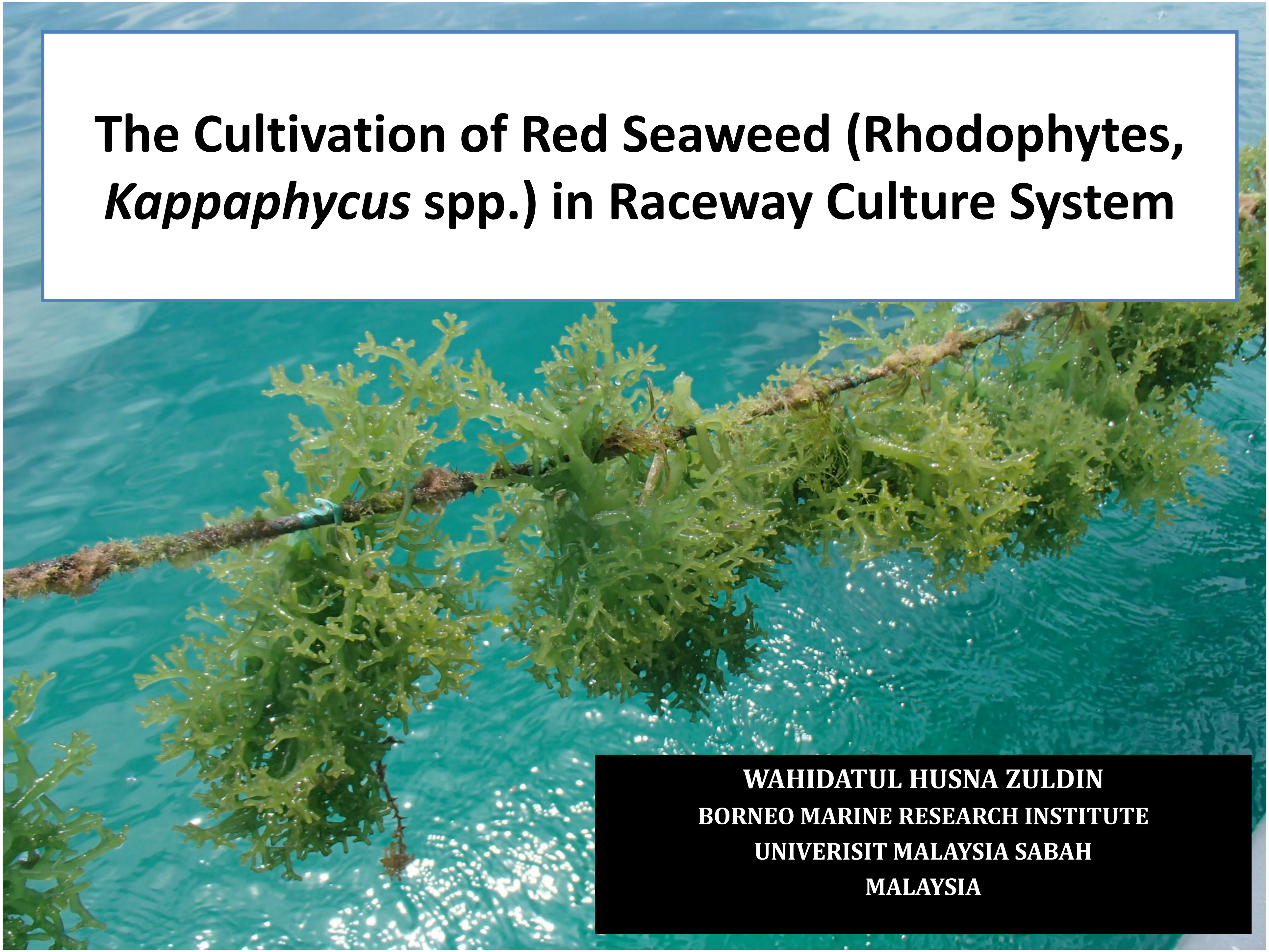
The Cultivation of Red Seaweed (Rhodophytes, *Kappaphycus* spp.) in Raceway Culture System



