrick and Walker, 1994).

4.2.5. Remote-sensing image processing

The WV-2 digital numbers were first converted to top of atmosphere surface reflectance values with the procedure given by Updike and Comp (2010). The Fast Line-of-sight Atmospheric Analysis of Spectral Hypercubes (FLAASH) algorithm was then used for atmospheric correction. Then, the variable depth model derived by Lyzenga (1981) was employed for water-column corrections of the WV-2 imagery (Lyzenga, 1981). High-resolution panchromatic data (0.5-m) were fused with the lower resolution multispectral bands (2-m) to create a colorized high-resolution dataset.

4.2.6. Accuracy assessment

Overall classification accuracy was calculated by dividing the total number of calculated pixels by the total error pixel in the classification process; overall classification accuracy was measured in units of percent. Index error (error matrices) was used to calculate the user's and producer's accuracy. In particular, the error matrix (user’s accuracy) was used to determine the accuracy of the object’s classification and Cohen’s kappa (Ҡ) index of n parameter estimation.

4.3. RESULTS

In this section, we describe the marine SAV types, spectral reflectance characteristics, mapping SAV distribution, and results of accuracy assessment.

4.3.1. Marine submerged aquatic vegetation distribution substrates

According to field surveys, the distribution area of SAV at selected sites in WA coast is mainly distributed on the limestone substrates within depths ranging from 0.2 to 3.5 m. The major substrates are sandy and limestone rock.

4.3.2. Spectral reflectance characteristics

The results of the PCA analysis measuring the surface spectral reflectance of 19 SAV groups and three substrate types showed that all SAV groups in shallow coastal waters (<3 m) can be divided into four main groups: brown macroalgae, red macroalgae, green macroalgae, and benthic substrate groups. The seagrass group has two dominant species, *Posidonia* sp. and *Amphibolis* sp. The results from *in air* spectral measurements showed that the sand and limestone substrates were completely different from the reflectance spectra of the other SAV species (Fearns *et al.*, 2011). The peak spectral reflectance values of brown macroalgae species were usually at 550 nm. Brown and red macroalgae usually have longer wavelengths and could have more than one maximum point (580 and 650 nm) (Figure 4.2). Regression between *in situ* and WV-2 satellite-derived spectral reflectance, based on the classification results PCA and we found that all species of SAV and substrates can be classified into four main groups. Therefore, in the present study, four major substrate habitats including seagrass, mixed SAV, turf algae, canopy algae, and two main benthos substrate types including bare limestone and sand were selected for habitat mapping.

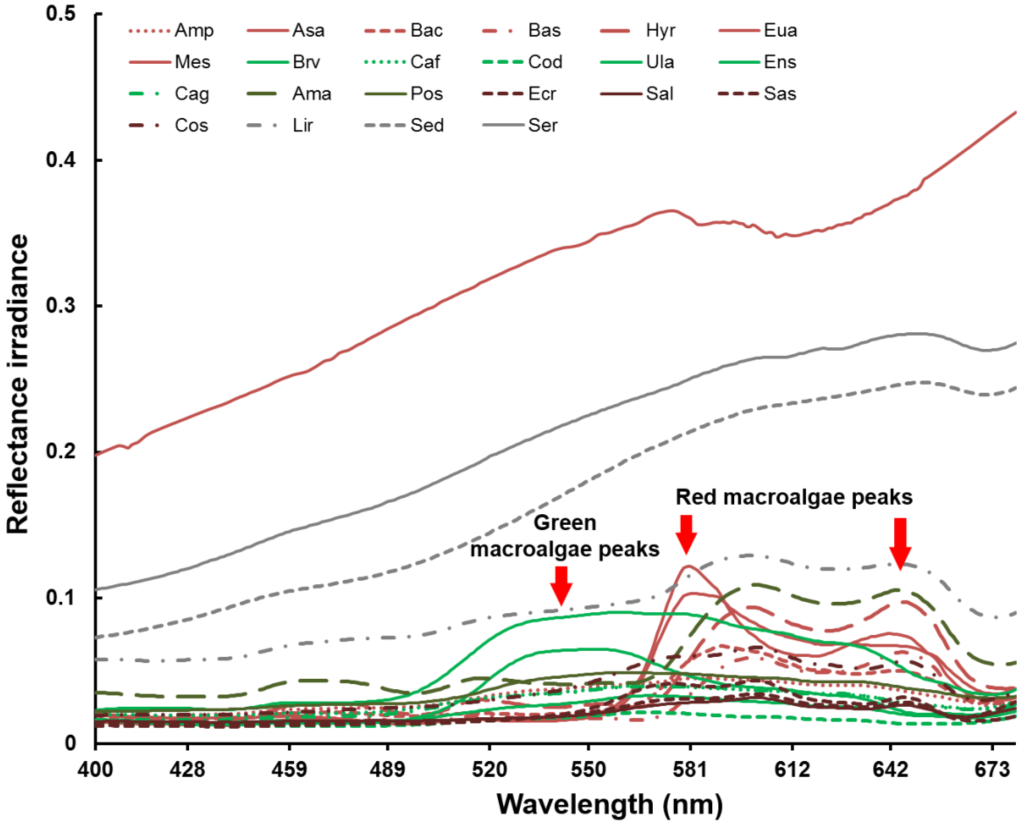

Figure 4.2 The *in air* spectral signatures of the main inter- and subtidal SAV species and substrates in WA. Note: *Sal* = *Sargassum longifolium*, *Sas* = *S. spinuligerum*, *Ecr* = *Ecklonia radiata*, *Cos* = *Colpomenia sinuosa*, *Asa* = *Asparagopsis armata*, *Hyr* = *Hypnea ramentacea*, *Bas* = *Ballia* sp., *Amp* = *Amphiroa anceps*, *Eua* = *Euptilota articulata*, *Bac* = *Ballia callitrichia*, *Mes* = *Metagoniolithon stelliferum*, *Ula* = *Ulva australis*, *Ens* = *Entermorpha* sp., *Cod* = *Codium duthieae*, *Cag* = *Caulerpa germinata*, *Caf* = *C. flexis*, *Brv* = *Bryopsis vestita*, *Ama* = *Amphibolis antartica*, *Pos* = *Posidonia* sp., Sed = Sediment, Ser = Sediment/Rubble, Lir = Limestone rocks with red coralline algae covering.

4.3.3. Mapping the distribution of marine submerged aquatic vegetation

The results of the MDiP, MiD and SAM classification methods revealed that the distribution area differed with each method (Figure 4.3 – Figure 4.4). The MDiP classifier showed that the mixed SAV was in deep waters. Seagrasses were identified as the dominant habitat and were usually distributed in the shallow waters where a sandy bottom is the most dominant. With the MiD classifier, seagrass was also identified as the highest distributed habitat that included the deep water areas.

Canopy algae, algae turf, and sand were also interpreted similarly to the results of the MDiP method. Likewise, the SAM classifier expressed the sketchy interpretation results and unclear pattern of some seagrass, mixed SAV, and sand. Canopy algae and algae turf were not presented clearly either. At Point Peron, fresh biomass of *Sargassum* at the inter-tidal zone reached 5651.7 ± 754.5, 5218.9 ± 192.6, 1136.6 ± 526.4, and 3472.2 ± 434.2 gram per square meter (g m^{-2}) for spring, summer, fall, and winter, respectively.

4.3.4. Accuracy assessment

In Rottnest Island, the confusion matrix showed that the highest overall accuracy was achieved with the MiD classification method (90.93%), followed by the MDiP classification method (90.66%). The lowest overall accuracy was found in the SAM classification method with a value of 49.93%. In particular, the MiD classification methods had user’s accuracy ranging from 86.9% for mixed SAV to 100% for the bare limestone class. In the MiD classification methods, all identified classes had a user’s accuracy greater than 70%.

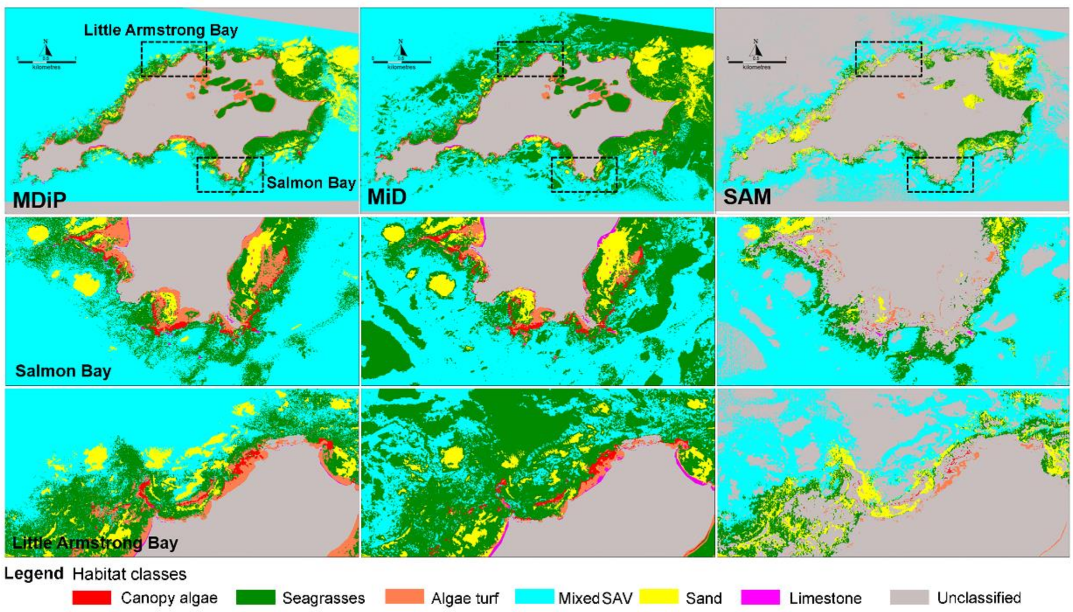

Figure 4.3 Comparison of classification results from three different classifier methods, MDiP, MiD, and SAM, at Rottnest Island study sites.

Producer’s accuracy ranged from 43.8% for the canopy algae class and 100% for both seagrass and sand classes. Five out of six classes had a producer's accuracy greater than 70%, excluding the canopy algae class with 43.8%. User's accuracy of the MDiP classifier gave only the seagrass class less than 70% (66.6%), while the remaining classes were greater than 70%. Producer's accuracy of canopy algae was 45.7%, while the remaining classes had values greater than 70%. The user's accuracy of SAM had an accuracy value greater than 70%. Two out of the six classification classes had producer's accuracy of less than 70%, including canopy algae and sand.

At Point Peron, the overall accuracy of the MiD classifier also reached the highest value of 97.13% (coefficient Ƙ = 0.94). The MDiP classifier with general accuracy value reached 94.16% (Ƙ = 0.88), and the lowest value SAM classifier a value of 88.43% (coefficient Ƙ = 0.74). In the classification results of the MiD classifier, only the producer's accuracy of the bare limestone class was 68.58%, none of the classes had classification results less than 70% in terms of both user's and producer's accuracy. Similarly to the MDiP classifier, only the user's accuracy of the canopy algae class was valued at 66.7%; the remaining classes in terms of both user’s and producer’s accuracy were greater than 70%. The SAM classifier gave two out of six classes user’s accuracy values lower than 70% including canopy algae and sand. The remaining results for both user’s and producer’s accuracy were above 70%.

4.4. DISCUSSION

Rehabilitation and development of SAV habitats is currently one of the priority activities in ecosystem conservation, including freshwater ecosystems (Harwell and Sharfstein, 2009; Herrera-Silveira and Morales-Ojeda, 2009; Yuan and Zhang, 2008). However, assessing the growth and mapping the current state of distribution of SAV on a large scale is very time-consuming and labour-intensive not only because of geographical issues but also because of seasonal and weather variations (Yuan and Zhang, 2008). The results of this study can be compared with those of the recent studies that used high-resolution satellite imagery for mapping of shallow coastal vegetation. Fearns *et al.* (2011) used the Hyperspectral Imager for the Coastal Ocean (HICO) in mapping marine vegetation that showed the optical model could classify 80% of the image pixels. Of those, approximately 50% of pixels were distinguished as seagrass and sand, and 90% were classified as macroalgae (Fearns *et al.*, 2011). Regarding the optimum depth for classification SRSI, this present study showed a similar trend to that of Reshitnyk and colleagues (2014) as results suggested that WV-2 imagery can provide the finest interpretation of eelgrass, and brown and green macroalgae habitats at depths above < 3.0 m.

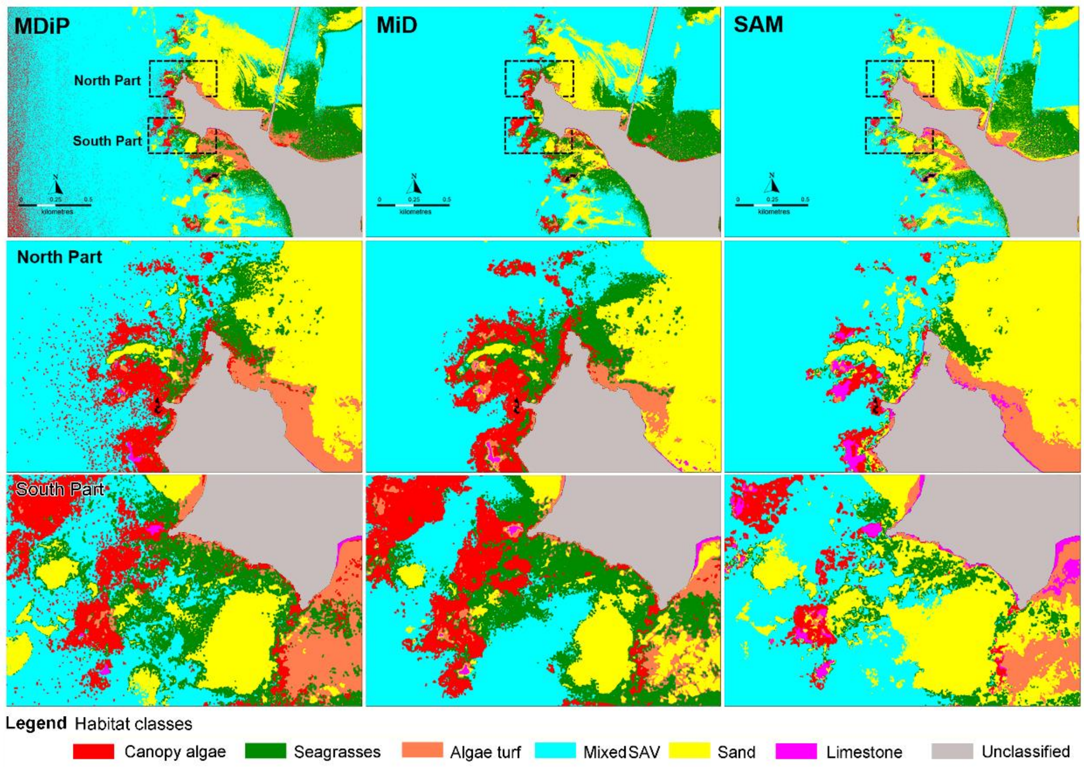

Figure 4.4 Comparison of classification results of three different classifier methods, MDiP, MiD, and SAM, at Point Peron study sites.

The overall accuracy of the classification outcomes in the present study is higher than that of the study by Kumar and colleagues (2015) when using support vector machine (SVM), artificial neural network (ANN), and SAM classification methods for classifying crop and non-crop canopy in India. The highest overall accuracy found in SVM and ANN algorithms was 93.45% and 92.32%, respectively. Likewise, the SAM method has low accuracy among the classification methods (74.99%) (Kumar *et al.*, 2015). In this study, MDiP and MiD methods demonstrated potential for identifying and mapping SAV with WV-2 multispectral high-spatial resolution (MHSR) satellite imagery. This was validated by the highest overall accuracy (greater than 90%) for both study sites in WA. The MDiP method showed that mixed SAV was in deep waters rather than shallow coastal waters.

This study supports the feasibility of previous studies suggesting that MHSR satellite data (e.g. QuickBird, WV-2) would be suitable for mapping benthic macroalgae cover in regions of very high heterogeneity (Vahtmäe and Kutser, 2007). In addition, the MHSR imagery data combined with field surveys in coastal shallows are a perfect fit. However, hyperspectral airborne imagery (HAI) is an advantageous imagery source which have the abundantly information to assess coastal marine habitats due to its hyperspectral characteristic bands, regardless data acquire price. Hyperspectral airborne imagery is still very expensive compared with MHSR as it is collected by separate regions for each study purpose (Yuan and Zhang, 2008).

The similarity of reflectance spectra of seagrass species to those of other macroalgae groups is owed to their biological characteristics. A study of seagrass distribution by remote-sensing in Bourgneuf Bay (France) for *Zostera marina* and *Z. noltii* species showed reflectance spectra distinct from micro and macroalgae, particularly in the wavelength used NIR band (Barillé *et al.*, 2010). Our results for *in air* measurement and reflectance spectra of 22 SAV species showed that two seagrass species, *Posidonia* sp. and *Amphibolis* sp., had reflectance spectra characterized by green and red macroalgae groups, respectively. As either they can have many different emphytes algae on the leaves' surface or they were on older stages in the life cycle when the leaves' pigments was changing. A similar pattern was found by Yuan and Zhang (2008), who found that the spectral reflectance ratios of submerged aquatic species decreased with the aging SAV species (Yuan and Zhang, 2008). This can be explained by the aging vegetation, decaying, and appearing of emphytes organisms on their leaves, branches, and thallus' surface. Therefore, spectral reflectance not only reflects the host species but is also affected by many other fouling organisms.

In conclusion, this work demonstrates a case study using high-spatial resolution satellite images in evaluating SAV identification and distribution in shallow coastal waters. The major advantages of increasing the number of spectral bands and spatial resolution are better detection ability and SAV distribution map with clear water column as WA coastal waters. The results revealed that both MDiP and MiD classification methods showed better evidence for the greater accuracy of SAV classification results than the SAM classification method. Classification results also showed a full representation of the distribution of SAV groups in shallow coastal areas.

Chapter 5. REMOTE-SENSED MAPPING OF *SARGASSUM* SPP. DISTRIBUTION AROUND ROTTNEST ISLAND, WESTERN AUSTRALIA USING HIGH SPATIAL RESOLUTION WORLDVIEW-2 SATELLITE DATA

Paper published in the *Journal of Coastal Research.* doi: 10.2112/jcoastres-d-15-00077.1 (in press) *(Appendix 3)*

5.1. INTRODUCTION

The marine brown algae *Sargassum* spp. is an ecologically important genus, which has a worldwide distribution, and is especially dominant in tropical and shallow sub-tropical waters (Hanisak and Samuel, 1987; Mattio *et al.*, 2008; Mattio and Payri, 2011). As a living renewable resource, *Sargassum* spp. also has economic value, including potential use in medicines, fertilizer, bio-fuel/energy resources, and a carbon offset, whereby it has the ability to both fix and sequester CO_2 from the atmosphere and distribute it among the different layers of the ocean (Aresta, Dibenedetto, Carone, *et al.*, 2005; Gellenbeck and Chapman, 1983; Hong *et al.*, 2007). As such, there is an increasing need to map the density and spatial distribution of *Sargassum* spp. beds, to quantify better the total biomass of this valuable resource.

Marine habitat mapping is usually undertaken using ground surveys and direct visual observations, side-scan sonar, and free-diving. All of these techniques are extremely time-consuming, expensive and are often unfeasible for large areas (Fearns *et al.*, 2011; Komatsu *et al.*, 2002; Tecchiato *et al.*, 2011). A more cost-effective method that is often employed is satellite remote-sensing imagery (SRSI). Satellite remote-sensing imagery requires fewer field surveys, and with the cost of imagery decreasing with concurrent improvements in spatial and temporal resolution, there has been a rapid increase in the use of SRSI for various marine-mapping applications.

Satellite remote-sensing imagery has been successfully applied for mapping marine habitats in shallow coastal waters, especially in clear water with good light penetration, where it is easy to carry out field observations (Green *et al.*, 2000). A range of different satellite imagery has been used for mapping the spatial and temporal distribution of macroalgae and their associated habitats, including MERIS (Gower *et al.*, 2005), IKONOS (Andréfouët *et al.*, 2004; Sagawa, Mikami, *et al.*, 2012; Sagawa *et al.*, 2008; Stumpf *et al.*, 2003), SPOT-2/4 (Carle *et al.*, 2014; Le *et al.*, 2009), Landsat (Vahtmäe and Kutser, 2007), CHRIS-PROBA (Casal *et al.*, 2011b), and ALOS AVNIR-2 (Hoang *et al.*, 2009; Phauk *et al.*, 2012; Sagawa *et al.*, 2012).

The recent launch of the commercial WV-2 satellite has further increased the spatial and spectral resolution of SRSI, with images having a 0.5-m spatial resolution for the single panchromatic band (450–800 nm) and a 2-m resolution for the eight multispectral bands. In addition to the four standard colors: blue, green, red and near–infrared 1, WV-2 includes four new colors, *i.e.* coastal band (400–500 nm), yellow band (585–625 nm), red–edge (705–745 nm) and near–infrared 2 (NIR2: 860–1,040 nm), which are particularly useful for coastal ecosystem studies (DigitalGlobe, 2013; Updike and Comp, 2010).

The WV-2 satellite has been effectively used to map SAV, seagrass, and macroalgae, since 2010 (Cerdeira-Estrada *et al.*, 2012; Chen *et al.*, 2011; Maheswari, 2013; Midwood and Chow-Fraser, 2010; Seoane *et al.*, 2012; Soo Chin and Chew Wai, 2012). However, no systematic study into the utility of WV-2 imagery for the remote-sensing of macroalgae has been performed, particularly of *Sargassum* spp. Fearns *et al.* (2011) reported the application of a hyperspectral remote-sensing model for coastal substrate mapping around Rottnest Island and along the WA coast, using the airborne hyperspectral sensor data, HyMap, which was collected along shallow areas of the WA coast during April 2004. Harvey (2009) also used hyperspectral remote-sensing in the study of Rottnest Island's substrate habitats. No studies have used multispectral satellite remote-sensing to map marine habitats in general or to map canopy macroalgae along the WA southwest coast. Therefore, assessing the distribution of *Sargassum* beds using high spatial resolution satellite images WV-2 from this study could be considered the first such approach.

On the shallow subtidal and intertidal reefs around the WA coast, *Sargassum* spp. form a dominant brown macroalgae group that shows strong seasonal variation (Kendrick and Walker, 1991). The highest biomass and density of reproductive thalli is recorded in spring (September to November) (Kendrick, 1993; Kendrick and

Walker, 1994). According to Kendrick (1993), *S. spinuligerum* in the subtidal has an increased density of vegetative thalli from autumn (March to May) to winter (June to August). *S. spinuligerum* reproduces in spring (September to November) and summer (December to February), when it reaches the greatest biomass. In this study, we have obtained high-resolution satellite imagery in spring (October 2013) that aimed to capture the highest biomass and density of *Sargassum* spp. surrounding Rottnest Island.

The main objective of this chapter was to test the utility and ability of WV-2 imagery in the mapping, monitoring, and classification of *Sargassum* beds and associated habitats around Rottnest Island. This was achieved through the combination of ground truth validation and the methodological development of image processing techniques. The present study is expected to contribute to a better understanding of the distribution of *Sargassum* spp. beds and their potential impact on broader ecosystem functions.

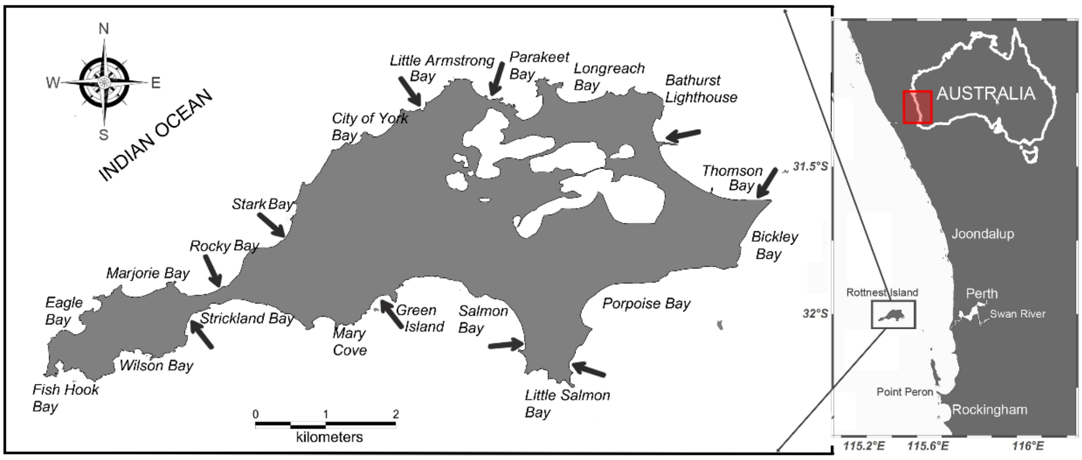

Figure 5.1 Map of the study area, Rottnest Island (32°00'S−115°30'E), off the WA coast about 19 kilometers west of Fremantle. One of the largest A Class Reserve in the Indian Ocean.

5.2. METHODS

This study integrates field observations and high spatial resolution WV-2 imagery processing techniques to provide an assessment of coastal marine *Sargassum* beds around Rottnest Island, WA. Field observation methods included free-diving, monitoring transects, quantify quadrats, and underwater photography techniques. The satellite remote-sensing processing techniques are described below.

5.2.1. Study area

Rottnest Island is 11 km long and 4.5 km at the widest part, with a total area of about 1900 hectares (Figure 5.1) – see Section 2.5.3 – marine environments around Rottnest Island for more details of sampling area.

5.2.2. Ground truth of *Sargassum* beds

Ground truth validation of SRSI was carried out using photoquadrat snorkel surveys. A total of 98 random ground truth points (GTPs) were collected along ten 100 m long transects. Field data were collected over a 2-day trip on September 21–22, 2013, with two trips after classification or validation being carried out on November 24, 2013 and on February 14, 2015.

Each transect was surveyed for species composition, percentage cover, and thallus height of *Sargassum* spp. (Figure 5.2). Transects were designed to cover the major bays, points, reefs, and sanctuaries around the island (sand, coral, and rocky habitats), as well as to cover a range of water depths. Along each transect line, five 0.5 × 0.5 m quadrats were randomly placed within a 10- to 20-m distance between each quadrat, at a bottom depth ranging between 0.5 to 3.5 m. Within each quadrat, the percentage cover of all macroalgae groups including *Sargassum* spp. were measured, as well as the length of five randomly selected *Sargassum* thalli for mean thallus length (MTL), which was measured from the base to the tip of the selected branches. The percentage cover was calculated based on the percentage cover of macroalgae in a quadrat. The quadrat was divided into 25 small square boxes, with each box representing 4% of the cover. For instance, if *Sargassum* covered 10 small boxes within a quadrat, it was indicated with a 40% cover. The mean value of canopy cover for each transect represent data from random quadrats. Numerous fresh *Sargassum* spp. were collected at these selected study sites and were then stored in plastic bags and transported to CARL.

5.2.3. Satellite data acquisition

Currently, WV-2 satellite imagery is one of the highest spatial resolution commercial imagery in the world. The WV-2 satellite was launched into orbit in mid-October 2009 and was fully operational on January 6, 2010 (DigitalGlobe, 2013). The WV-2 imagery used in this study was obtained at 02:38:58 Greenwich mean time (GMT) on October 28, 2013. The WV-2 image was collected during the season that that has shown historically high *Sargassum* spp. biomass cover in the region (Kendrick, 1993; Kendrick and Walker, 1994). The selection of WV-2 images was based on two key factors: (1) captured time that coincided with high *Sargassum* spp. distribution periods (late spring/early summer), and (2) the highest-quality images during cloud-free coverage.

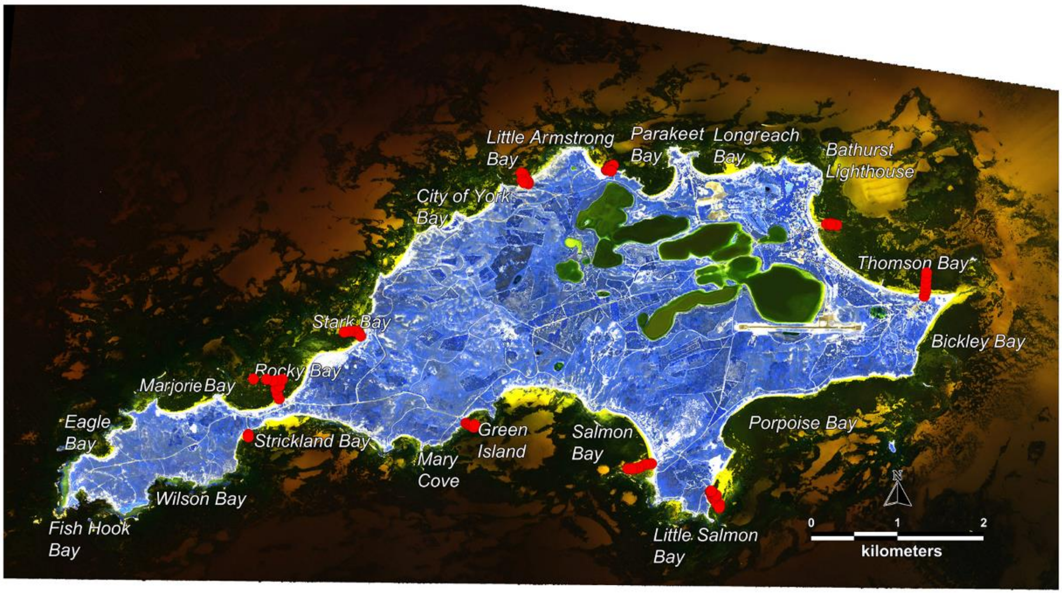

Figure 5.2 The composite image of WV-2 of Rottnest Island, WA, captured at 02:49 h (GMT) on October 28, 2013. One-hundred-m field survey transects with ground truth locations (red points ●) around Rottnest Island.

The WV-2 image data are composed of eight bands, six of which are visible and the other two are near-infrared bands with a 2-m spatial resolution (Table 5.1). Notably, the WV-2 satellite carries a sensor with spectral bands of the coastal band (400–450 nm), which is capable of penetrating into the shallow water column (DigitalGlobe, 2013; Seoane *et al.*, 2012; Updike and Comp, 2010). The WV-2 image was georeferenced in the Universal Transverse Mercator (UTM) World Geodetic System 1984 (WGS84), zone 50 south (50S), which used a cubic convolution method for resampling.

5.2.4. WorldView-2 image pre-processing methods

A flow chart of the analysis processes of mapping macroalgae, other macroalgae groups and their associated benthic habitats using high-resolution WV-2 imagery is shown in Figure 5.3.

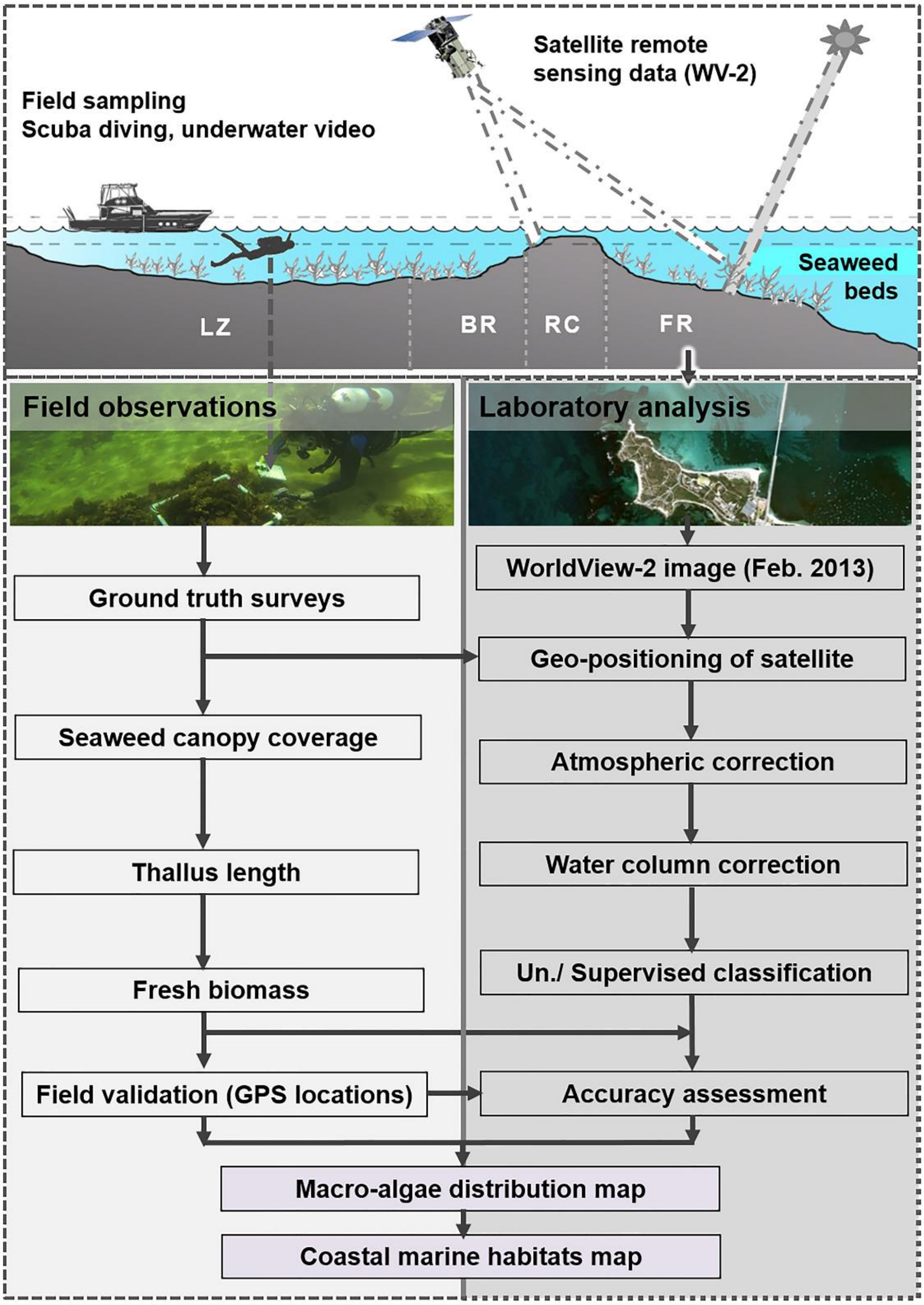

Figure 5.3 Diagram presenting the methodology used to map macroalgae distribution and the associated benthic habitats at Point Peron using high-spatial resolution satellite imagery and field survey data. Sites: LZ = Lagoon zone, BR = Back reef, RC = Reef crest, and FR = Fore reef zone.

