Optimising the fishery of the under-exploited edible Sea Urchin, *Centrostephanus rodgersii*

by

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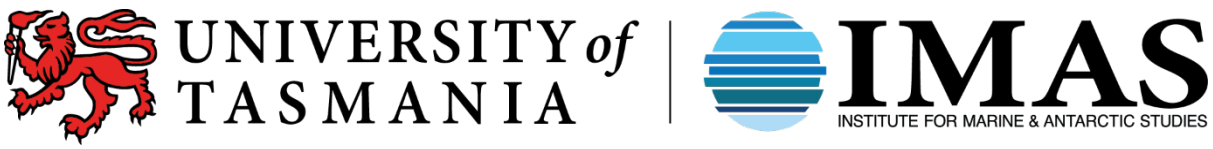
Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University of Tasmania

December 2021



Statements and declarations

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Acknowledgements

First of all, I would like to thank my academic supervisors Dr Craig Mundy, Dr John Keane, Dr Nigel Swarts and Prof. Caleb Gardner. My immense gratitude goes to Dr Craig Mundy for the advice, guidance and continued support throughout every step of this Thesis, for the regular meetings that didn't let me astray, for the many things that I learnt. I thank Dr John Keane for making possible the several urchin sample collections, the help during sample processing and the founding support for the various analyses. I want to thank Dr Nigel Swarts for supervising me on the development of the project involving the use of urchin waste as fertiliser; the field was completely new to me and it wouldn't have been possible without his support and advice. I am also especially thankful for my primary supervisor Prof. Caleb Gardner who accepted this project and provided the funding to cover the tuition fees and leaving allowance, without that I could not have complete this Thesis.

I would like to express my deepest gratitude to Mrs Lisette Robertson, her help was invaluable and her presence at the institute is priceless, the many times and many ways she is been helpful go beyond the expertise of laboratory manager.

A special thank goes also to Dr Basseer Codabaccus and Dr Bryan Choa for the help and for sharing their knowledge in lipid analysis and Prof. Quinn Fitzgibbon as my GRC; to Dr David Nichols for Fatty Acids analysis and Dr Christian Dietz for Isotopes analysis; to Mr Phil Andrews for his help and advice at the Horticulture Centre and Ms Caroline Claye for help and advice in the analysis of tomato fruits; to all the people that helped me during the fieldwork, the processing, the trials, among which Sarah-Jane Pike, Jane Ruckert, Olivia Johnson, Gabby Walley, Dr David Moreno, Komeil Razmi, Moha Esmaeili, Ehsan Mousavi, Edward Forbes, Ruari Colquhoun, Graeme Ewing, Antony Cave, Mike Porteus, Sam Foster.

I would also like to thank the two examiners of my Thesis and the Chair of Examiners Dr. Scott Hadley.

I thank Ms Gail Eagle and Ms Lynnette Baily for the kindness and availability in solving administrative problems.

I am grateful for all the people I met, for the company, the conversations, the beers, the memories. In particular for Dr Audrey Tuzan for supporting me throughout my four-year

journey even though she is far far away, and for allowing me the use of her Netflix account and for other things.
Thanks to Paola Petrelli and Patrick for hosting me when I was homeless and Spoon and Kim for including me in swimming session, beers wine and pizzas.
I thank for the company and conversations the colleagues at the Taroona Institute whom with I shared the journey: Shuangyao Wang, David Moreno, Kate Fraser, Barrett Wolfe, Komeil Razmi, Ehsan Mousavi, Freddie Heather, Daniela Farias, Marion Angelini, Lincoln Wong, Patricia Peinado, Renzo Tascheri, Darryn Sward.

Finally, I am really glad for the friendship with my long-time housemates Floriaan Devloo-Delva, Sahan Jayasinghe and Phillip Hardstaff and for the whole crew always around: Romain, Pauline, Tina, Ole, Claudio, Cristina, Emiliano, Noémie, Nicolò and Dalila, Samantha and Matt, Rita and Eric, Alessandro and Rosa, Sandra, Habacuc, Leyla, Fernando, Guillome, Jake, JC, Olly, Vanni, Camilla and many others.

General abstract

Climate change in the Southern Ocean is posing problems in many coastal areas where changes in ocean currents affect dispersal, water temperature and nutrients so that the ecological balance of the rocky reefs is adjusted. Although changes have occurred in the past as a normal part of marine ecosystems, the current rapid rate of change introduces special challenges. Research on the negative effects of climate change on marine ecosystems is most common, although the arrival of new species through range extension can also be positive where this brings new commercial opportunity. The overall objective of this thesis was to guide the development of best practices to optimise the emerging commercial fishery of the range extending edible sea urchin, *Centrostephanus rodgersii*. By optimising the fishery, a cost-effective method can be developed to combat the climate-driven negative impacts of *C. rodgersii*, which include the formation of extensive urchin barrens and subsequent negative impacts on lucrative traditional fisheries. The objective was achieved via pre-harvest investigations into the natural influences on gonad biochemistry as well as roe enhancement opportunities, and post-harvest research on handling stress and waste utilisation. Economic returns in urchin roe fisheries are highly dependent on roe quality, with 10-fold increases in value from low to high-quality roe based on macroscopic characteristics. As such even small advancements in fishery processes can lead to large economic rewards.

In *Chapter 2*, the effect of seasonality, feeding habitat and sex on urchin gonad physical parameters and biochemical composition is described. In this study, *C. rodgersii* showed a pattern of gonad growth and biochemical composition similar to other echinoids, while only minor differences were found between gonad of animals from kelp bed and barrens habitat. Protein was the main gonad macronutrient followed by lipids, with males being higher in proteins and females in lipids. Triacylglycerol was the main class of lipids in both sexes. No differences in gonad colour were found between urchins in kelp and barrens while it was observed a higher accumulation of lipids in urchins feeding in kelp areas compared to the barren ones. Gonad of both sexes had good texture and firmness between September and June during recovery and maturation, while were softer and difficult to handle and process at the spawning stage. It has long been held that urchins from barrens

habitat have poor roe quality and should be avoided by urchin fishers, whereas I was able to demonstrate that urchins living in barrens possess not only gonad of acceptable commercial yield but also good fatty acids and amino acids profile. Urchins living in the barrens represent an unfished stock available to be targeted by the fishery which by doing so would alleviate the urchins fishing pressure on the kelp beds while at the same time reducing the urchins' population density in the barrens allowing the recovery of algae cover.

In *Chapter 3* an experimental feeding trial was undertaken to examine the potential for supplementary feeding to enhance the quality of *C. rodgersii* gonad. Growth of gonad in urchins provided with the formulated feed pellets was similar to growth of a wild control, whereas there was little growth of urchin gonad in the fresh algae diet treatments. There was however a pattern of accumulation in biochemical components from the algae to the gonad. These preliminary results show that gonad yield enhancement through urchin farming is at the moment unnecessary given the large availability of urchins with good yield along the Tasmanian eastern coast in both barrens and kelp habitats. However, it provides a starting point for further studies aimed at improving the quality aspects of the gonad, namely colour, texture and flavour. Specifically, results showed a selective accumulation of sweet taste amino acids in gonad of urchins fed the algae *Ulva spp*. Further development should be addressed in gonad sweet and umami taste enhancement in short time aiming at the production of high-priced A-grade roes.

In *Chapter 4,* the effect of post-harvest handling techniques on urchin stress was explored by a simulated harvest and post-harvest storage experiment that mimicked current harvest practice in the commercial fishery. Live urchins collected during the fishing season with mature gonad were exposed to different environmental conditions to investigate the effect of air exposure and storage during fishing, as well as storage post-fishing, on gonad quality prior to processing. High temperature, wind and storage time were shown to increase stress and premature death of animals, as did storage at low temperatures. Protecting the urchins from direct wind and sunlight, storing them at ambient seawater temperature and minimising shell damage reduced stress and mortality and preserved gonad quality. *Centrostephanus rodgersii* survival after the harvesting is greatly determined by air exposure, storage

conditions and time prior to processing. The results show that optimising post-harvest handling techniques can vastly improve roe quality and economic returns. Both fishers and processors should employ practices aimed at reducing stress and mortality from the moment of harvesting to the moment of processing with the ultimate goal of preserving maximum freshness and gonad quality.

In *Chapter 5* the waste of sea urchin processing was analysed for its nutrient content and applied as an organic fertiliser in an experimental growth trial with greenhouse tomato plants. Dried urchin shell powder at the highest rate of addition produced vegetative growth comparable to a control fertiliser solution but resulted in only half of the fruit production due to macronutrient exhaustion. Results show the urchin waste product has a distinct profile of both macro and micronutrients with high bioavailability for uptake by plants, indicating potential commercial development. The fertiliser trial demonstrated that urchin waste, which is the majority of biomass landed, has the potential to be used as an organic soil ameliorant and plant fertiliser. In doing so it will reduce the environmental impact and costs of waste disposal while simultaneously promoting the economic viability of the urchin fishery and maximising the economic return creating economic value of the waste product.

This thesis provides some clear directions for changes in current fishery practice and provides also a clear direction for further research on maximising roe quality and utilisation of urchin waste products. Utilising the key findings of this thesis the commercial *C. rodgersii* harvest fishery will be able to be optimised with increased profitability. Collecting urchins from barrens habitat would reduce the population density and therefore reduce the grazing pressure on the rocky reef allowing the recovery of algae cover. The recovery of the Tasmanian rocky reef would benefit the ecosystem and other compartments of the fishery like Abalone and rock lobsters. A better understanding of *C. rodgersii* population dynamic related to gonad quality will improve the management of the resource and the urchin fishery in the long term.

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Chapter 1 General introduction

1.1 Sea Urchins biology and ecology

1.1.1 General urchin biology

Sea urchins are free-moving marine invertebrates that belong to the phylum Echinodermata, class Echinoidea. More than 1000 species are described (Kroh & Mooi 2013) living in all the oceans from the intertidal zone to the great depths of the abyssal plain, representing an important component of marine benthic communities in hard substrates (rocks) and soft bottomed habitat (Furman & Heck 2009). Many species are primarily herbivorous (Lawrence 1975), however as reported by (Vance 1979) on the diadematid *Centrostephanus coronatus* urchins can also feed upon a wide range of invertebrates like sponges, bryozoan, ascidian, crustaceans, annelids, and other echinoderms. Sea urchins are spherical, oval or flattened and covered with spines. A digestive tract, five gonad, and a very specialized water vascular system with its external appendages, the tube or ambulacral feet characterize their anatomy (Ziegler et al 2008). The space between the major organ systems is composed of the main body cavities (the perivisceral coelom) that are filled with coelomic fluid. Sea urchin skeleton is made up of interlocking calcium carbonate plates and spines. The test is enclosed by the epidermis and is therefore an endoskeleton. The mouth, on the underside of the body, has a complex dental apparatus called Aristotle's lantern, which is also made of calcium carbonate enriched in magnesium (Ma et al 2009). The teeth of Aristotle's lantern grow continuously and are typically extruded to scrape algae and other food from rocks. The reproductive cycle of sea urchins can be generally divided into four main phases. In both males and females these phases are: (1) a major spawning period; (2) a period of post-spawning or recovery; (3) a period of gonad weight increases due to enlargement of the nutritive phagocytes; (4) a period of intense gametogenesis when the gonad rapidly develop gametes in preparation for spawning (Byrne 1990, Byrne et al 1998, James et al 2018, Kelly 2000). Depending on the species, several seasonally-variable environmental factors such as photoperiod, temperature and food availability play an important role in regulating

the different stages of gametogenesis (proliferation, growth and maturation) and breeding (Kennedy & Pearse 1975).

The life history strategy of urchin's varies depending on the habitat, food availability and disturbance caused by water energy and predators (Lawrence 1990). In the deep seas and Antarctic waters, disturbance from predators is low (Aronson et al 2007) and high water energy is absent but food quality and availability is low (Thistle 2003). In these environments stress-tolerant slow-growing species able to withstand long periods of starvation are present. At deeper depths in the tropical reef sea urchins find shelter from the energy of the waves in the crevices of the rocks and corals, their density is limited by competition for space and the poor diet consisting of reef seaweed and much sediment, the production is low and survival is high (McClanahan 1998). Tropical seagrass beds are characterized by high primary productivity (Williams 2001). In this environment, climatic conditions can be extreme and predation intense. Sea urchins of tropical sea bed grow rapidly, mature early and have a short life span (Ebert 1982). In kelp forests sea urchins feed on macroalgae assemblages and on the seabed rich in drift produced by kelp (Harrold & Pearse 1987), to avoid predators and water turbulence they adopt cryptic behaviour finding shelter in crevices and under the rocks. In these seas, production is high; urchins have access to high-quality food, but they might compete for the limited shelters (Andrew 1993). In shallow water and on the continental shelf many predators like durophagous brachyuran crabs, lobsters, sharks, rays, and teleosts can prey on sea urchins (Lawrence 2013b). The removal of target predators can lead to an increase in urchin populations allowing grazing to reduce algal biomass (Steneck et al 2003). At higher densities, sea urchins can considerably modify the composition of subtidal communities (Hereu et al 2012). Complex, biodiverse benthic communities turn into flats dominated by encrusting algae, known as "barrens" through the urchins overgrazing activity (Wright et al 2005). Removal of top predators has repeatedly been shown to indirectly cause overgrazing of marine plants by for instance sea urchins (Harrold & Pearse 1987, Steneck et al 2003).

1.2 Centrostephanus rodgersii

1.2.1 Distribution

Centrostephanus rodgersii is a large echinoid, abundant in shallow, subtidal, rocky habitats along the southeast coast of Australia. In the 1960s the southern distribution limit of *C. rodgersii* was reported to be the islands in eastern Bass Strait and extended by the late 1970s to northeastern Tasmania where is known with the common name of Longspined sea urchin. Over time the southern limit extended south along the east coast of the island to the Tasman Peninsula, with individuals now reported across the southern edge of the state (Edgar et al 2005, Ling 2008). The transport and settlement of *C. rodgersii* larvae in the colder water off the Tasman Sea has been driven by the East Australian Current (EAC) extension, (Ridgway 2007), with eddies and warm water pockets extending further south due to climate change (Walker & Wilkin 1998).

The northern limit of *C. rodgersii* in Australia is reported for the Solitary Islands, New South Wales, where it lives on algae and hard corals, while at its southern limit it is found with cold-water algae such as *Phyllospora comosa* and *Ecklonia radiata* (Andrew & Byrne 2007). In New South Wales, *C. rodgersii* can be found abundant in shallow water, becoming less dense at depths of ca. 20 m where individuals found shelter in crevices and fractures of the rocks (Andrew & O'Neill 2000, Andrew & Underwood 1989). In Tasmania, *C. rodgersii* is found in slightly deeper water, with highest abundances between depths of 15 and 25 m (Ling & Johnson 2009, Ling & Keane 2018, Stuart-Smith et al 2010). Offshore, at a depth of 50 m in the New Zealand Star Bank, and NE Bass Strait high-density populations of *C. rodgersii* inhabit granite outcrops in association with sea whips (*Primnoella australasiae*) and sponges (Beaman et al 2005, Perkins et al 2015).

1.2.2 Biology

Centrostephanus rodgersii is described as fast-growing species compared to other echinoids (Ebert 1982), the maximum test diameter recorded in Tasmania is 133mm (Ling & Johnson 2009). Larger, faster-growing individuals of *C. rodgersii* with thicker tests and shorter spines are found in macroalgae habitat, while urchins living in barrens are reported to be smaller with thinner test (Ling & Johnson 2009). *C.*

rodgersii adjusts its body growth with food availability and in barrens habitat quantity and quality of food is limited (Ling & Johnson 2009). Studies aimed at estimating growth and age, shows that *C. rodgersii* can reach 50 mm test diameter (TD) at 4-5 years of age and urchins with test diameter between 110-130 mm can be 25-35 years old (Ling et al 2009a, Pecorino et al 2012). Andrew (1993), reported growth of 40mm in the first year in a population from New South Wales. Comparison of urchin populations from different locations showed that in New South Wales and New Zealand growth is faster than in Tasmania, suggesting the colder water temperature in southern Australia may influence the growth rate (Pecorino et al 2012). Density of *C. rodgersii* can be very high in barrens and subsequently food limited. Reduction in the urchin density can improve growth through an increased supply of food to those that remain (Blount & Worthington 2002). A New South Wales manipulative field experiment showed that improvements in both colour and yield of barren urchin gonad occurred after three months when density was reduced by as little as 33% (Blount et al 2017). A significant increase in yield occurred when density was reduced by 66%.

1.2.3 Feeding behaviour

Like other species of Diadematidae (Carpenter 1984, Ogden et al 1973) *C. rodgersii* is light sensitive and shows nocturnal feeding habit (Andrew & Byrne 2007, Andrew 1993, Andrew 1994, Flukes et al 2012, Ling & Johnson 2012). After grazing, and before dawn, *C. rodgersii* tend to return to the same crevice to avoid daylight predators (Flukes et al 2012, Jones & Andrew 1990, Nelson & Vance 1979).

Centrostephanus rodgersii is omnivorous and forage on a wide range of algal species like *Ecklonia radiata, Phyllospora comosa* and *Sargassum* spp. (Hill et al 2003, Jones & Andrew 1990, Strain & Johnson 2009), as well as other organisms attached to the rock surface. Foliose kelp, sponges, erect bryozoa, and tunicates are the main choice while encrusting coralline algae and encrusting bryozoa are avoided (Vance 1979). The tropical species *Centrostephanus coronatus*, also feed upon a wide range of invertebrates (Vance 1979).

1.2.4 Predators

The wrasses *Achoerodus viridis* and *Notolabrus tetricus* are reported to pray on *C. rodgersii* (Andrew & Underwood 1989, Gillanders 1995, Nelson & Vance 1979). Day-active predatory fishes do not remove urchins from their shelters, but attack and consume urchins placed in normal feeding locations during the daytime, as also reported for the wrasse *Pimelometopon pulchrum* on *C. coronatus* (Nelson & Vance 1979). Shark *Heterodontus* portusjacksoni and rock lobster *Jasus edwardsii* are nocturnal predators and can actively feed on *C. rodgersii* (Ling et al 2009b, McLaughlin & O'Gower 1971). Durophagous brachyuran crabs, octopi, rays, and other teleosts that co-occur on rocky reefs can also predate on small adults and juveniles of *C. rodgersii* (Lawrence 2007). Both rock lobsters and wrasses, including *Achoerodus viridis* feed by flipping *C. rodgersii* onto their aboral side, so exposing the vulnerable peristome to attack (Byrne & Andrew 2013).

1.2.5 Reproductive Cycle

The annual reproductive cycle of *Centrostephanus rodgersii* is typical of diadematoids, with a period of gonad growth where reserves accumulate in the nutritive phagocytes followed by mobilisation of the reserves during gametogenesis prior to spawning. Sexes are separate but occasionally hermaphrodites are encountered (King et al 1994, O'Connor et al 1978). Gametogenesis is influenced by photoperiod and commences in April-May when day-length decreases. Spawning occurs during the austral winter; however, the length of spawning is variable lasting nearly a month in northern New South Wales ending by July (Byrne et al 1998, O'Connor et al 1978). A longer spawning period is reported for urchin populations in the south coast of New South Wales, Tasmania and New Zealand (Byrne et al 1998, Ling et al 2008, Pecorino et al 2013). The spawning follows a latitudinal trend being longer where the sea temperature remains cool. Reproductive output is also dependent on the amount of reserves that can be accumulated in the gonad, which derive from food quantity and quality (Andrew 1986, Byrne 1990, Ebert 1968). The timing of gametogenesis is reported similar in barrens and fringe habitats, however reproductive output differs markedly between locations and habitats. Fringe urchins on the edge of barrens are reported to have significantly

larger gonad than their barrens conspecifics, hence the production of gametes is greater and extend over a longer period (Byrne et al 1998).

1.3 Links between habitat and urchin roe quality

1.3.1 Kelp forest and Barrens

Kelp forests are described as phyletically diverse, structurally complex and highly productive components of cold-water rocky marine coastlines (Steneck et al 2003). Factors linked to climate change and the periodic event of El Niños (Tegner & Dayton 1991) along with overfishing and other human activities can cause measurable impacts on kelp forest ecosystems, however well-developed kelp forests are most threatened by herbivory, usually from sea urchins (Steneck et al 2003). Deforestation events caused by the overgrazing of sea urchins have occurred more and more frequently in the last 20-30 years. Cases are reported for *Strongylocentrotus* spp. in central California kelp forest (Watanabe & Harrold 1991), North East Atlantic (Hagen 1995) and Eastern Canada, Nova Scotia (Breen & Mann 1976). A review of at least 16 events of sea urchin overgrazing of seagrasses is reported by (Eklöf et al 2008). The outcome of the overgrazing is the formation of barrens described as benthic communities on rocky subtidal reefs that are dominated by urchins and coralline algae (Filbee-Dexter & Scheibling 2014). Barrens are characterized by low primary productivity and low food-web complexity relative to kelp communities and are generally considered a collapsed state of the kelp ecosystem (Filbee-Dexter & Scheibling 2014).

In New South Wales almost 50% of the reef area has already been converted to barren habitat due to *C. rodgersii* catastrophic overgrazing (Andrew & O'Neill 2000). In eastern Tasmania urchin barrens are expanding, in 2002 a survey reported 3% of barrens formation while a resurvey conducted in 2017 report a 15% increase of barrens area with extended barrens in the north-east and small patches of “incipient barrens” more common in the south-east (Johnson et al. 2005, (Ling & Keane 2018). However, small barrens areas are thought to be precursors for the development of more extensive barrens habitat (Byrne & Andrew 2013, Johnson et al 2005).

C. rodgersii is capable of maintaining the state of barren indefinitely with relatively few individuals able to prevent the algae cover from re-establishing (Andrew 1993), and the scarcity of food in barrens does not pose a problem to urchin survival (Ling et al 2014). In addition, the greater depth at which barrens occur in Tasmania, (Perkins et al 2015), makes more difficult any control measures.

The small home-range and limited movement of *C. rodgersii* lead to a gradual process of barren creation, by progressive increase in local barrens from individual urchins, with larger barrens forming with increased densities of urchins (Andrew & Byrne 2007). This mechanism of barrens formation differs from that described for *Strongylocentrotus* spp. that start a feeding front of kelp in aggregation with other conspecifics during day-night activity (Rogers-Bennett 2007). Observations and experimental studies have shown that sea urchins are primarily responsible for causing and maintaining the barrens habitat they occupy (Fletcher 1987). Where mass mortality of sea urchins caused by disease, storms or changes in salinity occurred, changes in reef habitat structures followed (Andrew 1991). A study conducted in Tasmania demonstrated that the removal of *C. rodgersii* from patches of "incipient barrens" resulted in a rapid recovery of macroalgal habitat (Ling 2008).

1.3.2 Sea urchin barrens: problems and control methods

In Tasmania, the two most valuable species for the fishery, black-lipped abalone (*Haliotis rubra*) and southern rock lobster (*Jasus edwardsii*), with a combined value of $150-170M, are strictly dependent on shallow rocky reefs where the highly productive seaweed beds with high diversity of associated other invertebrates provide both shelter and food source (Johnson et al 2011).

The substantial biomass of the longspined sea urchin (*Centrostephanus rodgersii*) is a threat to the reef productivity since the 'barrens' habitat result in reduced invertebrate biomass and diversity, unable to support commercial fisheries for abalone or rock lobster (Johnson et al 2005). In the early 1970s, a population explosion of green urchins in Nova Scotia and the subsequent formation of many coralline barrens co-occurred with a dramatic decline in commercial landings of American lobsters (*Homarus americanus*) (Garnick 1989).

Removal of *C. rodgersii* from barrens resulted in recovery of seaweeds (Andrew & Underwood 1993, Hill et al 2003), however where some methods were demonstrated to be effective on a small scale, the application on extensive coastal areas might be unfeasible.

Chemical control includes the use of toxic substances (e.g., formalin copper sulphate, hydrochloric acid and ammonia). Quick-lime or calcium oxide CaO has been used to kill *Asterias amurensis* (Goggin 1998), to reduce the green sea urchin *Strongylocentrotus droebachiensis* in the Northwestern Atlantic (Bernstein & Welsford 1982) and in the management of urchins in kelp beds in Southern California (Strand et al 2020, Wilson & North 1983).

The use of quicklime is proved to be effective in achieving high killing rates of sea stars and sea urchins (Bernstein & Welsford 1982, Rolheiser et al 2012), but it requires long exposure in contact with animals epidermis. The application on *C. rodgersii* would involve operations at a considerable depth, making the work slow, physically difficult and potentially expensive (considering the time that a diver can spend underwater, the number of treated specimens and the cost of diver per hour), however, an engineering solution that does not involve divers but plans to pump down the quicklime is proposed. Quicklime is known to be harmful to echinoderms but much less harmful to other organisms (Shumway et al 1988). Nonetheless, small scale trials in Tasmanian waters should investigate the possible detrimental effect on other marine life before a broad scale application in order to consider the practice environmentally and socially ethical.

Species invasions that occur at geographic scales often preclude complete eradication (Green et al 2017). Culling activities contribute to local invasion suppression and alleviate invasion effects (Côté et al 2014). Systematic culling on *C. rodgersii* has given excellent results by drastically reducing the density of urchins and patches of barrens were quickly recolonised by canopy-forming kelps (Tracey et al 2015). Even though it has proved an effective method in restricted areas, the application has limits set by both high costs and logistics (Sanderson et al 2016). Larger barrens occur at depth between 15-30m (Johnson 2013) and this limits the ability of divers to control on sea urchins due to limited dive times at these depths.

The over-exploitation and eradication of highly valuable vertebrate apex predators have often triggered an increase in the population of herbivores, leading to widespread deforestation of algae (Strong & Frank 2010). The drastic reduction of sea urchin predators such as sea otters in the North Pacific and cod in the North Atlantic has allowed these populations of herbivores to grow out of control (Estes et al 1978, Hagen 1983). However, a rapid and extensive modification of algal species composition and a dramatic increase in kelp biomass was observed following the return of sea otters in some areas (Duggins 1980).

In Australia, the large rock lobster *Jasus edwardsii* is a key predator of sea urchins (Pederson & Johnson 2006) and several studies showed a direct relationship between development of sea urchin barrens and fishing of their predators (Sala et al 1998). Rock lobsters have nocturnal feeding habits and therefore have access to a wider size range of sea urchins than do the day-active fishes (Ling & Johnson 2012). Large rock lobsters (≥ 140 mm carapace length, 1.4 kg weight) are the most important predators of *C. rodgersii*, and likely the only predators capable of preying on the largest sea urchins (Ling & Johnson 2012). In Tasmania, experimental work in marine reserves showed that natural control of urchins' population can be achieved by protecting their principal predators. Marine reserves have higher abundance of larger sized, rock lobsters, as well as other large predators such as the wrasse *Notolabrus tetricus*, resulting in fewer sea urchins and consequently reduced patches of barrens (Edgar & Barrett 1999, Ling & Johnson 2012). This is consistent with the formation of large barrens along eastern Tasmanian reefs where rock lobster populations are at very low levels (Ling et al 2009b).

A manipulative study has been conducted aimed to increase the predation of lobsters on *C. rodgersii* either by translocating large rock lobster in incipient barrens and by removing fishing pressure on sea urchin predators (Johnson 2013). On a small pilot scale, the measures adopted demonstrated effectiveness in limiting sea urchin population expansion and facilitated some restoration of algae cover in areas of small incipient barren. However, on extensive barrens predation on urchins by lobster was unable to restore habitat (Johnson 2013).

Commercial harvesting of sea urchins for urchin control is feasible. Internationally, urchins are collected for the characteristic taste of their gonad and in the Asian

market, especially in Japan, premium quality gonad are sold at high prices (Sun & Chiang 2015). Some countries where sea urchins were considered pests 30 years ago are now applying stock recovery programs due to excess harvesting (Harris & Tyrrell 2001).

In Australia, a small fishery for sea urchins exists but the harvest is insufficient to control urchin density. Economic limitation resides in the variability of the gonad's quality (Byrne & Andrew 2013). Only premium gonad reach high prices and characteristics of good quality are the colour, yield and texture (Reynolds & Wilen 2000). It has been reported that sea urchins that graze in kelp bed generally have larger roe than sea urchins of a similar size in barrens (Byrne et al 1998), relating this to the availability of better quality food.

Commercial fishermen have interest in harvesting only sea urchins from kelp beds or the fringe of barrens, as a result, population of urchins in extensive barrens persists at high density and inhibit the recovery of algae cover. This abundance of urchins represents a substantial potential resource if a mechanism can be found to improve the economic viability of urchins from barrens.

Natural stocks of sea urchins in some regions of the world have been overexploited due to the constant and increasing demand for urchin's roe (Pais et al 2007), therefore, research and industry have had a strong interest in the development of sea urchin aquaculture (Carboni et al 2014, Liyana-Pathirana et al 2002a) and in the utilisation of unfished wild stocks of sea urchins on barrens. Studies aimed to improve urchin gonad yield and quality feeding algae or artificial diet have been performed on *Strongylocentrotus droebachiensis* (Carrier et al 2017, Siikavuopio et al 2007a), *Mesocentrotus nudus* (Takagi et al 2017), and *Evechinus chloroticus* (Phillips et al 2010).

The development of a suitable artificial diet able to increase urchin gonad yield in relative short time preserving the quality characteristics of colour and taste has been investigated for decades (Hammer et al 2004, Hammer et al 2006a, Hammer et al 2006b, Hammer et al 2012, Liyana-Pathirana et al 2002a, Pearce & Robinson 2010, Pearce et al 2002a, Pearce et al 2002b, Pearce et al 2002c, Pearce et al 2004, Phillips et al 2010, Senaratna et al 2005). Fresh algae as diet source have often resulted ineffective in increasing gonad yield compared to formulated diets (Carrier

et al 2017, McBride et al 2004, Shpigel et al 2005, Siikavuopio et al 2007a, Woods et al 2008). Artificial diets on the contrary, allowed a rapid gonad's increase but produced light-coloured and soft gonad (Barker 1998, Pearce et al 2002a, Watts et al 1998).

Along the coast of Tasmania, there is a great availability of sea urchin *Centrostephanus rodgersii*, but the quality is variable and dependent on several factors. It is necessary to understand better, how seasonality and the environment affect the quality characteristics to turn the sea urchins into profitable seafood through aquaculture practices.

1.3.3 Sea urchin fishery

Worldwide, the sea urchin fishery is the most important among the commercial exploitation of the echinoderms. Sea urchins gonad, usually called "roe" or "uni", are culinary delicacies in many parts of the world (Stefansson et al 2017). The roe of sea urchins is considered a prized delicacy in Asian, Mediterranean and Western Hemisphere countries and is a sought-after luxury food in Japan. The world production of sea urchin fishery is difficult to estimate because apart from the commercial fishery, artisanal sea urchin fisheries along the coasts of many tropical countries are largely unrecorded (Andrew et al 2002).

The global commercial sea urchin fishery is centred on the Japanese market, which consumes more than 80% of the world's production (Sonu 2003). Japan import urchin roe from at least 13 countries (Sloan 1985), among which USA, Chile, South Korea and Canada are the largest. France is the world's second-largest consumer of sea urchin roe (Hagen 1996).

Production of roe progressively increased until 1995 when 120,306 t of landed sea urchins have been recorded (Andrew et al 2002). In some countries, however, concentrations of fishing effort and lack of management system have led to depletion of natural populations and worldwide production of roe has been declining since 1995. In 1998, the total catches were estimated at 90,257 t and since then worldwide sea urchin fishery fluctuate around 70,000-80,000 t with over half of this catch supplied from the Chilean fishery for *Loxechinus albus* (Andrew et al 2002).

There are several edible species of sea urchins and some are economically important for the urchin fishery (Lawrence 2001). Following depletion through intensive fisheries for some species, restocking programs have been established in place in countries like Japan, Canada, France, Philippines (Couvray et al 2015, Juinio-Menez et al 2008, Unuma et al 2002).

Among the edible sea urchins, some commercially important are the red sea urchin *Strongylocentrotus franciscanus*, common along the West Coast of North America from Baja California to the Aleutian Archipelago (Tegner & Dayton 1981), the green sea urchin *Strongylocentrotus droebachiensis* which is handpicked by divers in the coastal waters of British Columbia and Maine, but smaller fisheries are prosecuted in Alaska, Washington and Iceland (Perry et al 2002). Japan harvests six species of sea urchins in its waters: *Strongylocentrotus nudus*, *Strongylocentrotus intermedius*, *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus*, *Anthocidaris crassispina* and *Tripneustes gratilla*, a further nine species are consumed but catches are small and restricted to local areas (Kawamura 1993). In Chilean waters is collected the red sea urchin *Loxechinus albus* (Vásquez 2007). *Paracentrotus lividus* is common in the Mediterranean Sea and the Atlantic east coast and is one of the most intensely harvested benthic invertebrate species for commercial and recreational purpose (Fernández-Boán et al 2012, Guidetti et al 2004, Pais et al 2012). The sea urchin *Evechinus chloroticus* is widely distributed around New Zealand but attempts to establish a commercial fishery have not succeeded because of the poor product quality and low recoveries (Barker 1998, McShane et al 1994). On the Australian east coast, small fisheries exist for three species of sea urchins, the Longspined (black) Sea Urchin *Centrostephanus rodgersii*, the Shortspined (white, purple) Sea Urchin *Heliocidaris erythrogramma* and the red urchin *Heliocidaris tuberculata* (Blount et al 2003).

The economic viability of sea urchin fisheries is greatly dependent on the marketable condition of the roes (Blount et al 2017). Gonad yield and colour are often variables among wild populations, and some animals present roes that do not meet commercial markets requirements (Blount & Worthington 2002, James & Heath 2008).

Gonad quality can be affected when sea urchins are exposed to sudden environmental changes. Some urchin species are less tolerant than others to increases in water temperature, turbulence, hypoxia (Siikavuopio et al 2007b). For instance, the fishing activity can generate metabolic stress in sea urchins due to handling procedures, emersion, exposure to environmental factors, transport, vibrations and overall delay from the moment of collection to the processing phase (Reynolds & Wilen 2000, Warren & Pearce 2020).

The coelomic fluid of echinoderms is an indicator of metabolic stress since particular groups of cells present in the fluid called coelomocytes are activated to contrast inflammatory process (Matranga et al 2005, Matranga et al 2000, Pinsino et al 2007). A group of cells called red spherule coelomocytes have anti-inflammatory properties and when they are released in great numbers the coelomic fluid from transparent become brownish and turbid, this could potentially affect the quality and colour of the gonad (Coates et al 2018). The stress response of *C. rodgersii* to collection and handling could be assessed monitoring variations in coelomic fluid parameters.

Variation in the size and colour of roe observed in wild populations has also been shown to be related to the availability of food (Meidel & Scheibling 1998). *Centrostephanus rodgersii* is the most abundant species in NSW (Blount et al 2017). This is reflected in commercial catches, which have been dominated by *C. rodgersii* since 2001. Difficulties with the reliable harvesting of good condition roe, and therefore, the costs of harvesting and processing sea urchins, have slowed the fishery's development. Annual catches in NSW have fluctuated around 50 tonnes and from 1998 to 2018 the total catches accounted for 806 tonnes, despite (Worthington & Blount 2003) indicating that sustainable catches of *C. rodgersii* of 200–1000 tonnes could be possible. In the state of Victoria catches for *C. rodgersii* around the same period were less consistent and are reported around 328 tonnes.

In Tasmania, the sea urchin fishery started in the 1980s with the harvest of the 'Shortspined' sea urchin *Heliocidaris erythrogramma*. The commercial fishery for the Longspined sea urchin *C. rodgersii* started in 2008 in St. Helens on the northeast coast of the State. The total annual catch started and remained at 100 tonnes

or less until the 2017/18 season, when the catch increased to 185 tonnes, then tripling in 2018/19 to 560 tonnes (Cresswell et al 2019, Keane et al 2019).

Sea urchin fishery harvests worldwide will probably remain around 100,000 tonnes per year or decline (Andrew et al 2002). When wild stocks decline, the demand created in the marketplace's raises the price of the product and, consequently, culturing is more likely to become economically viable (Kelly 2005).

1.4 Biochemical composition

1.4.1 Role of proteins, lipids, carbohydrates, fatty acids and amino acids in urchin roe quality.

The macro and micro composition of sea urchin gonad is important both biologically and nutritionally. Proteins have a structural role and serve as energy substrate especially in male gametes (Marsh & Watts 2007b). Major yolk protein (MYP) accumulates in developing gonad nutritive phagocytes of both male and female sea urchins before gametogenesis (Unuma et al 2003). Protein metabolism will also influence lipid metabolism since some transport proteins and enzyme activity are responsible for supplying the essential fatty acids for gamete development (Cook et al 2007).

