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Ocean Grown Abalone

THE UNIVERSITY OF WESTERN AUSTRALIA

Investigating critical biological issues for commercial greenlip abalone sea ranching in Flinders Bay, Western Australia

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Front Cover: Abalone on their ‘abitats’ or abalone habitats, and drift macroalgae where it has collected at the base of the abitats.

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1 Preliminaries

1.1 Acknowledgements

We would like to acknowledge the help of the diving staff at Ocean Grown Abalone, in particular Mark Wall, in collecting and often delivering to our laboratory, the algae and abalone samples used in this study.

Several staff/students at Curtin University and the University of Western Australia used parts of this project towards their thesis assignments. Their names appear in this report as lead and co-authors of their work. Other students assisted with sorting, weighing and other tasks associated with this project – we thank in particular, Anthony Cole and Patrick Stirling for their assistance in this regard.

There are other acknowledgements of a more specific nature for various components of this project. Those acknowledgements have been incorporated into some of the Chapters included in this report.

1.2 Executive Summary

1.2.1 What the report is about

The Ocean Grown Abalone Pty Ltd sea ranch is the first abalone sea ranching venture to have been commercialised in Australia. The abalone are grown on patented concrete structures that the company has placed on the seabed on their lease site in Flinders Bay, Augusta, Western Australia. In 2016 the farm exported 10 t of product and this volume is set to rapidly increase as the farm expands its artificial habitat structures and reaches full production. It is projected that ~200 t of abalone will be produced by 2022. The company has undertaken feasibility studies in other areas within Australia that they have identified as suitable for abalone ranching and is looking to expand in the near future. This report deals with research into the drift macro algae and seagrass that forms the food source of abalone on the farm. It examines the movement of the drift algae in Flinders Bay and specifically across the Ocean Grown Abalone lease sites. It then moves from a hydrological focus, to one more biologically centred, by examining the quantity and quality of drift algae and seagrass on the sea ranch and how that changes seasonally through the year. Only one part of the report deals specifically with abalone; it uses different physiological stress indicators as a way of predicting the health/condition of wild-caught and ranched abalone in Flinders Bay.

1.2.2 Background

Feasibility studies investigating different artificial habitat structures and growth and survival of the greenlip abalone on the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay were conducted and peer reviewed in 2012 and 2013 (FRDC project 2012/220). This project was initiated to undertake the next phase of research for the Flinders Bay abalone sea ranch venture.

Abalone on the Flinders Bay sea ranch, just as in the wild, trap and feed on drift macro-algae and seagrass and no artificial food or food supplements are fed to them. Given their dependency on the drift algae and seagrass, it is important for farm managers to understand the quantity and characteristics of this food supply and its movement as the algae is carried by the water currents across the farm. Not only this, but given that greenlip abalone are selective feeders, it was also important to know what species of algae and seagrass drift across the sea ranch and their nutritive value as a food source for the abalone.

1.2.3 Aims/objectives

(i) To understand the source and seasonal movements of drift algae across the aquaculture lease in Flinders Bay and relationships to local wind-wave climate

(ii) To evaluate the seasonal variations in the biomass, species composition and nutritive value of the drift algal species that form the main food source greenlip abalone on the lease site

(iii) To evaluate the health status of ranched greenlip abalone by using various physiological stress indicators and to use this status to predict the future health/condition of the abalone

1.2.4 Methodology

The source and seasonal movements of the algae were modelled. A hydrodynamic model was developed using current and wave fields and it was used to drive a dispersal model that predicted the movements of the drift algae. To evaluate the seasonal variations in the biomass and species composition and time-dependent functional properties of the drift algal species, seaweed were collected monthly by divers. The algae and seagrasses collected were identified, wet/dry weights were recorded and their nutritional values (protein, energy, moisture and ash) were determined. Samples of abalone were collected at the same time as the seaweeds and they were analysed for protein and moisture content. To evaluate the health status of the ranched and wild abalone, samples were collected in two different seasons and were evaluated using a range of physiological stress indicator tests (total haemocyte count, phagocytic rate, neutral red retention, lactate level, osmoregulatory capacity and glucose level). These indicators were examined at point of capture and then later, after the animals had been subjected to the simulated stress of being transported out of water.

1.2.5 Results/key findings

The hydrodynamic/dispersal modelling work showed seasonal differences in water circulation within Flinders Bay and across the lease site. This had implications for the model-inferred transport of drift algae across the sea ranch lease and within the Bay. Wave induced currents were experienced year-round and had a major influence on the movement of drift algae. During summer, south-easterly winds combined with the wave driven currents and created a clockwise circulation in Flinders Bay and in so doing, could be predicted to move drift algae across the lease site and towards the shore and into the Bay. In winter, currents were shown by the modelling work to generally move particles eastward and then northwards over the lease site. Wind was less influential in winter, and the larger swells in these months were predicted by the model to be responsible for moving more particles across the lease site and by implication therefore, funnelling more drift algae through the site.

There were a large number of species of drift algae and seagrasses identified on the lease site. The majority (66 %) were red algae, then brown (22 %) with the remainder comprising seagrass and green algae taxa. Brown algae dominated through the year in terms of their volume as a proportion of the algae and seagrasses sampled, but they dropped off in summer in favour of red algae and seagrasses. Red algae are considered from other published work to be the preferred food source for greenlip abalone. There were differences in the proportion of species sampled at one of the sites on the lease compared to the other two sites that were sampled. This result was somewhat unexpected given that sites on the lease were within a few hundred metres of each other.

Data on the nutritional parameters of drift algae and seagrasses showed that they had significantly higher crude protein levels in autumn and winter than in spring and summer. Red algae, which is the main food source of greenlip abalone, had higher crude protein levels than the other sea weeds. Energy levels of the different sea weed taxa sampled were mostly within a similar range. We did examine the protein and moisture content of the farmed abalone through the year as a more direct way of tracking the quality of the food. Meat protein remained relatively constant though the year, except for a brief and sharp increase in summer. Some reasons for this unexpected result have been suggested in the report.

Physiological stress indicators used to examine the health status of ranched compared to wild caught abalone, found differences between the two seasons sampled. Indicators showed that the abalone were more responsive to stress during the winter samples. In general, there was no difference in stress response between wild and ranched abalone in either of the seasons sampled. Where differences were measured, they were not consistent for the wild-caught and ranched abalone sampling sites and they therefore did not point to any immediate concern that ranched abalone at the study site were any differently stressed to those in the wild.

1.3 Implications for relevant stakeholders

The study has showed the benefits of

(i) Identifying seasonal circulation patterns prior to siting an abalone sea ranch. This provides the ability to site the farm in the best possible position for maximising drift algae and sea grass and also provides important information on the likely circulation patterns in the event of an oil spill, or similar natural disaster.

(ii) Having a thorough knowledge of the seasonal and spatial variation of the drift algae and seagrasses making up the food source to the farm. Without this baseline data, there would be no way of tracking any changes to the food source in the future.

(iii) Having baseline knowledge on the drift algal species composition of the food source to the farm. This information would become critical to managers should there be changes to the species composition of seaweed that is carried onto the farm. This information would be critical to explaining the likely effect that such change might have on the abalone farm stock.

(iv) Having baseline information on stress indicators for ranched and wild-caught animals. So that their condition can be monitored into the future, thereby providing one means of alleviating concerns about ranched abalone being any differently stressed to those in the wild and therefore any more susceptible to disease compared to those in the wild.

1.4 Recommendations

Good information is now available on the species of drift algae and sea grasses that are available for the abalone on the sea ranch in the different seasons. However, a link does need to be made between what species are available and what are selectively consumed by the abalone. Identifying the species of algae and seagrass eaten by the abalone on the ranch using visual gut content analyses proved in this study, not to be possible. Using molecular analysis of the gut contents to determine their food preferences is suggested as being a superior and more reliable way of gaining knowledge on what the abalone are eating.

Monitoring the stress levels of animals is considered to be a useful as a potential alert for disease. When the population is stressed, it becomes susceptible to disease. Baseline data has been established in this project, but that might be considered for extension into the future to build up a time series of the ‘health’ of the farmed population compared to wild caught abalone in Flinders Bay.

There is the potential for doing a lot more work in developing methods for the live transportation of abalone. There is little doubt in our view, that methods could be developed to minimise mortalities during live transport. This might potentially open up markets that are presently not being fully tapped.

1.4.1 Keywords

Hydrodynamic, dispersal modelling, drift algae, nutritional value, health, physiological stress

2 General Introduction

Commercial production of abalone by sea ranching is a new concept in Australia and when this project was first conceived, there had been little tangible success to date. There had been some small-scale attempts which had either failed to progress beyond the research phase (James, 2007; James, 2005; Shepherd, et al., 2000), or become commercially viable (Shepherd, et al., 2000). The venture in Flinders Bay, Augusta, Western Australia, upon which the research in this report has focussed, was very much in its infancy. At the start of this project it had succeeded in producing small quantities of greenlip abalone (*Haliotis laevigata*) for the local and export market and production was set to rapidly expand (Adams, 2015; Adams pers. comm.).

Prior to commercialisation, trials were undertaken at the sea ranch at Flinders Bay to determine growth and survival rates of different year classes on several different types of artificial reef structures (Melville-Smith, et al., 2013). The research objectives in this report are amongst the outcomes that were identified for further development by that research.

It was recognised that there was a need to investigate water circulation within Flinders Bay so that the artificial habitats could be positioned so as to maximise the volume of drift algae being carried by water moving across the sea ranch. This has formed the first objective (see Objectives in 2.2) and Chapter 3 of this report.

Knowing the circulation patterns in the Bay and how they change under different conditions and at different times of the year is important. Equally so, it is important to understand any seasonal differences in the species composition of drift algae that provide the food source of the abalone on the farm. This question has partly formed the second objective (see Objectives in 2.2) of this report and is dealt with in Chapter 4.

The other part of the second objective in this report was to understand the functional properties of the drift algae that form the food source for the greenlip abalone on the farm. The outcomes of this research have been reported on in Chapter 5.

Lastly, it was considered important to evaluate the health status of abalone on the ranch (see Objectives in 2.2). It is recognised that the health/condition of the abalone may vary in response to many different factors (e.g. water temperature, food quantity and quality, swell and surge and more). In Chapter 6, the condition of farmed and wild abalone during live transport has been compared using various physiological stress indicators. The thinking behind this as an evaluator of health, has been that abalone in a different states of health would be expected to respond differently to stress and the results are discussed with this in mind.

The results in Chapter 5 on the functional properties of drift algae in Flinders Bay through the year, can also be considered as contributing to knowledge on the health/condition of the abalone on the ranch. Obviously changes in the quality of the food source are likely to impact the health of the farmed abalone. Accordingly, Chapter 5 has taken the seasonal examination of the functional properties of the drift algae one step further, by also examining the moisture and protein content of the ranched abalone. These results have been related to the nutritional quality of the available drift algae collected through the year.

The objectives in this report have therefore been addressed as a series of four separate chapters, each with its own set of authors. It is important to note that in one case (Chapter 3), the chapter is in the form of a thesis and this will likely be revised at some point into one or more publications aimed at peer reviewed journals. Chapters 4, 5 and 6 were used as projects in Masters by coursework degrees and they are in draft form for submission to peer reviewed journals.

2.1 References

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2.2 Objectives

1. To understand the source and seasonal movements of drift-algae across the aquaculture lease in Flinders Bay and relate it to local wind-wave climate.
2. To evaluate the seasonal variations in the biomass, species composition and functional properties of the drift algal species that form the main food source for greenlip abalone on the lease site.
3. To evaluate the health status of ranched greenlip abalone by using various physiological stress indicators and use this status to predict health/condition of the greenlip abalone.

Modified from a thesis submitted in fulfilment of the requirements for the degree of Master of Philosophy of the University of Western Australia

3 Hydrodynamic and dispersal modelling of drift algae in Flinders Bay, Western Australia

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3.1 Abstract

An aquaculture lease in Flinders Bay (Augusta, south-west Australia) produces adult greenlip abalone (*Haliotis laevigata*) through sea-ranching, where juvenile abalone from onshore hatcheries are released into artificial habitats in the open ocean and harvested when grown. The survival and growth rate of the released abalone is therefore highly dependent on the availability of food at the release site. The primary food source of the abalone is drifting marine algae. A dispersal model was configured for this study region to determine the source and seasonal variations in drift algae movement throughout the area. The dispersal model is driven by current and wave fields generated by a hydrodynamic model, which was constructed using the modelling system SCHISM, and forced with local winds, water elevation from OzROMS and wave data from PACCSAP's WW3 wave model. Several scenarios which represent summer and winter conditions were simulated to identify key differences in seasonal drift algae movement.

Strong wave induced currents along a chain of islands extending southeast of Cape Leeuwin was shown to have a major influence on the movement of drift-algae within Flinders Bay. These currents occurred all year and were caused by large oceanic swells generated in the Southern Ocean. During periods of easterly winds, these currents combined with wind driven currents to form a clockwise circulation in Flinders Bay. This effect is most commonly seen during the summer when south-easterly winds are dominant. Drift-algae passing through the lease site in the winter was shown to originate from the south, driven by the strong wave induced currents along the island chain. Summer conditions setup the clockwise water circulation within the bay, allowing particles from the west to reach the site. The number of particles reaching the lease site was higher during winter simulations. This is to be expected with larger south-westerly swells common during winter months providing more energy for algae detachment from reefs and transport by the hydrodynamics.

3.2 Acknowledgements

Firstly, I would like to thank Chari for the many opportunities he has provided me over the last seven years. His advice and guidance has been crucial to my learning and I have made it this far thanks to him. Thanks to Wije and Ivica for all you have taught me, and giving up your own time whenever I had a question or needed a hand. I would like to thank Paul for the countless morning teas, and for being a good friend with a solid understanding of sports. Our conversations played a big part in keeping me sane over the years. Cheers to my office mates past and present (in no particular order): Moritz, Dani, Hadi, Mirjam, Tanziha, Miaoju, Sarik, Ali, Jen, Yasha, Shari, Cyprien, Asha, Soheila, Tharanga and Julia. Other friends at the OI who deserve a special thanks include Ivan, Michele, Ana, Ylva, Flo, Claire, Andrew, Jim, Jim, Good Paul, Bad Paul, Christine, Arne, Thomas, Chantae, Jeremie, Sammy, Phillipa, Luciana, Anas, Liah, Raph, Marion and Anne. Thank you to Mia whom I love, and my parents, who have always supported me, I am eternally grateful. This research was funded by the Fisheries Research and Development Corporation.

3.3 Introduction

3.3.1 Overview

There currently exists an aquaculture lease in Flinders Bay, southwest Australia for the purpose of sea-ranching greenlip abalone. These marine molluscs are raised in onshore hatcheries and released into the wild as juveniles. Once released, they survive off wild food stock within the lease site and are harvested once grown. Their survival and growth rate is therefore very dependent on the availability of drift-algae at the lease site, which is the primary food source for abalone. The overall aim of this research is to determine the seasonal variabilities in the movement of drift-algae in Flinders Bay, and identify the most likely source of drift-algae passing through the aquaculture lease. Movement of drift-algae can be simulated through the use of a dispersal model, which is driven by water current and wave data fields. Therefore, the specific objectives of the study are:

1. Construct a hydrodynamic model of the Flinders Bay region capable of reproducing water circulation and waves accurately.
2. Calibrate a dispersal model to simulate the movement of drift-algae particles within Flinders Bay.
3. Identify seasonal variabilities in the movement of drift-algae and define areas which supply drift-algae to the aquaculture lease.

The structure of this thesis is as follows. 3.4 introduces the context of the study and outlines the current state of research relevant to the project. This includes a brief background on the study site, aquaculture and abalone, macroalgae in Western Australia, hydrodynamic numerical modelling and dispersal modelling. Methods used during this project are described in detail in 3.5, including information on field work conducted as part of this research. The results of the hydrodynamic and dispersal modelling are outlined in 3.6. A discussion of this research and its key findings are presented in 3.7, with conclusions given in 3.8.

3.3.2 Significance

Dispersal models are a valuable tool used to improve understanding on particle movement in coastal and oceanic settings. Their ability to simulate a large range of particles such as drift-algae, plastics and debris, makes them a versatile solution for many problems in coastal management, research and emergency services. Drift-algae movement in the coastal zone is of particular concern to the aquaculture industry as it is a source of food to many high-value products, such as abalone. With the stabilisation of capture fisheries production in recent decades, aquaculture provides opportunities for sustainable growth in both capture and aquaculture industries. This thesis provides crucial information on the movement of drift-algae in Flinders Bay, specifically in regards to an aquaculture lease in the region. The information will assist in the management of an abalone farm in Flinders Bay, while providing a methodology to assess drift-algae movement in the coastal zone.

3.4 Background

This chapter provides a context for the research outlined by this thesis through a review of relevant literature. The study site is described in Section 3.4.1, including information on the wind and wave climate of Flinders Bay. Section 3.4.2 explains the importance of aquaculture and the methods currently being employed in Flinders Bay. A brief description of greenlip abalone is also included in this section. Macroalgae assemblages in southern Western Australia are the topic of Section 3.4.3 whilst hydrodynamic numerical modelling is outlined in Section 3.4.4 and dispersal modelling in Section 3.4.5.

3.4.1 Study Site

Flinders Bay lies to the south of Augusta on the southwest tip of Australia. It includes the area of ocean east of Cape Leeuwin and is bound by offshore reefs to the south. Sandy areas with extensive seagrass beds make up the majority of the bay with rocky reefs located along its perimeter (Melville-Smith, et al., 2013). A chain of small islands extend south east from Cape Leeuwin, providing the bay with some protection large Southern Ocean swells.

Granite headlands and sandy beaches exist along most of the southern WA coastline, where large oceanic swells are common due to the narrow continental shelf and the coast’s exposure to the Southern Ocean (Kendrick, 1999). Granite reefs along the southern coast are interspersed with limestone reefs from Cape Leeuwin to Esperance (Wernberg, et al., 2003). The waters are generally cool but experience warm influxes from the Leeuwin Current which is strongest during autumn and winter. Prevalent southerly winds during summer months slow the Leeuwin Current and force a countercurrent close inshore known as the Cape’s Current (Pearce and Pattiaratchi, 1999). A map of the study site is shown in Figure 3.1 below.

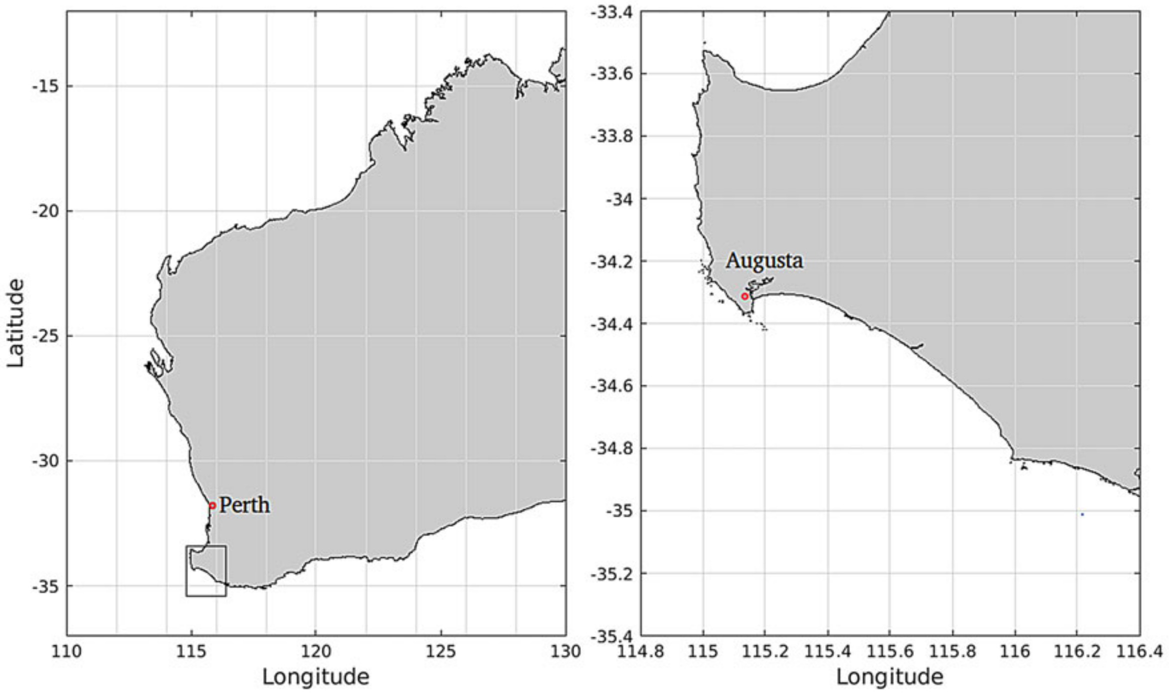


Figure 3.1 Flinders Bay is located south of Augusta on the southwest tip of Australia

The wave climate in Flinders Bay is heavily influenced by the Southern Ocean. Large swells are generated in the latitudes 40 °S – 60 °S where strong winds are common (‘roaring forties’, ’furious fifties’), and travel northeast to the southern coastline of Australia (Kendrick, 1999). During the winter, monthly significant wave heights near Flinders Bay average between 3.5 m and 4.5 m, and events with swells in excess of 6 m are not uncommon. Average significant wave height is reduced during summer months to ~2.5 m, with single events rarely reaching heights greater than 5 m.

Summer months experience prevalent southeasterly winds that generate seas of up to 2 m. Winter seas are more erratic due the varying winter wind conditions. Strong westerly and southwesterly winds are driven by large winter storms that travel eastward in the mid latitudes (30 °S – 60 °S). Periods between storm events experience moderate to strong northeasterly winds.

3.4.2 Aquaculture and greenlip abalone

Aquaculture is the cultivation and farming of aquatic organisms, and relies on outside intervention into rearing to enhance production (FAO, 2014). Globally, per capita fish consumption rose by more than 70 % from the 1960s to 2000 whilst production from capture fisheries has stabilised (FAO, 2014). As global population grows, aquaculture development provides opportunities for sustainable growth in both aquaculture and capture fisheries, by supplementing production from capture fisheries and enhancing wild stocks (Bell, et al., 2008; Delgado, 2003).

In Australia, per capita consumption of fish rose by about 1 % annually from 2003 – 04 to 2013 – 14, despite a reduction in production from fisheries of 19 % over the same period. Aquaculture accounted for 33 % of total Australian fisheries production in 2013 – 14 (ABARES, 2015). The Western Australian aquaculture industry attains the majority of its value from pearls (83 %), whilst edible products (specifically marron, mussels and fish) make up 17 %. Value derived from the edible sector of the WA aquaculture industry decreased in value by 26 %, mainly due to the reduction in the value of farmed fish species (ABARES, 2015).

There currently exists an aquaculture lease in Flinders Bay on the southern coast of WA for the purpose of commercial production of greenlip abalone (Melville-Smith, et al., 2013). Greenlip abalone (*Haliotis laevigata*) are a species of marine gastropod endemic to Australia and are the target of commercial and recreational fishing due to their high value. They are generally found on open rock adjacent to sandy areas in depths of 10 m to 30 m. Their diet consists of macroalgae (commonly referred to as seaweed) with a preference for red species over brown or green (Shepherd, 1973).

Due to their high value, attempts to increase production of abalone through aquaculture is not a new idea. Japanese hatcheries have been producing juvenile abalone to enhance wild stocks for several decades (Honma, 1993; Imamura, et al., 1999), whilst experimental releases of juveniles have occurred elsewhere, including Australia (Shepherd, et al., 2000), the US (Tegner and Butler, 1985), South Africa (Sweijd, et al., 1998) and New Zealand (Roberts, et al., 2007).

Bell, et al. (2008) define three processes where cultured juveniles are released into wild settings. These are restocking, stock enhancement and sea-ranching. Restocking aims to return wild populations to a level where they are able to provide regular and substantial yields; stock enhancement optimizes harvest by increasing the natural supply of juveniles; and sea-ranching allows juveniles to grow in unenclosed environments for future harvest. The work conducted in Flinders Bay falls under the sea-ranching category (Melville-Smith, et al., 2013).

Ocean Grown Abalone (OGA) are the group conducting sea-ranching of greenlip abalone in Flinders Bay. OGA are employing both methods of stock enhancement suggested by Hilborn (1998): the restoration or construction of habitats; and the stocking of juveniles. OGA have constructed artificial concrete habitats that provide shelter for juvenile abalone and have sufficient surface area for colonisation. A habitat unit can be seen in Figure 3.2. The habitat is designed to avoid sand submergence and to circulate water through a central opening that traps algae, two characteristics reported by Melville-Smith, et al. (2013) that improve survival rate of seeded abalone. Currently (August 2017), 7,000 habitats have been deployed in Flinders Bay. The artificial habitats are then seeded by OGA divers with juvenile abalone that were raised in onshore hatcheries in Bremer Bay (Melville-Smith, et al., 2013). These abalone are then left unattended until they reach a size suitable for harvest, feeding primarily on particles of macroalgae that pass through the area.

Figure 3.2 An artificial habitat constructed by OGA. The design prevents sand submergence, traps algae, provides shelter for seeded juvenile abalone and contains sufficient surface area for colonisation

3.4.3 Macroalgae assemblages in southern Western Australia

Macroalgae are macroscopic marine algae that are commonly referred to as ‘seaweed’. They derive their energy through photosynthesis but differ from marine plants, lacking roots, flowers and leafy shoots. They grow in rocky areas, attached to reefs and other hard substrates. This section briefly describes the state of macroalgae assemblages along the southern coastline of Australia, specifically the area surrounding Flinders Bay.

The southern coast of Australia is home to a diverse range of subtidal organisms. This is due to the mixing of cool temperate waters of the Southern Ocean and warm tropical waters brought southward by the Leeuwin Current (Kendrick, 1999). This mixing creates a region where macroalgae species from both tropical Damperian and temperate Flindersian biogeographic regions can coexist (Womersley, 1990). This unique environment creates macroalgae assemblages that are rich in diversity with high levels of endemism (Kendrick, et al., 2004; Phillips, 2001). A recent study identified 289 species of macroalgae from three regions on the west and south coastlines of southern WA (Smale, et al., 2011).

Ecklonia radiata is a species of brown macroalgae that forms large kelp forests along much of the southwest coast of WA, including Flinders Bay (Kendrick, 1999; Phillips, 2001; Wernberg, et al., 2003). These forests exert a strong influence on the structure of the local assemblages, with high heterogeneity of assemblages linked to the density of the kelp forest's canopy (Kendrick, et al., 1999). Where *Ecklonia* is less dominant, other brown species such as *Sargassum* or *Cystophora* form kelp forests with dominant canopies (Kendrick, 1999; Wernberg, et al., 2003). The understory of these canopies contains a large range of smaller macroalgae species (Kendrick, 1999).

Drift algae refers to particles of macroalgae and seagrass that become detached from the sea bed. This can include individual leaves or entire plants removed from their footing. In this study, drift algae refers to particles of macroalgae that move within the water column, excluding seagrasses. Drift algae are a source of food for many marine organisms including abalone. Drift algae collected at the study site were found to have undergone little decomposition, suggesting that the particles had not been detached for long (less than 3 – 4 days).

3.4.4 Hydrodynamic numerical modelling

Numerical techniques to solve for ocean circulation problems have been employed since the 1960s (Bryan, 1969). With rapid development of computer technologies over the past two decades, hydrodynamic numerical models have become an extremely useful tool in improving understanding of ocean processes (Flather, 2000).

The Navier-Stokes equations form the basis of most computational hydrodynamic models (Reeve, et al., 2004). These equations describe the movement of fluids and are derived from Newton's second law of motion. Due to the continuous nature of the Navier-Stokes equations, discretization is used to approximate a solution for any boundary driven problem. There are many different methods of solving these equations that employ discretization, and this is reflected in the wide range of hydrodynamic modelling systems currently available. These methods can be grouped into two classes defined by their discretization of the problem into a model grid; structured and unstructured grids. A number of current modelling systems are available which fall under these two classes, including structured grid models; ROMS (Shchepetkin and McWilliams, 2005) and NCOM (Barron, et al., 2006); and unstructured; Mike21 (Warren and Bach, 1992); ELCIRC (Zhang, et al., 2004); and SELFE (Zhang and Baptista, 2008). Advantages of a structured grid models include less complex formulations and more efficient model algorithms, however unstructured grids allow for varying degrees of resolution without the need for nesting.

SCHISM (Semi-implicit Cross-scale Hydroscience Integrated System Model) is a 3D modelling system which is an updated and enhanced version of the original SELFE model (Zhang, et al., 2016). The model domain is discretized into triangular (or quadrangular) elements in the horizontal with a hybrid SZ coordinates vertical grid. Water velocities are calculated in the horizontal using the finite element method (FEM) and in the vertical using the finite volume method (FVM). The development of SCHISM was undertaken to improve the baroclinic capabilities of unstructured grid models in cross-scale domains (Zhang, et al., 2016).

3.4.5 Dispersal modelling

The pathways of biological and non-biological particles have been studied extensively through the use of dispersal models. These models have been used to track the movement of debris (Pattiaratchi and Wijeratne, 2014), plastic (Reisser, et al., 2013), larvae (Nahas, et al., 2003), turtle hatchlings (Shillinger, et al., 2012) and drift algae (Pattiaratchi, et al., 2011), among others. These models rely on stochastic methods to simulate dispersal of particles, that is particles move according to a behaviour determined from observed data and some random element, generally a diffusivity coefficient.

Pattiaratchi, et al. (2011) constructed a dispersal model to simulate the accumulation and removal of wrack (seagrass and macroalgae particles) on beaches in Port Geographe, WA. This model is driven by depth-averaged current fields along with wave data. Particles move when they are suspended in the water column which occurs when bed stress is greater than some specified critical stress. Once suspended, these particles are forced by water currents, stokes drift and eddy diffusivity in the horizontal. They also move in the vertical according to a sinking velocity and eddy diffusivity.

3.5 Methods

This chapter presents the methods employed throughout this study. Fieldwork conducted as part of this research is outlined in Section 3.5.1. Construction and validation of the hydrodynamic model is described in Section 3.5.2, while the dispersal model is covered in Section 3.5.3. Final simulations are given in Section 3.5.3.3.

3.5.1 Fieldwork

Several experiments were conducted in the field to gather data for this research. This includes two ADCP deployments and algae critical stress experiment. The methods used and the purpose for this fieldwork is presented in this section.

3.5.1.1 ADCP deployments

Two ADCP (Acoustic Doppler Current Profiler) deployments were carried out in 2015. The first occurred on the 30th March and the second on the 24th of August. Both deployments were conducted using a Workhorse Sentinel ADCP from Teledyne RD Instruments, and hourly measurements of water height, currents and wave conditions were collected over a two month period.

An ADCP utilizes the Doppler effect to measure water currents over a certain depth range. Transducers on the ADCP emit an audio pulse and are able to measure the frequency shift of the returning echo in ‘beams’ above the transducers. Water velocities can then be calculated from these frequency shifts. The Workhorse Sentinel ADCP deployed during this project also allows for wave measurements. Distance to the water surface is measured several times over a short period, an estimation of wave height is then found using a peak estimation algorithm. Wave direction can then be determined through cross-correlation of wave height and water velocities.

For the first deployment, the ADCP was bolted to a concrete mooring onshore with help from the OGA habitat construction team. The mooring was then lowered to the sea bed at the most southern and western point of the OGA lease site. An image of the ADCP attached to the mooring can be seen in Figure 3.3. The ADCP was recovered after two months by an OGA diver. For the second deployment, the ADCP was reattached to the mooring by a diver as the mooring had remained on location. Recovery of the second deployment was handled in the same way as the first.

Figure 3.3 An ADCP attached to a concrete mooring at the OGA habitat construction yard

Both deployments were setup to measure horizontal and vertical currents in 0.5 m intervals above the ADCP, water heights and wave data, including wave heights, periods and directions. Wave data was collected at one hour intervals and all other data was collected at 50 minute intervals. These sample rates provided high resolution data without draining battery reserves before the end of the two month deployment period.

3.5.1.2 Critical stress experiment

An experiment was conducted to determine the critical stress exerted on certain macroalgae species required to raise them into suspension within the water column. Bottom stress induced by water currents can be calculated using the following equation:

$$t = \frac{(u^2 + v^2)k^2}{\ln^2(0.37\, h/z_0)} \tag{3.1}$$

where, t is the shear stress, u and v are depth averaged horizontal velocities, k is the von Karman coefficient (0.41), h is the mean water depth and z_0 is the roughness length. By observing drift algae samples, and measuring the water velocity at the moment of suspension, the critical shear stress can be determined.