5.2.4.1 Geometric correction

The raw WV-2 satellite images were registered in UTM-50S and converted to geographical longitude and latitude WGS84. The satellite images were delivered as a level LV3D product to ensure that they were sensor corrected, radiometrically corrected, and orthorectified (DigitalGlobe, 2013; Eckert, 2012; Liang *et al.*, 2012).

5.2.4.2 Converting WorldView-2 data to reflectance

Two steps are involved in the conversion of WV-2 data to reflectance values. The first step involves converting the digital number (DN) in the range from 0 to 255 into radiance values, and the second step converts the radiance in watts per square meter per steradian per micron (W m^{-2} sr^{-1} μm^{-1}) into reflectance values. This process requires relevant input information, such as the distance between the sun and the earth (in astronomical units), the day of the year (Julian date), and solar zenith angle.

Table 5.1 Characteristics of WV-2 multispectral eight band and panchromatic images acquired at Rottnest Island, WA.

Imagery parameters	**Multispectral**
Acquired dates	October 28, 2013
Acquired times/ local time	02:49:25 GMT/10:49:25 Australia/Perth
Top left coordinate	352350.00mE,462504.00mN
Rows/Columns	3974/6826
Path	one swathe, 91 km^2
Nadir/Off-Nadir	Nadir/20° off-nadir
Projection/Datum	SUTM50/WGS84
Processing level	Ortho-Ready Standard Level 2A
Resampling method	Cubic convolution
Data storage format	Geo TIFF
Mean Sun elevation/ Azimuth	65.0^o/ 45.6^o
Cloud Cover	0%
Band and spatial resolution	2.00 × 2.00 m

5.2.4.3 Digital number to radiance

To convert DN values to radiance values, the gain and offset method was used with these values from the metadata file (Table 5.2) (Chavez, 1996). The formula to convert DN to radiance using gain and bias values is:

$$L_{\lambda} = gain * DN + offset \qquad (1)$$

where L_{λ} is the cell value of the satellite spectral radiance for a given spectral band (W m^{-2} sr^{-1} μm^{-1}); the DN is the digital number of 0 to 225 for a given cell; gain is the gain value for a given specific band, and offset is the bias value for the given specific band. This processing stage was analyzed using the WorldView Radiance calibration toolbox in the environment for visualizing images (ENVI) software package (Exelis, 2014).

Table 5.2 Spectral information, spatial resolution, gain-offset and band-averaged solar spectral irradiance of the WV-2 in Rottnest Island acquired on 28 October 2013.

Spectral Bands	**SB***	**SI****	**EB*****	**Rottnest Island**		**SR********	
				Gain	Offset	Nadir	20° off-nadir
Panchromatic	450 – 800	1580.8140	284.6			0.46	0.52
Eight multispectral band							
MS1 – NIR1	770 – 895	1069.7302	98.9	0.026229	0.00	1.85	2.07
MS2 – Red	630 – 690	1559.4555	57.4	0.013780	0.00		
MS3 – Green	510 – 580	1856.4104	63.0	0.026081	0.00		
MS4 – Blue	450 – 510	1974.2416	54.3	0.017990	0.00		
MS5 – Red-edge	705 – 745	1342.0695	39.3	0.017990	0.00		
MS6 – Yellow	585 – 625	1738.4791	37.4	0.017990	0.00		
MS7 – Coastal*	400 – 450	1758.2229	47.3	0.017990	0.00		
MS8 - NIR2	860 – 1040	861.2866	98.9	0.017990	0.00		

*SB: Spectral Band edges (nm), SI: Spectral Irradiance with unit (W m^{-2} μm^{-1}), EB: Effective bandwidths $\Delta\lambda$ (nm), SR: Sensor Resolution (m) (Updike and Chris, 2010).

5.2.4.4 Radiance to Top of Atmosphere reflectance

As mentioned in the above section, the distance between the sun and the earth (in astronomical units), the day of the year (Julian date), the mean solar exoatmospheric irradiance, and solar zenith angle were used to calculate reflectance (NASA, 2011). The top of atmosphere (ToA) is the spectral radiance entering the WV-2's telescope at an altitude of 700 km (Chander *et al.*, 2009; Updike and Comp, 2010). The following formula for calculating reflectance was used.

$$P_{\lambda} = \pi . L_{\lambda} . d^2 \Big/ ESUN_{\lambda .} \cos(SZ) \qquad (2)$$

where ρ_λ = the unit-less planetary reflectance; L_λ = the spectral radiance for a given spectral band (from equation 1); d is the earth–sun distance in astronmoical units (Table 5.3); $ESUN_\lambda$ is the mean solar exoatmospheric irradiance for the given spectral band, and SZ is the solar zenith angle (extracted from metadata files). The step of converting the radiance to ToA can be processed by calibrated radiance at the data specific utilities in spectral toolbox in the ENVI 4.7 software (Exelis, 2014).

Table 5.3 Earth-Sun Distance in Astronomical Units

Julian Day	Distance	Julian Day	Distance	Julian Day	Distance	Julian Day	Distance	Julian Day	Distance
1	.9832	74	.9945	152	1.0140	227	1.0128	305	.9925
15	.9836	91	.9993	166	1.0158	242	1.0092	319	.9892
32	.9853	106	1.0033	182	1.0167	258	1.0057	335	.9860
46	.9878	121	1.0076	196	1.0165	274	1.0011	349	.9843
60	.9909	135	1.0109	213	1.0149	288	.9972	365	.9833

5.2.4.5 Atmospheric correction

The WV-2 satellite spectral data were atmospherically corrected, using the dark substrate method, to reduce haze and other influences of atmospheric and solar illumination. The major principle of the dark substrate method is to calculate the mean value of deep water pixels (Gordon and McCluney, 1975; Liang *et al.*, 2012; Stumpf *et al.*, 2003). The atmospheric correction was carried out using the dark substrate tool box in ENVI software (Exelis, 2014).

5.2.4.6 Water column corrections

This study made use of the variable depth model derived by Lyzenga (1981) for water-column corrections and is one of the most popular methods when using satellite remote-sensing images for the mapping the marine coastal ecosystems. The depth invariant index (*DII*) was calculated as follows:

$$DII_{ij} = \ln(L_i) - \frac{k_i}{k_j} * Ln(L_j) \qquad (3)$$

where L_i is the measured radiance of a given band i; L_j is the measured radiance of given band j, and k_i/k_j is the ratio of the attenuation coefficient that is derived from the slope of the log-transformed plot at numerous different depths.

5.2.4.7 Masking

To eliminate the spectral variability that is affected by terrestrial and deep water areas, the vector layer of these areas was masked to the satellite images.

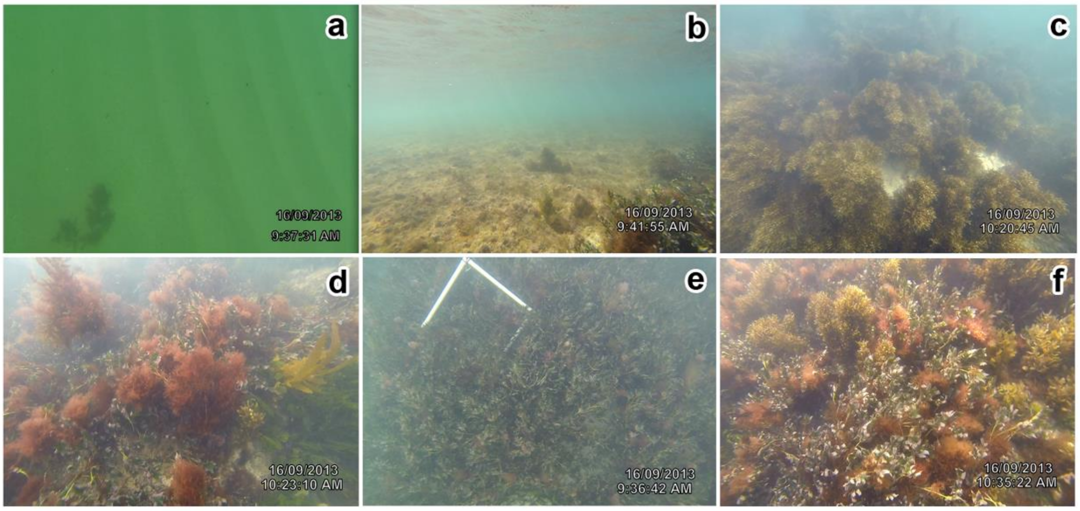

Figure 5.4 The ground truth survey images that taken from the study sites. (a) Sand substrate, (b) Limestone substrate, (c) *Sargassum* spp. habitat, (d) Red algae, (e) Seagrass (*Amphibolis australis*), (f) Algae turf habitat.

5.2.5. Image classification and data analysis

Field survey data on macroalgae distribution and abundance were processed using the ENVI 4.7 Integrated Development Language remote-sensing software. Four classifiers–the minimum distance (MiL), Mahalobis (MaH), K-means (KM), and Parallelepiped (PaR) classifiers–were chosen for this study, because they are the most commonly used classifiers in marine coastal habitat mapping studies to date (Andréfouët *et al.*, 2004; Belgiu *et al.*, 2014; Benfield *et al.*, 2007; Carle *et al.*, 2014; Ghosh and Joshi, 2014; Muslim *et al.*, 2012). The coastal habitat around Rottnest Island can be categorized into six classes: rocky substrate, sandy, canopy macroalgae (*Sargassum* spp. and *Ecklonia* spp.), red macroalgae, seagrass, and mixed vegetation. The whole analysis process is presented in a diagram in Figure 5.4 and the scheme for habitat classification is supplied in Table 5.4.

5.2.6. Accuracy assessment

Error matrices and Cohen's kappa (Ҡ) were used to assess the accuracy of the classification results. These are helpful models to understand the accuracy of the classification scheme. The Ҡ coefficient can be employed to assess the agreement between classification results and reality (Congalton, 1991). Cohen's kappa is calculated as follows:

$$\mathcal{K} = \frac{\mathrm{N}\sum_{\mathrm{i}=1}^{\mathrm{r}} \mathrm{x}_{\mathrm{ii}} - \sum_{\mathrm{i}=1}^{\mathrm{n}} (\mathrm{x}_{\mathrm{i}+} \times \mathrm{x}_{+\mathrm{i}})}{N^2 - \sum_{i=1}^{n} (x_{i+} \times x_{+i})} \qquad (4)$$

The producer and user accuracy were also calculated for each classification result (Congalton and Green, 2009). The producer and user accuracy are one of the most popular indices to evaluate classification outcomes. The producer accuracy is an index that measures the possibility that the classifier fitted the image pixel in class A similar to the ground truth in class A. The producer accuracy index is also related to the errors of omission (exclusion) (Congalton, 1991; Congalton and Green, 2009). From the user perspective, the user accuracy is an index to measure the possibility that the classifier labeled the image pixel in class A. The overall accuracy and the Ƙ coefficient for the four classification techniques was also evaluated.

Table 5.4 A scheme used for habitat classification based on ground truth data.

Class 1	**Class 2**	**Description**	**Ground truth image**
Unvegetated	Sandy substrate	Fine sand with bared substrates	Fig. 5.4a
	Limestone substrate	Either bared substrates or some coralline on its surface.	Fig. 5.4b
Vegetated	Canopy macroalgae (*Sargassum* spp. & *Ecklonia* spp.)	Most abundant *Sargassum* spp., commonly found in tidal and sub-littoral zone and mostly attached to the limestone rock.	Fig. 5.4c
	Red macroalgae	Red algae (*Gracilaria* sp.) and coralline algae often found in limestone rock, sand, and mud.	Fig. 5.4d
	Seagrass	*Amphibolis* sp. often found on sandy substrates in the sub-littoral zone. Also found on gravel and firm, clay banks.	Fig. 5.4e
	Algae turf	The community of green, red, and brown algae	Fig. 5.4f

5.3. RESULTS

This study is the first of its kind to use WV-2 high-resolution multispectral satellite imagery to map *Sargassum* spp. distribution. Eight *Sargassum* spp. have been identified in intertidal and subtidal beds around Rottnest Island. Among these *Sargassum* spp., *S. spinuligerum, S. distichum,* and *S. podacanthum* are most abundant (Kendrick, 1993). However, because of the similarities in morphological structure, including thallus color and length, holdfast shapes, stipe, and primary branches (Kendrick, 1993; Mattio *et al.*, 2008), individual *Sargasum* species could not be distinguished or separated spectrally. Therefore, *Sargassum* spp. distribution was defined at the genus level.

5.3.1. *Sargassum* spp. percentage cover and thallus length from field-survey data

5.3.1.1 Percentage cover of Sargassum spp. and other macroalgae groups

In the period between September and November 2013, the spatial distribution of *Sargassum* spp. showed statistically significant differences (P = 0.008) in percentage cover between monitored transect lines around Rottnest Island. The highest percentage cover of *Sargassum* spp. was found on the inshore reefs at depths of 0.5 to 3.5 m in the areas around Salmon Bay, Rocky Bay, and Armstrong Bay, with values of 61.6 ± 11.5, 53.3 ± 13.1, and 48.3 ± 14.3%, respectively. The percentage cover in Parakeet Bay and Green Island area was 45.9 ± 24.0 and 18.9 ± 10.5%, respectively. The lowest percentage cover of *Sargassum* spp. was found in the Parker Point area (15 ± 5.7%) and Thomson Bay had a cover of 4.3 ± 0.0% (Figure 5.5).

For other macroalgae groups, *Ecklonia* sp. in particular, statistically significant differences (P = 0.041) were observed between monitored transect lines. The greatest percentage cover was found in Green Island, Thomson Bay, Parker Point, and Rocky Bay, with values of 77.8 ± 10.2, 59.3 ± 0.0, 51.3 ± 13.4 and 42.8 ± 13.2%, respectively. The lowest percentage cover of other macroalgae groups were found in the Parakeet Bay, Armstrong Bay, and Salmon Bay, with values of 40.9 ± 24.0, 36.7 ± 9.4 and 17.9 ± 6.4%, respectively.

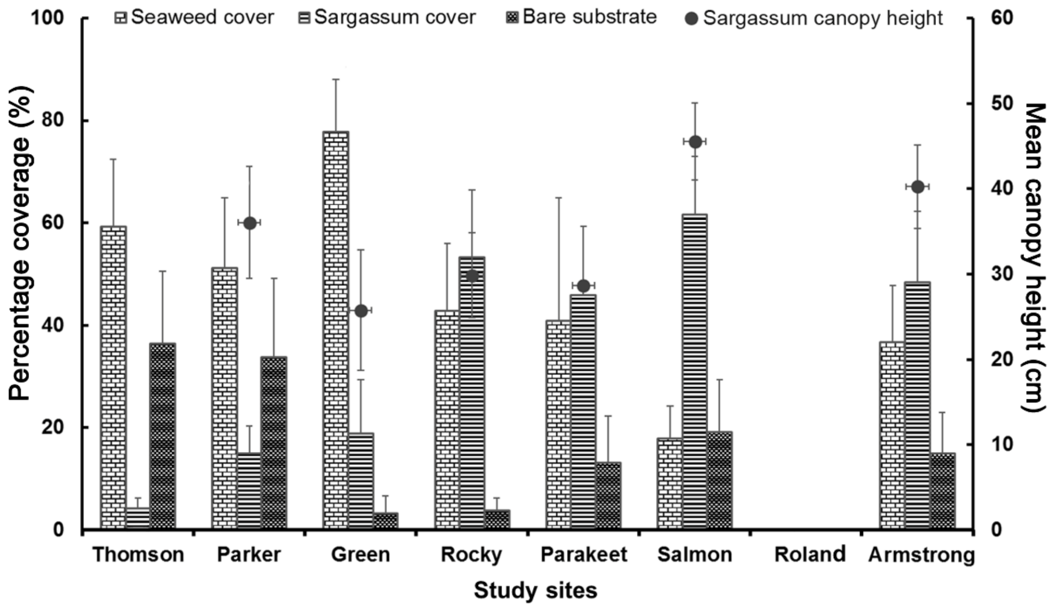

Figure 5.5 The percentage coverage of *Sargassum* spp. observed from different sites during spring season 2013. Thomson = Thomson Bay, Parker = Porpoise Bay, Green = Green Island, Rocky = Rocky Bay, Parakeet = Parakeet Bay, Salmon = Salmon Bay, Rollan = Strickland Bay, Armstrong = Little Armstrong Bay. Each column shows the mean and standard error of five observed quadrats (0.5 × 0.5 m).

5.3.1.2 Thallus length

There were no statistically significant differences (*P* = 0.069) between the monitored transects in terms of mean thallus length (MTL). The MTL of *Sargassum* spp., was highest along the monitored transect in Salmon Bay (45.5 ± 4.5 cm). Armstrong Bay, Parker Point, and Rocky Beach had MTL values of 4.9 ± 40.3, 36.1 ± 6.54, and 29.8 ± 4.9 cm, respectively. The lowest MTL of 25.8 ± 0.6 cm was found in Green Island.

5.3.2. **Separating *Sargassum* spp. and other macroalgae groups**

Spectral profile analysis of the major substrate types and biological communities around Rottnest Island showed that there was a significant difference between limestone and sand substrates, and *Sargassum* spp., *Ecklonia* sp., algae turf, and seagrass communities (Figure 5.6). It is the characteristically different spectral profiles between the major substrate types and biological communities that enable the *Sargassum* spp. distribution to be mapped.

Comparing the spectral profiles of bare sand and limestone rock, sand has a higher and increasing reflectance intensity across the 400- to 750-nm spectrum than does limestone rock, for which the reflectance decreases after 650 nm and has peak at 500, 550, and 650 nm. In addition, it is rare to encounter bare limestone rock, and the reflectance value is probably influenced by encrusting and turf algae. The substrate reflectance components are significantly different when compared with the spectral reflectance of biological communities, such as *Sargassum* spp., *Ecklonia* sp., algal turf, and seagrass (Figure 5.6a, b).

Sargassum spp. and *Ecklonia* sp. have a similar bimodal spectral reflectance pattern, with a first weak-intensity peak at 600 nm and the lowest value at 650 nm, with the change of reflection direction. The major difference between the two species is the overall intensity; *Ecklonia* sp. exhibit higher peak values of 0.05 at the 600-nm wavelength, whereas *Sargassum* spp. have the highest spectral reflectance value of 0.02 at 600 nm which has a lower peak value than for *Ecklonia* sp. (Figure 6e, f).

Algae turf communities can include coralline algae, red algae, green algae, and brown folios algae. The spectral reflectance values of the algae turf communities on Rottnest Island are often dictated by the coralline algae, which have a high cover abundance and irradiance value peaking between 550 and 600 nm (Figure 6c).

The three main seagrass species distributed around Rottnest Island are *Posidonia* sp., *Amphibolis* sp., and *Halophila* sp., among which *Posidonia* sp., and *Amphibolis* sp. have the most abundant distributions. Because of the similarity of spectral profiles in the present study, we classified all three seagrass species into one group. These species have highest reflectance irradiance values between 500 and 550 nm (Figure 5.6d).

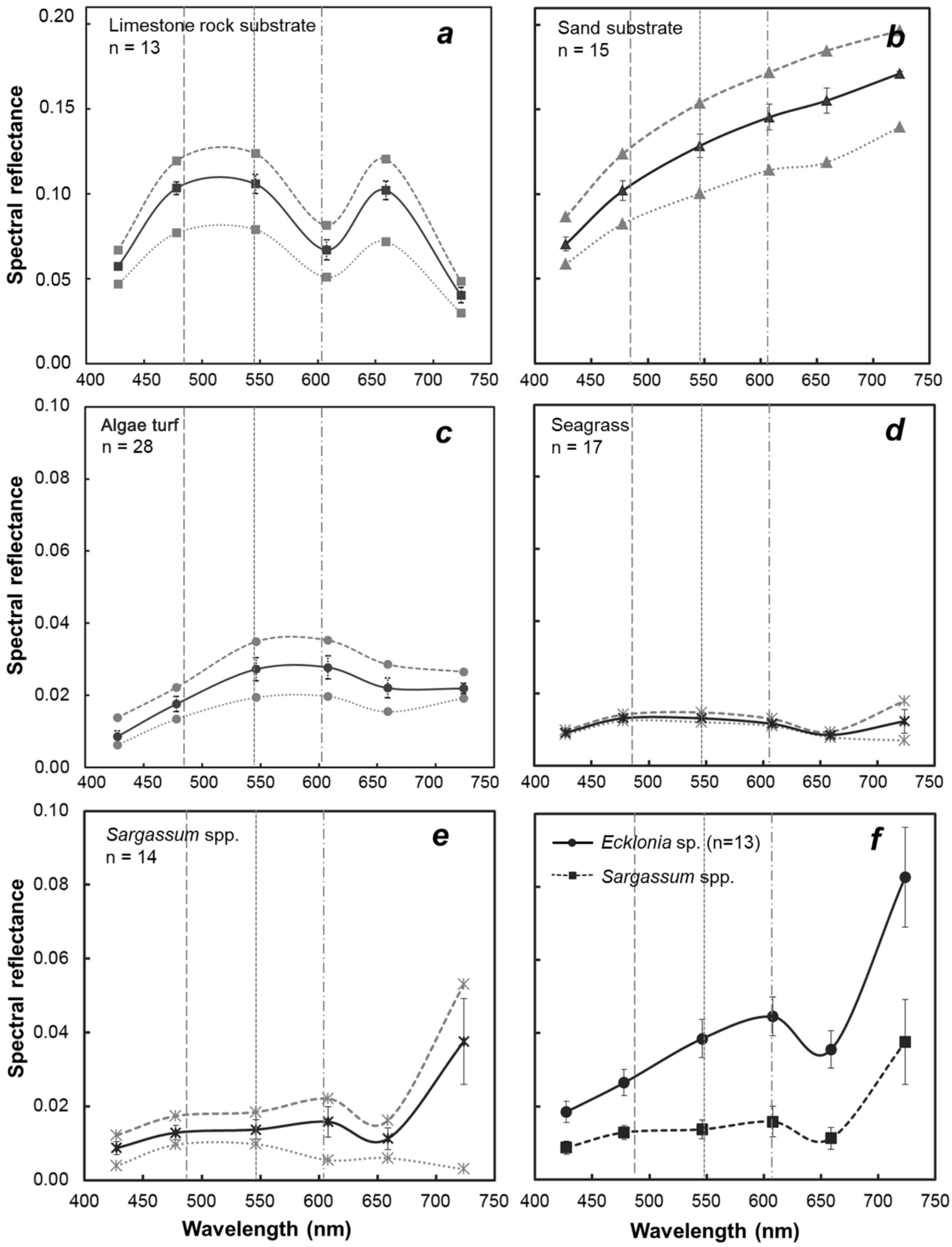

Figure 5.6 Spectral profiles of the majority of benthic components extracted from WV-2 imagery. (a) Limestone substrate; (b) Sand substrate; (c) Algae turf (Coralline algae, red algae, green algae, and brown algae); (d) Seagrass; (e) *Sargassum* spp.; (f) *Ecklonia* sp. vs. *Sargassum* spp.

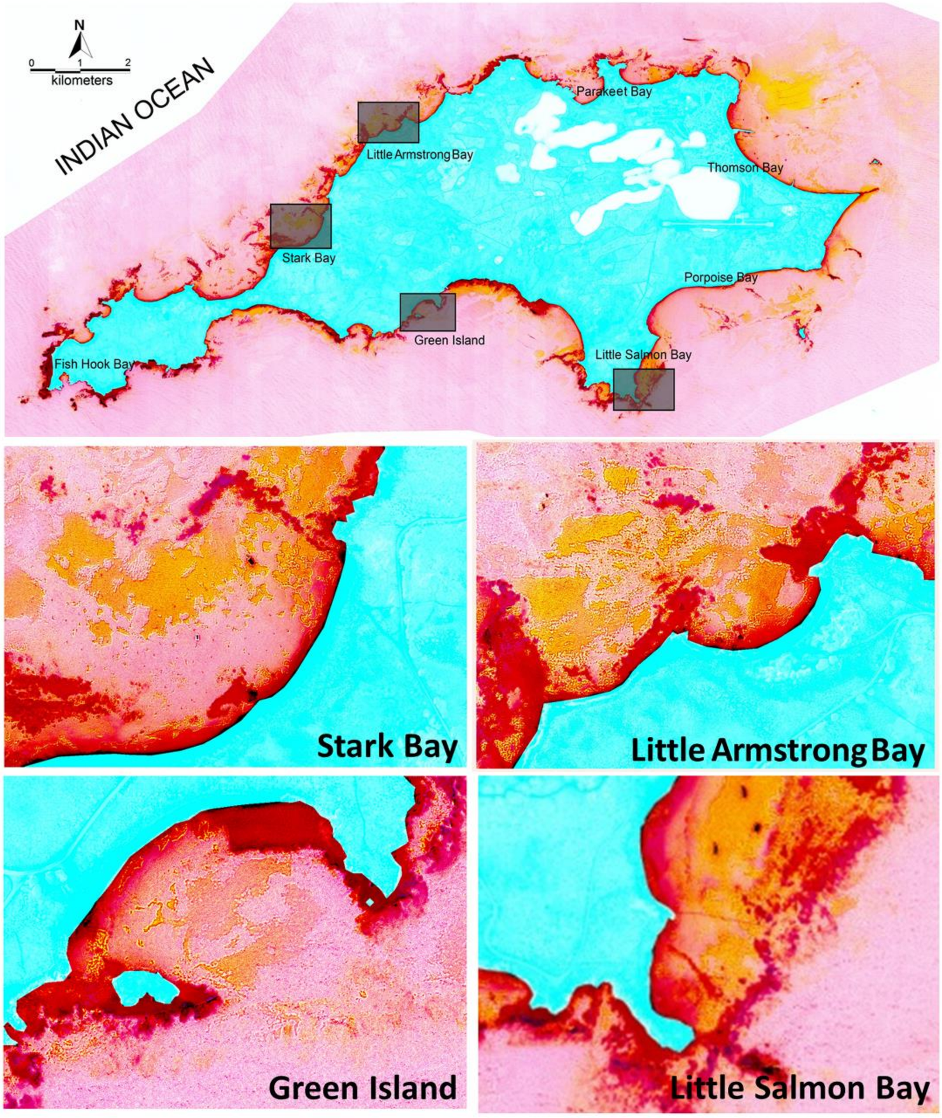

Figure 5.7 The result of spectral math of three bands red-edge, red-edge, and yellow bands showed the visibly observed distribution of *Sargassum* spp. beds with yellow pixels and red color for submerged reefs around the study sites.

5.3.3. Spatial distribution of *Sargassum* spp. from satellite remote-sensing data

The combined results of the three-band match with red-edge, red-edge, and yellow bands showed that the macroalgae beds with a majority of *Sargassum* spp. were detected by yellow and orange patterns. The following environmental and biological factors helped to detect the *Sargassum* spp. bed boundaries: (1) the beds are found in clear waters and in shallow intertidal and subtidal zones (0.5–10 m); (2) *Sargassum* spp. and other brown macroalgae (*Ecklonia* spp., *Cystoseira* spp.) show a preference for rocky substrates, i.e. not sandy, which cannot be confused with seagrass on a sandy substrate; and (3) *Sargassum* spp. have different spectral characteristics compared to other macroalgae groups that are highly distinct in the high-resolution WV-2 imagery (2-m) (Figure 5.7).

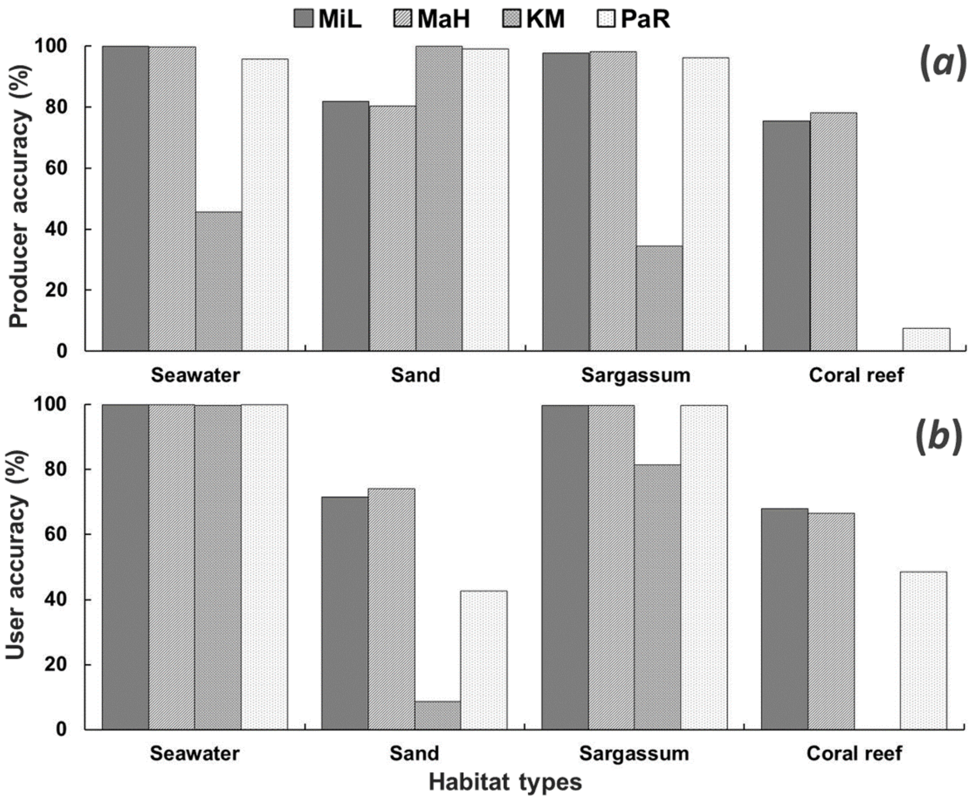

Figure 5.8 Validation accuracy for each habitat type from different classifiers. Producer accuracy results (a) and User accuracy (b). Minimum distance (MiL), Mahalanobis (MaH), the K-means (KM), and parallelepiped (PaR) classifiers.

Satellite remote-sensing WV-2 data showed the highest densities of *Sargassum* spp. around Rottnest Island along the shallow subtidal zone on limestone substrates. The area around Green Island contained patches of high *Sargassum* spp. coverage, but the distribution of *Sargassum* spp. in this area was not even and was instead concentrated mainly around fringing reefs (Figure 5.7). In Little Armstrong Bay and Stark Bay areas, the distribution of macroalgae was also high and was concentrated in large meadows on the right side and the outside of the fringing reefs. The area had red color patterns, which supposedly symbolize submerged coral reef platforms.

5.3.4. Accuracy assessment

The accuracy assessment method was employed to test four classifiers: the Minimum distance, Mahalanobis, K-means, and parallelepiped (PaR) methods. The minimum distance method produced the greatest classification accuracy results for the seawater class followed by accuracy rates for *Sargassum* spp., sand, and coral reef of 99.8, 97.8, 81.8, and 75.5%, respectively. The highest accuracy rates obtained by the Mahalanobis classification method were found in the seawater class (99.6%), followed by *Sargassum* spp. (98.1%), sand (80.3%), and coral reef (78.1%). The classification results produced by the parallelepiped classification method were 95.7% for the seawater class, 99.1% for sand, 96.1% for *Sargassum* spp., and 7.5% for coral reef. Of the four classification methods, the K-means method produced the lowest accuracy rates for seawater (45.6%), *Sargassum* spp. (34.5%), and coral reef (0%), but yielded the highest accuracy rate for sand (100; Table 5.5).

Table 5.5 Confusion matrix for WV-2 classification of Rottnest Island Reserve using four classifiers. The accuracy assessment was based on selected main habitats from WV-2 images.

Classes	Classifiers	Test areas/ ground truth (Percentage)				
		Seawater	Sand	*Sargassum*	Coral reef	Total
Unclassified	MiL	0	0	0	0	0
	MaH	0	0	0	0	0
	KM	0	0	0	0	0
	PaR	4.3	0.9	2.9	3.3	3.9
Seawater	MiL	**99.8**	0	0.2	0.1	70.6
	MaH	**99.6**	0	0.1	0.3	70.4
	KM	**45.6**	0	0.5	0.1	32.3
	PaR	**95.7**	0	0	0.1	67.6
Sand	MiL	0	**81.8**	0	21.2	2.0
	MaH	0	**80.4**	0	18.5	1.9
	KM	0.3	**100**	65.2	99.7	20.9
	PaR	0	**99.1**	0.2	85.7	4.1
Sargassum	MiL	0	0	**97.8**	3.2	24.4
	MaH	0	0	**98.1**	3.2	24.5
	KM	2.8	0	**34.4**	0.3	10.5
	PaR	0	0	**96.1**	3.5	24.0
Coral reef	MiL	0.2	18.2	2.1	**75.5**	3.0
	MaH	0.4	19.7	1.8	**78.1**	3.2
	KM	51.4	0	0	**0**	36.3
	PaR	0	0.1	0.8	**7.5**	0.4
Total	MiL	100	100	100	100	100
	MaH	100	100	100	100	100
	KM	100	100	100	100	100
	PaR	100	100	100	100	100

Minimum distance (MiL), Mahalanobis (MaH), the K-means (KM) and parallelepiped (PaR)

5.4. DISCUSSION

To date, no studies on the use of WV-2 images for mapping *Sargassum* spp., particularly along the Australian coast, have been performed. This study showed a high level of accuracy in classifying *Sargassum* spp. using WV-2 imagery, highlighting the utility of SRSI in mapping shallow marine environments. The integrated study of high-resolution satellite data provides useful and valuable information for natural resource managers to respond to questions of seasonal change in *Sargassum* spp. biomass and the typical substrate utilised by *Sargassum* spp. (Andréfouët *et al.*, 2004).