Carbohydrates in the guts and gonad of sea urchins are mostly present in form of glycogen (Taylor et al 2017). During gamete development, glycogen accumulates in the nutritive phagocytes, contributing to increases in gonad weight (Lawrence et al 1966) (Giese 1966). Glycogen content decreases before spawning as it is used for the synthesis of egg components mainly lipids and glycoproteins (Doezemcl 2012). Much of the glycogen of the gonad is found in the gametes themselves, mostly in the eggs but also in sea urchin sperm (Unuma et al 2003).

Lipids are a major source of metabolic energy for urchins and provide essential materials for the formation of cell and tissue membranes (Matson et al 2012). They are essential for the physiology and reproductive processes providing hormones, vitamins, and pigments for body function and development (Castell et al 2004). Lipids have been found to be dominant in the gonad of *Psammechinus miliaris* and *Paracentrotus lividus* (Cook & Kelly 2007, Montero-Torreiro & Garcia-Martinez

2003), *Strongylocentrotus droebachiensis* (González-Durán et al 2008, Liyana-Pathirana et al 2002a, Liyana-Pathirana et al 2002c), *Anthocidaris crassispina* and *Salmacis sphaeroides* (Chen et al 2010). Neutral lipids represent the major lipid fraction (Cook et al 2007, González-Durán et al 2008, Zárate et al 2016), which in sea urchin consist predominantly of triacylglycerols. Free fatty acids, sterols and polar lipids serve as oxidative substrates to provide energy (ATP) or for incorporation into phospholipids (Marsh & Watts 2007b). Phospholipids are the building blocks for the lipid bilayer, the universal component of all animals' cell membrane.

Fatty acid composition is important as it influences flavour and storage characteristics (Archana & Babu 2016). Animals accumulate lipids from their diets and the type of diet can influence or alter the fatty acids composition. For instance, the presence of specific fatty acids or their ratio in urchin gonad tissue can provide information on their diets. This information can be useful in aquafarming to help formulate a feed that meets the requirement of the species. In urchin gonad, the fatty acids profile is also important as it determines the nutritional value. Monounsaturated and Polyunsaturated FAs are considered healthy components and long-chain PUFAs Omega 3 have been studied extensively for their anti-inflammatory and antioxidant activity and prevention of cardiovascular disease. In sea urchins, fatty acids are an important energy source, and, in addition to their possible use during gametogenesis, they are needed by spermatozoa for swimming (Mita & Nakamura 1998); in ova, they can be important for larval development and survival during dispersal (Sewell 2005, Unuma et al 2003). Moreover, polyunsaturated fatty acids have important structural roles in membranes and are needed for the synthesis of eicosanoids (active biological compounds that are known to be implicated in reproduction) (Archana & Babu 2016).

The amino acid composition of urchin gonad is important in two aspects, namely nutrition and flavour (Hall 1992). The composition of total amino acids (TAA) affects the nutritional value of the food, while free amino acids affect the flavour. Komata (1964) found that the characteristic amino acids in sea urchin are glycine, alanine, valine, glutamine, and methionine, with glycine and alanine contributing to sweetness, while valine was responsible for bitterness and glutamine to the umami

taste of sea urchin roe. Dincer and Cakli (2007) found glycine to be the dominant FAA in sea urchin gonad of *P. lividus* in spring and summer. Glycine was also found the major FAA in the gonad of green sea urchin *S. pulcherrimus* (35 to 41%) of total FAA and *S. droebachiensis* (18–60%) of total FAA (Komata et al 1962, Lee & Haard 1982). According to Lee and Haard (1982), *S. droebachiensis* contained the highest FAA content when the gonad were fully matured and ready to release gametes, while amino acid reserves, in general, are depleted following spawning. In addition to glycine, there are several key FAA such as arginine, lysine, alanine, serine, glutamic acid, and methionine which are important for the taste profile of urchin roe (Lee & Haard 1982). Amino acids are the building blocks of proteins and enzymes that may act as catalysts for energy production. Deficiency or excess of one or more of the amino acids is known to limit protein synthesis, growth, or both (Murai 1992). Therefore, a balanced assimilation of amino acids in the body tissues is necessary to support optimum growth, development, and health. FAAs are also known to play a role in osmoregulation neutralizing the dehydrating effect of the saline environment (Diehl 1986).

1.4.2 Seasonal variation

Several studies have documented seasonal variation in the biochemical composition of urchins (Liyana-Pathirana et al 2002c, Murata et al 2020, Rocha et al 2019, Symonds et al 2009, Verachia et al 2012b, Zárate et al 2016) and the allocation pathway of the components in body tissues (Beddingfield & McClintock 1998, Guillou et al 2000, Schram et al 2018). The biochemical component of sea urchin soft tissues (gonad and guts) appears to vary seasonally, while the mineral structure (test, spines, and jaws) remain stable (Montero-Torreiro & Garcia-Martinez 2003).

Accumulation and mobilization of macronutrients in the gonad follow an annual pattern controlled by the reproductive cycle (Byrne et al 1998). This reproductive cycle is linked to seasonal changes in temperature and photoperiod (Walker et al 2007), although the quantity and quality of food can also influence gonad growth (Marsh & Watts 2007a). Generally, carbohydrates accumulate during gonad development but decrease in the last months of gonad maturation (Fernandez 1998). Proteins usually increase during gonad growth and maturation stages, decreasing with spawning, whereas glycogen accumulates in nutritive phagocytes but declines

when gametogenesis initiates (Marsh & Watts 2007a). Changes in lipid content are less clear but tend to be similar to that of glycogen, with higher levels during the growing phase and a decrease before spawning (Fernandez 1998, Montero-Torreiro & Garcia-Martinez 2003). The accumulation of protein and carbohydrate in the nutritive phagocytes confer gonad' texture and firmness relative to the percentage of moisture, with increasing moisture negatively affecting texture and firmness.

1.5 Objectives

The primary objectives of this study were:

To investigate the effect of seasons, site, feeding habitat and sex on the proteins, lipids, carbohydrates, ashes, and moisture content in the *C. rodgersii* gonad.

To provide information to the fishery on nutritional quality and best season to harvest *C. rodgersii*.

To study the potential of *C. rodgersii* as an aquaculture species, in particular the possibility of gonad enhancement through selective feeding in the short term.

To assess the conditions to which *C. rodgersii* is exposed during the collection for commercial purpose and how environmental and human factors contribute to the loss of quality of the gonad prior to the processing.

To repurpose the waste produced from the *C. rodgersii* urchin fishery, utilising it as fertiliser for agricultural crops.

Chapter 2 Effect of season, site, feeding habitat, and sex on the biochemical composition of *Centrostephanus rodgersii* gonad

2.1 Abstract

The Longspined sea urchin, *Centrostephanus rodgersii,* is an edible marine species targeted to capture fisheries activity due to its gonad or "roe". The abundance of local *C. rodgersii* is high and remains underexploited. The market value of sea urchins is corresponding to the quality of the gonad. However, due to lack of information on this species, development of the industry has become challenging. Within this context, the present study assessed the annual cycle of gonadal growth, nutrients storage and examined the effects of seasonal changes on *C. rodgersii* gonad appearance and biochemical composition. Specimens (n=420) were randomly collected from kelp beds and barrens grounds on two locations (Sloop Rock and Elephant Rock) at the east coast of Tasmania on seven occasions between March 2018 and February 2019. The gonad were mature during summer and autumn, followed by gametogenesis, and spawning occurred throughout the winter. In spring, gonad were spent on nutrients and followed a period of recovery. Ovaries and testes showed differences in colour and the biochemical components and a clear seasonal variation related to the gonad cycle. Male gonad showed a brighter yellow colour with more whiteness during gametogenesis. Testes were higher in proteins, carbohydrates, sweet taste amino acids, and important fatty acids; C18:0, C20:1n9, DHA, ARA and Σω6. Female gonad showed a more intense yellow colour and greater levels of lipids and umami taste amino acids with major fatty acids were C16:0, C18:1n9, EPA; SDA and LA. In general, *C. rodgersii* gonad presented high levels of carbohydrates and proteins, triacylglycerol (TAG) was the dominant component of lipid classes, fatty acids were rich in PUFAs, and major amino acids were Arginine, Glycine, Lysine, Glutamic Acid and Aspartic Acid. These findings demonstrate that the quality of *C. rodgersii* gonad is associated with seasonal variation suggesting June and December as the best periods for the commercial harvest as the gonad were at the developing and matured stages rich in nutrients in both sexes and attractive colour. Gonad quality traits decreased from the commencement of spawning (July) through the recovery period. Moreover, findings

of this study demonstrated that the quality of gonad from urchins living in the barrens is comparable with that of animal collected in kelp beds in terms of appearance, yield and nutritional aspects, therefore harvesting from the barrens is possible.

2.2 Introduction

The Longspined Sea Urchin, *Centrostephanus rodgersii* (A. Agassiz, 1864), is abundant in shallow, subtidal, rocky habitats along the southeast coast of Australia (King et al 1994). Over the past three decades, the species has extended its range southward (Edgar et al 2005, Johnson et al 2005, Ling et al 2008) as transport and settlement of larvae in the warming water off Tasmania was facilitated by the strengthening East Australian Current (EAC), (Ridgway 2007). *Centrostephanus rodgersii* is an edible species that is targeted by fishers for the commercialisation of the gonad or "roe". Abundance is locally very high and remains largely underexploited commercially. The Tasmanian commercial sea urchin fishery began in the 1980s with the harvest of the 'Shortspined' sea urchin *Heliocidaris erythrogramma*. The fishery for *C. rodgersii* started in 2008 on the Northeast Coast (St. Helens) but lack of familiarity with the species has made difficult the development of this industry and over the years the catch rates have remained low. Between 2008 and 2015 less than 100 tonnes were collected annually, however, a subsidy program introduced in 2016 encouraged the fishery and over 1000 tons have been harvested from 2018 to August 2020.

Scepticism in developing the *C. rodgersii* sea urchin fishery resided in the observation of inconsistency in gonad colour, shape, granularity and taste that resulted in some animals not suitable for the market. The fishery of sea urchins focuses on the export of gonad to the Asian markets which have high-quality standards and roes are graded according to specific criteria, with corresponding acquisition prices. The high costs inherent in the harvesting, transport and processing of animals and the potential results of an aliquot of non-marketable gonad or low-grade roes, initially discouraged the idea to pursue this business as it did not seem economically feasible.

Several studies have investigated the ecology and reproductive cycle of *C. rodgersii* in Australia (Andrew & Byrne 2007, Byrne & Andrew 2013, Byrne et al 1998, King et al 1994) and New Zealand (Pecorino et al 2012, Pecorino et al 2013), as well as the range extension and larvae settlement in Tasmanian waters (Johnson et al 2011, Johnson et al 2005), and the overgrazing impact on the kelp forest and consequent barrens formation (Ling & Johnson 2009, Ling et al 2009b, Ling et al 2014).

At present, little is known about *C. rodgersii* gonad' biochemical composition, the proportions in macro components as well as the profile in fatty acids and amino acids that characterize the species, how the accumulation of nutrients define the gonad quality traits and if these are influenced by different feeding habitat and seasonality. Seasonal changes in urchin gonad biochemical composition have been investigated in other commercially exploited urchin species, including *Paracentrotus lividus* (Arafa et al 2012, Martinez-Pita et al 2010, Mol et al 2008, Montero-Torreiro & Garcia-Martinez 2003, Rocha et al 2019), *Strongylocentrotus droebachiensis* (Liyana-Pathirana et al 2002b), *Evechinus chloroticus* (Verachia et al 2012b), *Arbacia dufresnii* (de Vivar et al 2019, Zárate et al 2016), *Tripneustes gratilla* (Chen et al 2013), *Psammechinus miliaris* (Cook et al 2000).

Sea urchins, in preparation for the reproductive season, go through a phase of accumulation of reserves (protein, lipid and glycogen) that are stored in the nutritive phagocytes (Byrne et al 1998, Fernandez 1998). During this period gonad increases in size until the gametogenesis take place (Marsh & Watts 2007a). When gametes 'sperm and ova' are produced, the glycogen decline as it's utilized for energy source (Marsh & Watts 2007a). Other macronutrients are hydrolysed; fatty acids from lipids are stored in eggs while amino acids from protein are mostly allocated in sperm. At the spawning stage gonads are soft and gametes are released in the environment; at the end of spawning gonad are spent, small depleted of nutrients. A phase of recovery follows in preparation for the next reproductive season (Montero-Torreiro & Garcia-Martinez 2003).

Most commercially harvested urchins species follow a similar pattern of reproductive development with both male and female gonad increasing in size during the accumulation of reserves in the nutritive phagocytes (Walker et al 2007). The consumer market prefers gonad rich in nutrients, heavier in weight and bigger

in volume with a firmer texture and pleasant taste attributed to carotenoids, lipids and amino acids. Harvesting ceases at the beginning of spawning and recommences after recovery. Urchin roes spent of nutritive resources are small, dark in colour and bitter in taste hence not palatable or acceptable to the premium market.

The attributes that determine gonad quality and marketability are the colour, size and shape, texture, granulometry and flavour. While some quality attributes are affected by the seasonal changes of the reproductive cycle, food source and availability also play an important role in defining the biochemical composition and qualitative traits such as colour, texture and flavour (Phillips et al 2010).

Sea urchin gonad taste is mainly determined by the presence in certain amount of known amino acids (Murata et al 2002), different group of amino acids are classified whether they confer a sweet taste (Fuke & Konosu 1991), a bitter or umami taste (Komata 1964, Komata et al 1962, Murata et al 2001). The umami taste is widely present in seafoods and is attributed to the amino acid glutamate which enhance the overall palatability of the food (Komata 1990). Differences in gonad taste were also found between sexes, with ovaries having a higher proportion of amino acids conferring bitter taste and testes presenting a higher proportion of amino acids that determine sweet taste (Murata et al 2020, Osako et al 2007, Osako et al 2006).

Information on biochemical composition is important to assess urchins' nutrient requirements, which is relevant in aquaculture for feed formulation, and also to define market-related traits potential for commercialization. Moreover, a profitable fishery with a long-term sustainable harvest of sea urchins would be beneficial in reducing the number of urchins along the coasts of Tasmania, which with their overgrazing activity have already reduced to barrens part of the once productive rocky reef.

The regrowth of kelp was observed after removal of the sea urchin *Strongylocentrotus droebachiensis* from barrens ground in Northern Norway (Carlsson & Christie 2019). In New South Wales, the removal of *C. rodgersii* from barrens resulted in recovery of seaweeds (Andrew & Underwood 1993, Hill et al 2003). The collection of urchins from barrens areas and the enhancement of gonad quality traits through aquaculture practices (ranching) is also explored in Norway

with the sea urchin *Strongylocentrotus droebachiensis* (Dale et al 2005), in California with the sea urchin *Strongylocentrotus purpuratus* (Gardner et al 2021), in Japan with *Mesocentrotus nudus* (Takagi et al 2019) and Australia with Heliocidaris erythrogramma (Pert et al 2018).

This investigation aimed to determine the annual cycle of gonadal growth and nutrient storage as well as to document the effects of seasonal changes in the appearance and biochemical composition of *C. rodgersii* gonad from contrasting habitats on the east coast of Tasmania. The findings intend to constitute a baseline of information for the fishery of *C. rodgersii* and the potential enhancement/conditioning of *C. rodgersii* gonad in aquafarming.

2.3 Material and Methods

Sea urchins, *Centrostephanus rodgersii,* of commercial processing size (> 85 mm) were collected by SCUBA-divers in St. Helens on the northeast coast of Tasmania, Australia. Two locations about 3.5km apart (Sloop Rock and Elephant Rock) were selected and in each location 15 urchins were collected from both kelp beds habitat (5-12m depth) and extensive barrens habitat (with more than 20m of distance from any kelp plants and deeper than the 20m contour). A total of 60 sample animals per month were harvested, on 7 occasions between March 2018 and February 2019. Animals were processed immediately after the collection and biometric parameters (total wet weight, test diameter, drained weight, and gonad wet weight) were recorded. Subsequently, the urchins were cracked open and drained of coelomic fluid for three minutes, then re-weighed to obtain the total drained weight. Gonad were removed from the test with a spoon, damp-dried with blotting paper, and the wet weight of gonad (WW) recorded. The test and guts and jaws were discarded, and gonad separated. A piece of one lobe of the five gonad was cut and preserved in 10% formaldehyde, acetic acid, and calcium chloride for histology and sex determination. The rest of the gonad were sealed in labelled plastic bags, frozen in liquid nitrogen, and stored at -30˚C until analysis.

2.3.1 Biological parameters

The whole animal wet weight was recorded to the nearest 0.5 gram and test diameter measured with a Vernier calliper in millimetres to one decimal place. The gonad index of each sea urchin was calculated as the gonad wet weight (GWW) divided by the wet weight of the intact animal (TW) and multiplied by 100: GI = (GWW / TW) *100. Gonad colour intensity was recorded with a colour meter (Konica Minolta Chroma Meter CR-400) to identify parameters of colour associated with gonad quality comparable with literature data; the use of colour meter allows an objective measurement of gonad colour change during seasons and in relation to different sites and habitats. Three replicate measurements on one gonad lobe per urchin were recorded and values averaged. The system used to record the colour was the international standard CIEL*a*b* that expresses colour as three values: L* for the lightness from black (0) to white (100), a* from green (−) to red (+), and b* from blue (−) to yellow (+). Hue and Chroma were then calculated from each measurement using the following formulas:

$$Hue = \arctan\left(\frac{b}{a}\right)$$

$$Chroma = (a^2 + b^2)^{0.5}$$

2.3.2 Gonad moisture and ash content.

After recording gonad wet weight (WW), samples were lyophilised using a freeze-dryer (Labconco FreeZone 4.5L Benchtop, United States) until constant weight. Dried tissue was weighed (DW) and the moisture content percentage (MC) was calculated by the formula:

$$MC\% = \frac{WW - DW}{WW} \times 100$$

Dry matter content percentage was determined by the formula:

$$DMC\% = \frac{DW}{WW} \times 100$$

Ash content was obtained after combustion of roughly 1gr. of dry tissue at 550˚C for 12 h in a muffle furnace (AOAC 942.05).

2.3.3 Biochemical analysis

Temporal changes in biochemical properties of gonad were determined in order to characterise the influence of reproductive stage on roe quality and identify the underlying mechanisms linked to those changes. Proximal analysis was conducted to identify patterns of accumulation of the main nutrient components such as lipids, carbohydrates and proteins which are important as structural material and energy source. Accumulation of these macro components is not only determinant to reach a commercial gonad yield, but also influences the nutritional value of the gonad. Moisture content is important as it affects physical aspects of the gonad such as texture, shape and brightness of colour. Lipid components and amino acids were quantified in order to further characterise the nutritional profile of the gonad and to identify changes that might influence quality aspect like colour or flavour in relation to different habitats.

2.3.4 Proximal Analysis

Lyophilised samples were homogenised and then analysed to establish the main biochemical components. Total lipid content was determined gravimetrically with a modification of the (Bligh & Dyer 1959) method. The concentration of soluble proteins was measured by the Bradford method (Bradford 1976), using the Bio-rad protein assay (Bio-rad, USA) with bovine serum albumin (BSA) as a standard. The total Carbohydrate content of each sample was determined by difference summing the other constituents (lipids, proteins, ashes, and water) and subtracting them from the total weight of the sample, with the following formula: 100 - (weight in grams [protein + fat + water + ash] in 100 g of sample).

2.3.5 Isotope analysis

The stable isotopes $\delta^{13}C$ and $\delta^{15}N$ ratio of urchin gonad was determined to identify possible differences in feeding source between urchins living inside the kelp canopy and urchins present in the barrens. The stable isotopes were analysed in mass spectrometry performed with an Isoprime100 mass spectrometer coupled to an Elementar vario PYRO cube elemental analyser at the Central Science Laboratory (CSL) of the University of Tasmania (UTAS). Samples were lipid extracted, dried and homogenised, then an aliquot between 0.4 and 0.6 mg of sample was weighted for the analysis.

2.3.6 Analysis of sea urchin nonpolar lipid classes via Iatroscan (TLC–FID).

Lipid classes were determined by Thin Layer Chromatography (TLC-FID). The crude lipids obtained from the Bligh and Dyer (1959) extraction were chromatographed on silica gel coated Chromarods-S III and then analysed on an Iatroscan MK-5 (Iatroscan Laboratories Inc., Tokyo, Japan) analyser equipped with a flame ionization detector (FID) connected to a computer loaded with TSCAN software (Scientific Products and Equipment, Concord, ON) for data handling. A hydrogen flow rate of 160 ml per min and an airflow rate of 2000 ml per min were used in operating the FID. The scanning speed of rods was 30 s per rod. For the analysis of sea urchin non-polar class of lipids, the total lipids extracted were diluted in Dichloromethane (DCM) to obtain a concentration between 10 to 20 mg lipid per ml. The Chromarods were spotted with 1µl aliquot of sample. The Chromarods were then developed for 33 minutes in a chamber with the solvent systems hexane/diethyl ether/acetic acid (70:10:0.1 v/v/v) used for separation of non-polar lipids (Christie 1982). Chromarods were then dried at 80˚C for 10 min and scanned completely by Flame Ionization Detector (FID) to reveal non-polar lipids.

2.3.7 Fatty Acids Methyl Esters

Fatty Acid Methyl esters were determined by separation in Gas Chromatography-Mass Spectrometry (GS-MS). The following esterification procedure was applied to the lipids extracted from the dried gonad sample material before the injection in GS-MS for the determination of single fatty acids. An aliquot between 1-2 mg of lipids

was taken and added to a new vial followed by the addition of 100 µl of C19 FFA surrogate standard solution for quantitative analysis (500 ug/mL C19 FFA in DCM). The solvent was evaporated to dryness under a stream of nitrogen gas. Approximately 3 mL of Methylation reagent 10:1:1 (v/v/v) MeOH:DCM: conc HCL was added to the evaporated lipid. Vials were heated for 1 hour at 80˚C and once at room temperature, mixed with 1 ml of deionized water. To the methylation reaction mixture was added ca. 1.5 mL 4:1 (v/v) Hexane: DCM. After the separation of phases, the upper phase containing the fatty acid methyl esters (FAME) was transferred to a GC vial and evaporated to dryness under nitrogen and made to a final volume 1000 µL of DCM with C23 FAME internal standard solution for quantitative analysis (50 ug/mL C23 FAME in DCM).

FAMEs were analysed using a Varian CP-3800 gas chromatograph coupled to a Bruker 300MS triple quadrupole mass spectrometer (Bruker Corporation, Massachusetts, USA) fitted with an Agilent DB-5MS column (30 m x 0.25 mm; 0.25 um film thickness). Helium was used as the carrier gas with a flow rate of 1.2 mL / min. The Injector was set to 290^{O}C and the Transfer line to 310^{O}C. Samples were injected at 50 ^{O}C in splitless mode. After 1 min, the oven was programmed from 50 to 150 ^{O}C at 30 ^{O}C / min, then at 2 ^{O}C / min to 250 ^{O}C, and finally 5 ^{O}C / min to 300 ^{O}C, which was held for 15 min. Electron ionization mass spectra were recorded in full scan mode over the range (m/z) 40 to 400. Individual FAMEs were identified based on comparison of retention times and MS data of laboratory standard FAMEs, together with the use of the NIST2017 Mass Spectral Library (National Institute of Standards and Technology, USA). Data were processed using Agilent MS Workstation Version 7.

2.3.8 Amino Acids

The gonad amino acids profile was studied on a small pool of the collected samples and over four months of the annual reproductive cycle; the maturity phase (April, Autumn), the spawning (July, winter), the post-spawning (September, Spring) and the recovery phase of the gonad (December, Summer). For each of the two sites of collection (Elephant and Sloop), five gonad samples per Habitat (Kelp and Barrens) were analysed, for a total of 20 samples per month and 80 samples overall.

Amino Acids were derivatized in Reversed-Phase High-Pressure Liquid Chromatography (RP-HPLC). Dried samples were sent for the determination of standard amino acids analysis (Auspep Pty Ltd, Victoria, Australia). Amino acids were analysed using the Waters Pico-tag methodology (Heinrikson & Meredith 1984). A mass of sample (3-4mg) was accurately weighed, to which was added a known amount of hydroxyproline standard. The sample/standard mix was hydrolysed in 6N HCl at 1C for 60 minutes in vacuo. Samples were then neutralized with triethylamine (neutralizes residual HCl) and dried by lyophilization. The hydrolysed amino acids were derivatized with phenyl-isothiocyanate (PITC). Samples were then run on RP-HPLC against an amino acid reference mix. Recoveries of the individual amino acids were measured by comparison to the reference mix and quantitated with the HyPro standard. The technique provided the determination of 16 amino acids, Cysteine and Tryptophan were not detected. Aspartic acid and Asparagine were not separable and are reported together as Asx. Glutamic acid and Glutamine were not separable and are reported together as Glx.

2.3.9 Statistical analysis

Biometric measurements, gonad colour, proximate composition, isotopes, amino acids, lipid class and fatty acids were analysed separately using a multi-factor, multivariate approach to determine the effect of season and location effects on biochemical composition. A four-way PERMANOVA was used to examine the effect of time (7 levels), sites (2 levels), habitat (2 levels) and sex (Male/Female) on each dataset. All factors were treated as fixed effects, and each PERMANOVA used type 3 sum of squares and 9999 permutations and the unrestricted permutation of raw data as the permutation method. Each multivariate dataset was normalised and similarity matrices were calculated with Euclidean distance. The two main effects of interest were Time and Sex. If significant interactions involving Sex are present, the dataset is analysed separately for Male and Female. In the presence of further significant interactions with the two spatial factors Site and Habitat, the main effect of Temporal changes is interpreted in the context of temporal variation across sites/habitats. Pairwise comparisons are performed when relevant to compare significant differences in analysis results between monthly samplings. Similarity patterns in the data were visualised using multidimensional scaling (MDS) of group

centroids. Similarity percentage (SIMPER) analysis was conducted on the groups to determine the contribution of each variable to the average dissimilarity among groups (Clarke & Gorley 2015).

Multivariate routines were performed using PRIMER 7 Version 7.0.13 (Plymouth Routines In Multivariate Ecological Research), (Clarke & Gorley 2015) with the PERMANOVA+1 add-on (Anderson et al 2008).

The multivariate biometric dataset contained six variables (Total weight, Diameter, Test drain weight, Gonad wet weight, Gonad dry weight, Gonad Somatic Index), while the multivariate gonad colour dataset contained eight variables (L*, a*, b*, Hue, Chroma, WI, YI, BI), and the multivariate proximate dataset contained four variables (Protein, Lipid, Carbohydrates, Ashes), representing percentage in dry weight. The multivariate lipid classes dataset contained six variable (Hydrocarbons, Sterol Ester and Wax Ester were grouped to form one variable, Triacylglycerol, Free Fatty Acids, Sterol, Diacylglycerol, Polar Lipids), representing percentages of the lipid extract in dry weight. Multivariate datasets for fatty acids and amino acids contained 31 and 16 variables respectively, representing percentages of the total component in dry weight for each individual component.

2.4 Results

2.4.1 Biometric measurements

PERMANOVA analysis of urchin biometric measurements shows statistically significant interactions of the seasonal factor with Site and Habitat indicating that seasonal trends vary spatially, (Table 2.1, PERMANOVA, P<0.05). There was no statistical difference for the factor Sex (Table 2.1, $F_{(1,415)}$ = 0.650, P = 0.432), the test did not show other significant interactions between factors. SIMPER analysis identified the urchin's total weight (TW) as the variable accounting the most for the dissimilarity between temporal groups (>94%) while differences within locations appear to be driven by the gonad wet weight (GWW), (Table 2.2, SIMPER analysis).

Samples taken in the post-spawning months of September, December, and February clustered away from the pre-spawning and spawning months, and variation among sites appeared to be driven by differences in gonad wet weight (GWW) within each sample month (Figure 2.1). Samples from within Kelp at Elephant Rock tended to have higher GWW within each month, while samples from Barrens at Sloop had lower GWW, however this did not translate into a significant Month x Habitat x Site interaction (Figure 2.1).

Table 2.1. Results of 4-way PERMANOVA analysis of urchin's biological measurement tested for the factors Month, Site, Habitat, and Sex.

Source	df	MS	Pseudo-F	P-value	Unique perms
Month	6	91595	5.4014	**0.001**	997
Site	1	1.11E+06	65.432	**0.001**	999
Habitat	1	1.30E+06	76.744	**0.001**	998
Sex	1	11024	0.65011	0.432	999
MoxSi	6	1.36E+05	8.0114	**0.001**	999
MoxHa	6	37679	2.2219	**0.031**	999
MoxSe	6	15113	0.89121	0.539	999
SixHa	1	2947.8	0.17383	0.731	997
SixSe	1	25032	1.4762	0.232	999
HaxSe	1	14825	0.87423	0.329	997
MoxSixHa	6	23890	1.4088	0.178	997
MoxSixSe	6	17991	1.061	0.359	998
MoxHaxSe	6	7455.4	0.43965	0.881	998
SixHaxSe	1	504.81	0.029769	0.953	998
MoxSixHaxSe	6	9519.2	0.56135	0.786	997
Res	360	16958			
Total	415				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences (p <0.05) are indicated in bold.

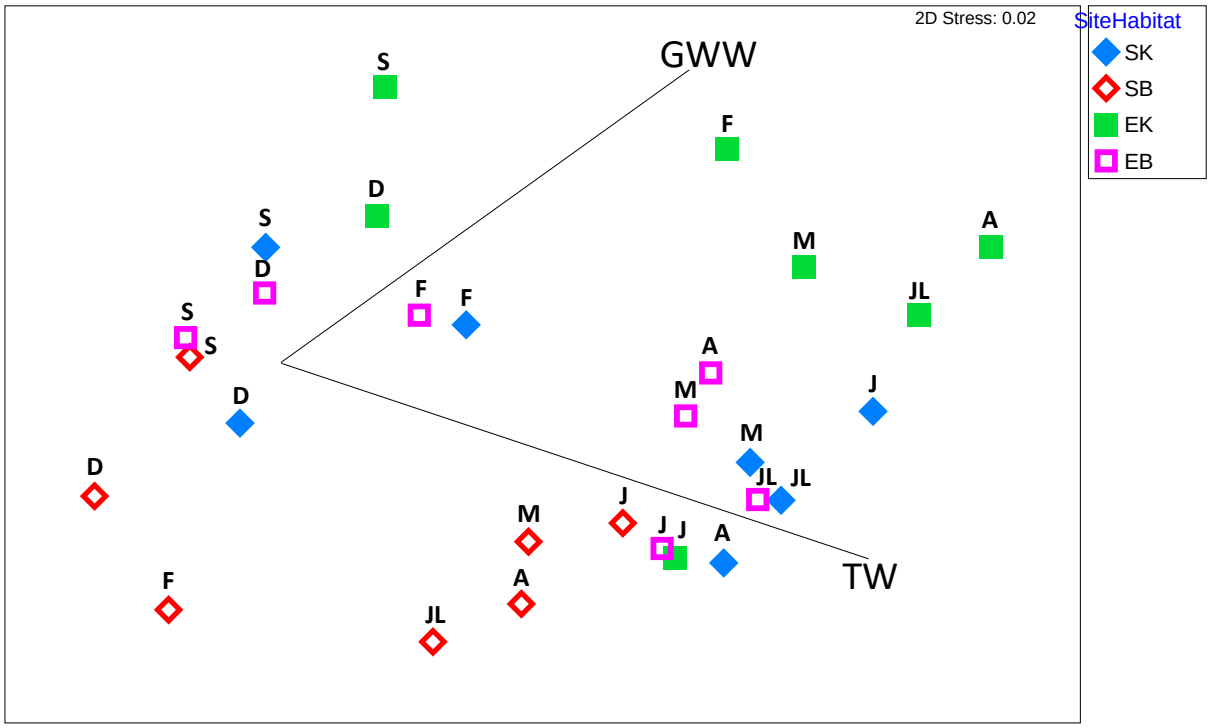

Figure 2.1. Non-metric multidimensional scaling (nMDS) of distance among group centroids for individual levels of the fixed main effect Month and the combined fixed effects Site and Habitat in the urchins' biological measurements data set. Monthly sampling: seven levels identified with letters above symbols; (M) March; (A) April; (J) June; (JL) July; (S) September; (D) December; (F) February. Site and Habitat: four levels identified by symbols of a different colour: Sloop Kelp (SK), Sloop Barrens (SB), Elephant Kelp (EK), Elephant Barrens (EB). The mapped variables Total Weight (TW) and Gonad Wet Weight (GWW) were chosen based on the major contributors to the dissimilarity among the groups identified by the SIMPER analysis. Plot Based on a resemblance matrix of Euclidean distances of normalised data.

Table 2.2. SIMPER analysis on the biological measurements of sea urchins collected at two locations (Sloop Rock and Elephant Rock) and feeding habitat (barrens and kelp) during seven annual samplings.

March			**April**			**June**			**July**		
Var.	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*
TW	475	95.52	TW	478	95.24	TW	456	94.82	TW	478	94.6
GWW	69.6	3.66	GWW	76.5	3.89	GWW	78.1	4.15	GWW	82.9	4.56
Sept.			**Dec.**			**Feb.**					
Var.	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*			
TW	482	98.76	TW	450	98.62	TW	477	97.99			
Dia	104	0.74	GWW	23.8	0.69	GWW	42.6	1.35			

Variables (Var.), contributing to the difference between groups. Total weight (TW), Gonad Wet Weight (GWW), Test Diameter (Dia), are shown along with the Average Value (Av.Val.), and their contribution to the dissimilarity (Cont%).

The urchins' Gonad Somatic Index (GSI) increased from September reaching a peak in July in both kelp and barrens habitat, with kelp animals showing a faster recovery of gonad between September and February and male presenting a greater GSI compared to female. In barrens ground the recovery was slower and differences

were not evident between male and female; at the mature stage barrens GSI was similar to the kelp one (Figure 2.2).

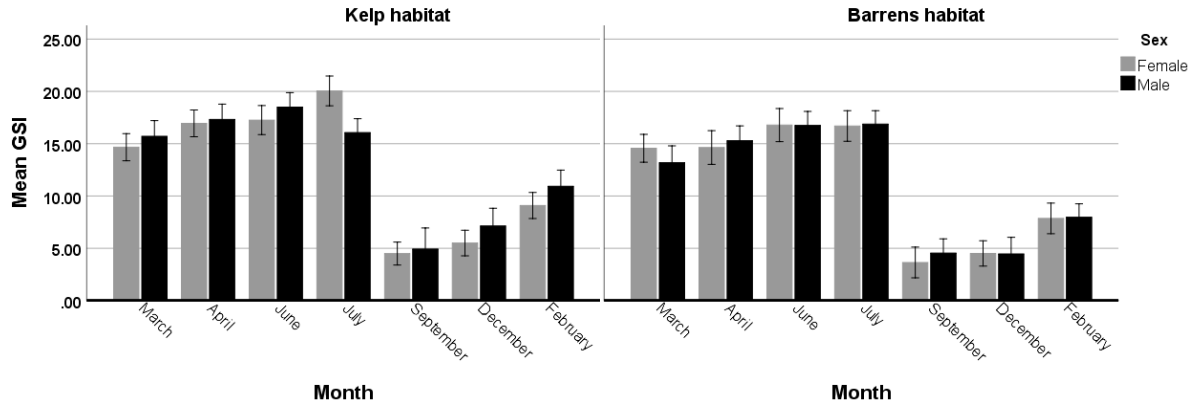

Figure 2.2. GSI of male and female sea urchins *C. rodgersii* collected in kelp and barrens habitat between March 2018 and February 2019.

2.4.2 Gonad colour

Statistically significant interactions between Month and the other three main effects of Site, Habitat and Sex, denote both a spatiotemporal variation in colour and a sex-related metabolic variation in colour over time (Table 2.3, $p < 0.01$). A significant three-way interaction (Month x Site x Habitat, $p < 0.05$) evidence that effects of colour changes varied across seasons and in different sites and feeding habitat (PERMANOVA, Table 2.3).

Table 2.3. Results of 4-way PERMANOVA analysis of urchin's gonad colour measurement tested for the factors Month, Site, Habitat, and Sex.

Source	df	MS	Pseudo-F	P-value	Unique perms
Month	6	165.19	40.976	**0.001**	999
Site	1	9.9715	2.4735	0.069	999
Habitat	1	22.007	5.459	**0.006**	997
Sex	1	211.81	52.542	**0.001**	998
MoxSi	6	13.886	3.4446	**0.001**	998
MoxHa	6	9.5445	2.3676	**0.007**	999
MoxSe	6	21.553	5.3464	**0.001**	998
SixHa	1	17.64	4.3756	**0.017**	999
SixSe	1	0.68948	0.17103	0.883	999
HaxSe	1	3.4517	0.85622	0.413	998
MoxSixHa	6	8.4153	2.0875	**0.021**	996
MoxSixSe	6	4.286	1.0632	0.371	997
MoxHaxSe	6	4.2517	1.0546	0.381	998
SixHaxSe	1	2.9214	0.72466	0.495	999
MoxSixHaxSe	6	5.1494	1.2773	0.224	998
Res	360	4.0314			
Total	415				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences (p <0.05) are indicated in bold.