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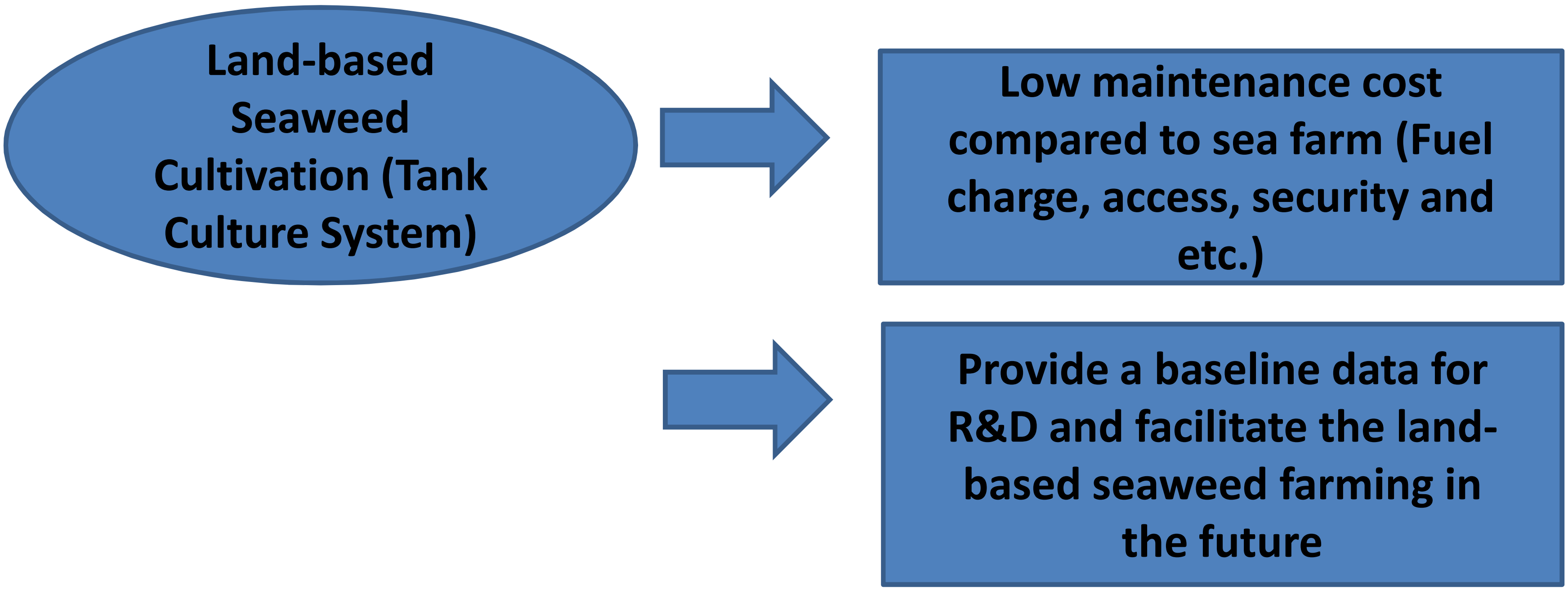
INTRODUCTION

- Seaweed farming is one of the top priorities set for development in Malaysia due to the increasing world demand for processed seaweed.
- Seaweed farming has been identified as one of the high impact aquaculture activities in Malaysia due to the increasing world demand for raw and processed seaweed with reported global world demand in 2012 of about 350,000 to 400,000 metric tonnes **(Yassir, 2012).**

TARGET: **Increment** of yield and total seaweed production up to 150,000 metric tonnes of high quality processed seaweed worth RM 46.7 million in **2020** by clustering of farms.


Land-based Seaweed Cultivation (Tank Culture System)
Low maintenance cost compared to sea farm (Fuel charge, access, security and etc.)
Provide a baseline data for R&D and facilitate the land-based seaweed farming in the future

Seaweed in Tank Culture System

- **Little is known** about the land-based **seaweed cultivation using tank culture method** especially in **Malaysia**.
- The cultivation of *Kappahycus* spp. **in land-based nursery system** such as in tank is very **uncommon in Malaysia**.
- The land-based seaweed cultivation or also known as on-shore tank cultivation of Gracilaria has been adopted in **Florida** (Hanisak, 1987), **Israel** (Friedlander, 1990) and **Chile** (Edding *et al*, 1987; Ugarte & Santelices, 1992) years ago.
- In 2009, researchers from **China** had invented a small-scale (65 X 45 X 35 cm) raceway tank for the cultivation of brown seaweed, ***Sargassum horneri*** in an indoor space **(Pang *et al*, 2009).** The brown seaweed was reported with the ability to survive in tank and accelerated reproduction rate under an optimum condition.
- A red algae, *Gracilaria* sp. was found to be viable in an integrated aquaculture tank in **Japan**. **(Naita *et al*, 1995).**
- Therefore, comparable experimental design could also be applied for *Kappaphycus* sp. in order to investigate the ability to grow in tank culture system.