However, benthic habitat mapping using the object-based and supervised classification technique is still subject to a number of limitations, *i.e.* the method requires the gathering of ground-truth data. Field data collection is relatively time consuming and expensive (Komatsu *et al.*, 2002; Muslim *et al.*, 2012; Vahtmäe and Kutser, 2013). In addition, field data do not always correlate with satellite image data, because of discrepancies in spatial resolution, time lag between photos and surveys, field survey techniques, and effects due to the environmental conditions of data collection (Kutser *et al.*, 2006). In terms of ground truth, this study found that a minimum of 10 transects with five survey quadrats per transect, for a total survey area of approximately 20 km^2, is required for a high-accuracy assessment. Moreover, this study recommends a survey quadrat size of 0.5 × 0.5 m, which is the standard method for general benthic habitat mapping studies (e.g. Duarte and Kirkman, 2001; Japar *et al.*, 2001; McKenzie *et al.*, 2001; Short and Duarte, 2001). We believe that this approach returns better accuracy assessments than numerous previous studies that merely employ the manta tow technique for the photography of the benthic substrate (<0.25 m^2 per site) (Noiraksar *et al.*, 2014).

In addition, this study highlights the importance of understanding seasonal variations in *Sargassum* spp. biomass and distribution before acquiring WV-2 image data. Spring was chosen for this study, because it is when the biomass and cover of *Sargassum* spp. peak in Rottnest Island. Thus, it is important to understand the seasonality of *Sargassum* spp., so that WV-2 imagery captures the period of highest biomass and facilitates the mapping of *Sargassum* spp. There is additional value in investigating the utility of WV-2 image data to track and understand the seasonal variations in *Sargassum* spp. biomass and distribution.

One limitation of this study is that it has yet to assess the comparable classification results of the VW-2 pan-sharpened and broad-spectral bands. Likewise, a potential error that might be encountered in the study of coastal ecosystems is the variation in the inherent optical properties (IOPs) of the water column. For example, coastal waters are often affected by runoff, upwelling, and water mixing, which can lead to high concentrations of phytoplankton, inorganic particles, and colored dissolved organic matter (CDOM). A change in the IOPs affects both the shape and magnitude of the reflectance spectra (Vahtmäe and Kutser, 2013). Therefore, further work is required to evaluate the differences in classification results between pan-sharpened and broad-spectral bands and other very high-resolution remote-sensing data (*e.g.*, hyperspectral images and aerial photography) and to assess the potential effect of changing the IOP conditions.

Table 5.6 Confusion matrix of the overall accuracy of the classification maps obtained from the WV-2 image.

	Overall accuracy (%)	**Kappa coefficient**
Minimum distance	98.32	0.96
Mahalanobis	98.30	0.96
K-means	42.50	0.22
Parallelepiped	93.50	0.86

The producer and user accuracies were employed and compared among the four classification techniques of this study (Figure 5.8). The advantages of the coastal (400–450 nm) and yellow (585–625 nm) bands were illustrated by the high classification accuracy of these types of habitats (Table 5.6). These are the unique bands that are only found in WV-2 images. With these bands, WV-2 images can penetrate through the clear water column and gather appropriate information about bottom habitats in the shallow waters. A similar study by Su *et al.* (2008) showed that in the water sector, green light (500–600 nm) can reach a maximum depth of 15 m, red light (600–700 nm) can penetrate to 5 m and infrared light (700–800 nm) to 0.5 m (Green *et al.*, 2000; Su *et al.*, 2008). The overall classification accuracies achieved in the present study are higher than those found by Noiraksar *et al.* (2014), who mapped *Sargassum* beds in Chon Buri province, Thailand, using ALOS AVNIR-2 images. They used minimum distance and supervised maximum likelihood classification methods, which resulted in overall accuracy rates of 67% and 69%, respectively. However, a study by Carle *et al.* (2014), which mapped the distribution of freshwater marsh species using WV-2 imagery, showed that maximum likelihood classifiers delivered the highest overall classification accuracy (75%). They also found that the coastal blue and red-edge bands played a major role in enhancing vegetation mapping.

Comparing the classification results of this study with those of the previous studies on the *Sargassum* group and other coastal habitats showed that our study results have a higher classification accurate ratio (Carle *et al.*, 2014; Heenkenda *et al.*, 2014; Malahlela *et al.,* 2014). This can be explained by two factors. First, as discussed earlier, this study was evaluated source imaging at high spatial resolution (2-m) and spectral resolution (eight bands). In particular, the coastal band is capable of deeply penetrating and gathering appropriate information from marine habitats. Second, our study area (Rottnest Island, which is situated 20 km offshore) has clear ocean waters with a relatively low influence of coastal sediments and nutrients. Consequently, the clearness of the water column is high because of low concentrations of phytoplankton, inorganic particles, and CDOM in the water. According to Vahtmäe and Kutser (2013), in clear ocean waters, optical remote-sensing can be limiting at a depth of 30 m. However, the spectrum of information that is most useful to separate the substrate components is achieved at a depth of 5 to 6 m. Likewise, another study in an open-sea area showed that benthic plants cannot be identified at a bottom depth below about 5 m (Vahtmäe *et al.*, 2012). In this study, therefore, the detection range of *Sargassum* spp. lies within the depth range from 0.5 to 3.5 m and is wholly useful spectral information. In addition, the findings of this study agree with those of Vahtmae and Kutser (2013), who showed that the classification outcomes from the object-based methods provide higher-quality benthic habitat maps than did the spectral library method (Vahtmäe and Kutser, 2013).

In summary, this study has increased understanding of the spatial distribution of *Sargassum* beds around coastal area of Rottnest Island, WA, using field survey and remote-sensing techniques. Based on the results, we conclude that eight-band high-resolution multispectral WV-2 satellite imagery has a great potential for mapping and monitoring *Sargassum* beds, as well as other associated coastal marine habitats. The results are relevant for coastal marine health monitoring and economic planning purposes on the WA coast using high-resolution satellite imagery with a large-scale coverage. However, further study to detect the biomass of *Sargassum* beds, using WV-2 in combination with *in situ* observation data, is required.

Chapter 6. THE SPATIAL DISTRIBUTION, LIFE CYCLE AND SEASONAL GROWTH RATE OF *SARGASSUM SPINULIGERUM* IN THE WESTERN AUSTRALIAN COAST

6.1. INTRODUCTION

The majority of *Sargassum* species are distributed in the northern and southern Pacific Ocean at the biodiversity hotspots including the Indian Ocean, Southeast Asia and Australia. Shimabukuro *et al.* (2008) had estimated that there are approximately 140 tropical and subtropical *Sargassum* species in the eastern Asian countries such as Japan, China and the Philippines (Shimabukuro *et al.*, 2008). On the shallow subtidal and intertidal reefs around the WA coast, *Sargassum* spp. form a dominant brown macroalgae group that shows strong seasonal variation (Kendrick and Walker, 1991). *S. spinuligerum* is widely distributed globally and is one of the most important brown macroalgae species in tropical and temperate regions (Kendrick, 1993; Kendrick and Walker, 1991; Mattio *et al.*, 2008). This species constitutes a canopy of habitats with high productivity and contributes to maintaining a healthy coastal ecosystem.

The growth and mortality rate of *S. spinuligerum* varies between seasons and life cycle stages and is dependent on the irradiance, water depth, salinity, pH, photosynthetic quantum yield, water temperature, and bioavailability of nutrients (Hanisak and Samuel, 1987; Hwang *et al.*, 2004; 2007a; Liu *et al.*, 2007; Pustizzi *et al.*, 2004; Schaffelke and Klumpp, 1998). The highest biomass and density of reproductive thalli is recorded in the spring (September to November) (Kendrick, 1993; Kendrick and Walker, 1994). According to Kendrick (1993), *S. spinuligerum* in the subtidal zone has an increased density of vegetative thalli from the autumn (March to May) to the winter (June to August). *S. spinuligerum* reproduces in spring (September to November) and summer (December to February), when it reaches the highest biomass.

So far, no studies have ever documented the ecological characteristics such as growth, reproduction, and life cycle of *S. spinuligerum,* in particular around the WA coast. The previous studies in Australia on the *Sargassum* species were only confined to taxonomy and distribution (Dixon and Huisman, 2010; Fulton *et al.*, 2014; Goldberg and Huisman, 2004; Kendrick, 1993; Kendrick and Walker, 1994; Vuki and Price, 1994) and the sexual and vegetative reproduction of various *Sargassum* species inhabiting different geographical areas, for example *S. thunbergia* at the Shandong Peninsula coast, China (Chu *et al.*, 2011), *S. muticum* at Friday Harbor, Washington, USA (Aguilar-Rosas and Machado, 1990; Baer and Stengel, 2010; Lawrence, 1984; Norton, 1977), *S. muticum* at Kurosima, Japan (Tsukidate, 1984), *S. horneri* at Maizuru Bay and Obama Bay, Japan (Choi *et al.*, 2009; Pang *et al.*, 2009; Uchida, 1993; Umezaki, 1984), *S. yezoense* at the coastal area of Oshika Peninsula, Japan (Yukio *et al.*, 2002), and *S. vachellianum* at Zhejiang province, China (Yan and Zhang, 2014), *S. polyceratium* at the coastal area of Venezuela (Engelen, Breeman, *et al.*, 2005).

In recent years, due to over-harvesting of natural stocks, pollution, and changing climate, a decline in *Sargassum* biomass and distribution have been reported (Díez *et al.*, 2012; Yu *et al.*, 2013). From 2012–2014, due to a number of extreme weather conditions such as the hottest and prolonged heat in the summer of 2014 and the lowest temperature in the winter of 2015 along the WA coast, the distribution and abundance of *S. spinuligerum* was affected (Fulton *et al.*, 2014; Singh and Singh, 2015). Therefore, the aim of this study was to document the spatial distribution, life cycle, and growth rate of *S. spinuligerum* based on the field study data.

6.2. MATERIALS AND METHODS

6.2.1. Site description

The study was carried out at two study areas off the WA coast, Point Peron and Rottnest Island. Point Peron belongs to Shoalwater Marine Park and is located off Rockingham City area, whereas, Rottnest Island is located about 18 km from the Perth city area (Figure 2.1). These two areas belong to the Southwest Australian eco-region which is one of 34 biodiversity hotspots globally based on the Conservation International report on the unique and highly threatened flora. In addition, the eco-region of south WA is one of the five Mediterranean eco-region biomes with an extremely high biodiversity but also is also under stress due to the anthropogenic causes (Morgan *et al.*, 2008).

6.2.2. Spatial distribution of *Sargassum* and other submerged aquatic vegetation

Spatial distribution of *Sargassum* spp. and other SAV were carried out at intertidal zones around Rottnest Island with water depth from 0.3 to 2.5 m. The distribution characteristics and canopy coverage (CC) were documented and surveyed at nine transects during three sampling trips. Canopy coverage percentage was monitored using a standard quadrat (0.5 × 0.5 m). The standard quadrat was divided into 25 small cells with each cell corresponding to 4% of the total quadrat. Along the monitored transects, a series of underwater videos was also recorded and stored to study and illustrate the spatial distribution.

6.2.3. Reproductive phenology studies

The study on the growth and reproduction phenology of *S. spinuligerum* was carried out at Point Peron from September 2012 to December 2014. The mean thallus length (MTL), CC, and the fresh biomass (FB) were measured at four permanent transect lines with a sampling frequency of once in every three months. The location of transect lines were recorded by hand-held GPS device (Garmin eTrex® 10).

The MTL was measured from the holdfast to the highest tip of the thallus. Five thalli were measured and then averaged out for the mean value with a standard error (S.E.). Canopy coverage was measured using the same presented methods in section 6.2.2 for Rottnest Island. The fresh biomass was quantified by randomly placing the quadrats along the defined transects. All *Sargassum* species were collected from inside the quadrats, dried and then weighed using a digital scale. The fresh biomass was counted for all *Sargassum* species regardless of how many species were collected in the quadrat. An amount of selected fresh *Sargassum* samples within monitored quadrats was collected in labeled polyethylene bags, stored in a cool-box and then transported to the CARL, Curtin University within three hours after sampling.

The samples at the CARL were washed with filtered seawater to remove any epiphytes and fouling organisms, and then stocked in the large composite holding tank (113-L) with constant aeration for further laboratory studies. During all field sampling trips, the presence of reproductive thalli and receptacles on the identified *S. spinuligerum* were observed and recorded at each sampling time and then examined by

SMZ1500 stereoscopic zoom microscopy (Nikon, Japan) in the CARL. The reproductive morphology specimens such as main thalli, receptacles, and young thalli were photographed and line drawn for each stage of the life cycle. The samples were morphologically identified by comparing with the previous studies (Garton, 1997; Noro *et al.*, 1994; Phillips, 1994b).

6.2.4. The specific growth rate of *Sargassum*

The length of the main thallus was measured at every sampling trip at Point Peron during the study period. Main thallus growth rate (cm d^{-1}) was measured to determine the apical growth rate using equation (1) (Hanisak and Samuel, 1987; Mai *et al.*, 2010).

Specific Growth Rate (SGR) = Change in height/ Change in time

where SGR is specific growth rate of the main thallus (cm d^{-1}), change in height is the mean thallus length of *Sargassum* between two sampling trips, and change in time is a period of time between two sampling trips. Unit of time is per days.

6.2.5. Data analysis

Field monitoring data were analyzed and processed by a statistical software IBM® SPSS® Statistics version 20 (New York, USA) and Microsoft Excel 2010. One way of analysis of variance (ANOVA) was employed and principally used to test any significant differences among surveyed seasons. Once a significant difference between treatments was observed, the Fisher's least significant difference (LSD) test was used for multiple mean comparisons. The statistical significance level was set at $P < 0.05$.

6.3. RESULTS

6.3.1. Spatial distribution of *Sargassum* around Rottnest Island

Field observations showed that there are three dominant *Sargassum* species, *S. spinurigerum, S. swartzii,* and *S. confusum,* in both of the surveyed study sites. *S. spinurigerum* had the widest distribution. In Point Peron, *S. spinurigerum* is usually found in shallow waters in the intertidal zone and up to 2-m in depth, while *S. swartzii,* and *S. confusum* are dominant in deeper areas over 2 m deep.

In Rottnest Island, the spatial distribution of *Sargassum* spp. in the monitored transects was divided into three main areas: *Sargassum*, seagrasses, and mixed seaweed zones (Figure 6.1). In Parker Point, the first *Sargassum* area was found approximately 80 m from the shore with a maximum depth of around 2.0 m (Figure 6.1a). *Sargassum* dominated with canopy coverage of approximately 90%, *Gracilaria* sp. covers about 5%, and the rest was covered by other macroalgae. Limestone is the dominant substrate in this area. At the end of the monitored transect line (~150 m), seagrasses are predominate (90%) with sand and rubble at depths of approximately 2.5 m. The red macroalgae group (i.e. *Gracillaria* sp.) and other algae groups accounted for about 5% with scattering distribution. At the furthest area from the transect, at a distance of 150 to 220 m from the shore, *Sargassum* dominated with a canopy coverage around 95%; the red macroalgae and seagrass groups accounted for 2% and 3%, respectively.

In the Green Island transect (Rottnest Island) (Figure 6.1b), the distribution pattern of marine submerged vegetation was divided into four main areas including in-shore *Sargassum*, seagrasses, off-shore *Sargassum*, and mixed areas. The first *Sargassum* area was found from the shore to 50 m further with a dominant limestone substrate. The maximum depth was found up to 1.8 m, and the *Sargassum* canopy coverage was reached 90%, red macroalgae and seagrasses occupied around 10%. The second area from the shore was dominated by seagrasses (90%), and the rest was *Gracillaria* spp. and other macroalgae. In this transect, the distribution pattern of marine submerged vegetation was also divided into four main zones: in-shore *Sargassum*, seagrass, off-shore *Sargassum*, and mixed macroalgae zones; in order from the shore to the further area, the abundant canopy coverage was over 90% for each group.

Overall, the survey results of the monitored transects showed that *Sargassum* was largely distributed on limestone and rubble substrates with depths ranging from 0.2 to 3.5 m. *Sargassum* can be found right from the shore or in the furthest areas of the transect. Likewise, seagrass was usually distributed in sandy bottoms with an average depth of 2.0 m. The red macroalgae and other macroalgae groups often had a scattered distribution on the limestone reef or were associated with seagrass and *Sargassum* spp.

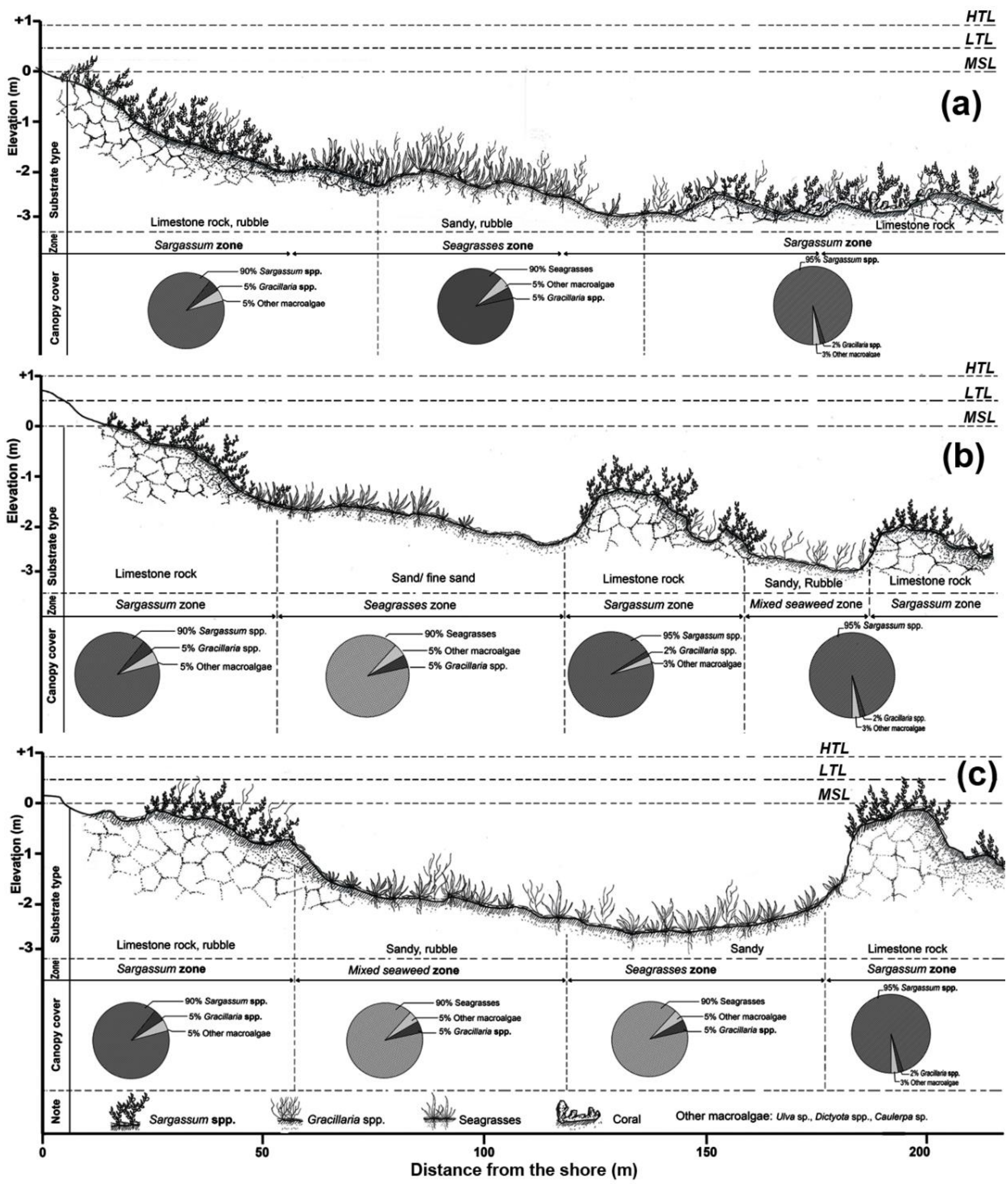

Figure 6.1 A typical spatial distribution and percentage coverage of *Sargassum* spp. and other coastal marine submerged vegetation along the transects (a) in the Parker Point (32^o 01.493S – 115^o 31.719E); (b) the Green Island area (Rottnest Island) (32^o 1'0.55"S – 115^o 29'55E); (c) Parakeet Bay (31^o 59.417S – 115^o 30.400E) in spring (Oct. 2013) in Rottnest Island, WA.

6.3.2. Life cycle studies of *Sargassum spinuligerum* in the WA coast

S. spinuligerum (Sargassaceae, Fucales) have a single life cycle/ history phase. The life cycle of *S. spinuligerum* in the subtidal zone of WA coast can be divided into five stages including *i*) dormancy; *ii*) the new growth season/regeneration/recruitment; *iii*) increasing biomass; *iv*) maturity and reproductive; and *v*) die back/senescent phase (Figure 6.2).

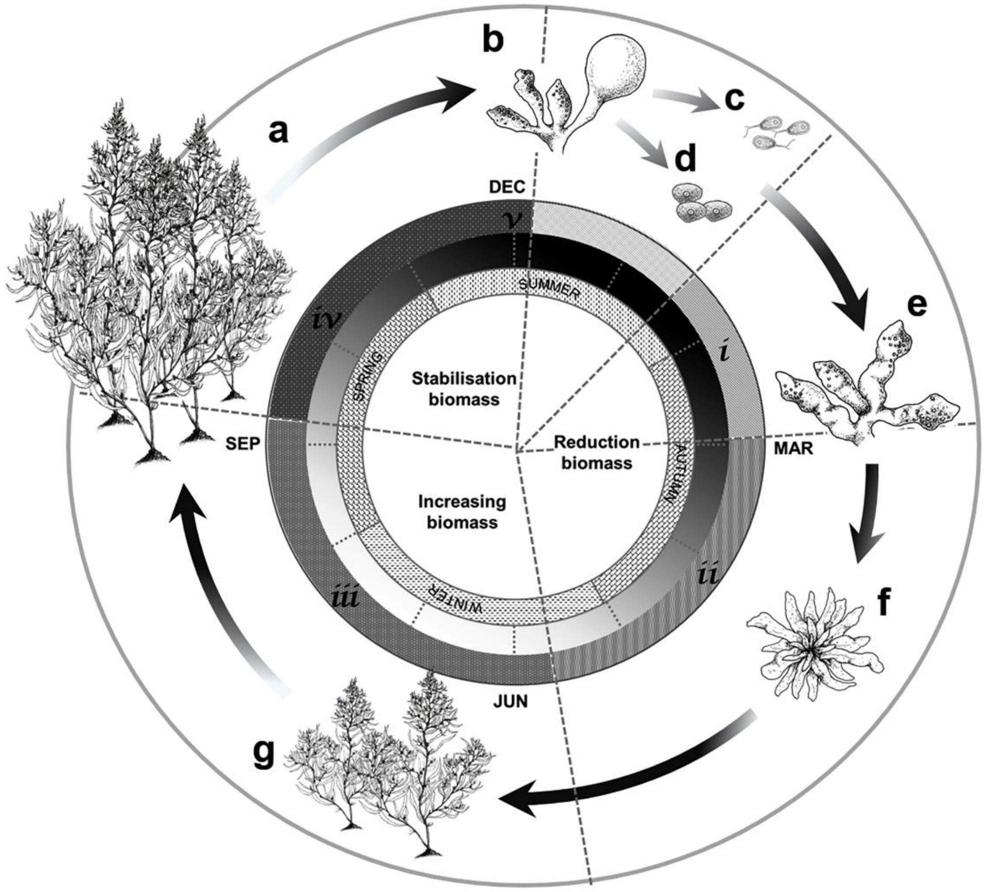

Figure 6.2 Schematic representation of the life cycle of *S. spinuligerum* sexual reproduction in relation to seasonal changes. (a) Reproductive thallus; (b) Receptacles; (c) Sperm cells; (d) Egg cells; (e) Oogonial/Zygotes develop on conceptacle; (f) Germling released and attached onto substrates to develop young thalli; (g) Fully growth thalli. Five stages including *i)* dormancy; *ii)* the new growth season/regeneration/recruitment; *iii)* increasing biomass; *iv)* maturity and reproductive; and *v)* die back/senescent.

i) Dormancy phase: This phase usually commences from the end of summer to early autumn (February–March). Dormancy is found after conceptacles reduce division/meiosis inside male and female conceptacles to produce spermatozoids and oospores. Spermatozoids are motile and oospores are non-motile. The fertilized eggs develop into young gametophytes that freely travel in the water bodies and then settle on the substrate surfaces. In the vegetative productive form, the dormancy phase is found from the broken thallus, from the remaining holdfasts on the substrates.

ii) The new growth season phase/recruitment: This phase often commences from early autumn to early winter (March–June). The young gametophyte starts growing and the holdfasts begin the recruitment of a new thallus from their old holdfasts.

iii) Maximum standing crop/increasing biomass phase: In WA, this phase starts from early winter and continues to the middle of spring (June–September). The thallus steadily increases in biomass in early spring (August–September).

iv) The maturity and reproductive phase: This phase runs from the middle of spring to early summer (September–December). *Sargassum* reach their maximum biomass and begin the second reproduction phase by developing receptacles. *Sargassum* biomass stabilizes for several months before shifting to the next phase.

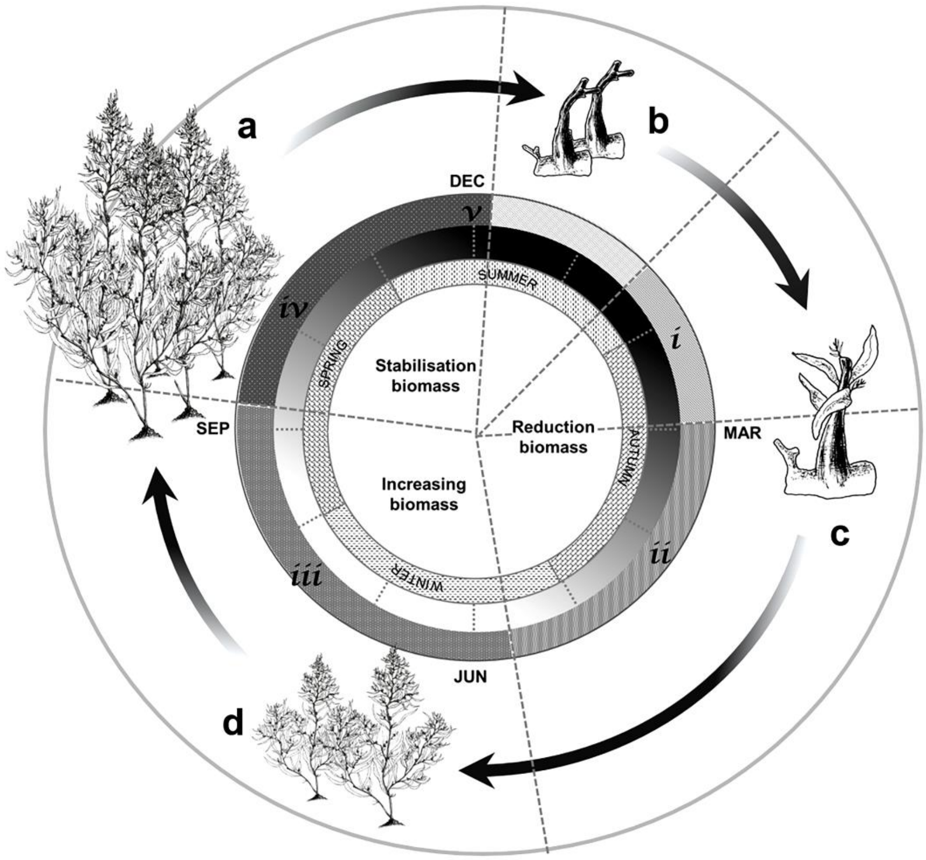

Figure 6.3 Schematic representation of the life cycle of *S. spinuligerum* vegetative reproduction in relation to seasonal changes. (a) Reproductive thalli; (b) Remaining holdfasts; (c) Recruitment new thalli from the remaining holdfasts; (d) Fully grown thalli. Five stages including *i)* dormancy; *ii)* the new growth season/regeneration/ recruitment; *iii)* Maximum standing crop/increasing biomass; *iv)* maturity and reproductive; and *v)* die back senescence.

v) The die back phase: This phase commences from the beginning until the end of summer (December–January). Mature *Sargassum* die off leaving only some remaining holdfasts.

Sexual reproduction is found in most *Sargassum* species in the coastal intertidal zones, while vegetative reproduction is also found in the majority of *Sargassum* species within the coastal subtidal zones (Figure 6.3). During vegetative reproduction, while the majority of the *Sargassum* thalli dies, the holdfasts often remains *(v)*. This holdfasts remains dominant during the warmer months *(i)*, and then begins to grow in the new growth phase *(ii)*, finally forming a new thallus in the colder months. The thallus increases in biomass *(iii)*, and then reproduces before senescence in the summer *(iv)*.

Table 6.1 The value of canopy cover, fresh biomass, mean thallus length and specific growth rate of *Sargassum* at difference seasons at Point Peron, WA.

Season	**Canopy cover (%)**	**Fresh biomass (g wt m^{-2})**	**MTL (cm)**	**SGR (cm d^{-1})**
Spring	78.83 ± 3.92[a]	1412.9 ± 177.36[a]	36.55 ± 6.41[a]	0.98 ± 0.41 [a]
Summer	70.03 ± 3.55[a]	963.2 ± 199.14[b]	34.46 ± 3.59 [a]	-0.79 ± 0.49 [b]
Autumn	30.69 ± 5.13[b]	284.14 ± 76.96[c]	15.76 ± 2.12 [b]	-0.74 ± 0.47 [b]
Winter	54.73 ± 8.64[c]	868.06 ± 103.44[b]	16.18 ± 1.23 [b]	0.38 ± 0.32 [ab]
F	15.127	8.275	5.901	3.085
P	< 0.001	< 0.001	0.002	0.04

6.3.3. Seasonality of *S. spinuligerum* growth rate in Point Peron

There were significant differences ($P < 0.005$) in CC, FB, MTL and SGR between seasons as determined by one-way ANOVA with $F(3, 45) = 15.127$, $F(3, 33) = 8.275$, $F(3, 45) = 5.901$, and $F(3, 37) = 3.085$, respectively (Table 6.1). The lowest values of CC, FB, and SGR were found in May and June. The highest value of CC and FB were found in December (2012, 2014), while the SGR reached the highest value in July (2013), December (2013) and September (2014). The lowest SGR occurred in February 2013 and January 2014 (Figure 6.4). Overall, the highest CC and FB occurred in the spring coinciding with increasing sun light and temperature. Meanwhile, the highest SGR was found in winter (Jun–July) and spring (September).

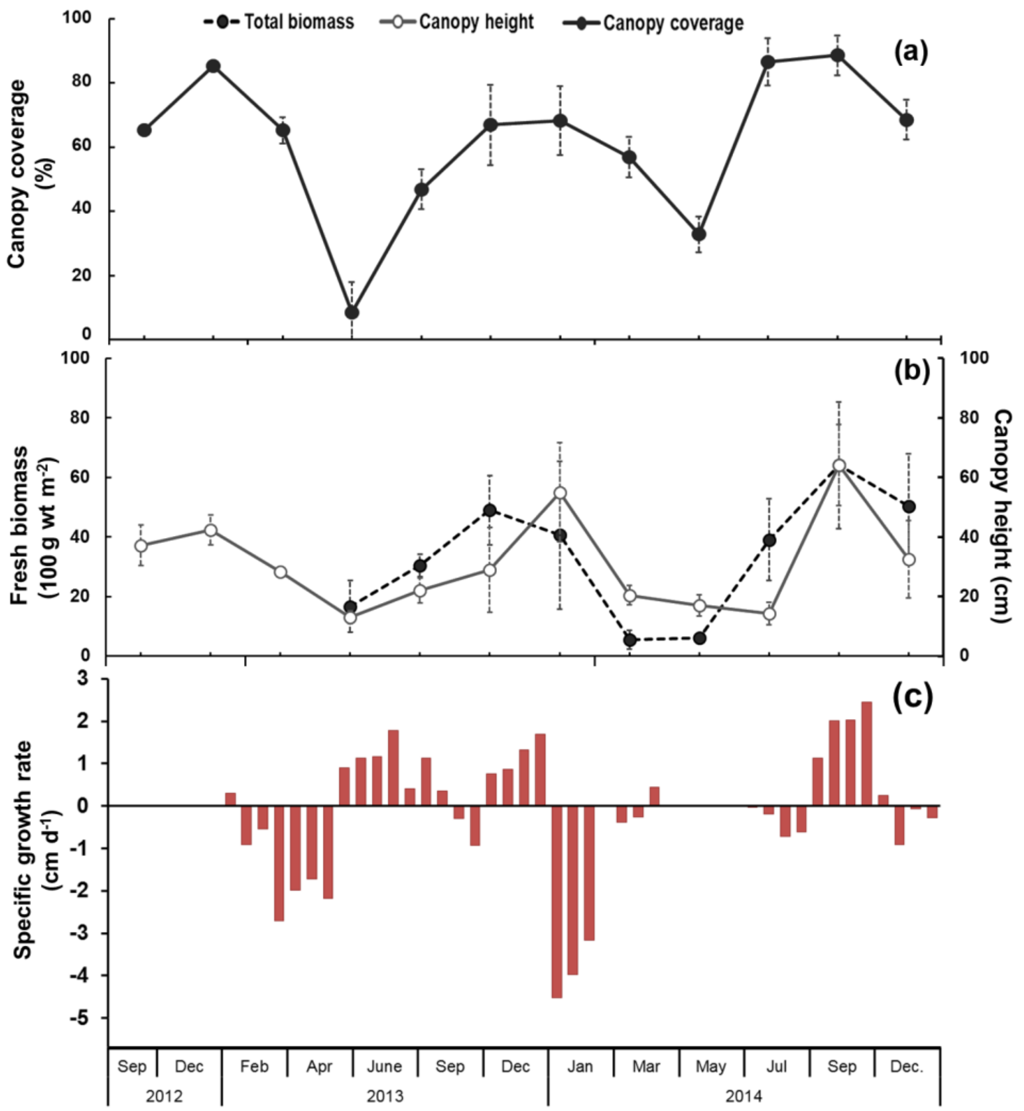

Figure 6.4 Inter-annual changes in (a) mean canopy cover (solid line), (b) fresh biomass weight (dotted line) and canopy coverage (light solid line), and (c) specific growth rate (cm per day) with the standard deviations (vertical bars) of the main thallus at different reef areas in Point Peron.