Given the significant interaction terms in the full model, the dataset was partitioned by sex and a three-way PERMANOVA analysis was done on gonad colour with an orthogonal design including Month, Site, and Habitat main effects separately for the Male and Female datasets. In this reduced dataset, the temporal effect is a major contributor to the variation and a significant spatiotemporal interaction in both sexes (Table 2.4, Month x Site, $p < 0.05$). A significant three-way interaction (Month x Site x Habitat, $p < 0.05$) is apparent only for Male gonad colour measurements.

Table 2.4. Results of three-way PERMANOVA of urchin's gonad colour measurements for samples grouped by factors Site and Habitat.

Male	**df**	**SS**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Month	6	751.13	125.19	37.436	**0.001**	999
Site	1	5.8782	5.8782	1.7578	0.158	999
Habitat	1	9.9054	9.9054	2.9621	0.07	999
MoxSi	6	54.81	9.135	2.7317	**0.003**	998
MoxHa	6	34.036	5.6726	1.6963	0.084	997
SixHa	1	13.969	13.969	4.1772	**0.027**	999
MoxSixHa	6	44.005	7.3342	2.1932	**0.019**	996
Res	164	548.43	3.3441			
Total	191	1528				
Female	**df**	**SS**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Month	6	516.59	86.098	11.982	**0.001**	999
Site	1	5.496	5.496	0.76488	0.464	999
Habitat	1	26.847	26.847	3.7363	**0.021**	999
MoxSi	6	93.862	15.644	2.1771	**0.011**	999
MoxHa	6	74.143	12.357	1.7197	0.055	999
SixHa	1	7.369	7.369	1.0255	0.364	997
MoxSixHa	6	48.149	8.0249	1.1168	0.348	999
Res	196	1408.3	7.1854			
Total	223	2230				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

Ordination (nMDS) plot on the full matrix reveals a seasonal trend (left to right) as well as a sex effect mostly determined by the separation of male samples during March, April, June and July (Figure 2.3). Sex differences are less clear in the cluster containing the post-spawning months. The primary variables identified by the SIMPER analysis as driving overall dissimilarity were the Yellow Index (YI), Luminosity (L*) and Whiteness Index (WI) (Table 2.5). The Yellow Index (YI) indicates a more intense yellow colour of the roe in the S, D and F months representing gonad in the post-spawning and recovery stage. Increasing Luminosity (L*) indicates an increase in moisture content in the lumen of gonad phagocytes which confer a brighter colour, and White Index (WI) indicate the appearance of whiteness in male gonad determined by the accumulation of male gametes which

are white in colour. Male gonad in M, A, J and JL months where gonad are at the mature to spawning stage are characterised by higher Whiteness Index and Luminosity. Despite March samples being mature, gonad colour presents greater variability and the group centroids for Site and Habitat effects are scattered. Colour is more homogeneous in April, June and July samples and group centroids are less spread. Female gonad present a more intense yellow colour than males and are more tightly clustered than males. In J and JL female gonad are characterized by higher grades of L* and WI relative to the post-spawning months, giving the gonad a brighter tone of colour. During the recovery phase, there is less distinction between sexes based on colour characteristics, and there were no significant pair-wise comparisons in December and February (Table 2.20, $p > 0.05$, in appendix). The two-way PERMANOVA pair-wise test for the interaction Month x Site for pair of levels of factor Site and within levels of factor Month was statistically significant for M, A, J, JL in males and significant only in March for females (Table 2.21, in appendix).

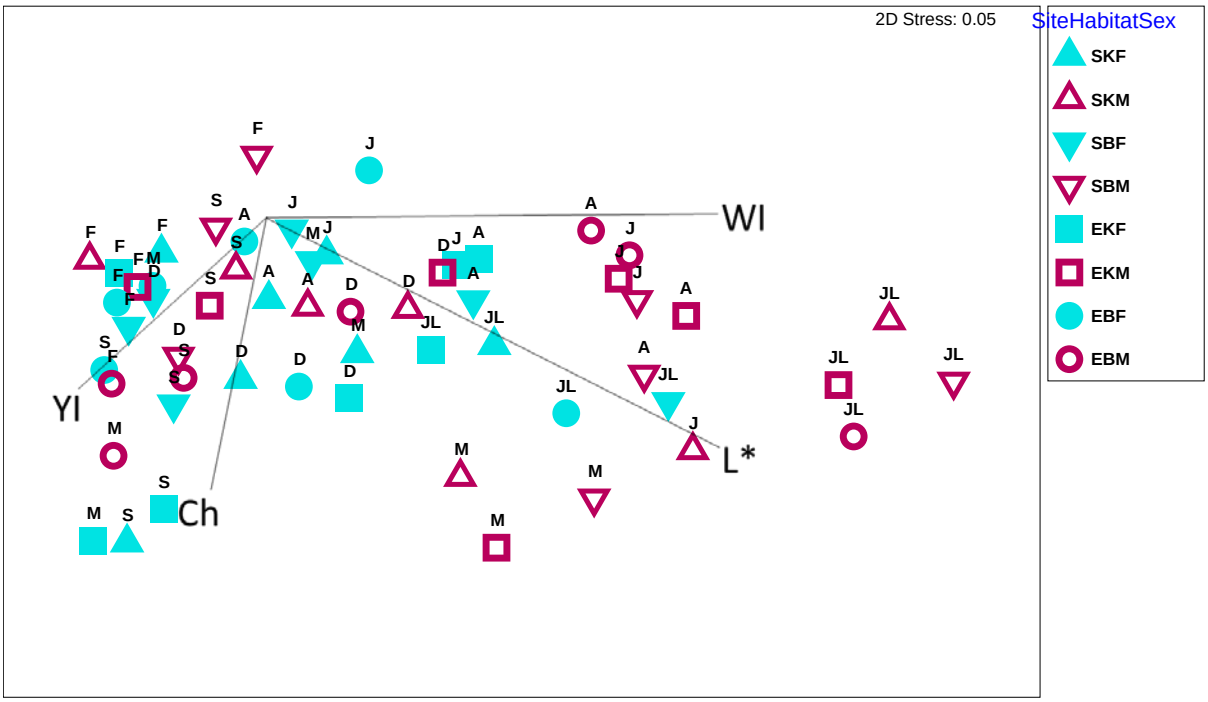

Figure 2.3. Non-metric multidimensional scaling (nMDS) of distance among the centroids for individual levels of the fixed main effects in urchins' gonad colour measurements data set. Month: seven levels identified with letters above symbols, (M) March; (A) April; (J) June; (JL) July; (S) September; (D) December; (F) February. The combined fixed effects Site, Habitat and Sex: eight levels identified with symbols of different shapes and colours, Sloop Kelp Male (SKM), Sloop Barrens Male (SBM), Elephant Kelp Male (EKM), Elephant Barrens Male (EBM), Sloop Kelp Female (SKF), Sloop Barrens Female (SBF), Elephant Kelp Female (EK), Elephant Barrens Female (EBF). The mapped variables Yellow Index (YI), Luminosity (L*), White Index (WI), Chromaticity (Ch) were chosen based on the major contributors to the dissimilarity among the groups identified by the SIMPER analysis. Plot based on a resemblance matrix of Euclidean distances of normalised data.

Table 2.5. SIMPER analysis of gonad colour coordinates of sea urchins during seven annual samplings.

March			**April**			**June**			**July**		
Var.	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*
YI	50.5	43.9	YI	48.8	43.36	YI	50.5	48.18	YI	42.9	46.65
L	71.9	17.86	L	76.5	20.55	WI	63.7	14.22	WI	66.5	18.33
WI	61.2	12.9	WI	64.4	18.92	L	76.6	11.84	L	75.8	15.52
b	25.4	12.43	Chroma	26.5	7.09	Chroma	27.6	11.21	Chroma	23.1	9.3
Sept.			**Dec.**			**Feb.**					
Var.	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*			
YI	50.3	54.42	YI	49.9	53.95	YI	54.8	43.17			
b	23.7	15.59	WI	61.5	11.96	L	70.6	19.63			
Chroma	24.9	13.2	L	71.8	11.77	b	27.1	12.98			
L	67.2	10.51	b	25	11.16	Chroma	28.1	11.76			

Variables (Var.), contributing to the difference between groups, Yellow Index (YI), Luminosity (L), White Index (WI), tones of yellow (b), are shown along with the Average Value (Av.Val.), and their contribution to the dissimilarity (Cont%).

2.4.3 Proximate Composition

PERMANOVA analysis of urchin gonad proximate composition indicated that seasonal trends were dependent on Sex, Site and Habitat with statistically significant two-way and three-way interactions of the temporal factor with Site, Habitat and Sex (Table 2.6, $p < 0.001$). The three-way interaction (Month x Site x Habitat) was only marginally significant, (PERMANOVA, Table 2.6, $p = 0.043$).

Table 2.6. Results of four-way PERMANOVA analysis of urchin's gonad proximate composition tested for the factors Month, Site, Habitat, and Sex.

Source	df	MS	Pseudo-F	P-value	Unique perms
Month	6	2202.4	50.423	**0.001**	997
Site	1	373.92	8.561	**0.001**	998
Habitat	1	1401.5	32.087	**0.001**	998
Sex	1	8292.5	189.850	**0.001**	999
MoxSi	6	102.85	2.355	**0.006**	997
MoxHa	6	181.01	4.144	**0.001**	999
MoxSe	6	185.18	4.240	**0.001**	999
SixHa	1	24.517	0.561	0.619	999
SixSe	1	23.887	0.547	0.593	998
HaxSe	1	61.43	1.406	0.235	999
MoxSixHa	6	45.073	1.032	0.436	998
MoxSixSe	6	49.284	1.128	0.328	999
MoxHaxSe	6	74.783	1.712	**0.043**	998
SixHaxSe	1	9.4621	0.217	0.867	999
MoxSixHaxSe	6	28.988	0.664	0.812	999
Res	360	43.678			
Total	415				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

To address the significant interaction between seasonal and sex main effects, the proximate composition dataset was tested separately for male and female gonad in three-way PERMANOVA analyses. A weak significant interaction for the factor Month with Site and Habitat in Males and only a Month with Habitat but stronger interaction in Females (Table 2.7, $p < 0.01$). The sex effect is represented in multidimensional space (nMDS) with a clear separation between male and female group centroids across seasons, locations and habitats (Figure 2.4). The Month with Site interaction within the Male group is determined by the significant distance among the centroids of Sloop and Elephant sites only for the March collection (PERMANOVA pairwise, $p < 0.01$). In both Male and Female groups, significant differences between habitat are more evident from September to March.

Table 2.7. Results of three-way PERMANOVA of urchin's gonad Proximate Composition for group tested by factors Month, Site and Habitat.

Male	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Month	6	1003.3	23.747	**0.001**	998
Site	1	132.67	3.1402	**0.038**	999
Habitat	1	473.31	11.203	**0.001**	999
MoxSi	6	78.316	1.8537	**0.038**	999
MoxHa	6	81.653	1.9327	**0.033**	998
SixHa	1	19.634	0.4647	0.673	998
MoxSixHa	6	27.564	0.6524	0.837	999
Res	164	42.248			
Total	191				
Female	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Month	6	1420.5	31.655	**0.001**	997
Site	1	278.04	6.1959	**0.002**	999
Habitat	1	1039.9	23.173	**0.001**	998
MoxSi	6	69.825	1.5560	0.082	999
MoxHa	6	198.60	4.4257	**0.001**	996
SixHa	1	13.830	0.3082	0.795	998
MoxSixHa	6	46.209	1.0297	0.438	998
Res	196	44.875			
Total	223				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

The SIMPER analysis found lipids as a major component contributing to the dissimilarity across the seven annual samplings followed by carbohydrates and proteins (Table 2.8). Lipid was the principal macro component that contributed to the dissimilarity between female and male gonad, while carbohydrates and proteins separate spawning and post-spawning. Overall, the seasonal trend shows a greater proportion of lipids and carbohydrates in post-spawning and recovery gonad with a steady decline towards maturation and spawning, while proteins show the opposite trend being in lower proportion in September and peaking in June (Table 2.22, in appendix). No major differences in the accumulation of gonad macro components were observed between different sampling sites except for the proportion of lipids of female gonad proceeding from barrens area in September, March and April. The ordination plot shows that pointed out symbols Sloop kelp female and Elephant kelp female group centroids are located together for each month (Figure 2.4). In Elephant Rock barrens the amount of lipids was higher than in Sloop Rock barrens, the outcome could be attributed to a different hydrodynamic at Elephant Barrens that provides more drift algae.

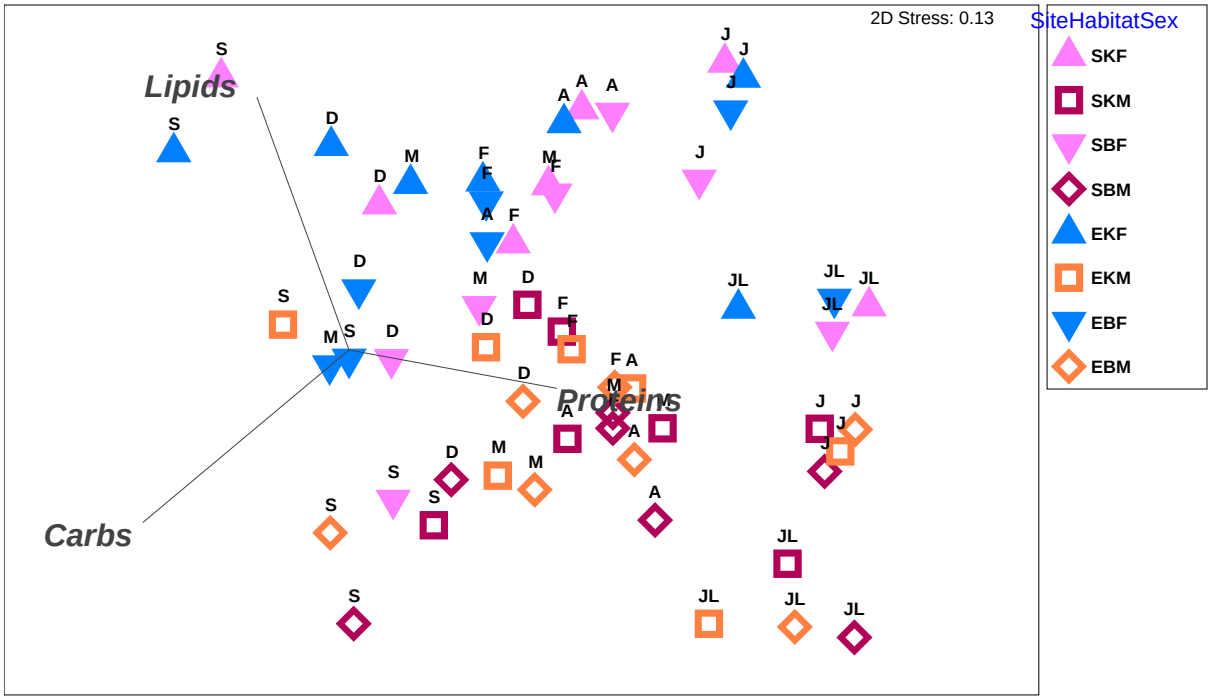

Figure 2.4. Non-metric multidimensional scaling (nMDS) of distance among the centroids for individual levels of the fixed main effects in urchins' Proximate Composition data set. Month: seven levels identified with letters above symbols, (M) March; (A) April; (J) June; (JL) July; (S) September; (D) December; (F) February. The combined fixed effects Site, Habitat and Sex: eight levels identified with symbols of different shapes and colours, Sloop Kelp Male (SKM), Sloop Barrens Male (SBM), Elephant Kelp Male (EKM), Elephant Barrens Male (EBM), Sloop Kelp Female (SKF), Sloop Barrens Female (SBF), Elephant Kelp Female (EKF), Elephant Barrens Female (EBF). The mapped variables Lipids, Protein and Carbohydrates were chosen based on the major contributors to the dissimilarity among the groups identified by the SIMPER analysis. Plot based on a resemblance matrix of Euclidean distances of normalised data.

Table 2.8. SIMPER analysis of sea urchins gonad proximate composition during seven annual samplings.

March			**April**			**June**			**July**		
Var.	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*
Lipid	26	36.68	Lipid	26.7	47.76	Lipid	27	50.74	Lipid	21.7	46.7
Carbs	34.4	31.78	Carbs	30.7	30.16	Carbs	25.9	24.05	Protein	34	25.14
Protein	33.1	28.29	Protein	35.6	18.11	Protein	36.4	14	Carbs	30.9	22.68
Sept.			**Dec.**			**Feb.**					
Var.	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*	*Var.*	*Av.Val.*	*Cont%*			
Lipid	29.3	54.48	Lipid	28.8	44.91	Lipid	26.8	38.31			
Carbs	37.8	26.9	Carbs	35.6	27.19	Carbs	32.2	29.84			
Protein	24.8	15.45	Protein	29.3	23.38	Protein	35.1	27.52			

Variables (Var.), contributing to the difference between groups are shown along with the Average Value (Av.Val.), and their contribution to the dissimilarity (Cont%). Averages are across Site, Habitat and Sex

2.4.4 Lipid Classes

The multivariate PERMANOVA analysis of lipid classes in urchins' gonad found seasonal patterns varied by Sex and by Habitat with significant interactions between Month and Habitat, and, Month and Sex (Table 2.9). The three-way interaction (Month x Site x Habitat, $p = 0.044$) was only marginally significant (Table 2.9).

Table 2.9. Results of four-way PERMANOVA analysis of urchin's gonad classes of lipids tested for the factors Month, Site, Habitat, and Sex.

Source	df	MS	Pseudo-F	P-value	Unique perms
Month	6	3640.3	43.715	**0.001**	999
Site	1	122.31	1.4688	0.223	998
Habitat	1	2083.7	25.023	**0.001**	999
Sex	1	2361.1	28.354	**0.001**	998
MoxSi	6	106.03	1.2733	0.271	998
MoxHa	6	391.08	4.6964	**0.001**	999
MoxSe	6	798.43	9.5883	**0.001**	999
SixHa	1	122.08	1.466	0.183	999
SixSe	1	33.908	0.4072	0.616	998
HaxSe	1	420.92	5.0547	**0.022**	999
MoxSixHa	6	171.1	2.0548	**0.044**	998
MoxSixSe	6	26.548	0.31881	0.965	999
MoxHaxSe	6	129.84	1.5593	0.147	998
SixHaxSe	1	26.117	0.31364	0.688	998
MoxSixHaxSe	6	118.85	1.4273	0.193	999
Res	360	83.272			
Total	415				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences (p <0.05) are indicated in bold.

Given the significant interactions, the lipid class dataset was tested separately for male and female in a three-way PERMANOVA analysis. In both sexes, seasonal patterns in lipid class varied by Habitat (Table 2.10, $p < 0.01$), and by Site and Habitat for females (Table 2.10, $p < 0.05$).

Representation of results in multidimensional space (nMDS) shows evidence of the temporal and sex interaction effect with separation between male and female at each temporal collection (Figure 2.5). The Habitat effect is apparent in the female group with significant distinction between Kelp and Barrens ground at each temporal sampling except for June, while from the ordination plot (nMDS) the Habitat effect within the male group may not be evident, the pairwise comparison confirms statistical differences in April, July, September and December (Table 2.24, in appendix). The Site and Habitat interaction within the Female group seems determined by statistical differences between Sloop Kelp and Elephant Kelp ($p <$

0.05), while no significant differences were recorded between Sloop Barrens and Elephant Barrens ($p = 0.133$).

SIMPER analysis identified Triacylglycerol and Polar Lipids as major contributors to the dissimilarity between groups (Table 2.11). TAG determined the seasonal trend being in higher proportion in September and declining towards July; TAG and PL were always more abundant in female urchins compared to males while males showed higher proportion of PL and ST (Table 25, in appendix). Finally, in both sexes, TAG was found in greater proportion in kelp area while the amount of PL and ST was generally higher in barrens (Table 2.11).

Table 2.10. Results of three-way PERMANOVA of urchin's gonad classes of lipids for group tested by factors Month, Site and Habitat.

Male	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Month	6	55.627	23.775	**0.001**	999
Site	1	5.1341	2.1943	0.08	999
Habitat	1	15.238	6.5125	**0.001**	998
MoxSi	6	2.8679	1.2257	0.202	997
MoxHa	6	6.4919	2.7746	**0.001**	999
SixHa	1	2.7475	1.1743	0.312	999
MoxSixHa	6	2.2288	0.95257	0.533	999
Res	164	2.3398			
Total	191				
Female	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Month	6	83.463	29.25	**0.001**	997
Site	1	2.7714	0.97127	0.457	998
Habitat	1	30.182	10.578	**0.001**	999
MoxSi	6	3.7167	1.3026	0.147	997
MoxHa	6	10.848	3.8019	**0.001**	999
SixHa	1	10.699	3.7495	**0.010**	999
MoxSixHa	6	3.0785	1.0789	0.344	999
Res	195	2.8534			
Total	222				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

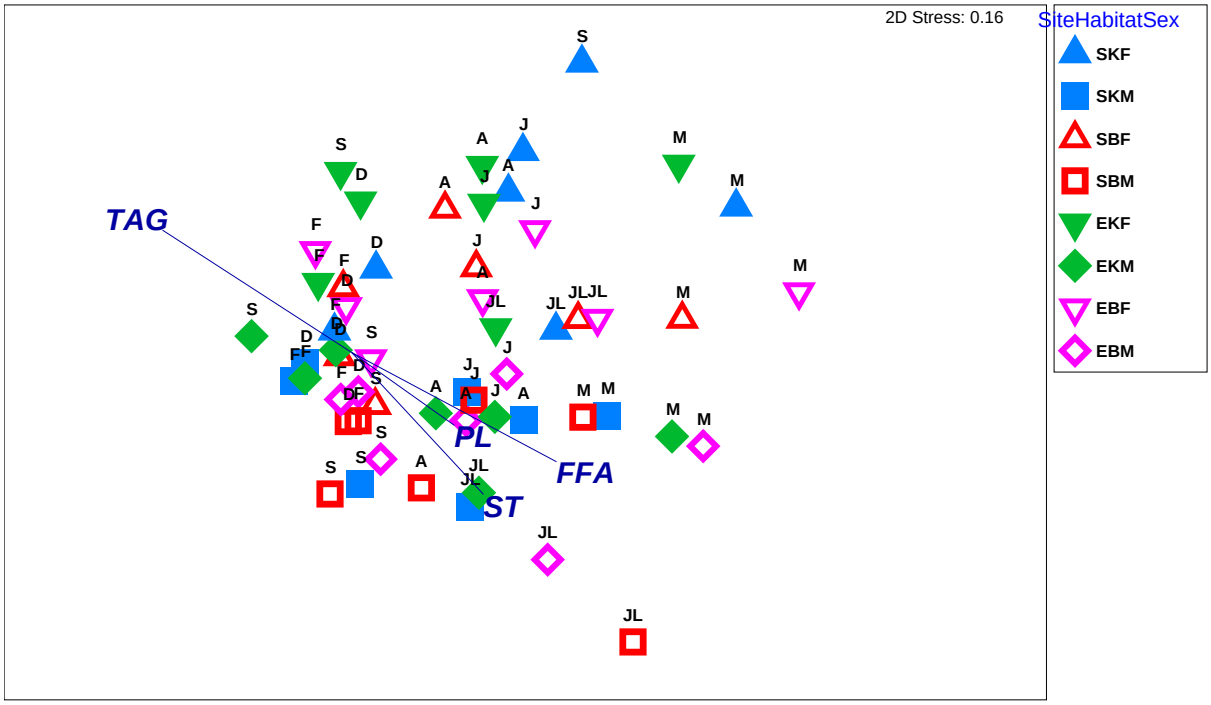

Figure 2.5. Non-metric multidimensional scaling (nMDS) of distance among the centroids for individual levels of the fixed main effects Site, Habitat and Sex in gonad classes of lipids data set. Month: seven levels identified by letters above symbols, (M) March; (A) April; (J) June; (JL) July; (S) September; (D) December; (F) February. The combined fixed effects of Site, Habitat and Sex: eight levels identified with symbols of different shapes and colour, Sloop Kelp Male (SKM), Sloop Barrens Male (SBM), Elephant Kelp Male (EKM), Elephant Barrens Male (EBM), Sloop Kelp Female (SKF), Sloop Barrens Female (SBF), Elephant Kelp Female (EKF), Elephant Barrens Female (EBF). The mapped variables Triacylglycerol, Polar Lipids, Sterol (ST) and Free Fatty Acids were chosen based on the major contributors to the dissimilarity among the groups identified by the SIMPER analysis. Plot based on a resemblance matrix of Euclidean distances of normalised data.

Table 2.11. Results of SIMPER analysis of gonad classes of lipids contributing the most to the dissimilarity between months for each Habitat (K = Kelp, B = Barren).

March_K	*Av.Value*	*Contrib%*	***March_B***	*Av.Value*	*Contrib%*
TAG	48.8	51.14	TAG	46.5	51.15
PL	34.8	34.86	PL	35	36.13
FFA	1.87	4.9	FFA	3.34	7.59
April_K	*Av.Value*	*Contrib%*	***April_B***	*Av.Value*	*Contrib%*
TAG	51.1	56.32	TAG	50.6	50.57
PL	35.1	33.52	PL	35.8	37.71
ST	9.09	5.14	ST	8.81	6.96
June_K	*Av.Value*	*Contrib%*	***June_B***	*Av.Value*	*Contrib%*
TAG	52.5	54.93	TAG	51	59.66
PL	33.3	37.31	PL	34.5	34.57
ST	9.95	5.51	ST	10	4.22
July_K	*Av.Value*	*Contrib%*	***July_B***	*Av.Value*	*Contrib%*
TAG	52.7	63.31	TAG	38.4	63.83
PL	29.7	28.21	PL	37.5	23.29
ST	12.3	7.21	FFA	4.77	7.56
Sept_K	*Av.Value*	*Contrib%*	***Sept_B***	*Av.Value*	*Contrib%*
TAG	66.4	59.47	TAG	63.8	45.95
PL	24.3	35.4	PL	25.9	41.01
ST	4.56	2.28	ST	6.79	10.81
Dec_K	*Av.Value*	*Contrib%*	***Dec_B***	*Av.Value*	*Contrib%*
TAG	61.8	50.71	TAG	57.6	52.7
PL	30.3	45.04	PL	33.7	41.5
ST	5.51	3.56	ST	6.79	5.29
Feb_K	*Av.Value*	*Contrib%*	***Feb_B***	*Av.Value*	*Contrib%*
TAG	59.9	50.92	TAG	56.4	51.25
PL	32.7	43.57	PL	35.1	43.7
ST	6.07	4.92	ST	6.47	3.98

Variables contributing to the difference between groups are shown along with the Average Value and their contribution to the dissimilarity (Cont%). Combined effect Month and Habitat, Kelp (K), Barrens (B). Triacylglycerol, Polar Lipids, Sterol (ST), Free Fatty Acids (FFA), Hydrocarbons, Wax Esters, Sterol Esters (HC_WE_SE).

Overall, eight classes of lipids were detected in the lipid extract of urchin gonad, five classes of energy lipids Triacylglycerol (TAG), Free Fatty Acids (FFA), Diacylglycerol (DAG), Hydrocarbons, Wax Esters and Sterol Esters (HC_WE_SE) and two classes of structural lipids Polar Lipids (Saran & Kumar) and Sterol (ST). TAG was the most abundant component accounting for between 50% to 60% of total lipid classes followed by PL 25% to 35% and ST 6% to 13% other classes were minor components of gonad lipids (Table 2.25, in appendix).

2.4.5 Fatty Acids

The multivariate PERMANOVA analysis of urchin gonad fatty acids profile found that seasonal trends were affected by Sex and by the location terms (Site, Habitat) with significant two-way interactions between Month and Site, Habitat and Sex (Table 2.12, $p < 0.01$). A significant three-way interaction was also present (Month x Site x Habitat, $p < 0.01$), indicating that the assimilation of different FAs in the gonad tissue varied across seasons, location and feeding habitat.

Table 2.12 Results of four-way PERMANOVA analysis of urchin's gonad Fatty Acids profile tested for the factors Month, Site, Habitat, and Sex.

Source	df	MS	Pseudo-F	P-value	Unique perms
Month	6	462.64	37.464	**0.001**	998
Site	1	123.46	9.9979	**0.001**	999
Habitat	1	327.27	26.502	**0.001**	998
Sex	1	435.52	35.268	**0.001**	996
MoxSi	6	74.846	6.0609	**0.001**	997
MoxHa	6	60.212	4.876	**0.001**	999
MoxSe	6	38.763	3.139	**0.001**	999
SixHa	1	39.144	3.1699	**0.007**	999
SixSe	1	19.951	1.6156	0.098	999
HaxSe	1	6.8057	0.55112	0.877	999
MoxSixHa	6	26.133	2.1162	**0.001**	999
MoxSixSe	6	16.416	1.3294	0.072	998
MoxHaxSe	6	16.258	1.3165	0.059	999
SixHaxSe	1	12.568	1.0178	0.396	998
MoxSixHaxSe	6	15.597	1.263	0.096	999
Res	224	12.349			
Total	279				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences (p <0.05) are indicated in bold.

Given the significant interactions, the fatty acids dataset was tested separately for male and female in a three-way PERMANOVA analysis. The test showed a significant two-way interaction for the temporal factor Month with Site and Habitat in both sexes (Table 2.13, $p < 0.01$). The multivariate analysis also showed the significant interaction of Site with Habitat ($p = 0.027$) and the three-way interaction (Month x Site x Habitat; $p < 0.01$) in the female group.

Table 2.13. Results of three-way PERMANOVA of urchin's gonad Fatty Acids profile for group tested by factors Month, Site and Habitat.

Male	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Mo	6	247.11	19.066	**0.001**	998
Si	1	60.781	4.6898	**0.002**	997
Ha	1	157.65	12.164	**0.001**	999
MoxSi	6	49.042	3.784	**0.001**	999
MoxHa	6	29.103	2.2456	**0.001**	996
SixHa	1	24.76	1.9104	0.066	997
MoxSixHa	6	18.067	1.3941	0.058	999
Res	96	12.96			
Total	123				
Female	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Mo	6	273.84	23.031	**0.001**	996
Si	1	85.631	7.2017	**0.001**	999
Ha	1	179.01	15.055	**0.001**	998
MoxSi	6	46.36	3.899	**0.001**	998
MoxHa	6	48.779	4.1024	**0.001**	997
SixHa	1	27.253	2.2921	**0.027**	999
MoxSixHa	6	25.873	2.176	**0.001**	997
Res	128	11.89			
Total	155				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

Distance among the group centroids for each individual level of the fixed main effects shows a seasonal trend and two major groupings. September, December and February were distributed to the left representing gonad at the post-spawning and recovery phase, while March, April and June were distributed to the right representing mature gonad. The July group centroids are located between the pre- and post-spawning groupings. The seasonal interaction with sexes is evidenced by the distinct separation of females (top) from males (below) at each temporal collection (Figure 2.6), and with less overlap between group centroids of Male and Female samples in the pre-spawning months (except July).

The male group centroids are more dispersed suggesting higher variability, however lack of statistical differences between the sites (Sloop and Elephant) was observed in February ($p = 0.249$) and March ($p = 0.142$) and no statistical differences were found in March, April and June for the comparison between feeding habitats (Table 2.26, PERMANOVA pairwise, $p > 0.05$, in appendix).

On the opposite, female centroids appear to group tighter at each seasonal collection except in July (Figure 2.6). Pairwise comparisons revealed always statistical differences between locations at each monthly collection, while no statistical

significance emerged from the comparison between kelp and barrens habitats in the months of April and June (Table 2.26, $p > 0.05$, in appendix)

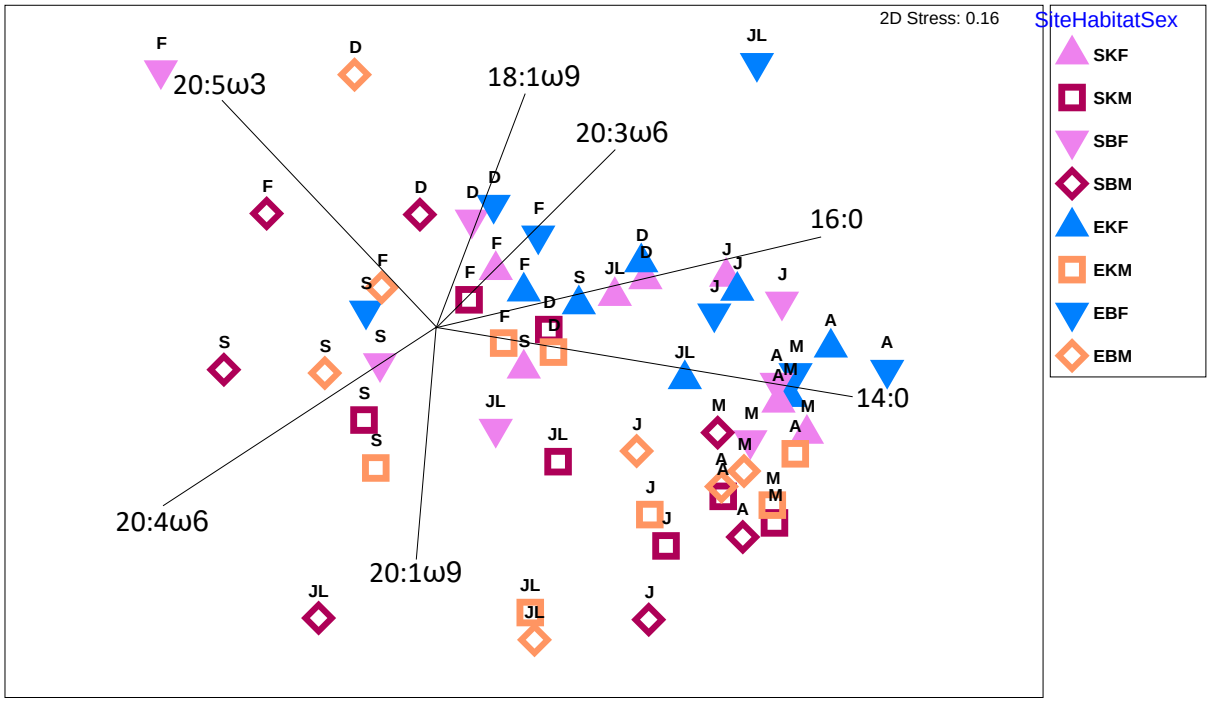

Figure 2.6. Non-metric multidimensional scaling (nMDS) of distance among the centroids for individual levels of the fixed main effects Month, Site, Habitat and Sex in gonad Fatty Acids profile data set. Month: seven levels identified by different colours (M) March; (A) April; (J) June; (JL) July; (S) September; (D) December; (F) February. The combined fixed effects of Site and Habitat: four levels identified with symbols of different shapes, Sloop Kelp (SK), Sloop Barrens (SB), Elephant Kelp (EK), Elephant Barrens (EB). Sex: two levels identified by letters above the symbols, Male (M), Female (F). The mapped variables were chosen based on the major contributors to the dissimilarity among the groups identified by the SIMPER analysis. Plot based on a resemblance matrix of Euclidean distances of normalised data.

SIMPER analysis identified the FAs with the highest contribution to the dissimilarity between sex at each sampling time (Table 2.14). The MUFAs Eicosenoic acid C20:1ω9 and the Arachidonic acid (ARA) C20:4ω6 were predominant in male gonad, while the Palmitic acid C16:0, the Oleic acid C18:ω9, the EPA C20:5:ω3 and the Homo γ Linolenic C20:3:ω6 were in greater proportion in female gonad. The C14:0 showed the highest contribution in the seasonal structure with similar proportion in both sexes (Table 2.14). These FAs determined also the distinction between Sites and Habitat since the two SFAs C14:0 and C16:0 were in greater amount in kelp assemblages at both Sites and EPA, ARA and C20:1:ω9 were more abundant in barrens habitat at Sloop and Elephant.

Table 2.14. Results of SIMPER analysis of identified Fatty acids that contributed the most to the dissimilarity between sexes at each of the seven-annual samplings.