An apparatus was constructed that allowed drift algae samples to be observed on the sea bed while measuring the surrounding water currents. Figure 3.4 shows an image of the apparatus, constructed from a metal base and fitted with an ADCP and a GoPro camera. The ADCP (Nortek Aquadopp HR Profiler) was setup to measure water velocities in 2.5 cm bins from the seabed to its transducers. Drift algae samples are placed below the ADCP and are filmed by the GoPro camera. A wrist-watch was also attached to the apparatus and was synced with the ADCP. When the algae sample is suspended in the water, as seen on the video image, the surrounding water velocities at that time can then be determined by referring to the ADCP data.

Figure 3.4 An apparatus designed to measure water currents while observing the suspension of drift algae samples. Included in the setup are a Nortek Aquadopp HR Current Profiler, a GoPro Camera and a stop watch.

Four species of algae were used for this experiment, each taken from the lease site at Flinders Bay. These species were *Ecklonia radiata*, *Amphibolis griffithii*, *Platythalia angustifolia* and *Echinothamnion sp*. The results of the experiment are presented in Table 3.1 below, including the measured velocities at time of suspension and the critical stress determined from these values.

Table 3.1 Critical stress required to suspend drift-algae particles. Values were determined through experimentation

Algae Species	**Water Velocity (ms^{-1})**	**Critical Stress ($\times 10^{-5}$ Nm^{-2})**
Ecklonia radiata	0.06	4.2
Amphibolis griffithii	0.07	5.6
Platythalia angustifolia	0.08	7.4
Echinothamnion sp.	0.07	5.6

3.5.2 Hydrodynamic Modelling

To generate current and wave fields required to run the dispersal model, a hydrodynamic numerical model was constructed using the SCHISM modelling system. SCHISM is described in Section 3.4.4.

3.5.2.1 Model construction

The model grid was constructed using coastline data collected using the NOAA Coastline Extractor. The grid boundaries were aligned to intersect data points of PACCSAP's (Pacific-Australia Climate Change Science and Adaption Planning programme) WW3 model, which supplied wave data to force the hydrodynamic model. The grid included approximately 100 km of fetch from Flinders Bay to accurately capture the effect of southeasterly winds. The model has a resolution of 1.5 km, increasing to 10 m in Flinders Bay. The model grid can be seen in Figure 3.5.

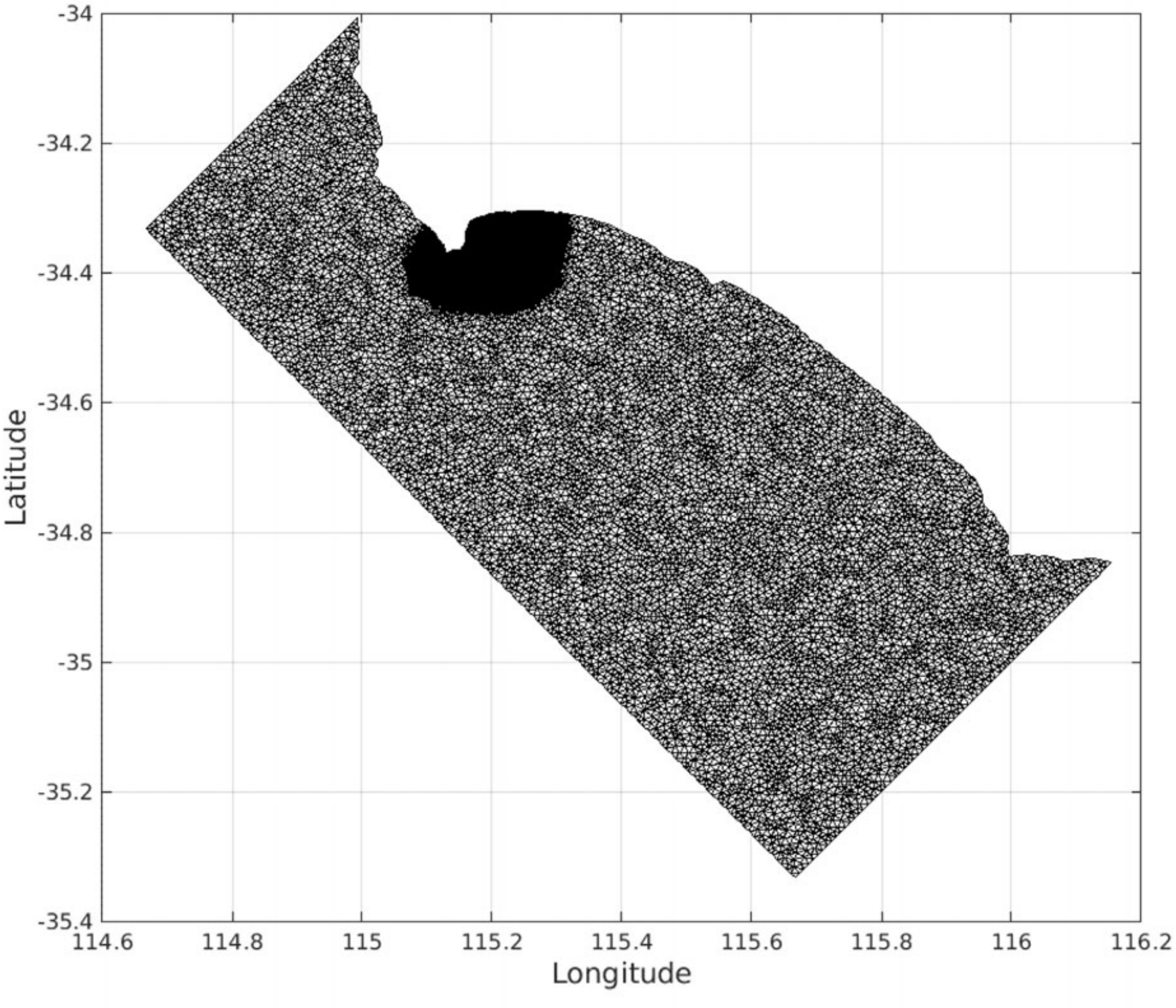


Figure 3.5 The unstructured grid used for the hydrodynamic numerical model

Bathymetry data was supplied by Geosciences Australia with a resolution of 250 m. Higher resolution hydrographic survey data was provided by the Department of Transport (Spatial Information Branch). This data was interpolated onto the grid to complete model construction. Model bathymetry is shown in Figure 3.6. The model was forced with water elevation obtained from OzROMS, local wind measured at the Cape Leeuwin meteorological station and wave data from PACCSAP's WaveWatchIII model.

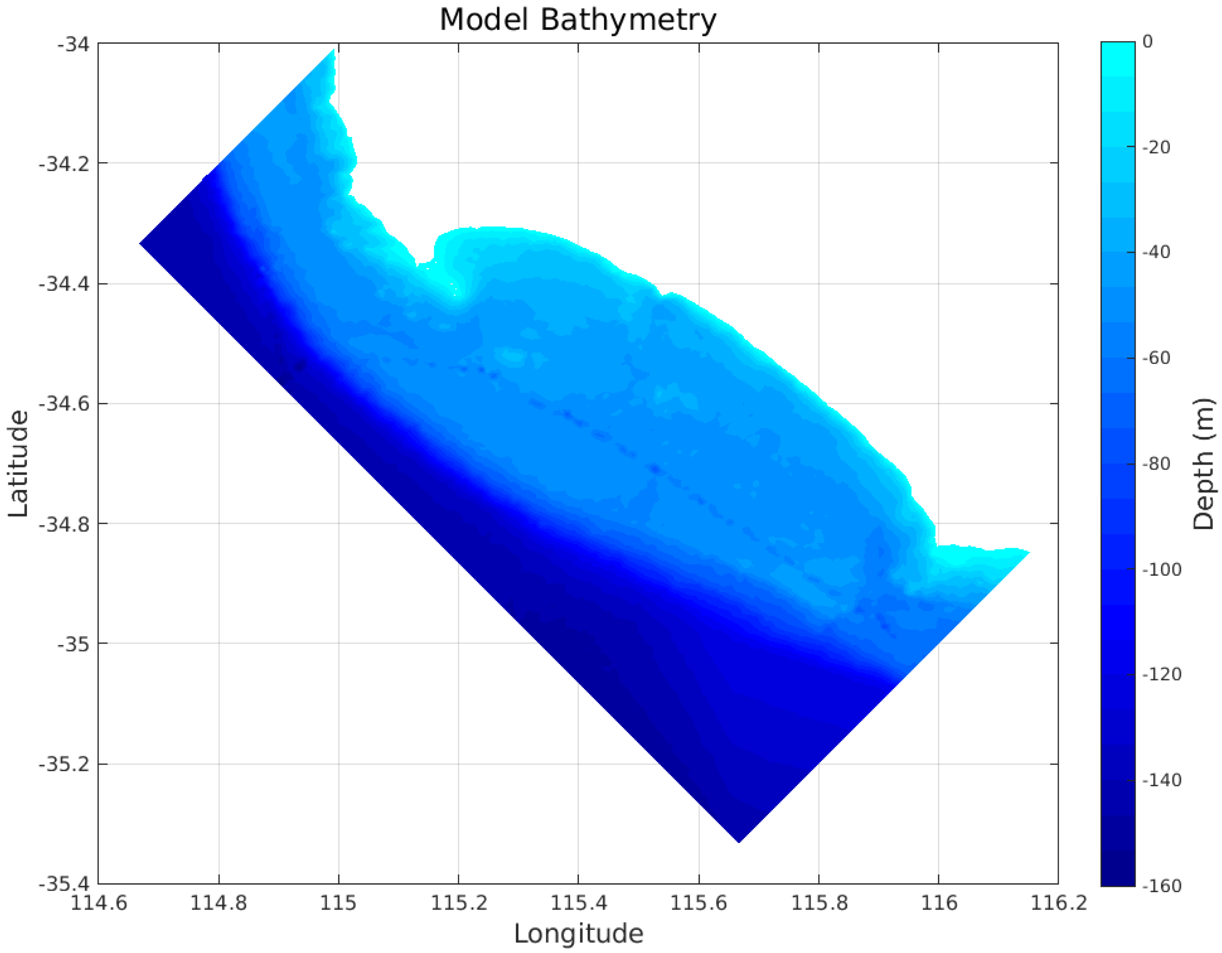


Figure 3.6 Model bathymetry

3.5.2.2 Model validation

The model was run for the period covering the first ADCP deployment. Water elevation, currents and significant wave height predicted by the model were compared to measured data collected by the ADCP. This was done to ensure model simulations were representative of actual conditions in Flinders Bay. These comparisons are shown in Figure 3.7. A correlation coefficient was calculated for each validation which represents the model skill. This value was calculated using the following equation:

$$Skill = 1 - \frac{\sum |X_M - X_O|^2}{\sum (|X_M - \bar{X}_O| + |X_O - \bar{X}_O|)^2} \qquad 3.1$$

where X_M is the model predicted variable, X_O is the observed data and the overbar represents time-averaging. A model skill of 1 would denote perfect correlation and 0 would denote no correlation.

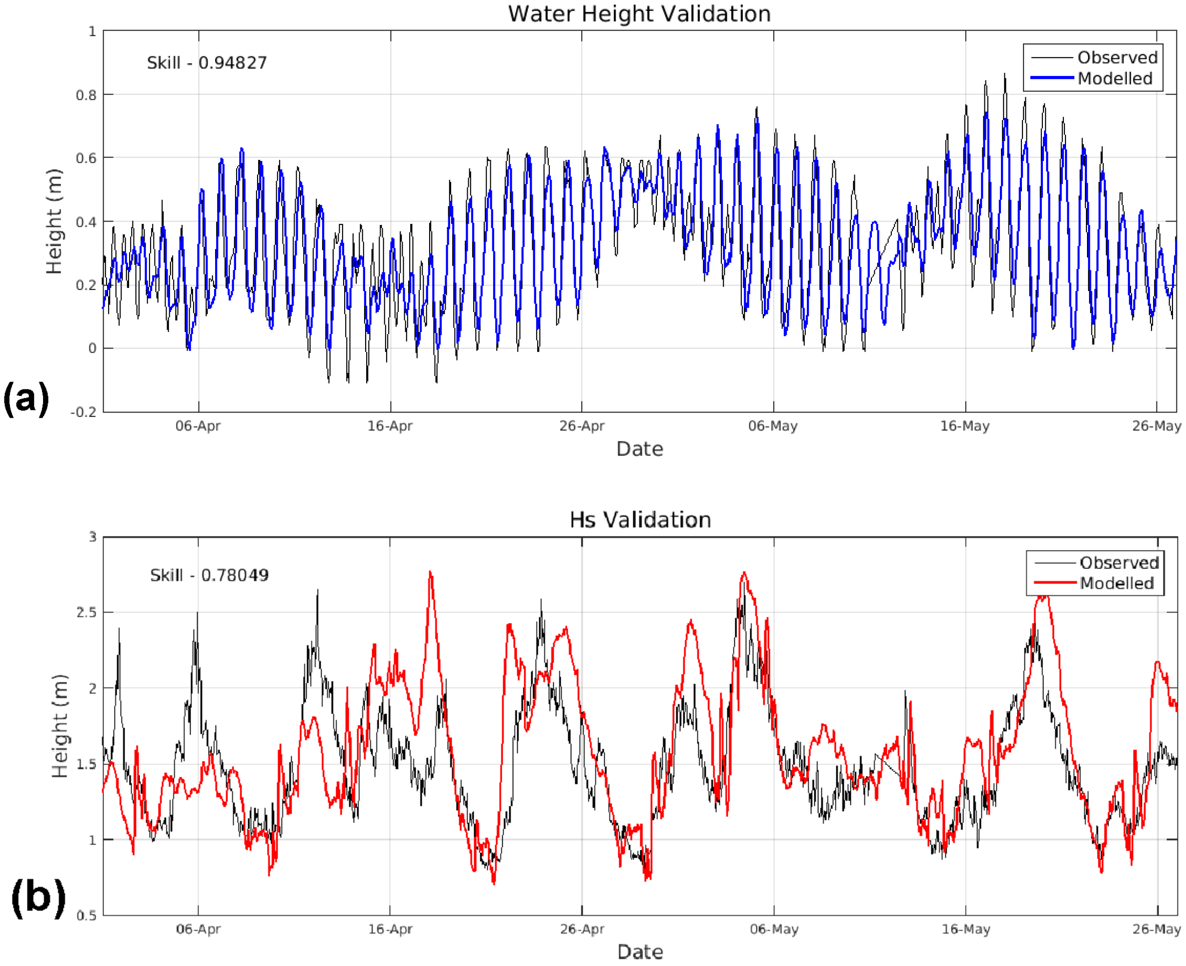


Figure 3.7 Model validation of water height (a) and significant wave height (b). Measured data is shown in black with water height in blue and significant wave heights in red

Overall the model does exceptionally well at capturing the water elevation, reproducing water heights with a skill of ~0.95. Some individual peaks during high tides are missed but the model does well at capturing the residual element created by storm surges and shelf waves. The significant wave height is also well represented by the model with a skill of ~0.78.

3.5.2.3 Simulations

An analysis of wind and wave data was conducted to distinguish metocean characteristics that typify summer and winter conditions. Five periods between June 2014 and July 2015 were identified as being representative of these typical conditions. Simulations varied in length from 12 to 18 days. This allowed for sufficient drift algae movement as samples taken on site were very fresh, suggesting they had not been shed in more than a few days past. Three winter and two summer periods were modelled, covering six winter storms and two periods of summer southeasterly winds. These simulations are hereafter referred to as W1, W2, W3, S1 and S2, where ‘W’ and ‘S’ signify winter and summer.

3.5.3 Dispersal modelling

This section outlines the methods used to model the dispersal of drift algae in Flinders B.

3.5.3.1 Dispersal model concept

The dispersal model employed for this study was constructed by Pattiaratchi, et al. (2011) for their research into wrack accumulation and removal on beaches near Port Geographe. The model is forced with depth-averaged currents and wave data. The model concept is shown in Figure 3.8.

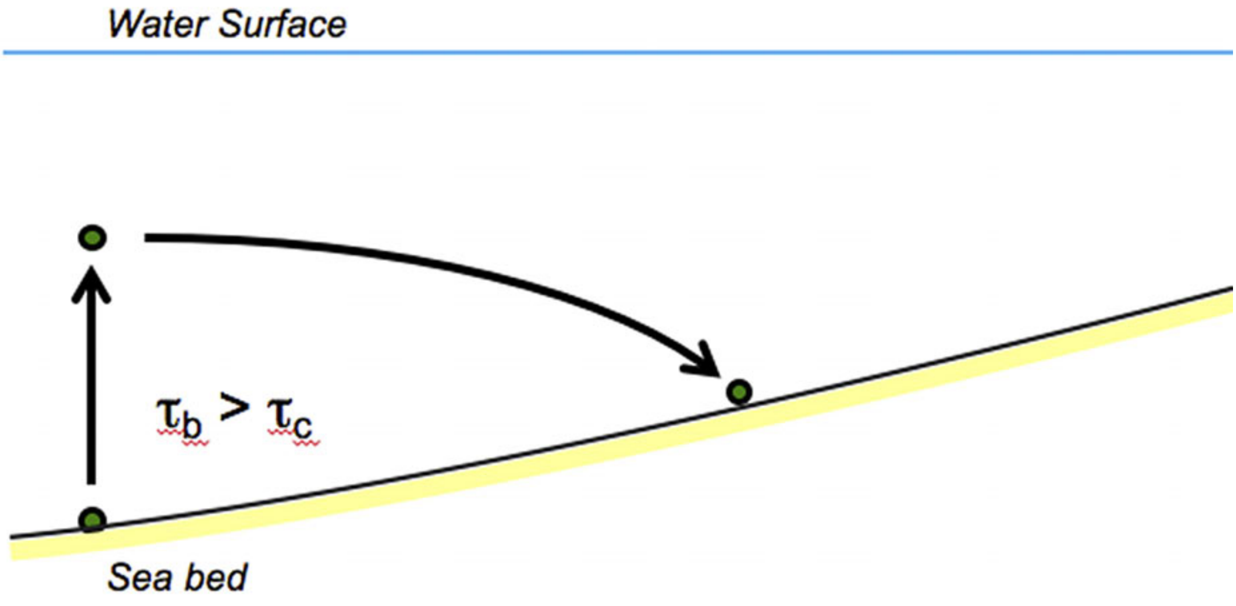


Figure 3.8 Dispersal model concept. Particles are suspended in the water column when bed stress is greater than critical stress (a). Once suspended, particles are driven by water currents, stokes drift and eddy diffusion in the horizontal, and sinking velocity and eddy diffusion in the vertical (b)

The dispersal model runs on the grid constructed in Section 3.5.2.1. Particles are seeded within the model on the sea bed. These particles are then suspended within the water column when bed stress surpasses a pre-defined critical stress. Bed stress is determined for each grid element using equation 2.1.

Once suspended, particles move based on the following equations

$$\delta x = u_z dt + u_s dt + a\sqrt{6E_h dt} \tag{3.3}$$

$$\delta z = w_s dt + a\sqrt{6E_v dt} \tag{3.4}$$

where, δx and δz are movement in the horizontal and vertical planes, u_z is the depth based current velocity, u_s is Stokes drift, a is a normally distributed random number, w_s is the particle's sinking velocity and E_h and E_v are Eddy diffusivity in the horizontal and vertical respectively. Velocity due to Stokes drift (u_s) is calculated using the equation below:

$$u_s = \frac{1}{8}\omega k H^2 \frac{\cosh(-2kz)}{\sinh^2(kh)} \tag{3.5}$$

where, ω is the angular frequency (rads^{-1}), k (radm^{-1}) is the wave number and H is the wave height (m). The model does not take into account leaf shedding or uprooting once particles are seeded. That is, the model assumes that all seeded particles are free to move and are not attached to the sea bed.

3.5.3.2 Drift algae particle seeding locations

Particles were seeded in the model based on an analysis of macroalgae beds in Flinders Bay. Satellite imagery from Nearmap and Google Earth was examined to identify macroalgae habitats. Further, Hovey and Bellchambers present a detailed map of coastal habitats in southwest Australia, including Flinders Bay. These resources were used to generate a map of likely macroalgae habitats within the model domain. Particles were then seeded randomly within these areas. A map of the particle seed locations over the model are shown in Figure 3.9.

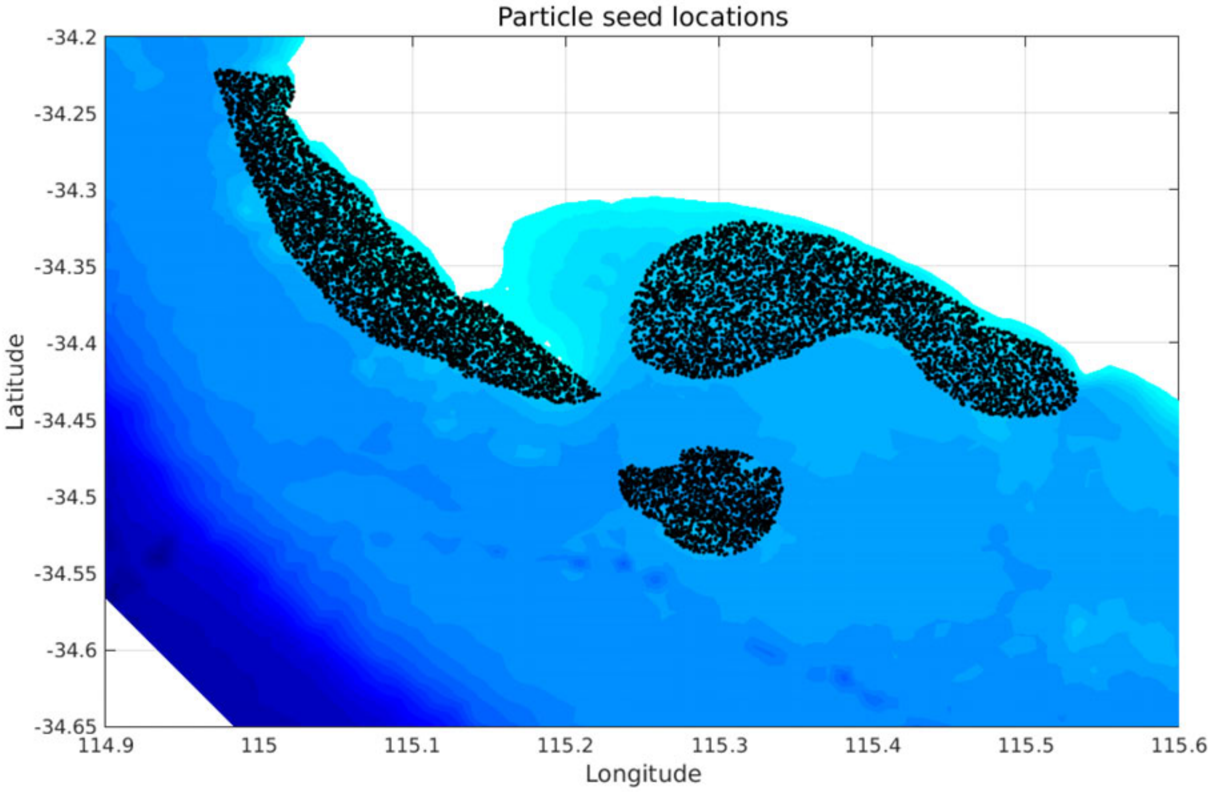


Figure 3.9 Particle seed locations within the model domain.
Black areas show seeded particles over the model bathymetry

3.5.3.3 Model setup and simulations

The model was run for each simulation specified in Section 3.5.3, and was forced by two-dimensional depth-averaged water currents produced by the hydrodynamic model. Significant wave heights and peak wave periods and directions from the hydrodynamic model were also used to force Stokes drift. 15,000 particles were seeded every day for the duration of each simulation in locations shown in Figure 3.9. Critical shear stress was taken as 5×10^{-5} N^{-2}, a mean value determined by experiments described in Section 3.5.1.2 and similarly reported by Pattiaratchi, et al. (2011). Sinking velocity was taken as 0.18 m^{-1}, which was determined by Pattiaratchi, et al. (2011) in laboratory conditions. Eddy diffusivity was set to 0.002 m^2s^{-1} for both horizontal and vertical values.

3.6 Results

This section presents an overview of the results obtained from this study. All simulations are referred to by an abbreviated form, as specified in Section 3.5.2.3.

3.6.1 Summary

For each simulation, strong northward currents were generated across the chain of islands to the southeast of Cape Leeuwin. These were more intense during winter events where significant wave heights were larger. Winter simulations saw large amounts of particles travelling from the western seed location through the lease site. This was highest during storm events and lowest when northeasterly winds occurred. During summer simulations, southeasterly winds and wave action across the islands resulted in a clockwise circulation of water within Flinders Bay. This circulation swept the majority of particles to the west of the lease site, except for those particles seeded to the east. During periods of decreased circulation strength, particles passing through the lease site were more likely to have originated from the western seed location. A planned extension to the main lease site was analysed to identify areas of high drift-algae volume in the new lease area.

3.6.2 Hydrodynamic model results

Summer simulations experienced a clockwise circulation of water in Flinders Bay despite strong southeasterly winds. This circulation appeared to be forced by wave induced currents along the chain of islands extending from Cape Leeuwin. To test this hypothesis, wave forcing was removed and the simulations were run again. Water currents were then compared to initial simulations which contained wave forcing. This comparison is shown in Figure 3.10 (a) with wave simulation and (b) without wave simulation.

The Figure shows significantly stronger currents throughout the model domain, especially at the chain of islands, with waves included Figure 3.10 (a). Clockwise circulation did not occur in summer simulations where wave forcing was removed (Figure 3.10 (b). This confirms that the circulation experienced during the two summer simulations is driven by the combination of currents, forced by southeasterly winds, and wave action along the chain of islands.

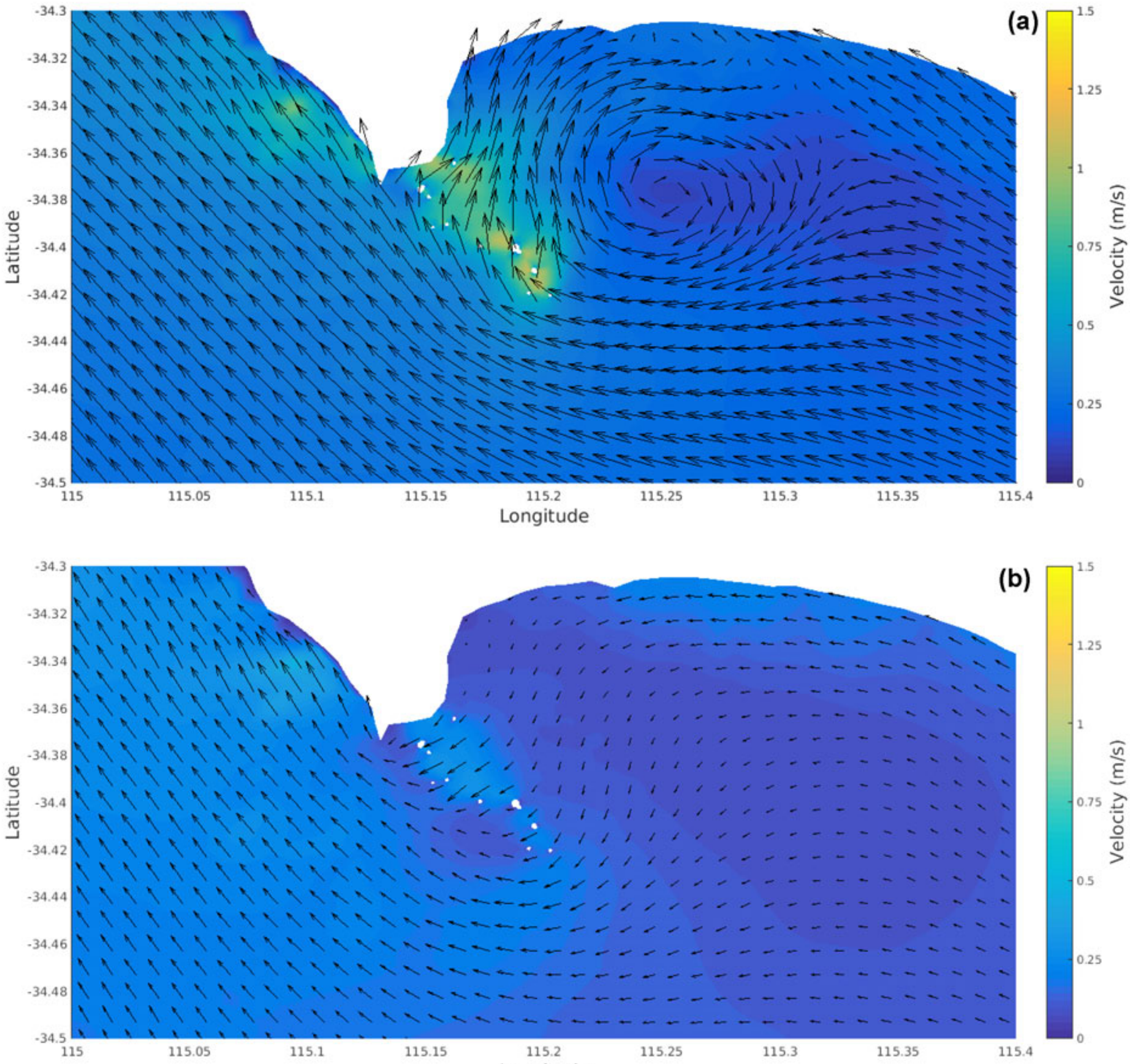


Figure 3.10 Comparison of currents in Flinders Bay,
Western Australia, simulated with waves (a) and without (b)

Winter conditions predicted by the model were as expected, with currents moving eastward with high velocities over the shallow island chain. One simulation experienced the clockwise circulation experienced for both summer simulations. The mean currents for the winter simulations are shown in Figure 3.11.

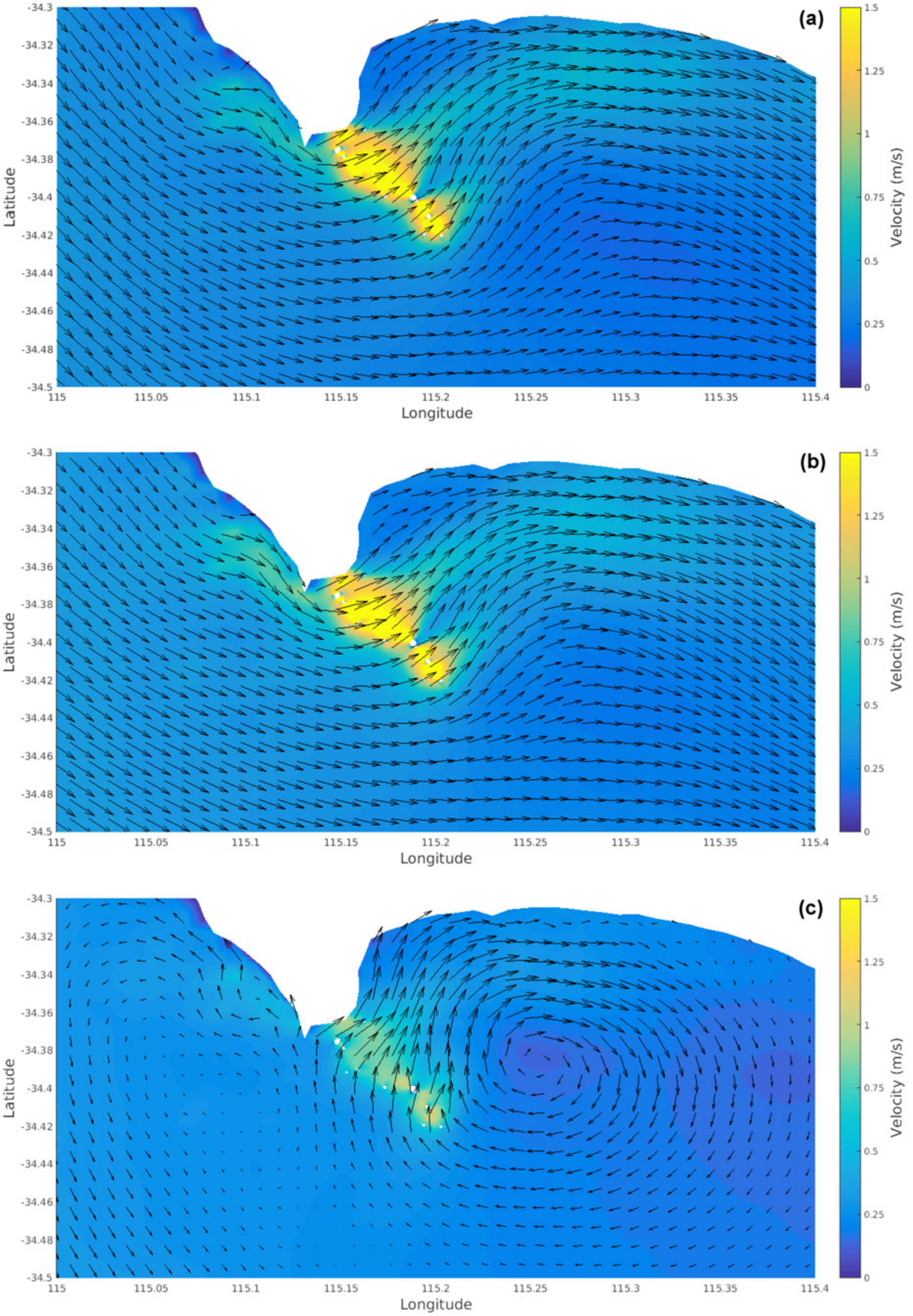


Figure 3.11 Mean currents for winter simulations W1 (a), W2 (b) and W3 (c)

The circulation in W3 is similar to that experienced in both summer simulations, and is caused by an extended period of easterly winds, which are not common during the winter (Figure 3.11). Strong currents across the chain of islands extending southeast from Cape Leeuwin are common for all simulations, less so in summer. These wave-induced currents, when combined with easterly winds, cause a clockwise circulation within Flinders Bay. This circulation is most common in summer months when prevalent southeasterly winds occur.