6.4. DISCUSSION

This present study could be considered one of the first on the spatial distribution, life cycle, and growth rate of *S. spinuligerum* along the WA coast. The study has documented the main growth and development stages of *S. spinuligerum* in association with inter-season variations in water quality. The previous study on the growth and development of an invasive *Sargassum* species (*S. muticum*) showed that there was a strong relationship between the length and width of germlings, and the number of produced rhizoids during the first development stages (Norton, 1977). A study on the reproduction of *S. muticum* at La Jolla, California (USA) showed that a higher ratio of thalli carrying fertilized receptacles was usually found from late December to March, and also coincided with SST values from 13.1−15.0^{o}C (Lawrence, 1984).

Sargassum is one of the genera of brown macroalgae that is difficult in taxonomic classification due to the large variation of morphology in its life cycle (Kantachumpoo *et al.*, 2015). There are various receptacles and morphological structures of *Sargassum* species during their various life cycles, as well as surrounding environmental conditions such as waves, currents, and the availability of nutrients. For instance, thalli develop more branches in strong wave conditions than in quiet waters (Gillespie and Critchley, 1997). A study on *S. lapazeanum* species in the south-western Gulf of California showed that this species is dioic; there are male and female conceptacles on the same receptacle but with different maturation times (Gabriela and Rafael, 2011). *S. spinuligerum* have both male conceptacles (antheridia) and female conceptacles (monoecious) on the same thallus as similar to *S. filipendula* (Redmond *et al.*, 2014; Scrosati, 2002).

The reproduction morphology of *Sargassum* is called receptacles and is usually found either at the secondary or tertiary of the main thalli. The surface of the receptacles usually have many little pouches called conceptacles which carry gametangia (Gillespie and Critchley, 1997). According to morphology, receptacles are classified into three different types including terate (*S. elegans*), three-cornered (*S. heterophyllum/S. incisifolium*), and twisted (*Sargassum* sp.). Of those species, the three-cornered type have the largest number of conceptacles per area of unit. The terate receptacles have an average number of conceptacles per area unit (Gillespie and Critchley, 1997). In some rare cases, it was found that the process of sexual reproduction can disappear particularly in those species with the floating life cycle (free-floating forms) in seas such as the Sargasso Sea. Here, the process of vegetative reproduction occurs by developing new crops from the thallus segments (Maria *et al.*, 2012). Furthermore, in vegetative reproduction, the *Sargassum* species in coastal areas often regenerated by the development of new thalli from old holdfasts from the previous year that remained on the substrate (Figure 6.3). Some previous studies have shown that the growth rate of thallus regeneration from holdfasts is often greater than sexual reproduction where development occurs from a zygote (Kendrick, 1993; Kendrick and Walker, 1994).

In WA coastal area, *S. spinuligerum* is an abundant species that forms dense populations with the canopy coverage area of over 80% at the study sites. Therefore, the morphology of this species makes it quite easy identify in the field. In this study, the classification of species in the *Sargassum* genus was mainly based on the traditional morphological taxonomy that relies on the thalli, vesicles, leaves, and receptacles characteristics. This classification method generally produces accurate results for common species with distinct morphological characteristics. However, genetic analysis is needed to assess the *Sargassum* species that have morphological similarities in WA waters. A study on the systematics of *Sargassum* in Thailand showed that based on Nuclear Ribisomal Internal Transcribed Spacer 2 (ITS2) data gives the relationship between species with the phylogenetic tree constructed from genetic analysis data found in coastal regions in Thailand (Kantachumpoo *et al.*, 2015).

A pilot study of the growing *S. naozhoue nse* species in the Leizhou canal, Guangdong, China showed that this species can be grown from seeds after six months cultivation in tanks to achieve a size of up to 5 cm in length. Then, the cultivating plants were moved into the sea conditions for a further 95 cultivation days. The biomass obtained after cultivation periods reached 1750 kg wet wt. km^{-1} per rope. In addition, this study also found that this species has high heat resistance and the ability to survive in water temperatures up to 33.7°C (Xie *et al.*, 2013). However, another study on *S. muticum* at Baja California Peninsula in Mexico showed that the highest number of the reproduction structures was present in the late spring and early summer (May–July).

The lowest number of reproduction structures was usually found at the end of winter (March) in the Southern Hemisphere (Aguilar-Rosas and Machado, 1990). According to the biomass results, the length and density of the main thallus of *S. yezoense* in Oshika Peninsula, its life cycle and the development of this species was divided into three main phases; *i*) germination phase: the biomass and thallus length with the smallest size (< 10 cm), usually taking place in the period from August to November;

ii) the development stage: the biomass increases by increasing of the main thallus, usually takes place in the period from November to December; and *iii)* maturation phase takes place in the period from June to August, when the thalli are reaching their maximum length (Yukio *et al.*, 2002). In our study, the germination phase occurred from early autumn to winter, the development stage from winter to spring and the maturation phase from spring to early summer. The new growth season phase/recruitment phase coincides with short light day (~11h) and low temperature months (~18^{o}C) (Dawes, 1987). The maximum standing crop/biomass phase has been found to have a strong relationship between increasing biomass and an availability of nutrients in the water bodies.

In conclusion, *Sargassum* communities in WA have several distinct growth phases, varied growth rates, seasonal reproduction and an annual life cycle. There are three main species of *Sargassum* found in coastal WA (*S. spinuligerum, S. swartzii, S. confusum*), of which *S. spinuligerum* has the greatest distribution and is usually found in the intertidal zone, at a depth of up to 2 m. The growth rate under matural conditions is highest in the cooler months, and canopy cover and biomass were highest toward the end of the growing period in spring to summer. In the survey areas, *Sargassum* was widely distributed on limestone and rubble substrates, with a high canopy coverage in these areas. *S. spinuligerum* have a single annual life cycle beginning with the dormancy phase. Afterwards, the young thalli undergo the recruitment phase when growth begins from zygotes and remaining holdfasts for sexual and vegetative reproductive respectively, then the maximum biomass phase, the maturity and reproductive phase, and finally the die back or senescence phase. This study provides novel and up-to-date information on the morphology, growth, reproduction and life cycle of *S. spinuligerum* of WA coastal waters.

Chapter 7. SEASON CHANGES IN WATER QUALITY AND *SARGASSUM* BIOMASS IN WESTERN AUSTRALIA

Paper publised in the *Marine Ecology Progress Series*. 551, 63–79. doi:10.3354/meps11735

(Appendix 3)

7.1. INTRODUCTION

Sargassum spp. are brown macroalgae with a global distribution, and are especially dominant in shallow tropical and sub-tropical waters (Hanisak and Samuel, 1987; Mattio *et al.*, 2008; Mattio and Payri, 2011). *Sargassum* spp. are commonly attached to rocks, but can also have floating life forms. In coastal areas and surrounding offshore islands they form dominant communities playing vital ecological roles for marine ecosystems by providing feeding grounds for sea birds and sea lions and providing essential nursery habitats for invertebrates, larval and juvenile fish surrounding these islands (Tyler, 2010; Wells and Rooker, 2004). *Sargassum* spp. also represents a living renewable resource that is used in medicines, and for the production of fertilisers, alginate, and bio-fuels (Arenas and Fernández, 2000; Chengkui *et al.*, 1984; Hong *et al.*, 2007; Rivera and Scrosati, 2006).

Approximately 46 *Sargassum* species are found along the WA coast (Herbarium, 2013) and the majority of these have been studied to determine their taxonomic affiliation, including the molecular basis of identification (Dixon and Huisman, 2010; Dixon *et al.*, 2012; Goldberg and Huisman, 2004; Kendrick, 1993; Kendrick and Walker, 1994; Rothman *et al.*, 2015), and physiology (De Clerck *et al.*, 2008; Huisman *et al.*, 2009; Kumar *et al.*, 2011; Muñoz and Fotedar, 2011; Staehr and Wernberg, 2009). However, few studies have been performed on the impact of seasonality on *Sargassum* along the subtropical and temperate coastal zone of WA (Kendrick, 1993; Kendrick and Walker, 1994). Previous studies have shown that the growth, development and distribution of *Sargassum* beds are strongly influenced by physiochemical water parameters (Mattio *et al.*, 2008; Payri, 1987; Ragaza and Hurtado, 1999), which play an important role in influencing nutrient-uptake via photosynthesis (Nishihara and Terada, 2010). Seasonal variations in the physiochemical parameters of seawater strongly influence changes in *Sargassum* canopy structure, which in turn, affect the density of the local populations (Ang and De Wreede, 1992; Arenas and Fernández, 2000; Ateweberhan *et al.*, 2009; Rivera and Scrosati, 2006).

In recent years, satellite remote-sensing studies have successfully been applied to benthic marine habitat mapping, and more specifically, have been used to estimate macroalgae biomass in the coastal waters (Andréfouët and Robinson, 2003; Benfield *et al.*, 2007; Casal *et al.*, 2011a; Fearns *et al.*, 2011; Kutser *et al.*, 2006; Maheswari, 2013; Vahtmäe and Kutser, 2007). However, the most clear and direct method for marine habitat mapping is visual observations, also termed ground truth data, using either SCUBA or snorkelling, which provides an essential input to remote-sensing observations (Komatsu *et al.*, 2002). A methodology for mapping *Laminariales* (Kelp) in turbid waters of the Seno de Corcubión (Northwest Spain), using SPOT-4 satellite images was developed, which showed that the mapping of *Sargassum* beds could be improved through the application of higher spectral images, increasing the spatial and radiometric resolution and performing new field calibrations simultaneously with the acquisition of images (Casal *et al.*, 2011a). Lower resolution Landsat (30 m) and higher resolution QuickBird (2.4 m) satellite images have been used to estimate the spatial distribution of 11 *Sargassum* beds in South West Lagoon, New Caledonia (Mattio *et al.*, 2008). Nevertheless, only a few studies have been carried out to assess of the spatial distribution of *Sargassum* and their temporal biomass variations in marine coastal areas using high-resolution satellite remote-sensing data (Hoang 2015; Noiraksar *et al.*, 2014).

The WV-2 satellite images provide one of the highest available spatial and spectral resolutions (eight spectral sensors ranging from 400–1040 nm) (DigitalGlobe, 2013; Lee *et al.*, 2011). However, no detailed mapping studies of *Sargassum* have been performed using high-resolution satellite images, such as WV-2. In addition, a direct visual approach that is integrated into high spatial resolution remote-sensing observations could represent a robust approach to minimize costs and increase the accuracy of detection and distribution patterns of *Sargassum* shallow coastal waters.

The aim of this study was to investigate the effects of seasonal changes in water quality on canopy cover, mean thallus length and the *Sargassum* biomass at a fringing limestone reef in Point Peron, WA. We have used *in situ* observations and remote-sensing methods to study the seasonal variation in physico-chemical water parameters with changes in mean thallus length (MTL), canopy cover (CC), and fresh biomass (FB) of the *Sargassum* community and determined how these changes impact the broader spatial distribution of *Sargassum.*

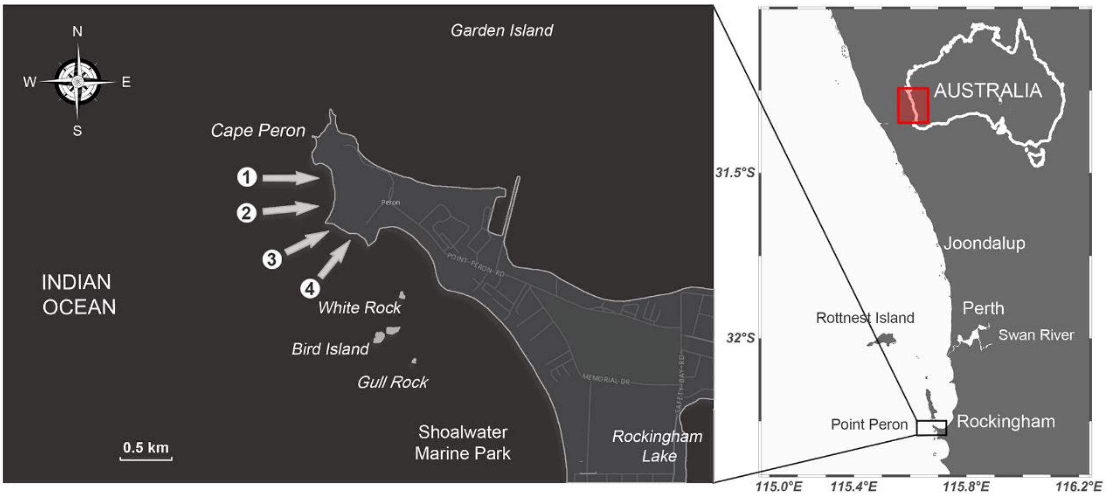

Figure 7.1 Study area, with sampling sites shown by arrows. Point Peron is located approximately 50 kilometers south of Perth City, WA.

7.2. MATERIALS AND METHODS

7.2.1. Study sites

The demonstration site was selected to be Point Peron, WA, which is a small peninsula located within the Shoalwater Islands Marine Park, an area of approximately 67 km^2, west of Rockingham city, 50 kilometers south of Perth City, WA (Figure 7.1). The point is approximately 930 m wide and 1450 m long and is surrounded by a chain of limestone reefs and islands, including Garden Island to the north. As part of the Shoalwater Islands Marine Park, Point Peron has a high diversity of marine fauna and flora (DEC, 2011).

The study area includes a chain of limestone reefs approximately 450 m offshore (32°14'–32°17'S and 115°39'–115°42'E). The coastal area of Point Peron was divided into four zones: the Lagoon zone (LZ), Back reef (BR), Reef crest (RC), and Fore reef (FR) zone with the distance approximately 100 m between each zone (Rützler and Macintyre, 1982) (Figure 7.2). The field studies were carried out from September 2012 to December 2014 during four well-defined seasons; summer

(December to February), autumn (March to May), winter (July to August), and spring (September to November) (BoM-Australia, 2013).

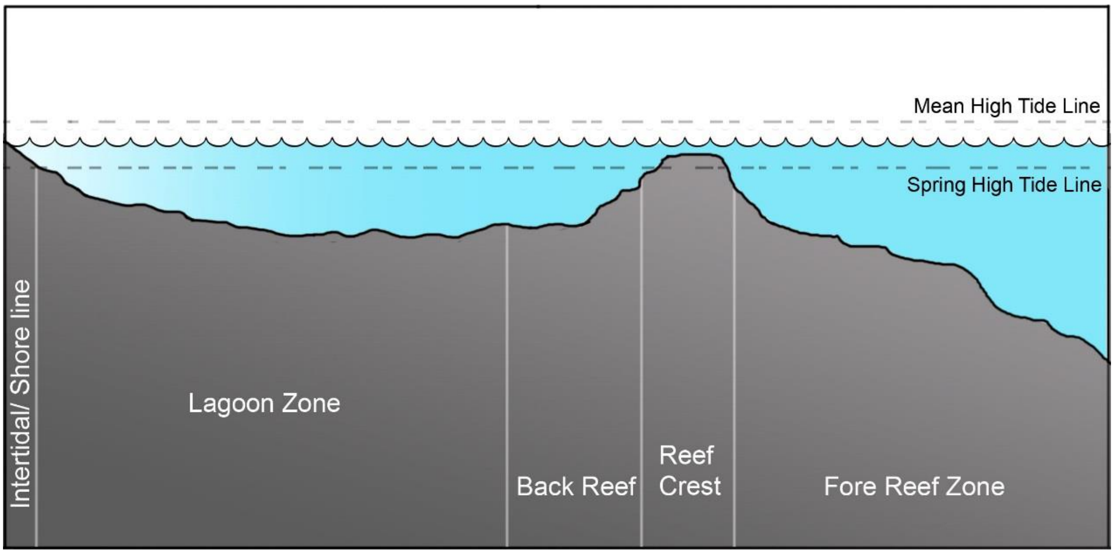

Figure 7.2 The diagram of one transect which including four reef zones: Lagoon zone (LZ), Back reef (BR), Reef crest (RC) and Fore reef (FR) zone. Adapted with modification from Rützler and Macintyre (1982)

7.2.2. Field sampling methods

7.2.2.1 Sampling frequency

The total duration of the trial was two and half years wherein summer and spring were represented three times and winter and autumn were represented twice. At least one sampling trip was carried out per season, however, we could sample twice during summer and spring seasons which were then averaged out. During every trip, four 400 m long transects were sampled. The average depth along each transect ranged from 0.3 to 2.5 m. For water quality analysis, one sample was collected from every transect. For canopy cover, fresh biomass, and mean thallus length of *Sargassum* spp., every transect was further monitored from four reef zones by deploying random quadrats (0.5 × 0.5 m), one for each reef zone. The distance between the quadrats ranged from 20 to 80 m. The above protocol provided four samples for water quality analysis and 16 (4 transects × 4 quadrats) samples for *Sargassum* measurements per season.

7.2.2.2 Sampling description

The transects were selected based-on the actual study site's topography and covering a range of different habitats. Using SCUBA survey techniques, we monitored and sampled *Sargassum* spp. along four predefined transects extending from the coastline to offshore. From each transect the seawater samples were collected in a 1-L polyethylene bottle. The *Sargassum* spp. within each quadrat was collected, stored in labelled polyethylene bags and brought to the CARL, Curtin University, WA. The locations of the sampling quadrats were recorded by a hand-held GPS (Garmin eTrex® 10). The collected *Sargassum* samples were retained in fibreglass tanks with seawater under natural sunlight. The samples were provided with constant aeration till further measurements. Fresh specimens were photographed immediately after arrival at the CARL. The holdfasts, blades, vesicles, and receptacles were also examined and photographed. *Sargassum* specimens were identified based-on taxonomic references (Garton, 1997; Guiry and Guiry, 2014; Huisman and Walker, 1990; Noro *et al.*, 1994; Phillips, 1994a). A morphological study of *Sargassum* samples was under taken on dried specimens. Herbarium specimens were stored at the CARL. Underwater video and photographs were captured along the monitored transects from five sampling trips in June and September in 2013, and January, March, and July in 2014. These data were used for ground truth and classifying the marine habitats.

7.2.3. Meteorological data and environmental parameters

Meteorological data, including the maximum (MaxAT, ^{o}C), mean, and minimum (MinAT, ^{o}C) air temperature and monthly rainfall for each season, were acquired from the nearest Bureau of Meteorology weather station, at Garden Island (32°14'24"S–115°40'48"E), 2 km north of Point Peron (BoM-Australia, 2013). Euphotic depth (ED) (m), sea level pressure (SLP) (hPa), colored dissolved organic matter (CDOM) index, photosynthetically active radiation (PAR), sea surface temperatures (SSTs), and chlorophyll-*a* concentration (Chl-a) (mg m^{-3}) in the study area (32°12'–32°17'S, 115°38'–115°42'E) were extracted from MODIS satellite data. The northward wind (NW) (m s^{-1}) was extracted from the Modern Era Retrospective-analysis for Research and Applications (MERRA) flat form in the Giovanni system, developed and maintained by the National Aeronautics and Space Administration (NASA) (Acker and Leptoukh, 2007).

In situ sea water temperature (i-SST), conductivity, and pH were measured in each season using a portable waterproof ^{o}C/mV/pH meter (CyberScan pH 300, Eutech Instruments, Singapore). Salinity was measured using a hand-held refractometer (Atago® RHS-10ATC, Japan) in practical salinity units, and dissolved oxygen (DO) was determined with a digital DO meter (YSI®55, Perth Scientific, Australia). Four seawater samples were collected during each sampling season for the analysis of nutrients; nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}). All samples were stored in 250-mL bottles and kept in a cold container (approximately 10^{o}C) in the dark. Samples were transferred to the CARL for analysis within 48 h following collection and followed the methods described in Standard Methods for the Examination of Water and Wastewater (APHA 1998). Nitrate and nitrite were measured using a Hach DR/890 Colorimeter (Hach, Loveland, CO, USA) with the cadmium reduction method (Method 8171) and diazotization method (Method 8507), respectively (APHA 1998). Phosphate concentration was analysed by Ascorbic acid method (Standard Method 4500-PE) and ammonium was determined by using Aquanal™ test kits (Sigma-Aldrich®, Germany).

7.2.4. Satellite remote-sensing data and processing

WV-2 satellite images at a 2-m spatial resolution were acquired on February 7, 2013 (austral summer), which was a period of high biomass and areal extent of *Sargassum* beds. Satellite remote-sensing WV-2 images were adjusted to pseudo-color composite images prior to the classification process, to enhance the image contrast to detect the *Sargassum* beds.

The acquired WV-2 images from DigitalGlobe® were registered into Georeferenced−the global geodetic system 1984 for latitude and longitude. The ground truth data were acquired and confirmed using *in situ* field checks from five survey trips in 2013 and 2014. The ENVI environment for visualizing images was used to mask out the land area that was not used for classification at the study area (Exelis, 2014). Methods of K-means unsupervised classification and maximum livelihood supervised classification were employed for image classification as these are the most commonly used classifier in reef studies (Benfield *et al.*, 2007; Hoang *et al*., 2015b). A toolbox in ENVI was employed for the classification and to count the number of pixels of the WV-2 satellite image that was used to detect the distribution of *Sargassum* beds. After classification, the data were converted from raster to vector format and were edited in geographical information systems software packages. The complete diagrammatic processing imagery is presented in Figure 5.3.

7.2.5. Data analysis

Seaweed distribution and abundance data were processed using statistical software, IBM® SPSS Statistics 20 and Microsoft® Excel 2010. One-way ANOVA and general linear models were employed to test for significant differences between seasons in seawater quality. A two-way ANOVA was carried out to test the effects of seasons and distribution sites on the *Sargassum* CC and MTL. The multiple comparison, least significant difference (LSD's) post hoc test, was also implemented to test for the statistical significance among treatments. The statistical significance level was set at 0.05. Principle component analysis (PCA) was employed to evaluate the interaction between the physical, chemical and biological parameters and their effect on *Sargassum* spp. Results from the PCA were acquired based on the correlation matrix of the mean values of water quality parameters against sampling times. Principle component analysis was prepared by using the latest XLSTAT 2015.1.01 (Addinsoft™, France) package for Microsoft® Excel. All the results were presented as means ± S.E. (standard error), unless otherwise stated.

7.3. RESULTS

7.3.1. Temporal variation in environmental conditions

The analysis of air temperature over the three study years (2012–2014) indicates that the monthly mean temperature was highest in the summer months (December–February). Temperatures then decreased in autumn (March–May) and were lowest in winter (June–August) and finally increased in spring (September–November). In the summer months, the maximum monthly mean temperature reached 28.2 ± 0.6^{o}C and in autumn, it reached 24.1 ± 0.9^{o}C. In winter and spring, the maximum monthly mean temperatures were 18.5 ± 0.2 and 21.7 ± 1.3^{o}C, respectively. In 2012, the mean air temperature reached a maximum in January (30.5^{o}C) and was lowest in July (9.9^{o}C) (Figure 7.3a).

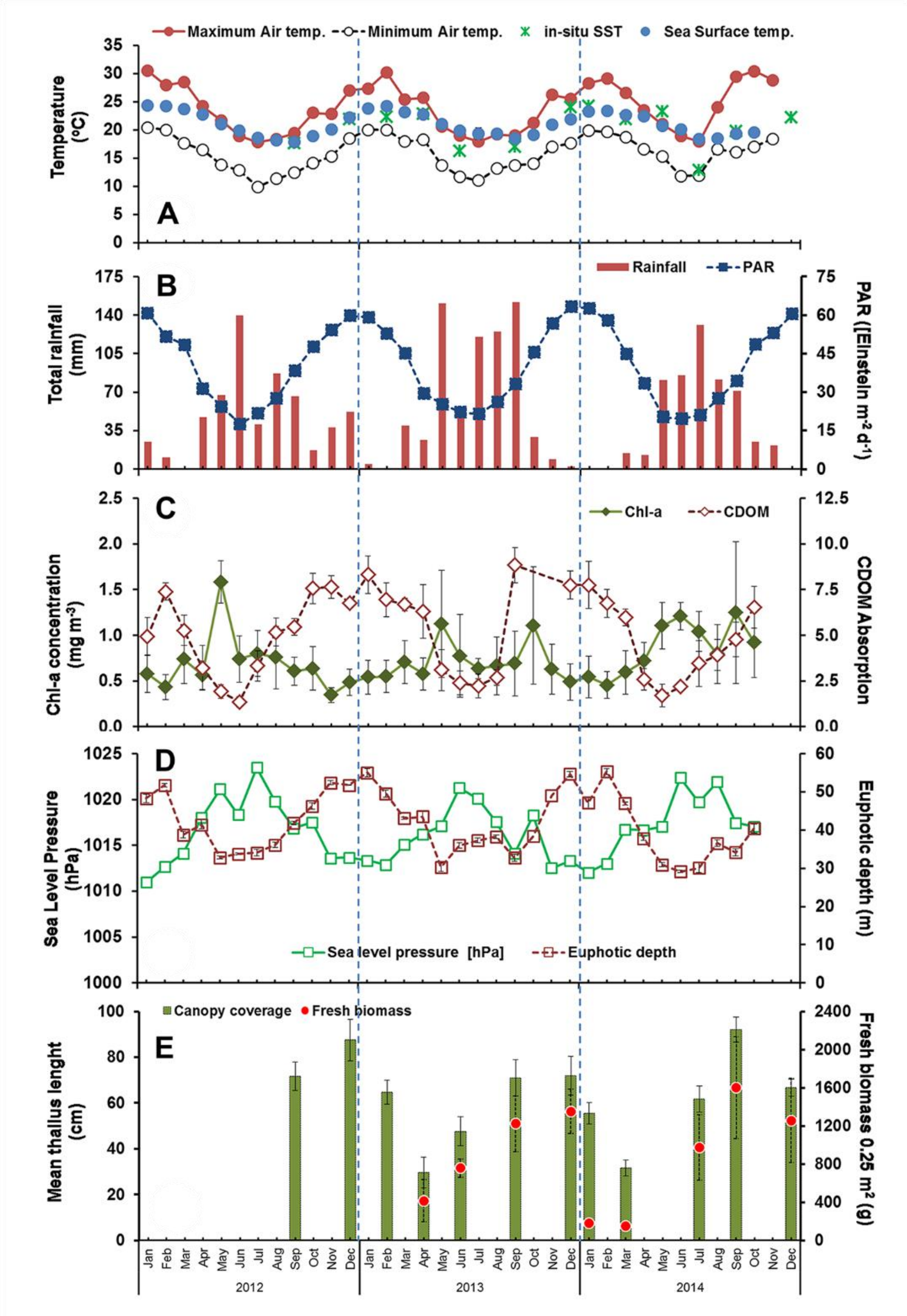

Figure 7.3 Seasonal changes in (a) air temperature (maximum and minimum value) and sea surface temperature, (b) PAR and rainfall, (c) Chl-a and CDOM index, (d) Sea level pressure and euphotic depth, (e) *Sargassum* canopy cover and fresh biomass at the study sites. *Sargassum* fresh biomass was not available for the sampling trips in September, December 2012 and February 2013. The air temperature and rainfall data were obtained from the Garden Island weather station, Bureau of Meteorology, Australian Government. The euphotic depth, CDOM, PAR, SST, Sea level pressure, and Chl-a in the study area (32°12'–32°17' S, 115°38'–115°42' E) were extracted from the Giovanni online data system, developed by NASA.

Sea surface temperatures also showed a seasonal pattern, with values ranging from 12.9 to 24.1°C. There were significant (ANOVA, $F(9, 37) = 551.23$, $P < 0.001$) differences in SSTs between the seasons, except for between winter and spring. Rainfall and PAR usually showed an inverse pattern and both showed a strong seasonal variation. The PAR reached the highest value in the summer at 58.5 ± 1.5 Einstein m^{-2} d^{-1} (a maximum in December 2013 of 63.2 Einstein m^{-2} d^{-1}) (Figure 7.3b). Although the monthly rainfall was only 2.6mm, mean summer rainfall was 11.9 ± 6.4mm. In contrast, the PAR was the lowest in the winter months at 22.8 ± 3.6 Einstein m^{-2} d^{-1} (17.5 Einstein m^{-2} d^{-1} in June 2013) and the highest mean rainfall of 95.5 ± 11.9mm was reached in winter (the maximum value of 151.6 mm was in September 2013 (Figure 7.3b).

Table 7.1 Seasonality of physico-chemical parameters (mean ± S.E.) observed at Point Peron, WA. SSTs = Sea surface temperatures, DO = Dissolved oxygen.

Year	Month	Season	Salinity (psu)	pH	Cond. (ECs)	SSTs (°C)	DO (mg L^{-1})
2012	Sep	Spr.	36.5 ± 0.29	8.1 ± 0.08^{ab}	-65.9 ± 2.89^{a}	17.6 ± 0.2^{d}	7.53 ± 0.28^{c}
	Dec	Sum.	35.8 ± 0.31	8.1 ± 0.05^{ab}	-98.8 ± 0.28^{d}	22.1 ± 0.1^{f}	6.07 ± 0.08^{b}
2013	Apr	Aut.	35.5 ± 0.20	8.1 ± 0.06^{ab}	-92.5 ± 6.13^{c}	22.8 ± 0.3^{h}	6.08 ± 0.02^{b}
	Jun	Win.	35.5 ± 0.29	8.0 ± 0.06^{b}	-87.8 ± 0.25^{bc}	16.3 ± 0.3^{b}	8.27 ± 0.13^{d}
	Sep	Spr.	35.8 ± 0.25	8.1 ± 0.11^{ab}	-88.0 ± 0.00^{bc}	17.0 ± 0.0^{c}	7.75 ± 0.25^{c}
	Dec	Sum.	35.7 ± 0.14	8.0 ± 0.02^{b}	-82.1 ± 2.79^{b}	24.1 ± 0.0^{z}	5.92 ± 0.40^{b}
2014	Mar	Aut.	35.8 ± 0.18	7.8 ± 0.14^{c}	-83.8 ± 2.95^{b}	22.6 ± 0.1^{gh}	5.99 ± 0.05^{b}
	Jul	Win.	35.5 ± 0.29	8.2 ± 0.01^{ab}	-87.0 ± 0.58^{bc}	12.9 ± 0.2^{a}	5.39 ± 0.01^{a}
	Sep	Spr.	35.4 ± 0.24	8.2 ± 0.01^{ab}	-69.7 ± 0.28^{a}	19.7 ± 0.1^{e}	5.84 ± 0.03^{ab}
	Dec	Sum.	35.8 ± 0.17	8.2 ± 0.02^{a}	-68.3 ± 0.50^{a}	22.2 ± 0.1^{fg}	7.33 ± 0.11^{cd}
		F	1.43	3.32	17.01	551.23	30.05
		P	0.224	0.007	< 0.05	< 0.05	< 0.05

The mean in the same column with different superscript letter are significantly different at the 0.05 level.

Seawater salinity in the study area ranged from 35.4 to 36.5 among the seasons, but the differences were not significant (ANOVA, $F(9, 37) = 1.43$, $P = 0.224$). The electrical conductivity of seawater in the study area also differed significantly between the sampled seasons (ANOVA, $F(9, 37) = 17.01$, $P < 0.001$), with conductivity values ranging from -98.87 to -65.87 ECs. Dissolved oxygen (ANOVA, $F(9, 37) = 30.05$, $P < 0.001$) and pH (ANOVA, $F(9, 37) = 3.32$, $P = 0.007$) were significantly different between the seasons and ranged from 5.39 to 8.27 mg L^{-1}, and 7.82–8.21, respectively (Table 7.1).

Significant differences were observed in all nutrient levels among seasons during the study period at Point Peron as determined by one-way ANOVA where NO_2^- (ANOVA, $F(3, 36) = 12.05$, $P < 0.05$), NO_3^- (ANOVA, $F(3, 36) = 13.38$, $P < 0.05$), NH_4^+ (ANOVA, $F(3, 32) = 5454$, $P < 0.05$), PO_4^{3-} (ANOVA, $F(3, 36) = 7.38$, $P = 0.001$). In particular, the concentration of nitrite was relatively low, ranging from 2.2–17.4 µg L^{-1} during the study period. The nitrate concentration reached its highest value in spring 2014 (0.48 ± 0.06 mg L^{-1}) and lowest value in summer 2013 (0.02 ± 0.001 mg L^{-1}). The concentration of ammonium during the study period ranged from 0.6–2.0 mg L^{-1} and that of phosphate ranged from 0.08–0.72 mg L^{-1} and reached the highest value in spring 2014 and lowest value in summer 2013. In general, the nutrient concentrations were lowest in autumn and highest in spring throughout the study period (Table 7.2 Seasonality of the mean nutrient concentrations in collected seawater during the study period at Point Peron, WA. All data represent the means from four replicates (± S.E.).

Table 7.2 Seasonality of the mean nutrient concentrations in collected seawater during the study period at Point Peron, WA. All data represent the means from four replicates (± S.E.).