	March_F	**March_M**			**April_F**	**April_M**	
Variable	*Av.Value*	*Av.Value*	*Contrib%*	*Variable*	*Av.Value*	*Av.Value*	*Contrib%*
C20:5ω3	14.8	12.2	27.46	C20:4ω6	7.24	10.7	20.02
C14:0	12.3	12.8	17.68	C14:0	15.2	13.8	18.76
C20:1ω9	12.3	14.2	13.59	C16:0	18	16.2	13.34
C20:4ω6	7.84	9.16	10.1	C20:5ω3	11.4	11.6	13.23
C20:3ω6	7.62	7.15	9.74	C20:3ω6	8.25	6.34	9.8
	June_F	**June_M**			**July_F**	**July_M**	
Variable	*Av.Value*	*Av.Value*	*Contrib%*	*Variable*	*Av.Value*	*Av.Value*	*Contrib%*
C14:0	14.7	16.4	33.15	C14:0	11.9	8.39	26.56
C20:5ω3	12.8	10	19.71	C20:4ω6	8.98	13.5	24.56
C20:4n6	9.3	10.5	9.76	C20:5ω3	12.8	13.1	10.11
C18:1ω9	8.53	6.21	9.45	C16:0	15.8	14.6	8.77
	Sept_F	**Sept_M**			**Dec_F**	**Dec_M**	
Variable	*Av.Value*	*Av.Value*	*Contrib%*	*Variable*	*Av.Value*	*Av.Value*	*Contrib%*
C14:0	12.8	11.5	30.49	C14:0	11.2	11.5	27.8
C20:4ω6	8.3	10.5	17.1	C20:5ω3	13.7	14	21.4
C16:0	17	15.4	15.7	C20:4ω6	8.76	9.81	14.28
C20:5ω3	10.4	9.79	12.18	C16:0	17.9	16.7	7.56
	Feb_F	**Feb_M**					
Variable	*Av.Value*	*Av.Value*	*Contrib%*				
C14:0	13.3	12.2	33.19				
C20:5ω3	11.9	11.3	22.52				
C20:4ω6	6.9	8.92	13.61				
C20:3ω6	6.35	4.94	8.49				

Variables contributing to the difference between groups are shown along with the Average Value and their contribution to the dissimilarity (Cont%). Combined effect Month and Sex, Female (F), Male (M).

Table 2.15. Fatty Acids profile of urchin gonad as percentage of total fatty acids detected.

Fatty acids	March	April	June	July	September	December	February
C14:0	12.48 ± 0.36	14.51 ± 0.42	15.62 ± 0.58	9.87 ± 0.58	12.38 ± 0.52	11.25 ± 0.46	12.80 ± 0.49
Ci15:0	0.23 ± 0.01	0.23 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.34 ± 0.01	0.21 ± 0.01	0.30 ± 0.01
C15:0	1.02 ± 0.02	1.02 ± 0.04	1.45 ± 0.05	1.13 ± 0.03	1.72 ± 0.05	1.04 ± 0.03	0.93 ± 0.02
C16:0	16.26 ± 0.20	17.09 ± 0.34	17.75 ± 0.18	15.15 ± 0.36	16.48 ± 0.37	17.46 ± 0.22	16.00 ± 0.23
C17:0	0.89 ± 0.06	0.50 ± 0.02	1.20 ± 0.14	0.42 ± 0.05	0.30 ± 0.01	0.31 ± 0.01	0.26 ± 0.01
C18:0	2.95 ± 0.13	3.44 ± 0.16	3.20 ± 0.15	3.23 ± 0.18	2.74 ± 0.12	2.90 ± 0.09	3.19 ± 0.05
C20:0	0.66 ± 0.02	0.57 ± 0.03	0.53 ± 0.01	0.65 ± 0.03	0.73 ± 0.02	0.62 ± 0.02	0.80 ± 0.02
C16:1n5	0.08 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.10 ± 0.01
C16:1n7	0.18 ± 0.01	0.20 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	0.16 ± 0.01	0.22 ± 0.01
C16:1n9	2.80 ± 0.11	2.83 ± 0.11	3.37 ± 0.13	2.88 ± 0.14	3.57 ± 0.11	3.65 ± 0.13	3.60 ± 0.07
C18:1n7	4.29 ± 0.07	4.18 ± 0.11	3.53 ± 0.05	4.69 ± 0.21	4.20 ± 0.09	4.04 ± 0.09	4.18 ± 0.07
C18:1n9	8.02 ± 0.17	8.06 ± 0.23	7.25 ± 0.25	6.92 ± 0.20	8.20 ± 0.20	8.18 ± 0.24	8.58 ± 0.16
C20:1n7	0.34 ± 0.02	0.25 ± 0.01	0.34 ± 0.01	0.52 ± 0.03	0.63 ± 0.03	0.51 ± 0.02	0.72 ± 0.02
C20:1n9	12.99 ± 0.28	13.16 ± 0.29	11.46 ± 0.24	14.33 ± 0.26	13.60 ± 0.22	11.15 ± 0.22	11.19 ± 0.18
C24:1n9	0.07 ± 0.01	0.08 ± 0.01	0.12 ± 0.01	0.21 ± 0.01	0.25 ± 0.01	0.10 ± 0.01	0.23 ± 0.01
C16:4n3	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.22 ± 0.01	0.54 ± 0.03	0.47 ± 0.03	0.56 ± 0.04
C18:4n3	0.99 ± 0.05	0.66 ± 0.04	1.07 ± 0.05	0.96 ± 0.03	1.29 ± 0.05	1.69 ± 0.06	1.60 ± 0.08
C20:4n3	0.16 ± 0.01	0.22 ± 0.01	0.34 ± 0.02	0.34 ± 0.01	0.35 ± 0.01	0.57 ± 0.02	0.56 ± 0.02
C20:5n3	13.85 ± 0.40	11.47 ± 0.37	11.26 ± 0.40	13.00 ± 0.39	10.20 ± 0.34	13.82 ± 0.40	11.66 ± 0.41
C22:5n3	0.62 ± 0.02	0.43 ± 0.02	0.63 ± 0.02	1.18 ± 0.04	1.05 ± 0.04	0.87 ± 0.03	1.13 ± 0.04
C22:6n3	1.82 ± 0.10	1.97 ± 0.10	2.82 ± 0.14	3.35 ± 0.22	3.94 ± 0.14	3.85 ± 0.17	4.21 ± 0.14
C16:2n6	0.13 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	0.10 ± 0.01	0.32 ± 0.01	0.25 ± 0.01	0.28 ± 0.02
C16:3n6	0.09 ± 0.01	0.13 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.29 ± 0.02	0.32 ± 0.03	0.41 ± 0.04
C18:2n6	0.56 ± 0.04	0.43 ± 0.01	0.52 ± 0.02	0.37 ± 0.02	0.62 ± 0.03	0.75 ± 0.04	0.75 ± 0.03
C18:3n6	0.74 ± 0.04	0.45 ± 0.02	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.19 ± 0.01	0.32 ± 0.01
C20:2n6	1.13 ± 0.03	0.90 ± 0.05	0.68 ± 0.03	0.99 ± 0.04	0.43 ± 0.02	0.50 ± 0.03	0.55 ± 0.03
C20:3n6	7.44 ± 0.26	7.29 ± 0.28	5.05 ± 0.19	5.68 ± 0.29	4.97 ± 0.18	5.19 ± 0.19	5.72 ± 0.22
C20:4n6	8.34 ± 0.25	8.97 ± 0.36	9.94 ± 0.31	11.61 ± 0.51	9.04 ± 0.36	9.12 ± 0.33	7.81 ± 0.27
C22:4n6	0.32 ± 0.01	0.24 ± 0.01	0.33 ± 0.02	0.53 ± 0.04	0.63 ± 0.02	0.44 ± 0.01	0.62 ± 0.01
C22:5n6	0.29 ± 0.01	0.16 ± 0.01	0.28 ± 0.01	0.59 ± 0.04	0.61 ± 0.04	0.35 ± 0.02	0.54 ± 0.02
ΣSFA	**34.52 ± 0.42**	**37.39 ± 0.65**	**40.05 ± 0.66**	**30.73 ± 0.77**	**34.73 ± 0.78**	**33.81 ± 0.54**	**34.30 ± 0.61**
ΣMUFA	**28.80 ± 0.23**	**28.90 ± 0.38**	**26.33 ± 0.32**	**29.81 ± 0.43**	**30.73 ± 0.38**	**27.90 ± 0.31**	**28.86 ± 0.24**
ΣPUFA	**36.67 ± 0.54**	**33.64 ± 0.62**	**33.61 ± 0.74**	**39.45 ± 0.82**	**34.62 ± 0.76**	**38.47 ± 0.61**	**36.83 ± 0.71**
Σn3	17.55 ± 0.44	14.84 ± 0.41	16.24 ± 0.48	19.02 ± 0.51	17.34 ± 0.46	21.24 ± 0.49	19.65 ± 0.58
Σn6	19.05 ± 0.30	18.69 ± 0.35	17.21 ± 0.35	20.29 ± 0.51	17.07 ± 0.36	17.02 ± 0.31	16.88 ± 0.27
n6:n3	1.11 ± 0.03	1.29 ± 0.04	1.09 ± 0.03	1.09 ± 0.03	0.99 ± 0.02	0.81 ± 0.02	0.88 ± 0.02

Data are showed with their average value and standard error. Month (n=40)

2.4.6 Isotope analysis

$\delta^{13}C$ and $\delta^{15}N$ values were similar between urchin gonad collected in contrasting habitats (kelp and barren) at each location and sampling period (Figure 2.7). The present study did not analyse $\delta^{13}C$ and $\delta^{15}N$ isotopic signature of different feed

source from contrasting habitat, hence for reference are reported isotopic values of brown and red algae present in the same habitat of *C. rodgersii*, analysed in a previous study (Guest et al 2008).

The range of urchin gonad δ^{13}C and δ^{15}N values fall between those of brown kelp which reflect availability of that type of seaweed compared to red algae. The similar isotopic signature between gonad from kelp and barrens suggest that sea urchins in both habitats feed on the same algae source but animals in the barrens rely on drift algae.

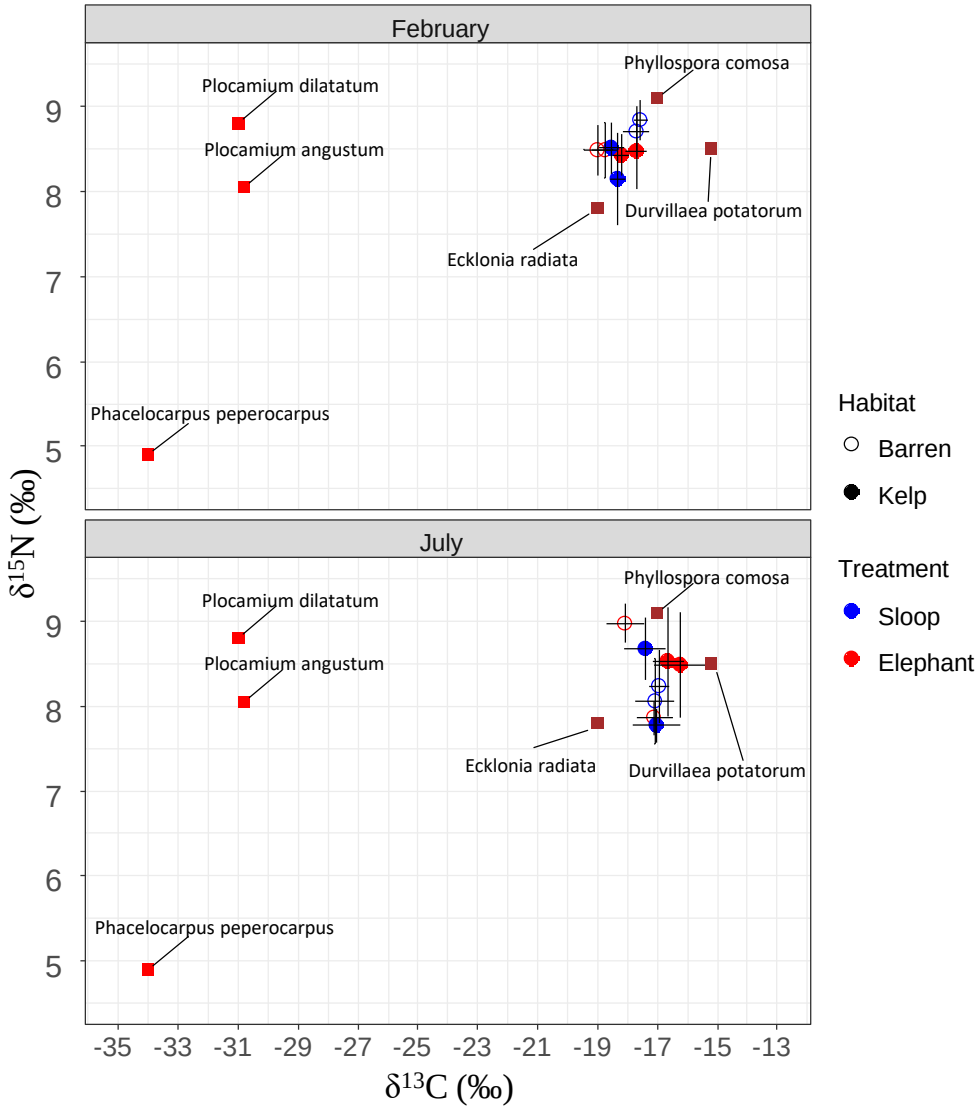

Figure 2.7. Mean (± 1 SE) carbon and nitrogen isotope values of sea urchin gonad, *Centrostephanus rodgersii* collected from two locations (Sloop and Elephant) and contrasting habitat at each location (Kelp and Barren) during February (top) and July (below). Red and brown squares represent mean (± 1 SE) carbon and nitrogen isotope values of red algae, *Phacelocarpus peperocarpus*, *Plocamium dilatatum*, *P. augustum*; and brown algae, *Durvillaea potatorum*, *Phyllospora comosa*, *Ecklonia radiata* from (Guest et al 2008) Figure 2.

2.4.7 Amino Acids

PERMANOVA analysis of urchin gonad Amino Acids profile on a reduced set of months found a significant interaction between Month and Sex (Table 2.16, $p < 0.01$).

Table 2.16. Results of four-way PERMANOVA analysis of urchin's gonad Amino Acids profile tested for the factors Month, Site, Habitat, and Sex.

Source	df	SS	MS	Pseudo-F	P-value	Unique perms
Month	3	314.03	104.68	15.996	**0.001**	998
Site	1	2.8559	2.8559	0.43643	0.793	999
Habitat	1	15.139	15.139	2.3135	0.059	999
Sex	1	96.312	96.312	14.718	**0.001**	998
MoxSi	3	17.753	5.9175	0.90429	0.522	996
MoxHa	3	24.349	8.1165	1.2403	0.248	998
MoxSe	3	123.88	41.292	6.3101	**0.001**	999
SixHa	1	9.5445	9.5445	1.4585	0.191	999
SixSe	1	2.9584	2.9584	0.45209	0.789	999
HaxSe	1	11.348	11.348	1.7342	0.151	999
MoxSixHa	3	32.404	10.801	1.6506	0.098	998
MoxSixSe	3	23.29	7.7633	1.1864	0.293	998
MoxHaxSe	3	22.083	7.361	1.1249	0.326	998
SixHaxSe	1	4.1671	4.1671	0.63679	0.625	999
MoxSixHaxSe**	2	11.766	5.8831	0.89902	0.5	999
Res	49	320.65	6.5439			
Total	79	1264				

Data normalised and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences (p <0.05) are indicated in bold.

The ordination (nMDS) plot reveals a pattern of differences between and within clusters group that highlight the temporal variation and the sex effect (Figure 2.8). The distinction between temporal groups (monthly collection) is supported by significant pairwise comparisons for all paired groups (Table 2.23, in appendix). Differences between sexes were highly significant in July ($p < 0.01$) and showed little significance in September ($p = 0.044$), no statistical differences between sexes were present in December and April (Table 2.23, in appendix) and despite the ordination plot (nMDS) showing a clear separation of the April group in two clusters, overlapping of males and females results in each subgroup (Figure 2.8).

The SIMPER analysis identified the AAs with a greater contribution to the data structure (Table 2.17). September and December were characterised by a higher proportion of Arginine (Arg) in gonad of both sexes compared to April and July. Males showed higher levels of Glycine (Gly) during April and especially July while

Females had a greater proportion of Glutamic Acid (Glx), and Threonine (Thr), (Table 2.17).

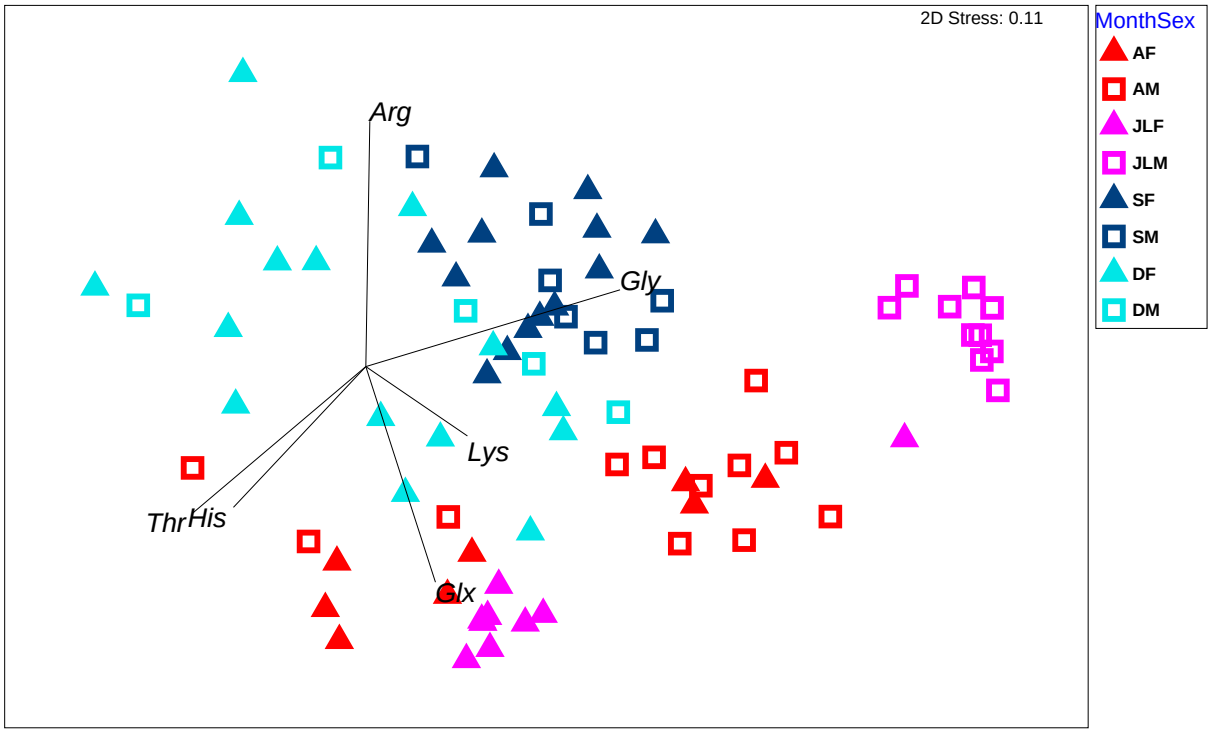

Figure 2.8. Non-metric multidimensional scaling (nMDS) for individual levels of the fixed main effects Month and Sex in gonad amino acids data set. Month: four levels identified by different colour (A) April; (JL) July; (S) September; (D) December. Sex: two levels identified with symbols of different shapes, Male (M), Female (F). The mapped variables Arginine (Arg), Glycine (Gly), Lysine (Lys), Glutamic Acid (Glx), Threonine (Thr) and Histidine (His) were chosen based on the major contributors to the dissimilarity among the groups identified by the SIMPER analysis. Plot based on a resemblance matrix of Euclidean distances of normalised data.

Table 2.17. Results of SIMPER analysis on gonad Amino Acids profile of samples collected in April, July, September and December 2018.

April_F	*Av.Value*	*Contrib%*	**April_M**	*Av.Value*	*Contrib%*
Gly	6.34	19.99	Gly	7.37	22.5
Thr	6.76	11.05	Ala	5.41	9.39
Lys	9.66	10.24	Val	6.15	6.66
July_F	*Av.Value*	*Contrib%*	**July_M**	*Av.Value*	*Contrib%*
Gly	6.08	35.89	Gly	14.9	30.22
Asx	10.5	4.82	Lys	9.43	4.88
Arg	7.37	3.45	Pro	4.56	2.57
Sept._F	*Av.Value*	*Contrib%*	**Sept._M**	*Av.Value*	*Contrib%*
Gly	7.66	24.99	Glx	10.8	14.95
Lys	7.78	12.64	Lys	9.29	12.39
Asx	9.08	7.66	Arg	12.5	11.47
Dec._F	*Av.Value*	*Contrib%*	**Dec._M**	*Av.Value*	*Contrib%*
Glx	10.2	17.99	Glx	10.3	17.21
Thr	6.53	8.48	Thr	6.14	13.43
Tyr	3.9	7.24	Gly	6.55	10.68

Variables contributing to the difference between groups are shown along with the Average Value and their contribution to the dissimilarity (Contrib%). Arginine (Arg), Glycine (Gly), Lysine (Lys), Glutamic Acid (Glx), Threonine (Thr) and Histidine (His)

Overall, Glutamic Acid (Glx), Aspartic acid (Asx), Arginine (Arg), Glycine (Gly) and Lysine (Lys) were the most abundant AAs by proportion in all the temporal sampling; a higher quantity of non-essential AAs (NEAAs) compared to the essential ones (EAAs) was also measured. NEAAs were higher in July during spawning then declined, conversely EAAs were lower in July and increased during recovery, the seasonal variation in the level of AAs is more pronounced in the male gonad rather than females suggesting that AAs are highly required during male gametogenesis and the EAAs are accumulated in male gametes. Moreover, bitter taste AAs were found in greater proportion followed by the sweet and umami taste AAs (Table 2.18). Bitter taste AAs were found in higher proportions in female gonad only during July when sweet taste AAs instead increased in male gonad. Umami taste AAs increased in female gonad and were higher than male during maturation but there was no difference between sex during gonad recovery.

Table 2.18. Amino Acids profile as a percentage of total AAs in Male and Female urchin gonad collected in April, July, September and December 2018.

AA	AM	AF	JLM	JLF	SM	SF	DM	DF
Asx	8.58 ± 0.17	9.32 ± 0.24	7.31 ± 0.07	10.51 ± 0.29	8.17 ± 0.22	9.08 ± 0.18	7.89 ± 0.36	8.28 ± 0.24
Glx	11.16 ± 0.19	11.48 ± 0.13	10.4 ± 0.08	12.61 ± 0.12	10.82 ± 0.38	10.86 ± 0.17	10.3 ± 0.74	10.22 ± 0.38
Ser	5.49 ± 0.23	6.18 ± 0.29	4.85 ± 0.03	6.04 ± 0.12	5.22 ± 0.08	5.32 ± 0.09	5.55 ± 0.27	5.91 ± 0.20
Gly	7.36 ± 0.49	6.34 ± 0.54	14.87 ± 0.37	6.07 ± 0.80	8.10 ± 0.60	7.65 ± 0.33	6.55 ± 0.58	5.92 ± 0.23
His	3.46 ± 0.16	4.22 ± 0.32	2.27 ± 0.02	4.07 ± 0.87	2.94 ± 0.08	2.73 ± 0.03	2.96 ± 0.15	2.87 ± 0.06
Arg	10.53 ± 0.56	8.57 ± 0.57	10.99 ± 0.50	7.36 ± 0.24	12.46 ± 0.33	13.27 ± 0.37	12.98 ± 1.07	12.14 ± 0.49
Thr	6.24 ± 0.18	6.75 ± 0.40	4.86 ± 0.07	6.29 ± 0.22	4.76 ± 0.07	5.17 ± 0.07	6.14 ± 0.65	6.52 ± 0.26
Ala	5.40 ± 0.31	4.89 ± 0.31	7.31 ± 0.09	5.35 ± 0.19	4.67 ± 0.15	4.31 ± 0.07	4.13 ± 0.25	4.09 ± 0.11
Pro	3.95 ± 0.12	3.98 ± 0.11	4.56 ± 0.11	4.41 ± 0.09	3.75 ± 0.09	3.76 ± 0.07	3.26 ± 0.19	3.29 ± 0.10
Tyr	2.54 ± 0.15	2.62 ± 0.12	3.06 ± 0.02	3.10 ± 0.07	3.95 ± 0.23	3.92 ± 0.10	3.93 ± 0.43	3.89 ± 0.24
Val	6.15 ± 0.26	6.32 ± 0.23	4.37 ± 0.02	5.68 ± 0.13	5.72 ± 0.16	5.70 ± 0.10	6.59 ± 0.28	6.65 ± 0.17
Met	1.94 ± 0.07	2.00 ± 0.05	2.03 ± 0.01	2.29 ± 0.07	3.01 ± 0.13	3.14 ± 0.07	2.54 ± 0.13	2.67 ± 0.08
Ile	4.77 ± 0.18	4.94 ± 0.13	3.64 ± 0.02	4.79 ± 0.12	4.95 ± 0.11	4.91 ± 0.07	5.24 ± 0.18	5.38 ± 0.12
Leu	7.67 ± 0.17	7.97 ± 0.21	6.49 ± 0.03	8.07 ± 0.14	7.60 ± 0.10	7.54 ± 0.11	8.19 ± 0.20	8.34 ± 0.14
Phe	4.36 ± 0.05	4.69 ± 0.07	3.48 ± 0.03	4.99 ± 0.12	4.51 ± 0.06	4.78 ± 0.05	4.77 ± 0.09	5.05 ± 0.13
Lys	10.32 ± 0.21	9.65 ± 0.38	9.42 ± 0.15	8.29 ± 0.15	9.29 ± 0.34	7.77 ± 0.23	8.90 ± 0.16	8.69 ± 0.16
EAA	44.94 ± 0.94	46.58 ± 0.92	36.60 ± 0.18	44.50 ± 0.88	42.82 ± 0.69	41.78 ± 0.50	45.36 ± 1.35	46.22 ± 0.59
NEAA	55.05 ± 0.94	53.41 ± 0.92	63.39 ± 0.18	55.49 ± 0.88	57.17 ± 0.69	58.21 ± 0.50	54.63 ± 1.35	53.77 ± 0.59
E_NE	0.82 ± 0.03	0.87 ± 0.03	0.57 ± 0.0	0.80 ± 0.02	0.75 ± 0.02	0.71 ± 0.01	0.83 ± 0.04	0.86 ± 0.02
Bitter	51.77 ± 0.69	51.02 ± 0.37	45.79 ± 0.46	48.68 ± 0.63	54.47 ± 1.13	53.81 ± 0.51	56.14 ± 1.60	55.73 ± 0.94
Sweet	28.47 ± 0.61	28.16 ± 0.44	36.48 ± 0.43	28.18 ± 0.85	26.52 ± 0.79	26.24 ± 0.36	25.65 ± 0.81	25.75 ± 0.49
Umami	19.74 ± 0.30	20.8 ± 0.27	17.72 ± 0.12	23.12 ± 0.38	19.00 ± 0.55	19.94 ± 0.32	18.20 ± 1.06	18.50 ± 0.61

Data are showed with their average value and standard error. Month (n=20), Sex (n=10).

2.5 Discussion

This study documented seasonal changes in the biochemical composition of *C. rodgersii* gonad in relation to sex, location and feeding habitat. Mature gonad of commercial size were found during Summer and Autumn and early winter when nutrients are stored in the phagocytes of ovaries and testes in preparation for the gametogenesis and spawning which occur throughout the Winter. During Spring gonad were small, spent of nutrients and in a period of recovery. Urchin sex was the strongest factor contributing to variation in biochemical composition within months. The variability in urchins' biometric parameters (body size, body weight, gonad weight) revealed both a seasonal and location effect but not an effect of sex. Differences in the size and total weight of animals were found between sampling sites, but not between feeding habitats. Whereas feeding habitat rather than site influenced nutrient accumulation, especially lipids in female gonad. Nutrient accumulation and composition of gonad was dependent on season and sex, with sex differences in amino acid profile between ovaries and testes evident only during gametogenesis and spawning. The pattern of development and biochemical changes in longspined sea urchin gonad was similar to patterns documented for other echinoids.

2.5.1 Habitat and Site effects on Urchin size structure

Urchin body size varied between sites and habitat, reflecting differences in harvesting pressure and food availability, respectively. Within each site, urchins collected from kelp areas presented heavier body weight and bigger gonad compared to urchins from the barrens (Figure 1). Dissimilarity in body size and body mass in sea urchins *C. rodgersii* from the Kelp and Barrens habitat in Tasmania were previously described by (Ling & Johnson 2009), who found animals of relatively large size and thick test grazing in macroalgal beds while urchins on the barren habitat were smaller. We also observed larger and heavier urchins at the Elephant Rock site compared to the Sloop Rock site. These two sites have a similar aspect and physical environment, but Elephant Rock is a low-intensity fishing area, while Sloop Rock is intensively harvested. Rather than a site related effect on mean

size, we assume differences in population size structure between the two sites is driven by different levels of commercial exploitation.

In barrens habitat, we observed a delay in gonad recovery after spawning and no differences for GSI and gonad weight between sexes, while in kelp assemblages' gonad recover faster and gonad across seasons were larger in males than females.

In this study, the highest Gonad Somatic Index (GSI) value (20%) was recorded in July in female urchins collected in kelp habitat, the average GSI of mature gonad in both sexes and habitats was around 16%. Despite the differences in gonad weight between habitat, the GSI of urchins collected in barrens was greater than expected and within the market target values of 10 - 15% (Sun & Chiang 2015). Various authors report small poor-quality gonad from sea urchins found in the barrens ground area as a consequence of limited food availability (Blount & Worthington 2002, Byrne et al 1998). In contrast, several large female urchins' presenting gonad of irregular shape, coarse granularity and brown or pale colours (i.e. low roe quality score) were found in kelp habitat, mostly at the Elephant Rock site, which is consistent with the observation that quality of the gonad declines with age (Agatsuma et al 2005).

2.5.2 Habitat effect on gonad GSI and quality.

The $\delta^{13}C$ and $\delta^{15}N$ isotopic signature of urchin gonad collected in contrasting habitats (kelp and barren) at each location were similar, confirming that urchins in barrens relied on a similar feed source as urchins in kelp habitat. If urchins on barrens were reliant on turfing algae, corallines and other ephemeral species of algae or invertebrates, we should expect the isotopic signature to differ between the two habitats. The lack of difference in isotopic signature suggests a reliable provision of drift algae from the fringe area and explains the higher-than-expected GSI of urchins in barrens. This also explains the lack of difference in gonad colour between the two habitats since urchins feeding on the same algae are able to synthesize carotenoid pigments from the same precursors. Moreover, we observed that the isotopic signature matches with that of brown algae rather than red algae, indicating a higher availability of this type of kelp or selective retention of nutrient components especially long-chained PUFAs from brown kelp.

2.5.3 Seasonal changes in macro-composition and colour of urchin gonad

The biochemical composition of *C. rodgersii* gonad showed a clear seasonal variation with a significant Sex effect. In both sexes, proteins and carbohydrates were the major components of gonad with an inverse trend during maturity and recovery. Proteins increased from March to June while carbohydrates decreased; in July (likely month of spawning), proteins and carbohydrates were at similar levels, then towards September proteins decreased and carbohydrates increased. Throughout the season, monthly protein content in testes was greater than in ovaries, whereas lipid content in males and females showed an opposite trend. The major yolk protein (MYP) is the dominant form of protein in urchin gonad and accumulates in the nutritive phagocytes of immature gonad in both male and female before gametogenesis (Unuma et al 2003). The mean protein content in wet weight across sexes varied between 9.4% from February to June (harvest period) and 6.7% from July to December (spawning and recovery). A similar protein content was measured in *P. depressus* (11.2%-7.5%) (Unuma et al 2003), *T. gratilla* (9%) (Chen et al 2013), *P. lividus* (9.2% - 11.7%) (Dincer & Cakli 2007). MYP in testis assume particular importance for the production of male gametes, while the lipids stored in the nutritive phagocytes of ovaries is transferred to the eggs during the oogenesis (Byrne et al 1998)

The accumulation of biochemical macro-components did not differ greatly between sites and habitats with the exception of lipids which were found in greater proportion in kelp beds compared to the barrens and at Elephant Rock compared to Sloop. Kelp assemblages are an important source of lipids for herbivores (Hurd et al 2014, Steneck et al 2003); in barrens, ground grazers feed upon incrusting coralline algae and drift algae and the provision of lipids is likely to be limited relative to the availability of lipids in kelp habitat.

Table 2.19. Urchin gonad proximate composition of different urchin species in comparisons with *C. rodgersii*.

Urchin species	Lipids	Proteins	Carbohydrates	Ashes	Moisture	Authors
C. rodgersii	6.78 ± 0.10	8.25 ± 0.10	8.15 ± 0.09	2.00 ± 0.02	74.80 ± 0.22	Present study
T. gratilla	3.00 ± 0.60	9.00 ± 1.80	3.20 ± 2.00	2.80 ± 0.30	82.10 ± 1.60	Chen et al, 2013
S. variolaris	4.98 ± 0.36	12.10 ± 0.41	1.63 ± 0.18	3.76 ± 0.25	77.53 ± 0.80	Archana et al, 2016
S. droebachiensis	4.55 ± 0.70	14.44 ± 1.00	3.90 ± 0.40	1.90 ± 0.30	77.60 ± 3.20	Mamelona et al, 2010
E. chloroticus	3.60 ± 0.00	8.40 ± 0.00	2.10 ± 0.00	0.90 ± 0.00	85.30 ± 0.00	Woods et al, 2008
S. purpuratus	5.25 ± 1.17	11.87 ± 1.04	5.29 ± 1.19	0.01 ± 0.00	77.58 ± 1.42	Gomez et al, 2016
P. lividus	3.05 ± 0.50	12.03 ± 1.26	2.80 ± 2.41	2.25 ± 0.24	79.87 ± 1.43	Mol et al, 2008

Data expressed as mean percentages in wet weight.

Colour is an important quality factor that determines gonad marketability (McBride et al 2004, Robinson et al 2002, Shpigel et al 2005). The desired bright yellow-orange colour is primarily derived from the accumulation of carotenoid pigments that are incorporated with the diet (Shpigel et al 2005, Shpigel et al 2006, Symonds et al 2007). The presence of different kelp assemblages or the absence of it could lead to the assumption of variability in sea urchin gonad colour between contrasting habitat given the different availability of the food source. In the present study, it appears that feeding habitat has less importance, and sex and progression through the reproductive cycle drive differences in colour across the seasons. The difference in gonad colour between sex is less pronounced in the post-spawning and recovery stage; in September gonad were small, spent of nutrients and low in moisture (Figure 2). Moisture accumulates over time in correlation with gonad development; the water content in the lumen of the phagocytes confer brightness which highlights the difference in colour intensity between ovary and testes and this becomes more evident when gonad are mature and during the gametogenesis. For instance, we report a more intense yellow in ovaries and a lighter yellow in testes with also a similar trend between the value of Luminosity and the GSI (Figure 2 and 3). The increase in GSI through the spawning seasons was matched by an increase in gonad luminosity, yellowness and whiteness. This is consistent with observations in *Paracentrotus lividus* (Cook & Kelly 2007, Rocha et al 2019) who also found a positive correlation between the colour values L* and a* and the increase of gonad yield. Comparisons of the values L*, a*, b*, with those proposed by Cook and Kelly (2009) as references for good quality gonad colour is not possible here given the different characteristics of the two urchin species. In *P. lividus* gonad colour

discriminates clearly between sexes as female presents red-orange gonad whereas male gonad are yellow-mustard in colour. These translate to low values of L* and high values of a* (redness) and b* (yellowness). *Centrostephanus rodgersii* females do not present the same red-orange pigmentation of the gonad's, values of L* are higher, while a* is lower and b*is higher. Nonetheless, in this study, the values of *C. rodgersii* gonad colour recorded between February and June are consistent with attributes of good quality (McBride et al 2004, Robinson et al 2002).

Blount et al (2017) reported that *C. rodgersii* gonad present high variability in gonad colour and texture and a corresponding perception that variable gonad colour would occur mostly in barrens area due to the scarcity of food. Here, we found that while gonad in barrens are slightly smaller than those in kelp beds, there was relative consistency across the habitats in gonad colour. However, it should be noted that the variability in gonad colour in animals collected in kelp beds is similarly to be attributed both by the presence of a variety of foliose algae which present a different concentration of carotenoid pigments and by sea urchins of different age classes, some of which are very old.

2.5.4 Seasonal and sex effects on urchin gonad Lipid composition

2.5.4.1 Lipids class

The lipid class and FA profile of *C. rodgersii* gonad showed a clear influence of seasonality and a distinction between sex demonstrating a different allocation of lipid components between male and females. Habitat linked variation in lipid and FA profiles suggest differences in food availability are also important in addition to sex and season. Hughes et al (2006) suggested that different lipid composition of *P. miliaris* gonad was influenced by the feeding habitat and sex, given that the gonad serve both as a reproductive and storage organ.