1. Introduction

1.1 Australian Cultivated Seaweed – A New and Emerging Industry

This is a new conference project to support the development of a seaweed industry body for Australian cultivated seaweed. Industry groups have previously met with Rural Industries Research and Development Corporation (RIRDC) and advocated their support for the formation of an Australian seaweed industry organisation and the development of strategic industry and research plans.

RIRDC has supported a number of seaweed related research projects (eg, Winberg et.al.,2009, Lee, 2008, Lee and Momdjian, 1997) and these projects have confirmed the growth of markets for seaweed-based products and the associated growth of the industry. However, Australian and State Government environmental legislation limit the potential for large, sea-based seaweed cultivation close to the coast. McHugh and King (1998) have previously reported that for developed countries, tourism, recreation and environmental concerns would out-compete the need for sea-based seaweed culture in most cases. While sea-based cultivation of seaweed should not be ignored, industry development and research is required to develop alternate land-based sources of cultivated seaweed. These sources shall potentially provide seaweed products with a consistent source of supply, food safety integrity and traceability.

RIRDC has supported research for the cultivation of seaweed as it is potentially a new and emerging industry which shall provide economic, community and environmental benefits for regional Australia. These include:

- The inclusion of seaweed in the Australian diet as a functional food for health and nutrition
- Exports of bioactive substances extracted from seaweeds
- Potential replacement of seaweed imports
- Sustainable management of (aquaculture) resources in industry and the environment
- Support of indigenous community groups.

(RIRDC, 2010)

Reporting period 01/02/2010 to 31/07/2010

The intensive work designed to avoid spore crashes was completed without providing clear guidance on how to avoid the problem. However, this problem was largely overtaken by another because the majority of the seeded material that was deployed in the sea during the winter season failed to grow satisfactorily, and the ongrowing techniques that have resulted in good growth in previous years seemed not to work this year. Consequently, it was decided to test a new approach to the aquaculture of *Palmaria* in the final year of the project, and to set up tanks on land, which are stocked with small thalli from culture or natural populations. This would be in addition to a further attempt to grow good crops in the sea.

Reporting period 01/08/2010 to 31/05/2011

Tank trials were established at three hatcheries (DOMMRS, MRI Carna and QUB Portaferry) and at Cartron Point Shellfish Ltd. to see how *Palmaria* would perform. Different tank sizes were used, and growth in both natural and nutrient enriched water was compared. The advantage of this cultivation method is that the nursery

phase is omitted because harvestable biomass of *Palmaria* is grown vegetatively from an initial stock of *Palmaria* collected from the shore. Once the initial biomass is growing in tanks the surplus material is harvested at frequent intervals throughout the year. High growth rates were observed between early Spring and Autumn resulting in high biomass production per unit of tank surface area. Addition of fertilisers was found to enhance the growth of *Palmaria*. Trial results indicate that, at a stocking density of 4 kg m^{-2}, *Palmaria* doubles in weight every four weeks.

3.2 Conclusions from work undertaken on *Palmaria palmata*

In conclusion, in the time that the Project Team had (i.e. 2008 to mid 2011), it was not possible to demonstrate a consistent year-on-year successful culture methodology to achieve *Palmaria* sporulation – settlement on string – sea deployment – grow-out to harvest. Tank cultivation provides another means of cultivating *Palmaria*. Higher growth rates are observed with increasing light. Addition of fertilisers improves growth rate but may also encourage fouling. The main advantage of this cultivation method is that a hatchery is no longer required and biomass can be produced continuously at an accessible land based site.

(Irish Sea Fisheries Board, 2007)

Significance of Study

- Providing baseline data for R&D and land-based seaweed farming.
- A “blue-economy” strategy.
- Lead to the latest study on land-based seaweed culture in Malaysia.