Year	Month	Season	NO_2^- (µg L^{-1})	NO_3^-(mg L^{-1})	PO_4^{3-}(mg L^{-1})	NH_4^+(mg L^{-1})
2012	Sep	Spr.	6.33 ± 1.86^{b}	0.33 ± 0.08^{cd}	0.45 ± 0.08^{c}	1.97 ± 0.12^{de}
	Dec	Sum.	13.25 ± 2.07^{c}	0.05 ± 0.02^{a}	0.14 ± 0.02^{a}	1.70 ± 0.13^{cd}
2013	Apr	Aut.	2.00 ± 0.41^{a}	0.02 ± 0.00^{a}	0.24 ± 0.03^{b}	0.73 ± 0.09^{a}
	Jun	Win.	4.50 ± 0.65^{ab}	0.02 ± 0.00^{a}	0.14 ± 0.02^{a}	1.55 ± 0.06^{c}
	Sep	Spr.	3.50 ± 0.65^{ab}	0.17 ± 0.00^{bc}	0.20 ± 0.01^{ab}	2.00 ± 0.06^{e}
	Dec	Sum.	10.50 ± 1.56^{c}	0.09 ± 0.01^{ab}	0.26 ± 0.03^{b}	1.11 ± 0.04^{b}
2014	Mar	Aut.	2.75 ± 0.48^{a}	0.02 ± 0.01^{a}	0.19 ± 0.02^{ab}	0.55 ± 0.10^{a}
	Jul	Win.	3.00 ± 0.58^{ab}	0.02 ± 0.00^{a}	0.22 ± 0.06^{ab}	1.53 ± 0.09^{c}
	Sep	Spr.	3.33 ± 0.33^{ab}	0.42 ± 0.04^{d}	0.72 ± 0.05^{d}	2.03 ± 0.09^{e}
	Dec	Sum.	5.00 ± 0.67^{ab}	0.28 ± 0.09^{c}	0.17 ± 0.03^{ab}	
		F	12.05	13.38	7.38	54.54
		P	< 0.05	< 0.05	0.001	< 0.05

The data is presented as the mean ± S.E. of four replicates per sampling period. The different superscript letters are significantly different means of environment parameters in the same column. The mean difference is significant at the 0.05 level.

7.3.2. Seasonal pattern of *Sargassum* canopy cover

The mean values of *Sargassum* CC in the selected quadrats at the four sites were higher during the warmer months (spring and summer) than in the cooler months (autumn and winter). The mean value of *Sargassum* CC for the whole area was highest (91.7 ± 2.6%) in spring 2014 and was lowest (29.7 ± 10.1%) in autumn 2013 at all sites. Thus, a two-way ANOVA revealed that both seasons and reef sites did affect the *Sargassum* CC which differed significantly between sampling seasons (ANOVA, $F(9, 26) = 9.88$, $P < 0.001$) and reef sites (ANOVA, $F(3, 26) = 5.86$, $P = 0.03$) from spring 2012 to summer 2014 (Figure 7.4a).

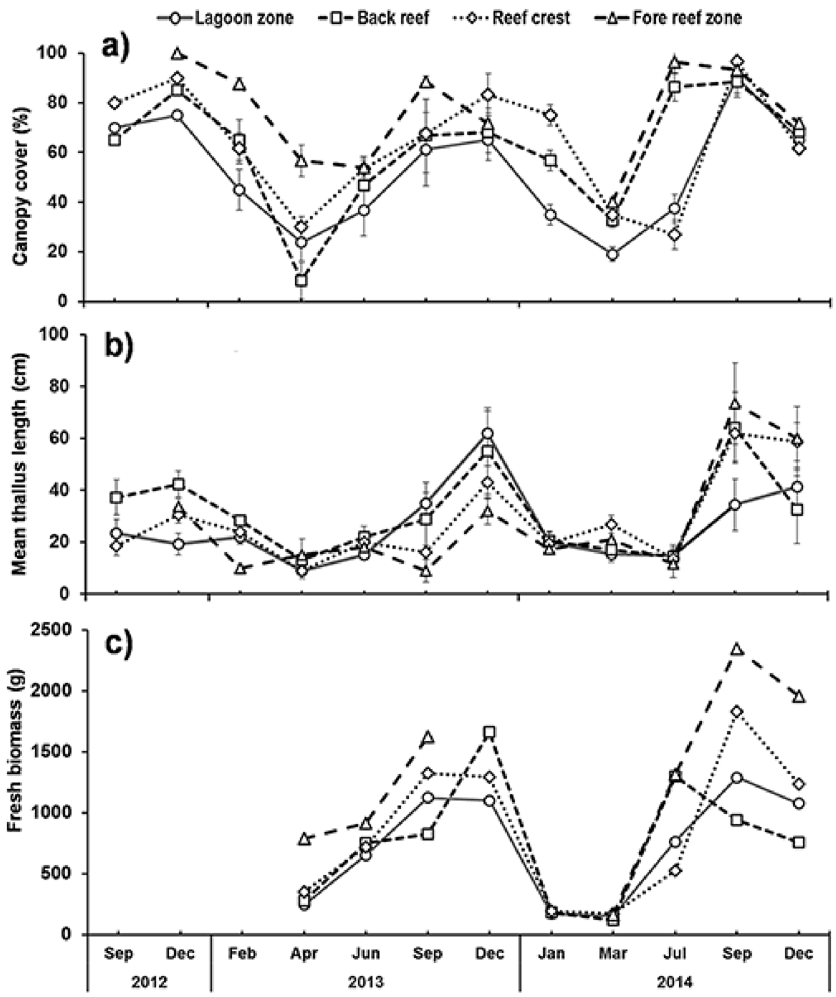

Figure 7.4 Seasonality of (a) percentage canopy cover, (b) mean thallus length, and (c) fresh biomass of *Sargassum* observed in four different areas during spring 2012 to 2014. Each column for (a) and (b) present the mean and standard error, and (c) the fresh *Sargassum* biomass samples. Three replicated quadrats (0.5 × 0.5 m) (n = 3) and four replicates (n = 4) were measured for CC and MTL, respectively.

7.3.3. The mean length of *Sargassum* thalli

The mean length of the seasonally harvested *Sargassum* thalli from randomized quadrats at each site is shown in Figure 5. The longest thalli were found in months with higher temperatures (summer 2013 and spring to summer 2014). The MTL for all sampling sites was highest in spring 2014 (53.5 ± 9.6 cm). In a similar pattern of coverage, the MTL was also lowest in the cold months, when the mean length ranged from 11.5 ± 1.5 cm and 13.6 ± 0.7 cm for autumn 2013 and 2014 winter, respectively (Figure 7.4b).

In terms of spatial distribution, the BR sites had the longest *Sargassum* thalli during all seasons (31.3 ± 4.7cm). The height of *Sargassum* thalli in the FR averaged 28.4 ± 6.9cm in all seasons. The shortest thalli were present in the LZ (25.9 ± 4.3cm). The two-way ANOVA revealed that reef sites did not affect the *Sargassum* MTL (ANOVA, $F(3, 26) = 0.59$, $P = 0.628$), but the seasonal changes did have an effect (ANOVA, $F(9, 26) = 10.868$, $P < 0.001$) from spring 2012 to summer 2014.

7.3.4. The distribution of *Sargassum* beds and associated marine habitats

The *Sargassum* CC was widely distributed around Point Peron. The highest coverage of *Sargassum* was recorded at the FR, followed by the RC, BR and LZ sites, with values of 75.9 ± 6.5%, 63 ± 6.7%, 61.4 ± 6.7%, and 51.9 ± 6.4%, respectively (Table 7.3). However, no differences ($P > 0.05$) were found between sampling sites. The surveyed data showed that three dominant *Sargassum* species were present in the study area; *S. spinuligerum, S. swartzii,* and *S. confusum.* In addition, *S. longifolium* was less abundant in the FR zone than the other species.

Table 7.3 Multiple comparisons of canopy coverage (%) and thallus length (cm) between the sites ($n = 46$)

Sites	**Canopy coverage (%)**		**Thalli length (cm)**	
	Mean	**± S.E**	**Mean**	**± S.E**
Lagoon zone	51.9	6.4	25.9	4.3
Back reef	61.4	6.7	31.3	4.7
Reef crest	63.5	6.7	28.5	4.9
Fore reef zone	75.9	6.5	28.4	6.9

The classification of the benthic habitat was confirmed using WV-2 satellite images. *Sargassum* was mainly distributed on the coral reefs and submerged limestone substrates from Gull Rock to Bird Island, White Rock and further west from Point Peron, extending to the area further south of the Shoalwater Islands Marine Park. Field studies showed that the bottom depth of the *Sargassum* distribution area was relatively shallow (between 1.5 and 10 m). A sandy bottom and hard coral substrates were frequently found around *Sargassum* beds, and the boundaries between

Sargassum and seagrass beds were detected with a high spatial resolution (2-m). Five bottom types were identified, including macroalgae (*Sargassum* sp. and *Ecklonia* sp.) canopy, seagrass, sand, muddy sand, and bare substrate, which were classified by the K-means unsupervised classification method (Figure 7.5).

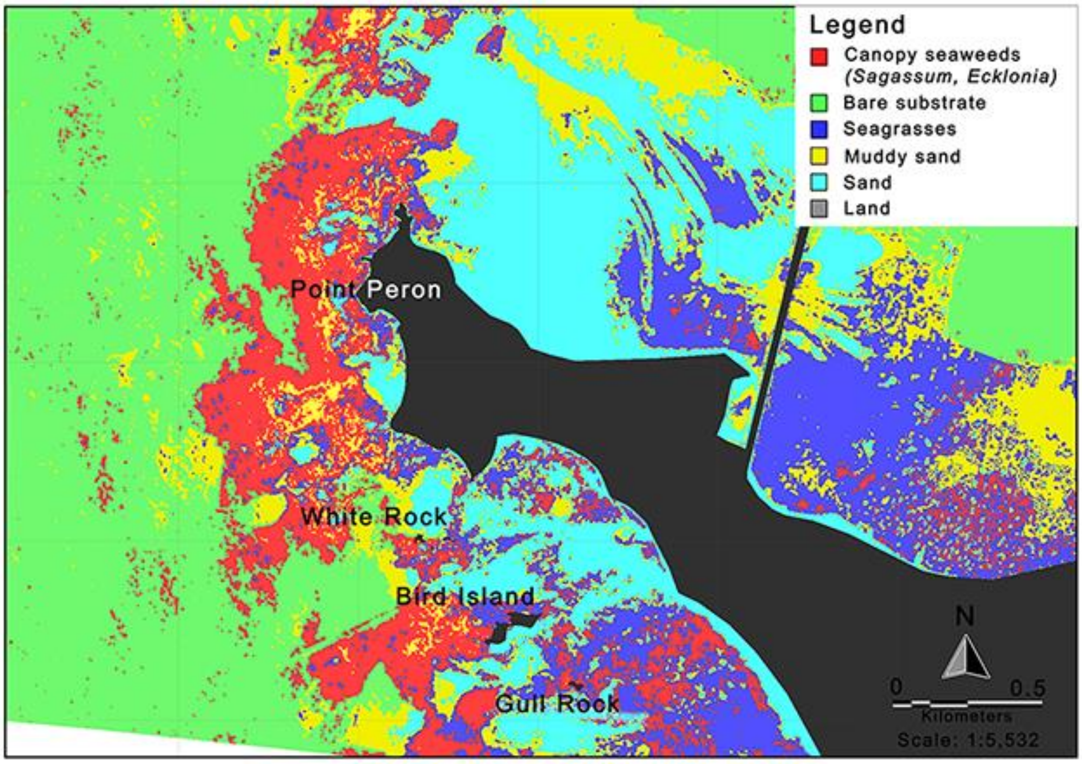

Figure 7.5 Map of the benthic habitat from satellite image classifications showing the canopy macroalgae beds (*Sargassum* species), their distribution and associated sublittoral habitats (seagrass, sand, and muddy sand) around Point Peron in summer (February 7, 2013).

7.3.5. Multivariate analysis

The principle component analysis (PCA) to establish multi-dimensional relationships among the studied parameters showed that there were four first principle components that accounted for 88.6% of the total variation. The first principle component accounted for over 43.3% of the total variation between sampling seasons and consisted of the physiochemical parameters PAR, SSTs, SE, ED, MinAT, MaxAT, CDOM, salinity, and NW. The second principle component accounted for 28.3% of the variation and included nutrient parameters such as MLT, CC, NO_3^-, PO_4^{3-}, FB, conductivity, NH_4^+, and Chl-a. The third principle component explained 9.7% of the total variation, and included DO, NH_4^+, rainfall, NO_2^-, PAR, and CC. The fourth principle component explained 7.4% of the total variation, and consisted of salinity, rainfall, and conductivity parameters; 6.6% of the total variation was explained by the fifth principle component and 4.8% of the variation of the sampling seasons by the sixth component.

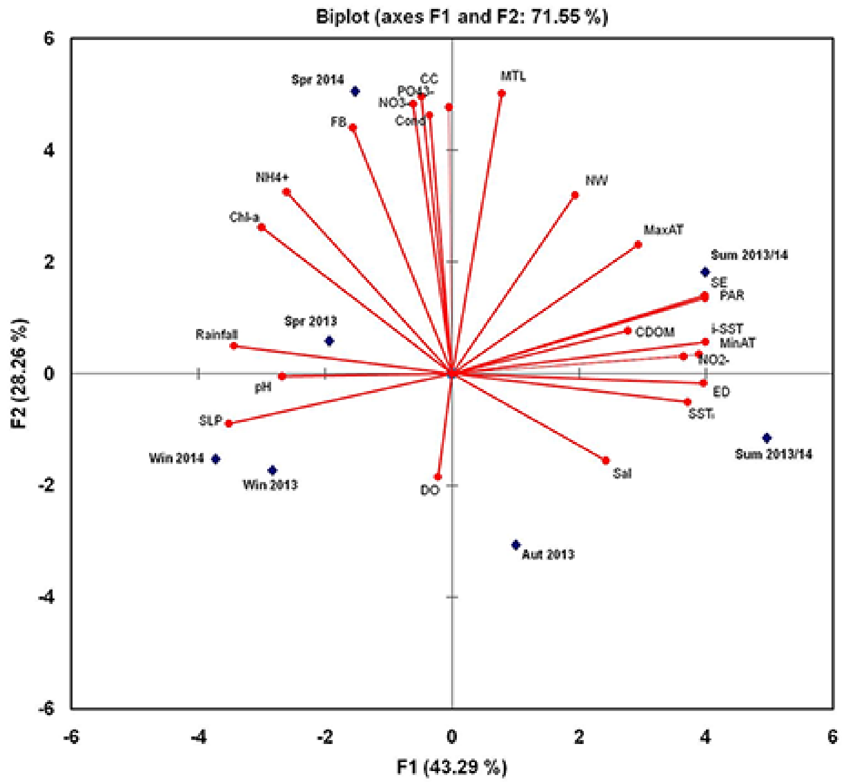

Figure 7.6. Principal Component Analysis biplot showing the relationship between *Sargassum* sampling time, CC, MTL, fresh biomass, and the physico-chemical parameters: FB represents fresh biomass (g 0.25 m^2); Cond. represents conductivity (mS m^{-1}); CC represents canopy coverage (%); MTL represents mean thallus length (cm); NW represents a northward wind (m s^{-1}); MaxAT represents maximum air temperature (^oC); SE represents solar exposure (MJ m^{-2}); CDOM represents colored dissolved organic matter; i-SST represents *in situ* sea surface temperatures; MinAT represents minimum air temperature (^oC); ED represents euphotic depth (m); SSTs represents satellite-derived sea surface temperatures (^oC); Sal represents salinity (psu); DO represents dissolved oxygen (mg L^{-1}); SLP represents sea level pressure (hPa).

The bi-plot chart of the first and second components explained 71.6% of the total variation in the environmental parameters during the sampling time. The results showed that nutrient composition (NO_3^-, PO_4^{3-}, NH_4^+) and *Sargassum* community structure (CC, FB, and MTL) were encountered at the spring sampling times. The PAR, salinity, and SSTs were key parameters during the summer. The *Sargassum* population structure was typically explained by rainfall, SLP, and pH parameters during the winter months (Figure 7.6).

7.4. DISCUSSION

7.4.1. Seasonal growth trends in *Sargassum* beds

This study investigated the ecology and seasonal growth trends in the brown algae, *Sargassum* spp. at Point Peron, WA for the first time. *Sargassum* biomass increased during the winter and early spring, and stabilized during late spring and early summer, before decreasing during the late summer and early autumn. This pattern of (*i*) increase; (*ii*) stabilization; and (*iii*) reduction in biomass is linked to the five main stages of the *Sargassum* life cycle, including: recruitment and growth (increase in biomass), senescence and reproduction (stabilization of the biomass), and regeneration (reduction in biomass) (Gillespie and Critchley, 1999). Here, we investigated which of the key environmental parameters, including SSTs, nutrients availability, and irradiance are responsible for regulating the timing of the *Sargassum* life cycle events (Figure 7.7a).

Table 7.4 Correlation matrix between different physiochemical parameters at the study sites. The level of significance is $P < 0.05$.

Variables	CC	MTL	FB	PAR	Rain	SST	Chl	pH	DO	NO_2^-	NO_3^-	PO_4^{3}	NH_4^+
CC	**1**												
MTL	**0.82**	**1**											
FB	**0.83**	0.76	**1**										
PAR	0.21	0.39	-0.10	**1**									
Rain	0.31	-0.18	0.42	-0.65	**1**								
SST	-0.23	0.08	-0.43	0.70	-0.72	**1**							
Chl-a	0.55	0.34	0.53	-0.57	0.53	-0.49	**1**						
Sal	-0.18	-0.16	-0.35	0.54	-0.38	0.65	-0.38						
pH	-0.11	-0.02	0.40	-0.72	0.41	-0.37	0.33	**1**					
DO	-0.33	-0.43	-0.55	-0.11	-0.04	-0.27	-0.16	-0.43	**1**				
NO_2^-	0.05	0.21	-0.31	0.90	-0.73	0.58	-0.53	-0.87	0.15	**1**			
NO_3^-	**0.80**	**0.82**	**0.70**	0.02	0.16	-0.10	0.66	0.13	-0.21	-0.17	**1**		
PO_4^{3-}	**0.73**	**0.90**	**0.69**	0.11	-0.07	0.05	0.57	0.18	-0.37	-0.07	0.95	**1**	
NH_4^+	**0.74**	0.35	**0.65**	-0.34	0.75	-0.69	0.70	0.10	0.12	-0.42	0.66	0.43	**1**

7.4.1.1 Increase in biomass

This study showed that *Sargassum* biomass began to increase in early winter from new recruits and remaining holdfasts, increased throughout winter and accelerates during spring. The highest nutrient concentrations, including NO_3^-, PO_4^{3-}, NH_4^+ were measured during winter and early spring, which coincided with the increase in biomass and the highest growth rates. Considering that these high nutrient values occurred in the winter and spring, which is a high rainfall season for southwest WA rain water run-off from the land probably played a vital role in the accelerated growth phase of *Sargassum* spp. Notably, this phase of high growth was negatively correlated with SSTs, i.e. the fastest growth rates occurred during the period with the lowest SSTs and irradiance (r = -0.43) (Table 7.4) and only a weak correlation was observed between PAR and *Sargassum* spp., and this trend was also observed in other studies (Fulton *et al.*, 2014; Sangil *et al.*, 2015).

7.4.1.2 Stabilization of biomass

Following the growth phase, *Sargassum* biomass stabilized, with little or no observed change in MTL or CC between early spring (September) to mid-summer (January). This period is characterized by higher SSTs, longer day lengths, and relatively high nutrient concentrations and primary productivity. Higher concentrations of ammonium were found at Point Peron during the late spring and were strongly correlated with the increase in CC and fresh biomass (r = 0.74 and 0.65, respectively).

7.4.1.3 Reduction in biomass

Following the reproductive stage, there was a reduction in *Sargassum* biomass beginning in late summer (February) through the end of autumn (April to May). Die-off occurred towards the end of summer, when some holdfasts remained and regenerated into new thalli in autumn and winter (Arenas *et al.*, 1995; Hoang *et al.*, 2015a). The decomposition of *Sargassum* thalli might lead to an increase in nitrite concentrations in the summer and autumn months.

In general, the timing of the *Sargassum* life cycle is geared so that full plant maturity is reached by late spring or early summer for the plant to take advantage of the highest levels of sunlight to redirect energy towards sexual reproduction. Towards the end of spring, the growth rates of *Sargassum* spp. begin to slow and cease as they enter the reproductive stage (Kendrick and Walker, 1994). The reproductive activity of *Sargassum* spp. occurs mainly in mid-summer via the release of ova and sperm into the water column (Gillespie and Critchley, 1999).

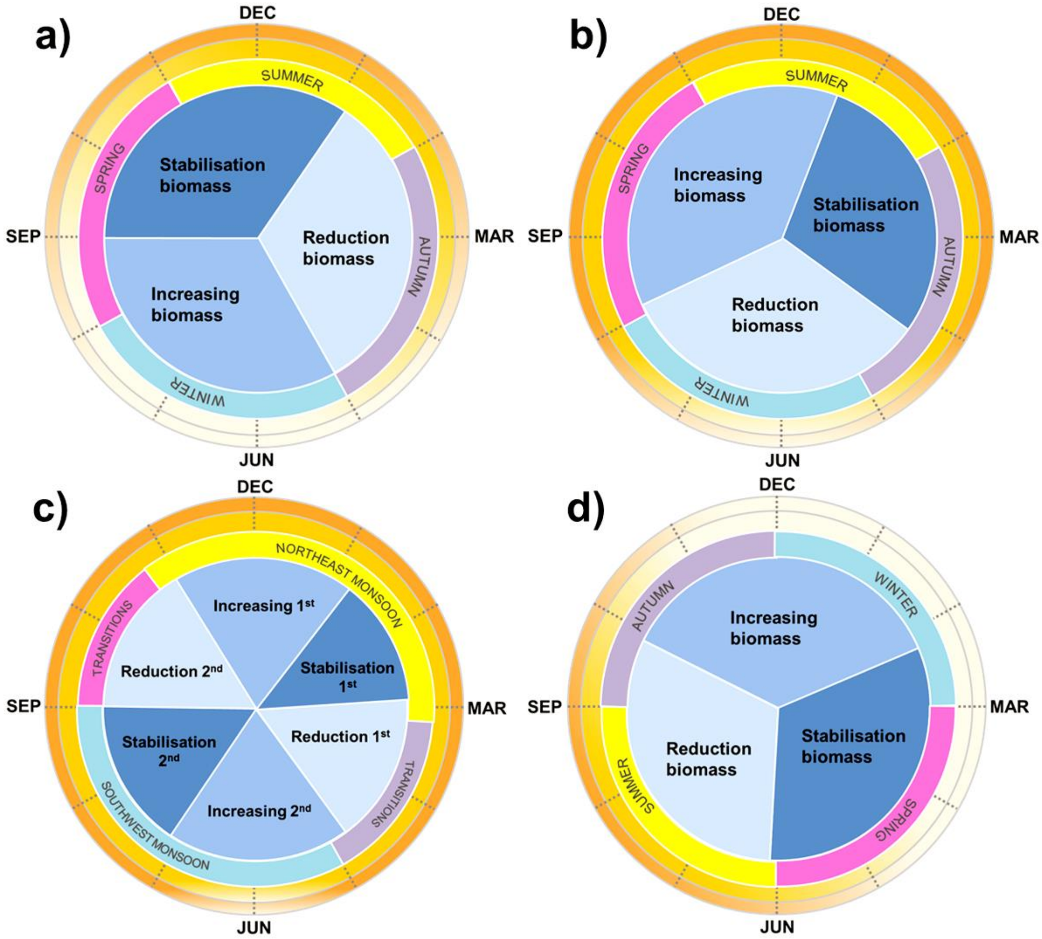

Figure 7.7 Diagram showing the seasonal variation in *Sargassum* biomass in different climate zones across Australia and other geographical areas. (a) Point Peron, WA with a Mediterranean climate; (b) Magnetic Island, Australia with a humid continental climate; (c) Pock Dickson, Malaysia with a tropical rainforest climate; and (d) Cape Peñas, Spain with and oceanic climate. The phase of increasing biomass includes recruitment and growth up stages. The stabilization biomass phase includes the late growth and reproduction stages. The reduction phase consists of senescence and regeneration periods. The outer ring and second ring represent SST and solar exposure, respectively. The light color represents months with a low temperature and the darker color represents those with a high temperature.

7.4.2. Comparison in the seasonality of *Sargassum* biomass between Point Peron and other localities

To further understand how environmental parameters such as nutrients, SSTs, and irradiance drive the *Sargassum* spp. growth cycle, we compared the seasonal results from Point Peron to other geographic regional studies in Australia and overseas.

7.4.2.1 Point Peron and Magnetic Island in Australia's Great Barrier Reef region (Fig. 7b)

Magnetic Island is located 8 km off the North Queensland coast at about 22˚S experiences a tropical savanna-type climate, with a distinct wet summer and dry winter (opposite to the WA). The increase in *Sargassum* biomass on Magnetic Island occurs at the beginning of spring to mid-summer, stabilization occurs between mid-summer and mid-autumn, and reduction occurs between mid-autumn and the start of spring (Vuki and Price, 1994). The increasing biomass on Magnetic Island occurs during cooler SSTs towards the end of the dry season, and increasing irradiance, stabilization and growth of reproductive organs occurs during the period of highest irradiance and SSTs. In contrast to Point Peron, reproduction on Magnetic Island occurs several months later, with the reduction in biomass occurring during the high SSTs, whereas on Point Peron it occurs during the lowest SSTs and irradiance levels. *Sargassum* beds in Magnetic Island, Australia do not reach their highest MTL until autumn (Vuki and Price, 1994). However, a similar relationship between CC and mean thallus length was observed at both study sites. A positive correlation was found between CC and MTL in Magnetic Island ($r = 0.73$), and a strong correlation was also present in the Point Peron study ($r = 0.82$). When the MTL was high, the *Sargassum* spp. in the selected quadrats also had a greater density, in turn resulting in a high biomass.

The difference in the *Sargassum* growth cycle can be explained by high rainfall during the summer (December to February, 624.9 ± 275.3 mm), which coincides with high nutrient concentrations from run-off, which provide optimum conditions for *Sargassum* growth (Vuki and Price, 1994). The later growing stage of *Sargassum* beds in Magnetic Island might be caused by the irregular, high rainfall and lower radiation in summer than in spring and winter, due to the higher cloud cover at this time, or a difference in *Sargassum* species composition.

7.4.2.2 Point Peron and Pock Dickson, Malaysia with a tropical forest climate (Fig. 7c)

Tropical regions near the equator experience high SSTs and high rainfall throughout the whole year, with little difference between the wet and dry season. Several seasonality studies have been performed on *Sargassum* in tropical regions, such as Pock Dickson in Malaysia, the northern part of the Philippines, and New Caledonia. Due to the effect of two strong monsoons, the *Sargassum* beds reveal two periods of increasing biomass rates (January to February and June to July) and decreasing biomass rates (April and September) (May-Lin and Ching-Lee, 2013). Thus, the growth cycle depends on seasonal changes in the monsoon, the species of *Sargassum* and the existing nutrient availability (Schaffelke and Klumpp, 1998). The highest biomass can occur in the wet season for some species (e.g. *S. binderior*) or the dry season for others (e.g. *S. siliquosum*). In these tropical areas, the seasonality of *Sargassum* beds can be more dependent on changes in SSTs and rainfall (i.e. tropical monsoons).

A study in New Caledonia in the Indo-Pacific region showed that *Sargassum* spp. have a high MTL in the summer months due to higher rainfall at this time, which causes an increased nutrient concentration and growth (Mattio *et al.*, 2008). However, in the Philippines, *Sargassum* biomass is highest in the dry season, which possibly coincides with high SSTs (Ang, 1986). Thus, equatorial climates can also experience a range of seasonal effects on *Sargassum* spp., although this might be less pronounced than in more temperate climates such as that at Point Peron.

7.4.2.3 Point Peron and studies in the Northern Hemisphere (Fig. 7d)

Cape Peñas (Asturias, Spain) is located at latitude 43.4^{o}N and has a similar Mediterranean climate to Point Peron, and experiences warm dry summers and cool wet winters. The summer season occurs from June to September, with a mean daily high air temperature above 20°C. The increase in *Sargassum* biomass on Cape Peñas occurs from mid-autumn to late-winter, stabilization with peak biomass occurs between the end of winter and the end of spring, and reduction occurs between early summer and mid-autumn. Growth increases during the winter until spring, when higher SSTs increase photosynthesis and productivity and provide optimum growth conditions, followed by senescence from early summer to mid-autumn (Arenas and Fernández, 2000). Seasonal changes in temperature are also thought to drive the growth of *Sargassum* spp. at La Palma, and in the Canary Islands, Spain. The biomass of *S. flavifolium* reaches its maximum in spring to summer and is similar to that of *Sargassum* spp. in this study, coinciding with an increase in the SST and day length (irradiance) (Sangil *et al.*, 2015). The growth and development of *Sargassum* in the study sites in Spain and WA share a similar seasonal pattern, which can be explained by similar climate zones. However, they occur at different times of the year due to the reverse timing of seasons in the Northern and Southern hemispheres.

7.4.3. Distribution of *Sargassum* spp. from both *in situ* and space observations

The distribution of *Sargassum* beds was restricted mainly to shallow water habitats, similar to the results of others (Hanisak and Samuel, 1987a; Mattio *et al.*, 2008; Mattio and Payri, 2011). Because the holdfasts grow on limestone rock substrates, the beds were widely distributed throughout these habitats, but not on sandy substrates, where seagrass was dominant. A similar study in New Caledonia found that *Sargassum* was dominant on rubble substrate and rocky bottoms, ranging from to 12 m deep (Mattio *et al.*, 2008). In this study, biomass increased as depth increased along the transects, and showed some variation in reef zones from the LZ to the FR. This represents a trend, suggesting that the biomass of *Sargassum* beds increases at greater depths, until light becomes a limiting factor (Ang, 1986; Rützler and Macintyre, 1982; Vuki and Price, 1994).

The highest MTL of *Sargassum* in all seasons is related to its distribution area and was found in the BR zone, which is protected by the RC zone further offshore, where the waves and currents are broken down and their kinetic energy reduces before they approach the shoreline. The lowest MTL value was found in the LZ. The length of thalli in the LZ reflects the shallow depth here, as well as the high heat absorption from the sun, which causes higher SSTs than at other study sites. At Point Peron, the mean MTL of *Sargassum* species is similar to that found for *S. ilicifolium* and *S. subrepandum* in the southern Red Sea, which was 38.71 cm and 32.65 cm, respectively (Ateweberhan *et al.*, 2009). The MTL here is also similar to that from a phenology study of *Sargassum* species in Tung Ping Chau Marine Park, Hong Kong (48.2 ± 29.9 cm) (Ang, 2007). However, the MTL of *Sargassum* in Point Peron is shorter than that found in previous studies in Rottnest Island, WA (10–95 cm) (Kendrick, 1993), in the middle reef flat of Magnetic Island, North-Eastern Australia (Vuki and Price, 1994) and in other studies in Malaysia (May-Lin and Ching-Lee, 2013; Wong and Phang, 2004).

The present study was initially applied using WV-2 satellite remote-sensing data to determine the spatial distribution of *Sargassum* and associated marine benthic habitats in the study area. This study can be considered as an original approach for the region when using more advantageous satellite remote-sensing data, with higher spatial and spectral resolution, than the previous studies in Thailand with ALOS AVNIR-2 images (10 m spatial resolution) (Noiraksar *et al.*, 2014), New Caledonia with Landsat images (30 m) and QuickBird (2.4 m) (Mattio *et al.*, 2008).

Thus, the further studies could apply the recent archived results for identifying and mapping *Sargassum* beds for the WA region (Garcia *et al.*, 2015; Hoang *et al.*, 2015b, c). The results of spatial distribution characteristics of *Sargassum* beds play an important role in providing information on regional natural resource management and a better understanding of the distribution characteristics, areas, and seasonality of *Sargassum*, in terms of the highest biomass.

However, a limitation does exist in this study due to the inadequate WV-2 satellite remote-sensing data sources in evaluating the brown canopy macroalgal distribution. The current satellite remote-sensing image only reflects the distribution of brown canopy macroalgal (*Sargassum* sp. and *Ecklonia* sp.) in the peak biomass season, spring. However, if there were more than one satellite remote-sensing images during another season available at the study region that would markedly illustrate the seasonal variation in the distribution area.

In summary, this study provides primary and novel information on *Sargassum* spp. at Point Peron using a combination of *in situ* and satellite remote-sensing observations. The results show that the *Sargassum* beds demonstrated a seasonal variation pattern in CC and MTL, which was significantly influenced by the nutrient concentration (PO_4^{3-}, NO_3^-, NH_4^+), Chl-a, and rainfall ($P < 0.05$). This seasonal variation pattern is similar to that found in areas with a temperate or Mediterranean climate, such as Rottnest Island, Australia and Cape Peñas, Spain (Arenas and Fernández, 2000; Kendrick and Walker, 1994). The highest peaks in *Sargassum* biomass generally occurred between late spring and early summer. This seasonal pattern was also found in *Sargassum* CC and MTL. The seasonal variation in *Sargassum* biomass, CC and MTL at Point Peron was closely associated with seasonal changes in the PAR and nutrient concentration. These results provide essential information for coastal marine management and conservation, as well as for the sustainable utilisation of this renewable marine resource.

Chapter 8. EFFECT OF NUTRIENT MEDIA AND INITIAL BIOMASS ON GROWTH RATE AND NUTRIENT-UPTAKE OF *SARGASSUM SPINULIGERUM* (SARGASSACEAE, PHAEOPHYTA)

Paper has been submitted to the *Springer Plus* – An Open Access journal (Under revision)

8.1. INTRODUCTION

The genera *Sargassum* belongs to the family Sargassaceae (Phaeophyta), and is one of the most diverse genera in the family, with over 336 species (Guiry and Guiry, 2014). *Sargassum* is distributed globally in both temperate and warm waters, and is most abundant in subtidal areas of the Indo-west Pacific and Australia (Tseng *et al.*, 1985). In WA, there are 46 *Sargassum* species (Huisman and Walker, 1990). The *Sargassum* species are a great source of polysaccharides and phenolic compounds (Keusgen and Glombitza, 1995, 1997) for cosmetics, pharmaceutical, and biofuel extraction industries (Gao and Hua, 1997; Gellenbeck and Chapman, 1983; Hanisak and Samuel, 1987; Murase *et al.*, 2000; Pang *et al.*, 2009).