Eight classes of lipid were detected in the gonad of *C. rodgersii:* six energy lipid classes - HC, WE, SE, TAG, FFA, DAG and two structural lipid classes - ST and PL. Triacylglycerol, PL and ST were the most abundant components; with TAG being the main energy reserve found in both male and female gonad. TAG is also identified as the principal lipid component in gonad of other sea urchins i.e., *Strongylocentrotus droebachiensis*, *Psammechinus miliaris* and *Paracentrotus*

lividus (Carboni et al 2013, Cook et al 2007, Liyana-Pathirana et al 2002c, Montero-Torreiro & Garcia-Martinez 2003). In our study, the difference in lipid profile among sex was determined by the higher concentration of TAG in female gonad which was consistent in all seasonal collection. A greater energy requirement for reproductive effort in females requires higher allocation of TAG in the phagocytes that is later transferred to the eggs, as previously reported for female echinoderms (Fenaux et al 1977, Raymond et al 2007, Unuma et al 2003). Polar lipids and ST were found in greater proportion in male gonad compared to females; structural lipids are constituents of the cell membrane and as already reported for other urchins (de Vivar et al 2019, Martínez-Pita et al 2010) these lipid classes are incorporated in male gametes as a source of energy for spermatozoa motility (Kozhina et al 1978, Mita & Nakamura 1998). TAG was more abundant in urchin gonad collected in kelp beds while the structural lipids PL and ST were higher in barrens ground. In principle, urchins in barrens ground are less likely to direct the same allocation of resources to reproduction as urchins in kelp beds, and this is reflected in all urchins' body components. Nonetheless, these results in combination (GSI, lipid class etc) suggest that there is sufficient food to sustain growth and metabolic functions, but reasonable to assume that the reproductive output could be reduced compared to urchins in kelp habitat and that may affect females more than males.

2.5.4.2 Fatty acids profile

Overall, the lipid fraction of *C. rodgersii* gonad was dominated by polyunsaturated fatty acids (PUFAs, 33.5 to 39.5%) and SFAs (31 to 40%) and finally, MUFAs (26 to 31%), similar proportions were found in other temperate sea urchins (Angioni & Addis 2014, Arafa et al 2012, Carboni et al 2013, De La Cruz-García et al 2000, de Vivar et al 2019, González-Durán et al 2008, Liyana-Pathirana et al 2002a, Martinez-Pita et al 2010, Martínez-Pita et al 2010, Zárate et al 2016). Saturated Fatty Acids (SFAs) increased from September to June and drastically decreasing in July during spawning. Whereas the Monounsaturated Fatty Acids (MUFAs) remained stable across summer and autumn, slightly decreasing in Winter then peaking in Spring. Polyunsaturated Fatty Acids (PUFAs) shows greater seasonal variability, reaching their maximum in July, decreasing in September, increasing

again in December and then steadily decreasing until June (Table 17). PUFAs are allocated in gametes which are largely released during spawning. The high content of Omega 3 and 6 in July was probably driven by mature gonad still retaining most of the gametes; in September gonad are reduced in volume after the spawning season and this is reflected in the low level of PUFAs (Table 17). The peak of SFAs in June corresponds with the lowest content of MUFAs and PUFAs, while the peak of PUFAs in July corresponds with the lowest amount of SFAs (Table 17). The annual trend is also similar between males and females, but the amplitude of the fluctuation is much bigger in males.

We report a different FAs profile between ovaries and testes. Females showed higher levels of C16:0, C16:1ω9, C18:1ω9, EPA, LA, GLA, MUFAs, whereas males presented higher levels of C18:0, C20:1ω9, DHA, ARA, total Omega 6 and PUFAs. The discrimination between sexes was consistent in kelp beds and barrens ground.

To our knowledge, there are no other studies that investigated the lipids profile of *C. rodgersii* gonad and here we compare our results with those described in the literature for other echinoids species. The FAs C14:0, C16:0, C16:1ω9, C18:1ω9, LA, SDA were consistently found at higher levels in the ovaries, whereas C18:0, C20:1ω9, DHA, ARA were higher in testes of *P. lividus*, *A. lixula*, *P. miliaris* and *A. dufresnii* (Hughes et al 2005, Hughes et al 2006, Martínez-Pita et al 2010, Zárate et al 2016). In contrast, we did not find differences between sex for C14:0 and SDA. The long-chained PUFA, EPA in *P. lividus* and *P. miliaris* was greater in males while in *C. rodgersii* similarly to *A. lixula* EPA was higher in females. The different fatty acids profile between ovaries and testes can be attributed to specific metabolic requirements of the organs during the production of male and female gametes (Martínez-Pita et al 2010). As we previously reported in section (4.3.1) *C. rodgersii* testes were higher in phospholipids (structural lipids) and was showed that phospholipids have a higher level of C18:0, ARA and EPA while TAG in ovaries has a higher proportion of C14:0, C16:0 and C16:1n7 (Kozhina et al 1978). Our results appear similar to those reported for ovaries and testes in *P. lividus* and *A. lixula* (Martínez-Pita et al 2010).

Modifications in the diets from diverse habitats underpin variation in tissue FA profiles of wild populations of sea urchins (Hughes et al 2006). This study also found differences in gonad FAs composition between the feeding habitat irrespective of the sex; the C14:0, C16:0, C18:1n9, C20:1n9, GLA, SFA and Omega 6 were in higher concentration in kelp beds while C18:0, C20:0, C16:1n9, SDA, EPA, LA, ARA, PUFAs and Omega 3 were in higher levels in barrens. This is consistent with different FA profiles observed in two distinct populations of *P. lividus*, one Mediterranean and the other from the Atlantic coast of Spain, which was assumed to be a function of the food source (Martínez-Pita et al 2010). Spatial variation in the lipid and FA profiles of *P. miliaris* was also attributed to differences in the food available in each location (Hughes et al 2005).

2.5.5 Seasonal variation in urchin gonad Amino acid profiles

The amino acid composition contributes along with fatty acids to the characterization of the flavour, while also determine the nutritional value of the food items (Hall 1992). The amino acid composition of *C. rodgersii* gonad was influenced by sex and seasonal change of reproductive cycle, but unlike for other biochemical parameters, no effect of site or feeding habitat was detected. The AA profile in male and female gonad was similar during the early reproductive phase but started to diverge in April when gametogenesis commences and is more evident at the peak or the reproductive phase in July (Figure 5). Specifically, from April to July the umami taste AAs (Glx and Asx) increased in ovaries at expense of the bitter taste AAs (Arg, Leu, Ile and Tyr), and with no change in the proportion of the sweet AAs (Gly, Ser Thr, Pro). During the same period the sweet taste AAs Gly, Ala, and Pro increased in testes at expense of both bitter and umami AAs. In September and December when gonad were spent, there was no clear distinction between sex, and both ovaries and testes were low in sweet AAs and in general of EAA which are allocated to gametes (Lee & Haard 1982). During post-spawning and recovery NEAA accumulates, as well as bitter-tasting AAs particularly Arg, Leu, Ile, Met.

In this study Glx, Arg and Lys were the most abundant amino acids with Glx being more important in April and July and Arg becoming dominant in September and December. Tyrosine and Glx were found in greater proportion in the gonad of the

sea urchin *Stomopneustes variolaris* (Archana & Babu 2016). Gly also accounted for 13 - 16% of total AAs in the gonad of *S. droebachiensis* and was the dominant AA during the early stages of harvesting, whereas Tyrosine assumed more importance in later reproductive stages (Liyana-Pathirana et al 2002b). Dincer and Cakli (2007) also report that Gly represented 18 – 20% of total AAs in *P. lividus* gonad. We found that *C. rodgersii* contained 6 – 8% of Gly during gonad recovery and maturation with a peak to 15% in July male gonad during spawning.

In *S. variolaris* and *S. nudus* the percentage of essential amino acids was 32.1% (Archana & Babu 2016, Xu et al 2009). The essential (EAA) to non-essential amino acid (NEAA) ratio was 0.50 in *S. variolaris* similar to *P. lividus* 0.58 (Mol et al 2008). In this study, the percentage of EAA was 43% and the ratio EAA: NEAA was 0.78. In marine foods, EAA: NEAA ratios greater than 0.5 indicate a useful source of dietary proteins (Mamelona et al 2010).

2.6 Conclusions

This study clearly highlighted that most of the characteristics assessed associated with quality attributes were principally dependent on sea urchin seasonal variation as primary factor. The study also showed differences in colour and accumulation of nutrients between sexes. The results confirm on the basis of colour, texture and nutritional values, the best period for the commercial harvest of *C. rodgersii* is between December and June. Over this time, gonad result at the growing and mature stages of gametogenesis with the nutritive phagocytes full of stored nutrients represented by high levels of carbohydrates, proteins, essential amino acids, lipids, Omega 3 and Omega 6 PUFAs and TAG content, in both males and females. The gonad colour was found more yellow and brighter during the maturation period irrespectively of the sex and the feeding habitat. Nonetheless, from the commencement of spawning (July) through the recovery period, gonad quality traits were diminished; however, farming practices could shorten the recovery time of gonad. The seasonal patterns in biochemical profiles identified in this study could be used to formulate a feed suited for the nutritional requirement of *C. rodgersii* to achieve specific outcomes such as enhance colour and taste to meet market preference. Opposite to the general idea that urchins from barrens habitat have poor

gonad quality and should be avoided by urchin fishers, results of the study report only minor differences between gonad from barrens and kelp. Gonad's recovery was slower in barrens ground between December and February but at maturation, the yield reached commercial acceptance. The total amount of lipids in gonad from the barrens was slightly lower compared to the kelp ones but no differences in visual quality characteristics and nutritional attributes were detected so to discourage the harvesting, on the contrary, fishing urchins from the barrens is possible.

2.7 Appendix

Table 2.20. Results of two-way pairwise PERMANOVA within factor Month and for pair of levels of factor Sex in urchin gonad colour measurements.

Month	Groups	t	P-value	Unique perms
March	F, M	3.4894	**0.001**	999
April	F, M	2.4687	**0.004**	998
June	F, M	5.659	**0.001**	999
July	F, M	7.9203	**0.001**	999
Sept.	F, M	2.4233	**0.007**	999
Dec.	F, M	1.6189	0.073	999
Feb.	F, M	1.6389	0.073	998

Table 2.21. Results of two-way pairwise PERMANOVA within factor Month and for pair of levels of factor Site for Male (left) and Female (right) urchin gonad colour measurements.

Male	Site	t	P-value	Uniq perms	Female	Site	t	P-value	Uniq perms
March	S, E	2.0155	**0.036**	998	**March**	S, E	2.639	**0.006**	997
April	S, E	2.0238	**0.018**	998	April	S, E	0.564	0.694	999
June	S, E	1.9193	**0.044**	999	June	S, E	1.411	0.154	999
July	S, E	1.9625	**0.046**	999	July	S, E	1.436	0.143	999
Sept.	S, E	0.6913	0.675	998	Sept.	S, E	0.433	0.833	997
Dec.	S, E	1.0176	0.367	996	Dec.	S, E	1.748	0.071	997
Feb.	S, E	1.4211	0.132	998	Feb.	S, E	0.546	0.774	998

Table 2.22. Seasonal Proximate Composition of *C. rodgersii* urchin gonad.

Prox. Co.	March	April	June	July	Sept	Dec	Feb
Moisture%	71.52 ± 0.34	73.42 ± 0.25	76.41 ± 0.39	80.67 ± 0.23	78.06 ± 0.38	72.34 ± 0.50	71.01 ± 0.52
Lipids %	26.98 ± 0.68	26.67 ± 0.80	26.96 ± 0.73	21.73 ± 0.76	29.32 ± 1.09	28.76 ± 0.73	26.75 ± 0.57
Proteins %	35.26 ± 0.59	35.55 ± 0.49	36.38 ± 0.38	34.01 ± 0.56	24.75 ± 0.58	29.30 ± 0.53	35.08 ± 0.48
Carbs %	31.21 ± 0.63	30.71 ± 0.64	25.85 ± 0.50	30.94 ± 0.53	37.76 ± 0.77	35.56 ± 0.57	32.22 ± 0.50
Ashes %	6.53 ± 0.20	7.06 ± 0.23	10.79 ± 0.34	13.31 ± 0.26	8.15 ± 0.26	6.37 ± 0.23	5.93 ± 0.19

Table 2.23. Results of pairwise PERMANOVA for the factor Month (left) and two-way pairwise PERMANOVA within factor Month for pair of levels of factor Sex (right) on urchin gonad Amino Acids profile.

Month	t	P-value	Unique perms	Month	Sex	t	P-value	Unique perms
A, JL	3.425	**0.001**	999	April	F, M	1.188	0.244	998
A, S	3.595	**0.001**	998	**July**	F, M	6.626	**0.001**	996
A, D	3.362	**0.001**	998	**Sept.**	F, M	1.548	**0.044**	998
JL, S	4.369	**0.001**	998	Dec.	F, M	0.839	0.483	998
JL, D	5.145	**0.001**	999					
S, D	2.203	**0.008**	998					

Table 2.24. Results of two-way pairwise PERMANOVA within level of factor Month and for pair of level of factor Habitat in the lipid class data set of Male (left) and female (right) gonad.

Male	Habitat	t	P-value	Unique perms	Female	Habitat	t	P-value	Unique perms
March	K, B	0.228	0.997	999	**March**	K, B	2.109	**0.003**	998
April	K, B	1.790	**0.008**	998	**April**	K, B	1.798	**0.014**	999
June	K, B	1.291	0.134	999	June	K, B	1.417	0.088	998
July	K, B	2.836	**0.001**	998	**July**	K, B	1.637	**0.022**	999
Sept.	K, B	1.804	**0.029**	998	**Sept.**	K, B	3.213	**0.001**	999
Dec.	K, B	2.025	**0.019**	999	**Dec.**	K, B	2.131	**0.003**	997
Feb.	K, B	1.336	0.112	999	**Feb.**	K, B	1.952	**0.013**	998

Table 2.25. Composition of classes of lipids in urchin gonad lipid extract.

Lipid class	March	April	June	July	Sept.	Dec.	Feb.
SE_WE_HC	5.54 ± 0.2	3.81 ± 0.17	2.66 ± 0.11	3.78 ± 0.17	3.15 ± 0.17	1.8 ± 0.09	1.01 ± 0.06
TAG	47.68 ± 0.8	50.85 ± 0.64	51.76 ± 0.84	45.51 ± 1.88	65.07 ± 0.93	59.69 ± 0.98	58.12 ± 0.82
FFA	2.59 ± 0.29	0.62 ± 0.06	1.03 ± 0.09	3.2 ± 0.52	0.54 ± 0.11	0.04 ± 0.01	0.24 ± 0.06
ST	7.95 ± 0.19	8.95 ± 0.21	9.99 ± 0.24	13.48 ± 0.5	5.67 ± 0.31	6.15 ± 0.29	6.27 ± 0.23
DAG	1.34 ± 0.06	0.31 ± 0.05	0.66 ± 0.04	0.42 ± 0.05	0.43 ± 0.03	0.28 ± 0.03	0.41 ± 0.05
PL	34.87 ± 0.65	35.44 ± 0.52	33.86 ± 0.66	33.58 ± 1.14	25.12 ± 0.76	32 ± 0.89	33.92 ± 0.74

Table 2.26. Results of two-way pairwise PERMANOVA within level of factor Month and for pair of level of factor Site (on top) and Habitat (below) for the Fatty Acids profile data set of Male (left) and female (right) gonad.

Male	Site	t	P-value	Unique perm	Female	Site	t	P-value	Unique perm
March	S, E	1.171	0.142	999	March	S, E	1.943	**0.001**	999
April	S, E	1.770	**0.007**	999	April	S, E	1.384	**0.035**	998
June	S, E	2.840	**0.001**	999	June	S, E	2.420	**0.001**	998
July	S, E	2.903	**0.001**	998	July	S, E	2.604	**0.001**	999
Sept.	S, E	1.540	**0.037**	993	Sept.	S, E	1.824	**0.003**	998
Dec.	S, E	2.211	**0.003**	998	Dec.	S, E	1.876	**0.001**	997
Feb.	S, E	1.153	0.249	999	Feb.	S, E	2.542	**0.001**	999
Male	**Habitat**	**t**	**P-value**	**Unique perm**	**Female**	**Habitat**	**t**	**P-value**	**Unique perm**
March	K, B	1.058	0.307	997	March	K, B	1.630	**0.003**	998
April	K, B	1.217	0.128	997	April	K, B	1.252	0.078	998
June	K, B	1.028	0.412	998	June	K, B	1.344	0.074	999
July	K, B	2.153	**0.002**	999	July	K, B	2.494	**0.001**	997
Sept.	K, B	1.830	**0.028**	996	Sept.	K, B	2.942	**0.001**	999
Dec.	K, B	3.099	**0.001**	998	Dec.	K, B	3.616	**0.001**	999
Feb.	K, B	2.416	**0.001**	998	Feb.	K, B	3.322	**0.001**	998

Chapter 3 Effect of natural and formulated diet on proximate composition, colour, amino acids, fatty acids and lipid classes of sea urchin *Centrostephanus rodgersii* gonad

3.1 Abstract

Providing supplementary feed to *Centrostephanus rodgersii* was trialled as an approach to improve key quality characteristics of the gonad. Here we assessed the effect of a formulated diet and three monospecific natural diets on the somatic growth, gonadal index and biochemical composition of specimens of the sea urchin *Centrostephanus rodgersii* collected in barren grounds. After a 12-week feeding trial, the animals fed formulated diet showed a significant increase in test diameter compared to the initial control and had a considerable increase in GSI which was statistically similar to the GSI of animals collected as a wild control at the end of the trial. GSI and test diameter were significantly larger than urchins in the natural diet treatments and the start control. There was no significant effect of diet (formulated feed, natural feed) on gonad colour.

3.2 Introduction

Sea urchin gonad are very much sought after in the international market and can reach prices as high as US$131.6/kg (Sun & Chiang 2015). The market price is dependent on the qualitative measures of gonad quality (Reynolds & Wilen 2000). Many wild populations of sea urchins around the world have been overexploited due to the strong market demand (Andrew et al 2002). In other regions, however, sea urchins have become invasive and the overgrazing activity has led to the formation of barrens ground, areas of reef devoid of the canopy of algae (Flukes et al 2012). While *Centrostephanus* can persist in barrens habitat, the absence of seaweed is thought to result in a poor state of the gonad, with both low yield and low quality (Blount & Worthington 2002). These stocks of urchins on barrens area are avoided by fishers, hence the interest in adopting sea urchin aquaculture practices to enhance in a short period of time urchin roe quality of animals collected in barrens area (Pert et al 2018, Takagi et al 2017, Unuma et al 2015).

Characteristics of good urchin roe quality are yield, gonad colour, texture and flavour (Pearce et al 2002c).

The effects of different feeding regimes on the yield and quality of urchin gonad using both natural and artificial diets was investigated in *Strongylocentrotus franciscanus, Strongylocentrotus droebachiensis, Paracentrotus lividus* (McBride et al 2004, Pearce et al 2004, Shpigel et al 2005, Siikavuopio et al 2007a). The use of fresh kelp often resulted in a low gonad yield (Machiguchi et al 2012, Pearce & Robinson 2010, Walker et al 2015) while the use of formulated feed has shown that the gonad somatic index can be enhanced in a short period of time (Prato et al 2017, Rubilar et al 2016, Suckling et al 2011). The addition of dried macroalgae *Ulva sp*. in the formulated diet, however, improved gonad colour and feed intake in the sea urchin *Tripneustes gratilla* (Cyrus et al 2013), and digestible energy of formulated diet with correct carbohydrates: proteins ratio resulted determinant for gonad productions in juveniles *Lytechinus variegatus* (Taylor et al 2017). In Australia, urchin gonad quantity and quality enhancement was attempted in experimental trials with the use of formulated diets in wild barrens populations of *Heliocidaris erythrogramma* (Pert et al 2018, Senaratna et al 2005) and by reducing density or transplanting individuals of *C. rodgersii* from barrens habitat to kelp beds (Blount et al 2017).

The Longspined Sea Urchin *C. rodgersii* is an invasive species in Tasman coastal waters where it is now locally abundant on the east coast and Bass Strait Islands to the north. Its grazing activity has led to the formation of extensive barrens areas with associated loss of biodiversity and habitat of many species, some of particular commercial interest like abalone and southern rock lobsters (Ling 2008, Ling et al 2014). The Longspined Sea Urchin is commercially harvested along the east coast of Tasmania predominately in kelp habitat while a large portion of the population remains unexploited in barrens ground. In the present study, *C. rodgersii* specimens from a barren habitat were collected, kept in aquaria tanks and fed three monospecific algae diet and a manufactured feed diet during a 12-week experiment. The purpose of the study was to evaluate the yield of gonad during the spawning season and to identify the effect on roe quality by diets (colour, texture, proximate

composition, fatty acids, amino acids) compared to those of natural populations from the same barren habitat.

A study on the biochemistry of the gonads is informative over many aspects. Gonads are edible parts; they are usually eaten in many countries and their taste is determined by the overall nutrients composition which act synergically to confer a particular flavour (Komata 1964, Lourenço et al 2019, Siikavuopio et al 2007a). For instance, whether amino acids are mostly responsible for the taste, lipids are equally important to confer a creamy and smooth flavour that increase the palatability (Ning et al 2022). Some urchins gonad can present unpleasant flavour, and this can be determined either by their diet or by their own composition genetically determined (Agatsuma et al 2005, Murata et al 2001, Murata et al 2002, Murata et al 2020).

Urchin that lives in the barrens have scarcity of good quality food and this can affect the taste, however providing them formulated feed can result in good quality gonad (Agatsuma et al 2005, Pert et al 2018, Takagi et al 2019). On the contrary, if a sea urchin produces endogenous metabolites that confer a bitter, unpleasant flavour this cannot be changed replacing the diet (Murata et al 2001). Information on the biochemical composition of urchin gonad is therefore important specially to set the base of a productive fishery or aquaculture activity.

The primary objective of this study was to determine whether there were quantifiable improvements in roe quality of urchins from barrens habitat, and the underlying biochemical changes, with a view to increasing the marketability of *C. rodgersii* harvested from urchin barrens.

3.3 Material and methods

Sea urchins *C. rodgersii* were collected in summer when gonad are in the recovery phase after the spawning season. Around 200 individuals of the longspined sea urchin were hand-collected on the 11th of January 2019 from the centre of a ca. 5000 m^2 barren in Fortescue Bay, Tasman Peninsula on the south-east coast of Tasmania, at a depth of 13 meters. The urchins were held in cooler boxes filled with seawater and transported within four hours of collection to the Institute for Marine and Antarctic Studies (IMAS) aquarium facility in Taroona, Hobart. All animals were held in a 3000 litre acclimation tank and starved until no more release of

aegesta (faecal pellets) was observed (four weeks), to ensure the guts were empty at the commencement of the experiment. The tank was provided with running seawater and aeration. The acclimation period served also to monitor mortality and exclude damaged and weak animals; a high mortality rate was observed in the following three weeks after collection, and at the end of the acclimation period 30% mortality, (59 urchins of the 198 collected died) was recorded in the acclimation tank. After four weeks of starvation, *C. rodgersii* specimens were placed in 12 replicates circular 150L polyethylene aquaria and exposed to a natural photoperiod in a covered outdoor open flow circulating system. The experiment was conducted between the 1st of February and the 3rd of May 2019. The replicate aquaria were supplied with aeration and unfiltered running seawater, with water flow, maintained at 10L/min. Water pH (8.16) and temperature (14-15°C) during the experimental period.

The experiment consisted of four feeding regimes with 3 replication tanks per treatment (n=3) at a stocking density of 11 sea urchins per tank. During the trial a water flow failure caused the death all the animals in Tank 11 (Feed pellets treatment), at end of trial Feed pellets treatment consisted in two replication tank (n=2). The four feeding treatments were: (1) Brown algae (*Ecklonia radiata*); (2) Red algae (*Plocamium dilatatum*); (3) *Ulva spp*.; (4) Formulated feed pellet (Urchinomics, Japan). The animals were fed ad libitum and the tanks siphoned weekly to remove aegesta and waste food. The experimental tanks were monitored every day for the duration of the trial, sick or dead animals were removed immediately, and mortality recorded.

At the beginning of the trial (February 1st) and after the four week starvation period (week zero) nine animals randomly selected from the stocking tank (acclimation tank) were dissected for initial assessment as a Tank Control Start (TCS) treatment, to set a starting point of comparison. Three urchins from each tank were dissected and material preserved for biochemical analysis, providing nine replicate urchin gonad per treatment. At the end of the experiment (week 12) between 11 and five urchins remained alive in each experimental aquaria (Table 3.1). The remaining animals were sacrificed, biometric data recorded, and the condition of the gonad assessed. At week 12, further nine long-spined sea urchins were collected from the

wild at the same source location and habitat for the urchins used in the experiment. These urchins were used as an additional wild control (wild control end) to compare against the feeding regimes and the TCS and are referred to as WCE.

Table 3.1. Diagram representing the experimental set up

Tanks	1	2	3	4	5	**6**	7	8	9	10	**11**	12
Treatments	Eck	FP	PD	Ulv	FP	PD	Ulv	Eck	PD	Ulv	FP	Eck
Urchins at start	11	11	11	11	11	11	11	11	11	11	11	11
Urchins at end	11	11	6	9	11	5	11	10	9	11	0	10
Dead urchins	0	0	5	2	0	**6**	0	1	2	0	**11**	1
Mortality 9.8%												

Eck (*Ecklonia sp*.), FP (Feed Pellet), PD (*Plocamium sp*.), Ulv (*Ulva sp*.). Death of animals in Tank 11 (n=11), and Tank 6 (n=4) out of 6, was due to a waterflow issue and are not included in the total mortality due to harvesting/handling stress.

3.3.1 Biological parameters

Weight measurements were recorded on a digital scale to the nearest 0.5 gram and diameter measured with a Vernier calliper in millimetres to one decimal place. Urchins were then sacrificed by cracking open the test and drained of coelomic fluid for three minutes, then re-weighed to obtain the drained weight. Gonad were removed from the test with a spoon, damp-dried with blotting paper and the wet weight (WW) determined in order to calculate the gonad index. Gonad colour was determined against a Yolk Fan chart, with a colour range from a very pale yellow (value 1) to an intense orange (value 16). Colour intensity was also recorded with a colour meter (Konica Minolta Chroma Meter CR-400) with three replicate measurement on each gonad lobe and values averaged. The system used to record the colour was the international standard CIELAB that expresses colour as three values: L* for the lightness from black (0) to white (100), a* from green (−) to red (+), and b* from blue (−) to yellow (+). Hue and Chroma were then calculated from each measurement using the following formulas:

$$Hue = \arctan\left(\frac{b}{a}\right)$$

$$Chroma = (a^2 + b^2)^{0.5}$$

The rest of the gonad were sealed in labelled plastic bags and stored at -30˚C for later analysis.

3.3.2 Gonad index, moisture and ashes.

The gonad index of each sea urchin was calculated as the gonad wet weight (gonad WW) divided by the wet weight of the intact animal (total WW) and multiplied by 100: GI = (gonad WW / total WW) *100. The gonad moisture was calculated following samples lyophilization, frozen samples were freeze-dried using a freeze-dryer (Labconco FreeZone 4.5L Benchtop, United States) until constant weight. Dried tissue was weighed (DW), and the moisture content percentage (MC) was calculated by the formula:

$$MC\% = \frac{WW - DW}{WW} * 100$$

Dry matter content percentage was determined by the formula:

$$DMC\% = \frac{DW}{WW} * 100$$

Ash content was obtained after combustion of roughly 1gr. of dry tissue at 550˚C for 12 h in a muffle furnace (AOAC 942.05).

3.3.3 Biochemical analysis

Total lipid content was determined gravimetrically with a modification of the (Bligh & Dyer 1959) method. Total proteins were calculated after determination of total Nitrogen content by analysis of carbon and nitrogen stable isotopes ratio in mass spectrometry performed with an Isoprime100 mass spectrometer coupled to an Elementar vario PYRO cube elemental analyser at the Central Science Laboratory (CSL) of the University of Tasmania (UTAS). Total nitrogen was converted into proteins using a conversion factor of 5.60 as proposed by (Mariotti et al 2008). Total carbohydrate content of each sample was determined by difference summing the other constituents (lipids, proteins, ashes and water) and subtracting them from the total weight of the sample, with the following formula: 100 - (weight in grams [protein + fat + water + ash] in 100 g of sample). Lipid classes were determined by Thin Layer Chromatography (TLC-FID), Fatty Acid Methyl esters were determined by separation in Gas Chromatography Mass Spectrometry (GS-MS) and Amino Acids derivatized in Reversed Phase High Pressure Liquid Chromatography (RP-HPLC).

3.3.4 Analysis of sea urchin non-polar lipids classes via Iatroscan (TLC–FID).

The crude lipids obtained from the Bligh and Dyer (1959) extraction were chromatographed on silica gel coated Chromarods-S III and then analysed on an Iatroscan MK-5 (Iatroscan Laboratories Inc., Tokyo, Japan) analyser equipped with a flame ionization detector (FID) connected to a computer loaded with TSCAN software (Scientific Products and Equipment, Concord, ON) for data handling. A hydrogen flow rate of 160 ml per min and an airflow rate of 2000 ml per min were used in operating the FID. The scanning speed of rods was 30 s per rod. For the analysis of sea urchin non-polar class of lipids, the total lipids extracted were diluted in Dichloromethane (DCM) in order to obtain a concentration between 10 to 20 mg lipid per ml. The Chromarods were spotted with 1µl aliquot of sample. The Chromarods were then developed for 34 minutes in a chamber with the solvent systems hexane/diethyl ether/acetic acid (70:10:0.1 v/v/v) used for separation of non-polar lipids (Christie 1982). Chromarods were then dried at 80˚C for 10 min and scanned completely by Flame Ionization Detector (FID) to reveal non-polar lipids.

3.3.5 Fatty Acids Methyl Esters

The following esterification procedure was applied to the lipids extracted from the dried gonad sample material for injection in GS-MS for the determination of single fatty acids. An aliquot between 1-2 mg of lipids was taken and added to a new vial followed by the addition of 100 µl of C19 FFA surrogate standard solution for quantitative analysis (500 ug/mL C19 FFA in DCM). The solvent was evaporated to dryness under a stream of nitrogen gas. Approximately 3 mL of Methylation reagent 10:1:1 (v/v/v) MeOH:DCM: conc HCL was added to the evaporated lipid. Vials were heated for 1 hour at 80˚C and once at room temperature, mixed with 1 ml of deionised water. To the methylation reaction mixture was added ca. 1.5 mL 4:1 (v/v) Hexane: DCM. After the separation of phases, the upper phase containing the fatty acid methyl esters (FAME) was transferred to a GC vial and evaporated to dryness under nitrogen and made to a final volume 1000 µL of DCM with C23 FAME internal standard solution for quantitative analysis (50 ug/mL C23 FAME in DCM).

FAMEs were analysed using a Varian CP-3800 gas chromatograph coupled to a Bruker 300MS triple quadrupole mass spectrometer (Bruker Corporation, Massachusetts, USA) fitted with an Agilent DB-5MS column (30 m x 0.25 mm; 0.25 um film thickness). Helium was used as the carrier gas with a flow rate of 1.2 mL / min. The Injector was set to 290^{O}C and the Transfer line to 310^{O}C. Samples were injected at 50 ^{O}C in splitless mode. After 1 min, the oven was programmed from 50 to 150 ^{O}C at 30 ^{O}C / min, then at 2 ^{O}C / min to 250 ^{O}C and finally 5 ^{O}C / min to 300 ^{O}C, which was held for 15 min. Electron ionisation mass spectra were recorded in full scan mode over the range (m/z) 40 to 400. Individual FAMEs were identified based on comparison of retention times and MS data of laboratory standard FAMEs, together with the use of the NIST2017 Mass Spectral Library (National Institute of Standards and Technology, USA). Data were processed using MS Workstation Version 7.

3.3.6 Amino Acids

Dried samples were sent for the determination of standard amino acids analysis (Auspep Pty Ltd, Victoria, Australia). Amino acids were analysed using the Waters Pico-tag methodology (Heinrikson & Meredith 1984). A mass of sample (3-4mg) was accurately weighed, to which was added a known amount of hydroxyproline standard. The sample/standard mix was hydrolysed in 6N HCl at 1C for 60 minutes in vacuo. Samples were then neutralised with triethylamine (neutralizes residual HCl) and dried by lyophilization. The hydrolysed amino acids were derivatised with phenyl-isothiocyanate (PITC). Samples were then run on RP-HPLC against a laboratory standard amino acid reference mix. Recoveries of the individual amino acids were measured by comparison to the reference mix and quantitated with the HyPro standard. The technique provided the determination of 16 amino acids and Cysteine, Tryptophan were not detected. Aspartic acid and Asparagine were not separable and are reported together as Asx also Glutamic acid and Glutamine were not separable and are reported together as Glx.

3.3.7 Statistical analysis.

3.3.7.1 Biochemical composition of diets

In order to understand the effect of any changes in gonad composition relative to the two control treatments (TCS, WCE), it is necessary to quantify differences if any, in the nutritional makeup of the feed used in each of the diet treatments. Two statistical approaches were used to examine the effect of diet on biochemical composition of urchin gonad: a multivariate analysis to look at broader shifts in biochemical composition and univariate analyses to examine specific components within each of the biochemical composition groups.

3.3.7.2 Multivariate analyses of feed components and of the diet experiment

The biochemical composition (proximate composition, amino acids, fatty acids, lipid class) of the feed material were compared across the four diet groups using multidimensional scaling (nMDS) on Euclidean distance (Clarke & Gorley 2015) of untransformed data. Similarity percentage (SIMPER) analysis was conducted on the groups to determine the contribution of each variable to the average dissimilarity among groups (Clarke & Gorley 2015).

PERMANOVA was used to test for statistical differences among treatments in multidimensional space (Anderson et al 2008), using the % dry weight of each component as the dependent variable with the following primary hypothesis.

H0: diets do not differ in their multivariate biochemical composition

H1: diets differ in their multivariate biochemical composition.

Planned contrasts were used rather than ad hoc pairwise comparisons, as there were specific comparisons of interest identified *a priori*:

Contrast 1 - FP vs Algae: there is difference in the biochemical composition between the formulated feed pellet and the natural algae diet.

Contrast 2 - ECK vs PD: there is difference in the biochemical composition between the brown algae (*Ecklonia sp.*) and the red algae (*Plocamium sp.*). Both algae are present in the same habitat, are normally part of *C. rodgersii* diet, are considered to offer different nutritional benefits to the urchin diet.

Contrast 3 - ULV vs ECK, PD: there is difference in the biochemical composition between the green algae (*Ulva sp.*) and brown and red algae. *Ulva sp*. is usually not present within *C. rodgersii* feeding ground and may have different characteristics from the brown and red algae.

For proximate analysis, Fatty acids and lipid classes three subsamples from each diet were analysed. The amino acids profile was determined in a single sample per diet.

The statistical analysis of urchin gonad composition used a similar strategy to that outlined for the diets above. The experimental design consisted of a single fixed main effect of feeding regime, comprising six treatments: four feeding regime treatments (formulated feed, brown algae, red algae, green algae), and two control treatments (TCS, WCE). For each data group (biometrical measurements, proximate composition, Amino Acids, Fatty Acid and lipid class), variables were analysed as follows:

PERMANOVA+ was applied to test the main hypotheses of interest

H0: there is no effect of treatment on biochemical properties of urchin gonad

H1: there is an effect of treatment on biochemical properties of urchin gonad

A series of *a priori* planned contrasts were used to explore specific comparisons among treatments:

TCS vs Feeding treatments: there is an effect of treatments between feeding regimes and initial control on gonad biochemical properties.

WCE vs Feeding treatments: there is an effect of treatments between feeding regimes and wild control on gonad biochemical properties.

FP treatment vs Algae treatments: there is an effect of treatments between formulated feed and natural diet on gonad biochemical properties.

FP treatment vs WCE: there is an effect of treatments between formulated feed and wild control on gonad biochemical properties.

PD treatment vs ECK treatment: there is an effect of treatments between red and brown algae on gonad biochemical properties.

Ordination via nMDS on Euclidean distance was used to detect underlying structure in the datasets and linear relationships to specific biological variables. Similarity percentage (SIMPER) analysis was conducted on the treatment groups to determine the contribution of each variable to the average dissimilarity among groups (Clarke & Gorley 2015).

3.3.7.3 Univariate analysis of specific feed components and effects of diet on biochemical composition

The one-way analysis of variance was used to investigate statistically significant differences between variables mean values of diets biochemical composition. Tukey's HSD multiple comparison test was used to evaluate pairwise means of diets variables when significant differences ($P>0.05$) were determined. We used Pairwise Tukey test in addition to PERMANOVA contrasts to test the differences between all pairs of groups which in PERMANOVA was reduced by the limited degrees of freedom that determine the number of contrasts.