Research Aim and Objectives

Aim

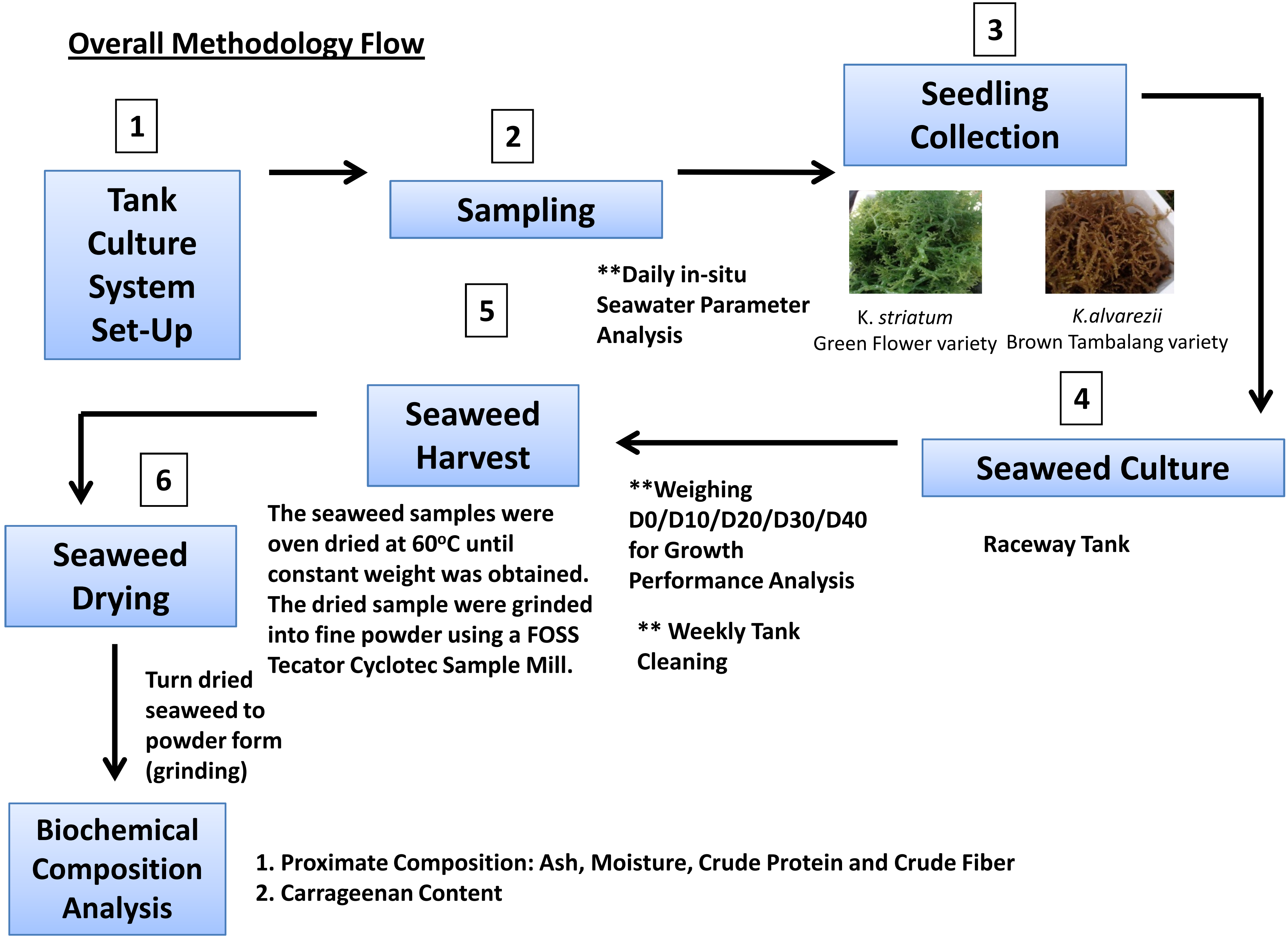
- To determine the seaweed **growth performance** in land-based tank culture system

Objectives:

- To determine the growth and biochemical composition of *K. alvarezii* and *K. striatum* cultivated in raceway tank.
- To design a tank culture system for optimum production of *Kappaphycus* spp.