Temperature, light intensity, nutrient availability, salinity, and initial stocking biomass (ISB) are the most important environmental factors to ensure a high growth rate of culturing macroalgal species (Mendes *et al.*, 2012; Michael and Ami, 1991). The nutrient-uptake capacity is one of the vital parameters to accelerate macroalgae growth and increase the productivity of *Sargassum* spp. Several studies have shown that the nutrients, particularly nitrogenized forms, are essential for algae growth (Nelson *et al.*, 2001; Yang *et al.*, 2005; 2006). The macroalgae can use the nutrients in the water to increase its photosynthetic ability and then convert them into productivity (Bezerra and Marinho-Soriano, 2010; Marinho-Soriano *et al.*, 2009). For instance, the red algae *Gracilaria tikvahiae,* can uptake ammonium-nitrogen relatively fast, and can increase the nitrogen content in the tissue within eight hours or less (Ryther *et al.*, 1981). After a four-week culture, the nutrient uptake capacity of *G. birdiae* for PO_4^{3-}, NH_4^+, and NO_3^- decreases by 93.5%, 34%, and 100%, respectively (Marinho-Soriano *et al.*, 2009). Determining the nutrient-uptake and photosynthesis of green algae, *Ulva prolifera*, under different conditions revealed that there was a limit to nitrate-uptake, but an increase in phosphorus. The N/P or NO_3^-/NH_4^+ ratio affects the uptake rate, and at the N/P of and 7.5, *U. prolifera* achieves the highest N P uptake rates (Guimaraens, 1999). However, there are a limited number of studies on the optimum ISB required, nutrient-uptake, and optimum cultivation environment of *S. spinuligerum* under laboratory conditions (Hanisak and Samuel, 1987).

Global production of macroalgae occurs mainly in marine and brackish waters (FAO, 2012; Muñoz *et al.*, 2011). The volume of farmed macroalgae production has increased from 3.8 million tons in 1990 to 19 million tons in 2010 and 26.1 million tons in 2013. However, only 4.5% of the total macroalgae production in 2010 came from cultivation; the remaining production was harvested from the wild (FAO, 2012). According to the Food and Agriculture Organization (FAO) of the United Nations' (2014), there are a few dominant macroalgae species that constitute 98.9% of the world production such as *Laminaria japonica*, *Eucheuma* sp., *Gracilaria* sp., *Porphyra* sp., and *Undaria pinnatifida.* China, which produces the largest amount of cultivated macroalgae, manufactured 13.5 million tons in 2013 (FAO, 2014). *Sargassum* still remains a small percentage of total macroalgae production and is considerably lower than its potential production and market demand.

To date, the *Sargassum* species has been proposed as a potential candidate for marine culture in large scale macroalgae farms (Gao and Hua, 1997; Pang *et al.*, 2009). However, there are a limited number of studies on the cultivation conditions and ecology of *S. spinuligerum* (Hanisak and Samuel, 1987). The objectives of this study were to investigate the effect of different commercially available fertilizers and different quantities of ISB on the growth rate and nutrient-uptake of *S. spinuligerum* in outdoor cultivation conditions.

8.2. MATERIALS AND METHODS

8.2.1. Plant collection and preparation

S. spinuligerum was collected from Point Peron, Shoalwater Islands Marine Park, WA (32°16.32'S−115°41.25'E) on September 2013. Specimens were collected using free-diving techniques. All holdfasts of macroalgae were collected, immediately transferred into sampling buckets with fresh seawater and then relocated to the CARL, Curtin University within three hours of collection.

In the CARL, the samples were well rinsed with filtered seawater to remove all epiphytes such as diatoms, red macroalgae (*Gracilarria* sp.), detritus and any decapods or snails attached to the receptacles (Hanson, 1977; Muñoz and Fotedar, 2010). The samples were then stored in the stocking tanks for acclimation with similar salinity to that of seawater (~36 psu) for two days. Four ISBs were used for culture as four different treatments from ISB_1 to ISB_4 with mean values of 15.35 ± 1.05 g, 18.77 ± 1.04 g, 28.07 ± 1.37 g, and 40.91 ± 2.25 g (n = 48, F = 51.56, P < 0.05), respectively. The selected ISBs were based on the previous trials.

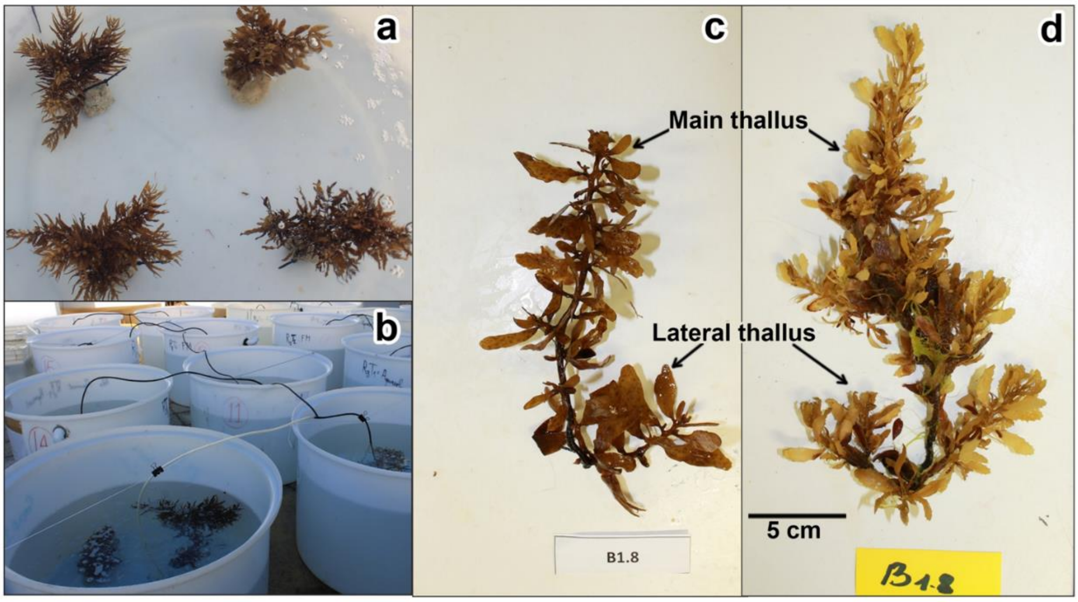

Figure 8.1 The diagram shows the four replicated *Sargassum* biomass in cultivation media (a) in the designed 113-L experiment tanks at outdoor conditions at the field trial area of CARL (b). The total of 16 tanks were randomly employed for three difference nutrient concentration treatments and control condition (seawater). Every nutrient and control treatment had four replicates. The initial biomass is shown at (c) the beginning of the experiment and (d) after seven weeks.

8.2.2. Experiment protocol and enriched cultivation media

Cultivation nutrient media: The seawater was collected from Hillary Harbor, WA (31°49'35S−115°44.16'E) and then transferred to the CARL by truck. Seawater was filtered through a 5 µm filter to eliminate phytoplankton and organic suspended materials. Sixteen 113-L round plastic tanks (40 × 60Ø cm) were used as containers with aeration. The cultured nutrient media was randomly enriched at the experiment tanks with an amount of 1325 mL of three dissolved commercial available fertilizers Hortico® (Yates, Australia), Seasol® (Miracle-Gro, USA), and Aquasol® (Yates, Australia). The initial nutrient concentration of cultured media was based on established requirements for other *Gracilaria cliftonii* species (Kumar *et al.*, 2011) (Figure 8.1).

Experimental procedure: The experiment was conducted in the CARL in Perth, Australia, from September 21 to November 6, 2013. These cultured tanks were maintained at outdoor field trial area conditions with an average temperature of 22^{o}C, salinity at 36 psu, and natural sunlight with a 12 h light and dark cycle (sunrise/sunset−6:20 am/6:05 pm) (Hanisak and Samuel, 1987).

8.2.3. Data collection and analysis

Environmental parameters: Water temperature (WT), salinity, dissolved oxygen (DO), conductivity, and pH were measured weekly. WT was automatically monitored and recorded every hour using a submerged HOBO Pendant® Temperature Data Logger 64K (OneTemp, Australia) for seven weeks. Salinity was maintained between 35 and 36psu, and regularly checked by a hand held refractometer (Atago® RHS-10ATC, Japan). DO was measured with a DO meter (YSI®55, Perth Scientific, Australia). Conductivity and pH were determined with a digital pH meter (Cyber scan, pH 300, Eutech Instrument, Singapore).

Nutrient concentration: Nutrients in the cultivation media were analyzed fortnightly. Ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), and phosphate (PO_4^{3-}) were determined using AQUANAL™ test kits (Sigma-Aldrich®, Germany) - plus ammonium (NH_4^+) 0.2–8.0 mg L^{-1}, nitrate (NO_3^-) 1–50 mg L^{-1} and phosphate (PO_4^{3-}) 0.02–0.4 mg L^{-1}, respectively.

Specific growth-rate (SGR): The fresh weight, length, and number of lateral branches were measured at the commencement, the middle (three weeks) and the end of the experiment (after seven weeks). Then, samples were dried at 95^{o}C for 48 hours in an oven to obtain dry weights. From the initial time, the SGR (%) was calculated using the following equation (1) (Hanisak and Samuel, 1987; Mai *et al.*, 2010):

$SGR = (100 \ln(W_t/W_o))t-1)$ (1)

where SGR is specific growth rate (% g d^{-1}), W_o is ISB fresh weight, and W_t is the final weight of macroalgae after the experiment (day).

Main and lateral thalli growth rates (% d^{-1}) were measured to determine apical growth rate using the following equation (2):

$AGR = ((Ln(L_f) – Ln(L_i))/t \times 100$ (2)

where AGR is apical growth rate of main and lateral thalli (% d^{-1}), L_f is final length of the thalli, L_i is initial length of the thalli, and t is cultivation time in days.

Nutrient-uptake rate: Nutrient-uptake rate (NUR) was calculated using the following equation 3 (Fan *et al.*, 2014; Ryther *et al.*, 1981):

$NUR = (C_0 – C_t)V/DW/t$ (3)

where NUR is the nutrient-uptake rate of *Sargassum* (mg nutrient g^{-1} DW h^{-1}); C_0 and C_t are the nutrient concentration at the beginning and at time (*t*) of the experiment (mg L^{-1}); respectively; *t* is time between two measures (days); V is the water volume (L); and DW is the *Sargassum* dry weight (gram).

8.2.4. Statistical analysis

The data were statistically analyzed using IBM® SPSS® Statistics 22 for Windows (IBM Corporation, Chicago, USA) and Microsoft Excel 2013. A one-way ANOVA was employed to test the significance of the variance between treatments. The multiple comparisons, least significant difference (LSD) post hoc test, was also implemented to test for statistical significance among treatments. The statistical significance level was set at 0.05, and the results were presented as means ± standard error (S.E.) unless otherwise stated.

8.3. RESULTS

8.3.1. Water quality parameters

The water quality was fairly constant during the experimental period. The averaged DO, WT, and pH were 6.13 ± 0.12, 21.5 ± 0.3 ^{o}C, and 8.28 ± 0.04, respectively.

Conductivity and salinity were -93.5 ± 1.87 ECs and 36.2 ± 0.8 psu, respectively. There were significant differences during the experimental period in terms of DO (df = 107, F = 5.08, P = 0.002), pH (df = 108, F = 22.67, P < 0.05) and conductivity (df = 107, F = 16.15, P < 0.05). However, there were no significant differences in temperature and salinity during the experiment period (Table 8.1).

Table 8.1 Overall means of water parameters of different cultivated media during seven cultivation weeks.

Parameters	**Enriched nutrient treatments/ Cultured media**				***P-value***
	SW	**N1**	**N2**	**N3**	
DO (mg L^{-1})	6.13 ± 0.12[a]	6.34 ± 0.11[a]	6.83 ± 0.13[b]	6.46 ± 0.14[a]	0.002
WT (^{o}C)	21.5 ± 0.3	21.6 ± 0.3	22.1 ± 0.3	21.2 ± 0.3	0.194
pH	8.28 ± 0.035	8.25 ± 0.03	8.69 ± 0.04[a]	8.41 ± 0.05[b]	< 0.005
Cond. (ECs)	-93.54 ± 1.87[cb]	-89.23 ± 3.38[c]	-114.10 ± 2.38[a]	-100.11 ± 2.85[bc]	< 0.005
Salinity (psu)	36.2 ± 0.8	36.8 ± 0.9	35.5 ± 0.9	36.6 ± 0.9	0.697

WT represents water temperature (^{o}C); Cond. represents conductivity (ECs); values represent mean ± S.E. of three replicates per treatment. Alphabetical superscript letters in the same row show significant differences at α = 0.05 using the LSD test.

8.3.2. The effect of cultured nutrient media on the SGRs

The SGR of *S. spinuligerum* was influenced by cultured nutrient media. *S. spinuligerum* had the highest growth rate in all cultured nutrient media during the first three weeks of the experiment. Of those, the SGR of Hortico® treatment reached the highest percentage value 1.61 ± 0.38% g per day. Meanwhile, the main thallus reached the highest value in the Aquasol® treatment with the value of 0.82 ± 0.16% per day. There were significant (P < 0.05) differences between the cultured nutrient media treatments regarding main thallus growth. However, there was no statistical difference in the SGR and lateral thallus of *S. spinuligerum* during the cultivated period.

At the end of the experimental period (after seven weeks), there were significant differences in the growth rate of *S. spinuligerum* main thallus (df = 58, F = 5.41, P = 0.002). However, there was no difference between the cultured nutrient media treatments in the SGR and lateral thallus. The average growth rate of their highest SGR in the Aquasol® reached 1.31 ± 0.31% g per day and the lowest value in Hortico® treatment reached 0.54 ± 0.09% g per day (Figure 8.2a). The main and lateral thalli reached the highest value in the Aquasol® treatment with a value of 0.59 ± 0.11 and 1.46 ± 0.39% g per day, respectively (Figure 8.2b, c).

8.3.3. The effect of ISB on the SGR

There was a significant difference ($P < 0.05$) between ISB treatments on the SGR of *S. spinuligerum*, and the SGR reached the highest value in the ISB_4 treatment: 1.70 ± 0.26 (% g per day) after three weeks of cultivation (Figure 8.3a). Meanwhile, the main and lateral thalli reached the highest values in the IBS_1 treatment: 0.75 ± 0.16 and 2.37 ± 0.84 (% per day), respectively (Figure 8.3b, c). However, there was no significant difference ($P > 0.05$) between ISB treatments on main and lateral thalli growth rates.

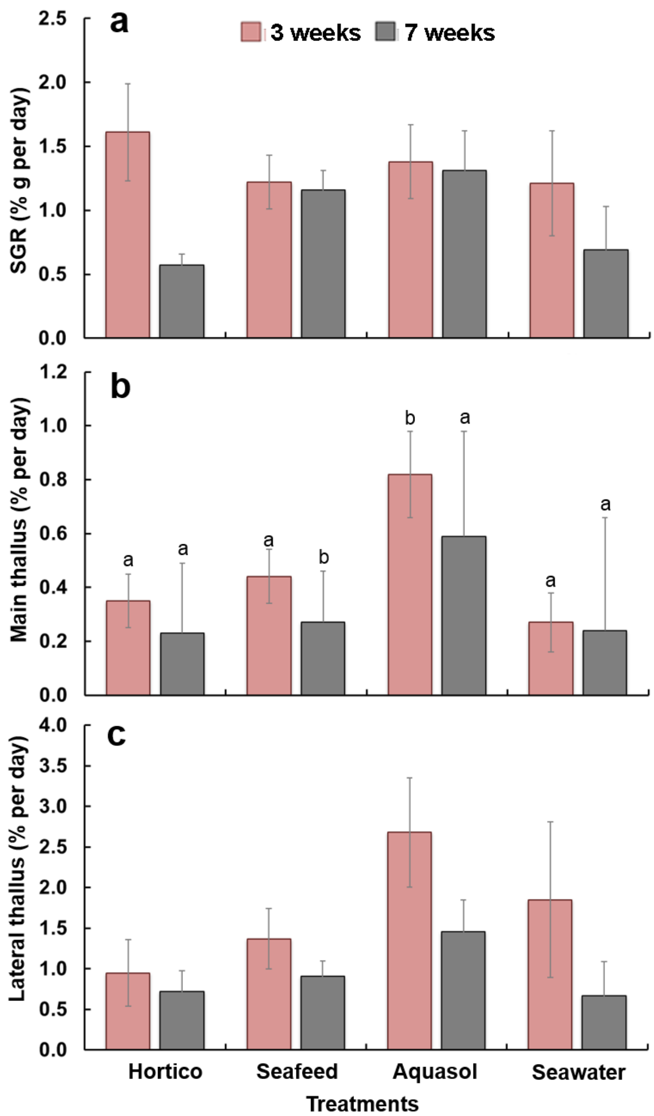

Figure 8.2 Specific growth rate (SGR), apical growth rate from main (Main) and lateral branches (Lateral) between treatments (mean ± S.E.) in outdoor cultivation conditions. (a) SGR after three and seven weeks of cultivation (% g per day); (b) main thallus growth rate (% per day); and (c) lateral thallus growth rate (% per day).

After seven weeks of culture, the SGR and main thallus were significantly different ($P < 0.05$) among the ISB treatments. The entire SGR, main, and lateral thallus reached the highest value in the ISB_1 treatment with 1.54 ± 0.19, 0.49 ± 0.11, and 1.19 ± 0.30% g per day (Figure 8.3).

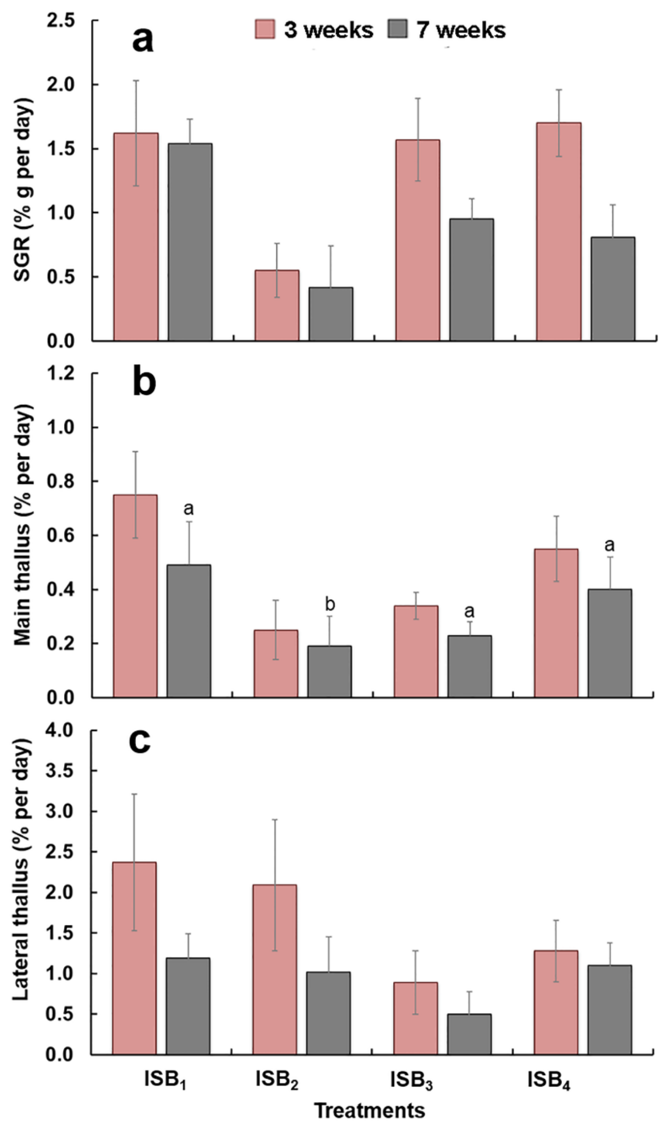

Figure 8.3 Specific growth rate (SGR), apical growth rate from main and lateral thallus between ISB treatments (mean ± S.E.) in outdoor cultivation conditions. ISB_1 equivalent with an averaged value of 15.35 g; ISB_2 equivalent with an averaged value of 18.77 g; ISB_3 equivalent with an averaged value of 28.07 g; and ISB_4 equivalent with an averaged value of 40.91 g. (a) SGR after three and seven weeks of cultivation (% g per day); (b) main thallus growth rate (% per day); and (c) lateral thallus growth rate (% per day).

8.3.4. The effect of different nutrient supplies on NUR

During the first two weeks of the experiment, there was a significant difference ($P < 0.05$) in nitrate concentration between the cultured nutrient media treatments. Meanwhile, there were no statistical differences in phosphate, nitrite and ammonia between treatments ($P > 0.05$).

There were significant differences ($P < 0.05$) in NO_3^- uptake-rates between the cultured nutrient media treatments over the cultivation period. For PO_4^{3-}, there were significant differences between the cultured nutrient media treatments after the first, second and fourth weeks ($P < 0.05$), and there was no significant difference in PO_4^{3-} uptake-rate after seven weeks of cultivation (Figure 8.4, Table 8.2).

Table 8.2 Mean (± S.E.) uptake rate of NO_3^- and PO_4^{3-} of *S. spinuligerum* in different nutrient media for seven cultivation weeks ($n = 4$).

Media	NO_3^-				PO_4^{3-}			
	2 weeks	4 weeks	6 weeks	7 weeks	2 weeks	4 weeks	6 weeks	7 weeks
Hortico	4.6 ± 0.89	4.1 ± 0.92	3.1 ± 0.52^{a}	2.8 ± 0.42^{a}	3.9 ± 1.04	2.3 ± 0.43^{a}	1.3 ± 0.28	0.4 ± 0.26
Seasol	4.1 ± 0.61	2.6 ± 0.19	2.0 ± 0.14^{b}	1.8 ± 0.10^{b}	3.7 ± 1.06	2.0 ± 0.51^{a}	1.3 ± 0.32	0.5 ± 0.32
Aquasol	3.4 ± 1.33	2.5 ± 0.46	2.1 ± 0.38^{ab}	1.8 ± 0.29^{b}	2.1 ± 0.25	1.3 ± 0.10^{b}	0.8 ± 0.06	0.1 ± 0.09
Seawater	-1.6 ± 0.88^{a}	-0.9 ± 0.33^{a}	0.3 ± 0.05	-0.1 ± 0.08	1.2 ± 0.44^{a}	0.9 ± 0.20^{ab}	0.7 ± 0.15	0.1 ± 0.09

Means in the same column with different superscript letters signify significant differences at P < 0.05.

8.4. DISCUSSION

The results of the present study indicate that *S. spinuligerum* could be grown in outdoor conditions with WT ranging from 17.3 to 25.3°C (averaged 21.5 ± 0.3°C), salinity at 36.2 ± 0.8 psu, pH ranging from 8.25 to 8.69, with the optimum ISB at 15.35 ± 1.05 g per 113-L. Regardless of the cultured nutrient media treatments, the ISB_1 (~ 15 g) had the highest value for *S. spinuligerum* growth. The Aquasol® resulted in the highest values of SGR, main, and lateral thalli after seven weeks of cultivation. This combination had the same pattern/results at combination number nine when we combined ISB_1 and Aquasol® (Table 8.3). There was a significant difference ($P < 0.05$) between ISB treatments on the SGR of *S. spinuligerum,* and SGR was highest in the ISB_4 treatment with 1.70 ± 0.26 (% g per day) after three weeks of cultivation. The highest SGR in all of the study experiments was 2.54 ± 0.28 (% g per day), which is similar to results found in a study on *S. baccularia* in the central Great Barrier Reef, Australia, where the maximum value reached 2.71 ± 20.76 (% per day) under a continuous nutrient supply (Schaffelke and Klumpp, 1998). Our results were also similar to the SGR of *S. horneri,* with values of 2.7 ± 0.75 (% per day, in length) and 3.28 ± 1.03 (% per day, in fresh weight) after being cultivated for 25 days in an enriched nutrient media of 10 mg KNO_3 + 1mg KH_2PO_4 (Pang *et al.*, 2009).

Table 8.3 Specific growth rate of *S. spinuligerum* under different combinations of initial stocking biomasses and nutrient supplies (mean ± S.E.). Negative growth rate presents mortality.

Combination number	Nutrients	Biomass (g)	SGR	Main	Lateral
1	Hortico	15	0.88 ± 0.09	0.35 ± 0.15	1.20 ± 0.77
2	Hortico	20	0.42 ± 0.08	0.26 ± 0.09	0.45 ± 0.32
3	Hortico	30	0.53 ± 0.24	0.17 ± 0.04	0.28 ± 0.11
4	Hortico	40	0.46 ± 0.18	0.25 ± 0.09	1.00 ± 0.74
5	Seasol	15	1.63 ± 0.27	0.29 ± 0.08	0.70 ± 0.07
6	Seasol	20	0.87 ± 0.09	0.26 ± 0.13	0.87 ± 0.13
7	Seasol	30	0.68 ± 0.25	0.22 ± 0.09	0.82 ± 0.09
8	Seasol	40	1.46 ± 0.31	0.30 ± 0.16	1.26 ± 0.16
9	Aquasol	15	**2.54 ± 0.28**	**1.08 ± 0.22**	**1.25 ± 0.42**
10	Aquasol	20	1.76 ± 0.27	0.29 ± 0.09	**2.51 ± 0.80**
11	Aquasol	30	1.28 ± 0.35	0.33 ± 0.06	0.71 ± 0.96
12	Aquasol	40	-0.35 ± 0.43	0.68 ± 0.14	1.35 ± 0.94
13	Seawater	15	1.12 ± 0.25	0.26 ± 0.10	1.62 ± 0.92
14	Seawater	20	-1.34 ± 0.48	-0.28 ± 0.00	-1.97 ± 0.00
15	Seawater	30	1.31 ± 0.32	0.20 ± 0.13	0.20 ± 0.38
16	Seawater	40	1.65 ± 0.19	0.41 ± 0.06	0.87 ± 0.53

Conversely, our SGR results were lower than another previous study on integrated macroalgae (*Sargassum*) - prawn culture system (ISP) (Mai *et al.*, 2008). The ISP results showed that after 30 days of cultivation, *Sargassum* sp. grew rapidly in a monoculture media and integrated with prawn as 5.70 ± 0.82 and 0.74 ± 3.16 (% g per day), respectively (Mai *et al.*, 2010). Meanwhile, our SGR results on *S. spinuligerum* were much higher than other published studies on various *Sargassum* species in Florida, US: *S. cymosum* (0.094 ± 0.011% per day), *S. filipendula* (0.107 ± 0.003% per day), *S. fluitans* (0.109 ± 0.003% per day), *S. natans* (0.073 ± 0.005% per day), *S. polyceratium* (0.078 ± 0.015% per day), and *S. pteropleuron* (0.112 ±

0.008% per day) (Hanisak and Samuel, 1987). One possible explanation for the difference in SGR from the previous studies might relate to different cultural conditions and nutrient media (Guimaraens, 1999; Pickering *et al.*, 1993).

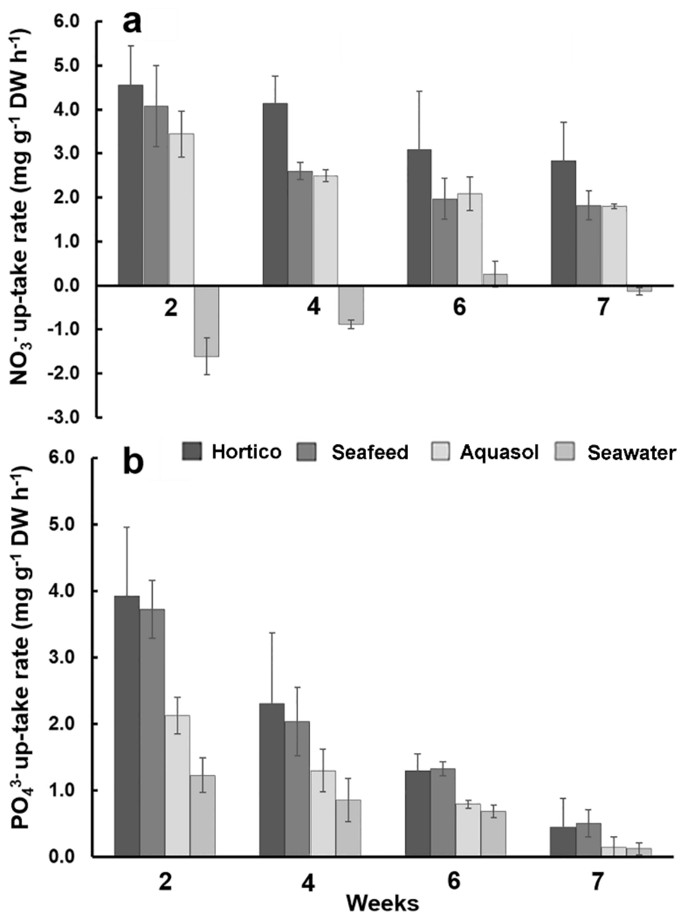

Figure 8.4 Change in NO_3^- and PO_4^{3-} uptake rates under different nutrient media of *S. spinuligerum* at different treatments and over the different cultivation times. Values are means ± S.E. (n = 4).

To assess the nutrient-uptake ability of *S. spinuligerum*, in this study, we reserved the cultivation media from the initial cultivation until the end of the experiment. During the experiment period, we only removed and adjusted salinity by adding fresh water into the tanks. It is believed that different species not only have different growth rates and nutrient requirements but also depend on the cultivation media exchange scheme of the experiment (Pedersen and Borum, 1996). In a study on *S. baccularia* from the Great Barrier Reef in a continuous flow culture system, the growth rate reached the highest values with supplied nutrient concentrations at 208 mg L^{-1} and 29.4 mg L^{-1} for N-NH_4/L and P-PO_4, respectively. However, the growth rate of this species decreased when the nutrient concentration increased (Schaffelke and Klumpp, 1998).

N and P concentrations in *S. baccularia* tissue increased when nutrient concentrations increased, but the tissue N and P concentration became saturated when N and P reached around 2.5% and 0.22% (dry weight), respectively.

However, the nutrient concentration of this study was lower than a study on *S. horneri* using 10 mg of KNO_3 L^{-1} and 1 mg of KH_2PO_4 L^{-1}. The cultivation media was renewed once every two days (Pang *et al.*, 2009). While previous studies have been conducted on the nutrient requirements of macroalgal, such as *Lamina saccharina* (Hsiao, 1972), *Enteromorpha intestinalis* (Kamer and Fong, 2001), and *S. baccularia* (Schaffelke and Klumpp, 1998) the cultivation media was changed either weekly or once every two weeks.

This study indicated that the main thallus reached the highest value in the Aquasol® treatment at 0.82 ± 0.16% per day. The different cultivation media significantly influenced the growth rate of *S. spinuligerum* during the first three weeks of the experiment. There was a statistical difference between cultivation media treatments on main thallus growth ($P < 0.05$). Our experimental results showed that the P uptake rate steadily decreased over cultivation time, and the concentration of PO_4^{3-} in the cultivate media was reduced to 38.9% and 59.6% after the fourth and sixth weeks, respectively. After the sixth cultivation week, PO_4^{3-} and NH_4^+ concentrations began to increase in all the experiments due to the mortality of *Sargassum* thalli. Then, they released into the cultivation media a quantity of PO_4^{3-} and NH_4^+ that created an advantageous environment for the opportunistic fast-growing algae; *Ulva* sp. (Fan *et al.*, 2014), *Porphyra* sp. (Hafting, 1999; Israel *et al.*, 1999; Liu *et al.*, 2010; Varela-A'lvarez *et al.*, 2007), *Chaetomorpha linum* (Xu and Lin, 2008), and *Enteromorpha intestinalis* (Kamer and Fong, 2001) grew in the tanks as well as attached to the *Sargassum* thallus. In the experiments with lower growth rates or thalli mortality, the growth of *Porphyra* sp. algae was higher than in the other experiments. In comparison with the fast-growing macroalgae species, *Ulva* sp., *Porphyra* sp., and *Enteromorpha* sp. have N demand per biomass that is 30 times higher than in the slow-growing *Sargassum* species. The reason is that the growth rate of the fast-growing species is ten times faster and their N requirements are three times higher than in the slow-growing species (Pedersen and Borum, 1996).

In summary, the different cultivation media significantly influenced growth rates of *S. spinuligerum* in both the SGR and NUR. The laboratory experiment on growth rate revealed that *S. spinuligerum* is significantly affected by stocking biomass in culture conditions where a lower initial biomass can yield better growth rates. The study indicated that *S. spinuligerum* could be cultivated for biomass in outdoor conditions with the optimum ISB at 15.35 ± 1.05 g per 113-L, enriched with Aquasol®, which contributed to a relatively higher SGR than other tested commercial fertilizers. The use of Aquasol® is recommended as a supplying nutrient to enhance the highest SGR of *Sargassum* cultivation. Further studies could investigate the growth ability in coastal environments where the effects of river flow could cause eutrophication. Additional research may be required on the physiological response of *S. spinuligerum* when cultivated under changing key environmental parameters such as increasing seawater temperature and ocean acidification.

Chapter 9. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1. INTRODUCTION

The *Sargassum* genus is considered one of the key groups of SAV that has high ecological and economic value in the coastal zone of tropical and subtropical regions (Chopin and Sawhney, 2009; Noro *et al.*, 1994; Trono, 1992). The present study was focused on investigating the temporal and spatial distributions, abundance and diversity of the *Sargassum* genus due to seasonal changes around Point Peron and Rottnest Island, WA.