A one-way analysis of variance was used to investigate differences between mean values of treatments and controls on the sea urchin biometrical measurements and gonad biochemical composition. Tukey's HSD multiple comparison test was used to evaluate pairwise means of diets and controls when significant differences ($P>0.05$) were determined. Univariate ANOVA tests were performed on a select set of variables to enable comparison with previously published work. The univariate analyses were performed using SPSS (IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp.)

3.4 Results

3.4.1 Univariate comparison of feed component nutrition

3.4.1.1 Proximate Composition of feed treatments

The proximate composition of formulated feed was significantly different from natural feed for all components (Table 3.2, Tukey's test, α=0.05). FP was lower in carbohydrate, higher in protein and lipids than natural algae, while ash weight was intermediate between the natural algal treatments. *Plocamium spp.* was much higher in protein than the other natural feed treatments, and lower in ash weight (Table 3.2).

Table 3.2. Proximate composition of diets fed to *C. rodgersii* during the experimental trial.

	FP	**ECK**	**PD**	**ULV**
Ashes%	16.10 ± 0.03b	21.84 ± 0.02d	12.34 ± 0.08a	17.58 ± 0.16c
Lipids%	5.91 ± 0.25c	3.86 ± 0.12b	3.71 ± 0.08b	2.92 ± 0.01a
Proteins%	18.08 ± 0.02d	6.08 ± 0.01b	10.28 ± 0.13c	5.58 ± 0.13a
Carbs%	59.91 ± 0.24a	68.22 ± 0.10b	73.67 ± 0.13c	73.93 ± 0.19c

Data presented as mean values with standard error of the mean (n = 3). Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05. Data are expressed as percentages in dry weight basis. FP (Feed pellets); ECK (*Ecklonia radiata*); PD (*Plocamium dilatatum*); ULV (*Ulva australis*).

3.4.1.2 Amino Acids composition of feed treatments

The amino acid content of diets was analysed on a single sample per each diet source (Table 3.3), for that reason the data showed in the table are purely informative of the potential AA content of each diet type but the lack of within diet variability prevents any reasonable comparisons between diets, for which further analyses are needed.

Table 3.3. Amino Acids composition (%) of diets fed to *C. rodgersii* during the experimental trial

	%	FP	ECK	PD	ULV
EAA	*His*	2.84	2.56	2.75	1.68
	Ile	4.07	4.33	5.16	4.50
	Leu	7.63	8.01	7.10	7.11
	Lys	4.94	5.07	7.76	3.96
	Met	1.24	2.22	1.03	1.35
	Phe	4.57	5.18	6.01	5.37
	Thr	3.96	6.68	6.91	8.61
	Val	4.87	5.99	6.57	7.20
NEAA	*Arg*	15.73	4.60	6.68	5.35
	Asx	4.57	10.54	9.16	9.86
	Ser	4.95	5.38	7.13	6.83
	Glx	18.01	14.08	12.73	12.23
	Pro	8.16	6.08	6.00	4.78
	Gly	6.45	6.32	6.13	7.46
	Ala	6.42	10.29	7.27	12.07
	Tyr	1.61	2.69	1.60	1.64
EAA		34.11	40.03	43.28	39.78
NEAA		65.89	59.97	56.72	60.22
E:NE ratio		0.52	0.67	0.76	0.66
Bitter AA		47.49	40.64	44.66	38.16
Sweet AA		29.93	34.74	33.45	39.76
Umami AA		22.58	24.62	21.90	22.09

Values represent a single measurement (n=1). Data are expressed as percentage of total amino acids detected in dry weight basis. FP (Feed pellets); ECK (*Ecklonia radiata*); PD (*Plocamium dilatatum*); ULV (*Ulva australis*); EAA (Essential Amino Acids); NEAA (Non-Essential Amino Acids).

3.4.1.3 Fatty acids composition of feed treatments

The fatty acids composition (as percentage of total fatty acids) varied considerably across the four diet treatments. The major Saturated FAs (C16:0, C14:0, C18:0) were significantly more abundant in PD than the other natural feeds and FP (Table 3.4).

Mean MUFAs content differed significantly across all feeding treatments (Table 3.4), with ULV having the highest level of MUFAs, and PD the lowest (Table 3.4). Unlike SFA, component MUFAs did not reflect the overall trend of total SFs across feed groups. For example, the dominant MUFA C18:1ω9 was significantly higher in FP, while C20:1ω9 was higher in PD. Levels of C18:1ω7 were between 5 and 10 times higher in ULV than the other three feed treatments.

The mean PUFAs content also showed variability in the four feeding regimes (Table 3.4); ECK presented the highest level of PUFAs among the natural diets and PD the lowest, FP showed an important proportion of these lipids with levels intermediate between ECK and ULV. The brown algae ECK differed from the other feeding regimes for the remarkable quantity of the Omega 6 FAs C20:4ω6 ARA and the Omega 3 FAs C18:4ω3 SDA and C20:5ω3 EPA. Levels of the important C18:2ω6 LA and C22:6ω3 DHA were instead significantly higher in FP. Noticeably, ULV presented a better ratio between Omega 6 and Omega 3 (Table 3.4).

Table 3.4. Fatty acids composition (%) of diets fed to *C. rodgersii* during the experimental trial.

% DM	**FP**	**Eck**	**PD**	**Ulv**	**F**	**p-value**
C14:0	2.07 ± 0.09b	2.87 ± 0.07b	8.55 ± 0.38c	0.15 ± 0.001a	F(3,8) =322.038;	p =<.001;
Ci15:0	0.01 ± 0.001a	0.01 ± 0.001a	0.89 ± 0.08b	0.03 ± 0.03a	F(3,8) =96.616;	p =<.001;
C15:0	0.13 ± 0.01a	0.18 ± 0.001a	1.25 ± 0.06b	0.11 ± 0.02a	F(3,8) =315.279;	p =<.001;
C16:0	21.10 ± 0.49a	19.03 ± 0.13a	53.86 ± 1.62c	32.40 ± 1.31b	F(3,8) =221.808;	p =<.001;
C17:0	0.10 ± 0.001a	0.11 ± 0.01a	1.20 ± 0.03c	0.44 ± 0.01b	F(3,8) =1075.307;	p =<.001;
C18:0	3.42 ± 0.08c	0.95 ± 0.01b	3.71 ± 0.07d	0.26 ± 0.01a	F(3,8) =954.229;	p =<.001;
C20:0	0.31 ± 0.03b	1.35 ± 0.01d	0.78 ± 0.03c	0.06 ± 0.001a	F(3,8) =412.266;	p =<.001;
ΣSFA	27.14 ± 0.44a	24.49 ± 0.18a	70.23 ± 1.9c	33.45 ± 1.31b	F(3,8) =323.667;	p =<.001;
C16:1ω9	0.78 ± 0.02a	1.59 ± 0.1b	0.89 ± 0.01a	2.52 ± 0.09c	F(3,8) =127.434;	p =<.001;
C16:1ω7	0.04 ± 0.001a	0.28 ± 0.01c	0.1 ± 0.01b	0.03 ± 0.001a	F(3,8) =156.667;	p =<.001;
C16:1ω5	0 ± 0a	0.13 ± 0.01b	0.02 ± 0.01a	0.56 ± 0.02c	F(3,8) =234.83;	p =<.001;
C18:1ω9	27.37 ± 0.21d	18.98 ± 0.28b	4.39 ± 0.41a	23.83 ± 1.01c	F(3,8) =309.758;	p =<.001;
C18:1ω7	0.97 ± 0.04b	0.05 ± 0.01a	2.13 ± 0.22c	12.25 ± 0.32d	F(3,8) =814.313;	p =<.001;
C20:1ω9	1.77 ± 0.08b	0.32 ± 0.01a	6.17 ± 0.55c	0.13 ± 0.01a	F(3,8) =103.076;	p =<.001;
C20:1ω7	0.06 ± 0.01a	0.01 ± 0.01a	0.73 ± 0.12b	0.14 ± 0.01a	F(3,8) =28.008;	p =<.001;
C24:1ω9	0.11 ± 0.01b	0.1 ± 0.01b	0.09 ± 0.01ab	0.06 ± 0.01a	F(3,8) =10.569;	p =0.004;
ΣMUFA	31.14 ± 0.31c	21.47 ± 0.21b	14.52 ± 1.06a	39.54 ± 1.39d	F(3,8) =149.755;	p =<.001;
C16:2ω6	0.01 ± 0.01a	0.02 ± 0.01a	0.02 ± 0.01a	0.49 ± 0.02b	F(3,8) =487.135;	p =<.001;
C16:3ω6	0.07 ± 0.01ab	0.15 ± 0.01bc	0.23 ± 0.06c	0 ± 0a	F(3,8) =9.56;	p =0.005;
C18:2ω6	25.92 ± 0.11d	3.44 ± 0.01b	1.69 ± 0.22a	7.39 ± 0.12c	F(3,8) =6519.843;	p =<.001;
C18:3ω6	0.31 ± 0.01d	0.27 ± 0.02b	0.02 ± 0.01a	0.54 ± 0.02c	F(3,8) =153.359;	p =<.001;
C20:2ω6	0.34 ± 0.01b	0.08 ± 0.01a	0.45 ± 0.07b	0.03 ± 0.01a	F(3,8) =30.533;	p =<.001;
C20:3ω6	0.22 ± 0.01a	0.81 ± 0.01a	6.55 ± 0.46b	0.37 ± 0.02a	F(3,8) =172.533;	p =<.001;
C20:4ω6	2.67 ± 0.01c	25.61 ± 0.33d	1.74 ± 0.11b	0.53 ± 0.02a	F(3,8) =4550.641;	p =<.001;
C22:4ω6	0.03 ± 0.01a	0.11 ± 0.01a	0.26 ± 0.03b	0.47 ± 0.01c	F(3,8) =127.095;	p =<.001;
C22:5ω6	0.16 ± 0.01b	0.19 ± 0.01b	0.07 ± 0.01a	0.17 ± 0.01b	F(3,8) =39.486;	p =<.001;
Other PUFA	0.07 ± 0.01a	0.01 ± 0.01a	1.19 ± 0.12b	0 ± 0a	F(3,8) =94.714;	p =<.001;
C16:4ω3	0.04 ± 0.01a	0.06 ± 0.01a	0.19 ± 0.02a	1.23 ± 0.11b	F(3,8) =100.274;	p =<.001;
C18:4ω3	0.96 ± 0.02a	14.32 ± 0.23c	0.41 ± 0.06a	10.89 ± 0.68b	F(3,8) =371.38;	p =<.001;
C20:4ω3	0.24 ± 0.01a	0.79 ± 0.01a	0.54 ± 0.15ab	0.79 ± 0.06b	F(3,8) =10.151;	p =0.004;
C20:5ω3	4.46 ± 0.04c	7.98 ± 0.11d	1.58 ± 0.11b	0.71 ± 0.03a	F(3,8) =1526.766;	p =<.001;
C22:5ω3	0.5 ± 0.01a	0.17 ± 0.01a	0.06 ± 0.01a	3.19 ± 0.25b	F(3,8) =135.714;	p =<.001;
C22:6ω3	5.68 ± 0.17b	0.1 ± 0.01a	0.18 ± 0.01a	0.14 ± 0.01a	F(3,8) =991.689;	p =<.001;
ΣPUFA	41.72 ± 0.14c	54.03 ± 0.31d	15.23 ± 0.86a	27.00 ± 0.41b	F(3,8) =1110.914;	p =<.001;
Σω3	11.9 ± 0.22b	23.34 ± 0.21d	2.97 ± 0.26a	16.98 ± 0.44c	F(3,8) =1283.792;	p =<.001;
Σω6	29.75 ± 0.07b	30.68 ± 0.31b	11.07 ± 0.52a	10.03 ± 0.18a	F(3,8) =817.877;	p =<.001;
ω6/ω3	2.51 ± 0.05c	1.31 ± 0.01b	3.74 ± 0.17d	0.59 ± 0.02a	F(3,8) =219.93;	p =<.001;

Data presented as mean values with standard error of the mean (n = 3). Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05. Data are expressed as percentage of total fatty acids in dry weight basis. FP (Feed pellets); ECK (*Ecklonia radiata*); PD (*Plocamium dilatatum*); ULV (*Ulva australis*)

3.4.1.4 Lipid class composition of feed treatments

Three classes of lipids were detected from the lipid extract of the feeding regimes (Table 3.5). The proportion of lipids in the formulated feed was significantly different from the natural diets for each class of lipids detected. Levels of TAG and ST were significantly higher in FP, while PL was the major lipid class in all algae diets (Table 3.5).

Table 3.5. Proportion of major class of lipids in diets fed to *C. rodgersii* during the trial.

	FP	**ECK**	**PD**	**ULV**
TAG	41.36 ± 2.84b	0.82 ± 0.02a	4.74 ± 0.10a	1.79 ± 0.23a
ST	14.45 ± 0.95c	6.55 ± 0.13b	0 ± 0	1.46 ± 0.17a
PL	44.19 ± 1.92a	92.64 ± 0.14b	95.26 ± 0.10b	96.75 ± 0.39b

Data presented as mean values with standard error of the mean (n = 3). Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and $P < 0.05$. Data are expressed as percentage lipid class in the total lipids in dry weight basis. FP (Feed pellets); ECK (*Ecklonia radiata*); PD (*Plocamium dilatatum*); ULV (*Ulva australis*). TAG (Triacylglycerol), ST (Sterol), PL (Polar Lipid).

3.4.2 Biochemical composition of natural and formulated feed diets

3.4.2.1 Multivariate comparison of nutritional content of feed components

The biochemical composition of the diets provided to sea urchins during the feeding trial was significantly different in multivariate space for all major nutritional components (Proximate Analysis, Fatty Acids, Lipid Class) (Table 3.6). Results from the a priori planned contrasts were variable across nutritional groups. The formulated diet FP was significantly different from the natural algae diets for all nutritional groups. The green algae ULV was statistically different from ECK and PD only on the basis of lipid classes, while no significant differences between nutritional composition of diets were found in the contrast between ECK and ULV (Table 3.6).

Table 3.6. Results of PERMANOVA analysis with contrasts of diets biochemical components (Proximate analysis, Fatty Acids and Lipid Classes), data untransformed, Euclidean distance.

Proximate analysis					
Source	df	SS	MS	F	P
Main Effect - Diets	3	842.83	280.94	1378.5	**0.001**
FP v Algae	1	602.91	602.91	24.96	**0.003**
Eck v PD	1	206.53	206.53	1964.8	0.098
Ulv v Eck, PD	1	546.38	546.38	1806.2	0.111
Res	7	1.6305	0.20381		
Total	10	844.46			
Fatty Acids					
Source	df	SS	MS	F	P
Main Effect - Diets	3	6871.2	2290.4	394.1	**0.001**
FP v Algae	1	2175	2175	4.5861	**0.016**
Eck v PD	1	3526.9	3526.9	594.62	0.101
Ulv v Eck, PD	1	1182.1	1182.1	207.67	0.089
Res	7	46.494	5.8118		
Total	10	6917.7			
Lipid class					
Source	df	SS	MS	F	P
Main Effect - Diets	3	16.284	5.4281	74.205	**0.001**
FP v Algae	1	15.079	15.079	84.204	**0.006**
Eck v PD	1	0.12658	0.12658	4.2789	0.102
Ulv v Eck, PD	1	9.7367	9.7367	83.422	0.104
Res	7	0.5852	0.07315		
Total	10	16.869			

Data untransformed and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

The PERMANOVA analyses are clearly visible in multivariate space (nMDS). Diet groups were visually distinct from each other on the basis of all nutritional components (proximate analysis, lipid class, fatty acids and amino acids). For Proximate composition, Amino Acids and Lipid class, there was a clear separation between the formulated feed and the natural feed treatments (Figure 3.1, 3.2, 3.3), whereas fatty acids had distinct clusters based on feed type, there was no evidence of a major cluster of natural feed treatments (Figure 3.4).

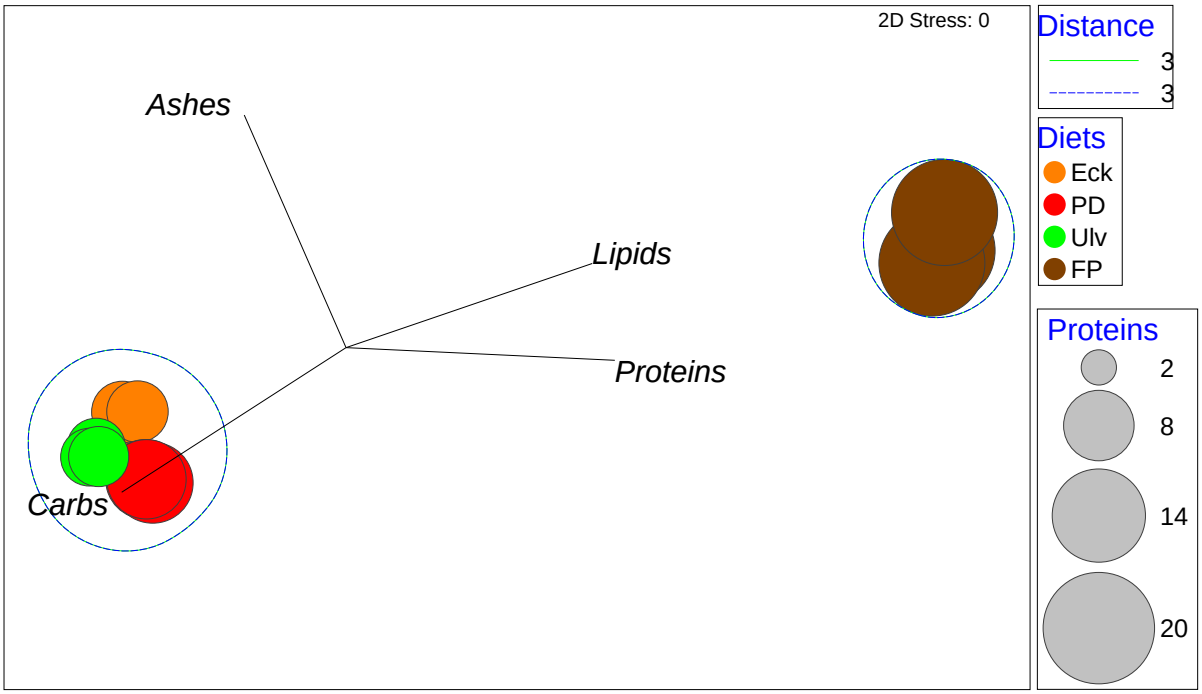

Figure 3.1. Non-metric multidimensional scaling (nMDS) plots of Euclidian distance showing percentage components of Proximate analysis of ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva australis*) and FP (feed pellets), (n=3), fed to *C. rodgersii*.

SIMPER analysis on the identified clusters evidences the importance of carbohydrates in the algae group while protein and lipids in FP contribute greatly to the separation between the formulated feed and algae (Figure 3.1, Table 3.7).

Table 3.7. SIMPER results of diets proximate composition.

Treatment	**Prox. Co.**	**Mean %**	**Cont (%)**
FP	Lipids	5.91	52.84
	Carb	59.9	45.91
ECK	Lipids	3.86	60.05
	Carb	68.2	37.65
PD	Carb	73.7	37.93
	Proteins	10.3	34.16
ULV	Carb	73.9	45.35
	Ashes	17.6	32.78
	FP	**Algae**	
Variables	**Av.Value**	**Av.Value**	**Cont%**
Carbs	59.9	71.9	51.49
Proteins	18.1	7.31	40.84

FP (feed pellet); Eck (*Ecklonia sp.*); PD *(Plocamium sp.*); Ulv (*Ulva sp.*); Carbs (Carbohydrates).

The amino acids composition of diets represented in the nMDS plot reveal a higher similarity between ECK and ULV and separation from PD but again clustering between algae and dissimilarity from FP (Figure 3.2).

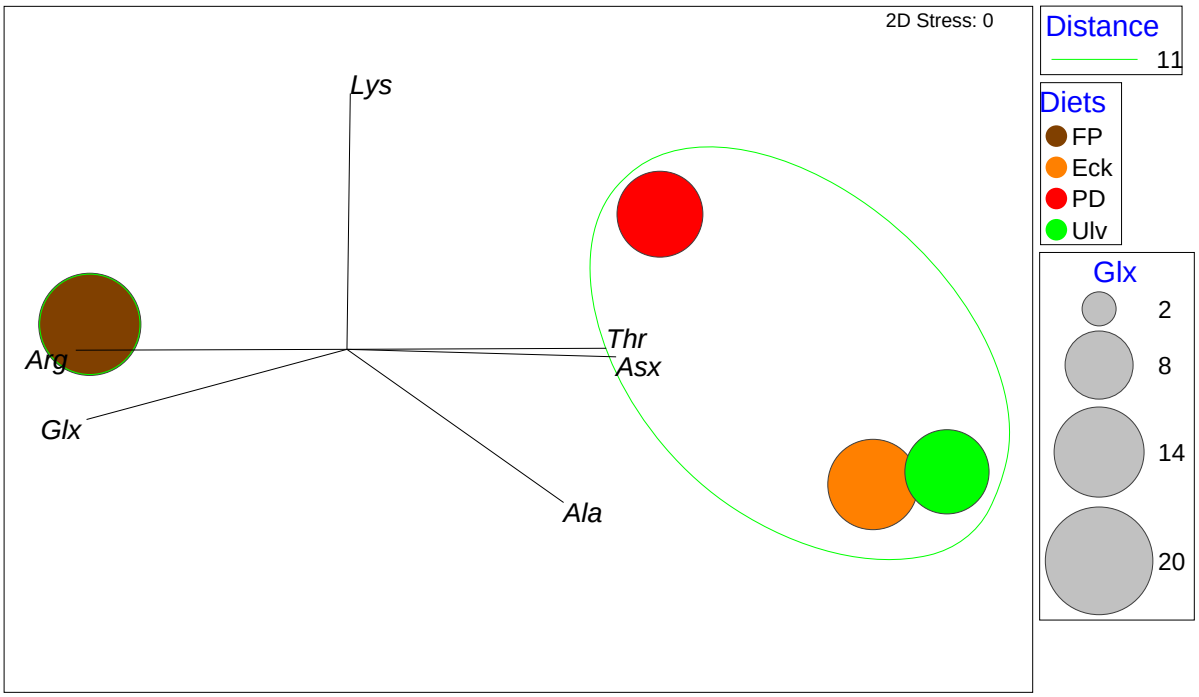

Figure 3.2. Non-metric multidimensional scaling (nMDS) plots of Euclidian distance showing percentage components of Amino Acids of ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva australis*) and FP (feed pellets), (n=3), fed to *C. rodgersii*.

The representation in the multidimensional space (MDS) of diets class of lipids composition shows clear separation between the formulated feed pellet and the three algal diets (Figure 3.3). The SIMPER analysis shows TAG as major component of lipids in FP that determined the dissimilarity between the groups (Table 3.8); PL was the principal component that contributed to the similarity between algae however, in ULV diet TAG contribute to a minor separation between the green algae and ECK and PD (Figure 3.3, Table 3.8).

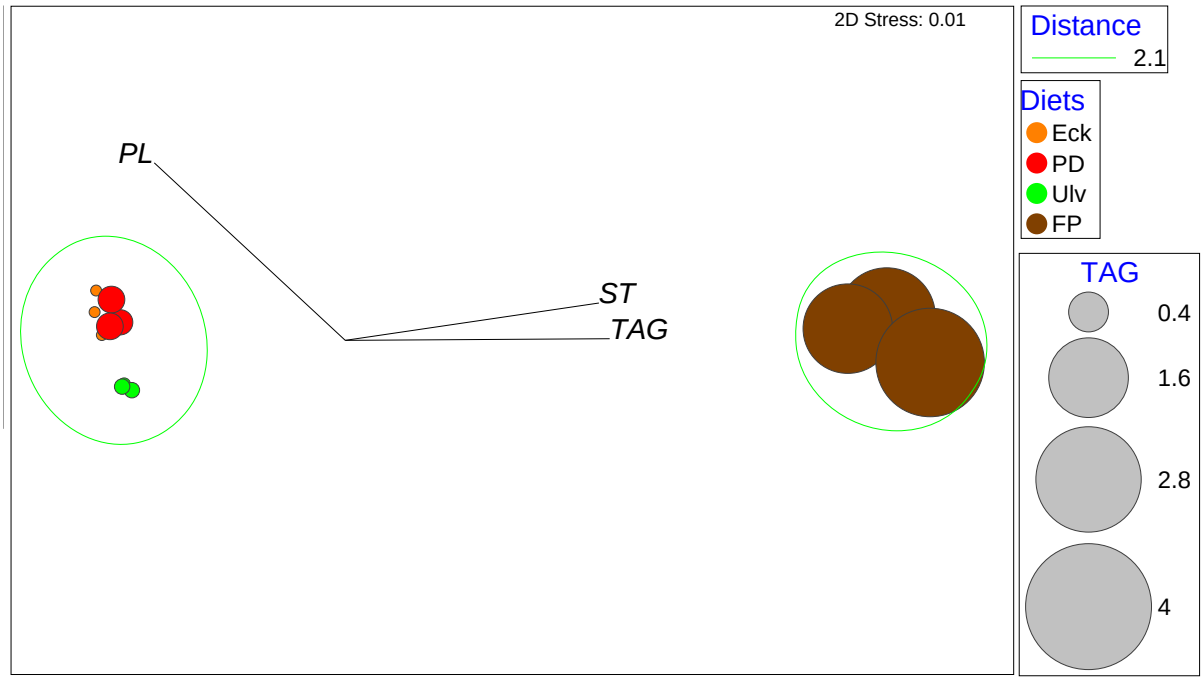

Figure 3.3. Non-metric multidimensional scaling (nMDS) plots of Euclidian distance showing percentage components of Lipid Classes of ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva australis*) and FP (feed pellets), (n=3), fed to *C. rodgersii*.

Table 3.8. SIMPER results of diets Class of lipids composition

Treatment	L.Class	Mean %	Cont (%)
FP	TAG	2.46	98.74
ECK	PL	3.57	99.76
PD	PL	3.53	99.25
ULV	PL	2.82	86.11
	TAG	0.0533	11.11

FP (feed pellet); Eck (*Ecklonia sp.*); PD (*Plocamium sp.*); Ulv (*Ulva sp.*); Carbs (Carbohydrates); TAG (Triacylglycerol); ST (Sterols); PL (Polar Lipids).

The MDS plot of diets fatty acids revealed a different lipids composition between the algae sources as each algae type clustered separated from the others (Figure 3.4). SIMPER analysis identified FAs contributing to the differences between the diets. The highest proportion of saturated 14:0 and 16:0 and monounsaturated 20:1ω9 greatly contributed to the separation of the red algae PD from the other algae and FP. The brown algae ECK was characterized by a great proportion of the important polyunsaturated fatty acids ARA 20:4ω6, EPA 20:5ω3 and SDA 18:4ω3. FP and ULV grouped together, the identified FAs Oleic acid 18:1ω9, Linoleic acid 18:2ω6, DHA 22:6ω3 contributed to the separation of this group to the red and brown algae (Table 3.9).

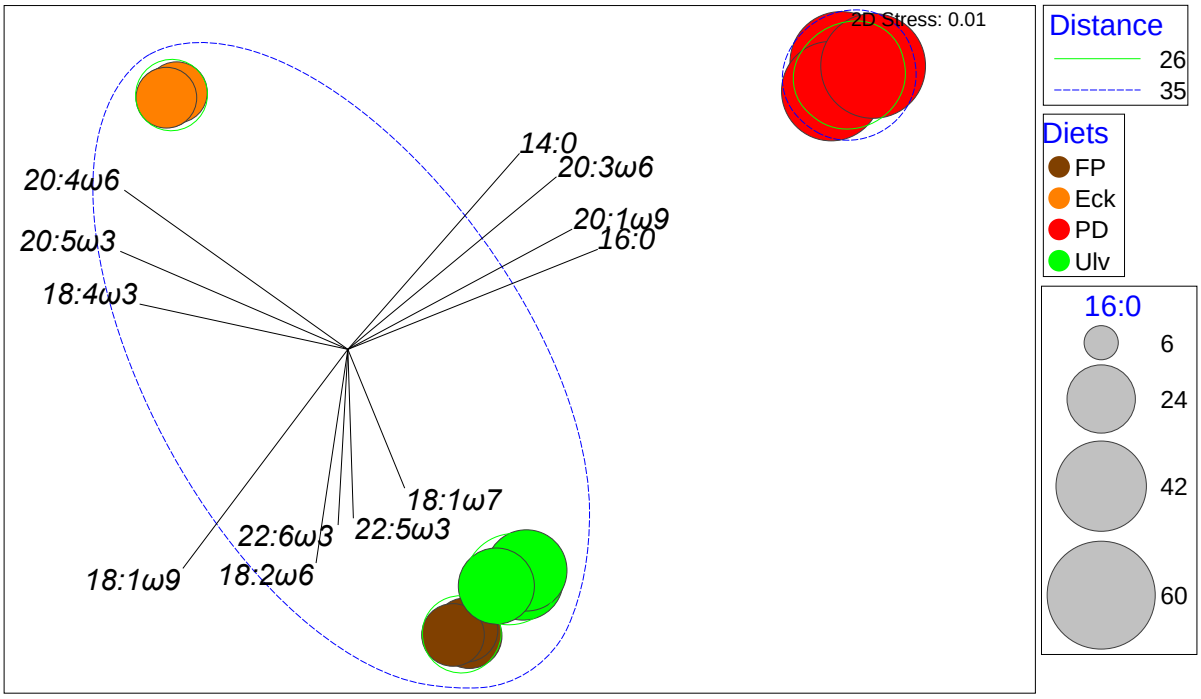

Figure 3.4. Non-metric multidimensional scaling (nMDS) plots of Euclidian distance showing percentage components of Fatty Acids of ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva australis*) and FP (feed pellets), (n=3), fed to *C. rodgersii*.

Table 3.9. SIMPER results of diets fatty acids profile

Treatment	FA	Mean FA%	Cont (%)	Treatment	FA	Mean FA%	Cont (%)
FP	16:0	21.1	67.61	PD	16:0	53.9	71.46
	18:1ω9	27.4	12.49		20:1ω9	6.17	8.19
	22:6ω3	5.68	8.74		20:3ω6	6.56	5.89
	18:2ω6	25.9	2.9		18:1ω9	4.39	4.6
	14:0	2.07	2.49		14:0	8.55	4.04
ECK	20:4ω6	25.6	38.59	ULV	16:0	32.4	50.06
	18:1ω9	19	27.09		18:1ω9	23.8	29.84
	18:4ω3	14.3	18.34		18:4ω3	10.9	13.77
	16:0	19	5.57		18:1ω7	12.3	3.09
	20:5ω3	7.98	4.54		22:5ω3	3.19	1.88

FP (feed pellet); Eck (*Ecklonia sp.*); PD (*Plocamium sp.*); Ulv (*Ulva sp.*).

3.4.3 Univariate Examination of specific components of interest of urchin gonad fed different diet treatments.

3.4.3.1 Somatic growth, gonad somatic index and gonad colour.

During the course of the experiment, mortality was sporadic and randomly distributed between the replicate treatment tanks and attributed to animals unable to recover from collection injury or stress post collection. At the end of the trial 21% mortality was recorded (28 urchins of the 132 distributed in the replica tanks).

The somatic growth of urchins fed formulated feed increased significantly compared to TCS treatment, but no statistical differences are observed in total weight, test diameter and drain weight between feeding treatments and WCE. The gonad wet weight of TCS recorded 14.8gr and no considerable increase in wet weight was observed in gonad from animals fed natural diets after the three month experiment while the treatment fed artificial diet FP had a clear significant increase in gonad wet weight (33.50gr) statistically similar to that of the wild control at the end of the trial (WCE 33.80gr), (Table 3.10, Tukey's test, α=0.05). The gonad somatic index (GSI) reflects the gonad wet weight with FP being statistically similar to WCE (10.80gr and 10.85gr) respectively and both statistically higher than TCS and the algae diets. Averages values for gonad colour coordinates CIE L* a* b* Hue and Chroma did not vary significantly among the feeding treatments and the two controls and no statistical differences were observed whatsoever (Table 3.10, Tukey's test, α=0.05).

Table 3.10. Biological parameters of sea urchins and colour coordinates of gonad among different diet treatments and wild controls.

	TCS	WCE	FP	ECK	PD	ULV
Tot. W.	265.80±7.36a	313.77±16.85ab	335.1±35.00b	278.44±21.81ab	323.07±24.82b	304.18±23.73ab
Dia.	78.44±1.42a	86.77±1.44ab	92.00±3.87b	84.33±2.74ab	88.33±2.83ab	87±2.33ab
Drain W.	202.11±5.35a	249±13.31b	240.90±18.4b	206.66±10.64ab	247.82±16.41b	225.16±14.79b
G. W.W.	14.8±0.81a	33.82±2.01b	33.50±2.07b	14.62±0.96a	16.6±2.44a	18.8±2.29a
G. D.W.	6.13±0.32ab	10.79±0.61c	8.60±0.54b	4.35±0.29a	4.3±0.67a	4.83±0.62a
GSI	5.55±0.21a	10.85±0.57b	10.80±1.49b	5.41±0.38a	5.09±0.58a	6.29±0.6a
L*	54.85±0.45	57.46±0.44	54.26±1.5	53.99±1.18	53.4±1.71	54.13±1.08
a*	9.39±0.27	9.43±0.48	8.89±0.46	9.87±0.39	9.42±0.4	9.05±0.34
b*	50.33±0.76	51.51±1.67	47.53±1.49	51.28±1.98	44.89±2.31	48.51±1.61
Hue	79.44±0.21	79.66±0.26	79.36±0.54	79.09±0.24	77.84±1.02	79.39±0.37
Chroma	51.2±0.78	52.37±1.72	48.37±1.5	52.23±2.01	45.91±2.23	49.35±1.62

Means that do not share a letter indicate significant different among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05. Values in parenthesis represent standard error of the mean (n = 9). W.W. (wet weight), D.W. (dry weight), GSI (gonad somatic index). TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp*.).

3.4.3.2 Urchin gonad proximate composition.

All treatment diets and WCE recorded a significant increase in gonad moisture and proteins compared to TCS (Table 3.11, Tukey's test, α=0.05). The lipid content decreased in all treatments compared to TCS with statistical difference only between FP and TCS. The carbohydrate level did not change significantly between TCS and treatments diets but was significantly lower in ULV treatment gonad compared to WCE and FP. No statistical differences were found between treatments for ash content; however, the lowest content was found in WCE while the highest in ULV.

Table 3.11. Proximate composition of sea urchin gonad in tank and wild controls and feeding treatments.

	TCS	WCE	FP	ECK	PD	ULV
G. Moist%	58.48±0.96a	68±0.44b	74.57±0.78c	70.19±0.8b	74.13±0.89c	74.49±1.02c
Ashes%	8.66±0.72	7.58±0.31	8.23±0.60	9.08±0.90	9.15±0.65	9.85±0.80
Lipids%	29.58±1.65b	24.84±0.90ab	23.22±0.94a	26.33±1.06ab	26.27±1.42ab	27.10±1.59ab
Proteins%	29.70±1.01a	34.62±0.81b	35.55±0.95b	33.97±0.88b	35.50±0.65b	34.86±1.03b
Carbs%	32.09±0.81ab	32.97±0.98b	33.00±1.54b	30.60±0.85ab	29.08±0.72ab	28.19±1.06a

Means that do not share a letter indicate significant different among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05. Values in parenthesis represent standard error of the mean (n = 9). G. Moist (gonad moisture), Carbs (carbohydrates). TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp*.).