Research Methodology

Overall Methodology Flow



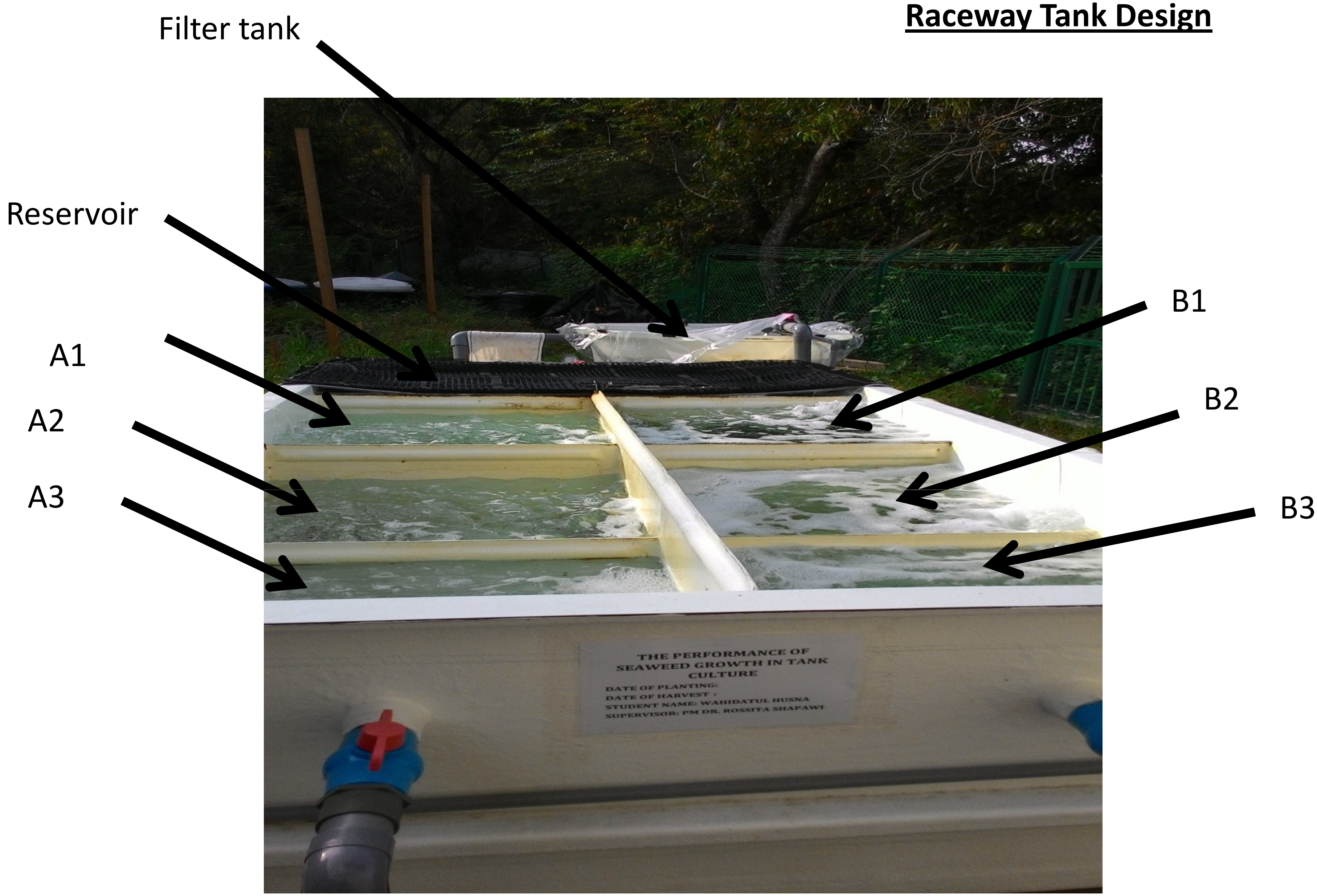
In this experiment, **Red Seaweed (Rhodophytes)** were used for tank culture.

- Two different species of Rhodophytes were used.