The results demonstrated that *Sargassum* have strong seasonal variation and are significantly influenced by the environmental parameters in the WA waters. The spatial distribution of SAV is a combination of environmental parameters and the composition of substrate, such as bathymetry, substrate morphology and structure, benthic biota, and water currents (Baker and Harris, 2012; Harris, 2012a). Therefore, the distribution maps for SAV are typically used in integrated coastal zone management programs by the local government authorities and for scientific surveys in coastal monitoring programs (Harris, 2012b). Accurate data on the distribution of SAV in general and *Sargassum* spp. habitats in particular, are important for marine managers to establish sustainable management plans of the coastal marine ecosystems (Baker and Harris, 2012).

This chapter provides an overview and discusses the research findings that were presented from chapters 3 to 8. The end of the chapter provides conclusions and summary of recommendations for the future research. In the first three research objectives of this project, we attempted to collect and construct a spectral reflectance library for the majority of SAV species including *Sargassum* in WA waters, providing vital inputs for the satellite remote-sensing studies (Objective 2), identification and mapping the SAV habitats (Objective 3), and evaluating the distribution of *Sargassum* along the WA coastal waters (Objective 4).

The present study is considered a novel approach in marine benthic ecology as the study has applied the high-spatial and spectral resolution to access the distribution of *Sargassum* and SAV habitats in shallow coastal waters (Objective 4 and 5) (Hoang *et al.*, 2015b; Hoang *et al.*, 2016). In addition, this study documents the reproduction, growth rate and life stages of *S. spinuligerum* in the WA coast (Objective 6 and 7). Noteworthy, this is the first study which also provides an understanding of the effects of environmental parameters on the abundant of *Sargassum* biomass in WA waters (Objective 8) (Hoang, O'Leary, and Fotedar, 2015). The present study has contributed background data on the growth and productivity of *Sargassum* spp. both under outdoor and laboratory conditions (Objective 9).

9.2. MARINE HABITATS MAPPING BY SATELLITE REMOTE-SENSING IMAGERIES

The structure of benthic communities is determined by the group of organisms and their associated benthos (Hochberg and Atkinson, 2003). Quantifying the structure of benthic communities is a first step towards understanding the ecological functions of coral reefs, seagrass, and macroalgal communities (Kinsey, 1985). The SAV habitats may be constituted by mono-species or multi-species which range from a few centimeters up to a hundred meters (Buddemeier and Smith, 1999). Similarly to coral reefs, the different benthic substrates play a vital role in distribution and development of SAV species including macroalgae and seagrasses. In addition, the SAV community structure is always in state of temporal variation (Buddemeier and Smith, 1999) and spatial heterogeneity (Hochberg and Atkinson, 2003). Instead of spatial variation, SAV communities such as seagrasses and macroalgae also have strong fluctuation over their life cycle from a few months to several years (Hoang *et al.*, 2015a, b).

Therefore, identifying and accessing the temporal and spatial variations of SAV habitats are very important in understanding the SAV community structure in the coastal waters. However, the assessment of SAV community structure is relatively difficult when using conventional/ecological methods such as diving surveys, *in situ* quadrats, underwater photos, and transects (Miller and Müller, 1999). Moreover, these ecological methods are highly labor-intensive and time-consuming. On the other hand, the satellite remote-sensing is one of the most powerful and cost-effective techniques to collect the spatial and temporal data of the SAV ecosystems in both regional and global scale (Hochberg and Atkinson, 2003; Mumby *et al.*, 1999). In the present study, high-resolution satellite remote-sensing imageries (i.e. WV-2) were coupled with the SAV spectral reflectance library, *in situ* quadrats, and transects to identify, classify, and access the spatial distribution of SAV communities in the selected areas along the WA coast.

9.2.1. Spectral reflectance library

The spectral reflectance patterns of SAV species are controlled by their pigment contents. SAV species in the coastal shallow waters can be classified into five major groups such as green, red, and brown macroalgae, seagrasses, and substrates (e.g. corals, sand, limestones, and rubble) and the difference between benthos substrates at different depths can be measured by subtracting the contrast of a typical substrate with another benthos at the same depth (Kutser *et al.*, 2006).

Recently, there have been numerous studies on the spectral reflectance of coastal marine species in general (e.g. Harvey, 2009; Harvey *et al.*, 2007) and of coral groups in particular (e.g. Hedley *et al.*, 2016; Miyazaki and Harashima, 1993; Russell *et al.*, 2016), but relatively a few studies are available on detailed measurements and on establishing a spectral reflectance library of SAV species (Garcia *et al.*, 2015). Therefore, the present study can be considered a contribution to bridge the data gap as well as to establish a comprehensive spectral reflectance library of SAV species, including *Sargassum* species.

The spectral shape of absorption of SAV relates to backscattering, absorption, and fluorescence of different species (Russell *et al.*, 2016). The study on coral spectral reflectance has shown that spectrum absorption is carried out by pigments both in photosynthetic symbionts and in host tissues (Russell *et al.*, 2016). The results of the 22 measured and analyzed spectral reflectance profiles of the major SAV species in the coastal waters showed that there was a statistical difference between the collected SAV species.

Correlation and Principle Component Analysis (PCA) methods were employed to evaluate the differences between SAV groups. The results have documented the spectral features of SAV and their associated habitats in Shoalwater Islands Marine

Park, WA, and developed a spectral library to distinguish among seagrass species and algae groups (green, red, and brown benthic macroalgal). The PCA results also indicated that SAV species were clearly separated between green, red, and brown macroalgal and associated substrates. The results of this study are similar to previous studies on healthy and bleached *Acropora* corals (Holden and Ledrew, 1999). However, there was no significant difference when comparing the reflectance spectral between the similar coral genera that were collected from the different distribution areas (Holden and Ledrew, 1999). The implications of this study will contribute to better estimate and detect the distribution and seasonal variation of SAV on a broader scale (Hoang *et al.*, 2016).

9.2.2. Mapping submerged aquatic vegetation habitats

During the next 30 years, it is predicted that more than half of the world's reef areas could be lost (Wilkinson, 2000); followed by favorable environmental conditions, the reefs will then become covered by macroalgae, and macroalgae species leading to an algal phase (Hochberg and Atkinson, 2003). Therefore, understanding the current status of reefs' structure in general and macroalgae cover in particular are an important area of study. The current spatial distribution of macroalgae on reefs can provide a base line data for future studies.

There were several comprehensive reviews on the history and current status of satellite remote-sensing in coral by Green *et al.* (1996; 2000) and Hedley *et al.* (2016). They reported that the majority of remote-sensing coral studies were using either Landsat or SPOT satellite images. However, their classification accuracy still requires further improvement. On the other hand, airborne sensors with high spatial and spectral resolutions provide more detailed information and have higher classification accuracy (Mumby *et al.*, 1998).

With regard to depth, the present study identified the distribution of SAV at depths ranging from 0.2 to 3.5 m (Table 9.1). The distribution depths of the present study are also similar to previous studies in ecology, and the distribution of *Sargassum* spp. in WA (Kendrick, 1993), Malaysia (May-Lin and Ching-Lee, 2013), Hong Kong (Leung *et al.*, 2014), Mauritius and Réunion (Mattio *et al.*, 2013).

Table 9.1 The habitat characteristics of monitored transects in the selected sites of WA coastal waters.

Transect characteristic	LiS	SaB	GiB	SB	RoB	SBa	LaB	PB	MaJ	ToB	PP_1	PP_2	PP_3
Length (km)	0.38	0.34	0.23	0.04	0.2	0.78	0.42	0.18	0.32	0.39			
Wide (km)	0.27	0.26	0.37	0.09	0.41	0.97	0.54	0.44	0.33	0.91			
Area (km^2)	0.102	0.088	0.085	0.004	0.082	0.756	0.227	0.079	0.106	0.35			
Habitat/Substrate													
Sandy	x	-	-	-	-	x	x	x	x	x	-	x	-
Life coral	x	-	-	-	-	-	-	-	-	-	-	-	-
Dead coral	x	x	x	x	x	-	-	-	-	-	x	-	x
Limestone	x	x	x	x	x	x	x	x	-	-	-	-	-
Depth (m)	0.5-2	0.2-3	0.5-2.5	0.5-3,5	0.3-2.5	0.2-2.5	0.2-3.0	0.2 - 2.5	3,0	1-3	2,5	2,0	2,5
Dominant habitats													
Seagrass	x	-	-	-	x	x	x	x	x	x	-	-	-
Canopy algae	-	-	x	x	-	-	-	-	-	-	x	x	x
Algae turf	-	-	x	x	-	-	-	-	-	-		x	x
Mixed MSAV	x	x	x	x	x	x	x	x	x	x	x	x	x
Canopy cover (%)													
Seaweed cover (%)													
Surveys period	9-11	9-11	9-11	9-11	9-11	9-11	9-11	9-11	9-11	9-11	10	10	10

Little Salmon Bay = LiS., Salmon Bay = SaB, Green Island Bay = GiB, Strickland Bay = SB, Rocky Bay = RoB, Stark Bay = SBa, Little Amstrong Bay = LaB, Paraket Bay = PB; Main Jetty = MaJ, Thomson Bay = ToB, PP1-3: Point Peron transects 1-3.

9.2.3. Mapping *Sargassum* sp.

Based on the classification results of SAV habitats in coupled with the spectral reflectance profiles library of *Sargassum*, *Ecklonia*, and other canopy macroalgal communities, a detailed classification *Sargassum* map has been established in the area around Rottnest Island. To date, the present study is considered the third study on mapping of submerged *Sargassum* habitats using satellite remote-sensing (Andréfouët *et al.*, 2004; Hu, 2011; Noiraksar *et al.*, 2014) and an improvement over the previous study in term of classification accuracy and interpreter mapping results. The current study acquired higher spatial and spectral resolutions (i.e. WV-2) and a relatively large number of ground truth data in both benthic habitats and environmental parameters relative to previous studies (Hoang *et al.*, 2015b).

The limitation of spatial and spectral resolutions of satellite sensors can lead to the ambiguous and incorrect classification results of benthic habitats (Garcia *et al.*, 2015). To improve the classification accuracy, it is necessary to enhance the spatial and spectral resolutions of satellite sensors as well as to increase the frequency of ground truth data collection. During the current research (Oct. 2012), we acquired the highest commercial satellite imagery data, WV-2, with high spatial and spectral resolutions. Therefore, this is a significant contribution for the classification results of *Sargassum* and other SAV groups with higher overall classification accuracy than the previous studies that have mapped *Sargassum* and SAV by using other satellite images (Noiraksar *et al.*, 2014). The processing procedure for marine habitat mapping from high spatial resolution WV-2 imagery is presented in Figure 9.1.

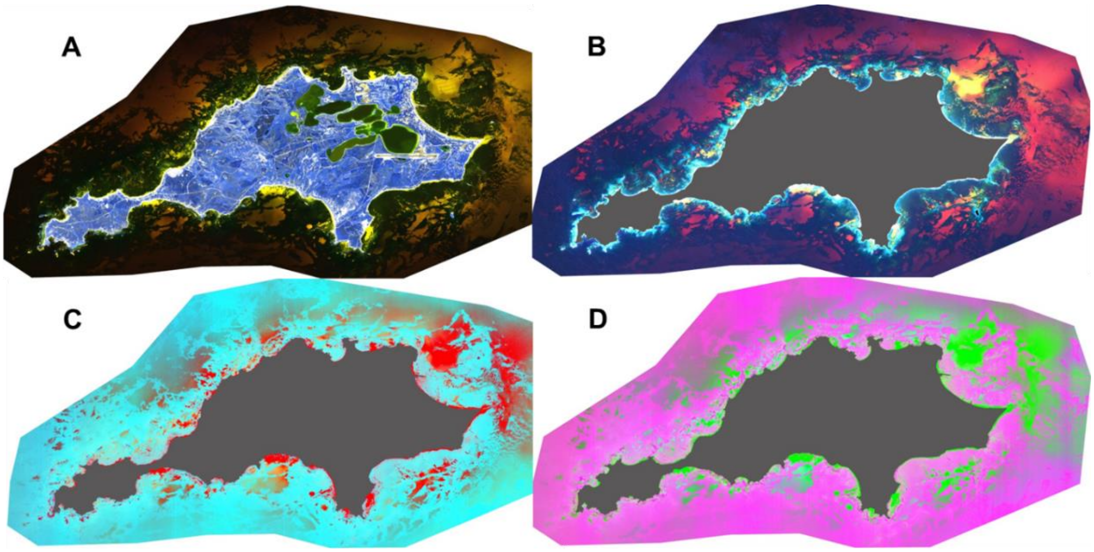

Figure 9.1 The processing procedure for marine habitat mapping by using *DII* method from WV-2 imagery. (A) False-color WV-2 after geometric and atmospheric corrections with combination of 237 bands; (B) Imagery after reflectance correction and ToA reflectance; (C) Vegetation have been performed with 687 and 768 bands (D) Bared sand substrate.

9.2.4. Comparison between different classifiers in mapping marine habitats

In the present study, the results of three classification methods such as Mahalanobis Distance (MDiP), Supervised Minimum Distance (MiD), and Support Angel Machine (SAM) classifiers were evaluated (Table 9.2). These classification methods are considered the most commonly and accurately used methods in the coastal ecosystems' classification (Andréfouët *et al.*, 2004; Belgiu *et al.*, 2014; Benfield *et al.*, 2007; Carle *et al.*, 2014; Ghosh and Joshi, 2014; Hoang *et al.*, 2015b; Muslim *et al.*, 2012). MDiP classification is a method for classifying the distance towards using the statistics of each class. This method is similar to the Maximum Likelihood method, but this method observes that all of the variables have equal value, so the processing time is faster. Meanwhile, the MiD method uses the average vector of each region of interest (ROI) and finds the Euclidean distance from each unknown pixel to the average vector of each class. All pixels are classified to the closest ROI class.

In addition, the WV-2 images were radiometrically calibrated according to the sensor surface that was publicized by DigitalGlobe® (Updike and Comp, 2010). The study areas are pure coastal waters and have an approximate distance from residential areas as well as belonging to protected marine parks. Hence, water quality is relatively high, so here we assume the influence of the irrherence optical properties (IOPs) of the water column are lowest.

Table 9.2 Comparison of MDiP, MiD, and SAM classification accuracy at Rottnest Island and Point Peron image

Classifiers	MDiP				MiD				SAM			
Accuracy indices	UA (%)		PA (%)		UA (%)		PA (%)		UA (%)		PA (%)	
Class	ROT	POP	ROT	POP	ROT	POP	ROT	POP	ROT	POP	ROT	POP
Seagrass	66.62	77.18	98.23	99.13	83.81	84.65	100	100	80.89	98.94	87.73	92.68
Canopy algae	95.8	66.7	45.71	96.3	93.17	96.22	43.8	99.16	100	66.84	7.98	94.15
Sand	100	99.46	100	85.69	99.25	95.2	100	96.05	97	21.02	44.7	88.82
Algae turf	83.31	73.35	100	90.56	87.47	96.87	93.74	93.82	93.33	86.8	76.67	72.5
Mixed SAV	95.31	99.47	91.75	95.81	86.93	100	99.92	98.06	99.98	99.06	86.87	89.36
Bare limestone	100	100	76.13	73.64	100	77.1	77.48	68.58	100	92.81	72.35	80.21
OA (%)	**90.66**	**94.16**			**90.93**	**97.13**			**49.93**	**88.43**		
Kappa	**0.849**	**0.884**			**0.853**	**0.942**			**0.389**	**0.740**		

*MDiP = Mahalanobis Distance, MiD = Minimum Distance, SAM = Spectral Angle Mapper, UA = User's accuracy, PA = Producer's accuracy, OA = Overall accuracy, ROT = Rottnest Island, POP = Point Peron.

9.3. SEASONALITY OF *SARGASSUM* BIOMASS

9.3.1. Seasonality as a driver of *Sargassum* biomass changes

This is the second study on the seasonal variation of *Sargassum* in subtropical region. The previous study was carried out at Rottnest Island, WA (Kendrick and Walker, 1991) (Table 9.4) which merely observed the seasonal growth and reproductive patterns of *Sargassum* under the field conditions and did not investigate the relationship between *Sargassum* and environmental variables. SSTs have strong effects on the *Sargassum* populations in tropical regions and could be one of main driving factors for the growth and senescence of *Sargassum* (Ang, 1985, 1986; Fulton *et al.*, 2014). In the subtropical regions, however, SST is not the primary factor in driving the seasonality of *Sargassum*, which instead is driven by the availability of nutrients in the water bodies. This finding was found by PCA and correlation test results between *Sargassum* and multi-variable environmental parameters. This is also similar with previous studies in the subtropical regions such as by Kendrick and Walker (1994).

This study also shows that *Sargassum* MTL reached the highest value in the period of decreasing SST (late autumn–winter). Similar growth patterns were also observed at other *Sargassum* species in tropical and temperate regions such as Caribbean of Colombia (Camacho and Hernandez-Carmona, 2012), and in the Red Sea (Ateweberhan *et al.*, 2009). In contrast, several previous studies in tropical regions of Malaysia also reported that nutrients were a driving factor for *Sargassum* growth (May-Lin and Ching-Lee, 2013; Wong and Phang, 2004). However, this study did not take into account other environmental parameters including nutrients in the context of multi-dimensional relationship. A study by Cortés *et al.* (2014) on the tropical *Sargassum* populations in Costa Rica showed that thalli length had a relationship with increasing SST. On the other hand, the minimum thalli length was found in April which coincided with the end of upwelling. Conceivably, the enrichment of nutrient source from upwelling enhanced the growth of *Sargassum* (Cortés *et al.*, 2014).

Meanwhile, in temperate and Mediterranean regions, the growth rate of *Sargassum* had an inverse pattern where the highest growth rates occurred during the warmest SST periods, such as in Spain (Arenas *et al.*, 1995), Japan (Yoshida, 1985), and France (Plouguerné *et al.*, 2006).

9.3.2. Life cycles of *Sargassum* spp. and climate zones

On a regional scale, life cycle of *Sargassum* is affected by the seasonal variations in the environmental parameters. In different geographical areas, the local climate conditions control the seasonal changes of water quality. Therefore, the life cycle of *Sargassum* could be strongly influenced by climatic conditions in its distribution areas. The life cycle of *Sargassum* in Point Peron, WA has been investigated and presented in the chapter 8 and compared with the life cycle of other *Sargassum* species in different geographic areas, including wet tropical, subtropical, temperate, and Mediterranean climates. The results showed that the growth and reproduction of

Sargassum in the subtropical climate (WA) has a similar pattern with Mediterranean conditions (Spain) (Arenas and Fernández, 2000) but a reverse seasonality pattern as they are distributed in two different hemispheres.

This is supported by the recruitment study on *Sargassum* in Rottnest Island by Kendrick (Kendrick and Walker, 1994). The present study on life cycle and reproduction of *Sargassum* in Point Peron is the second study in WA waters which aims to provide and improve the understanding of ecology and reproductive biology of *Sargassum*. However, in this research, the life cycle is limited to the field observation data over a period of 2.5 years.

9.3.3. Increasing water temperature and *Sargassum* abundance

Sea surface temperature has been rapidly increasing over recent decades in both the regional and global scales (Díez *et al.*, 2012; Haraguchi and Sekida, 2008; Nagai *et al.*, 2011). According to the IPCC fourth assessment report (2007), the SST had steadily increased by approximately 0.13^{o}C per decade over the last 50 years. However, SST was predicted to increase by 0.2^{o}C per decade within the next two decades. Several studies have found evidence that benthic organisms have shifted to higher latitude regions in relation to warming climate (Díez *et al.*, 2012).

Although Point Peron experiences a Mediterranean-style climate, Ningaloo, which is located 1,000 km to the north of WA at a latitude of about 22.5^{o}S, experiences a hot and dry sub-tropical climate. Ningaloo reefs experience higher SST (32.2 ± 0.5^{o}C annually) and drier conditions all-year-round, and the heat potential is among the highest on Earth. Comparable to at Point Peron, *Sargassum* biomass at Ningaloo is the lowest in winter and increases throughout spring and summer, driven by increases in SST and irradiance that increases *Sargassum* metabolism and photosynthesis (Fulton *et al.*, 2014). The increased biomass in *Sargassum* on Ningaloo reef occurs early in spring (November) to mid-summer, stabilization occurs between mid-summer and early autumn, and reduction occurs between mid-autumn and the start of spring. The increasing biomass on the Ningaloo reefs occurs during periods of increasing SST and irradiance. The biomass stabilization phase occurs during months with a high water temperature (36.6 ± 2.0^{o}C) and PAR. In Ningaloo, the high variation in SSTs might account for the strong influence of temperature on

Sargassum biomass. It is unlikely that nutrient concentrations drive growth in Ningaloo as significantly as in Point Peron, as nutrient run-off and upwelling are limited in this area (Fulton *et al.*, 2014). In addition, it is essential to take into account the impacts of the recent heatwave records in the relationship between growth and development of *Sargassum* populations in Australia (IOCI, 2009).

9.3.4. Factors impacting the *Sargassum* biomass

The present study showed that *Sargassum* canopy cover reaches the highest value in cooler months (autumn to winter) than in warmer months (spring to summer). The majority of *Sargassum* spp. generally become less common from late February to April. Numerous holdfasts remain behind and develop into new thalli during the following autumn and winter seasons. The disappearing of *Sargassum* spp. could be related to the length of summer days, leading to increased water temperatures.

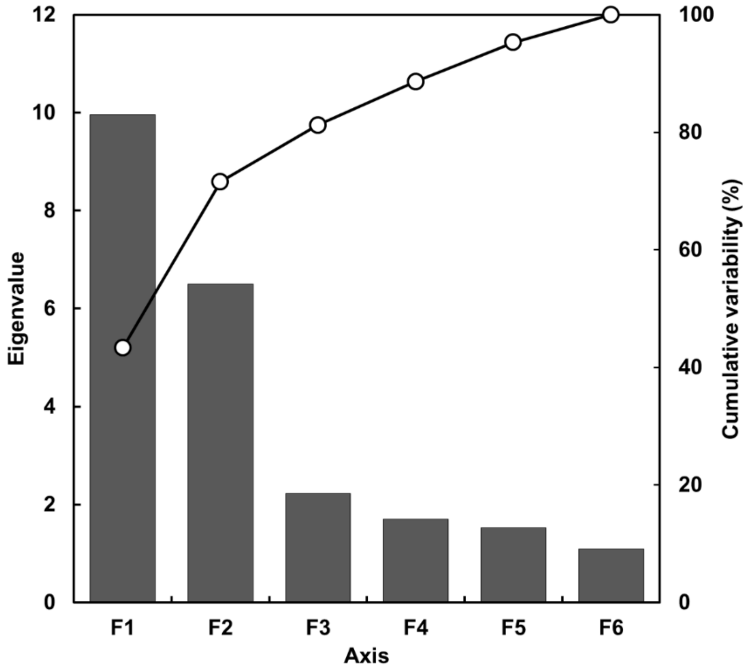

Figure 9.2 Results of PCA presents eigenvalues (grey bars) and cumulative variability (markers line).

The seasonal variation in growth and reproduction of *Sargassum* are different from the different populations and geographical areas. There are even differences in phenology between species in the same area. A study on seasonal variation of the *Sargassum* population in Hong Kong waters showed that two common species *S. hemiphyllum* and *S. siliquastrum* have different spatial and vertical variations in the water column. In particular, the growth and reproduction of *S. hemiphyllum* took place two months earlier than *S. siliquastrum* (Leung *et al.*, 2014). Therefore, environmental parameters are used control the phenological growth and reproduction of *Sargassum* species.

Table 9.3. Eigenvectors values of the principal components*.

	F1	F2	F3	F4	F5	F6
CC	-0.036	0.368	0.126	0.170	-0.049	-0.133
MTL	0.058	0.372	-0.108	0.008	-0.072	0.175
FB	-0.116	0.326	-0.042	0.229	0.138	0.217
SLP	-0.261	-0.066	-0.182	-0.065	-0.329	0.210
NW	0.144	0.239	0.312	-0.138	0.121	0.369
ED	0.293	-0.012	-0.099	0.083	-0.005	0.319
PAR	0.295	0.100	0.127	0.131	-0.045	0.000
Rainfall	-0.255	0.037	0.191	0.189	0.277	-0.280
i-SST	0.295	0.042	-0.118	-0.196	0.104	0.086
MaxAT	0.217	0.171	-0.234	-0.310	-0.069	-0.208
MinAT	0.288	0.026	-0.146	-0.179	0.115	-0.210
SST	0.275	-0.037	-0.270	-0.012	0.053	-0.259
Chl-a	-0.222	0.194	-0.155	-0.059	-0.236	-0.329
CDOM	0.205	0.057	0.218	0.076	0.527	-0.142
SE	0.294	0.104	0.119	0.133	-0.061	-0.008
Sal	0.179	-0.115	-0.135	0.495	-0.198	-0.266
pH	-0.198	-0.003	-0.375	0.000	0.367	0.288
Cond	-0.026	0.343	0.081	0.232	-0.262	0.127
DO	-0.017	-0.137	0.428	-0.492	-0.191	-0.020
NO_2^-	0.270	0.023	0.188	0.077	-0.344	0.047
NO_3^-	-0.045	0.358	-0.092	-0.234	0.037	-0.173
PO_4^{3-}	-0.004	0.354	-0.234	-0.193	-0.021	-0.020
NH_4^+	-0.193	0.241	0.286	-0.049	0.067	-0.227

*The physico-chemical parameters including CC represents canopy coverage (%); MTL represents mean thallus length (cm); FB represents fresh biomass (g $0.25m^{-2}$); SLP represents sea level pressure (hPa); NW represents a northward wind (m s^{-1}); ED represents euphotic depth (m); i-SST represents *in situ* sea surface temperatures; MaxAT represents maximum air temperature (^oC); MinAT represents minimum air temperature (^oC); SST represents satellite-derived sea surface temperatures (^oC); CDOM represents colored dissolved organic matter; SE represents solar exposure (MJ m^{-2}); Sal represents salinity; Cond. represents conductivity (mS m^{-1}); DO represents dissolved oxygen (mg L^{-1}).

Principle component analysis presented the correlation matrix of *Sargassum;* environmental parameters between seasons showed that four first components accounted for 88.6 % of the total variation. The corresponding eigenvectors are summarized in Table 9.3. The percentage cumulative variability and the actual eigenvalue are shown in Figure 9.2.

Previous studies have shown that application of PCA and CA methods are very useful in assessing water quality with a large dataset. Based-on the PCA and correlation analysis results, this study demonstrated that the availability of nutrients is an important resource as well as one of the main factors affecting the growth and reproduction of *Sargassum* in WA waters. Numerous studies have applied the PCA method to detect sources of pollution in the study areas. Principle component analysis and CA were employed in evaluating of pollution in Manchar Lake, Pakistan (Kazi *et al.*, 2009). Sheela *et al.* (2012) used PCA and CA to assess the main factors that affect the seasonal changes of water quality. Thirteen physico-chemical parameters were collected at 33 sampling sites in different areas of Lake Leusiedler See, Hungary to identify the primary process liable for the heterogeneity in several areas of the lake using PCA and CA techniques (Magyar *et al.*, 2013). In addition, PCA and CA were also recognized as powerful tools in the assessment of water quality in Lake Naivasha with three separate areas that have been clearly classified (Ndungu *et al.*, 2015).

In Asian–Pacific regions such as in the Philippines (Hurtado and Ragaza, 1999; Ragaza and Hurtado, 1999), French Polynesia (Payri, 1987), Mauritius and Réunion, Western Indian Ocean (Mattio *et al.*, 2008), and in New Caledonia, South Pacific (Mattio *et al.*, 2013), the seasonal variation in biomass and growth of *Sargassum* has been thoroughly studied. The research showed that a relative increase in biomass occurred during the colder months. A study by Wong and Phang (2004) showed that water temperature has a strong impact on the increase of biomass of *S. baccularia* in Cape Rachado, Malaysia. The increase in biomass of *S. binderi* was significantly correlated with an increase in sunshine, water temperature, and rainfall (Wong and Phang, 2004).

Therefore, the present study provides an overview of the development of *Sargassum* at different climate and geographical regions. In addition, the effects of seasonal changes on *Sargassum* are presented (Figure 8.8, Chapter 8). Thus, it is necessary to develop a procedure/set of standard to monitor and detect the water quality and health of the coastal ecosystems' using *Sargassum* as a biological indicator.

Table 9.4 The seasonal variation in *Sargassum* species and their correlation with the environmental parameters reported in tropical and subtropical waters.

Study site	Country	Climate	Species	Max MTL	Peak FB	Max CC	Nutrient	SST	PAR	Rainfall	Sub.	Depth (m)	Ref.
Point Peron	Australia	Csa	*Sargassum* spp.	Sp.-Su. (9–12)	Sp.-Su. (10–12)	Sp.-Su. (10–1)	✓	x	✓	✓	Rb, CR	1.5–10	(1)
Rottnest Isl.	Australia	Csa	*S.* spp.	Sp. (8–9)	Su. (1–2)	Su. (1–2)	-	-	-	-	S, Rb, CR	-	(2)
Ningaloo reef	Australia	Bwh	*S.* spp.	-	Su. (2)	-	-	✓	✓	✓	CR	1–5	(3)
Magnetic Isl.	Australia	Dfb	*S.* spp.	Au. (3–4)	Sp. (01)	Sp. (10)	-	-	-	-	CR.	-	(4)
Port Dickson	Malaysia	Af	*S. binderi*	Wet (1–2)	-	-	✓	x	x	x	CR	-	(5)
			S. siliquosum	Dry (6–7)	-	-	✓	x	x	x	CR	-	
The Northern	Philippines	Af	*S.* spp.	-	-	Dry (10)	-	✓	-	-	-	-	(6)
Tung-Ping C.	Hong Kong	Cwa	*S.* spp.	Au. (11–2)	-	-	-	-	-	-	-	10	(7)
New Caledonia	N. Caledonia	Af	*S.* spp.	Wet (12–3)	-	-	-	-	-	-	CR, Rb, S	20	(8)
Cape Peñas	Spain	Cfb	*S. muticum*	Wi. (12–1)	Sp.-Su. (4–6)	-	-	-	-	-	Rb	-	(9)
La Palma Isl.	Spain	Bwh	*S. flavifolium*	Sp.-Su. (5–7)	-	-	-	✓	✓	-	P, Rb, S	6–18	(10)
Massawa	Eritrea	Bwh	*S.* spp.	Su. (2–3)	-	-	-	-	-	-	CR	-	(11)
Gulf of Cali.	Mexico	Bwh	*S.* spp.	-	Sp. (4–5)	-	-	-	-	-	CR	-	(12)

Note: Climate zones (according to Köppen-Geiger climate classification): Af = tropical rainforest climate, Bwh = Hot desert climate, Cfb = Oceanic climate, Csa = Mediterranean climate, Cwa = Humid subtropical climate, Dfb = Humid continental climate. Sp. = spring (specific months), Su.= summer, Au.= autumn, Wi.= winter for oceanic climate and Mediterranean and Wet= wet months, Dry= dry months for the tropical climate zones. (-) = data not available, (✓) = affected/ correlated factors ($P < 0.05$), (x) = no correlated factors. Sub. = substrate types: C = cobbles, S = sand-covered, R = rock, Rb = rubble, CR = coral reef. Ref.= References; (1) This study, (2) Kendrick and Walker, 1994, (3) Fulton *et al*., 2014, (4) Vuki and Price, 1994, (5) May-Lin and Ching-Lee, 2013, (6) Ang, 1986, (7) Ang, 2007, (8) Mattio *et al*., 2008, (9) Arenas and Fernández, 2000, (10) Sangil *et al*., 2015, (11) Ateweberhan *et al*., 2009, (12) McCourt, 1984.

9.3.5. Seasonal changes in water quality and *Sargassum* biomass

As already discussed in the previous sections, there are many different physical, chemical and biological parameters that affect *Sargassum* populations (Figure 9.3). This study showed that water quality had a strong effect on the growth and reproduction of *Sargassum* (Hoang *et al.*, 2015a). The results of the present study revealed that instead of nutrient concentration of water bodies, rainfall has a significant effect on the seasonal growth pattern of *Sargassum* beds in WA waters.

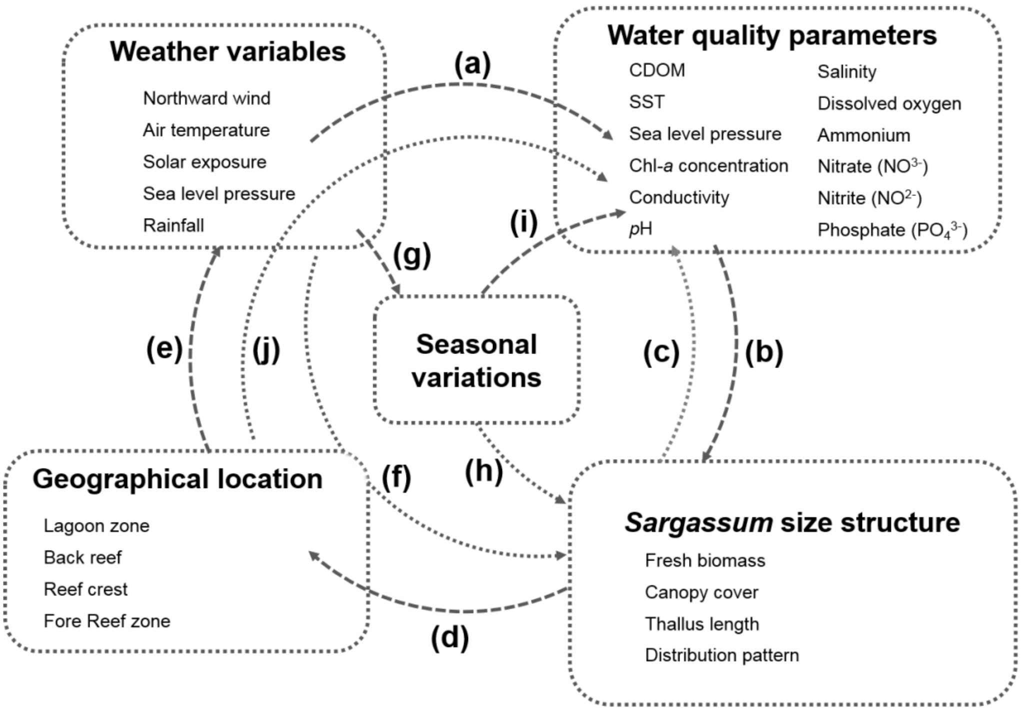

Figure 9.3 Conceptual framework of the interaction effect of seasonality, weather variables, water qualities and distributing geographic (reef zones) on canopy cover, thallus length and distribution of *Sargassum* community for Point Peron, WA coast.