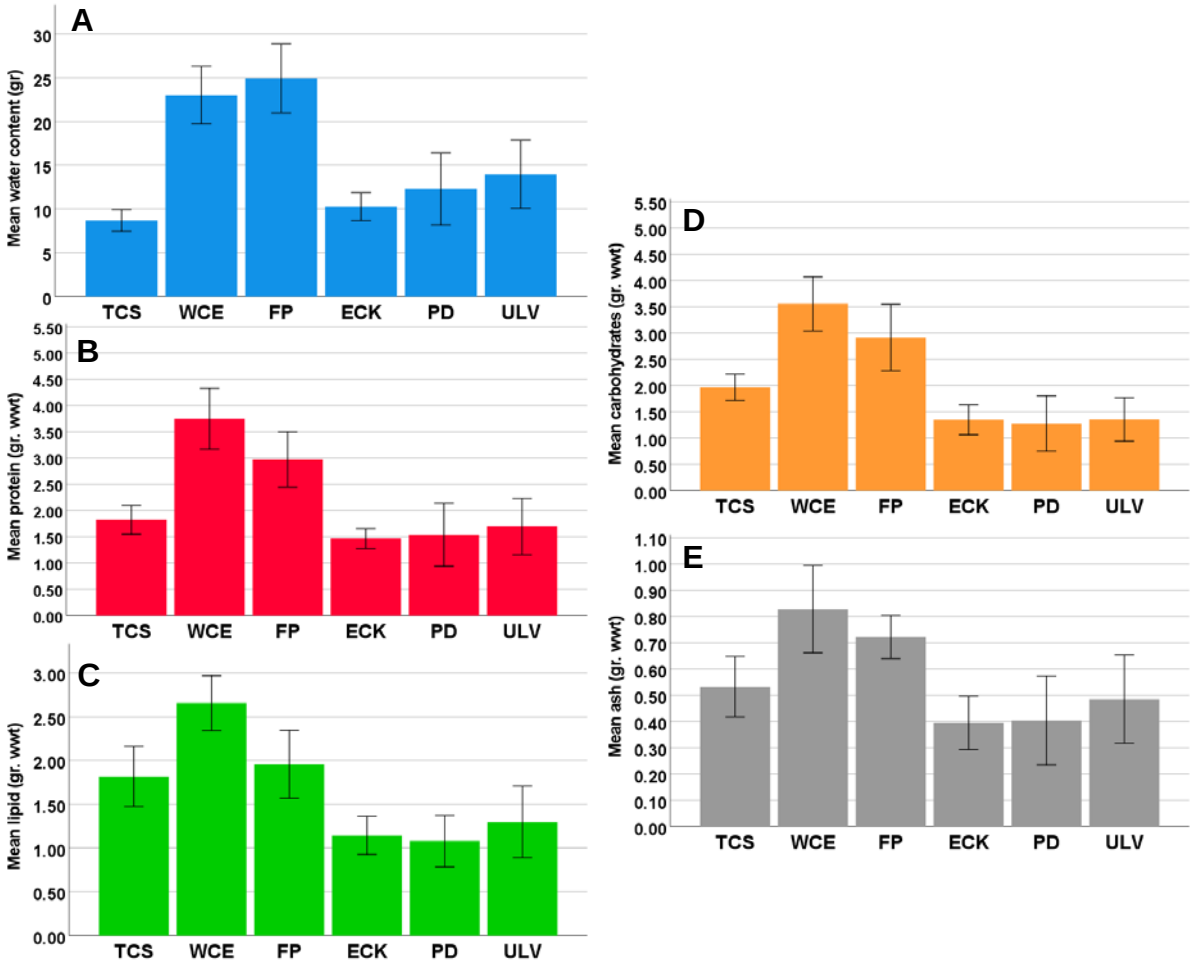

Figure 3.5. Bar charts of gonad proximate composition of sea urchin treatment diet and controls on wet weight basis. Gonad water content (A); Protein content (B); Lipid content (C); Carbohydrate content (D); Ash content (E). TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

3.4.3.3 Urchin gonad Amino Acids profile.

All treatment diets and WCE recorded a significant increase in NEAA and a decrease in EAA compared to TCS (Table 3.12, Tukey's test, α=0.05). Glutamic acids (Glx), Arginine, Aspartic acids (Asx) and Lysine were the most abundant amino acids in all treatments, followed by Leucine Glycine Threonine and Valine. Algae treatments and WCE show significant changes from the TCS for the amino acids Glx, Gly, Ala, Val, Ile, Leu, Lys. Gonad of urchins fed ECK present the highest proportion of Glx, statistically similar to PD and WCE and significantly different from FP and ULV. No statistical differences were observed between feeding treatments and controls for Aspartic acid, Histidine, Tyrosine and Phenylalanine. The EAA content in urchin gonad was significantly higher in TCS

and no significant differences resulted between the other treatments; conversely, the NEAA were in significant lower amount in TCS than the other treatments all sharing statistically similar values (Table 3.12, Tukey's test, α=0.05). TCS recorded a significantly higher amount of total bitter AAs compared to WCE and the algae treatments; the total sweet AAs were in higher proportion in ULV treatment and statistically different from TCS and ECK; the total umami AAs were higher in ECK significantly higher than FP and ULV (Table 3.12, Tukey's test, α=0.05).

Table 3.12. Amino Acids content (%) of urchin gonad of tank and wild controls and feeding treatments.

	TCS	**WCE**	**FP**	**ECK**	**PD**	**ULV**
Asx	9.64 (0.19)	9.35 (0.17)	9.12 (0.40)	10.2 (0.29)	9.99 (0.22)	9.37 (0.27)
Glx	11.58 (0.19)a	12.45 (0.10)bc	11.77 (0.23)ab	12.81 (0.28)c	12.50 (0.23)bc	11.02 (0.10)a
Ser	5.20 (0.12)ab	5.27 (0.13)ab	5.58 (0.21)b	5.31 (0.12)ab	5.16 (0.18)ab	4.85 (0.06)a
Gly	4.75 (0.16)a	7.48 (0.38)b	6.56 (0.52)ab	5.87 (0.54)ab	6.01 (0.35)ab	7.52 (0.56)b
His	3.37 (0.05)	3.36 (0.04)	3.61 (0.25)	3.34 (0.20)	3.71 (0.49)	2.97 (0.16)
Arg	9.55 (0.49)a	10.57 (0.25)ab	10.75 (0.43)ab	11.11 (0.49)ab	10.90 (0.53)ab	12.36 (0.34)b
Thr	6.63 (0.27)b	6.13 (0.20)ab	5.48 (0.20)a	6.31 (0.16)ab	6.31 (0.30)ab	6.80 (0.19)b
Ala	3.42 (0.12)a	4.76 (0.23)b	4.43 (0.26)b	4.05 (0.13)ab	4.21 (0.13)ab	4.92 (0.38)b
Pro	3.97 (0.11)a	4.34 (0.09)ab	4.45 (0.13)b	4.16 (0.06)ab	4.46 (0.17)b	4.06 (0.07)ab
Tyr	2.97 (0.19)	2.70 (0.15)	2.85 (0.20)	2.72 (0.10)	2.75 (0.20)	3.34 (0.14)
Val	6.65 (0.21)b	5.49 (0.13)a	5.90 (0.26)ab	5.26 (0.14)a	5.48 (0.20)a	5.12 (0.17)a
Met	2.89 (0.08)b	2.52 (0.08)ab	2.46 (0.12)a	2.46 (0.06)a	2.46 (0.13)a	2.48 (0.10)ab
Ile	5.63 (0.20)c	4.70 (0.14)ab	5.00 (0.19)bc	4.56 (0.13)ab	4.65 (0.11)ab	4.24 (0.12)a
Leu	8.59 (0.19)c	7.43 (0.17)ab	7.85 (0.26)bc	7.34 (0.24)ab	7.22 (0.11)ab	6.79 (0.12)a
Phe	4.89 (0.07)	4.56 (0.08)	4.77 (0.19)	4.96 (0.17)	5.04 (0.12)	4.67 (0.17)
Lys	10.29 (0.35)b	8.91 (0.16)a	9.44 (0.29)ab	9.56 (0.38)ab	9.17 (0.30)ab	9.51 (0.17)ab
EAA	48.93 (0.54)b	43.10 (0.61)a	44.50 (0.86)a	43.78 (0.47)a	44.03 (0.90)a	42.57 (0.77)a
NEAA	51.07 (0.54)a	56.90 (0.61)b	55.5 (0.86)b	56.22 (0.47)b	55.97 (0.90)b	57.43 (0.77)b
E:NE	0.96 (0.02)b	0.76 (0.02)a	0.80 (0.03)a	0.78 (0.01)a	0.79 (0.03)a	0.74 (0.02)a
Bitter	54.82 (0.76)b	50.24 (0.35)a	52.61 (0.75)ab	51.30 (0.48)a	51.37 (0.59)a	51.47 (0.59)a
Sweet	23.96 (0.47)a	27.97 (0.34)bc	26.50 (0.59)bc	25.69 (0.54)ab	26.15 (0.32)abc	28.14 (0.82)c
Umami	21.22 (0.38)abc	21.79 (0.18)abc	20.89 (0.62)ab	23.00 (0.55)c	22.49 (0.30)bc	20.39 (0.34)a

Means that do not share a letter indicate significant different among treatments. Grouping Information Using the Tukey Method and $P < 0.05$. Values in parenthesis represent standard error of the mean (n = 6). TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

3.4.3.4 Urchin gonad Fatty acids methyl-esters profile.

The fatty acids composition (as percentage of total fatty acids) of urchin gonad varied between the four feeding regimes and the two control treatments (Table 3.13). The proportion of saturated fatty acids (SFAs) in the two controls TCS and WCE was similar and significantly higher than the four diet treatments, while no significant differences are observed in the amount of SFA between feeding treatments (Table 3.13, Tukey's test, α=0.05). The Palmitic acid (C16:0) and the Myristic acid (C14.0) were the major SFAs in all treatments together accounting for around 80% of the total SFAs. The proportion of the Monounsaturated fatty acids (MUFAs) was significantly lower in WCE compared to TCS and the feeding regimes (Table 3.13, Tukey's test, α=0.05). The most abundant MUFAs were the Eicosenoic acid (C20.1ω9), Oleic acid (C18:1ω9) and the (C18:1ω7). The Polyunsaturated fatty acids (PUFAs) represented the highest fraction of FAs in urchin gonad. PUFAs content at the end of the trial increased in all treatments compared to TCS (Table 3.13, Tukey's test, α=0.05). The Arachidonic acid (C20:4ω6) and the Homo γ Linolenic acid (C20:3ω6) were the major omega 6 FAs in all treatments, but no statistical differences were observed for these components. The Linoleic acid (C18:2ω6) was significantly higher in FP gonad than the other treatments which were all similar (Table 3.13, Tukey's test, α=0.05). The SDA (C18:4ω3), the EPA (C20:5ω3) and the DHA (C22:6ω3) were the most important omega 3 FAs; all treatments increased the proportion of omega 3 in gonad lipids compared to the initial control with statistical differences between PD and ULV from TCS. No statistical differences were observed for total omega 6 and for the ratio omega 6 and omega 3 among treatments (Table 3.13, Tukey's test, α=0.05).

Table 3.13. Fatty acids content (%) of urchin gonad in tank and wild controls and feeding treatments.

	TCS	**WCE**	**FP**	**ECK**	**PD**	**ULV**
C14:0	8.81 ± 0.72ab	9.64 ± 0.66b	7.10 ± 0.33ab	7.27 ± 0.63ab	6.75 ± 0.67a	6.66 ± 0.56a
Ci15:0	0.37 ± 0.07	0.28 ± 0.03	0.20 ± 0.01	0.22 ± 0.02	0.26 ± 0.04	0.30 ± 0.04
C15:0	0.80 ± 0.07	0.77 ± 0.07	0.59 ± 0.07	0.63 ± 0.08	0.67 ± 0.06	0.73 ± 0.03
C16:0	15.05 ± 0.34a	14.39 ± 0.59ab	12.76 ± 0.39bc	12.85 ± 0.36bc	11.88 ± 0.24c	12.26 ± 0.37c
C17:0	0.34 ± 0.06	0.41 ± 0.03	0.32 ± 0.03	0.32 ± 0.02	0.37 ± 0.03	0.41 ± 0.02
C18:0	3.02 ± 0.10	3.26 ± 0.15	2.85 ± 0.09	2.89 ± 0.03	3.14 ± 0.17	3.09 ± 0.16
C20:0	1.13 ± 0.09	0.78 ± 0.07	0.96 ± 0.11	1.01 ± 0.09	1.04 ± 0.10	0.98 ± 0.07
ΣSFA	29.52 ± 1.00a	29.54 ± 1.13a	24.8 ± 0.52b	25.21 ± 0.85b	24.11 ± 0.72b	24.44 ± 0.65b
C16:1ω9	2.23 ± 0.12	2.74 ± 0.16	2.13 ± 0.20	2.14 ± 0.06	2.17 ± 0.09	2.27 ± 0.15
C16:1ω7	0.45 ± 0.07a	0.57 ± 0.03ab	0.53 ± 0.04ab	0.62 ± 0.02ab	0.71 ± 0.04b	0.71 ± 0.05b
C16:1ω5	0.02 ± 0.02	0.00 ± 0.00	0.06 ± 0.02	0.00 ± 0.00	0.07 ± 0.02	0.03 ± 0.03
C18:1ω9	13.06 ± 0.42a	9.57 ± 0.59c	12.04 ± 0.74abc	12.45 ± 0.26ab	11.43 ± 0.51abc	10.24 ± 0.74bc
C18:1ω7	3.90 ± 0.11a	4.34 ± 0.04ab	4.11 ± 0.15ab	4.12 ± 0.12ab	4.16 ± 0.15ab	4.53 ± 0.04b
C20:1ω9	14.13 ± 0.97	13.34 ± 0.49	14.14 ± 0.57	14.90 ± 0.32	14.77 ± 0.29	15.70 ± 0.52
C20:1ω7	0.25 ± 0.12a	0.40 ± 0.14ab	0.56 ± 0.02ab	0.50 ± 0.11ab	0.63 ± 0.11ab	0.75 ± 0.12b
ΣMUFA	34.07 ± 0.77a	30.99 ± 0.82b	33.59 ± 1.06a	34.75 ± 0.41a	33.96 ± 0.38a	34.24 ± 0.28a
C16:2ω6	0.19 ± 0.02a	0.05 ± 0.02b	0.10 ± 0.01ab	0.14 ± 0.01ab	0.10 ± 0.00ab	0.15 ± 0.04a
C16:3ω6	0.02 ± 0.01a	0.04 ± 0.00a	0.04 ± 0.01a	0.05 ± 0.01a	0.05 ± 0.00a	0.26 ± 0.10b
C18:2ω6	2.39 ± 0.08a	1.77 ± 0.13a	3.45 ± 0.50b	2.21 ± 0.08a	2.10 ± 0.16a	1.87 ± 0.15a
C18:3ω6	0.42 ± 0.02	0.35 ± 0.00	0.40 ± 0.03	0.42 ± 0.01	0.39 ± 0.02	0.33 ± 0.01
C20:2ω6	1.67 ± 0.16	1.59 ± 0.17	1.84 ± 0.30	1.53 ± 0.17	1.44 ± 0.19	1.77 ± 0.20
C20:3ω6	4.52 ± 0.28	4.86 ± 0.32	5.04 ± 0.27	4.98 ± 0.19	5.69 ± 0.41	5.64 ± 0.21
C20:4ω6	9.96 ± 0.30	10.69 ± 0.44	10.59 ± 0.64	10.59 ± 0.60	11.50 ± 0.50	10.86 ± 0.58
C22:4ω6	0.26 ± 0.06abc	0.47 ± 0.05c	0.30 ± 0.04abc	0.34 ± 0.08bc	0.17 ± 0.04ab	0.08 ± 0.05a
C22:5ω6	0.22 ± 0.04a	0.27 ± 0.02ab	0.29 ± 0.02ab	0.22 ± 0.04a	0.41 ± 0.04b	0.42 ± 0.02b
C16:4ω3	0.60 ± 0.06a	0.24 ± 0.06b	0.35 ± 0.07ab	0.39 ± 0.06ab	0.32 ± 0.06ab	0.43 ± 0.06ab
C18:4ω3	3.35 ± 0.16a	2.24 ± 0.20b	2.74 ± 0.19ab	3.45 ± 0.18a	2.90 ± 0.28ab	2.74 ± 0.17ab
C20:4ω3	0.62 ± 0.10a	0.84 ± 0.07a	0.78 ± 0.09a	0.78 ± 0.17a	0.42 ± 0.06ab	0.06 ± 0.03b
C20:5ω3	9.22 ± 0.69a	12.82 ± 0.68b	12.14 ± 0.83ab	11.81 ± 0.58ab	13.25 ± 0.79b	13.19 ± 0.40b
C22:5ω3	0.84 ± 0.06a	0.90 ± 0.05ab	0.90 ± 0.04ab	1.08 ± 0.06b	1.02 ± 0.04ab	1.07 ± 0.03ab
C22:6ω3	2.05 ± 0.20	2.30 ± 0.14	2.59 ± 0.23	1.96 ± 0.16	2.10 ± 0.09	2.40 ± 0.16
ΣPUFA	36.40 ± 0.99a	39.46 ± 1.11ab	41.6 ± 0.82b	40.02 ± 0.91ab	41.92 ± 0.95b	41.31 ± 0.8b
Σω6	19.49 ± 0.56	20.05 ± 0.36	21.97 ± 0.79	20.38 ± 0.70	21.78 ± 0.69	21.25 ± 0.72
Σω3	16.71 ± 0.77a	19.35 ± 0.87ab	19.52 ± 0.71ab	19.50 ± 0.66ab	20.03 ± 0.85b	19.90 ± 0.23b
ω6/ω3	1.17 ± 0.06	1.04 ± 0.04	1.13 ± 0.06	1.05 ± 0.05	1.1 ± 0.06	1.06 ± 0.03

Means that do not share a letter indicate significant different among treatments. Grouping Information Using the Tukey Method and $P < 0.05$. Values in parenthesis represent standard error of the mean (n = 6). TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

3.4.3.5 Proportion of lipid classes in urchin gonad

The proportion of different lipid classes in the content of urchin gonad lipids displayed minor differences among the two controls and the feeding treatments (Table 3.14). Triacylglycerol represented the major component of urchin lipids being significantly higher in the Tank Control (TCS) compared to the Wild Control (WCE) and FP treatments; the algae treatments presented similar values for TAG (Table 3.14, Tukey's test, α=0.05). No statistical differences were observed for PL, ST and the group hydrocarbons, wax esters and sterol esters (indicated as HC_WE_SE) among the treatments. Free fatty acids (FFA) and Diacylglycerol (DAG) were not detected in TCS and in general, were minor components of gonad lipids; in ULV both FFA and DAG were found in statistically lower amount than the other treatments which otherwise showed similar values (Table 3.14, Tukey's test, α=0.05).

Table 3.14. Proportion of lipid classes in urchin gonad lipid extract among controls and feeding treatments

	TCS	**WCE**	**FP**	**ECK**	**PD**	**ULV**
HC_WE_SE	0.64±0.09	0.38±0.05	0.35±0.08	0.5±0.09	0.57±0.08	0.48±0.11
TAG	19.42±1.39a	14.67±0.52b	14.51±0.98b	15.7±0.62ab	15.45±0.77ab	15.58±1.24ab
FFA	0±0	0.27±0.12a	0.25±0.06a	0.2±0.07a	0.3±0.1a	0.03±0.03b
ST	1.57±0.2	1.47±0.08	1.51±0.1	1.56±0.25	1.76±0.14	1.92±0.21
DAG	0±0	0.25±0.06a	0.23±0.04a	0.29±0.03a	0.28±0.08a	0.08±0.03b
PL	7.93±0.73	7.79±0.61	6.38±0.34	8.09±0.58	7.91±0.7	9.01±0.74

Means that do not share a letter indicate significant different among treatments. Grouping Information Using the Tukey Method and P<0.05. Values in parenthesis represent standard error of the mean (n = 9). Results express the percentage composition of lipid classes in the total lipid extract in dry weight basis. TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp*.).

3.4.4 Multivariate comparison of the biological and biochemical characteristics of gonad from urchins provided with specific feed treatments

There were significant differences between feed treatments for the biometrical measurements of the sea urchin and all biochemical profiles of the gonad tissue (PERMANOVA, Table 3.15). No significant differences were found for the colour parameters of the gonad tissue among treatments (PERMANOVA, P = 0.144). The planned contrasts found significant differences between TCS and the experimental diets for all the components studied with the exception of the colour parameters, revealing an effect of the feeding regimes on the gonad tissue and urchin body measurements (Table 3.15). The planned contrasts WCE versus Diets and WCE versus FP displayed both significant differences for the proximate composition and fatty acids profile whereas no differences in the amino acids profile, lipid classes distribution and biometrical data were found (Table 3.15). The contrast PD versus ECK showed significant differences only for the proximate composition between these two algae treatments, while no statistical differences appeared for any of the component analysed for the contrast between the formulated feed treatment FP and the algae treatments (Table 3.15).

Table 3.15. Results of PERMANOVA analysis with contrasts of urchin treatments biological measurements and biochemical analysis.

Factor	Biometric data	Proximate analysis	Amino Acids	Fatty Acids	Lipid Class
Main Effect	F=3.122; **P=0.016**;	F=26.874; **P=0.001**;	F=4.874; **P=0.001**;	F=4.006; **P=0.001**;	F=8.359; **P=0.001**;
TCS v Diets	F=7.094; **P=0.008**;	F=80.991; **P=0.001**;	F=8.057; **P=0.001**;	F=8.865; **P=0.001**;	F=35.97; **P=0.001**;
WCE v Diets	F=1.111; P=0.317;	F=14.406; **P=0.001**;	F=1.550; P=0.190;	F=6.505; **P=0.001**;	F=2.609; P=0.085;
FP v WCE	F=1.954; P=0.167;	F=23.007; **P=0.003**;	F=1.640; P=0.192;	F=3.838; **P=0.007**;	F=3.696; P=0.062;
PD vs ECK	F=1.543; P=0.238;	F=5.380; **P=0.005**;	F=0.329; P=0.873;	F=1.643; P=0.177;	F=1.493; P=0.243;
FP v Algae	F=1.055; P=0.319;	F=1.416; P=0.231;	F=2.139; P=0.089;	F=1.582; P=0.132;	F=1.204; P=0.259;

Data untransformed and analysed on a resemblance matrix of Euclidean distance. Analyses used fixed effects, with Type III sums of squares 999 permutations of data residuals to determine significance. Significant differences ($p < 0.05$) are indicated in bold.

Analysis results represented in the multidimensional space (MDS) shows that replicate samples from the initial tank control (TCS) clustered separately from all feed treatments for all data groups except gonad colour (Figure 3.6), and except for proximate composition and fatty acids, the WCS was more aligned with the experimental feed treatments. Separate clusters are also apparent for TCS and WCE, but in both cases replicates within each cluster were widely spaced.

Among the biometrical measurements the test diameter and drain weight were the variable identified by SIMPER analysis that greatly contributed to the dissimilarity between TCS, WCE and the treatment diets (Figure 3.6, Table 3.17).

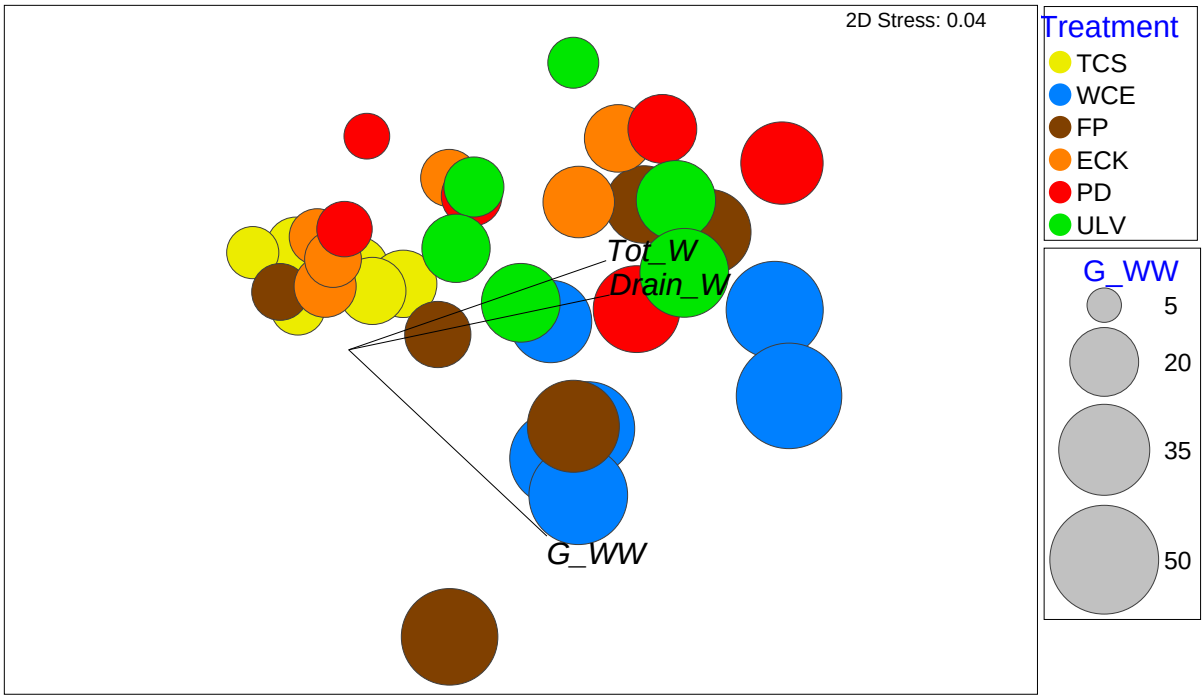

Figure 3.6. Non-metric multidimensional scaling (nMDS) plot of urchin biological measurements**.** TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp*.).

Table 3.16. Results of SIMPER analysis on biological measurement of experimental treatments.

Treatment	Bio. Val.	Av. Value%	Cont (%)	Treatment	Bio. Val.	Av. Value%	Cont (%)
TCS	Tot. Wt.	219	69.81	ECK	Tot. Wt.	269	79.45
	Drain Wt.	154	26.58		Drain Wt.	202	19.11
WCE	Tot. Wt.	325	61.46	PD	Tot. Wt.	321	65.78
	Drain Wt.	258	37.44		Drain Wt.	253	32.76
FP	Tot. Wt.	274	74.26	ULV	Tot. Wt.	336	72.84
	Drain Wt.	202	23.61		Drain Wt.	245	24.55

TCS (Tank Control Start), WCE (Wild Control End), FP (feed pellet); Eck (*Ecklonia sp*.); PD (*Plocamium sp*.); Ulv (*Ulva sp*.).

Gonad' moisture content was the most relevant component of proximate composition that characterized the grouping of diet treatments and WCE and the separation from TCS, while gonad lipid content was higher in TCS contributing to the separation of TCS to the other treatments (Figure 3.7, Table 3.17).

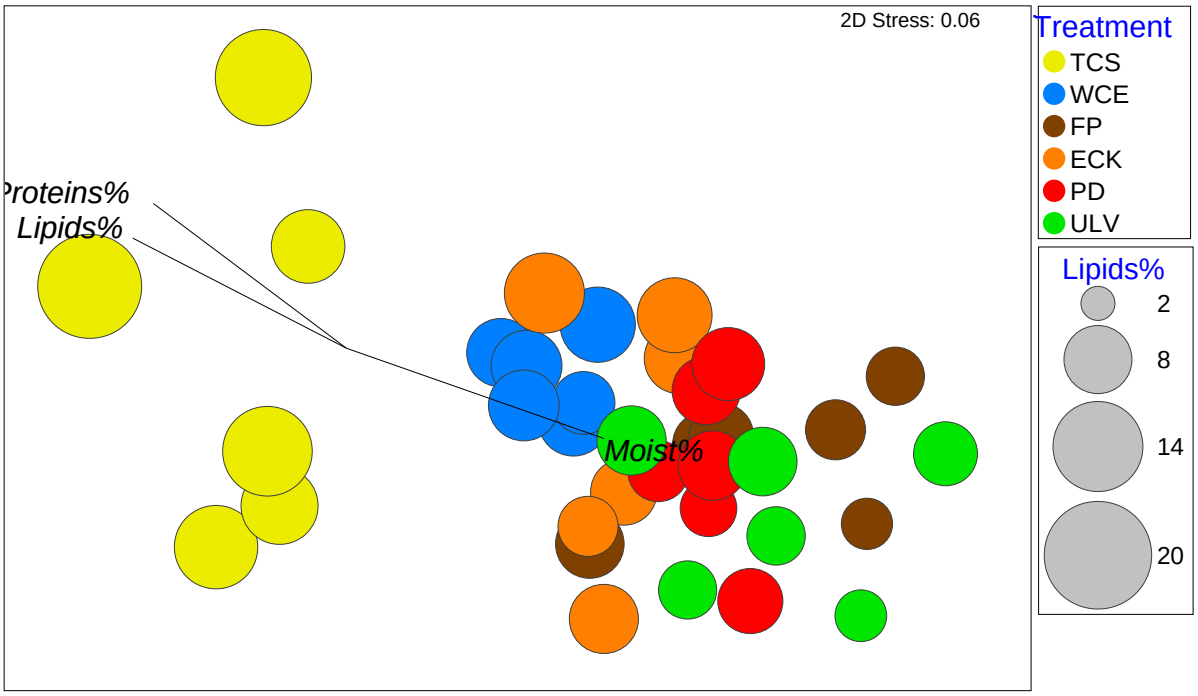

Figure 3.7. Non-metric multidimensional scaling (nMDS) plot of gonad proximate composition of experimental treatments. TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

Table 3.17 Results of SIMPER analysis on gonad Proximate Composition of experimental treatments.

Treatment	Prox. Comp %	Av. Value%	Cont (%)	Treatment	Prox. Comp %	Av. Value%	Cont (%)
TCS	Lipid	12.4	48.01	ECK	Moist	70.3	45.11
	Moist	59.2	35.86		Lipid	7.98	28.01
	Protein	12.1	8.88		Protein	10	13.61
WCE	Moist	68.3	42.19	PD	Moist	73.3	36.96
	Lipid	7.83	29.3		Lipid	6.95	34.13
	Protein	11.4	16.21		Protein	9.4	15.2
FP	Moist	74.8	69.62	ULV	Moist	75.5	66.26
	Lipid	6.11	12.05		Lipid	6.18	12.48
	Protein	9.16	9.37		Protein	8.83	10.52

TCS (Tank Control Start), WCE (Wild Control End), FP (feed pellet); Eck (*Ecklonia sp.*); PD (*Plocamium sp.*); Ulv (*Ulva sp.*).

SIMPER reveal also that the amino acid Arginine and Lysine contribute to the separation between TCS and WCE, FP and ECK being TCS lower in Arginine and higher in Lysine, while the treatments PD and ULV differ from TCS for the AAs Glycine and Valine in ULV and for Histidine and Glycine in PD (Figure 3.8, Table 3.18).

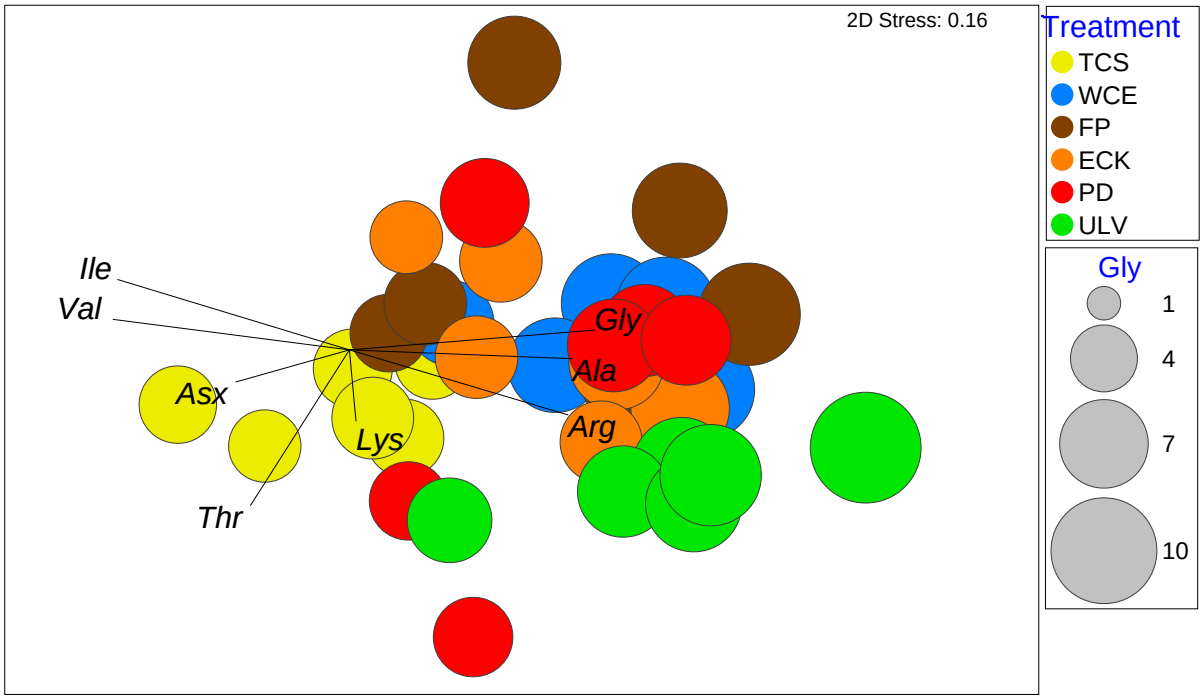

Figure 3.8. Non-metric multidimensional scaling (nMDS) plot of gonad gonad Amino acid profile of experimental treatments. TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

Table 3.18. Results of SIMPER analysis that identify the Amino Acids contributing to the dissimilarity between the experimental treatments.

Treatment	AA	Mean AA%	Cont (%)	Treatment	AA	Mean AA%	Cont (%)
TCS	Arg	9.55	32.6	ECK	Arg	11.1	22.55
	Lys	10.3	16.27		Lys	9.56	13.25
	Thr	6.63	9.98		Asx	10.2	7.79
	Val	6.65	5.76		Glx	12.8	7.19
WCE	Arg	10.6	12.3	PD	His	3.71	21.69
	Ala	4.76	10.32		Gly	6.01	10.82
	Thr	6.13	8.25		Thr	6.31	7.81
	Leu	7.43	6.12		Lys	9.17	7.76
FP	Arg	10.7	14.67	ULV	Gly	7.52	36.31
	Asx	9.12	12.94		Ala	4.92	16.67
	Lys	9.44	6.6		Arg	12.4	13.15
	Val	5.9	5.6		Asx	9.37	8.57

TCS (Tank Control Start), WCE (Wild Control End), FP (feed pellet); Eck (*Ecklonia sp.*); PD (*Plocamium sp.*); Ulv (*Ulva sp.*).

SIMPER identify TAG and PL as components responsible for the dissimilarity between TCS and the other treatments being both major classes in TCS and fairly similar in the other treatments (Figure 3.9, Table 3.19).

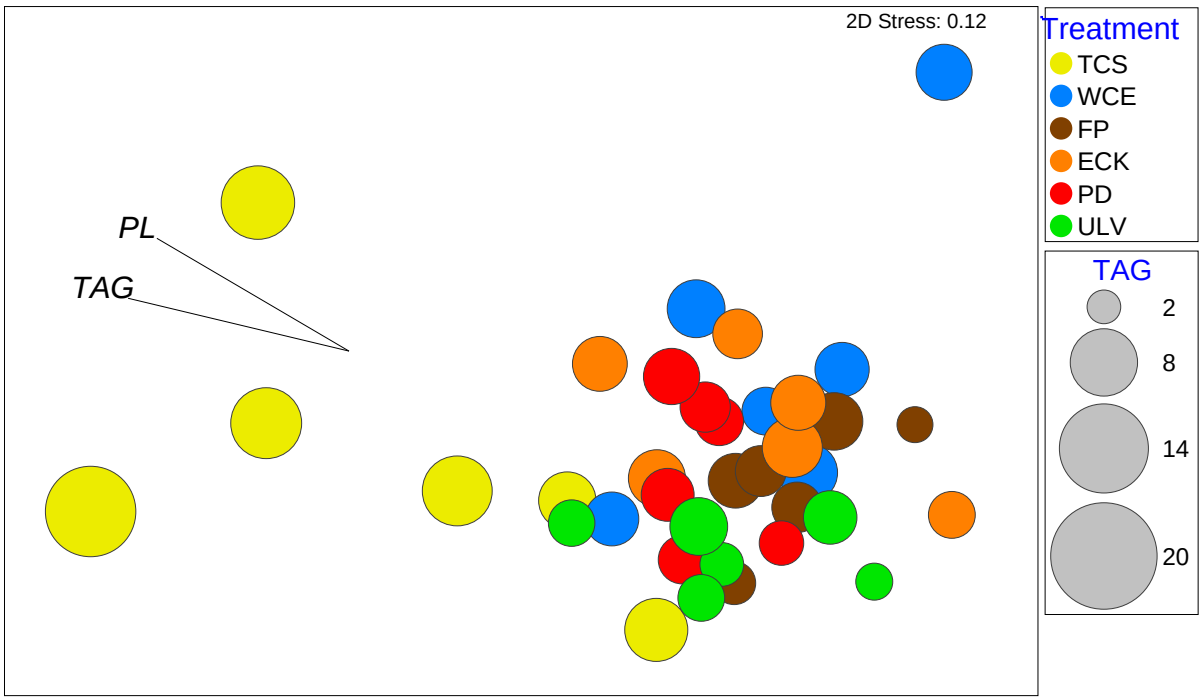

Figure 3.9. Non-metric multidimensional scaling (nMDS) plot of gonad Classes of lipids of experimental treatments. TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

Table 3.19. Results of SIMPER analysis that identify the classes of lipids contributing to the dissimilarity between the experimental treatments.