Summer and winter simulations show water flowing generally northwards over the lease site. The ADCP data was checked to see if this was indeed the case for the periods of the two deployments. A vector plot of depth-averaged currents was generated, essentially showing the movement of a particle experiencing currents equal to those measured by the ADCP. This plot can be seen in Figure 3.12.

Supporting the hydrodynamic model prediction, vector plots of observed currents show a northeasterly direction.

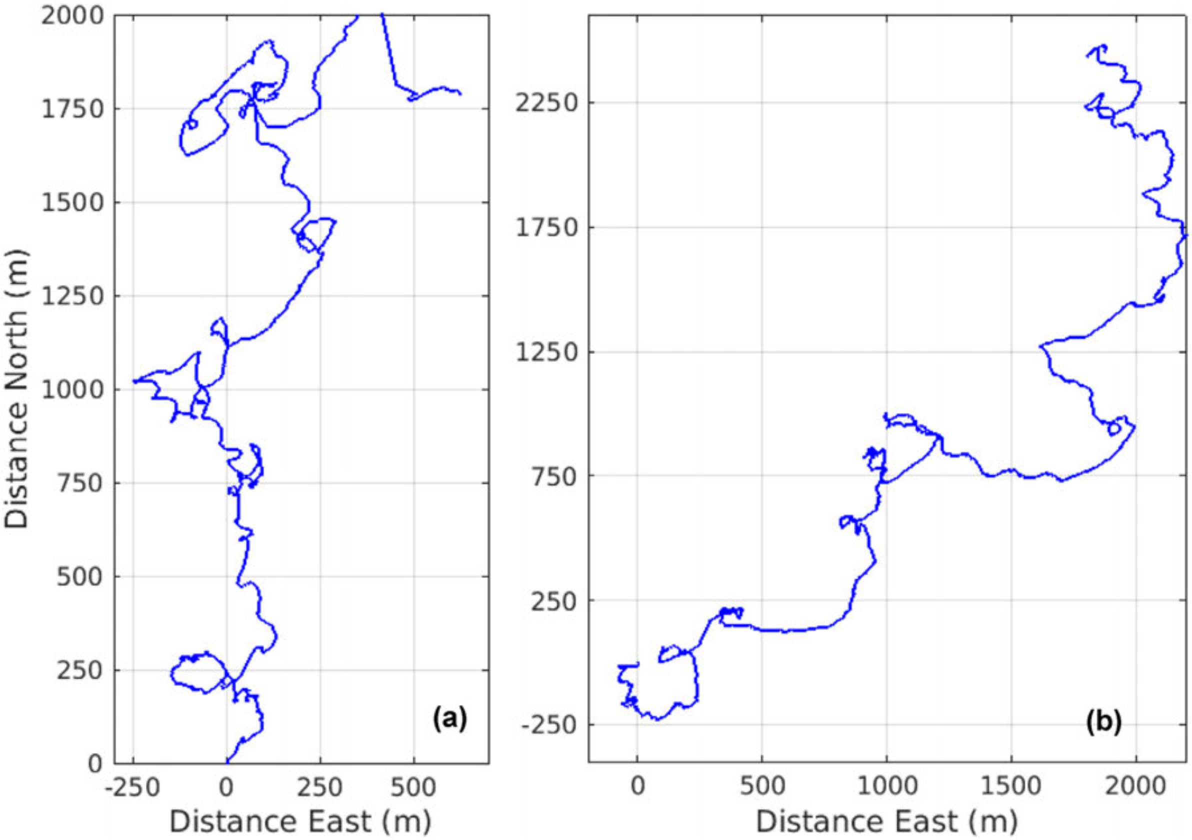


Figure 3.12 Vector plot for the two ADCP deployments. Measured currents flow to the north during the April/May deployment (a) and to the northeast during the September/October deployment (b)

3.6.3 Dispersal model results

The dispersal model results were analysed to identify particles which passed through the lease site. These particles were traced back to their original seeding locations to determine the source of drift algae. This section outlines the results from the dispersal model in two parts. Results from winter simulations are given in Section 3.6.3.1 and summer results are outlined in Section 3.6.3.2.

3.6.3.1 Winter simulations

Figure 3.13 shows the movement of particles within Flinders Bay, as predicted by the dispersal model for W1, highlighting those particles which passed through the main lease site. For the winter simulations, only particles seeded in the western most seeding location are examined. This was done as no particles seeded in the eastern or southern locations passed near the lease site during winter simulations

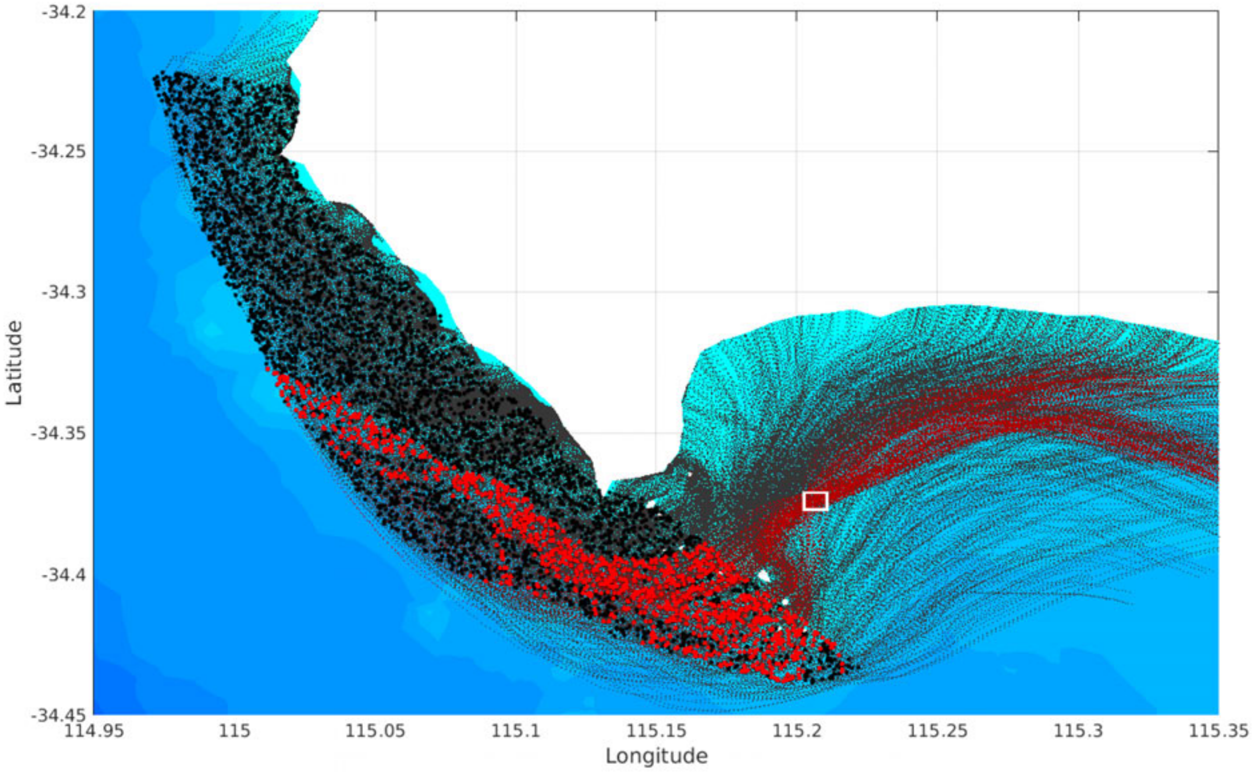
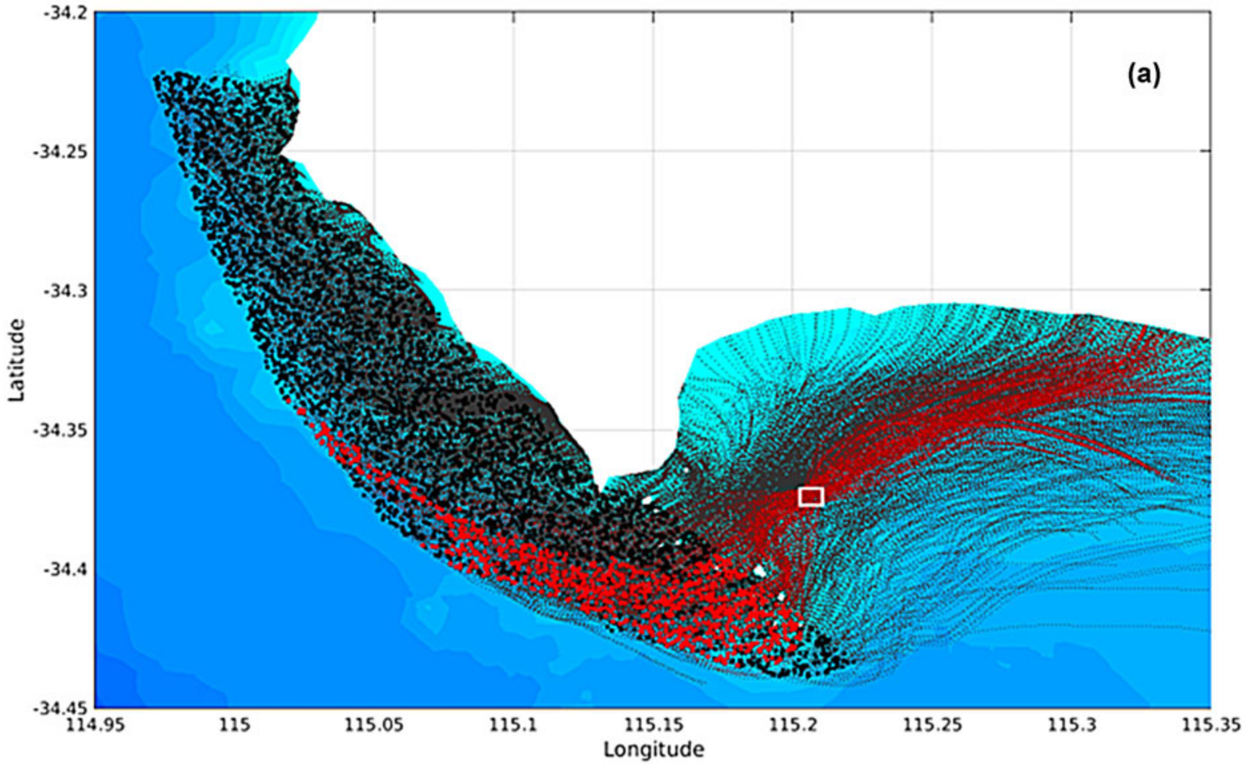


Figure 3.13 Particle movement predicted by the dispersal model for W1. Black dots represent the original seeding locations, with particle paths shown as black lines. Particles that passed through the lease site are highlighted in red

As seen in Figure 3.13, the majority of particles that pass through the lease site originate to the south of Cape Leeuwin. This is also the case for W2 and W3, plots of which can be seen in Figure 3.14.



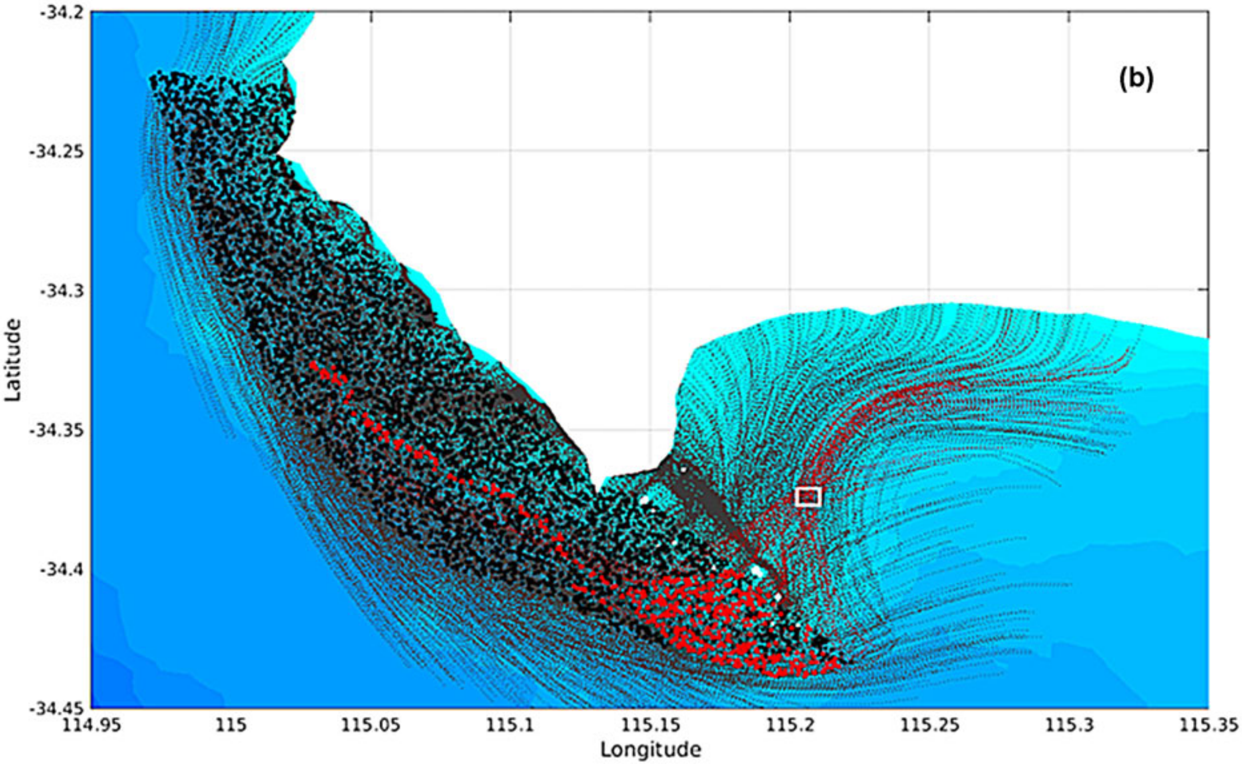


Figure 3.14 Particle tracks for W2 (a) and W3 (b)

Table 3.2 lists the percentages of seeded particles that pass through the lease site for each of the winter simulations. Seeded particles are grouped according to their original seeding location

Table 3.2 Percentage of seeded particles that pass through the lease site during winter simulations, grouped according to original seeding location

Simulation	Seed West	Seed South	Seed West
W1	2.88 %	0 %	0 %
W2	4.27 %	0 %	0 %
W3	1.54 %	0 %	0 %

The percentage of seeded particles that pass through the lease site vary greatly between winter simulations. To determine the cause of this difference, metocean conditions in Flinders Bay at the time of each simulation were compared. Winds experienced during each simulation are shown in Figure 3.15 and waves in Figure 3.16.

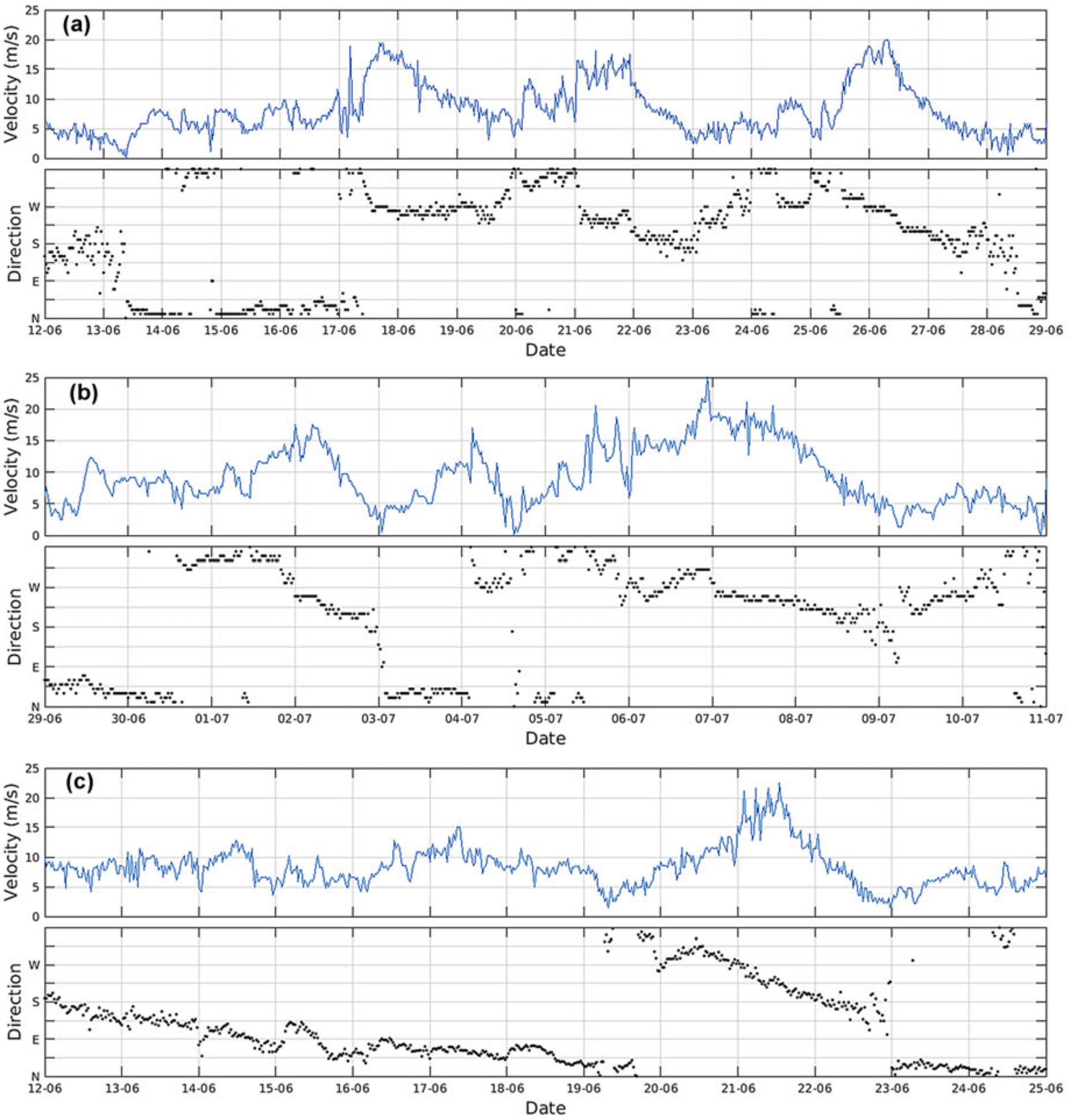


Figure 3.15 Wind velocities and directions for simulations W1 (a), W2 (b) and W3 (c). Wind velocities are plotted in blue with directions in black

Of the three winter simulations, W2 experienced the largest storm event with strong, predominantly west and southwesterly winds. W1 contained three large events, defined by westerly winds, whilst W3 saw one storm event amongst mostly strong easterly winds. Waves were most intense in W2 with mean heights inside Flinders Bay 10 – 20 cm larger than those in W1. Mean wave heights to the south of flinders were approximately 40 cm larger in W2 than W1. W3 experienced the smallest mean wave heights, approximately 1.8 m less than those predicted by W2 south of the islands and between 30 – 40 cm inside Flinders Bay. Figure 3.16 shows the differences in significant wave heights between W2 and W1; and W2 and W3.

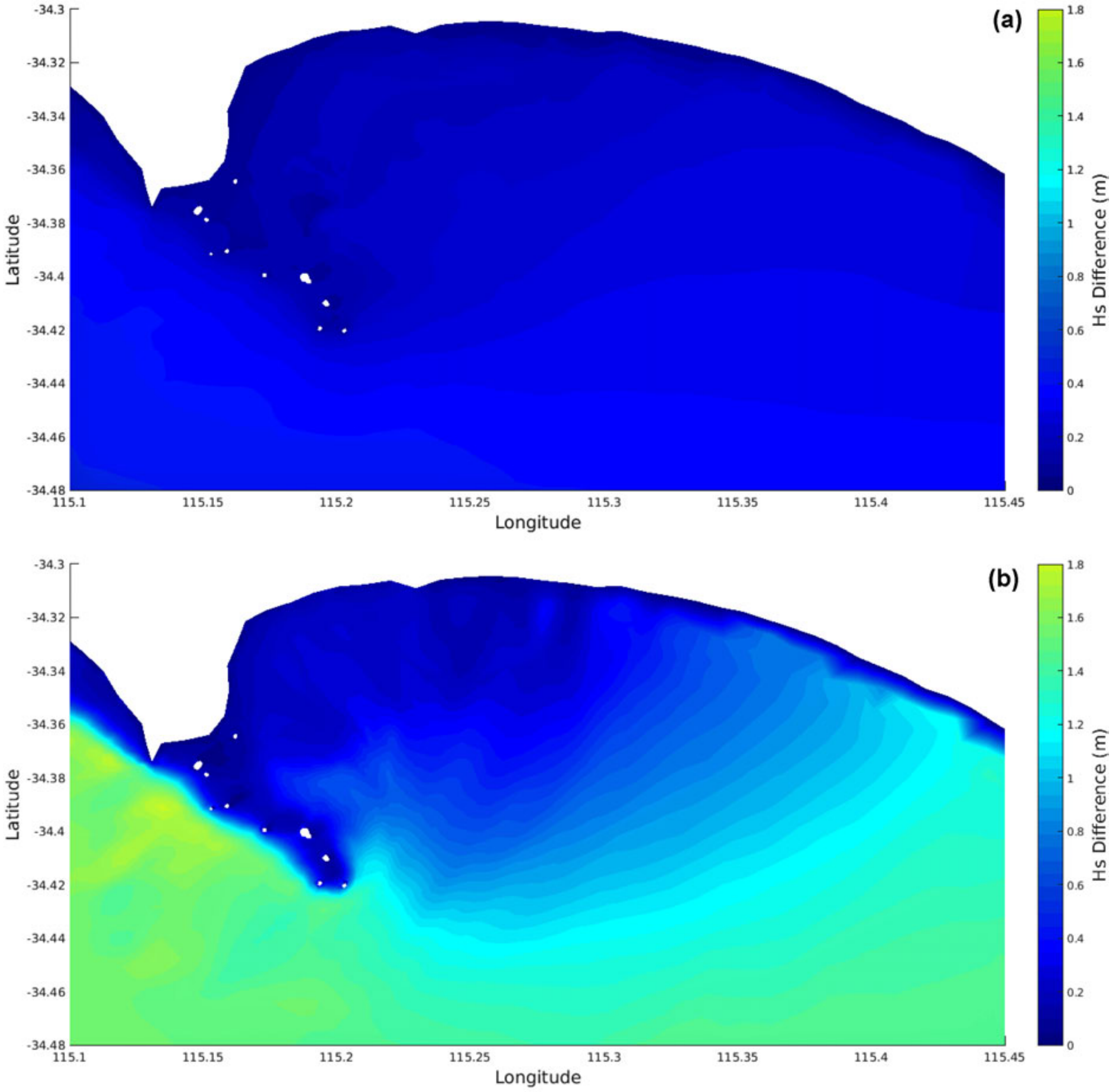


Figure 3.16 Mean significant wave height difference between W2 and W1 (a); and W2 and W3 (b)

The percentage of seeded particles which pass through the lease site is directly related to the intensity of the wind and wave conditions of the simulation. W2 had the strongest winds and largest wave heights, resulting in the largest volume of particles passing through the lease sight. Alternatively, the lowest percentage of seeded particles which passed through the lease site occurred in W3, which experienced the calmest winds and swells.

W3 experienced the lowest number of particles passing through the lease site, partly due to the predominantly easterly winds. These winds reduced the supply of particles travelling into Flinders Bay. Particles seeded to the south of the chain of islands were initially transported westward. This movement was reversed when a storm passed through the area, bringing westerly and southerly winds. Westerly seeded particles then behaved as those in W1 and W2. Particles seeded to the east moved slowly toward the lease site during the easterly winds, but were swept out of the area when the storm arrived.

Results from all three winter simulations were combined and used to map areas of ‘good supply’. That is, areas where seeded particles were able to travel through the lease site often. This map can be seen in Figure 3.17 below.

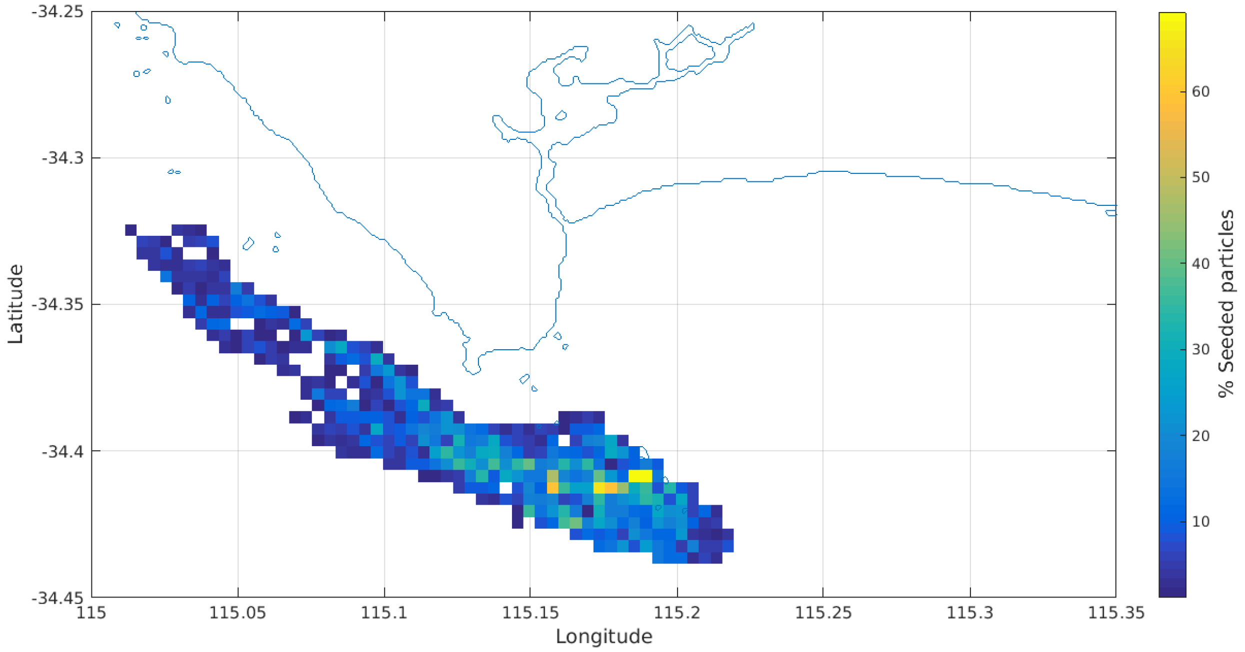


Figure 3.17 Heat map showing seeding areas of good supply for winter simulations. Colours represent the percentage of particles seeded in that area that passed through the lease site

The map above highlights areas which contribute a large percentage of their seeded particles to the supply of the lease site. The area just south of the largest island has a supply percentage of almost 70 %. This indicates that 70 % of the particles seeded in this location passed through the lease site at some point during the winter simulations. The most productive area is that south and west of the island chain. Areas to the west of Cape Leeuwin are less productive, as particles are more likely to accumulate on the western shoreline before reaching the islands.

A map of the lease site was also generated to see which areas observe the most number of particles passing through.

3.6.3.2 Summer simulations

Figure 3.18 shows the particle tracks for S1 and S2. Particles seeded in the southern seed location are not discussed in this section as they had no bearing on model results.

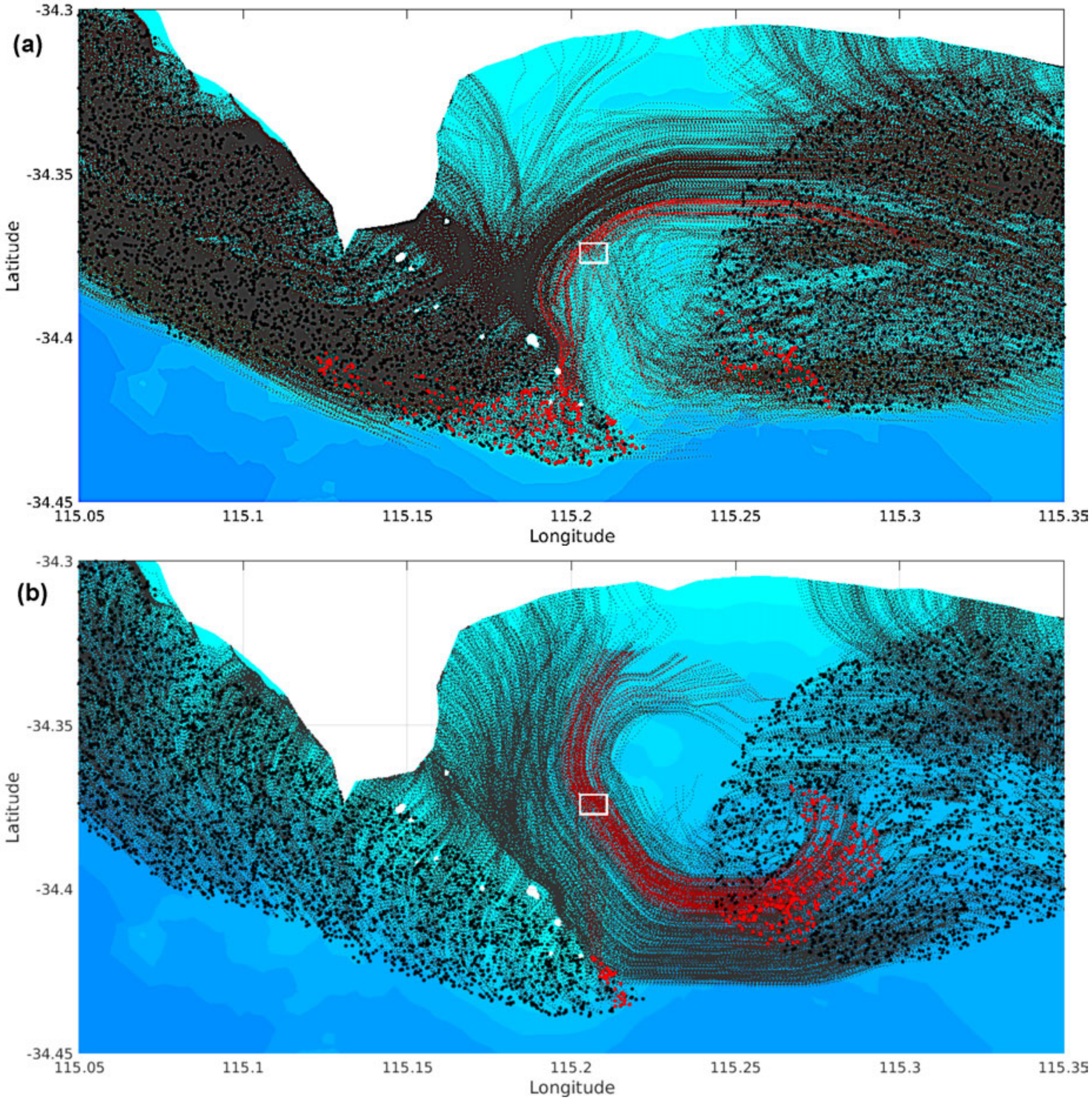


Figure 3.18 Particle tracks for S1 (a) and S2 (b)

Particles seeded to the east of Cape Leeuwin are now able to pass through the lease site due to water circulation within Flinders Bay. The proportion of seeded particles that pass through the lease site is approximately 2.5 times larger during S2 than S1, the majority of which originate to the east. These proportions are listed in Table 3.3 for both summer simulations

Table 3.3 Percentage of seeded particles which pass through the lease site during summer simulations

Simulation	Seed West	Seed South	Seed West
S1	0.85 %	0 %	0.23 %
S2	0.17 %	0 %	2.48 %

The water circulation in Flinders Bay appears to have a major effect on the source of particles passing through the lease site. S1 and S2 both show eastern seeded particles reaching the lease site. However, there remains a large difference in the number and source of particles between summer simulations. To determine the reason behind these differences, mean water currents for each simulation were compared. Figure 3.19 shows this comparison, where S1 mean currents were subtracted from those predicted by S2. Mean significant wave heights were also compared, a comparison of which is shown in Figure 3.20.