The weather variables such as (a) air temperature, radiation and rainfall affect water quality (b) the interaction of the *Sargassum* CC, MTL, FB, and distribution on water quality and (c) vice versa; (d) the variation of *Sargassum* community structure at difference geographical zones (reef zones); (e) the variation of water temperature, radiation and rainfall at difference geographical zones–study sites; (f) weather variables affecting *Sargassum* community structure as well as (g) the effects of season's conditions; (i) seasons are also drivers of water quality changes and (h) *Sargassum* community structure. In this study, we assessed the effect of environment parameters on *Sargassum* canopy cover; the effect of SST on the *Sargassum* and the relationship between nutrient concentration and *Sargassum* thallus length. However, how *Sargassum* communities affect water quality (c) and differences in water qualities at different geographical areas (d) have not yet been examined.

9.3.6. Nutrient and *Sargassum* biomass

Previous field trials and laboratory experiments have shown the important role of nutrient availability on *Sargassum* growth and reproduction (Mai *et al.*, 2010; Matanjun *et al.*, 2009; Schaffelke and Klumpp, 1998). The oligotrophic conditions in tropical regions where coral is distributed limits *Sargassum* growth. There are significant impacts of anthropogenic nutrients input on the growth and development of macroalgae, including *Sargassum* in coastal areas. In this study with the analysis of multi-parameters by PCA to find the interaction between various biological, physical and chemical factors and *Sargassum* parameters. The analysis outcome showed that the *Sargassum* CC, FB, and MTL were strongly affected by nutrient concentration (NO_3^-, PO_4^{3-}) in the study areas (Hoang *et al.*, 2015a). The enriched nutritional source in the water bodies can be added by various sources including the increase in rainfall in the late autumn and early winter, then increasing the amount of water runoff, and the mixing of coastal water.

In order to combine the field observation data and the analysis results from Chapter 8, we have conducted experiments on cultivating *Sargassum* with different supplements of commercial nutrients under laboratory conditions, demonstrating that there were significant differences in SGR between nutrient supplements compared with the control treatment that cultivated with seawater only. These results were similar to the findings of Schaffdke and Klumpp (1998b) when they cultivated *S. baccularia* and observed the growth of germlings. The experiment revealed that germling growth rate increased when supplied more nutrients. However, the nutrient supplement can become a limiting factor in the growth and development of *Sargassum* (Pedersen and Borum, 1996; Schaffelke and Klumpp, 1998).

The results also showed that the vegetative reproduction, *S. spinuligerum* in laboratory conditions grow faster than in the natural conditions. This result is also similar to the previous studies of Tsukidate (1984) which showed that the first/primary laterals grow faster than the secondary laterals, and that the secondary laterals grow quicker than the third laterals. There was no significant difference in reproductive rate between the secondary and the third laterals (Tsukidate, 1984). When comparing the growth rate of vegetation reproduction (thallus segment) of the present study with other studies which were grown from seed/spores collected from the wild, it was shown that the growth rate of thallus segments are much higher than plants grown from spores (Hwang *et al.*, 2007b; Pang *et al.*, 2009). The branch that has also been cultivated in the sea has the same growth rate as the laboratory studies. Results showed that culture usually has the highest growth rate in spring (Norton, 1977). The previous studies also found that most *Sargassum* species in tropical and subtropical conditions begin to thrive in the fall when water temperatures begin to decrease (Tsukidate, 1992).

At present, macroalgae and *Sargassum* in particular have been considered potential candidates for raw materials development in the biofuel production industry to combat climate change (Aitken *et al.*, 2014; Alvarado-Morales *et al.*, 2013; Borines *et al.*, 2011; Sahoo *et al.*, 2012). Therefore, it is essential to understand growth conditions, nutrients in the cultivation medium, and life cycle assessment of *Sargassum* species as these are the key information for *Sargassum* culture on a large scale (Aitken *et al.*, 2014; Alvarado-Morales *et al.*, 2013; Costa *et al.*, 2015; Hwang *et al.*, 2006; Redmond *et al.*, 2014).

9.4. PROJECT ASSUMPTIONS AND LIMITATIONS

Seasonal observation of herbivory rate data is one of the limitations of this study. The intraspecific competition between species, and the effect of tidal regime on the growth, reproduction and distribution of *Sargassum* are also the limitations of the present study. Therefore, the concept of this study (Figure 9.3) has been not completed yet with integration of all factors that are related to *Sargassum* populations.

Before this research was carried out the *Sargassum* genus was clearly identified as a priority species of this project, although other associated SAV groups and their benthic habitats at the study areas are also vital components of the coastal shallow habitats in the study area. However, a limitation of this project was that it was not conducted in deep taxonomy, especially the classification of *Sargassum* species composition in the study area. In the present study, we have attempted to identify several of the most common and abundant *Sargassum* species along the WA coast to carry out the experiments under both field and laboratory conditions.

Another limitation is the satellite remote-sensing database, although the present study only used the latest SRSI (2013) which had already secured high spatial and spectral resolution imagery (Hoang *et al.*, 2015b). However, due to a limitation in the project's budget, we could only map with the spatial distribution of *Sargassum* at their highest value period. This could be a limitation of the present study but also provide a new/potential research topic that needs to be investigated to expand research in the near future. In addition, the detailed description of the classifiers and introduce the classification algorithms are beyond the scope of this project.

9.5. CONCLUSIONS

The main conclusions of the study are summarized below:

1. The study has established a spectral reflectance library for SAV species and associated substrates for coastal marine habitat mapping by hyper- and multi-spectral remote-sensing data.

2. Eight-band high resolution multispectral WV-2 satellite imagery is an excessive potential for mapping and monitoring *Sargassum* spp. and other associated coastal marine habitats.

3. The MDiP and MiD classification methods provide evidence for the greater accuracy of SAV classification results than the SAM classification method.

4. The spatial distribution of *Sargassum* is strongly dependant on benthos morphology and geographical locations such as reef areas along the coastal line.

5. The *Sargassum* spp. beds are influenced by seasonal variations in canopy cover and mean thallus length which were significantly influenced by the nutrient concentrations (PO_4^{3-}) and rainfall.

6. *Sargassum* canopy cover reaches the highest value in the cooler months (autumn to winter) than in the warmer months (spring to summer).
7. The majority of *Sargassum* spp. generally become less common from late February to April.
8. Numerous holdfasts remain behind after summer and develop into new thalli during the following autumn and winter seasons.
9. Nutrient concentrations in water bodies, influenced by rainfall have significant effect on the seasonal growth pattern of *Sargassum* beds in WA waters.
10. The annual life cycle of *S. spinuligerum* in WA shows distinct seasonal growth rates, life cycle phases.
11. Different cultivation media and initial stocking biomass has a significant influence on SGR and NUR. *S. spinuligerum* can be cultivated under outdoor conditions with the optimum initial stocking biomass at 15.35 g per 113-L with a relatively higher SGR.
12. The enriched seawater enriched by Aquasol® can increase the specific growth rate of *S. spinuligerum* under outdoor cultivation conditions.
13. The spatial and temporal variation of *Sargassum* provides necessary information for coastal marine management and conservation and sustainable utilization of this marine resource.

9.6. RECOMMENDATIONS FOR THE FURTHER RESEARCH

The following recommendations for future research are suggested:

1. A long-term seasonal data of SAV spectral reflectance should be collected.
2. Develop the technology to quantify biomass of SAV and seaweed species from satellite imagery.
3. Extend mapping methods to include other sites in Australia and coastal regions.

4. Study into the impact of decreasing pH on biomass, diversity and health of *Sargassum* and other macroalgal species.

5. The influence of climate change variables on nutrient-uptake capacities of *Sargassum* and other SAV species needs to be quantified.

6. Conduct further study on marine plant taxonomy to evaluate the biodiversity of *Sargassum* and other SAV groups along the WA coast.
7. Detailed study on quantifying the male and female reproduction organs during reproductive phase of *Sargassum* spp. should be conducted.
8. The survival rate of reproductive organs of *Sargassum* spp. should be evaluated both under field and laboratory conditions during its life cycle.

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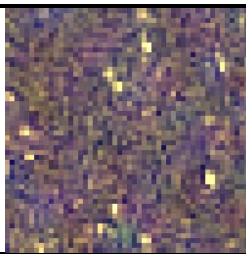
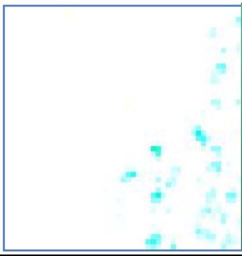
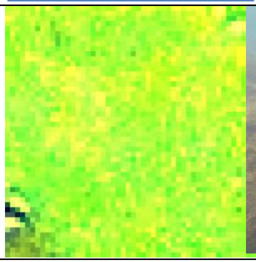
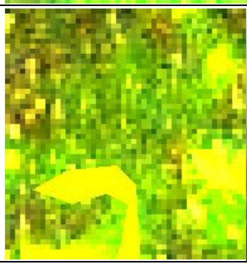
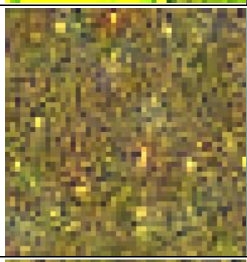
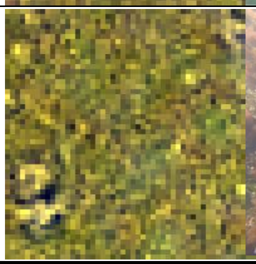
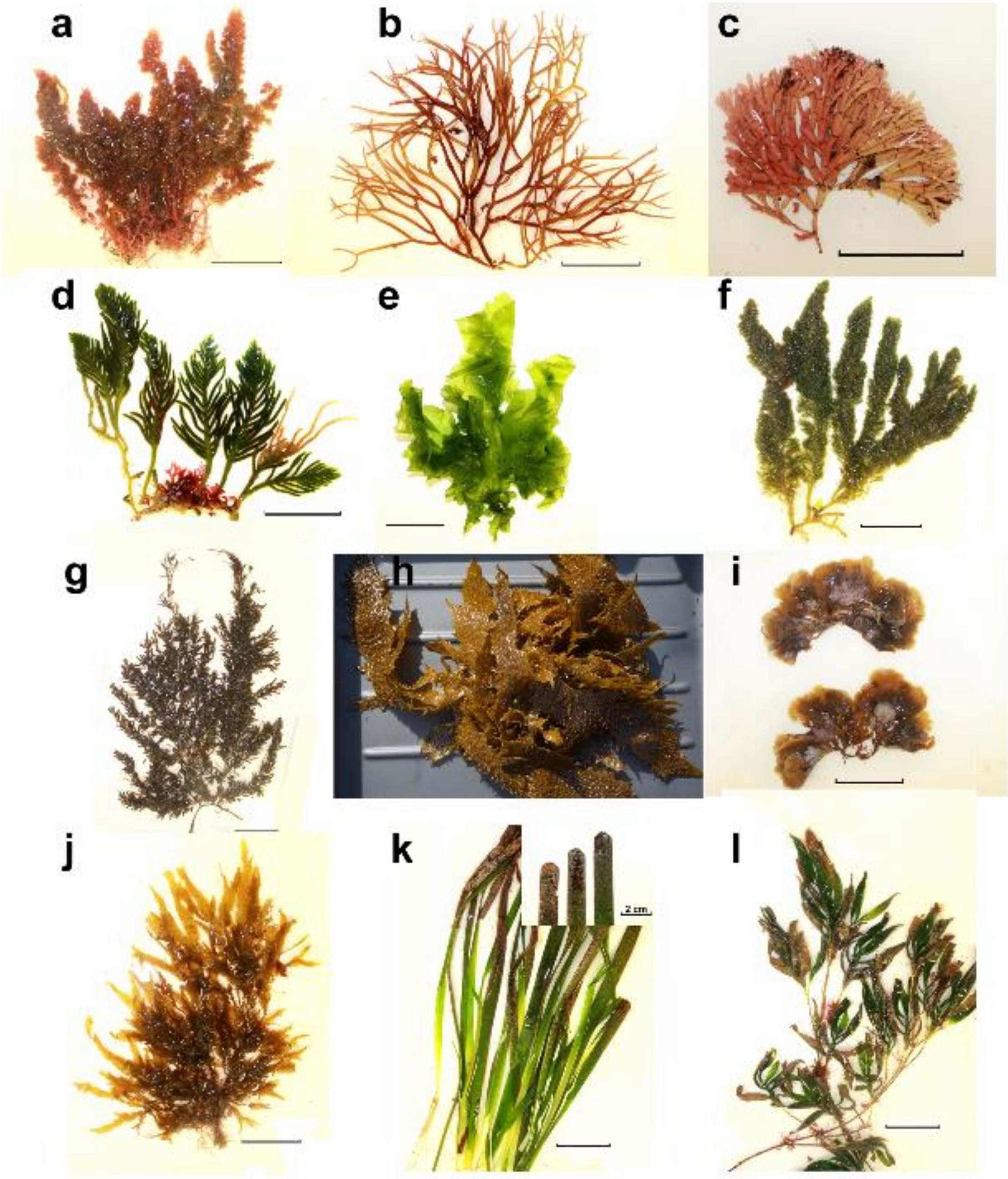
APPENDICES

APPENDIX 1. A scheme used for habitat classification based on ground truth data.

Class 1	Class 2	Description	WV-2 image texture	Ground truth photo
Un-vegetated	Rocky substrate	Either bared substrates or some coralline on its surface.		
	Sandy substrate	Fine sand with bared substrates		
Vegetated	Canopy macroalgal (*Sargassum* & *Ecklonia*)	Most abundant of *Sargassum* spp., commonly found in subtidal zone and mostly attached to the rocky reef substrates		
	Red macroalgae	Red algae (*Gracilaria* sp.) Rock, sand, mud, *Sargassum* and seagrass beds		
	Seagrass	*Amphibolis* sp. often found on sandy substrates in the sub-littoral zone. Also found on gravel and firm, clay banks.		
	Mixed Vegetation	The community of seagrass, coralline algae, Sargassum, Red macroalgae		

APPENDIX 2. An illustration of 12 dominance species have been recognized along the selected sites in WA.



a) *Asparogopsis armata*; b) *Gracilaria flagelliformis*; c) *Amphiroa anceps*; d) *Caulerpa flexilis*; e) *Ulva australis*; f) *Caulerpa obscura*; g) *Sargassum* sp.; h) *Ecklonia radiata*; i) *Zonaria* sp.; j) *Dictyopteris muelleri*; k) *Possidonia* sp.; l) *Amphibolis antartica*.
Scale bars: 5 cm.

APPENDIX 3. Copies of re-print articles that have been published or are in press at the time of submission this dissertation.

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Seasonal changes in water quality and *Sargassum* biomass in Southwest Australia

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ABSTRACT: *Sargassum* C. Agardh is one of the most diverse genera of marine macroalgae and commonly inhabits shallow tropical and sub-tropical waters. This study aimed to investigate the effect of seasonality and the associated water quality changes on the distribution, canopy cover, mean thallus length and the biomass of *Sargassum* beds around Point Peron, Shoalwater Islands Marine Park, Southwest Australia. Samples of *Sargassum* and seawater were collected every three months from summer 2012 to summer 2014 from four different reef zones. A combination of *in situ* observations and WorldView-2 satellite remote-sensing images were used to map the spatial distribution of *Sargassum* beds and other associated benthic habitats. The results demonstrated a strong seasonal variation in the environmental parameters, canopy cover, mean thallus length, and biomass of *Sargassum*, which were significantly ($P < 0.05$) influenced by the nutrient concentration (PO_4^{3-}, NO_3^-, NH_4^+) and rainfall. However, no variation in any studied parameter was observed among the four reef zones. The highest *Sargassum* biomass peaks occurred between late spring and early summer (from September to January). The results provide essential information to guide effective conservation and management, as well as sustainable utilisation of this coastal marine renewable resource.

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Spectral response of marine submerged aquatic vegetation: a case study in Western Australia coast

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***Abstract*— Marine submerged aquatic vegetation (SAV) plays a vital role as habitats, nursery and feeding grounds for a wide range of marine aquatic and terrestrial life. Recently, remote sensing techniques have been successfully applied in marine benthic mapping in coastal waters. However, the majority of these techniques have focused on either seagrasses meadows or coral reefs. There are a few studies that have been published validating a methodology for mapping SAV on brown macroalgae (*Sargassum* spp., *Ecklonia* spp.), seagrasses, and/or other macroalgae groups by spectral response from remote sensing. Hence, we studied the *in-situ* optical properties of living macroalgae, seagrasses, and rubble. The spectral characteristics of varied SAV groups were measured using the high resolution FieldSpec® 4 Hi-Res portable spectroradiometer. The study site selected was the Shoalwater Islands Marine Park, Rockingham, Western Australia as it is one of the fifteen biodiversity hotspots in Australia. Correlation and Principle Component Analysis were employed to evaluate the differences between SAV groups. The results have documented the spectral features of SAV and their associated habitats in Shoalwater Islands Marine Park, Western Australia, and developed a spectral library to distinguish among seagrass species and algae groups (green, red, and brown benthic macroalgae). The implications of this study will contribute to estimate and detect the distribution and seasonal variation of SAV on a broader scale.**

Keywords*— *spectral reflectance, macroalgae, coral rubble, SAV, marine submerged aquatic vegetation

I. Introduction

Marine submerged aquatic vegetation (SAV) is an important component of the coastal inter-tidal and sub-tidal ecosystems due to their ecological and conservational values [1], [2]. In coastal and estuarine areas, SAV is well-defined as a combination of seagrasses, oligohaline grass, benthic macroalgae, and floating macroalgae that covers from 10 to 100% substrates. In this study, we focused on the two main abundant SAV groups of seagrasses and benthic macroalgae, which play an important role in coastal marine ecosystems in Western Australia (WA). There are three basic groups of reef macroalgae that consist of turf algae, crustose calcareous algae, and fleshy macroalgae. Of those, turf algae and macroalgae are the main sources of carbon fixation and provide primary productivity for reef organisms [3]. Based on the characteristics of pigment composition we have divided the benthic macroalgae groups into three sub-groups consisting of red, green, and brown macroalgae.

However, the evaluation of large scale SAV distribution, seasonal fluctuations, and the effect of environmental factors is quite demanding due to the limited study areas and the combination of interdisciplinary knowledge [4]. Therefore, remote sensing has been used as a useful tool for SAV monitoring in both freshwater and saltwater environments [5]. A number of techniques have been widely adopted for the classification of marine habitats using *in-situ* substrate reflectance [6], [7]. There are two common techniques of remote sensing now includes access to specific study objectives and spectral reflectance. ***i)*** Understand the characteristics of spectral reflectance of SAV objects to distinguish them; ***ii)*** Based on the ground truthing data to extract the spectral reflectance from remote sensing data. The spectral reflectance of SAV has an important role in the analysis and interpretation of habitat classification. However, the collection of SAV specimens and their spectral reflectance, with measurements of the optical properties of water in the field are time and labor-intensive and may also be affected by weather conditions (e.g. [5], [8], [9]). Therefore, the establishment of a spectral reflectance library of SAV spectral is necessary to assess the distribution of coastal marine SAV in larger scale remotely sensing.

There have been a number of studies analyzing many spectral reflectance characteristics of marine benthos objects. However, these studies have focused on the spectral reflectance of the sea surface [9], [10], [11], [12], planktonic algae such as pelagic *Sargassum* [13], [14], [15], analyzing the spectral reflectance of coral reef benthos such as live and dead corals [7], [8], [16], [17], fresh water SAV [5], [6], [18], seagrasses [15], [18], [19], [20], [21] and terrestrial mangroves [22], [23], [24], [25]. At present, there are a few studies that document the spectral reflectance of the coastal substrate components including seagrass, macroalgae, and their substrates (sandy, coral, and limestone rocks) [8], [26]. Therefore, the major objective of the present study aimed to gather optical data of macroalgae (red, green, and brown), seagrasses, and sediment characteristics in coastal waters in order to support the selection of spectral bands and bandwidths for different environmental conditions such as clear water and high turbidity water bodies and to choose suitable satellite sensors for different benthos. This study may contribute as the primary spectral reflectance profile library for marine macroalgae with a comprehensive data on SAV species components and their associated substrates to provide input foundation information

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Article

A Method to Analyze the Potential of Optical Remote Sensing for Benthic Habitat Mapping

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Abstract: Quantifying the number and type of benthic classes that are able to be spectrally identified in shallow water remote sensing is important in understanding its potential for habitat mapping. Factors that impact the effectiveness of shallow water habitat mapping include water column turbidity, depth, sensor and environmental noise, spectral resolution of the sensor and spectral variability of the benthic classes. In this paper, we present a simple hierarchical clustering method coupled with a shallow water forward model to generate water-column specific spectral libraries. This technique requires no prior decision on the number of classes to output: the resultant classes are optically separable above the spectral noise introduced by the sensor, image based radiometric corrections, the benthos' natural spectral variability and the attenuating properties of a variable water column at depth. The modeling reveals the effect reducing the spectral resolution has on the number and type of classes that are optically distinct. We illustrate the potential of this clustering algorithm in an analysis of the conditions, including clustering accuracy, sensor spectral resolution and water column optical properties and depth that enabled the spectral distinction of the seagrass *Amphibolis antartica* from benthic algae.

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Remote-Sensed Mapping of *Sargassum* spp. Distribution around Rottnest Island, Western Australia, Using High-Spatial Resolution WorldView-2 Satellite Data

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ABSTRACT

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Satellite remote sensing is one of the most efficient techniques for marine habitat studies in shallow coastal waters, especially in clear waters where field observations can be easily carried out. However, such *in situ* observations have certain limitations: they are time consuming, have a limited ability to capture spatial variability, and require an interdisciplinary approach between marine biologists and remote-sensing specialists. The main objective of this study was to survey and map *Sargassum* beds around Rottnest Island, Western Australia, through a combination of high spatial resolution WorldView-2 imagery, using a validated depth invariant index model for water-column correction, and in-field observations. The combination of field survey data and four classification methods resulted in highly accurate classification outcomes that showed the distribution patterns of *Sargassum* spp. around Rottnest Island during the austral spring season (October 2013). Overall, the minimum distance and Mahalanobis classifiers yielded the highest overall accuracy rates of 98.32% (kappa coefficient, κ = 0.96) and 98.30% (κ = 0.96), respectively. The K-means classification method gave the lowest accuracy percentage of 42.50% (κ = 0.22). Thus, the primary results of this study provide useful baseline information that is necessary for marine-conservation strategic planning and the sustainable utilization of brown macroalgae resources around the Western Australian coast.

ADDITIONAL INDEX WORDS: *Brown macroalgae, satellite remote sensing, coastal habitat mapping.*

INTRODUCTION

The marine brown algae *Sargassum* spp. is an ecologically important genus that has a worldwide distribution and is especially dominant in tropical and shallow subtropical waters (Hanisak and Samuel, 1987; Mattio and Payri, 2011; Mattio *et al.*, 2008). As a living renewable resource, *Sargassum* spp. also has economic value, including potential use in medicines, fertilizer, and biofuel or energy resources and as a carbon offset, whereby it has the ability to both fix and sequester carbon dioxide from the atmosphere and distribute it among the different layers of the ocean (Aresta, Dibenedetto, and Barberio, 2005; Gellenbeck and Chapman, 1983; Hong, Hien, and Son, 2007). As such, there is an increasing need to map the density and spatial distribution of *Sargassum* beds to better quantify the total biomass of this resource.

Marine habitat mapping is usually undertaken using ground surveys and direct visual observations, side-scan sonar, and free diving. All of these techniques are extremely time consuming, expensive, and often unfeasible for large areas (Fearns *et al.*, 2011; Komatsu *et al.*, 2002; Tecchiato *et al.*, 2011). A more cost-effective method that is often employed is satellite remote-sensing imagery (SRSI). It requires fewer field surveys, and with the cost of imagery decreasing with concurrent improvements in spatial and temporal resolution, there has been a rapid increase in the use of SRSI for various marine-mapping applications.

The SRSI method has been successfully applied for mapping marine habitats in shallow coastal waters, especially in clear waters with good light penetration, where it is easy to carry out field observations (Green *et al.*, 2000). A range of satellite imagery tools have been used for mapping the spatial and temporal distribution of macroalgae and their associated habitats, including the Medium Resolution Imaging Spectrometer (Gower *et al.*, 2005), IKONOS (Andréfouët, Zubia, and Payri, 2004; Sagawa *et al.*, 2008, 2010, 2012a; Stumpf, Holderied, and Sinclair, 2003), Satellite Pour l'Observation de la Terre 2/4 (Casal *et al.*, 2011b; Hau, Son, and Mai, 2009), Land Satellite (Vahtmäe and Kutser, 2007), the Compact High Resolution Imaging Spectrometer/Project for On-Board Autonomy (Casal *et al.*, 2011a), and the Advanced Land Observing Satellite–Advanced Visible and Near Infrared Radiometer type 2 (ALOS–AVNIR-2; Phauk *et al.*, 2012; Sagawa *et al.*, 2012b; Tin, Tuan, and Son, 2009).

The recent launch of the commercial WorldView-2 (WV-2) satellite has further increased the spatial and spectral resolution of SRSI, with images with a 0.5-m spatial resolution for the single panchromatic band (450–800 nm) and a 2-m resolution for the eight multispectral bands. In addition to the four standard colors: blue, green, red, and near-infrared 1, WV-

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Identification and Mapping of Marine Submerged Aquatic Vegetation in Shallow Coastal Waters with WorldView-2 Satellite Data

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ABSTRACT

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Marine submerged aquatic vegetation (MSAV) naturally occurs on rubble and dead coral substrates in temperate and tropical coastal regions. During the growing season, MSAV develops to form dense canopy seaweed beds that play a vital role in coastal marine ecosystems and offer great potential to chemical, pharmaceutical, and bio-energy industries. At present, the total biomass and the distribution of the MSAV beds along the coast of Western Australia (WA) are not fully identified and quantified. Therefore, the application of satellite remote sensing data with high spatial resolution for examining the MSAV beds is required. The main objective of the present study was to assess and map the distribution of MSAV at two sites; Rottnest Island and Point Peron, Rockingham, WA, using WorldView-2 (WV2) satellite data. These study sites are important marine protected areas in WA waters with extraordinary documented biodiversity. By means of quantitative quadrat techniques, the MSAV canopy covers and fresh biomass data from the ground truth observations were assessed from September 2012 to December 2014. At Point Peron, the fresh biomass of *Sargassum* in the inter-tidal zone reached 5651.7±754.5, 5218.9±192.6, 1136.6±526.4, and 3472.2±434.2 g m^{-2} for spring, summer, fall, and winter, respectively. The overall accuracy of the minimum distance method was employed and yielded the highest accuracy rates of 90.93% (Kappa coefficient, $\kappa = 0.96$) and 97.13% ($\kappa = 0.96$) for Rottnest Island and Point Peron, respectively. The Mahalanobis classification with overall accuracy yielded 90.66% ($\kappa = 0.88$) and 94.16% ($\kappa = 0.85$) for Rottnest Island and Point Peron, respectively. The study results revealed that WV2 satellite data provided evidence of the high accuracy of MSAV classification.

ADDITIONAL INDEX WORDS: *clear shallow waters, marine habitat mapping, satellite remote sensing*.

INTRODUCTION

High spatial resolution satellite remote sensing is an effective tool for monitoring, evaluating and mapping biodiversity and natural resources in coastal areas (Green *et al*., 1996; Gibbons *et al*., 2006). There are numerous studies that have used high-resolution satellite images for identifying and mapping coastal habitats such as coral reefs (Benfield *et al*., 2007), sea grass meadows (Guimarães *et al*., 2011), mangroves (Heenkenda *et al*., 2014; Ibrahim *et al*., 2015), macroalgae (Garcia *et al*., 2015; Tin, O'Leary, and Fotedar, 2015), and freshwater/ salt marsh (Carle, Wang, and Sasser, 2014). However, these studies mostly utilized sensors with fewer than four spectral bands in the visible domain, which limited the detailed classification of vegetation (Feilhauer *et al*., 2013). To overcome the limitations, in October 2010, a WV2 satellite was successfully launched into orbit and began to acquire high spatial resolution images, 0.5-m for panchromatic and 2-m for multispectral images, and high spectral resolution (eight bands) including four additional spectral bands with additional near-infrared, coastal-blue, yellow, and red-edge bands (Updike and Comp, 2010).

Evaluation and validation of the feasibility of the new spectral bands of WV2 satellite data on identifying and mapping MSAV in coastal habitats are a necessity. This work not only contributes to scientific research but also provides useful information for managers, conservationists, and coastal planners, and is particularly relevant for marine conservation parks' authorities. The main objectives of the present study were: 1) validating the feasibility of the WV2 satellite data for identifying and mapping MSAV in coastal habitats; 2) evaluating three machine learning algorithms/classification methods, Mahalanobis distance (MDiP), supervised minimum distance (MiD), and spectral angle mapper (SAM), for mapping the diversity of MSAV.

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Seasonality and distributions of macro-algae *Sargassum* beds at Point Peron, Shoalwater Islands Marine Park, Western Australia *

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Sargassum C. Agardh is one of the most diverse genera of the marine macro-algae, is distributed worldwide, and is mainly dominant in tropical and sub-tropical shallow waters. There are about 46 *Sargassum* species found along the Western Australia (WA) coast (Herbarium 2013), with the large majority of studies on WA's macro-algae focusing on taxonomic and molecular works (e.g. Kendrick & Walker 1994; Goldberg & Huisman 2004; Dixon *et al.* 2012; Kendrick *et al.* 2012). A number of other studies have focused on seaweed physiology including green macroalgae (De Clerck *et al.* 2008) and red macro-algae (Huisman *et al.* 2009; Muñoz & Fotedar 2011). Up to date, there are a limited number of studies on seasonal variation on *Sargassum* communities that have been undertaken along the subtropical/temperate coastal zone of WA (Kendrick 1993; Kendrick & Walker 1994).

This study was carried out to investigate the seasonality of water qualities, canopy cover, thallus length and distribution of *Sargassum* beds around Point Peron, Shoalwater Islands Marine Park, WA. The aim was to improve our understanding of the seasonal abundance and distribution of *Sargassum* and the effects of water quality parameters on their biomass. Here we measured the seasonal variation in physico-chemical water parameters alongside changes in mean thallus length, density and total biomass of *Sargassum* and determined how this impacts the broader spatial distribution of *Sargassum* beds using *in-situ* observations and remote sensing methods.

The data on canopy cover, thallus length and distribution patterns were collected every three months from 2012 to 2014 at four different reef zones along monitored transects. Sampling was carrried out by either scuba or free diving techniques. Along these monitoring transects, a total of three sites were randomly selected within the lagoon, back reef, reef crest and fore reef zones. Measurements of fresh biomass, cover percentage, and thallus length were made by deploying 0.25 m^2 quadrats (0.5 x 0.5 metre), with a total of 12 tagged quadrat sites established. The tagged quadrats' locations were recorded with a hand held GPS (Garmin Etrex 10) for storage and easy renavigating during the following sampling season. *Sargassum* spp. within each quadrat were collected, stored in labelled plastic bags and carried to Curtin Aquatic Research Laboratory, Curtin University, WA for futher analysis and experiments. Meteorological data such as maximum, mean and minimum air temperature, monthly rainfall and monthly solar exposure for each season were acquired from the Garden Island HSF weather station, two kilometres north of Point Peron, Bureau of Meteorology, Australian Government (http://www.bom.gov.au/climate/data/). Euphotic depth, Coloured Dissolved Organic Matter (CDOM), Photosynthetically Available Radiation (PAR), Sea surface temperatures (SSTs), Sea level pressure, and Chlorophyll-a concentration (Chl-a) in the study area were extracted from the Moderate Resolution Imaging Spectroradiometer (MODIS) satellite data (Acker & Leptoukh 2007). Dissolved oxygen, salinity, *p*H, conductivity of seawater were *in-situ* measured from the field (YSI®55, Perth Scientific). Five seawater samples were collected for analysis of nutrients concentration including nitrate, nitrite, ammonia, and phosphate for each sampling season. High spatial resolution satellite images WorldView-2 with 2 m spatial resolution was acquired on February 7th 2013 (austral summer) and, along with the period of highest biomass of *Sargassum* beds, was used to estimate the spatial distribution pattern of *Sargassum*.

The results showed that the *Sargassum* beds in Point Peron showed remarkable seasonal changes in canopy cover and thallus length. There was a significant difference in *Sargassum* canopy cover between seasons. However, there were no significant differences between the reef zones. Results also show that the *Sargassum* spp. community demonstrated a seasonal variation pattern of coverage and mean thallus length which is significantly influenced by the nutrient concentrations (PO_4^{3-}), sun radiation, collecting zone, and collecting season ($P < 0.05$). There are many different physical, chemical and biological parameters that affect the *Sargassum* community. They might contribute to optimum conditions for *Sargassum* growth as well as limitation factors such as the effect of water temperature, radiation and rainfall on *Sargassum* canopy cover, thallus length and distribution; the interaction of the *Sargassum* communities on water quality and vice versa; the effect of geographical zone (reef zones) on water quality; the variation of water temperature, radiation and rainfall at different geographical zones (study sites); the effect of air temperature, radiation and rainfall factors on water qualities and the season's conditions; and the seasons also driving changing water qualities and influencing *Sargassum* growth. In this study, we were evaluating the effect of environment parameters on *Sargassum* cover; the effect of sea surface temperature on *Sargassum* community and the relationship between nutrient

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