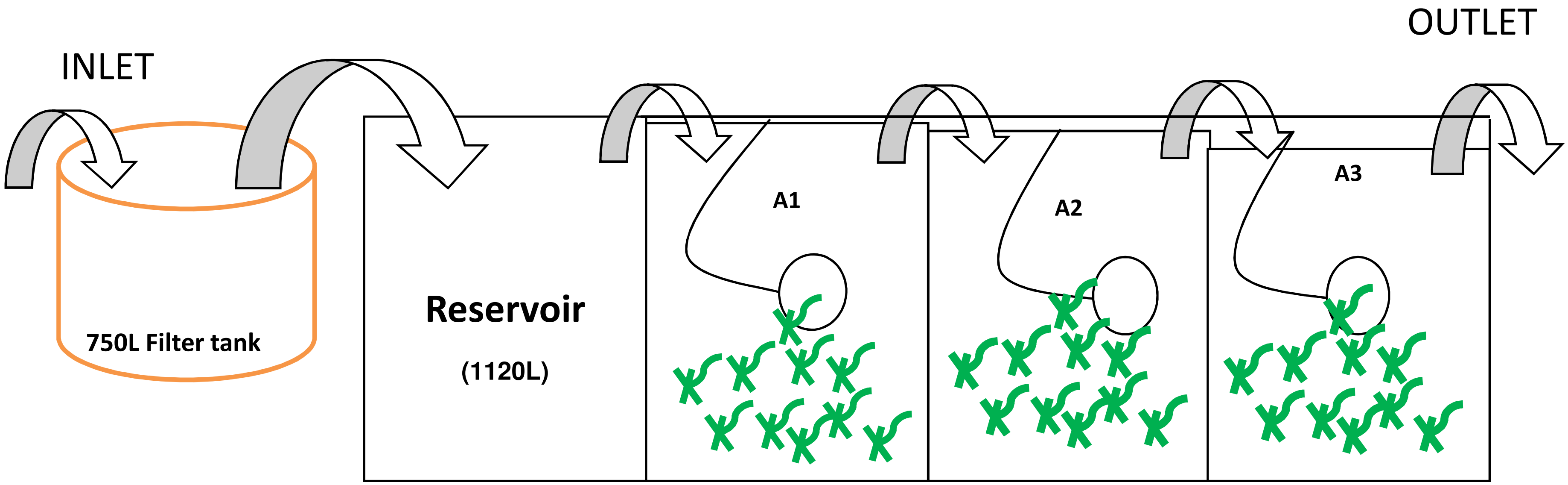
Species	Variety	Strength
K. alvarezii	*Brown Tambalang* (BT)	- High gel strength - High carrageenan content High growth rate
K. striatum	*Green Flower* (GF)	- High gel strength - High carrageenan content - High stress tolerant

(Neish, 2003)

Raceway Tank Design



How the seawater flow along the raceway?



Inlet Flowrate to Reservoir = 37.5 L per minute
Reservoir to A1 = 15 L per minute
A1 to A2 = 15 L per minute
A2 to A3 = 15 L per minute
Outlet Flowrate= 23L per minute

=> Method applied: Suspension Culture Method

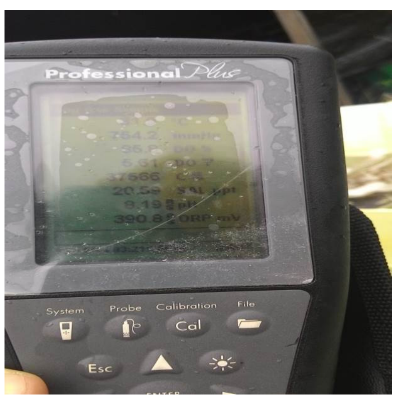
- Fixed Culture density: 1000g in each partitions (A1, A2, A3, B1, B2 and B3)

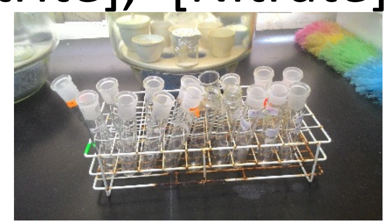
=> 3 cycles of seaweed culture were performed with 40 days culture period for each cycle.

Cycle #	Trial Period
1	10 Sept 2014 – 23 Oct 2014
2	23 Oct 2014 – 2 Dec 2014
3	28 Nov 2014 – 6 Jan 2015

Water Quality

- HANNA Multiparameter was used to measure the DO level, pH, Temperature and Salinity twice daily (8 am in the morning and 3 pm in the afternoon).
- Lux meter was used to measure the light intensity twice daily (8 am in the morning and 3 pm in the afternoon).
- The water nutrient content was measured twice during the culture cycle using colorimetric analysis ([Nitrite], [Nitrate] and [Ammonia])





Growth Performance

1) Seaweed Harvest

K. Striatum var. Green Flower

K. alvarezii var. Brown Tambalang

40-days cultivated seaweed were collected from the tank and weighed.

2) Seaweed Weighing Process

- Standard 1 decimal weighing scale was used.
- Seaweed was briefly strained first before weighing.
- Wet weight of the seaweed was measured.

3) Daily Growth Rate Calculation

**The growth was measured every 10 days before harvest (40 days per growth cycle) by calculating the daily growth rate (DGR) for each seaweed culture.