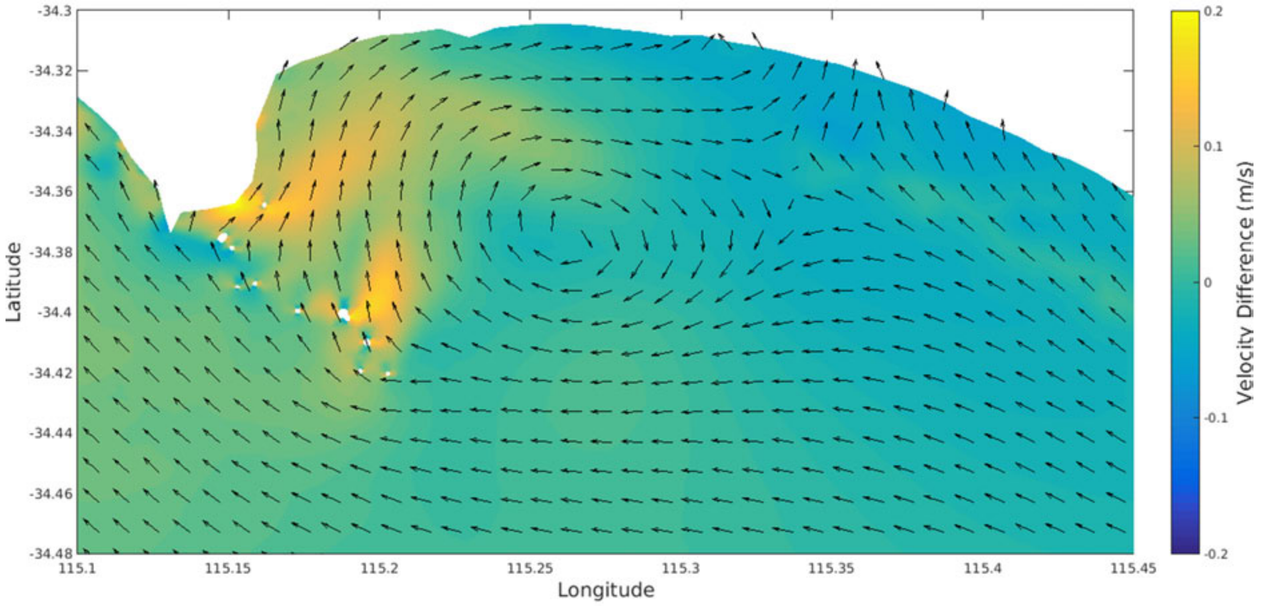


Figure 3.19 Difference in mean currents,
showing the increased clockwise circulation experienced in Summer 2 simulation

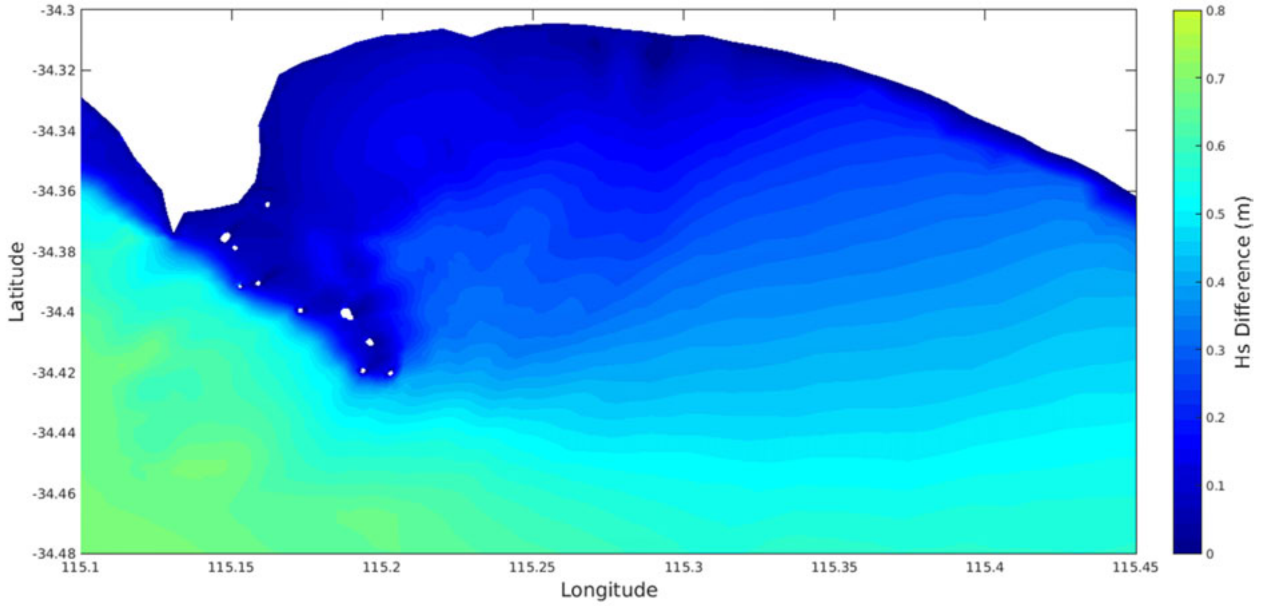


Figure 3.20 Comparison of mean significant wave heights.
Mean Hs from S1 was subtracted from S2, showing the larger wave heights experienced during S2

As shown in Figure 3.19, S2 was subjected to a stronger circulation. Larger wave heights in S2 caused increased water velocities moving northward from the chain of islands. These currents drive the circulation within Flinders Bay during periods of southeasterly winds, transporting seeded particles from the east to the lease site. The number of particles moving within this circulation is related to its strength, with an increased volume when circulation is strong, as seen in S2 compared to S1.

Particles seeded to the south of the island chain were less likely to pass through the lease site for the summer simulations. This was due to the circulation mentioned above. Particles which passed through the island chain were caught on the edge of the circulation and transported west of the lease site. However, when winds shifted westward, circulation strength decreased. This allowed particles from the south to pass closer to the lease site.

S2 experienced two days of light to moderate southwesterly and westerly winds. During this period, circulation strength within Flinders Bay decreased and particle tracks shifted eastward, allowing some particles to pass through the lease site. Three days of moderate to strong westerly winds occurred during S1, this not only weakened the circulation strength, but drove more southerly seeded particles towards the chain of islands, where they were carried into Flinders Bay by strong, wave driven currents. The combination of a weakened circulation and a greater supply of particles across the island boundary resulted in a greater number of western seeded particles passing through the lease site during .

Results from both summer simulations were combined to identify areas of good supply, as done with winter simulations.

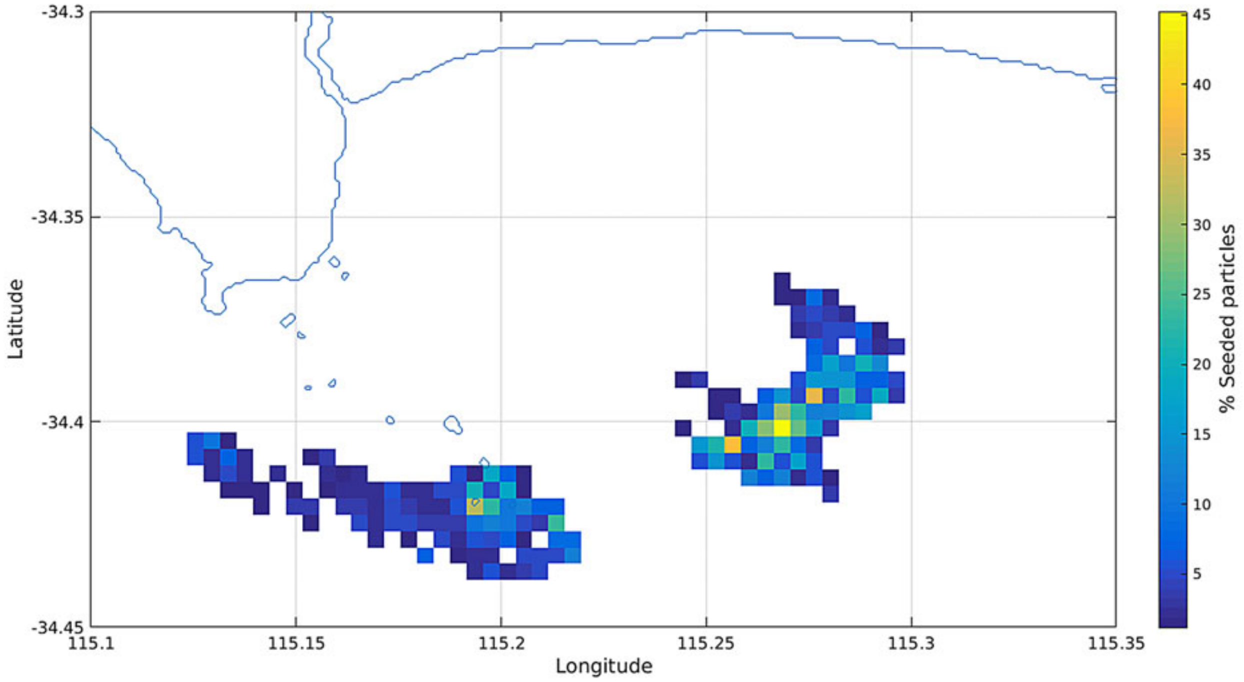


Figure 3.21 Heat map showing areas of good supply for summer simulations. Colours represent the percentage of particles seeded in that area that passed through the lease site.

3.6.4 Lease extension

The volume of drift-algae passing through the lease site was analysed to identify specific areas where food abundance might be higher. This analysis showed large volumes of drift-algae consistently passing through the northwest corner of the lease site. Further, many simulations showed large numbers of particles passing to the west. This was corroborated by OGA whose divers had noted western seeded abalone had significantly better survival and growth rates than those seeded to the east. Based on their observations, planning was undertaken to extend the lease. The larger planned lease site will hereafter be referred to as LS2. Model results from this study was used to identify areas within LS2 where food abundance would be high. Figure 3.22 shows particle abundance over the planned lease site for W2.

During winter simulations, there is a clear pathway through LS2 showing higher numbers of particles. As seen in Figure 3.22, W2 shows an abundance of particles through the middle of LS2, with reduced numbers to the southeast and northwest. This pathway is shifted slightly westward during W1. Pathways in W3 are less well defined as fewer particles pass through the site. However, from W1 and W2 it is clear that the most productive areas in terms of food abundance during winter conditions are located near the middle of the planned lease site.

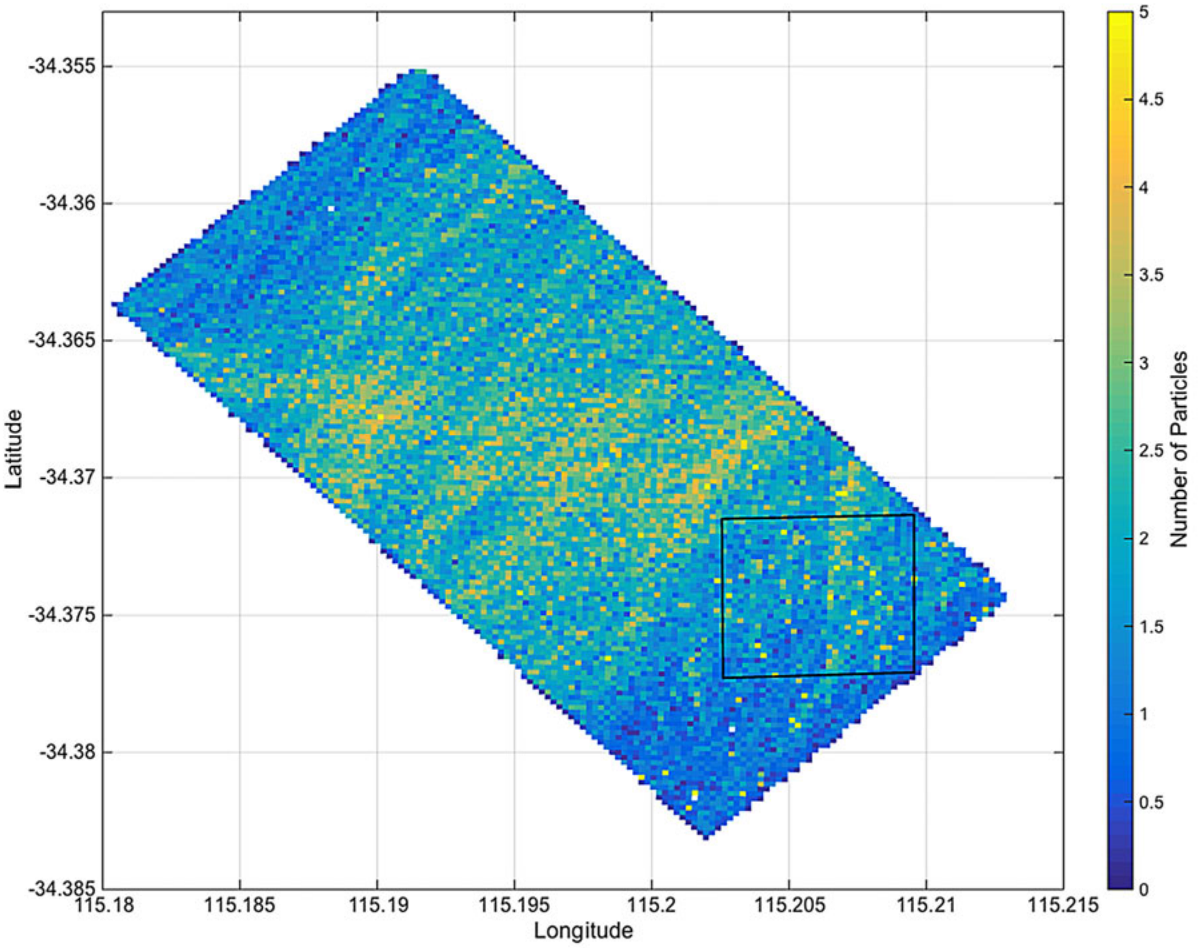


Figure 3.22 Heat map showing particle volume over LS2 for simulation W2. The original lease site is outlined in black

S1 produces similar results to the winter simulations, showing very clear pathways near the middle of the lease site. This can be seen in Figure 3.23 (a), with S2 shown in Figure 3.23 (b). Particle pathways predicted by W1 and W2 are clearly supported by the results of S1, where particles seeded to the south passed through LS2 under northwesterly winds. This pathway cannot be seen in S2, where the majority of particles originated to the east.

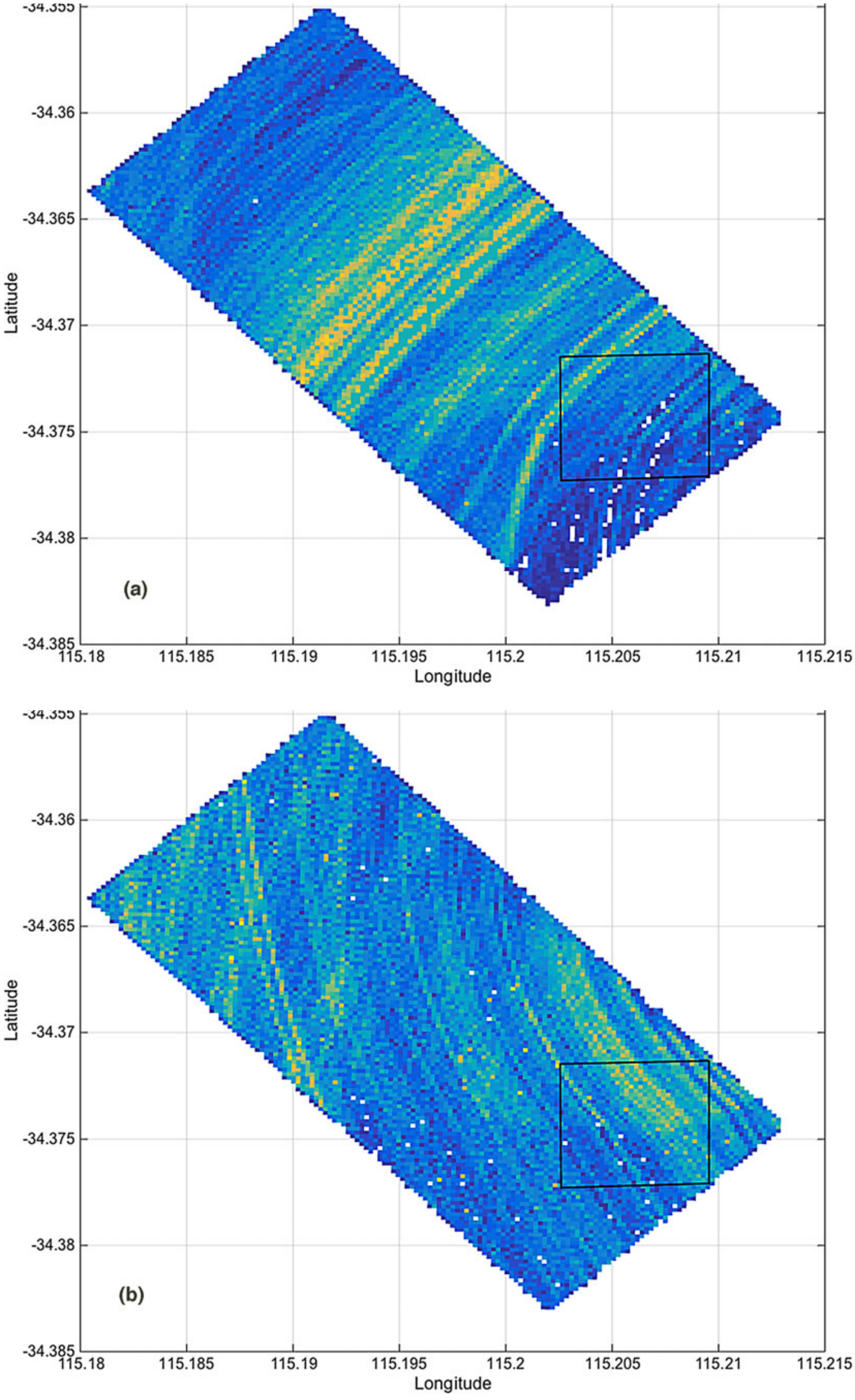


Figure 3.23 Particle abundance over the planned lease site LS2 during S1 (top) and S2 (bottom). Values show particle numbers, with the original lease site shown as a black rectangle

A comparison of the original seed locations of particles passing through the original lease site and LS2 was conducted and is shown in Figure 3.24 and Figure 3.25 for winter and summer simulations respectively. For each simulation, there is a larger area supplying particles to LS2 than the original lease site. However, this is to be expected considering the size difference between the two areas. Summer

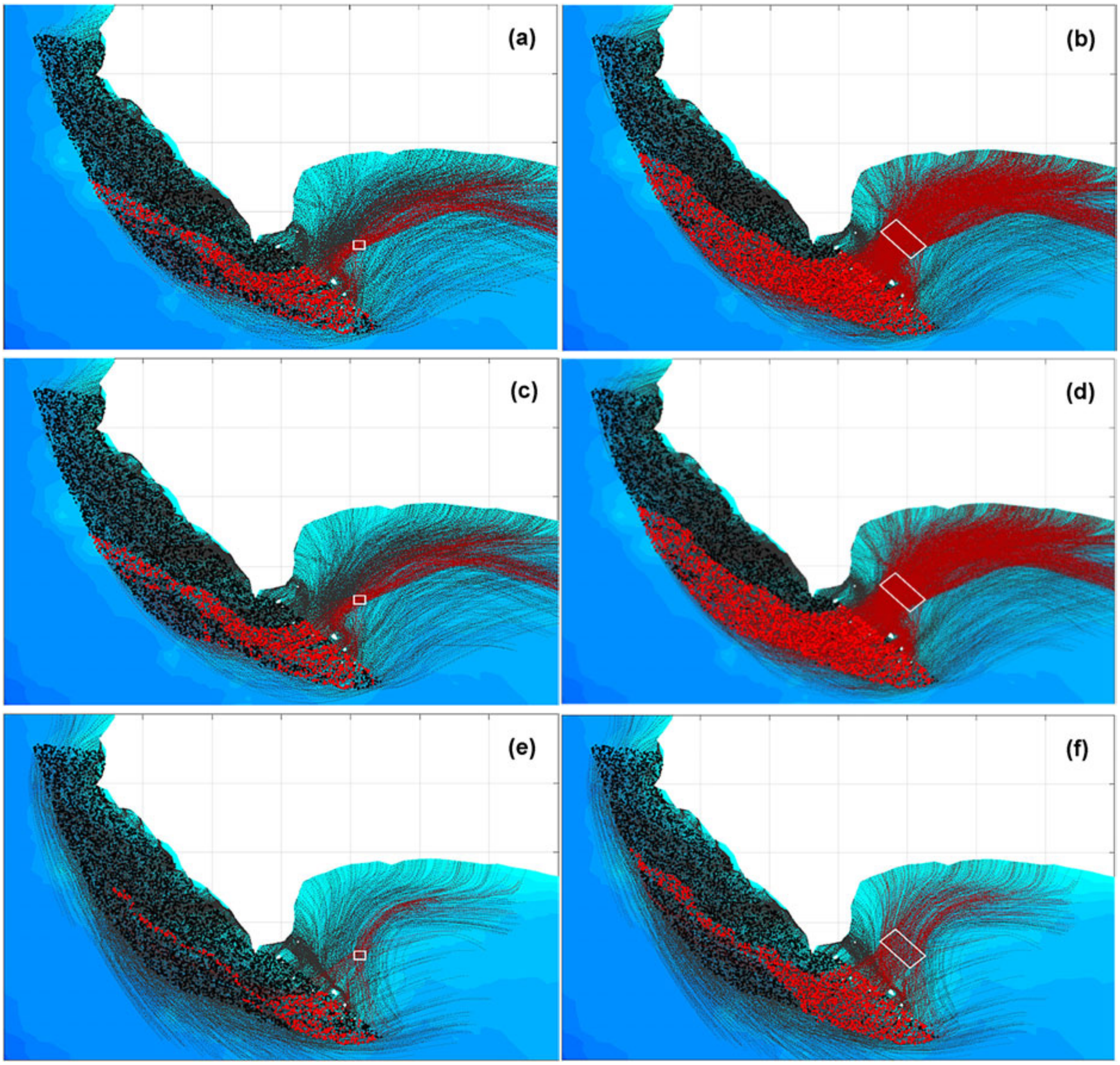


Figure 3.24 Particle tracks for W1 (a,b), W2 (c,d) and W3 (e,f), with a comparison between the original lease site (a,c,e) and LS2 (b,d,f). Original seed locations are shown as black dots with particle tracks as black lines. Particles that pass through the original lease site/LS2 are highlighted in red

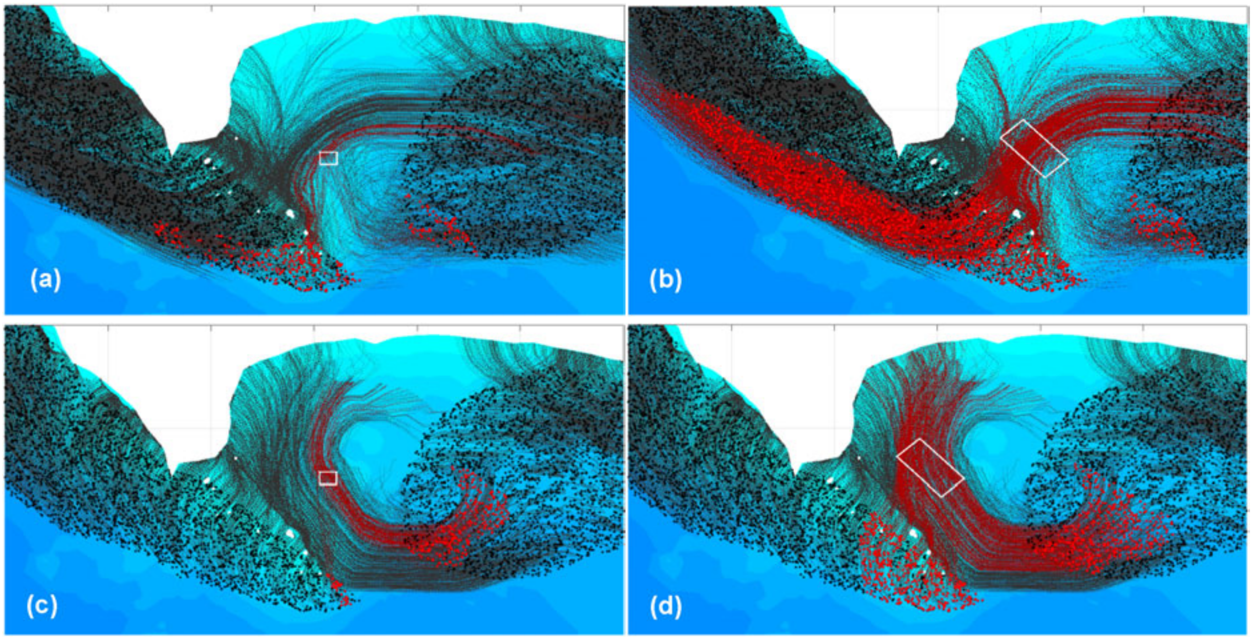


Figure 3.25 Particle tracks for S1 (a,b) and S2 (c,d), with a comparison between the original lease site (a,c) and LS2 (b,d). Original seeding locations are shown in black with particle tracks as black lines. Particles that pass through the original lease site/LS2 are highlighted in red

Particle supply to LS2 during S1 was much greater than the supply to the LS1, with substantially more particles seeded to the south and west able to reach LS2. As mentioned in 3.6.3, the clockwise circulation experienced in Flinders Bay sweeps many particles travelling from the south to the west of LS1. This can be seen in Figure 3.25 (a) and (c). LS2 however, extends far enough westward to capture many of these particles. An increase in western and southern seeded particle supply between the two sites can be seen for S2. However, supply to LS2 from the south and west remains relatively low, due to the sufficiently strong clockwise circulation experienced during the simulation

3.7 Discussion

This chapter discusses the overall findings on the source and seasonal variations in drift-algae in Flinders Bay, specifically in relation to the OGA aquaculture lease. The original contribution of this research is summarised in Section 3.7.1, followed by an outline of the key findings and their implications. Finally, Section 3.7.3 discusses possible future work that could be conducted to improve certain aspects of this research.

3.7.1 Original contribution

This study identified the seasonal variability in the movement of drift-algae within Flinders Bay. The source of drift-algae passing through an aquaculture lease site within the bay was also determined. A hydrodynamic numerical model was constructed to simulate water circulation of the Flinders Bay region using the modelling system SCHISM. This system included a fully coupled wave model and was forced by water elevation, wind and waves. Water currents and wave fields generated by the hydrodynamic model were then applied to a dispersal model to simulate drift-algae movement. Particle behaviour was described by stochastic equations, relying on observed drift-algae characteristics and random diffusivity terms. The main findings and original contribution of this research is outlined in the following paragraphs.

Water circulation in Flinders Bay was simulated using a hydrodynamic numerical model. Results showed strong wave induced currents along a chain of islands extending southeast from Cape Leeuwin. These northward currents were present in each simulation, and were driven by large southwesterly swells generated in the Southern Ocean. These swells were included in the hydrodynamic model through boundary forcing, using data from PACCSAP's WW3 model. ADCP measurements supported the model results, showing strong northward currents throughout both deployments.

A clockwise water circulation in Flinders Bay was present in several model simulations. This circulation occurred under easterly wind conditions, and its strength was proportional to wind velocity and wave heights. Circulation did not occur in simulations where wave forcing was excluded, suggesting that this circulation is caused by a combination of strong wave induced currents along the island chain and westward currents driven by easterly winds.

Strong wave driven currents to the south west of Flinders Bay had a large effect on the movement of drift-algae in the region, partly due to the formation of the clockwise circulation during periods of easterly winds. During winter months, large swells and intense winds created strong currents which forced particles into the bay from the south. The number of particles transported into the bay was proportional to the intensity of the weather conditions. These particles travelled north and east along the coast, exiting the bay to the east or being deposited on the beaches. Large numbers of particles were swept westward of the lease site by wave refraction around the island chain.

Summer conditions experienced in the bay allowed eastern seeded particles to travel towards the lease site. Extended southeasterly winds combined with large southwesterly swells to drive clockwise water circulation in the bay. This circulation carried particles from the east towards the lease site. However, the volume of particles transported under these conditions were significantly less than those driven by more intense weather, such as during winter storms. Also, the number of particles entering the bay from the south was reduced, with currents transporting large volumes of particles west of Cape Leeuwin. Particles that did enter the bay were caught in the circulation and were generally swept west of the lease site.

A planned increase to the size of the lease site was shown to cover areas where particle abundance is higher. During most simulations, the area west of the original lease site (LS1) saw substantially more particles passing through. This was corroborated by OGA divers who reported that western seeded abalone performed significantly better than those seeded to the east. To capture these particles, an extension of the lease was planned, covering a large area to the west of LS1. This new lease area is designated in this thesis as LS2.

Winter simulations show an abundance of drift-algae particles in the middle of LS2 and west of LS1. The performance of the lease sites during the summer simulations are very much dependent on the clockwise circulation strength within Flinders Bay. As seen in S1, where winds switched from southeasterlies to northwesterlies and circulation strength decreased, a large number of particles seeded to the south were able to enter the bay through the chain of islands. These particles were swept to the west of LS1 by the water circulation. LS2 however captures many of these particles. A larger number of eastern seeded particles pass through LS2 compared to LS1, however the increase is not significant for either summer simulation. This is partly due to the pathways of eastern seeded particles, which pass into LS2 along its shorter southeast face travelling northwest. This cross-section is not significantly larger than the southeast face of LS1.

3.7.2 Implications of key findings

3.7.2.1 Wave modelling in Flinders Bay

The inclusion of waves in the hydrodynamic model had a major effect on the simulation of water movement in the region. Large southwesterly swells generated in the Southern Ocean were included in the model through the use of PACCSAP's WW3 model data as boundary forcing. A small chain of islands extending southeast from Cape Leeuwin provides some protection to Flinders Bay from the large swells. However, it was shown that strong wave induced currents were generated along this island chain. These currents had a major influence on water circulation in the region, and therefore affected the movement of particles drastically. Under easterly winds, wave induced currents combined with wind driven currents to form a clockwise circulation in Flinders Bay. This circulation was shown to only occur in simulations with waves included. Simulations conducted without the inclusion of waves did not form this circulation, instead currents were reversed at the island chain and water flowed from east to west. Current strength within the bay was also shown to be reduced in simulations not including waves.

A reduction in the number of particles passing through the lease site is to be expected for simulations excluding wave calculations. Firstly, the number of particles being suspended in the water column would decrease due to lower bed stresses, preventing their movement within the model domain. Secondly, particle movement would slow due to the reduced current velocities. The source of these particles would also be affected by the exclusion of waves, with a reduced number of particles from the south travelling into the bay.

3.7.2.2 Seasonal variations in the supply of drift-algae

Some clear variations in the supply of drift-algae to the lease site in Flinders Bay can be seen between the summer and winter simulations. Furthermore, variations between individual seasonal simulations exist. Generally, a higher number of seeded particles passed through the lease site during winter simulations, however this was not always the case. The number of particles passing through the lease site was proportional to the intensity of metocean conditions in the region, referring to large wave heights and high wind velocities. Model results show that the most intense simulation (W2) experienced the highest percentage of seeded particles passing through the lease site, with the least intense (S1) experienced the smallest percentage.

Large storms frequently pass by Flinders Bay during winter months, bringing large waves and severe southerly and westerly winds. As shown in this study, these conditions provide the lease site with a good supply of drift-algae. Between storm events, northeasterly winds are common. Extended periods of northeasterly winds reduce the supply of drift-algae to the lease site due to the formation of a clockwise water circulation in the bay. Drift-algae supply from the south decreases when this circulation exists. This was shown in W3, where several days of northeasterly winds occurred. Large numbers of particles that entered Flinders Bay from the south were caught in the circulation and swept west of the lease site. Eastern seeded particles moved slowly towards the lease site but were eventually transported away when a storm occurred, destroying the clockwise water movement. Therefore, W3 predicted a reduced number of particles reaching the lease site compared to W1 and W2.

Southeasterly winds are dominant during summer months in Flinders Bay, and as shown in this study, these winds setup a clockwise water circulation. Both summer simulations contained extended periods of southeasterly winds, however S2 predicted significantly more particles passing through the lease site than S1. An analysis of water currents in Flinders Bay showed that circulation strength during S2 was greater than in S1. This was caused by larger wave heights at the island chain south of the bay, and stronger southeasterly winds. The combination of higher wave and wind induced currents resulted in a stronger circulation, able to suspend and carry more eastern seeded particles towards the lease site. This circulation, while increasing the supply of eastern seeded particles to the lease site, also decreases the supply of southerly seeded particles. Hence, a larger percentage of southern seeded particles are predicted to pass through the lease site in S1 compared with S2.

3.7.2.3 Drift-algae sources

The source of drift-algae supplied to the lease site is highly dependent on the metocean conditions. During winter months, this study predicts that drift-algae originating from the south makes up a large majority of particles that reach the lease site. During periods of northeasterly winds, it is possible that drift-algae from the east would pass through the lease site, however this was not seen throughout this study. Winter simulations predict that particles originating immediately south and west of the island chain would form the majority of the supply. Areas to the west of Cape Leeuwin were shown to contribute to supply, however in reduced volumes.

Summer months show a greater range of sources with contribution from areas located east of the lease site. The formation of the clockwise circulation within the bay is more common during the summer due to prevalent southeasterly winds. This allows drift-algae originating to the east to travel towards the lease site. The strength of this circulation affects the ratio of supply from east and south, with a stronger circulation resulting in a higher volume of drift-algae originating to the east and vice versa.

3.7.2.4 Lease extension

In most of the simulations conducted during this study, large numbers of particles passed to the west of the lease site. This feature was corroborated by OGA divers who had noted western seeded abalone in LS1 had performed much better than those seeded to the east. LS2 is a planned lease site which covers LS1 and extends further we.

The performance of LS2 compared to LS1, in terms of capturing areas of high drift-algae volume, is dependent on the direction of drift-algae movement. During much of the year, drift-algae travel north across the lease sites, carried by strong wave induced currents. As LS2 extends westward, a larger number of these particles travelling north are captured, with areas of high volume existing in the middle of LS2, just west of LS1. However, when the clockwise water circulation in Flinders Bay is strong, drift-algae particles travel northwest across the lease sites. This reduces the effectiveness of the lease extension as the southeast face of LS2 is similar to that of LS1. Therefore, a similar volume of drift-algae are captured by both lease sites.

3.7.3 Future work

3.7.3.1 Particle detachment included in dispersal model

The dispersal model used during this study relies on the assumption that all particles are detached from the seabed once seeded. As seeding was conducted for each simulation in the same way, drift-algae detachment was not taken into account. As detachment is dependent on stresses placed on algae, it follows that more intense conditions would result in higher rates of detachment, leading to higher volumes of drift-algae suspended in the water column. As this is not modelled in this study, it is likely a bias exists favouring simulations where conditions were calmer. This could be included in simulations by limiting seeding when conditions are calm, however little information exists on what processes cause detachment and at what rate detachment occurs. Experiments similar to that conducted in Section 3.5.1.2 could be carried out to determine stresses required for algae detachment. This process could then be included in an updated version of the dispersal model.

3.8 Conclusions

The overall aim of this thesis was to determine the source and seasonal variations of drift-algae supply to an aquaculture lease in Flinders Bay. This was achieved through the hydrodynamic modelling of seasonal events in the region, and particle tracking using a dispersal model. It was found that:

- Strong wave induced currents along a chain of islands extending southeast from Cape Leeuwin, greatly influence the hydrodynamics of Flinders Bay. During periods of easterly winds these currents interact with those driven by wind to form a clockwise circulation in the bay. This circulation greatly affects the source of drift-algae to the aquaculture lease. Strong circulation reduces southerly supply of drift-algae while increasing supply from the east.
- Areas to the southwest of Flinders Bay and Cape Leeuwin provide the largest supply of drift-algae to the lease site. They are most productive during winter months when wind and wave conditions are intense. Areas to the west of the lease site only supply drift-algae under easterly winds, when clockwise water circulation occurs.
- Larger volumes of drift-algae are supplied to the lease site during winter months when conditions are more intense. Larger wave heights and wind velocities result in increased particle suspension and movement.
- There exists an area west of the lease site which experiences a greater supply of drift-algae. This area is covered by a planned lease extension.

Overall this study shows that the supply of drift-algae to an aquaculture lease in Flinders Bay is higher during winter conditions, and areas southwest of Flinders Bay and Cape Leeuwin provide the majority of this supply.

Methodologies presented in this thesis can be applied to other areas and situations involving particle movement in oceanic and coastal settings

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Note: This is the draft of a research paper which has been submitted to the *Journal of Marine and Freshwater Research*

4 Seasonal and spatial variation of drift algae on an abalone (*Haliotis laevigata*) sea ranch in Flinders Bay, Western Australia

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4.1 Abstract

Greenlip abalone (*Haliotis laevigata*) are known to feed selectively on seaweeds. This study monitored changes in the species diversity and spatial variation of drift algae and seagrasses on an abalone sea ranch in Flinders Bay, Western Australia, between July 2015 and June 2016. A total of 67 taxa were identified, 52 to species and 15 to genus level. The majority of identified taxa were Rhodophyta (66 %) and Heterokontophyta (22 %). Two brown algae (*Scytothalia dorycarpa* and *Ecklonia radiata*), two seagrasses (*Zostera tasmanica* and *Amphibolis* spp.) and two red algae (*Pollexfenia pedicellata* and *Gracilaria flagelliformis*), were most consistently sampled over time. The species sampled showed significant (Pseudo-F = 7.37, P < 0.001) variation across ad hoc season descriptors (winter, spring, summer, autumn) at both the taxa and family level, with brown algae dominating in autumn, winter and spring (66 %, 50 % and 42 % respectively), but dropping off to only 18 % in summer, in favour of seagrasses (47 %). There was also a significant (Pseud-F = 3.51, P < 0.001) variation in assemblage structure across two sites sampled at both the species and family level, even though these sampling sites were within a few hundred metres of each other.

Additional keywords: ranching, macroalgae, artificial habitats, sustainable, greenlip, aquaculture.
Running Head: Seasonal and spatial variation in drift algae.

4.2 Introduction

Sustained pressure on abalone (*Haliotis* spp.) fisheries combined with increased market prices, has led to a search for alternative products to satisfy the high demand for these gastropod molluscs in south-east Asian markets (Cook & Roy Gordon 2010). In response to this, abalone sea ranching has been raised as an alternative to wild fisheries and land-based aquaculture (Bell et al. 2008). In Australia, small-scale sea ranching trials have been undertaken in Victoria and Tasmania (Shepherd et al. 2000, James 2005, James et al. 2007). However, these trials failed to lead to commercialisation due to the research concluding that environmental conditions in the study area were suboptimal for supporting commercial quantities of harvestable abalone stock. A new venture being developed at Flinders Bay, Augusta, Western Australia, has been more successful and has already succeeded in producing small quantities of greenlip abalone (*Haliotis laevigata*) for local and overseas markets (Adams, Ocean Grown Abalone Pty Ltd, pers. comm.).

Trials were undertaken prior to embarking on the Ocean Grown Abalone Pty Ltd sea ranching venture to determine growth and survival of the abalone on different types of artificial reef structures (Melville-Smith 2013). One of the outcomes identified by that research was the need to investigate seasonal and spatial differences in the species composition of drift algae that provide the food of the abalone on the ranch. This aspect has formed the basis of the research in the present study.

Abalone diets may change among species or even across different geographical locations (Shepherd & Steinberg 1992). Although some studies have concluded that abalone consume algae in proportion to the abundance that is available (Barkai & Griffiths 1986), others have shown that they do select for certain species (Angell et al. 2012). In the case of the greenlip abalone, the species has been shown to generally select red algae over green and brown algae and seagrasses (Shepherd 1973).

It is well known that the volume of drift algae fluctuates markedly across the year. This is no exception in Augusta on the south coast of Western Australia, where strong cold fronts over the Indian Ocean cross the country in the winter months (Bureau of Meteorology 2016). These fronts generate storm conditions at sea and the resulting swells dislodge vast quantities of algae and seagrasses and deposit them within Flinders Bay. There has been no research to date in the Flinders Bay area to identify whether there are seasonal differences in the species composition of the drift algae across the year. This is considered to be an important research gap given the selective feeding habits of greenlip abalone because the availability of suitable drift algal species is what will ultimately determine the growth, nutritional and health status of the stock held on the sea ranch. Therefore, the aim of this study was to monitor and record the seasonal and spatial variation of drift macroalgae and seagrasses on an abalone (*Haliotis laevigata*) sea ranch in Flinders Bay, Western Australia.

4.3 Materials and methods

4.3.1 Study area

Flinders Bay is a relatively protected bay subject to wind-driven circulation systems located within Augusta at the southwestern coast of Australia (Figure 4.1). There is a predominant north-south current following along the coastline during winter and the reverse in summer. In both seasons the current meanders into Flinders Bay creating a gyre during the summer months (MacPherson, unpubl. data).

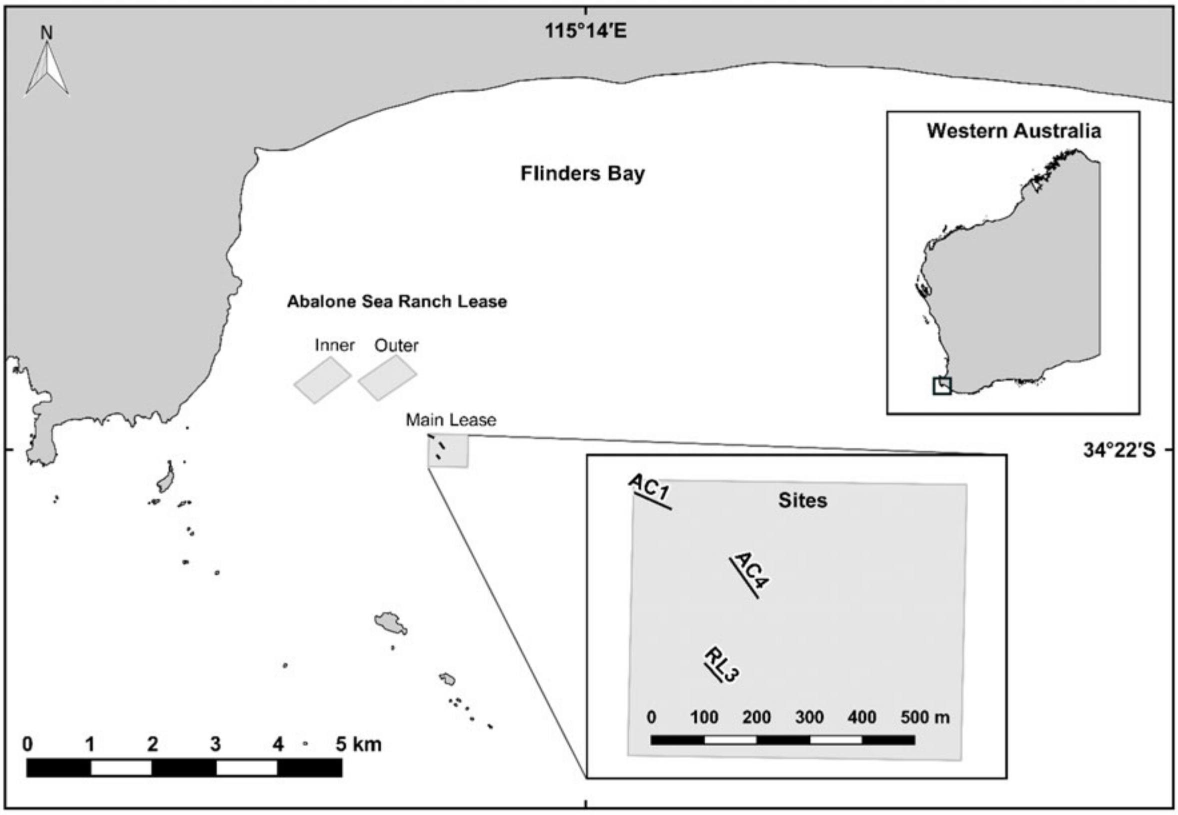

Figure 4.1 Flinders Bay region, showing the location of the Ocean Grown Abalone Pty Ltd sea ranch (the three shaded squares represent separate lease areas termed the inner, outer and main lease)

Currently, there are over 7 000 concrete structures distributed in three areas of the abalone sea ranch in Flinders Bay. These are termed the inner, outer and main lease areas (Figure 4.1). The artificial structures (defined hereafter as an abalone habitat or 'abitat') are arranged in a hierarchical spatial design in groups of six units, which together create a 'reef'. Likewise, a group of 15 – 20 reefs constitutes a 'site'.

4.3.2 Sample collection and processing

Three sites within the main lease area, namely AC1, AC4 and RL3 located at a depth of 18 – 20 m (Figure 4.1), were selected for sampling. The sites were distributed over an area of 36 hectares within the lease with minimum variability in depth or topography. Divers working on the lease were not aware of any obvious visual differences in the amount of drift algae accumulating in any particular site.

From July 2015 to June 2016, four randomly selected samples of drift macroalgae from each site were collected by hookah divers on a monthly basis to provide a total of 143 samples (one sample was missing for October 2015). The samples were collected at the base of the abitats in a semi-quantitative way. Quantities of drift algae and seagrass present against the abitats on any day, varied from nothing, to over a 0.5 m deep. Divers responsible for the sampling filled sample bags by taking handfuls of drift algae lying close to the abitat. During conditions when the drift algae was scarce, this required collecting any loose fronds or clumps of algae drifting around the abitat. However, when drift algae were in abundance around the abitats, a few handfuls randomly selected from close to the abitat was all that was required to fill a sampling bag. No attempt was made to accurately quantify the samples, because it is known that the drift algae pass through the ranch quite rapidly. In addition, quantities of drift algae around the base of the abitats can change within the course of a day (Adams, pers. comm.) and therefore any quantitative sample of drift algae would only have been representative of a relatively brief point in time. For this reason, we were more interested in the relative proportions by wet and dry weight of the different species that comprised the sample, rather than the overall biomass of the same.

The location of three sampling sites within the main lease (AC1, AC4, RL3) is shown in more detail in the lower inset. The upper inset shows the location of Flinders Bay at the southwestern corner of Western Australia.

The algal samples were placed in labelled plastic bags and kept in cool insulated containers on deck. Once ashore, the samples were frozen and transported to Curtin Aquatic Research Laboratories where once thawed, were separated into individual species. Wet weights were recorded to determine algal biomass. Unidentified specimens were later identified to species by Dr John Huisman (Department of Parks and Wildlife, Western Australian Herbarium, Bentley, Australia).

4.3.3 Statistical analyses

The wet weight of each algal and seagrass species >5 g was calculated as a proportion of the total wet biomass of the sample. Untransformed data was used to create a resemblance matrix through Bray-Curtis distances (Clarke et al. 2006). A dummy variable was added to compensate for the many zero cells resulting from abitats without drift algae that were recorded on some sampling days. A two-way crossed factorial design was created using site (three levels: AC1, AC4 and RL3) and season (four levels: summer, autumn, winter, spring) as fixed factors. Seasons were defined as follows: January – March: summer; April – June: autumn; July – September: winter; October – December: spring. These months were considered to better resemble the seasonal variation of temperature in Flinders Bay than the months that are more conventionally used to describe the Austral seasons based on land temperatures (Bureau of Meteorology 2016).

Permutational multivariate analyses of variance (PERMANOVA) were used for analyses because the relative abundances of algae were highly skewed and contained many zero cells. Traditional analyses such as MANOVA were not appropriate, as they assume normality distribution of the data (Anderson 2001). The analyses were performed at species and family levels using Type II sum of squares and 9999 permutations under the 'permutation of residuals under a reduced model' method (Clarke & Gorley 2006). PERMANOVA pairwise comparisons were performed for those factors with a significant result ($P < 0.05$).

In order to assess the suitability of the data for PERMANOVA (Anderson & Walsh 2013), permutational analyses of multivariate dispersions (PERMDISP) on the basis of deviations from medians were used to test for differences in dispersion among sites and seasons using 999 permutations. These tests revealed homogeneous dispersions (all $P > 0.05$) of the multivariate data. On the basis of this result, the potential differences between groups of samples of the PERMANOVA analyses can be attributed to variation in the location of the points within the cloud of the distribution of samples, rather than dispersion among them (Anderson & Walsh 20).

A similarity percentage analysis (SIMPER), was conducted to identify the contribution of each taxon to observed community dissimilarities (with the cut-off set to 90 %) between significant factors (Clarke 1993). Taxa were considered important if their contribution to percentage dissimilarity was ≥ 7 %. All statistical analyses were conducted using the PRIMER-E v6 statistical package (Clarke & Gorley 2006).

4.4 Results

A total of 67 macroalgae and seagrass taxa (52 identified to species, 15 to genus) were identified from the 143 samples collected over the course of the study (Table 4.1). The main contribution to the richness of taxa was from the phylum Rhodophyta, with the Gigartinales and Ceramiales being the richest orders represented (15 and 13 taxa respectively). Heterokontophyta was mostly represented by the order Fucales (11 taxa), whereas Chlorophyta was the least diverse phylum (3 taxa). Seagrasses (angiosperms in the order Alismatalese) were represented by five taxa, with Cymodoceaceae being the dominant family (comprising three of the five taxa) sampled.

Table 4.1 Drift algae and seagrasses identified on the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay, Western Australia, between July 2015 and June 2016

Division	Order	Family	Genus and species
Rhodophyta	Balliales	Balliaceae	*Ballia callitricha*
	Ceramiales	Dasyaceae	*Halydictyon arachnoideum*
			Heterosiphonia gunniana
			Thuretia quercifolia
		Rhodomelaceae	*Amansia serrata*
			Chondria sp.
			Coeloclonium tasmanicum
			Dictyomenia tridens
			Laurencia filiformis
			Laurencia sp.
			Lenormandia pardalis
			Pollexfenia pedicellata
			Polysiphonia decipiens
		Wrangeliaceae	*Griffithsia ovalis*
	Corallinales	Corallinaceae	*Amphiroa gracilis*
			Jania rosea
			Metagoniolithon stelliferum
	Gelidiales	Gelidiaceae	*Gelidium* sp.
			Pterocladia lucida
			Pterocladia pinnata
	Gigartinales	Acrotylaceae	*Claviclonium ovatum*
			Hennedya crispa
		Cystocloniaceae	*Hypnea filiformis*
			Hypnea sp.
			Rhodophyllis volans
		Dicranemataceae	*Dicranema revolutum*
		Gigartinaceae	*Gigartina disticha*
		Mychodeaceae	*Mychodea gracilaria*
			Mychodea ramulosa
			Mychodea sp.
			Nizymenia conferta
			Nizymenia sp.

Division	Order	Family	Genus and species
		Phacelocarpaceae	*Phacelocarpus sessilis*
			Phacelocarpus sp.
		Solieriaceae	*Erythroclonium muelleri*
	Gracilariales	Gracilariaceae	*Gracilaria cliftonii*
			Gracilaria flagelliformis
			Gracilaria sp.
	Halymeniales	Halymeniaceae	*Carpopeltis elata*
			Carpopeltis phyllophora
	Plocamiales	Plocamiaceae	*Plocamium mertensii*
			Plocamium sp.
	Rhodymeniales	Hymenocladiaceae	*Hymenocladia chondricola*
			Hymenocladia filiformis
Heterokontophyta	Dictyotales	Dictyotaceae	*Dictyota fastigiata*
			Lobospira bicuspidata
			Rugulopteryx radicans
	Fucales	Cystoseiraceae	*Cystophora polycystidea*
			Cystophora sp.
			Myriodesma sp.
			Myriodesma tuberosa
			Platythalia angustifolia
			Platythalia quercifolia
		Sargassaceae	*Sargassum fallax*
			Sargassum sp.
			Sargassum tristichum
			Sirophysalis trinodis
		Seirococcaceae	*Scytothalia dorycarpa*
	Laminariales	Alariaceae	*Ecklonia radiata*
Chlorophyta	Bryopsidales	Codiaceae	*Codium galeatum*
	Cladophorales	Cladophoraceae	*Cladophora* sp.
	Ulvales	Ulvaceae	*Ulva* sp.
Magnoliophyta	Alismatales	Cymodoceaceae	*Amphibolis antarctica*
			Amphibolis griffithii
			Syringodium sp.
		Posidoniaceae	*Posidonia australis*
		Zosteraceae	*Zostera tasmanica*

Patterns of variation in drift algae are depicted in the nMDS plot presented in Figure 4.2a. The Figure shows an apparent homogenous clustering of drift algae assemblages located in different sites and seasons. However, when these points are represented as distances among centroids of groups, both seasonal and spatial variations become evident (Figure 4.2b). Summer samples are clustered together in the far right of Figure 4.2b, while there is an assemblage of autumn samples to the bottom left of the Figure. Likewise, the seasons for site AC1 (specifically autumn, winter and spring) are generally more clustered than those representing site RL3 (Figure 4.2 b).

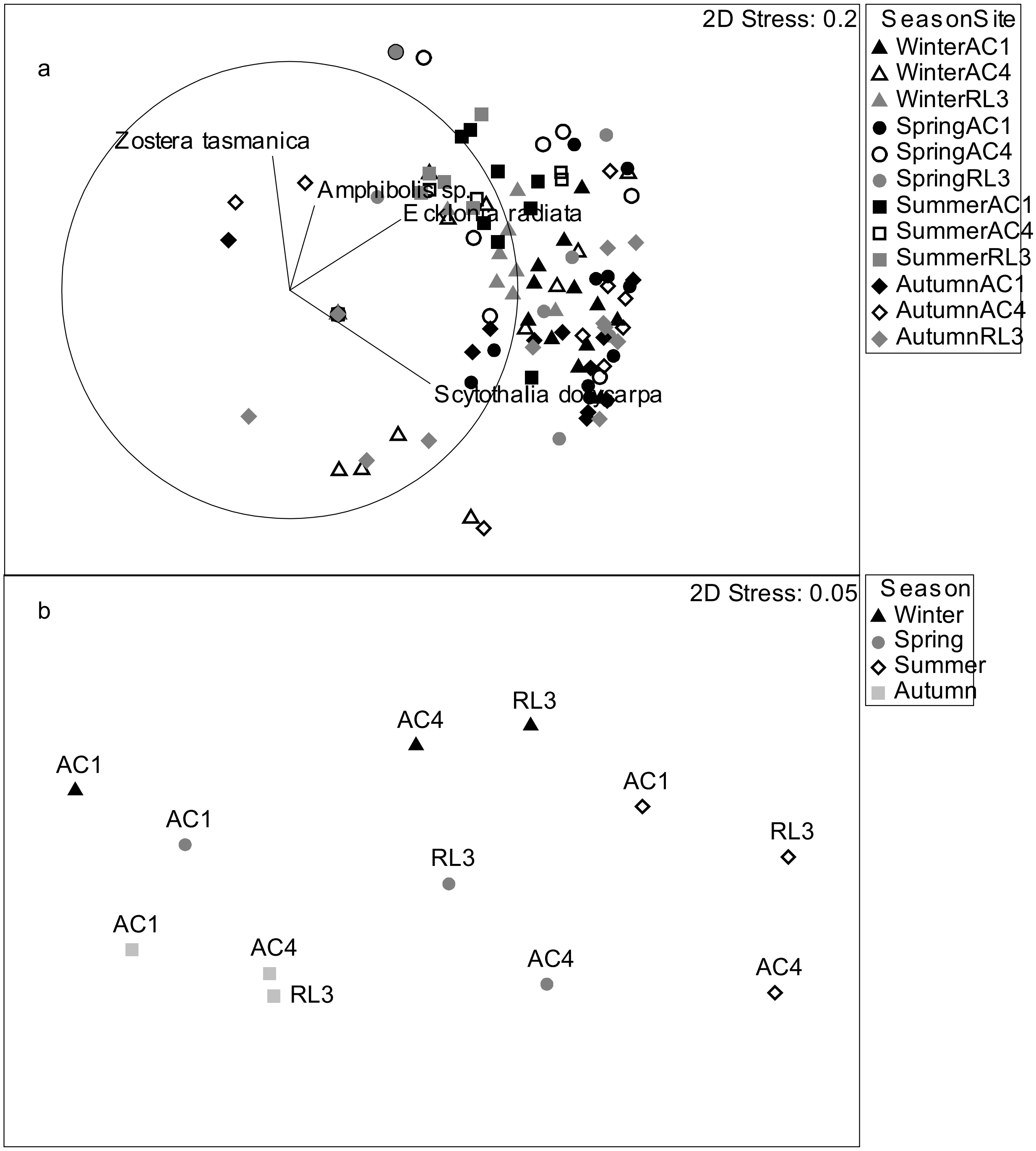

Figure 4.2 Non-metric multidimensional scaling (nMDS)
plots on the basis of Bray-Curtis similarities of untransformed data showing:
(a) relative abundance of drift algae across four seasons and three sites
at the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay, Western Australia. The plot is overlaid with taxa contributing to differences in drift algae assemblages (correlation > 0.38), and
(b) distances among centroids
for all samples across the three sampling sites (AC1, AC4 and RL3), by season

The same patterns of distribution are reflected in the results of PERMANOVA analyses (Table 4.2), where season and site have been shown to be significantly different (Pseudo-F = 7.37, P < 0.001 and Pseudo-F = 3.51, P < 0.001, respectively) in terms of their structure of drift algae assemblages (Table 4.2).

Table 4.2 PERMANOVA analysis based on Bray-Curtis similarities of untransformed relative abundance data for drift algae species and families sampled between June 2015 and July 2016 at the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay, Western Australia. Factors included are site, season and their interaction. Degrees of freedom (df), mean sums of squares (MS), Pseudo-F-statistics and the estimates of multivariate variation (Variation %) are also included

Source		Species-level data				Family-level data			
	df	MS	Pseudo-F	p(perm)	Variation (%)	MS	Pseudo-F	p(perm)	Variation (%)
Season	3	23,769	7.37	<0.01	26	22,621	7.08	<0.01	25
Site	2	11,305	3.51	<0.01	14	11,175	3.50	<0.01	14
Season×Site	6	2,941	0.91	0.62	0	2,839	0.89	0.67	0
Residual	131	3,224			61	3,196			61
Total	142				100				100

Pairwise comparisons between seasons (Table 4.3), showed that there were significant differences (P < 0.01) in the assemblage structure at both the species and family level for all season comparisons. The analysis between sites showed there to be significant differences (P < 0.01) in the assemblage structure at both the species and family level between sites AC1 and AC4 (t = 2.25) and AC1 and RL3 (t = 2.26) (Table 4.3) but not between AC4 and RL3 (P = 0.72 and P = 0.73 for species and family respectively (Table 4.3).

Table 4.3 PERMANOVA analysis using pairwise comparisons of species and families of drift algae sampled between June 2015 and July 2016 at the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay, Western Australia. Factors are site and season factors. The estimated sizes of average similarities between sites and seasons (AS) and pairwise t-statistics (t) are also included. Values in bold are significant at the < 0.05 level

Season						
	Species-level data			Family-level data		
Groups	t	P	AS (%)	t	P	AS (%)
Winter vs Spring	1.940	<0.01	16.4	1.997	<0.01	16.7
Winter vs Summer	3.266	<0.01	16.5	3.239	<0.01	17.2
Winter vs Autumn	2.217	<0.01	17.8	1.950	<0.01	19.8
Spring vs Summer	2.823	<0.01	16.5	2.792	<0.01	16.8
Spring vs Autumn	2.048	<0.01	15.4	1.922	<0.01	16.4
Summer vs Autumn	3.686	<0.01	11.8	3.680	<0.01	12.1
Site						
	Species-level data			Family-level data		
Groups	t	P	AS (%)	t	P	AS (%)
AC1 vs AC4	2.250	<0.01	16.0	2.247	<0.01	16.7
AC1 vs RL3	2.258	<0.01	16.6	2.259	<0.01	17.4
AC4 vs RL3	0.795	0.72	19.0	0.779	0.73	19.7

The taxa contributing to greatest within-site similarity for each of the three sites were very similar (Table 4.4). The brown algae *Scytothalia dorycarpa* and the seagrasses *Amphibolis* spp. ranked consistently as the first and second most abundant species at all sites. A second brown algae, *Ecklonia radiata* and red algae *Pollexfenia pedicellata*, were also substantial contributors among the three sites, reaching between 5 and 18 % to the total number of individuals. When compared among seasons, the seagrass *Zostera tasmanica* contributed 3 % and 2 % respectively, to the total similarity of the spring and summer samples (Table 4.4).

Patterns revealed by the SIMPER analysis (Tables 4.4 and 4.5) were related with those taxa that showed a high correlation in terms of their contribution to differences within drift algae assemblages on the Flinders Bay sea ranch (i.e. *Scytothalia dorycarpa*, *Ecklonia radiata*, *Zostera tasmanica* and

Table 4.4. Average relative abundance (Abun), average similarity (Sim), percentage contribution (%) and rank (Rk), by relative abundance of taxa recorded at each of the three sampling sites and four seasons at the Ocean Grown Abalone Pty Ltd sea ranch at Flinders Bay between July 2015 and June 2016. The five most common contributor taxa at each site have been shaded. Data are restricted to those taxa which contributed >1 % to the total number of individuals in any group.

Factor	Season																Site											
Level	Winter				Spring				Summer				Autumn				AC1				AC4				RL3			
Taxa	Abun	Sim	%	Rk	Abun	Sim	%	Rk	Abun	Sim	%	Rk	Abun	Sim	%	Rk	Abun	Sim	%	Rk	Abun	Sim	%	Rk	Abun	Sim	%	Rk
Scytothalia dorycarpa	23	11	46	1	18	6	40	1					37	15	76	1	34	18	61	1	12	3	27	1	13	4	32	1
Amphibolis spp.	19	6	26	2					17	7	53	1					11	4	15	2	9	2	20	2	11	3	24	2
Pollexfenia pedicellata	13	3	14	3	10	1	9	4									6	1	5	4	6	1	6	5	6	2	12	3
Ecklonia radiata	9	2	9	4	14	3	21	2					12	2	9	2	13	3	9	3	12	2	18	3	5	1	7	4
Zostera tasmanica					18	3	19	3	5	2	13	2									7	2	14	4	5	1	4	7
Ballia sp.						1	8	5													5	1	3	7				
Thuretia quercifolia									5	2	11	4													4	1	5	6
Amphiroa gracilis								2	1	4	5																	
Gracilaria flagelliformis													11	2	8	3									4	1	5	5
Platythalia angustifolia																					5	1	4	6				
Hymenocladia filiformis									5	2	11	3													2	1	3	8

Amphibolis spp.; correlation > 0.38) in the nMDS (Figure 4.2a). The analyses also revealed that six taxa (*Scytothalia dorycarpa*, *Amphibolis* spp., *Pollexfenia pedicellata*, *Ecklonia radiata*, *Zostera tasmanica* and *Gracilaria flagelliformis*) contributed consistently, but in different orders to the assemblage dissimilarities between seasons and sites.

Table 4.5 Similarity percentage analysis (SIMPER) summary for the Ocean Grown Abalone Pty Ltd sea ranch at Flinders Bay, Western Australia, showing taxa with the highest percentage contribution (reflected in the order of the taxa) contributing towards the dissimilarity between seasons and sites that showed a significant pairwise comparison result

Season	Winter	Autumn	Summer	Site	AC1
Spring	Avg. diss.: 84.19 *Scytothalia dorycarpa* *Amphibolis spp.* *Pollexfenia pedicellata* *Zostera tasmanica*	Avg. diss.: 87.21 *Scytothalia dorycarpa* *Ecklonia radiata* *Zostera tasmanica* *Gracilaria flagelliformis*	Avg. diss.: 94.36 *Zostera tasmanica* *Scytothalia dorycarpa* *Ecklonia radiata* *Amphibolis* spp.	AC4	Avg. diss.: 83.61 *Scytothalia dorycarpa* *Ecklonia radiata* *Amphibolis* spp. *Zostera tasmanica*
Summer	Avg. diss.: 91.39 *Amphibolis* spp. *Scytothalia dorycarpa* *Pollexfenia pedicellata* *Ecklonia radiata*	Avg. diss.: 95.97 *Scytothalia dorycarpa* *Ecklonia radiata* *Amphibolis* spp. *Gracilaria flagelliformis*		RL3	Avg. diss.: 81.37 *Scytothalia dorycarpa* *Amphibolis* spp. *Ecklonia radiata* *Zostera tasmanica*
Autumn	Avg. diss.: 83.18 *Scytothalia dorycarpa* *Amphibolis* spp. *Ecklonia radiata* *Pollexfenia pedicellata*				

The average relative abundance of the four Divisions of algae and seagrasses sampled over the course of the study is shown in Table 4.6.