Formula for getting DGR.
(Yoong, Thau & Anton, 2013)

$$\text{DGR (\%)} = (W_t/W_o{}^{1/t} - 1) \times 100$$

Where:
W_t = Final fresh weight at t day (g)
W_o = Initial Fresh Weight (g)
t = Number of culture days

Seaweed Sample Biochemical Composition Analysis

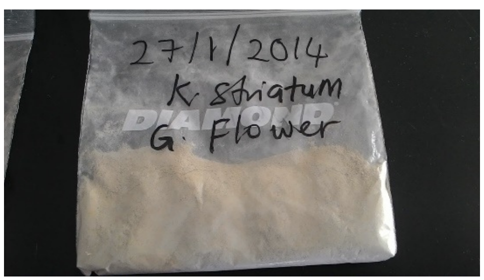
Proximate Analysis
(Standard AOAC 2000 Method)

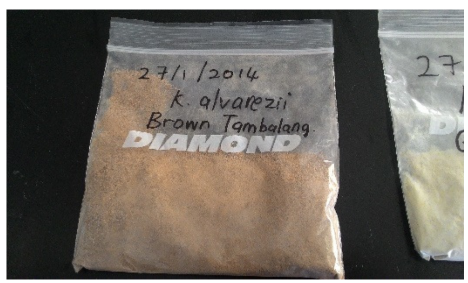
Crude Fiber- FOSS, Fibertec1020
Crude Protein- Kjeltec 2300 Protein Analyzer
Ash
Moisture



Carrageenan Content Analysis

Modified Protocol of Hurtado, Ponce & Umeski, 1988.






Statistical Analysis

- One-Way ANOVA statistical model Post-Hoc test was used to test any significant difference between the daily growth rate, water quality measurement and biochemical composition.
- T-test was used to test the significant differences on the daily growth rate between the two *Kappaphycus* spp. (*K. alvarezii* and *K. striatum*).

Results and Discussions

Daily Growth Rate, DGR

Table 1 The Average Daily Growth Rate of *Kappaphycus* spp. in Raceway Tank for Cycle 1, 2 and 3

Cycle #	Daily Growth Rate/ DGR (%day^{-1})	
	Kappaphycus alvarezii	**Kappaphycus striatum**
1	2.29±0.11[a]	2.81±0.06[b]
2	2.13±0.03 [a]	2.96±0.02 [b]
3	1.96±0.08 [a]	2.25±0.06 [b]

**There was significant differences on the growth rate between the *Kappaphycus* spp., where p values<0.05. *Different letters* indicated significant differences.

Figure 3 The Picture of seaweed Cultured in Raceway Tank taken at Day 5 and 35 during Trial 1

- The growth of *Kapppahycus* sp. **in raceway tank is still comparable with the open sea culture** since the reported daily growth rate of *K.striatum* at sea ranges from **1.75 to 3.5% (Ali, *et. al*., 2014)** depending on the culture density and the daily growth rate of *K.alvarezii* at sea is around **2.28 to 3.39% (Yassir, 2012).**

- It is very hard to culture seaweed in tank especially for acclimatization phase from sea to tank and longer trial period is needed to obtain the good results for growth performance compared to the seaweed cultured in sea farm.

Water Quality

Table 2 The "In-situ" Water Quality Analysis with average value of pH, DO level, temperature, salinity and light intensity taken in Raceway Tank during Trial 1, 2 and 3 within September 2014 until December 2014.

Water Parameters / Culture Cycle	pH Value	DO Level	Temperature (°C)	Salinity (ppt)	Light intensity (Lux)
1	8.00±0.07	5.43±0.09	30.56±0.02	32.32±0.05	6015.79±21.12
2	7.85±0.07	5.46±0.12	30.11±0.14	32.40±0.31	5981.72±20.54
3	7.80±0.05	5.45±0.13	30.49±0.29	32.17±0.09	6073.22±10.89

**There was no significant differences between the daily growth rate and pH, DO level, temperature, salinity and light intensity where the p values>0.05

Table 3 The Average Dissolved Inorganic Concentration (Nitrite, NO^{-}_{2}-N, Nitrate, NO^{-}_{3}-N and Ammonia, NH_3-N) recorded from Cycle 1, 2 and 3 in Raceway Tank.

Cycle	NO^{-}_{2}-N (Nitrite) μ/L	NO^{-}_{3}-N (Nitrate) mg/L	NH_3-N (Ammonia) mg/L
1	0.039±0.03	0.181±0.14	0.051±0.08
2	0.043±0.04	0.157±0.08	0.041±0.03
3	0.044±0.04	0.176±0.15	0.053±0.05

**There was no significant differences in the concentrations of nitrite, nitrate and ammonia between the culture cycles where the p values>0.05.