Table 4.6 Percentages of average relative abundances by season of the four Divisions of algae and seagrasess sampled on the Ocean Grown Abalone Pty Ltd sea ranch at Flinders Bay, Western Australia

Season	Heterokontophyta	Rhodophyta	Charophyta	Chlorophyta
Winter	50	26	22	2
Spring	42	33	24	1
Summer	18	34	47	0
Autumn	66	32	2	0

4.5 Discussion

The abitats on the abalone sea ranch in Flinders Bay have been placed in amongst seagrass beds. The drift seagrass leaves and rhyzomes that collect around the abitats are therefore likely to be from surrounding seagrass. The drift algae however, are transported from further afield. In a separate study, MacPherson (2017) has identified seasonal differences in currents that move drift algae onto the ranch from offshore reefs where they grow in abundance. The transport of detached algae from offshore reefs to seagrass beds has been shown by Wernberg et al. (2006) to produce substantial biomass volumes relative to that of attached algae on reefs >300 m away, in the process creating important trophic linkages between the reefs and seagrass habitats.

This study showed that the sea ranch supports a diverse drift algae and seagrass assemblage that is seasonally and spatially variable. A total of 67 distinct taxa were identified, with two brown algae (*S. dorycarpa* and *E. radiata*), two seagrasses *Z. tasmanica and Amphibolis* spp. (comprising *Amphibolis antartica* and *Amphibolis graffithii*) and two red algae (*P. pedicellata* and *G. flagelliformis*) dominating the taxa recorded. Altogether, these six taxa were responsible for the assemblage differences between seasons and sites on the sea ranch (Table 4.5).

Our results also demonstrated that, while the species of drift algae in Flinders Bay are markedly influenced by both season and site, it is the first of these two factors that is the most important. Season had almost twice the percentage of contribution to the total variation of taxa (Table 4.2) compared with site. In terms of the analysis for site, it is relevant that the ordination plot shown in Figure 4.2b depicts a transition from AC1 to AC4 with RL3 in the middle, confirming the result obtained in the pairwise comparisons between AC4 and RL3 (Table 4.3).

Differences between seasons are not unexpected because both the origin and the amount of drift algae and seagrasses in Flinders Bay are likely to be directly dependent on weather conditions. In particular, high-intensity weather events such as storms and large swells are likely to be important because they dislodge algae/seagrasses and the swells are likely to move drift algae substantial distances from their source reefs.

It was perhaps surprising to find significant differences in drift algae/seagrass species between the sites in this study. These differences were at a very small spatial scale of only hundreds of meters. This finding is important because it demonstrates that the positioning of artificial structures for maximising the retention of drift algae for the sea ranch is probably operating on a much finer spatial scale than has been recognised to date.

It has been shown that greenlip abalone prefer feeding on red algae over brown (Shepherd 1973). From this, it could be inferred that those seasons and sites with a higher prevalence of drift Rhodophyta would be most likely to produce the highest growth rates of abalone on the farm. This study has shown a relatively stable presence of red algae all the year round, with different species ranging between 5 and 13 % (Table 4.4). Autumn, winter and spring, were dominated by high percentage contributions of *S. dorycarpa* as well as *P. pedicellata* in winter and G. flagelliformis in autumn. The percentage of relative abundance of brown drift-algae species in summer (18 %) was remarkably low compared to the other seasons (Table 4.6). Seagrasses (Charophyta) were the dominating group in summer. The occurrence of the seagrass *Z. tasmanica* was the most conspicuous element in the transition between spring and summer (Table 4.5).

Although many studies have highlighted the dietary preference of red algae by *H. laevigata*, it has also been shown that this species accepts live seagrass blades and their epiphytes as a substantial component of its diet during the summer months (Shepherd 1973). The availability of drift seagrass is likely to contribute to the diet of *H. laevigata* in the summer season. However, Shepherd (1973) did record crop fullness of *H. laevigata* to be at its lowest level in the summer months and attributed that to the selective feeding behaviour of the species in these months.

It could be hypothesized that the highest peaks in growth for greenlip abalone on the ranch would be likely to occur during winter when red algae such as *P. pedicellata* and *G. flagelliformis* appear as one of the most important elements contributing to the dissimilarities among seasons (Table 4.5). Quarterly growth increments recorded on the Ocean Grown Abalone Pty Ltd sea ranch in 2012 are shown in Melville-Smith et al. (2013). They show growth to be consistently slower in spring compared to other seasons.

An important concern on an abalone sea ranch is the potential for the stock to move off the artificial abitat onto surrounding natural habitat. On the Flinders Bay abalone farm, this is not possible because the lease sites are on sand and seagrass beds (Adams, pers. comm.). However, a limited amount of movement of farmed stock from one abitat to another does take place (Melville-Smith et al. 2013). Shepherd (1973) considered it probable that abalone only move when their food supply becomes limited. He believed when drift algae is abundant that they cease moving altogether. If this hypothesis is valid, then the results from this study would suggest that the small and infrequent movements of stock from one abitat to another probably takes place in spring, when red algae are less dominant.

Given the consistent dominance of the brown algae *S. dorycarpa* across all sites and seasons except summer, it would be logical to investigate any specific level of preference of *H. laevigata* for this alga. The preference for red alga such as *P. pedicellata* has been supported by *in situ* observations by divers on the sea ranch, but the possible intake of some of the most abundant brown algae should be considered in further studies.

Overall, the results obtained in this study confirm our hypotheses concerning potential seasonal changes in the species composition of the drift algae and seagrasses. They have also drawn attention to the need for careful selection of locations to deploy artificial structures for abalone sea ranching, given that the species composition of drift algae may vary at even small scales (within hundreds of meters). One possible factor that may explain the variation at this scale is the presence of geographic elements that modify the flow of the currents which transport the drift algae. This could be the case in the main lease area where the islands located to the south of Cape Leeuwin have been shown to have a considerable effect on the hydrodynamic system of Flinders Bay (MacPherson, unpubl. data).

Another process that could be driving the variation among sites is a potential interaction between abitats. This could be explored using an experimental design modified to include more sites on the sea ranch, including on both the inner and outer lease areas. Such a design would generate a more comprehensive picture of the processes occurring on the sea ranch. A fully hierarchical design including replicates at the reef level would be useful to test whether drift algae composition can vary due to the arrangement of abitat structures.

This study has provided useful insights into spatial and seasonal differences in the composition of the algae and seagrasses on the Flinders Bay abalone farm. An important future direction for this research will be to use environmental DNA barcoding to positively identify the digested and partially digested algae and seagrass in the gut of the abalone.

Having a comprehensive understanding of abalone feeding preferences and the interactions they may have with their environment in a sea ranch may serve as a guide to replicate this enterprise in locations with similar conditions. In this sense, this study has shown that drift algae assemblage composition in Flinders Bay appears to reasonably match published *H. laevigata* feeding preferences.

4.6 Conflicts of Interest

Assistance was provided by staff of Ocean Grown Abalone Pty Ltd with diver collection of sample

4.7 Acknowledgments

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Note: This is the draft of a research paper which is destined to be submitted to a peer reviewed journal.

5 Seasonal variations in the protein:energy ratio of drift-algae and seagrass on sea-ranched abalone (*Haliotis laevigata*) in Flinders Bay, Western Australia

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5.1 Abstract

A newly developed sea ranch farming greenlip abalone (*Haliotis laevigata*), is operational in Flinders Bay, Augusta, Western Australia. The abalone are dependent for their food source, on drift algae and seagrass that are transported to the farm from offshore reefs. This study has evaluated seasonal variations in the nutritional value of the drifted seaweeds and seagrass.

Published research has shown that generally, optimal requirements for crude protein in abalone are higher than that which occurs in seaweeds. Drift seaweeds in all Divisions sampled, had significantly ($p<0.001$) higher crude protein levels in autumn and winter than in spring and summer Furthermore, red algae, which is the main food source of greenlip abalone, had significantly higher crude protein content than brown algae and seagrasses.

The energy levels of the different sea weed taxa sampled, were mostly within a similar range and showed no particular trend in terms of seasonal or taxonomic differences.

The abalone showed significant differences between seasonal crude protein levels in the foot muscle, with summer and to a lesser extent autumn, being higher than winter and spring.

There were significant differences in protein content of the abalone meat at the different sampling sites on the farm ($p = 0.002$) and in the different seasons ($p<0.001$). There were no significant differences in the meat moisture content across seasons, but there were significant differences ($p<0.001$) across sampling sites. These sites were within a few hundred metres of each other.

The significance of the nutritional biochemical composition of the drift seaweeds and the significance of seasonal and Divisional differences between them, is discussed. These have also been compared and contrasted with the meat moisture and crude protein levels in the abalone.

5.2 Introduction

Most abalone production in Australia is through commercial harvesting of wild stocks, with current catches ~4,500 t per annum (Abalone Council Australia Ltd, 2016). By comparison, the recent (2013) estimates of the global production of farmed abalone were 103,464 mt (Cook, 2014) and the growth of farmed abalone production over an eight year period preceding 2010 was over 750 % (Cook, 2014)

Abalone aquaculture has followed two different paths, namely intensive and extensive culture (Cook 2014). Intensive culture is where the farming takes place in man-made structures either on land or sea, whereas extensive culture takes place on artificial substrate or structures under water. Under intensive culture additional food is required, but extensive culture may, or may not require food supplementation.

Intensive culturing is commercially less important than fishing for abalone in the wild in Australia, but even so there are currently ~20 farms producing over 1,400 t of product per annum (2015 figure) (Anon., undated). Until recently, there was no commercially successful extensive culturing of abalone in Australia, however a greenlip (*Haliotis laevigata*) abalone sea ranch has now been established in Flinders Bay, Augusta, Western Australia. Harvest levels have been small and mostly experimental to date, but production is expected to expand rapidly in the future (Adams, 2015).

Juvenile greenlip abalone are hatchery reared at a land-based abalone farm, before being released onto the offshore farm in Flinders Bay. This process involves seeding the juveniles onto artificial reefs, termed abitats (a play on the words ‘abalone’ and ‘habitat’), allowing them to grow naturally without any artificial food. The end product is to all intent and purpose indistinguishable from wild-caught abalone (FRDC, 2014)

The growth, survival and overall quality of the abalone grown on the farm is highly dependent on the drift algae and seagrass that are their food source. This macro algal wrack is transported across the lease site by currents and swell action to finally wash up on the local beaches. Research on a hydrodynamic and dispersal model of drift algae in Flinders Bay is currently being finalised for publication (MacPherson, 2017), as is an analysis of changes in the species diversity of drift algae in the Bay (Fernandez et al. submitted). This study is a companion report to those by MacPherson (2017) and Fernandez et al. (submitted) and is aimed at examining seasonal changes in the nutritional value of the drift algae and seagrasses in Flinders Bay and implications that this might have on the condition of the abalone stock on the farm.

There are no data on the species of abalone that make up the diet of greenlip abalone on the Flinders Bay abalone farm. However, a study on the feeding behaviour of greenlip abalone in three areas of South Australia, each with a different kind of environment (Shepherd 1973), showed that greenlip abalone feed almost exclusively on drift algae and seagrasses and their epiphytes. Shepherd (1973) showed that greenlip abalone are selective in their food choice, feeding mainly on red algae in winter when these algae were most abundant. Green algae and most brown algae were rejected by greenlip ablone (Shepherd, 1973), but two brown algae species were consumed in summer and autumn when red algae were scarce. During these seasons, the greenlip abalone also consumed seagrasses and their epiphytes

While we are uncertain as to exactly which algae and seagrasses are consumed by the abalone on the Flinders Bay farm, we do know that their diet is determined by known seasonal patterns in the dominance of various drift algae and seagrass species. The aim of this study is to determine whether nutritional values of the drift algae and abalone meat change seasonally. The implications of any seasonal trend would be that this might be expected to show similar trends in the growth and overall health of the farmed abalone stock

5.3 Material and methods

Three sampling sites, AC1, RL3 and AC4 were selected on the main lease of the Ocean Grown Abalone Pty Ltd (OGA) aquaculture farm at Flinders Bay, Western Australia (Figure 5.1). There is very little difference in depth or topography over the lease site and the sites were therefore chosen to provide a spread of sampling across the lines of abitats that were in place when the project commenced. No sampling was done on the Inner and Outer lease sites, as these areas were not stocked with abalone when this project was initiated.

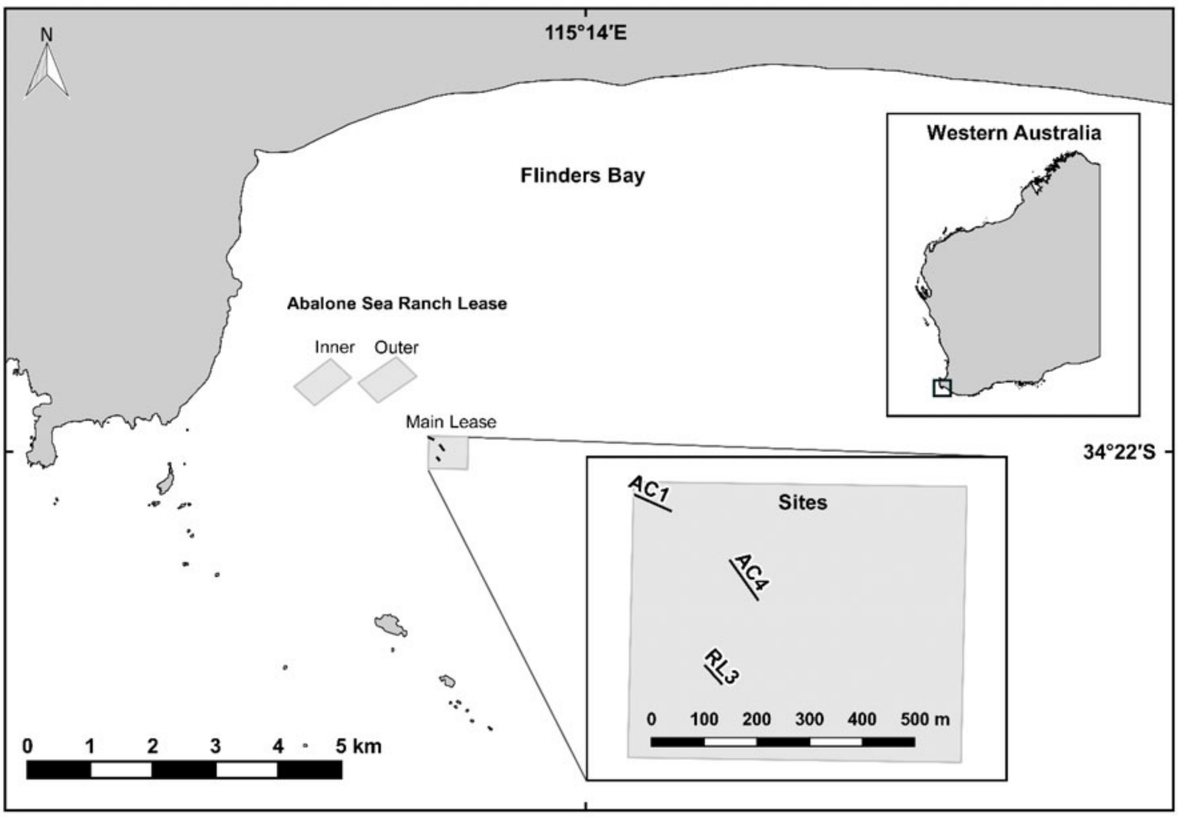

Figure 5.1 Map showing Greenlip abalone (*H. laevigata*) aquaculture lease sites on the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay, Augusta, Western Australia. The three sampling sites (AC1, AC4 and RL3) are shown in more detail are shown in more detail in the expanded view of the main lease site. The inset to the right of the Figure shows the location of Flinders Bay in relation to the rest of Western Australia

Samples were collected monthly. Four abitats were randomly selected at each of the three sampling sites and three abalone were taken from each abitat. In addition, a representative quantity of drift seagrass and algae found in and up against each of the four abitats, was also collected for later analysis. All samples were placed in labelled plastic bags and were frozen.

Before being processed, the samples were defrosted in sea water. The carapace length (CL) and width (CW) of each abalone was measured and the digestive tract was removed. The meat contents were cut into smaller pieces as possible using a scalpel and their wet weight was measured before being dried at 60 °C for 48 hours and reweighed. Differences between the wet and dry weights was used to provide the moisture content of the meat.

The algae and seagrass samples were cleaned thoroughly in seawater and separated by species prior to being identified. Wet weights of each species were recorded and they were then dried at 60 °C for 24 hours, to provide a dry weight. All samples >5 g (dry weight) were macerated in a blender for further analysis.

The protein, ash and calorific content of the seaweeds and seagrasses and protein content of the abalone meat were analysed according to standard guidelines for qualitative and quantitative analysis (Feldsine, Abeyta, & Andrews, 2002). Protein composition was determined using a ‘FOSS Kjeltec 8200 Auto Distillation Unit’. Inorganic ash content was measured by combusting the samples in a muffle furnace at 600 °C until constant ash weights were obtained, and calorific content of the samples was measured using an IKA Werke C2000 calorimeter.

5.3.1 Data analysis

Throughout this study, seasons have been defined as summer: January – March; autumn: April – June; winter: July – September and spring: October – December. While these months do not reflect the general monthly definitions for the terrestrial Austral seasons, they fit more closely to marine seasonal patterns of sea surface temperature in Flinders Bay (see Melville-Smith, Adams, Wilson, & Caccetta, 2013)

In the case of the algae and seagrasses, a total of 66 taxa (50 identified to species and 16 to genus) were identified over the course of the study (Fernandez et al. submitted). Many of the species did not occur regularly, but analysis showed that six taxa (*Scytothalia dorycarpa, Amphibolis* spp., *Pollexfenia pedicellata*, *Ecklonia radiata*, *Zostera tasmanica* and *Gracilaria flagelliformis*) contributed consistently across seasons and sites

Given the seasonal occurrence of the drift algae and seagrasses, it has not been possible to statistically analyse the nutritional value data of all species recorded, by season. All species have been considered when the nutritional values of the seaweeds have been examined over the one-year sampling programme. However, these data which have used mean values over the full year, are to some extent biased by ignoring seasonal effects.

Where seasonal differences in the nutritional value seaweeds have been examined, the data have considered only six taxa that occurred regularly through the year. Of those, two occurred in each of three Divisions; *Scytothalia dorycarpa* and *Ecklonia radiata* are Heterokontophyta, *Pollexfenia pedicellata* and *Gracilaria flagelliformis* are Rhodophyta, and *Zostera tasmanica* and *Amphibolis* spp. are Tracheophyta. These small number of phylogenetically distinct species have been arranged by Division over the year and for simplicity of presentation, the nutritional value data for the six taxa have been averaged within their three Divisions, for each month sampled. Results have been presented by the Division name, rather than the names of the individual taxa.

Data relating to the measured dimensions and weight of the abalone were recorded throughout the duration of the study, but these have not been compared in the analysis because of the potential for different year classes of the seeded abalone to be mixed in the samples. Parameters such as variations in meat protein and percentage of moisture content by month and season are less dependent on any uncertainty of the year class of the abalone sampled and these therefore have been used in the data analysis.

Data from a single abitat were combined by site in the case of abalone and across all sites for drift algae/sea grass. Mean moisture (%), protein (%), energy (KJ/g), and ash contents (%), as well as protein energy ratio (P/E ratio) were calculated for algae/seagrass. Mean moisture (%) and protein content values (%) were also calculated for the meat of ranched abalone taken from the Flinders Bay sea ranch. These data were all analysed to determined seasonal and Divisional similarities and differences using one- and two-way ANOVA (SPSS-20 and SPSS-24 software). To calculate the P/E ratio, protein content was converted to protein milligrams per gram, and energy from kilojoules per gram to kilocalories per gram, before dividing one by the other to calculate the ratio

For those factors with significantly ($p<0.05$) different F values, Scheffe post hoc tests were used to determine differences between the means. Levene's test was used to test the homogeneity of variance and Kolmogorov-Smirnov tests were used for to test for normality

To examine correlations between environmental temperature and meat moisture percentage, daily temperature data for Flinders Bay in 2012 from Melville-Smith et al. (2013) was averaged to calculate the mean monthly temperature for that year. The relationship between these monthly temperature values and monthly variation in meat moisture of the abalone was analysed using Pearson's correlation test ($\alpha = 0.05$).

Seasonal correlations, again using Pearson correlation tests, were also examined for the P/E content of a limited number of representative algae and seagrass species that occurred in all seasons and the protein content of the abalone sampled over the corresponding seasons.

5.4 Results

5.4.1 Algae and seagrasses

Seasonal changes in the moisture, protein, energy, ash and protein/energy ratio of Heterokontophyta, Rhodophyta and Tracheophyta sampled during the course of this study are documented separately below for each component of the proximate analysis. A summary or the results is presented in Table 5.1.

5.4.2 Moisture content

The percentage of moisture was significantly different between the three Divisions (Heterokontophyta, Rhodophyta and Tracheophyta) (F = 8.02, df = 2,72; p<0.001). The Rhodophyta had lower mean moisture content (11.14 ±2.5 %) than the other two Divisions, namely Tracheophyta (22.95 ±1.32 %) and Heterokontophyta (24.27 ±0.99 %). Moisture content was also shown to differ significantly across seasons (F = 3.09; df = 3,72; p = 0.032). However, there was no any interaction between season and Division (F = 0.45, df = 5,72; p = 0.99). Samples taken in summer had the highest moisture content (28.27 ±1.93 %), and this was not statistically different to autumn samples (23.33 ±2.38 %), but it was significantly higher than the spring (19.76 ±1.58 %) and winter (19.25 ±1.29 %) samples.

5.4.3 Protein content

Protein levels were generally higher for the Rhodophyta compared to the Heterokontophyta and Tracheophyta (Table 5.1). However, one family in the Rhodophyta, the Corallinaceae, recorded consistently low levels of protein of <2 % down to 0.4 %. The two species *Metagoniolithon stelliferum* and *Amphiroa gracilis* that recorded low overall mean values in Table 5.1, are both members of the Corallinaceae.

Table 5.1 The range of mean protein values (high, low and overall) for the three Divisions of seaweeds commonly sampled on the Flinders Bay abalone sea ranch, Western Australia during this study and the taxa that recorded those values

Division	Range	mean value (%)	Taxa
Rhodophyta	High	9.16 ±1.01	*Gracillaria* sp.
		8.36 ±0.88	*Pollexfenia pedicellata*
	Low	1.82 ±0.31	*Metagoniolithon stelliferum*
		1.28 ±0.13	*Amphiroa gracilis*
	Overall	6.03 ±0.32	
Heterokontophyta	High	7.89 ±1.69	*Myriodesma sp.*
		6.03 ±0.7	*Sargassum* sp.
	Low	3.49 ±0.15	*Scytothalia*
	Overall	4.62 ±0.17	
Tracheophyta	High	4.13 ±0.17	*Amphibolis sp.*
	Low	2.08 ±0.30	*Posidonia australis*
	Overall	3.87 ±0.13	

When examined over the full year using only species that occurred in all seasons (Table 5.5), there was no significant interaction in the mean percentage of protein between season and Division (F = 2.68; df = 5,72; p = 0.028), but when examined individually, protein levels were shown to differ significantly across both seasons (F = 16.07; df = 3,72; p<0.001) and Divisions (F = 11.78; df = 2,72; p<0.001).

Highest mean protein percentages were recorded in winter (Rhodophyta = 11 ±0.6 %) and lowest in summer (Heterokontophyta = 2.3 ±0.7 %) (Table 5.5). Protein percentages cycled seasonally. They were highest in winter and decreased through spring and summer, before increasing again in autumn. The mean protein content of the Rhodophyta (8.18 ±0.71 %) was significantly (F = 16.07; df = 3,72; p<0.001) higher than for the Tracheophyta (3.88 ±0.36 %) and Heterokontophyta (4.2 ±0.07 %) (Table 5.5).

5.4.4 Energy content

Energy content levels fell into similar ranges across taxa in all Divisions of seaweed (Table 5.2). The Rhodophyte species generally had slightly lower levels than the other two Divisions.

Table 5.2 Range of mean energy values (high, low and overall) for the three Divisions of seaweeds commonly sampled on the Flinders Bay abalone sea ranch, Western Australia during this study and the taxa that recorded those values

Division	Range	mean value (KCal/g)	Taxa
Rhodophyta	High	2.89 ±0.12	*Lenormandia pardalis*
	Low	0.67 ±0.03	*Metagoniolithon stelliferum*
	Overall	1.96 ±0.06	
Heterokontophyta	High	3.09 ±0.04	*Platythalia angustifolia*
	Low	1.48 ±0.27	*Myriodesma* sp.
	Overall	2.6 ±0.03	
Tracheophyta	High	2.84 ±0.09	*Amphibolis* sp.
	Low	1.85 ±0.24	*Posidonia australis*
	Overall	2.49 ±0.07	

Energy (KCal/g) values for those species that occurred in all seasons were significantly different across Division (F = 10.32; df = 2,72; p<0.001), but non-significant among season (F = 1.66; df = 3,72; p = 0.182). There was a significant interaction between those two factors (F = 3.65; df = 5,72; p = 0.005). In terms of Division, calorific levels in the Rhodophyta (1.8 ±0.15 KCal/g) were significantly lower KCal/g) (F = 10.32; df = 2,72; p<0.001), but there was no significant difference between levels in the later two Divisions (F = 10.32; df = 2,72; p<0.001) (Table 5.5).

5.4.5 Ash content

The ash content of the Rodophyte species were generally higher compared to seaweed taxa in the other two Divisions (Table 5.3). Differences in the ranges recorded for species in the Heterokontophyta and the Tracheophyta were less extreme.

Table 5.3 Range of ash values
(high, low and overall)for the three Divisions of seaweeds commonly sampled on the Flinders Bay abalone sea ranch, Western Australia during this study and the taxa that recorded those values

Division	Range	mean value (%)	Taxa
Rhodophyta	High	67.23 ±6.04	*Metagoniolithon stelliferum*
		61.6 ±1.2	*Thuretia quercifolia*
	Low	23.65 ±3.02	*Lenormandia pardalis*
	Overall	45.68 ±1.65	
Heterokontophyta	High	46.4 ±4.67	*Myriodesma* sp.
	Low	18.64 ±0.42	*Platythalia angustifolia*
	Overall	23.66 ±0.57	
Tracheophyta	High	29.79 ±1.19	*Amphibolis* sp.
	Low	20.2 ±1.67	*Zostera tasmanica*
	Overall	27.6 ±1.09	

Ash content of those species that occurred in all seasons, showed a significant interaction between season and Division (F = 3.22; df = 5,70; p = 0.017). Samples in summer showed a significantly lower percentage of ash (23.7 ±1.65 %), compared to those taken in spring (33.18 ±1.3) and winter (31.76 ±1.04 %), but the winter and spring samples were not statistically different to each other (F = 2.2; df = 3,70; p = 0.095) (Table 5.5). There was a significant difference in the percentage of ash across the three Divisions sampled (f = 39.52; df = 2,70; p<0.001). Rhodophyta samples had the highest percentage of ash (41.23 ±1.77 %), followed by Tracheophyta (30.72 ±1.1 %) and Heterokontophyta (23.45 ±0.79 %) (Table 5.5).

5.4.5 Protein/Energy Ratio

The range of P/E values recorded for different species in the Rhodophyta and Heterokontophyta was similar, but comparisons of all species within the two Divisions showed that P/E values were higher for the Rhodophytes. The mean P/E value for all species sampled was similar for the Heterokontophyta (17.5 ±0.77) and Tracheophyta (15.38 ±0.7) (Table 5.4).

Table 5.4 Range of mean P/E values (high, low and overall) for the three Divisions of seaweeds commonly sampled on the Flinders Bay abalone sea ranch, Western Australia during this study and the taxa that recorded those values

Division	Range	mean value (proportion)	Taxa
Rhodophyta	High	41.36 ±2.63	*Thuretia quercifolia*
	Low	13.87 ±1.04	*Lenormandia parda*
	Overall	32.96 ±1.45	
Heterokontophyta	High	53.16 ±1.68	*Myriodesma* sp.
	Low	13.11 ±0.55	*Scytothalia dorycarpa*
	Overall	17.5 ±0.77	
Tracheophyta	High	16.67 ±0.9	*Amphibolis* sp.
	Low	10.14	*Posidonia australis*
	Overall	15.38 ±0.7	

Protein and energy ratios for those species that occurred in all seasons showed significant interaction between season and Division (F = 3.77; df = 5,71; p = 0.004). The highest P/E value was recorded in autumn (Rhodophyta; 57.83 ±6.7) and the lowest in summer (Heterokontophyta; 8.75 ±2.7). There were significant differences in the P/E values across Division (F = 43.51; df = 2,71; p<0.001), with the Rhodophyta (44.05 ±2.7) recording a significantly higher P/E ratio than the other two Divisions (Tracheophyta 15.12 ±1.44 and Heterokontophyta 16.21 ±1.04) (Table 5.5). Differences in P/E values between the Tracheophyta and Heterokontophyta were not significant. P/E values were significantly different across the seasons (F = 19.74; df = 3,71; p<0.001). Winter (31.85 ±1.3) showed highest P/E values, followed by autumn (29.9 ±2.5), spring (16.9 ±1.67) and summer (10.08 ±2.03) (Table 5.5).

Table 5.5
Showing mean ± SE values for five different parameters and for three algae/seagrass Divisions, for each season and for the full year on the Flinders Bay sea ranch, Augusta, Western Australia

		Season				**Total**
Parameter	**Division**	**Summer**	**Autumn**	**Winter**	**Spring**	**Mean**
Moisture %	Tracheophyta	27.79 ±2.86	21.55 ±2.86	21.75 ±2.41	20.73 ±2.41	22.95 ±1.321
	Rhodophyta	na*	13.68 ±6.39	11.41 ±2.41	9.69 ±3.69	11.60 ±2.592
	Heterokontophyta	28.68 ±2.61	24.43 ±1.55	22.38 ±1.84	21.57 ±1.77	24.27 ±0.992
	Total	**28.23 ±1.93a**	**19.89 ±2.39ab**	**18.51 ±1.29b**	**17.33 ±1.58a**	
Protein %	Tracheophyta	3.33 ±0.79	4.01 ±0.79	4.86 ±0.67	3.35 ±0.67	3.89 ±0.371
	Rhodophyta	na	9.04 ±1.77	11.01 ±0.67	4.51 ±1.02	8.19 ±0.722
	Heterokontophyta	2.32 ±0.72	5.18 ±0.43	6.22 ±0.51	3.43 ±0.49	4.29 ±0.281
	Total	**2.82 ±0.54a**	**6.08 ±0.66b**	**7.36 ±0.36c**	**3.76 ±0.44ab**	
Calorie Kcal/g	Tracheophyta	2.88 ±0.17	2.13 ±0.17	2.42 ±0.14	2.68 ±0.14	2.53 ±0.081
	Rhodophyta	na	1.56 ±0.37	2.14 ±0.14	1.84 ±0.22	1.85 ±0.152
	Heterokontophyta	2.64 ±0.15	2.84 ±0.09	2.57 ±0.11	2.53 ±0.10	2.65 ±0.062
	Total	**2.76 ±0.11a**	**2.18 ±0.14a**	**2.38 ±0.08a**	**2.35 ±0.09a**	
Ash %	Tracheophyta	24.42 ±2.57	31.26 ±2.30	33.68 ±1.94	33.52 ±2.10	30.72 ±1.121
	Rhodophyta	na	na	37.82 ±1.94	44.64 ±2.97	41.23 ±1.773
	Heterokontophyta	23.15 ±2.10	25.47 ±1.25	23.79 ±1.48	21.40 ±1.42	23.45 ±0.802
	Total	**23.79 ±1.66a**	**28.36 ±1.31ab**	**31.76 ±1.04b**	**33.19 ±1.30ab**	
P/E ratio	Tracheophyta	11.42 ±3.01	13.51 ±3.37	20.95 ±2.55	12.86 ±2.55	14.69 ±1.441
	Rhodophyta	na	57.83 ±6.74	50.47 ±2.55	24.47 ±3.89	44.26 ±2.732
	Heterokontophyta	8.75 ±2.75	18.38 ±1.63	24.13 ±1.94	13.60 ±1.87	16.22 ±1.051
	Total	**10.09 ±2.04a**	**29.91 ±2.57ab**	**31.85 ±1.36c**	**16.98 ±1.67ab**	

Means followed by different subscript letters are significantly different among the seasonal mean and numbers are significantly different among division.
*na denotes unavailability of the data for particular season or division.

5.4.7 Meat Analysis

Seasonal changes in the moisture, and protein content of the meat of ranched abalone sampled during the course of this study are documented below and a summary or the results is presented in Table 5.6.

5.4.8 Meat moisture content

There was no significant interaction between season and sites when analysed for meat moisture content (F = 1.36; df = 6,131; p = 0.235). Meat moisture content was significantly different between sites (F = 10.754; df = 3,131; p<0.001), but not between the seasons (F = 1.64; df = 3,139; p = 0.182). Site AC4 had the highest mean meat moisture content (74.52 ±0.35 %), followed by RL3 (73.26 ±0.36 %) and AC1 (72.19 ±0.35 %) (Table 5.6). The mean meat moisture contents in summer, autumn, spring and winter were 73.97 ±0.41 %, 73.51 ±0.41 %, 73.19 ±0.41 % and 72.63 ±0.41 % respectively (Table 5.6).

5.4.9 Meat protein

Significant interactions in meat protein content were observed at all three sites during all seasons (F = 2.51; df = 6,131; p = 0.025). There were also significant differences between sites (F = 6.47; df = 6,131; p = 0.002) and season (F = 27.23; df = 6,131; p<0.001). Site AC4 showed significantly higher mean protein values (66.88 ±0.64 %) than AC1 (63.63 ±0.63 %) (Table 5.6), however meat protein content at site RL3 (65.21 ±0.65 %) was not significantly different compared to the other two sites. In terms of season, meat protein levels were significantly higher in summer than the other seasons

(F = 23.84; df = 3,139; p<0.001). The mean level of meat protein in summer was 70.91 ±0.75 % compared to autumn, spring and winter which were 64.68 ±0.74 %, 63.12 ±0.74 % and 62.3 ±0.74 % respectively (Table 5.6).

Table 5.6 Mean ± SE values for moisture and protein levels for the meat of sea ranched abalone sampled at Flinders Bay sea ranch, Augusta, Western Australia between July 2015 and June 2016

	Summer		Autumn		Winter		Spring		
Indicators	N	Mean±SE	N	Mean±SE	N	Mean±SE	N	Mean±SE	df

Percent Meat Moisture (%)	35	73.99 ±0.35a	36	73.51 ±0.49a	36	72.63 ±0.55a	36	73.21 ±0.34a	df = 3,139;
									f = 1.64;
									p = 0.182

Meat Protein (%)	35	70.91 ±0.62b	36	64.68 ±0.96a	36	62.30 ±0.62a	36	63.12 ±0.90a	df = 3,139;
									f = 23.84;
									p<0.000

Means followed by different subscript letters denotes significant differences of the indicators among season (one-way ANOVA; Scheffe post hoc test; P<0.05).

5.4.10 Correlations between seasonal P/E of algae and seagrass and the percentage protein content of abalone meat

There was no significant correlation between meat protein and drift algae and seagrass P/E for the five most abundant species sampled in winter: *Scytothalia dorycarpa* (r = –0.26, p = 0.272), *Pollexfenia pedicellata* (r = 0.101, p = 0.744), *Platythalia angustifolia* (r = 0.298, p = 0.437), *Ecklonia radiata* (r = –0.193; p = 0.548) and *Amphibolis* sp. (r = –0.127; p = 0.57), or in spring: *Scytothalia dorycarpa* (r = 0.072; p = 0.791), *Ecklonia radiata* (r = –0.219; p = 0.453) and *Amphibolis* sp. (r = –0.322; p = 0.28)

In summer, only one out of three species showed a significant positive correlation with meat protein. *Amphibolis* sp. was positively correlated with meat protein (r = 0.439, p = 0.025), but *Zostera tasmanica* (r = –0.339; p = 0.236) and *Hymenocladia filiformis* (r = –0.288; p = 0.39) were not.

In autumn, only one out of six drift algae and seagrass species was significantly correlated with meat protein. *Amphibolis* sp. was significantly correlated (r = 0.577; p = 0.039), but *Sargassum* spp. (r = 0.1; p = 0.873), *Scytothalia dorycarpa* (r = 0.035; p = 0.875), *Platythalia angustifolia* (r = 0.33; p = 0.267), *Ecklonia radiata* (r = –0.008; p = 977) and *Gracilaria flagelliformis* (r = 0.023; p = 0.96) showed no correlation.

5.5 Discussion

It is well known that nutritional properties can vary within and between species (Manivannan et al. 2009; Fleurence 1999,), both on an inter and intra-annual basis (Khairy and El-Shafay 2013) and can be influenced by a variety of factors including water temperature, light intensity and duration, salinity and nutrients (Manivannan et al 2009; Mæhre 2014). There is however, a greater degree of consistency in the nutritional values of algae when they are viewed across Divisions; for example, protein level and amino acid content is generally low in brown and green algae and highest in the reds (Mæhre 2014, Fleurence 1999, Dawczynski 2007, whereas fatty acid profiles are generally lowest in green algae, higher in the reds, and highest in the brown algae (Mæhre 2014). This general consistency in nutritional values of seaweeds considered at a Divisional level, was our motivation for combining those species that occurred in all seasons within a Division when examining seasonal trends in our results. Where the data were not considered across seasons, the analyses used individual species.

The algae and seagrass sampled on the study site were all detached fronds. Experiments by MacPherson (2017) using fronds from four seaweed species that commonly occur on the abalone sea ranch in Flinders Bay, showed the plant material to be easily suspended and swiftly transported by low velocity water movements. This, and the visual appearance of the drift weed, suggest that the material transported to the sea ranch is very fresh and therefore that the nutritional value is similar to what it would have been when it was attached to the growing plant.

Protein content of drift algal and seagrass species covered a wide range of values (Tables 5.1 and 5.2) and as has been found in many other studies (Rameshkumar et al. 2012; Dere et al. 2003; Fleurence 1999), the results showed that the red algae generally have higher protein content than brown.

Research undertaken at the same time as this study examining the species composition of algae and seagrasses through the year has been published elsewhere (Fernandez et al. submitted). That work showed that brown algae were dominant in all seasons except summer, at which time they were replaced by seagrasses. By comparison, red algae were proportionally very consistent in their abundance, in each season forming between 26 – 34 % of the dry weight biomass recorded on the farm (Fernandez et al. submitted). The same work also showed that the number of red drift algae taxa recorded on the Ocean Grown Abalone sea ranch in Flinders Bay, was very diverse through the year and that the Rhodophyta comprised 66 % of all the seaweed taxa that were sampled over the course of this study.

Red algae, though their consistent occurrence throughout the year and high protein content compared to other seaweed taxa, are likely to be a more nutritious food source for abalone than brown algae and seagrasses.

Shepherd and Steinberg (1992) found drift red algae to be seasonally abundant and when Rhodophyta were scarce in summer and autumn, that greenlip abalone consumed a limited number of brown algal species. In another area, Shepherd (1973) recorded greenlip abalone consuming mainly seagrasses and their epiphytes. He did note that because of this intense selection for red algae, that abalone consume less in months when red drift algae are scarce.

The regular supply of red algae recorded through the year in Flinders Bay (Fernandez et al., submitted) would suggest it is an optimal site for food supply suitable for greenlip abalone farming. The protein content of the seaweeds was variable through the year, peaking in autumn and winter, and this trend was particularly apparent for the Rhodophyta. Therefore, while food is available throughout the year for the abalone, it is not of a consistent quality.

If protein and amino acids are available in an animal's diet in sufficient quantity, then energy becomes the next most limiting factor in its growth (Bansemer et al. 2016).

The energy content values of the algae sampled in this study were in a similar range (albeit expressed in different units) to values recorded for algal species by other authors (Fleming 1996, Britz and Hecht 1997). Digestible energy values for different species of algae also tend to be similar between species (Fleming 1996), which suggests that this variable is unlikely to be as important as protein content in determining the nutritional advantages of one drift algal species over other.

Fleming (1996) showed for example, that growth of *Haliotis rubra* was fast on a diet of the red algae *Jeannerettia lobata* which has both high nitrate and energy content. However, it was slow when the abalone were fed the brown algae *Phyllospora comosa*, which has an even higher energy content than *J lobata*, but a very much lower digestible nitrogen content.

Ash provides a rough indication of the mineral content of the seaweeds. Ash content of the algae in this study was comparable with values measured in other seaweeds (Khairy and El Shafay 3013; Mæhre et al 2014).

In this, as with other studies comparing ash content in seaweeds by species and by season (Khairy and El Shafay 2002; Polat and Ozogul 2013), the levels of ash content recorded were high and variable across the seasons. Significant differences in ash content have been also been recorded across algal Divisions (Mabeau and Fleurence 1993; Ruburez 2002), but whereas Ruburez (2002) found mineral content to be higher in the brown algae than red, the reverse was true in our samples.

It is well recorded that levels of ash content in marine algae are higher than in terrestrial plants (Rupurez 2002), and are generally far higher than levels that are commonly found in commercial formulated abalone feeds. As an indication of commercial standards, abalone diets produced by Australian feed companies are generally ~35 % crude protein (Stone et al. 2014).

Britz and Hecht (1996) showed that growth rates of *Haliotis midae* of a size corresponding closest to those in this study, increased with increasing PE ratios to peak at their 44 % protein dietary treatment. These levels of protein are far higher than what are recorded in marine algae, but the relationship does show that abalone are more responsive to changes in protein in their diet than to the energy component.

The meat moisture content of the abalone sampled showed no seasonal trend, but there were differences between the three sampling sites. Further work would be necessary to categorically establish the cause of these differences in meat moisture at the sites, but it is likely that they relate to small-scale spatial differences in the quality and quantity of food available to the abalone across the farm. Variation in drift algal assemblage structure on the farm (Fernandez et al. submitted), has shown similar taxa to be present on the sampling sites, but in different proportions. These differences in the patterns of algal distribution between sites, followed the same progression sequence for the three sampling sites as the differences in moisture content of the abalone on those sites.

Stone et al. (2013) recorded an inverse relationship between meat moisture content in greenlip abalone and water temperature in animals held under experimental conditions, but even though summer temperatures in Flinders Bay are several degrees higher than winter temperatures (Melville-Smith et al. 2013), no seasonal differences were recorded in the meat moisture content of the animals sampled in this study.

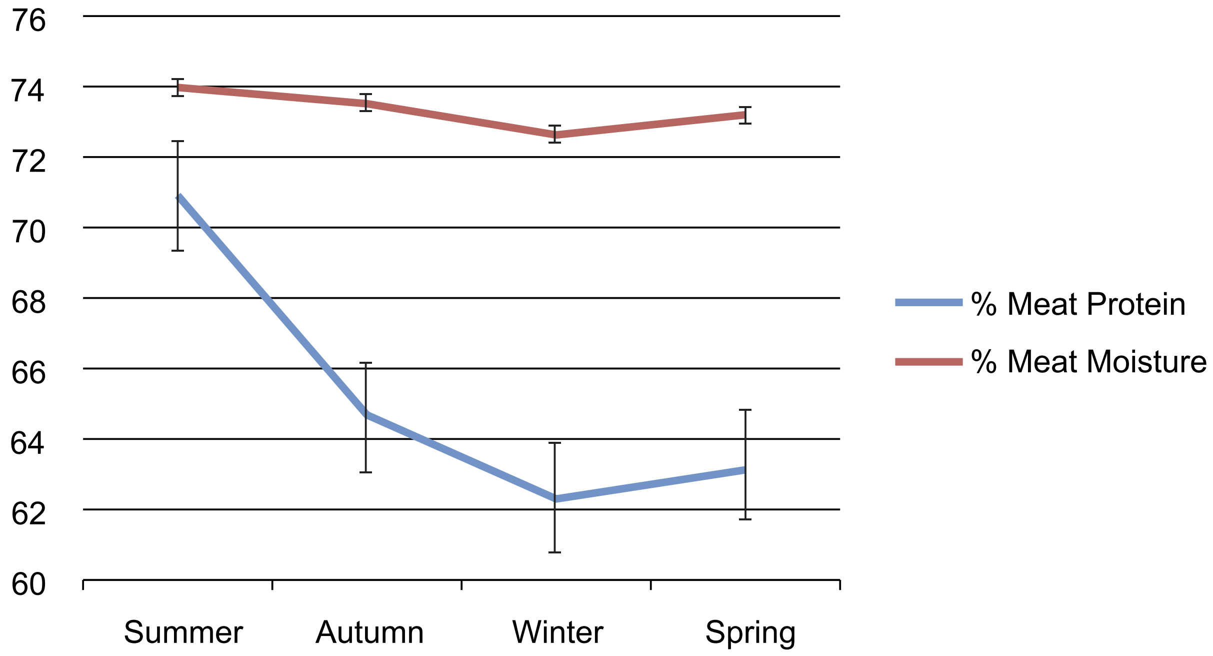

Figure 5.2 Comparisons of seasonal meat protein and moisture content for abalone sampled at Flinders Bay abalone farm, Augusta, Western Australia (error bar of SE)

Temporal changes in the dry and wet weight of muscle and viscera in *Haliotis diversicolor*, have been reported by Chiou et al. (2001) and these have been ascribed to variations in the accumulation of glycogen and protein through the year. Similarly, Dare and Edwards (1975) have shown seasonal changes in dry meat weight in the muscle (*Mytilus edulis*), which they related to changes in protein and carbohydrate levels in the animals through the year. Seasonal spawning cycles are considered to be important factors influencing changes in meat moisture (Chiou et al 2001), but this would not explain differences between sampling sites that were recorded in this study.

Optimal levels of dietary protein are variable for different species of abalone, different aged animals and for different water temperatures (Stone 2013), with ranges reported as low as 27 % in juvenile *H. laevigata* (Coote et al. 2000) to as high as 44 % in large *H. midae* (Britz and Hecht (1997). In all cases these are considerably higher than values that are generally recorded in marine algae. Seaweed taxa in this study that recorded high protein levels had mean protein values measured over the duration of the project that were <10 % (Table 5.1). The species with low protein content values recorded mean protein levels of ~1 % (Table 5.1).

In *H. laevigata*, optimum crude protein levels increase with increasing temperature and for animals of a size range comparable to those in those study, optimal dietary protein levels are between 24 % and 27 % (Stone et al. 2013). As noted by Kennish (1997), to maximise their fitness, herbivores should be maximising their protein intake by theoretically selecting for foods of high protein content.

The protein content of the abalone meat for animals sampled on the Ocean Grown Abalone sea ranch in Flinders Bay, was significantly higher in summer, which suggests that it may be related to environmental conditions. There is a lack of clarity as to optimal and maximum sea water temperatures for the survival of greenlip abalone. Gilroy and Edwards (1998), suggested the optimal maximum temperature to be 18.9 °C and the critical maximum 27.5 °C. However, Stone et al. 2013 has since shown the optimal temperature to be 22 °C. These discrepancies need clarification. They may be due to variations in different strains of *H. laevigata* that are adapted to either cold or warm water conditions over the range of the stock.

Bottom sea water temperatures increase to 22 °C on the Ocean Grown Abalone sea ranch in Flinders Bay over the summer months (temperature sensor data collected by Ocean Grown Abalone Pty Ltd during October 2014 to January 2016) and are therefore well within the reported optimal temperature tolerance limit of the species. However, Augusta is near the limit of the distributional range of the species (Hart et al. 1999). It is possible therefore, that the higher water temperature conditions in summer may be resulting in elevated protein reserves during those months. An unrelated study (Hart et al. 1999) has also shown that the condition of abalone in Flinders Bay, as measured by the whole weight of the animals/bled weight meat, is optimal in autumn and winter and less so in spring and summer.

Stone et al. (2013) showed protein deposition in greenlip abalone held under experimental conditions to be positively correlated with water temperatures in the range of 14 °C to 22 °C. While this may be the case in an experimental environment comparing responses to dietary protein and temperature, it is clearly far more complicated in the wild where there are many more variables involved such as food availability, food nutritional value, seasonal reproductive influences and more. All of these would play a part in determining the condition of the abalone.

As already noted, greenlip abalone are selective feeders favouring red algae. It has also been noted that all seaweed taxa in this study generally have higher protein and protein:energy levels in autumn and winter. These seasons of cooler water temperature are also those that show highest growth increments (Melville-Smith et al. 2013). Conversely, spring and to a lesser extent summer, are periods of slower growth (Melville-Smith et al. 2013), this despite the seaweeds generally having significantly higher protein content in those seasons.

It may be that even though the proportion of red algae compared to other seaweeds remained reasonably constant through the year (Fernandez et al. submitted), that larger quantities are available for the abalone in winter than summer. This would seem plausible, given that the winter months are periods when large numbers of cold fronts pass over the south coast of Western Australia (MacPherson 2017), causing storm surge which breaks off seaweed fronds and washes them inshore. Furthermore, MacPherson's (2017) work modelling particle transportation through the lease sites in the OGA sea ranch, confirmed that their movement was directly related to the intensity of the wind and wave conditions in the simulations.

A different, possibly related explanation for the seasonal responses to in protein content in the abalone, may be that the abalone not only have more food available in the winter months, but also a greater diversity of species available. It is generally accepted that abalone growth responds best to a mixture of algal species in their diet (Simpson and Cook 1998; Naidoo et al. 2006). The fact that abalone fed a mixed algal diet grow faster than those fed a single species diet, is considered to be because they benefit from the variety of different macronutrients in the different algal species making up the compound diet (Viera et al. 2015).

There are reasons other than differences in nutritional composition, that have been put forward to explain variations in the growth rate of abalone fed different algal taxa. Day and Cook 1995 and Foale and Day 1992 have for instance reported brown algae to be digested slower than red and green algae, possibly due to the high polyphenol content in the brown algal species retarding the digestion of those species. Other factors are also at play in determining digestibility of different algal species, the most obvious being whether the fronds are delicate or robust (McShane et al. 1994).

A perhaps unexpected result in this study, was that there were significant differences in both meat moisture and meat protein content of the abalone on the three different sampling sites. These sites were within a few hundred metres of each other. Circulation patterns (MacPherson 2017) also showed that for most simulations, the western margin of the lease (and areas to the west of that) received more drift algae particles than areas to the east. Similarly, Fernandez et al's. (submitted) research recorded significant differences in the assemblage structure of drift algae between sampling sites on the sea ranch.

The circulation pattern and their resulting impact on differences in the biomass and species composition of seaweeds transported onto the lease site, is therefore having an impact on the quality of food available to the abalone on abitats within only hundreds of metres of each other. This in turn produces different nutritional outcomes, as is reflected in the analyses of the meat protein content. It could be expected that this would further lead to fine-scale differences in the growth rates of the abalone across the lease site.

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Note: This is the draft of a research paper which is destined to be submitted to a peer reviewed journal.

6 The effect of transportation on immune modulation of ranched and wild greenlip abalone (*Haliotis laevigata*) from Flinders Bay, Western Australia

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Roy Melville-Smith, Nelson Fernandez and Subas Adhikari

6.1 Abstract

The effect of live transportation on the immune modulation of greenlip abalone (*H. laevigata*) was investigated in autumn and winter of 2016. Abalone were collected both on a sea ranch and in the wild in Flinders Bay, Augusta, Western Australia and were transported for six hours before being re-immersed in aerated water for approximately 12 hours before being resampled. Samples in the two seasons provided different physiological responses; the winter samples were more responsive to transportation than the autumn samples. Transportation stress on winter samples significantly increased total haemocyte count and osmoregulatory capacity, suggesting an immune-stimulation. This stress also triggered an immune-suppression, causing the phagocytic rate and lysosomal stability to significantly decrease after transportation. Lactate levels in the winter samples decreased significantly after transportation, possibly indicating the transition from a stressed to normal state, during the period of recovery. The constant level of glucose before and after transportation in both seasons, showed that it was the least sensitive indicator used in this study. In general, there was no difference between wild and ranched abalone in either of the seasons sampled. However, in the autumn samples there were significant differences in haemocyte count and osmoregulatory capacity indicators across sites in the wild and ranched samples. These differences did not form a consistent indicator trend between the sites from the two sample sources and therefore do not point to any immediate concern that ranched abalone at the study site were any differently stressed to those in the wild.

Keywords

Immune modulation; Abalone; Physiological responses; Transportation

6.2 Introduction

The growing demand for abalone worldwide has led to over-exploitation of wild stocks in many parts of the world, e.g. Southern California (Taniguchi, et al. 2013), South Africa (Troel, et al. 2006), and British Columbia, Canada (Campbell 1996). To counter declining stocks and to increase production, there has been world-wide growth in abalone aquaculture since the 1990s. China is the biggest contributor to farmed abalone in global markets followed by Korea and Chile (Cook 2014). In Australia, the production of farmed abalone is only around 1 to 2 % of China's annual production (Cook 2014).

In Western Australia (WA), abalone production is mostly fulfilled by the wild capture fishery. That industry focuses on three species, namely brownlip (*Haliotis conicopora*); greenlip (*H. laevigata*); and Roe's abalone (*H. roei*) (DoFWA 2013). The production of abalone from commercial and recreational fishing in the state in 2002, was around 322 tonnes, valued at about $14 million (DoEH 2014). Latest data in 2014 shows a decline in production to only around 240 tonnes (DoFWA 2016). Aquaculture of abalone in the state is still in its infancy. There is a land-based abalone hatchery in Bremer Bay on the south coast of WA as well as an approximately 80 ha sea ranching site in Augusta (WAFIC 2016).

The development of abalone aquaculture in WA, especially for commercial sea ranching, is constrained by the limitation of suitable sites and by stringent environmental regulations (Partridge and Furey 2002). Another challenge comes from post-harvest handling, particularly in live-transportation. It is well known that the market price for live abalone is much better than for frozen or canned product (Moltschaniwskyj, Mundy and Harris 2014). However, live transportation involves physical perturbations, such as temperature changes, oxygen availability, physical contacts and shaking and these trigger stress as a mechanism to preserve homeostasis (Malham, et al. 2003). The effects of physical perturbations on stress and immune responses of abalone have been widely studied across a range of abalone species, for example in *H. iris* and *H. australis* (Baldwin, et al. 1992; Behrens, et al. 2002; Wells and Baldwin 1995), *H. diversicolor supertexta* (Cheng, et al. 2004), *H. rubra* (Dang, Speck and Benkendorff 2012) and in hybrid *H. laevigata* and *H. rubra* (Hooper, et al. 2011). Stress hormones produced during stress exposure affect the immune functions indicating a consistent link between stress response and immune response (Adamo 2012; Lacoste, et al. 2001a; Lacoste, et al. 2001b; Malham, et al. 2003).

This study has aimed to investigate the effects of 6-hour live transportation, which simulates the time taken to transport live greenlip abalone from Augusta to Perth for shipment to overseas markets, on the immune modulation of the animals. The study has also examined the differences in stress and immune responses between ranched and wild greenlip abalone (*H. laevigata*) taken at the study site. This information can be used as a proxy for determining the health status of ranched abalone compared to wild stock. Some physiological changes, such as total haemocyte count, phagocytic rate, neutral red retention, lactate level, osmoregulatory capacity, and glucose level, were examined to assess stress responses of abalone.

6.3 Materials and Methods

6.3.1 Experimental set-up

Sixty abalone were collected randomly from three sea ranching sites on the main lease of Ocean Grown Abalone Pty Ltd and from two wild sites located within Flinders Bay (Figure 6.1). The samples were collected in two seasons, autumn (April) and winter (July) of 2016. The coordinates of two wild sites were 34°22.421 S, 115°10.214 E and 34°22.252 S and 115°29.935 E, and the depths of the two sites were 10 – 12 m and 5 m respectively. All the ranched abalone sampled were in the size range 61 – 103 mm shell length (SL), which corresponds to three-year post-settlement stock. Abalone sampled in the wild were larger (84 – 135 mm SL). The age of these animals is unknown.

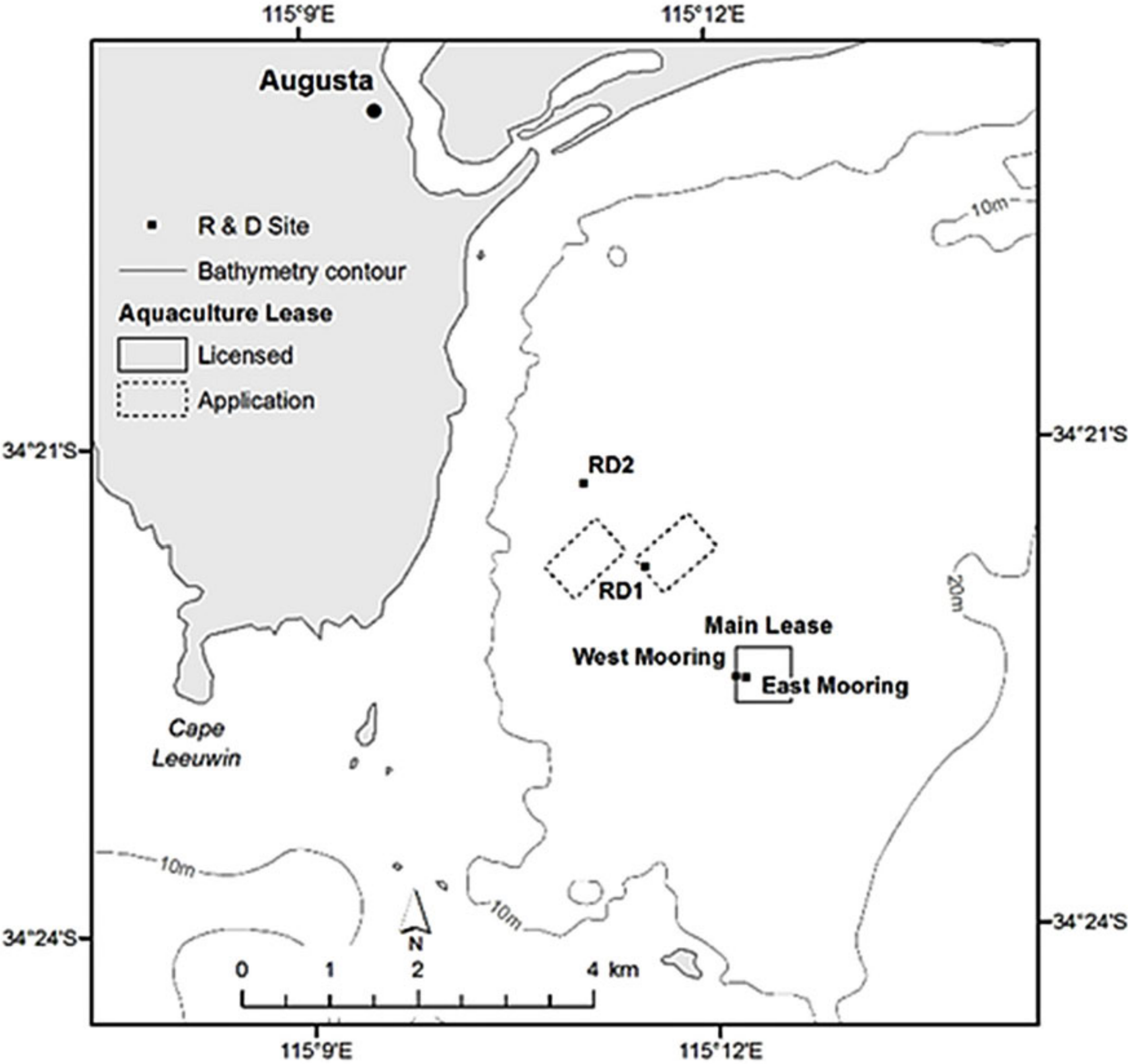

Figure 6.1 Map showing greenlip abalone (*Haliotis laevigata*) sampling sites at the Ocean Grown Abalone Pty Ltd sea ranch in Flinders Bay, Augusta

In order to measure immune responses of abalone due to transportation, haemolymph was taken before and after live-transport from three animals at each of the five sampling sites in both autumn and winter.

Immediately after being brought to the surface, the abalone sampled were temporarily placed into labelled plastic bags and put into a cool box. The autumn samples were chilled with ice and the winter samples with ice bricks. Once ashore, three abalone from each site were separated for haemolymph withdrawal. This first haemolymph withdrawal was used as a control and represented the normal immune modulation of the samples. All abalone were then packed into 40 L polystyrene boxes. Crushed ice was placed in the bottom of the box followed by wet sponge sheets to separate the ice from the abalone samples. More wet sponge sheets were used to cover the abalone and three ice packs were placed on top of the abalone. The wet sponge sheets were intended to keep the gills of the abalone moist, thereby allowing them to respire. The samples were live-transported for around 4 – 6 hours from the sampling site at Augusta, Flinders Bay, to the Curtin Aquaculture Research Laboratory (CARL) in Perth, Western Australia.

Immediately after arrived at the CARL, the abalone were transferred to a 70 L circulated and aerated fiber glass tank and allowed to recover. Visual observations on their condition were made after ~12 hours. At the same time, haemolymph was taken from those abalone from each site that had not previously had haemolymph extracted. All the haemolymph samples taken from before and after transportation were then subjected to several assays for immune indicators. These were total haemocyte count (THC), phagocytic rate (PR), osmolality, neutral red retention (NRR), lactate level, and glucose level tests.

6.3.2 Haemolymph withdrawal

Haemolymph was withdrawn before and after transportation. In each case, approximately 1 mL was taken from the cephalic sinus using a 3 mL sterile syringe and a 25 gauge one-inch sterile needle. The haemolymph was then placed into two Eppendorf tubes, with 200 µL added to 20 µL of 6 % Perchloric acid (PCA) for lactate assay purposes and the remaining haemolymph (approximately 800 µL) retained for other measurements.

The haemolymph samples that were taken before transportation were kept on crushed ice to maintain their quality until they could be stored in the laboratory freezer at –18 °C. The haemolymph samples taken after transport were also stored at –18 °C. Both samples were analysed within 24 hours of being frozen.

6.3.3 Survival rate

The effectiveness of the packaging systems used to transport live abalone has been measured by recording the percentage surviving after transportation.

6.3.4 Immune response measurements

Physiological responses of the abalone before and after transportation were analysed using six different indicators:

6.3.4.1 Total haemocyte count (THC)

Total haemocyte count (THC) was measured using the method described by Day, et al. (2010), with modification in the usage of cold-stored haemolymph. This modified method was not ideal, as previous studies (Day, et al. 2010; Ragg and Watts 2015) have suggested the use of "fresh" haemolymph taken five minutes prior to counting. However, it was unavoidable given the logistics of the fieldwork. The haemocytes were counted by adding haemolymph onto both sides of a standard Neuerbach Hemocytometer Counting Chamber under a microscope (Motic, China) at 100-fold magnification. The mean was then taken and multiplied by 5×10^4 cell per mL.

6.3.4.2 Phagocytic Rate (PR)

PR was measured using a slide-based assay which described the capacity of haemocytes to phagocytose foreign materials (Chen, et al. 2005; Dang, et al. 2013). A 40 µL sample of cold-stored haemolymph was smeared onto two Poly-LLysine® glass sides. The slides were incubated for a minimum of 10 minutes to allow the haemolymph to adhere. Excess haemolymph was then poured off the slides before adding a 40 µL of Zymosan solution (0.031 g Zymosan + 25 mL of sterile filtered seawater). The slides were re-incubated for 30 minutes before being fixed with seawater formalin for 20 min (10 % formaldehyde in filtered seawater). The hemocyte and zymosan were stained with May Grunwald Giemsa. Results were expressed as the percentage of phagocytosis (phagocytic haemocytes/100 adhered haemocytes × 100).

6.3.4.3 Neutral Red Retention (NRR)

The NRR assay followed a protocol described by Song, et al. (2007) and Ragg and Watts (2015). A 40 µL sample of haemolymph was smeared onto a Poly-LLysine® glass side and incubated in a dark chamber at a temperature of 10 °C. After incubation, excess haemolymph was poured off and 40 µL of working solution was added. The working solution was made from 20 µL of stock solution made from 2.28 mg NR powder diluted in 1 mL of Dimethyl Sulphoxide/DMSO and 1 mL of sterile filtered seawater. The stock solution was previously made from. Finally, the slide was covered with a coverslip and was re-incubated for 15 minutes. Observations under a microscope, were thereafter made at 15 minute intervals. The end-point of the assay was the time taken for the dye disappeared from the lysosomes.