****In natural aquatic ecosystems, 95% of the nitrogen which occurs as dissolved dinitrogen gas (N2), is not directly accessible to most photosynthetic-oxygen organisms.**
****Nitrate is the principal form of fixed dissolved inorganic nitrogen assimilated by organisms.**
****Nitrate constitutes the prevailing available nitrogen source for macroalgae in the marine environment.**

- According to (Preisig and Hans, 2005), the standard mariculture condition of *Kappaphycus* spp. at open sea includes the minimum water level of 1.0 meter depth, temperature ranges between 27^oC to 30^oC, salinity ranges between 30 to 33 ppt and pH that ranges from 7 to 8.5. In this study, most of the parameters were at the natural conditions in order to facilitate the seaweed adaptation towards new localities of hatchery-based tank culture system.

- The recorded environmental water quality parameters in the raceway culture tank fall within the range of optimum requirements for field-cultured *Kappaphycus* species.

- In terms of the seawater nutrient content in the raceway tank, the average concentrations of nitrite and ammonia in all treatment tanks were below 0.1 mg/L, indicating good water quality with low concentration of seaweed waste.

- The nitrate concentration is lower might be due to the nature of seaweed in assimilating nitrate as nutrient source in the form of fixed dissolved inorganic nitrogen.

- In natural aquatic ecosystems, 95% of the nitrogen which occurs as dissolved dinitrogen gas (N_2), is not directly accessible to most photosynthetic-oxygen organisms. Nitrate is the principal form of fixed dissolved inorganic nitrogen assimilated by organisms.

- Therefore, nitrate constitutes the prevailing available nitrogen source for macroalgae in the marine environment (Chow, 2012). Thus, lower toxicity of ammonia and nitrite in the tank might induce the growth performance of the *Kapppahycus* sp. in the tank culture system.

Table 4 The Comparison of Raceway tank-cultured and Field-cultured *Kappaphycus alvarezii variety Brown* Tambalang and *Kappapphycus striatum variety Green Flower* in terms of the Biochemical Composition and Daily Growth Rate.

Seaweed Properties	Raceway tank-cultured		Field-cultured (Secondary Data)	
	K.alvarezii var. BT	K.Striatum var. GF	K.alvarezii var. BT	K.Striatum var. GF
Daily Growth Rate, (%day^{-1})	2.13±0.17	2.67±0.3	2.28 -3.39% (Yassir, 2012)	1.75 – 3.5% (Ali et al, 2014)
Moisture Content (%)	79.51±0.98	79.97±0.79	79.78%±0.22 (Ahmad et al, 2012)	79.70%±0.70 (Ahmad et al, 2012)
Ash Content (%)	21.48±0.62	20.45±1.01	23.25%±0.08 (Ahmad et al, 2012)	22.99%±1.04 (Ahmad et al, 2012)
Crude Fibre Content (%)	5.05±0.14	5.41±0.05	4.50%±0.32 (Ahmad et al, 2012)	5.34%±0.03 (Ahmad et al, 2012)
Crude Protein Content (%)	5.90±0.46	5.62±0.11	5.35%±0.02 (Ahmad et al, 2012)	5.42%±0.47 (Ahmad et al, 2012)
Carrageenan Content (%)	54.25±1.11	41.68±1.97	51.50±21.0% (Yong et al, 2014)	38.10±2.60% (Hurtado, et al, 2009)

***There was no significant differences on the* percentage of moisture, ash, crude fibre and protein with the growth of *K.alvarezii* and *K.striatum where p values > 0.05.*

- There was no significant differences between the moisture, ash, crude fibre and protein of the raceway and field-cultured *Kappaphycus* species. The carrageenan content on both raceway-cultured *Kappaphycus* spp. was also fall within the range reported by Yong *et al*, (2014) and Hurtado *et al*, (2009). Therefore, the raceway culture system does not significantly affect the biochemical composition of the *K.alvarezii* and *K.striatum*.

Conclusion

- In summary, both *Kappaphycus* spp. (*K. striatum var. GF* and *K. alvarezii var. BT*) have high potentials to be cultivated in land based tank culture system.
- Indeed, the land-based seaweed cultivation might also create new opportunities for the industry to produce high quality seedlings in the hatchery using land-based tank culture system.
- Therefore, further research on different tank designs should be considered to explore more potential in land-based seaweed farming.

Acknowledgment

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PUBLICATION OUTPUT

Wahidatul Husna Zuldin and Rossita Shapawi, 2015. Performance of Red Seaweed (*Kappaphycus* sp.) Cultivated Using Tank Culture System. *Journal of Fisheries and Aquatic Science, 10: 1-12.*

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