6.3.4.4 Lactate level

Lactate is accumulated in haemolymph or muscles during transportation, or under hypoxia conditions. To measure this, haemolymph treated in 6 % PCA was centrifuged at 5,000 rpm at 4 °C for 10 minutes using a centrifuge (5804R, Eppendorf, Hamburg, Germany). The supernatant was then rinsed with 5 mol/L K_2CO_3 for one hour under chilled conditions to neutralise PCA. PCA was removed from the supernatant by re-centrifuging it at 5,000 rpm at 4 °C for 10 minutes. The ready-to-assay supernatant was stored frozen or used directly (Baldwin, et al. 1992; Behrens, et al. 2002). The assay was undertaken using a commercial reagent set kit (Pointe Scientific L7596). A 12 µL sample of haemolymph and 600 µL of reagent 1 were mixed and incubated at 25 °C for 30 s. Thereafter, a 400 µL of reagent 2 was added into the mixture and the sample was incubated at 25 °C for 5 min. Finally, the mixture was measured for absorbance at 546 nm using a UV-VIS Spectrophotometer (Shimadzu, Japan) and results were presented as mmol/L of lactate.

6.3.4.5 Osmoregulatory capacity

A 30 µL sample of cold-stored haemolymph was placed into a 0.5 mL Eppendorf tube. The osmolality of haemolymph was measured using a freezing point osmometer (Osmomat 030-D, Gonotec, Germany). Results were presented as Osmol/kg. Osmoregulatory capacity was determined by the difference of serum osmolality and the medium (seawater) osmolality.

6.3.4.6 Glucose level

The glucose level of haemolymph was examined using a liquid glucose (oxidase) reagent set (Pointe Scientific, Inc). A 1.0 mL quantity of working reagent was incubated at 25 °C for approximately 5 minutes, followed by the addition of 0.01 mL of haemolymph. The mixture was then incubated at 25 °C for approximately 10 minutes before measuring the absorbance at 500 nm using UV-VIS Spectrophotometer (Shimadzu, Japan). Results were presented as mmol/L.

6.3.4.7 Data analysis

Statistical analysis was performed using IBM SPSS statistics 22 at a probability level of 0.05. The mean difference of each immune response indicator before and after transportation in each sampling period was analysed using independent t-tests. The difference in immune response between sources (ranching and wild sites) was also analysed using independent t-tests. Differences in immune response between sites were analysed using one-way ANOVA and post hoc tests. In those cases, where the data were not normally distributed, they were transformed and if necessary, non-parametric statistical analysis (Mann Whitney U and Kruskall Wallis tests) was also conducted.

6.4 Results

6.4.1 Survival rate

Visual observations showed that all abalone from both the autumn and winter samples survived transportation to the laboratory. However, after transportation, some were in poor condition characterised by limp muscles and weak attachment to the tank wall.3.2

6.4.2 Effects of transportation

6.4.2.1 Total Haemocyte Count (THC)

The effect of transportation on THC was different between autumn and winter. An independent t-test indicated that there was no difference in THC before and after transportation in autumn (t = 0.564, df = 28, p = 0.577), but there were significant differences in winter (t = –2.109, df = 28, p = 0.044). THC significantly increased after transportation in winter from 483.067 ±33.833 to 644.8 ±61.185 (× 5 x 10^4 cell/mL) (Table 6.1).

Haemocyte counts taken before transportation are shown by site in Table 6.2 and for sites combined in Table 6.3. In autumn, there were significant differences in THC between sites (ANOVA, F = 3.341, df = 4, p = 0.025) (Table 6.2) and between the ranched and wild stock (t = –2.786, df = 28, p = 0.009) (Table 6.3). The THC of abalone collected from wild site 2 was significantly higher than ranching sites 2 and 3, but not ranching sites 1 and wild site 1 (Table 6.2). In contrast to that result, in the winter there were no differences in THC between sites (ANOVA, F = 1.484, df = 4, p = 0.237) (Table 6.2) and between ranched and wild stock (t = –0.939, df = 28, p = 0.356) (Table 6.3).

6.4.2.2 Phagocytic Rate (PR)

The effect of transportation on phagocytic activity between autumn and winter was similar. In both cases, an independent t-test showed that the PR was significantly different before and after transportation (autumn, t = 6.119, df = 28, p = <0.001; winter, t = 6.071, df = 28, p<0.001) (Table 6.1). The ability of haemocytes to phagocytose zymosan decreased from approximately 38 % to 18 % in autumn and from approximately 42 % to 24 % in winter (Table 6.1). Likewise, both sampling periods showed that there were no significant differences in PR between sites (autumn, ANOVA, F = 0.388, df = 4, p = 0.815; winter, ANOVA, F = 0.531, df = 4, p = 0.714) (Table 6.2) and between ranched and wild stock (autumn, t = 0.849, df = 28, p = 0.403; winter, t = 0.390, df = 28, p = 0.700) (Table 6.3).

Table 6.1 Mean ± SE of total haemocyte count × (5 × 10^4); phagocytic rate (%); neutral red retention (min); lactate level (mmol/L); osmoregulatory capacity (osmol/kg); glucose level (mmol/L) in the haemolymph of greenlip abalone (*H. laevigata*) before and after transportation in two different seasons. Common letters within the seasons sampled indicates statistical differences to not be significant

Indicators	**Autumn samples**		**Winter samples**	
	Before transport	**After transport**	**Before transport**	**After transport**
Total Haemocyte Count	491.5 ±151.3a	271.467 ±54.9a	483.067 ±33.8a	644.8 ±61.2b
Phagocytic Rate	38.067 ±3.0a	17.667 ±1.4b	42.333 ±2.4a	24.467 ±1.8b
Neutral Red Retention	186.0 ±4.3a	185.0 ±4.1a	281.6 ±7.2a	162.667 ±8.6b
Lactate Oxidase	0.386 ±0.0a	0.352 ±0.0a	0.393 ±0.0a	0.294 ±0.0b
Osmoregulatory capacity	0.128 ±0.0a	0.129 ±0.0a	–0.035 ±0.0a	0.046 ±0.0b
Glucose level	11.819 ±0.6a	14.956 ±1.6a	5.453 ±0.2a	6.007 ±0.4b

Table 6.2 Mean ± SE of total haemocyte count × (5 × 10^4); phagocytic rate (%); neutral red retention (min); lactate level (mmol/L); osmoregulatory capacity (osmol/kg); glucose level (mmol/L) in the haemolymph of greenlip abalone (*H. laevigata*) before transportation, between sites in two different seasons. Common letters within the seasons sampled indicates statistical differences to not be significant

Indicators	**Sites**				
	Ranch site 1	**Ranch site 2**	**Ranch site 3**	**Wild site 1**	**Wild site 2**
			Autumn samples		
Total Haemocyte Count	330.167 ±86.3ab	146.167 ±30.9a	157.583 ±55.6a	349.167 ±108.6ab	924.333 ±298.9b
Phagocytic Rate	25.167 ±4.1a	31.833 ±5.3a	31.833 ±7.9a	25.833 ±5.9a	24.667 ±5.2a
Neutral Red Retention	197.5 ±7.2a	180.0 ±6.7a	182.5 ±4.6a	185.0 ±8.4a	182.5 ±4.6a
Lactate Oxidase	0.370 ±0.0a	0.388 ±0.0a	0.397 ±0.0a	0.359 ±0.0a	0.330 ±0.0a
Osmoregulatory capacity	0.117 ±0.0a	0.142 ±0.0c	0.122 ±0.0ab	0.124 ±0.0abc	0.139 ±0.0bc
Glucose level	15.211 ±2.4a	15.221 ±2.4a	10.675 ±1.1a	13.826 ±2.3a	12.005 ±0.9a
			Winter samples		
Total Haemocyte Count	643.083 ±77.2a	499.25 ±53.9a	454.833 ±71.2a	532.917 ±79.2a	689.583 ±112.8a
Phagocytic Rate	28.667 ±3.9a	35.0 ±5.6a	38.667 ±6.4a	32.333 ±5.3a	32.333 ±3.7a
Neutral Red Retention	212.333 ±34.5a	227.5 ±33.2a	211.667 ±26.4a	246.667 ±13.2a	212.5 ±32.7a
Lactate Oxidase	0.351 ±0.0a	0.402 ±0.0a	0.316 ±0.0a	0.330 ±0.0a	0.319 ±0.0a
Osmoregulatory capacity	0.019 ±0.0a	–0.014 ±0.0a	–0.006 ±0.0a	0.0163 ±0.0a	0.013 ±0.0a
Glucose level	5.883 ±0.7a	6.45 ±0.5a	4.55 ±0.2a	5.683 ±0.4a	6.083 ±0.4a

Table 6.3 Mean ± SE of total haemocyte count × (5 × 10^4); phagocytic rate (%); neutral red retention (min); lactate level (mmol/L); osmoregulatory capacity (osmol/kg); glucose level (mmol/L) in the haemolymph of greenlip abalone (H. laevigata) sampled from all sites combined in the ranched and wild stock in two different seasons. Common letters within the seasons sampled indicates statistical differences to not be significant

Indicators	Autumn samples		Winter samples	
	Ranched abalone	Wild abalone	Ranched abalone	Wild abalone
Total Haemocyte Count	211.306 ±39.3a	636.750 ±174.7b	532.389 ±41.8a	611.25 ±69.8a
Phagocytic Rate	29.611 ±3.3a	25.250 ±3.8a	34.111 ±3.1a	32.333 ±3.1a
Neutral Red Retention	186.67 ±3.9a	183.75 ±4.6a	217.167 ±17.2a	229.583 ±17.6a
Lactate Oxidase	0.385 ±0.0a	0.344 ±0.0a	0.357 ±0.0a	0.325 ±0.0a
Osmoregulatory capacity	0.127 ±0.0a	0.132 ±0.0a	–0.0002 ±0.0a	0.015 ±0.0a
Glucose level	13.703 ±1.2a	12.915 ±1.2a	5.628 ±0.3a	5.883 ±0.3a

6.4.2.3 Neutral Red Retention (NRR)

Transportation only affected the immune modulation of abalone collected in winter. An independent t-test indicated that NRR before and after transportation in winter was significantly different (Mann Whitney U test, U = 2.00, p<0.001, N = 30) (Table 6.1). Transportation showed no measurable effect on lysosomal stability in the autumn samples (Mann Whitney U test, U = –0.246, p = 0.806, N = 30) (Table 6.1).

There were no statistical differences in NRR between sites (autumn, $\chi 2$ = 4.720; df = 4; p = 0.317; winter, χ^2 = 1.271; df = 4; p = 0.866) (Table 6.2) and between ranched and wild stock (autumn, U = –0.570, p = 0.569, N = 30; winter, t = –0.486, df = 28, p = 0.631) (Table 6.3) in either sampling period.

6.4.2.4 Lactate level

The lactate level in the haemolymph of samples was not affected by transportation in autumn (t = 1.298, df = 28, p = 0.205). However, the decrease in lactate level in winter from ~0.393 to ~0.294 mmol/L after transportation was shown by an independent t-test to be significantly different (t = 4.561, df = 28, p<0.001) (Table 6.1).

The differences in lactate level between sites (autumn, F = 0.788, df = 4, p = 0.544; winter, F = 1.313, df = 4, p = 0.293) (Table 6.2) and between ranched and wild stock (autumn, t = 1.566, df = 28, p = 0.129; winter, t = 1.110, df = 28, p = 0.278) (Table 6.3) was not significant for either of the seasons sampled.

6.4.2.5 Osmoregulatory Capacity (OC)

Mean osmoregulatory capacity was not affected by transportation in autumn (U = 106.500, p = 0.803, N = 30), but it did increase significantly from ~-0.035 to ~0.046 Osmol/kg (U = 0.00, p<0.001, N = 30) in winter (Table 6.1). The OC in all sites was similar in winter (χ^2 = 1.333; df = 4; p = 0.856), but the autumn samples showed significant differences between sites (χ^2 = 13.962; df = 4; p = 0.007) (Table 6.2). There was no significant difference in OC between ranched and wild stock for either sampling period (autumn, U = 82.000, p = 0.270, N = 30; winter, U = –92.500, p = 0.511, N = 30) (Table 6. 3).

6.4.2.6 Glucose level

There was no effect of transportation on haemolymph glucose level between sampling times (autumn, t = –1.443, df = 28, *p* = 0.160; winter, t = –1.183, df = 28, *p* = 0.247) (Table 6. 1), between sites (autumn, F = 1.065, df = 4, *p* = 0.394; winter, t = 2.156, d = 4, *p* = 0.104) (Table 6. 2), and between ranched and wild stock (autumn, t = 0.434, df = 28, *p* = 0.668; winter, t =-–0.525, df = 28, *p* = 0.604) (Table 6. 3) for either of the seasons sampled. The glucose levels ranged from ~12 to 15 mmol/L and ~5 to 6 mmol/L for autumn and winter samples respectively (Tables 6. 1 and 6. 2).

6.5 Discussion

Transportation-induced stress over the relatively short (~6 hour) period of travel monitored in this study, is considered to be at levels that can be physiologically managed by the animals. This was evidenced by the 100 % survival rate of the animals at the end of this transportation trial

As haemolymph is the centre of physiological responses during stress exposure, its observation is considered to be an effective way to demonstrate stress and immune responses of abalone and other molluscs (Hooper, et al. 2007). The haemolymph in this study had been stored on ice for 4 to 6 hours and in the freezer for 11 to 12 hours prior to use. This is at variance with recommendations made by Day et al. (2010) and Ragg and Watts (2015) who advocate using haemolymph immediately after withdrawal. However, results in this study showed that haemolymph was still suitable for all assays after a relatively long period of cool storage.

The significant increase in THC in the winter samples in this study, is a commonly reported stress response for abalone subjected to physical perturbations and pathogenic infections (Cheng, et al. 2004; Dang, Speck and Benkendorff 2012; Hooper, et al. 2011). A similar pattern has also been reported when abalone are heat stressed; in this case, the haemocyte count increases initially, before decreasing to control levels as the haemocytes infiltrate into the connective tissues (Hooper, et al. 2011). In contrast to these examples, other studies have recorded a decrease in the number of haemocytes when the animals are first subjected to a stressor. In a study reported by Malham et al. (2003), hemocyte counts initially decreased when abalone were shaken under experimental conditions, before gradually increasing during the recovery phase to a point beyond the basal line level, and finally declining back to the basal line level. It is hypothesized that the open circulatory system in abalone may allow migration of haemocytes from tissues to the circulatory system during stress exposure in the same way as occurs in bivalves (Cajaraville, Olabarrieta and Marigomez 1996).

In this study, the hemocyte count in the winter samples recorded a decrease during transportation, before increasing to levels beyond the pre-treatment value after ~12 hours of being re-immersed in water. The absence of any difference in the THC before and after transportation in the autumn samples may be evidence that the physiological responses of abalone can differ at different times of the year. It is possible that the higher amount of circulating haemocytes in the winter samples may indicate the animals had a higher response to stress and therefore better defence mechanisms in that season.

One possible reason for the animals having better response mechanisms in winter than autumn, is that their condition is likely to be better in winter. Storms caused by winter cold fronts passing across the site in winter cause big swells and result in abundant food in the form of drift algae being available to the abalone (Melville-Smith 2013, Fernandez et al., in prep.). The excess food also leads to high growth on the Ocean Grown Abalone sea ranch in the winter months (Melville-Smith 2013). This explanation for seasonal differences in stress response is similar to a pattern that has been noted and reported on in bivalves (Santarem et al 1992). Cajaraville, Olabarrieta and Marigomez (1996) and Santarem, et al. (1992) have reported that fluctuations in temperature, salinity, and food availability in different seasons is the likely cause of variation in physiological responses in bivalves.

The effect of lysosomal membrane stability (LMS) as measured through NRR assay, responded to translocation stressors in a similar way to that reported for the THC, in that only the winter NRR assay samples showed a significant response to values before and after transportation. Other studies have reported a similar reduction in lysosomal stability in molluscs due to factors such as heat stress (Hooper, et al. 2014); aerial exposure (Song, et al. 2007) and hypoxia and hyposalinity (Wang, et al. 2006).

Stress induced by transportation allows for faster release of neutral red dye from lysosomes into cytosol via the lysosomal membrane (Lowe, Soverchia and Moore 1995). The incapability of lysosomal membranes to retain dye reveals that the lysosomal membranes have limited capacity to prevent the degradative enzyme (hydrolase) located in the lysosome (De-duve and Wattiaux 1966), from leaking into cytosol, which in turn can lead to the lysosomal cell death (Aits and Jäättel 2013). These hydrolytic enzymes play an important role during phagocytosis, endocytosis and autophagy (Cheng, et al. 1975; Hu, et al. 2015) against some species of bacteria (Marin, et al. 2007).

Previous studies have indicated that physical stress induces the release of stress hormones (catecholamines), such as noradrenaline (NA) and dopamine into the haemolymph and that these can elevate the susceptibility of juvenile oyster (*Crassostrea gigas*) (Lacoste, Jalabert, et al. 2001a) and abalone *H. tuberculata* (Malham, et al. 2003) to pathogens. In this current study, the release of NA during transportation may have affected the immune responses of abalone by inhibiting the ability of haemocytes to phagocytose zymosan as a pathogenic agent. During both the autumn and winter sampling, the phagocytic rate after transportation decreased significantly, suggesting that this indicator is a sensitive tool for measuring immune response in abalone. All abalone, in both seasons sampled as well as in both the farmed and wild areas, showed a similar rate of phagocytosis ($p>0.05$). This implies that the detrimental effects of transportation to immunomodulation of abalone is universal.

As a facultative anaerobic animal, abalone can survive by utilising energy sources derived from anaerobic metabolism, which generates lactate as metabolite (Donovan, Baldwin and Carefoot 1999). The circulatory system in abalone may not necessarily allow the pedal muscle to receive enough oxygenated haemolymph and this can lead to anaerobic metabolism occurring in these muscles (Donovan, Baldwin and Carefoot 1999).

In the current study, lactate level in the haemolymph measured 12 hours after recovery were significantly below the control levels in the winter samples, but not during the autumn. This reduction in lactate level may indicate the transition of abalone from a stressed to a normal condition during the period of recovery. While in the recovery tanks abalone received sufficient oxygen, permitting oxidation of lactate into tissue glycogen during gluconeogenesis (Mustafa, et al. 1983). Well-oxygenated water allows all tissues including haemolymph to receive sufficient oxygen for aerobic metabolism. A drop in lactate level after stress has been reported in the haemolymph of the crab, *Cancer pagurus* (Barrento 2012). The slower anaerobic metabolisms during transportation may cause the low level of metabolites, including lactate, due to low temperatures. In that study, lactate level returned to control values 72 hours after recovery was initiated. A study on the same species by Lorenzon et al (2008) recorded lactate levels taking 96 hours to return to pre-stress levels.

Osmoregularity capacity (OC) is a commonly used indicator of stress. It has for example been used by Silverstre et al. (2005) to examine the effect of cadmium on the osmoregulation of the Chinese mitten crab (*Eriocheir sinensis*). It has also been used to examine the stress responses of abalone and crustaceans to inland salinity water (Fotedar et al. 2008; Prangnell and Fotedar 2006 etc etc). It does not appear to have previously been used as an indicator of transport stress.

In this study, the increase of OC after transportation in winter has shown an ability for greenlip abalone to osmoregulate during transportation. In contrast, the lack of any change in OC after transportation in autumn, suggests that the abalone were less able to maintain their osmoregulation on that occasion. This suggests that the abalone had better defence mechanisms in winter, possibly due to them being in better physiological condition in that season. Furthermore, the improved osmoregulating ability recorded in the winter samples was similar across all sites in both the ranched and wild stocks.

Glucose plays an important role in glycogen metabolism in molluscs (Rossi and Da Silva 1993). As an energy source, it allows the animals to maintain many physiological functions including immunity (Day, et al. 2010). During stress, glucose is metabolized anaerobically (O'Omolo, et al. 2003). O'Omolo, et al. (2003) have suggested for *H. midae*, that glycogen concentration could be expected to reduce significantly during transport simulation and exercise. Baldwin, et al. (1992), recorded a drop in the glucose level of *H. iris*, but an incrase in those levels after aerial exposure. In the current study, glucose levels remained constant before and after transportation in both sampling periods. This suggests that this indicator was less sensitive to transport stress than other indicators used in the study. Overall, this study has shown transportation to affect the immune functions of greenlip abalone as both an immune-stimulant and immune-suppressant. The increase in total haemocyte count recorded after transport showed the stress of this activity to stimulate an immune response. At the same time, the decreasing ability of haemocytes to phagocytose pathogenic agents and the decreasing stability of lysosomal membrane to protect red dye from leakage shows a suppressed immune response. Of the six indicators, glucose was the only one that did not respond to transportation, and therefore does not appear to be a useful tool for evaluating immune responses in greenlip abalone.

In general, the indicators did not show significant seasonal differences between sites or between ranched and wild stock. There were two exceptions to this; both the total haemocyte count and osmoregulatory capacity indicators showed significant differences across sampling sites in the autumn, but not the winter samples. Only in the case of the autumn haemocyte count difference was there any indication of ranched and wild animals showing statistically different response levels. These differences were driven by the results from two ranch sites and one wild capture site and did not form a consistent trend between the two sample sources. Similarly, the osmoregulatory capacity indicators did not form a consistent trend between the ranched and wild stock sites; in one case the wild stock sample had a lower and in the other a higher indicator value than were recorded for the three ranched stock samples. These results do not therefore point to any immediate concern that ranched abalone at the Flinders Bay sea ranching operation are any differently stressed to those in the wild.

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7 Conclusion

This project had ambitious objectives and for the most part, has succeeded in achieving its aims. As noted in the Introduction, the concept of abalone sea ranching is in its infancy in Australia and the operation in Flinders Bay will hopefully be replicated in other areas in the future. This, and the previous FRDC funded abalone sea ranching project (Melville-Smith et al. 2013), will provide good research information on which to build targeted investigations in the future.

The first objective in this project, identifying water circulation patterns within Flinders Bay and specifically across the sea ranch so as to better understand the source and seasonal movements of the drift algae, was one that had been identified in Melville-Smith et al. (2013). The techniques employed in this objective (i.e. modelling the hydrodynamics of Flinders Bay and simulating movement of drift algae) are ones that would be applicable for use in similar investigations in the future. However, the results from this study are quite specific to the abalone sea ranching operation in Flinders Bay.

The hydrodynamic/dispersal modelling work showed seasonal differences in water circulation within Flinders Bay and across the lease site. This had implications for the model-inferred transport of drift algae across the sea ranch lease and within the Bay. Wave induced currents were experienced year-round and had a major influence on the movement of drift algae. During summer, south-easterly winds combined with the wave driven currents and created a clockwise circulation in Flinders Bay and in so doing, could be predicted to move drift algae across the lease site and towards the shore and into the Bay. In these summer conditions, the model indicated that the supply of drift algae from the southerly part of Cape Leeuwin was reduced and that it was increased from the east.

In winter, currents were shown by the modelling work to generally move particles from areas southwest of Flinders Bay and Cape Leeuwin, eastward and then northwards over the lease site. Wind was less influential in winter, and the larger swells in this season were predicted by the model to be responsible for moving more particles across the lease site and by implication therefore, funnelling more drift algae through the site.

The modelling (Chapter 3) has succeeded in explaining the seasonal patterns of circulation within the Bay and the implications that that has on the source of drift algae to the sea ranch lease site. Of particular relevance to the farm, was the identification from model simulations, of an area to the west of the main lease site that appears to have stronger current circulation than what occurs though the main lease. This area slightly to the west of the main lease is predicted from the model simulations, to be a pathway for potentially higher drift algal abundance, which of course would translate into a potentially more productive area for farming abalone. Fortunately, this area to the west of the main lease is also allocated for abalone farming and is earmarked for the farms future expansion.

A word of caution does need to be added here. It is know from the comprehensive work of Shepherd (1973), that greenlip abalone occur in very different types of environments. In some cases, these areas of occurrence are inside of islands, headlands and reefs, in other cases in gutters at the base of granite cliffs. However, the common theme to where they are found, was shown by Shepherd (1973) to lie in them being in the path of where seaweed is either being conveyed, or where it settles. He noted that this species is very sedentary compared to other abalone species and that its feeding appeared to be triggered by the stimulus of moving water. However, he (Shepherd 1973) also showed that if water movement and surge is too rapid, it can inhibit the ability of greenlip abalone to trap seaweed. The point being made, is that while understanding water circulation patterns across the farm is important, there are other water movement patterns that are important to productive abalone farming areas.

In addition to the obvious reasons for having knowledge of circulation patterns within the Bay and through the farm – because this is what drives food for the abalone – there are other less obvious reasons. For example, for formulating contingency plans in the event of an oil spill or similar unforeseen disaster

The second objective of this research project was to evaluate seasonal variations in biomass, species composition and functional properties of drift algae on the farm. The seasonal variation in species composition of drift algae and seagrasses was examined in Chapter 4 The diversity of species proved to be far higher than had been anticipated prior to the start of the project. The drift red and brown algae and seagrasses followed a similar pattern of seasonal abundance to what has been published for South Australia (Shepherd 1973; Shepherd and Cannon 1988). Brown algae were the dominant drift sea weed on the farm in terms of relative abundance in all seasons bar summer, when sea grass dominated. Red algae, by comparison, maintained a relatively stable presence all year round, forming between 2 – 34 % of the proportional abundance of drift sea weeds sampled across the seasons

What was perhaps less expected were differences in drift algal species diversity between sampling sites. One of the three sites that was monitored showed significant differences compared to the other two – a result which was unforeseen given that the sites were not far apart. The implications of this finding are important from a stock management point of view, because they show that it is likely that there will be differences in food availability and quality operating at a localised scale. Such differences would be expected to result in corresponding localised differences in growth rates of the abalone.

Obtaining estimates of the biomass of drift algae and seagrass on the farm proved to be beyond the capability of the project. Diver estimates for the amount of drift algae on the seabed on a given diving day based on a 0 – 5 scale of abundance, were available for the farm. It had been considered that it might be possible to use these data to estimate drift algae biomass. However, we had not taken into account was how quickly algae can pass though the farm, and that scales of drift algal abundance can change both rapidly and markedly over the course of a day. Furthermore, diving does not occur on exceptionally rough days and it is on those days that algae gets dislodged and drifts across the farm, so not having information for those days would bias any estimate. In conclusion, it became evident that measuring drift algae biomass on the farm would be possible, but that it would require a dedicated study.

Prior to the commencement of the project, it had been anticipated that we would be able to identify the species of algae or seagrass that were being eaten by the abalone by examining their gut contents. This proved to be unsuccessful because the abalone on the farm were small (mostly in the 5 – 80 mm shell length size range) and the gut contents were very macerated. As a result, although we have excellent data for the species of drift algae and seagrass through the year, we have been unable to relate that to which species the abalone are eating. There are studies that have been more successful at identifying seaweeds consumed by greenlip (Shepherd 1973) and other species of abalone (Barkai and Griffiths 1986; Poore 1972), though probably not without sampling bias (Day and Cook 1995; Foale and Day 1992) and generally with larger abalone. Methods to address this question in the future is discussed further in 'recommendations for further development'.

The functional properties of the drift algae and seagrass have been discussed in Chapter 5 We have data for all the species that were sampled over the course of the study. However, many of them occurred seasonally. This absence of most species occurring in one or more seasons, precluded using much of the available data in the statistical analyses of the nutritional value of algae and seagrasses across the full year of sampling

The nutritional content of the red, brown and seagrass species collected over the course of the study, showed the Rhodophyta to generally be a more nutritious food source for the abalone than the other two Divisions.

An examination of the nutritional content of just those algal and seagrass species that occurred in all seasons, showed that protein content cycled seasonally across the Divisions; it was highest in winter and decreased through spring and summer before increasing again in autumn. This tracked the seasonal pattern of abalone growth on the farm (Melville-Smith, et al. 2013). However, it would be hard to tease out whether this pattern of growth is due to the greater quantity and quality of food available in winter and autumn, or whether it is due to environmental conditions such as the water movement and surge being more conducive for the abalone to feed, or temperatures that better suit the physiology of the animal

We did examine the protein (and moisture) content of the farmed abalone through the year as a more direct way of tracking the quality of the food. Meat protein remained relatively constant though the year, except for a brief and sharp increase in summer. This was unexpected, given that best growth rates are recorded in winter and autumn. It was also unexpected given that many of the animals were around the size at 50 % maturity for the Augusta area (Hart, et al. 2013). It is also know that the greenlip abalone spawn in spring and summer from October to March (Shepherd and Laws 1974), which coincides with the period when protein content in the meat increased. At least in fish (Basade et al. 2000; Mathana et al. 2012), spawning is considered to decrease rather than increase the protein content of meat.

The last objective was to evaluate the health status of the ranched abalone using physiological stress indicators. Separate to this, there is interest in optimising methods to transport abalone live from the farm to markets. We therefore chose to combine these two objectives by doing baseline monitoring of the stress levels of farmed and wild-caught animals immediately after capture and then comparing those levels after the animals were transported out of water

The experiment, which was run in two different seasons, provided different physiological responses to transport, with the experiment run in winter being more responsive than the one in autumn. Transportation stress on winter samples significantly increased total haemocyte count and osmoregulatory capacity, suggesting an immune-stimulation. This stress also triggered an immune-suppression, causing the phagocytic rate and lysosomal stability to significantly decrease after transportation. Lactate levels in the winter samples decreased significantly after transportation, possibly indicating the transition from a stressed to normal state during the period of recovery. The constant level of glucose before and after transportation in both seasons, showed that it was the least sensitive indicator used in this study.

In general, there was no difference between wild and ranched abalone in either of the seasons sampled. However, in the autumn samples there were significant differences in haemocyte count and osmoregulatory capacity indicators across sites in the wild and ranched samples. These differences did not form a consistent indicator trend between the sites from the two sample sources and therefore do not point to any immediate concern that ranched abalone at the study site were any differently stressed to those in the wild.

7.1 References

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7.3 Implication

While there are probably implications from this work that are not immediately obvious, those that have come to light are:

(i) The benefits of identifying seasonal circulation patterns prior to siting an abalone sea ranch. This provides the ability to site the farm in the best possible position for maximising drift algae and sea grass and also provides important information on the likely circulation patterns in the event of an oil spill, or similar natural disaster.

(ii) There are obvious benefits to having a thorough knowledge of the seasonal and spatial variation of the drift algae and seagrasses making up the food source to the farm. Without this baseline data, there would be no way of tracking any changes to the food source in the future. Such changes are unpredictable and could be the result of natural or man-made events. This part of the objective was achieved in this study. However, linking the species of drift algae and seagrass to what is eaten by the abalone was not achieved by the methods used and remains a need for further development (see Recommendations) using more sophisticated techniques

(iii) The project examined seasonal variations in the nutritional value of different drift algae and seagrass. As outlined in ii (above), it is important for managers to have as much baseline knowledge as possible of the food source supplying to the farm. The food source is natural and therefore beyond control of the managers, but if for whatever reason there are changes to the species composition of the drift algae and seagrass being swept onto the farm, this information would be critical to explaining the likely effect that such change might have on the abalone farm stock.

(iv) There have been concerns from some sectors about the possibility of abalone held on the sea ranch being a potential source of disease for abalone in the wild. There are protocols in place to prevent this possibility. However, if farm-held animals were more stressed than wild-caught animals, there would be a greater likelihood of them being a source of disease. Results from this study showed no consistent indicator trends to support concerns about ranched abalone being any differently stressed to those in the wild.

(v) There are local and overseas markets for live held greenlip abalone, but these are not being optimised because of difficulties in keeping the animals alive for extended periods and also the costs of live transportation. Addressing these issues is an important area for future research. This study has produced some preliminary findings.

7.4 Recommendations

One of the objectives of this project had been to identify species of drift algae and sea grass available for the abalone on the sea ranch in the different seasons. The aim had then been to determine though gut content analyses, which of those species was food for the abalone and whether this changed seasonally. As already noted, identifying drift algae and seagrass in the gut proved to not be possible. One recommendation for future research would be to use molecular analysis of the gut contents to determine their food preferences.

Monitoring the stress levels of animals is considered to be a useful as a potential alert for disease. When the population is stressed, it becomes susceptible to disease. Baseline data has been established in this project, but that might be considered for extension into the future to build up a time series of the ‘health’ of the farmed population compared to wild caught abalone in Flinders Bay.

There is the potential for doing a lot more work in developing methods for the live transportation of abalone. There is little doubt in our view, that methods could be developed to minimise mortalities during live transport. This might potentially open up markets that are presently not being fully tapped.

7.5 Extension and Adoption

Ocean Grown Abalone Pty Ltd and the Department of Fisheries are collaborators on this project, so communication with the direct users (industry and resource management) has been assured. The research in the Final Report is in the process of being published in peer reviewed scientific journals and this will ensure that it is available to the national and international scientific community.

8 Appendix 1: Intellectual Property

There is no new intellectual property arising from this project

9 Appendix 2: Staff

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