Treatment	Lipid Class	Mean %	Cont (%)	Treatment	Lipid Class	Mean %	Cont (%)
TCS	TAG	8.14	86.08	ECK	TAG	4.65	73.53
	PL	3.37	13.14		PL	2.31	21.77
WCE	TAG	4.77	69.84	PD	TAG	4.02	53.13
	PL	2.29	25.19		PL	2.18	45.05
FP	TAG	3.85	95.97	ULV	TAG	3.66	81.95
	PL	1.64	3.19		PL	1.95	15.96

TCS (Tank Control Start), WCE (Wild Control End), FP (feed pellet); Eck (*Ecklonia sp.*); PD (*Plocamium sp.*); Ulv (*Ulva sp.*).

The fatty acids mostly contributing to the identification of TCS from the feeding regimes were the C20:1ω9, C14:0 and C20:5ω3. The lipids of the feeding regimes were instead characterized by the C18:1ω9, C20:4ω3, 20:1ω9. Fatty acids contributing to the dissimilarity of WCE were the C14:0, C16:0 and C18:1ω9 while the difference between WCE and FP results determined by the importance of the saturated C14:0 and C16:0 in WCE and the higher content of C18:1ω9 in FP (Figure 3.10, Table 3.20).

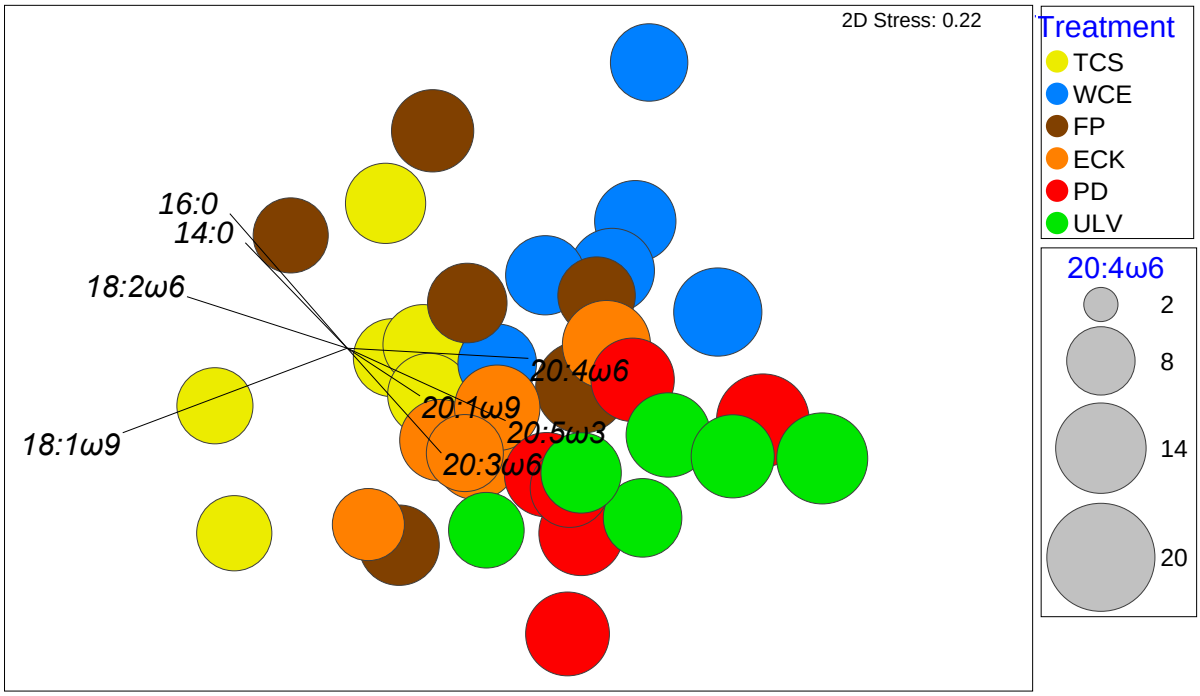

Figure 3.10. Non-metric multidimensional scaling (nMDS) plot of gonad Fatty acids profile of experimental treatments. TCS (Tank Control Start), WCE (Wild Control End), FP (Feed Pellets), ECK (*Ecklonia radiata*), PD (*Plocamium dilatatum*), ULV (*Ulva sp.*).

Table 3.20. Results of SIMPER analysis that identify the fatty acids contributing to the dissimilarity between the experimental treatments.

Treatment	FA	Mean%	Cont (%)	Treatment	FA	Mean%	Cont (%)
TCS	C20:1ω9	14.1	36.12	ECK	C20:4ω6	10.6	22.23
	C14:0	8.81	19.59		C20:5ω3	11.8	20.63
	C20:5ω3	9.23	18.26		C16:0	12.9	7.97
	C18:1ω9	13.1	6.73		C20:1ω9	14.9	6.44
	C16:0	15.1	4.57		C18:1ω9	12.5	4.3
WCE	C14:0	9.64	18.45	PD	C14:0	6.75	20.6
	C18:1ω9	9.58	15.07		C18:1ω9	11.4	12.03
	C16:0	14.4	14.69		C20:4ω6	11.5	11.84
	C20:1ω9	13.3	10.3		C20:3ω6	5.7	7.71
	C20:4ω6	10.7	8.45		C20:1ω9	14.8	4.04
FP	C18:1ω9	12	19.17	ULV	C20:4ω6	10.9	16.62
	C20:4ω6	10.6	14.4		C14:0	6.66	15.07
	C20:1ω9	14.1	11.42		C20:1ω9	15.7	13.57
	C18:2ω6	3.45	8.95		C20:5ω3	13.2	7.77
	C16:0	12.8	5.47		C16:0	12.3	6.64

TCS (Tank Control Start), WCE (Wild Control End), FP (feed pellet); Eck (*Ecklonia sp.*); PD (*Plocamium sp.*); Ulv (*Ulva sp.*).

3.5 Discussion

3.5.1 Handling and transport

The present trial recorded high mortality rate (29.8%) during the 4 week of acclimation after the collection. During the 12 week of the experiment, the mortality recorded (9.8%) and was distributed throughout the course of the trial. Urchins were handpicked by divers with a hook and stacked on the boat inside iceboxes. The day was characterised by a warm northerly wind and the trip back to the boat ramp was particularly rough. The bumpy transport and vibrations resulted in urchins cracking and test perforations. The mortality was high in the first three weeks of acclimation then gradually reduced; however, we consider mortality during the course of the experiment an effect of handling and transport stress. Sudden mortality occurred in two tanks for water flow issues and was not considered in the total count.

There is a possibility that some sub-lethal effects of stress on the remaining urchins compromised the results preventing the normal assimilation of nutrients during the feeding. Recommendations for future collections aimed at preserve urchins alive in captivity are to avoid test damages and care in stocking during transport as *C. rodgersii* specimens can easily spike each other, also avoid long boat trip in rough days since a smoother transport guarantee higher survival.

3.5.2 Gonad production

The present study demonstrated that gonad development and biochemical characteristics in *C. rodgersii* fed a formulated diet in captivity is comparable to gonad of wild animals over the matching time period. Dietary proteins and extruded carbohydrates were the major factors supporting gonad development in fed captive urchins. On the contrary, all three types of fresh monospecific algal diets proved to be ineffective in promoting gonad somatic growth in the short term. Wet weight, dry weight and gonad somatic index were significantly lower in fresh algae diets treatments compared to the treatment fed artificial diet and the wild control (Table 3.10). Feeding treatments and wild control at the end of the trial showed increased gonad moisture compared to the initial control, however gonad from urchins in the algae treatments had a lower macronutrient content compared to the wild control animals. Irrespective of the gonad indexes, pathways of assimilation from diet

source to gonad tissue could be identified for fatty acids and amino acids and are discussed in detail in section 3.5.6.

3.5.3 Biometric measurements

Urchins in all diet treatments increased in total body wet weight and diameter compared to the initial control. The weight gain in diet treatments is unsurprising as all urchins were starved for four weeks prior to commencement of the feeding trial. The TCS urchins all had empty guts and low gonad wet weight. At the end of the trial urchins of the FP treatment showed the largest test diameter (92mm) and animals of TCS the smallest (78mm). Since the urchin test diameter was not measured at the start of the trial (i.e. wild control start), it is not possible to conclude that samples in FP increased due to the effect of the provided diet. We can only speculate that increases may be attributed to artificial feed providing more energy (and a formulation with minerals including calcium carbonates and calcium phosphates) allowing the animals to allocate resources for the growth of all body components. The reabsorption of body parts and shrinking of the test is a phenomenon described in many Echinoids in response to a prolonged starvation period (Constable 1993, Guillou et al 2000, Lares & Pomory 1998, Levitan 1988) and could be the cause of the small test diameter in TCS. Urchins in algae treatments showed a larger test diameter compared to those in TCS but not a higher gonadal index. The gonad fresh weight of feed treatments did not display significant changes compared to TCS, but gonad moisture content increased while dry weight decreased compared to TCS. The mono-algal diets were not sufficient to meet the metabolic needs of the animals and sea urchins in this treatment appear to have mobilized nutritive resources of guts and gonad to survive. Flexibility in resource allocation in echinoids is primarily driven by quality and quantity of available food (Beddingfield & McClintock 1998) and food with insufficient nutritional requirements can lead to low somatic growth (Lawrence 1976). Some authors describe that marine invertebrates replace with water the loss of body mass during starvation (Krogdahl & Bakke-McKellep 2005, Wilcox & Jeffries 1976). In this study however, bodyweight and gonad moisture content in the starved control was very low. By comparison in algae treatments, the increased gonad moisture content and diameter can be attributed to water replacement and turgidity.

3.5.4 Proximate composition

Several studies observed sea urchins fed formulated diet in captivity often result in higher gonad yields than a natural macroalgae diet (Carrier et al 2017, McBride et al 2004, Shpigel et al 2005, Siikavuopio et al 2007a, Woods et al 2008). Improved gonad production is generally attributed to the high protein and carbohydrate content in the artificial feed (Hammer et al 2006a, Hammer et al 2012, Heflin et al 2012, Pearce et al 2002b), Schlosser et al (2005), (Taylor et al 2017). Dietary carbohydrates are mostly accumulated in the gonad in form of glycogen and mobilised to meet energy demands (Marsh & Watts 2007b). In marine macroalgae, carbohydrates are the predominant constituent serving different roles including storage, mucilage, and structural functions (Holdt & Kraan 2011). The high content in carbohydrates, however, does not correspond to high caloric values, since most of these carbohydrates exist as dietary fibres, i.e., polysaccharides that are not digestible or absorbed in the urchin guts (Chang et al 2005).

In this study, the artificial diet produced significantly larger gonad and while the energy content of the diets and urchin's energy intake was not measured, the feed pellets likely provided better energy intake than the algal feeds. The carbohydrates in extruded form allowed the assimilation of dietary proteins for gonadal growth. In contrast, urchins fed on the monospecific algal diets were not able to sustain gonad production to the same extent as the feed pellets and showed an overall decrease in dry mass as well as a decline in each macronutrient component. The dietary proteins and lipids in the algae diets were likely used by the urchins to meet metabolic requirements. Schlosser et al (2005) had similar results when *P. lividus* fed low carbohydrate algal diets had decreased gonad index compared to urchins fed a formulated diet with sufficient carbohydrate energy.

In general, the proximate composition of the seaweed provided in the experiment was similar to that reported in other studies (Angell et al 2015, Mebrahtu et al 2015, Miyashita et al 2013, Sánchez-Machado et al 2004), with non-starch polysaccharides as the most abundant component (Holdt & Kraan 2011), followed by high mineral content, low total lipid and acceptable level of proteins. Not surprisingly, the seaweeds showed some variation in the content of macro-components as they were species from three different orders, yet this variability was

not reflected in the proximal analysis of sea urchin gonad which showed little difference in the content of macro components per dry weight.

Adequate dietary protein content promotes protein storage in the gonad nutritive phagocytes (Fernandez 1997, Fernandez & Boudouresque 2000, Hammer et al 2004, Hammer et al 2006b). An excess of protein on the other hand can be detrimental for both gonad growth and quality (Komata et al 1962, Osako et al 2007, Pearce et al 2002b, Robinson et al 2002). Feeding the sea urchin *Lytechinus variegatus* diets formulated with different percentages of dietary proteins showed that the urchins fed with (20%) protein had higher gonad yield and larger test diameter than those fed with (9% or 31%) proteins (Hammer et al 2006b). Baião et al (2019) found that the optimal diet for feeding *P. lividus* contained (30%) proteins and (6%) lipids in dry weight, while an excess of (50%) protein diet resulted in a decrease of dry matter and energy intake resulting in low feed conversion ratio (FCR). In this study, the artificial feed was formulated with (18%) protein content and (6%) lipids. The highest energy intake of the FP treatment potentially contributed to the accumulation of nutrients not only of the gonad but also to the whole urchin body parts as animals in this treatment resulted in a larger test diameter and heavier total body weight. An increase in moisture level was also observed in sea urchin gonad fed artificial diet while the lipids content was the lowest. This pattern has also been reported by other authors (Agatsuma 1998, Liyana-Pathirana et al 2002a).

3.5.5 Gonad colour

The colour metrics CIE L*a*b*, Hue and Chroma were not significantly different among the feeding treatments and the two controls. Carotenoid pigments are primarily responsible for urchin gonad colour, and importantly, sea urchins cannot synthesize carotenoids *de novo* and must accumulate these pigments from diet or converted from other compounds through metabolic pathways (Liaaen-Jensen 1990, Maoka 2011, Symonds et al 2007). Carotenoids are lipid-soluble components and their concentration is linked with the presence of lipids in the gonad's nutritive phagocytes. Symonds et al (2009) found no difference in carotenoids content in gonad and guts of the sea urchin *Psammechinus miliaris* and, no differences were found for CIE L*a*b* measurements over the course of a season. Whereas

(Suckling et al 2011) demonstrated that *Psammechinus miliaris* gonad colour can be improved in a 12 weeks trial with artificial feed formulated with high carotenoids content.

Sea urchins fed artificial diet are often reported to increase in GSI but produce light-coloured gonad when formulated with insufficient levels of carotenoids pigments (Barker 1998, Pearce et al 2002a, Watts et al 1998). Improvement of GSI and gonad colour was achieved in *Paracentrotus lividus* feeding the animals with pellet diet for eight weeks followed by algal diet for other four weeks (Shpigel et al 2005). In *Strongylocentrotus droebachiensis* the addition of beta-carotene from the alga *Dunaliella salina* in a moist extruded diet performed better than a synthetic form of the same pigment in producing gonad with good colouration (Robinson et al 2002). Commercially acceptable gonad with good yield and colour were obtain feeding *Tripneustes gratilla* a protein-rich feed with 20% addition of the alga *Ulva sp.* (Cyrus et al 2013). In this study, the feeding trial with formulated feed did not decrease or worsen the colour of the gonad. The artificial diet used was formulated with seaweed meal that may have provided a natural form of carotenoids and also included a small addition (0.2%) of beta-carotene in synthetic form.

3.5.6 Effect of feeding regime on energy storage and structural lipids, FAs and AA.

3.5.6.1 Lipid classes

Three classes of lipids were detected in both the artificial feed and the algae diet (Triacylglycerol) TAG, (Sterol) ST, and (Polar Lipids) PL. The seaweed species showed higher concentrations of structural lipid PL and ST (>90% of total lipids) than energy lipid TAG (<10% of total lipids). The three seaweed species showed PL as the major component, while ST was not detected in *Plocamium sp.*, was low in *Ulva* and higher in *E. radiata* (Laminariales). TAG was found in trace amount in the brown algae *E. radiata* (< 1%) and this can explain the lowest gonad production in urchin fed the brown algae. Whereas the red algae *Plocamium sp* was higher in TAG (> 6%) which may have led to a better protein accumulation in the gonad compared to ECK treatment. In contrast, the formulated feed diet contained a high

concentration of energy lipids (TAG >40% of total lipids) and lower levels of structural (60%) compared to the seaweeds.

Eight classes of lipids were detected in the urchin gonad with energy lipids present in a higher concentration in all treatments than in the algal feed source. The energy lipid class components were HC_WE_SE, TAG, FFA, and DAG while ST and PL were the major structural lipids. In all feed treatments and controls TAG was the major gonad energy lipid component and PL the major gonad structural lipid component. FFA and DAG were not detected in gonad from the tank control TCS and were present only in trace amount in the green algae ULV treatment. No statistical differences were found between the four feeding treatments for the composition of lipid classes and no pattern of assimilation could be detected for TAG, despite the high concentration of TAG in the formulated feed, and very low TAG concentration in *E. radiata* and *Ulva sp*. Notably, the concentration of TAG was high and similar in gonad of both controls and feed treatments. TAG was also found to be the major lipid fraction in the gonad of other species, such as *P. miliaris, P. lividus, S. droebachiensis A. dufresnii* (Cook & Kelly 2007, González-Durán et al 2008, Liyana-Pathirana et al 2002c, Montero-Torreiro & Garcia-Martinez 2003, Zárate et al 2016). In contrast to TAG, the PL concentration was higher in *Ulva sp.* and this was reflected in PL within urchin gonad fed the green algae diet. Moreover, there were no differences in the PL concentration between gonad of the feeding treatments ECK and PD despite the brown kelp *E. radiata* having the lowest PL concentration among the algae diets. In this study, no clear pathway of assimilation from the diet to the gonad is observed for the different fractions of the polar lipids. The reserves of TAG found in the gonad of feeding treatments at the end of the trial were likely retained from the start of the experiment and appears to have been partially utilised to meet energetic requirements since TAG decreased in each feeding treatment compared to the initial control.

3.5.6.2 Fatty acids

This study found relatively little effect of diet on the fatty acids profile of the gonad. Both the artificial feed and the three algae diets had very different fatty acid profiles, but this did not translate into a different pattern of accumulation of the most abundant components in the gonad of sea urchins fed these diets. The three

months experimental feeding period may not be enough time to facilitate the gonad lipids turnover and in order to appreciate a selective accumulation of some FAs components a longer feeding period is required. Kelly et al (2008) observed modification of dietary FA in *S. droebachiensis* over a 14 week feeding experiment. Juveniles *S. droebachiensis* that were fed for 25 week showed differentiation in FA based on their experimental diet, however, the same modification occurred in 10 week in adult *S. purpuratus* (Schram et al 2018). Fatty acids in the gonad tissue of adult red urchins *Mesocentrotus franciscanus* were strongly differentiated when fed two kelp species over 17 week feeding experiment (Raymond et al 2014), but no dietary FA incorporation were detected in *S. droebachiensis* tissues in very short experiments (3 week duration) (Wessels et al 2012).

The red algae *Plocamium sp*. was highest in SFAs (70% of the total FAs) and reported the greatest content in C14:0 (8.55%) and C16:0 (53.86%). High content of the Saturated Palmitic acids (20-22%) and moderate levels of the Polyunsaturated ARA C20:4n6 (7 – 18%) and EPA C20:5n3 are reported in other species of the Phylum Rhodophyta (Blouin et al 2006, Gressler et al 2010, Schmid et al 2018). The green algae *Ulva sp*. had a considerable amount of C16:0 (32.40%) and showed the highest level of MUFAs (39.54% of total FAs) with high content in C16:1n9 (2.52%), Oleic acid C18:1n9 (23.83%) and C18:1n7 (12.25%), was low in total PUFAs content but LA C18:2n6 (7.39%) and SDA C18:4n3 (10.89%) were present at moderate level. *Ulva sp*. contained the lowest ratio w6/w3 (0.59), which is consistent with other studies (McCauley et al 2015, Schmid et al 2018). The brown algae *E. radiata* contained the greatest amount of PUFAs (54.03% of total FAs) among which ARA (25.61%), SDA (14.32%) and EPA (7.98%) were the most abundant components consistent with results reported by (Miyashita et al 2013, Schmid et al 2018). Several additional important FAs observed in *Ecklonia sp*. were the C16:0 (19.03%) and the C18:1n9 (18.98%). The artificial feed reported the highest amount in C18:1n9 (27.37%), C18:2n6 (25.92%) and DHA C22:6n3 (5.68%) and was also noticeable the amount of EPA (4.46%), ARA (2.67%) and C16:0 (21.10%).

The SFAs represented the lowest component of FAs by proportion in urchin gonad followed by MUFA and PUFA. The C14:0, C16:0 and C18:0 were the major SFAs

and this is in accordance with the FAs composition of other echinoids (Carboni et al 2013, Kelly et al 2008, Zárate et al 2016). The selective accumulation of lipid components from diet into sea urchin tissue has been demonstrated in several studies, for example, the gonad of *Psammechinus miliaris* fed a diet of formulated salmon feed contained high level of C22:6n3 derived from the fish meal incorporated in the feed. In contrast, *Psammechinus miliaris* gonad were lower in the same FAs when fed only the algae *L. saccharina* (Cook et al 2000). The fatty acid DHA C22:6n3 is abundant in the lipids of marine fauna generally and present only in trace amount in marine algae (Parrish 2013). In this study the content of DHA in algae and feed pellets is consistent with data reported in literature, regardless, DHA was found to be abundant and in similar quantity in the gonad of urchins from all treatments. While *C. rodgersii* is described as an omnivorous feeder, the DHA present in urchin gonad was likely to be self-synthesized from precursors rather than be accumulated from animal diet, as reported for other urchin species (Carboni et al 2013, Cook et al 2000, Prato et al 2018). The urchins accumulated FAs in the same quantity independently of the amount provided with the diets and provide clear evidence of selective accumulation of some nutritional components. Other FAs present in excessive amount may be converted or elongated into other compounds, for instance, the Δ9-desaturase activity is responsible for the production of palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9), from Palmitic acid (C16:0) and Stearic acid (C18:0), respectively (Monroig et al 2013). MUFAs and PUFAs can be synthesized or accumulated when their concentration in the feed source is low; C18:1n9 and C20:1n9 were low in the diet but higher in the gonad. The level of LA C18:2n6 was found to be considerably higher in the feed pellet and significantly higher in the FP gonad treatment, suggesting selective retention of this compound in urchin tissue.

At the conclusion of the experiment, *C. rodgersii* gonad tissue were found to have a significant amount of FAs that were likely accumulated from the natural diet in the wild prior to the experiment. The rate of nutrient turnover is unknown but three months of monospecific diet did not overtly modify the fatty acids profile of the gonad despite the different FA profiles of the diets. Schram et al (2018) report that trophic transfer of nutrients from monospecific diets to tissue occurs more rapidly in juveniles *S. droebachiensis* compared to adult *S. purpuratus*. All feeding treatments

and WCE increased their level of PUFAs compared to the TCS with a major increase in omega-3 fatty acids. These results suggest that level of LC-PUFA are low in the gonad at the recovery phase and accumulate or *de novo* synthesize in preparation for the gametogenesis (Cook et al 2000).

3.5.6.3 Amino acids

Specific Amino Acids can determine a characteristic taste if the level is above a threshold value (Murata et al 2002). The AAs glycine and alanine were found to confer a sweet taste in sea urchin gonad through an omission trial (Fuke & Konosu 1991). Komata (1964) also found that the essential taste of urchin gonad is attributed to umami tasting glutamic acid, sweet-tasting alanine and glycine, and bitter-tasting methionine and valine.

In the current study, glutamic acid, arginine and aspartic acid were the major AA in *C. rodgersii* gonad, followed by lysine, leucine, glycine, threonine, valine, alanine, serine, phenylalanine, isoleucine, histidine, methionine, proline, tyrosine. Several studies found glycine as the dominant FAA in *P. lividus* (Dincer & Cakli 2007), *S. droebachiensis* (Lee & Haard 1982, Liyana-Pathirana et al 2002b) and *Anthocidaris crassispina* (Osako et al 2006) gonad. Phillips et al (2010) found that sweet taste was significantly positively correlated to glycine in testes but not ovaries. Osako et al (2007) found that the ovaries of *Anthocidaris crassispina* had higher levels of the bitter-tasting AAs phenylalanine and histidine while testes had higher concentration of the sweet taste AAs Alanine.

The amino acid composition of urchin roe is reported to be important for the nutritional aspect defined especially by the proportion of essential amino acids and for the flavour they confer to the gonad (Dincer & Cakli 2007, Liyana-Pathirana et al 2002a). While formulated diets can improve gonad yield in relative short time, some studies found that feeds formulated with an excess of protein or a non-adequate AA composition confer to the gonad a pale colour and an unpleasant flavour with decreasing levels of sweet-tasting amino acids and increases in the bitter-tasting amino acids (Inomata et al 2016, Pearce et al 2002b, Phillips et al 2010, Walker et al 2015). In this study, the formulated feed protein content promoted gonad production compared to the algae diet but increased the level of

bitter taste AAs leucine, isoleucine and valine while decreased the umami taste glutamic acid and aspartic acid and is consistent with previous studies on other urchin species fed high protein diets (Lourenço et al 2019, Pearce et al 2002b).

Feeding trials on *M. nudus* were successful in improving gonad taste with a diet enriched in Alanine and Glutamic acid that enhanced the sweet and umami flavour respectively and resulted in a low arginine content (Takagi et al 2019, Takagi et al 2017). In *E. chloroticus* high amounts of glutamic acid and glycine in artificial feed produced sweeter gonad compared to those fed diets containing high amounts of valine and methionine (Phillips et al 2009). Takagi et al (2020) also reported an increase in alanine content in gonad of *M. nudus* fed the basal frond portion of *S. japonica* and suggest that the high content of glutamic acid in the algae could be converted to alanine through digestion. Takagi et al (2020) also observed that when fed to *M. nudus* the high alanine content in the sporophyll of *U. pinnatifida* is directly accumulated in the gonad. In this study we observe an increase of sweet taste amino acids alanine, glycine and threonine and a decrease of the bitter taste amino acids valine, isoleucine and leucine in the gonad of *C. rodgersii* fed the green algae *Ulva australis*. The treatment fed *E. radiata* increased the level of umami taste glutamic acid, and aspartic acid; the most abundant amino acids in brown seaweed are reported to be glutamic acid, aspartic acid and arginine (Dawczynski et al 2007, Fleurence 1999).

3.5.7 Feeding program directions

Given the abundant availability of urchins with commercially acceptable yield in Tasmania, farming practices should be primarily directed to gonad taste enhancement, a process of “finissage” aimed at increasing the ratio of sweet and umami amino acids that gives favourable taste in gonad. Moreover, with the purpose of increase profitability (given the high costs of farming activities) trials should investigate the feasibility of enhancing the gonad taste in the shortest period possible.

Furthermore, since in the present study the animals were fed monospecific algae diets, would be of interest to experiment with a mix of algae diets in different proportion. The brown algae *E. radiata* showed a high amount of the umami taste

AAs glutamic acid and aspartic acid and was very abundant in PUFAs while *Ulva sp*. showed a high content in sweet taste AAs and MUFAs. Both algae are abundant on the Tasmanian coast and the provision would be fairly inexpensive.

In this study, the urchins underwent a four-week period of acclimation and starvation, to eliminate confounding effects such as mortality caused by stress and damage during the collection and transport prior to the commencement of the feeding experiment, as well as provide a common base for all urchins in the study. At the time of collection, the urchin gonad were still in the recovery phase after the spawning season, small and depleted and the four weeks of starvation coupled with the stress of collection and new rearing conditions may have affected the prompt accumulation of nutrients in the phagocytes.

In future feeding program would be advisable a shorter period of starvation of one-two weeks and given the susceptibility of *C. rodgersii* to damage during harvesting, the collection of samples and the transport to the aquaria facility should be as less impacting as possible in an effort to ensure a faster acclimation and an active accumulation of nutrients in the gonad during the feeding period.

3.5.8 Conclusions

This experiment demonstrated that there is potential to modify *C. rodgersii* gonad nutritional and flavour components through a captive feeding program within a short time period. The provision of artificial feed with an adequate amount of energy and protein promoted gonad growth however, a shift towards the accumulation of bitter amino acids was noted. The natural diets did not promote gonad production but a greater accumulation of umami taste AAs with *Ecklonia sp*. and sweet taste AAs with *Ulva sp.* was observed. These results suggest that formulating a feed with a greater proportion of algae with umami and sweet taste AAs and the correct ratio protein/energy the gonad taste can be improved preserving or increasing the yield. Given the great availability of urchins with high GSI in both barrens and kelp habitat, the focus should be on modifying the flavour rather than somatic growth. Further development is required before commercial application.

Chapter 4 Post-harvest stress assessment on coelomic fluid parameters and gonad colour quality attributes of *Centrostephanus rodgersii*

4.1 Abstract

In this study, a post-harvest stress assessment was conducted to evaluate the impact of aerial exposure and processing delay on the coelomic fluid parameters and gonad colour quality attributes of the Longspined Sea Urchin *Centrostephanus rodgersii*. Urchins were held in air for 4hrs at two different temperatures 15˚C and 25˚C, and exposed to wind and no-wind treatments, before being held at 4˚C to mimic fishing, transport and processing operations. The treatments were compared with start and 4˚C controls. Subsamples (n=6) of urchins were processed immediately after the exposure period (4hrs) and at 16hrs and 28hrs. The coelomic fluid of urchins exposed at 25˚C and wind effect showed low volume, elevated turbidity and salinity and low luminosity. The treatment held at 15˚C and protected from the wind preserved stability of internal parameters for a longer period. After 28hrs from harvest, all urchins showed signs of decay in all parameters with no significant differences between treatments. The quality of gonad colour parameters was acceptable after the aerial exposure but during storage was recorded a decrease in luminosity and an increase in red/brown tones attributed to the contact with the turbid coelomic fluid. The results show that sea urchins develop metabolic stress due to harvesting and emersion, the degree of severity of which depends on the environmental conditions and length of exposure and processing delay. Recommendations for the industry would be limiting mechanical damages of urchins' test during harvesting and transport and reduce boating time. During transport and storage animals should be kept humid all the time and storage temperatures should be near the ambient water temperature of harvest.

4.2 Introduction

The fishery of sea urchins focuses on the commercialization of the gonad or “roe”, the only edible part of the animal (Andrew et al 2002, James et al 2016, Sonu 2003, Stefansson et al 2017). In countries where a traditional, domestic fishery is prevalent, urchins are sold fresh and whole shortly after the collection on the site of landing or at the local fish market. The product is consumed fresh within a few days and less attention is dedicated to the aspect of the product and to roe quality at the moment of acquisition. Commercial-scale processing around the world however aims to export the urchin gonad to locations where the demand and consumption are higher, typically the Asian market and mainly to Japan where the product is considered a delicacy and high-quality gonad reach remarkable prices (Sun & Chiang 2015). Gonad are graded based on visual quality traits such as good yield (gonad somatic index), shape, colour and texture/firmness, which usually correspond to a desirable flavour and overall preservation of freshness (McBride et al 2004). The quality of the fresh sea urchin gonad product strongly influences price (Unuma et al 2002); damaged gonad with irregular shape and colour are discarded or classified as a lower grade and sold at a lower price (Whitaker et al 1997).

Mechanical damage to the urchin such as cracks and punctures of the test can be caused by harvesting methods and handling, with air exposure, prolonged transport and storage time causing metabolic stress. Damage and stress result in increased mortality, reduced quality and may have an impact on gonad shelf life (Dale et al 2005, Verachia et al 2012a). When injured or exposed to adverse environmental conditions, urchins undergo an inflammatory reaction to which the coelomocytes, cells freely circulating in the coelomic fluid, respond as a defence mechanism (Matranga et al 2000). Coelomocytes keep the sea urchin free of microorganisms by binding and phagocytosing foreign materials (Smith et al 1992, Smith et al 2006). Among the different types of coelomocytes, the red spherule amoebocytes act as anti-inflammatory agents, including the naphthoquinone pigment, echinochrome A, which has antibacterial properties (Wardlaw 1984). In a normal state (homeostasis) the coelomic fluid appears clear like seawater but the activation of immune response triggers the releases of several cells in the coelomic fluid causing turbidity and the colour to appear brownish, with the potential to discolour the gonad.

Centrostephanus rodgersii commercial harvest starts in December, the summer season in the austral hemisphere and follows until the end of autumn when urchin gonad enter the spawning stage. The water temperature in the Tasmania east-coast at depth of collection during December is between 15-20˚C, while the summer atmosphere temperature can reach 30˚C+ on very hot days but can be as low as 15˚C on cloudy days with a southerly wind. Divers start harvesting in the morning collecting urchins with the use of a hook and a catch bag, when the catch bag is full, it is hoisted on the boat and left on the deck while the diver continues with the harvesting. Typically, divers return to land after 4-5 hrs and during that time the urchins collected are held in air on the boat deck and exposed to weather condition. Some fishers may cover the urchins with wet hessian sacks. Once landed the animals are loaded into a refrigerated truck, transported to the processing plant and placed in cool storage, with processing starting the following morning. It may be up to 30hrs after the harvest before the last individuals from a catch are processed.

The time elapsed for environmental exposure out of the water from the moment of collection to the moment of storage can be critical in the cumulative effects of metabolic stress that worsen the general condition of the gonad. Additional, when exposed to the sun the dark colour of *C. rodgersii* epidermis and spines absorb more sunlight and leads to a sharp increase in temperature on the surface of the animal with a consequent thermal shock in a short time. The association of wind in dehydrating the epidermis combined with other external factors (rough handling, shaking, accidental cracking) complementary to the collection and transport can cause considerable stress or premature death of the animals and therefore affect the quality of the final product, the urchin gonad or roe, or their shelf life.

The present study evaluated the effect of time, aerial exposure, temperature, and storage on the coelomic fluids of *C. rodgersii* recording data of temperature, pH, salinity, density, turbidity, colour, L-lactate, and volume of the coelomic fluid, as well as colour of the gonad. This experiment simulated the harvest, transport and storage conditions typical in the fishery at the time of the experiment.

The purpose of the study had two main objectives:

- Determine if and to what extent physiological stress accumulates during the time urchins are held on the boat exposed to weather conditions and which factors cause greater stress.
- Determine if the post-harvest cool storage temperature during transport is useful to stabilize the animals or if the deterioration continues over time until the moment of processing.

4.3 Materials and methods

In order to investigate the effect of post-harvest handling on stress and subsequently on the quality of sea urchin gonad, live urchins collected during the fishing season with gonad at the mature stage were exposed to different environmental conditions. The longspined sea urchin *Centrostephanus rodgersii* samples, roughly 150 individuals, were hand-collected by scuba-divers in Fortescue Bay, south-east coast of Tasmania on April 10th, 2019. During the transport urchins were placed inside crates at low density in order to minimise animals from injuring one another with the long spines and to reduce stress, the crates were held inside insulated 1000L tanks filled with marine water and provided with aeration. On arrival at the Institute for Marine and Antarctic Studies (IMAS) aquarium facility in Taroona, Hobart, the animals were removed from the crates and placed into two 3000L tanks provided with non-filtered running seawater and aeration. The urchins were acclimatised for four weeks and fed ad libitum a mixed kelp diet. The tanks were monitored daily, dead animals were removed, and mortality recorded. Tanks were also syphoned once a week to remove waste and food particles.

4.3.1 Experimental design

The effect of air exposure and handling during fishing and storage post-fishing was simulated, with parameters indicative of metabolic stress on urchin's coelomic fluid physical parameters recorded and analysed.

The experiment commenced after the four-week acclimation period, an initial control sample at time zero (T0) of six animals was processed immediately after being collected from the aquaria tank (water temperature was 12˚C). This treatment provided an experimental control for the initial physio-chemical parameters of urchin's coelomic fluid before stress-induced tests and is referred to as WA12 (Water 12˚C). Also, at T0 an additional 30 urchins were placed inside fish bins without water, covered with a humid hessian bag and immediately stored inside a walk-in fridge at 4˚C to serve as a control in storage conditions minimizing any effect of stress during the relocation of animals. To assess post-harvest stress conditions a further 96 urchins were allocated to four crossed treatments of temperature and wind. The air temperature inside different rooms was set at 15˚C

(representing mean air temperature) and 25˚C (representing a hot day), respectively. In each room, animals were further separated into two groups, one exposed to the effect of wind (simulated using a fan), while the other was protected from the wind effect and left inside the fish bin covered with a humid hessian bag. The combination exposure/temperature formed four treatments: Wind/15˚C (W15), No Wind/15˚C (NW15), Wind/25˚C (W25), No Wind/25˚C (NW25) and were designed to represent plausible scenarios of urchins held on the fishing vessel during harvest. After 4 hrs of exposure, six animals per temperature/wind treatment and six urchins from the fridge treatment at 4˚C (NW4) were processed (T1). The remaining 72 urchins in the four experimental treatments were then stored inside the walk-in fridge to simulate the effect of refrigerated storage after fishing. Samples from each temperature/wind treatment were kept in a separated and labelled box. After a further 12hrs (T2) and 24hrs (T3), six animals were processed from each temperature/wind treatment as well as from the fridge control treatment.

Table 4.1. Diagram of the experimental design

Treatment	**Exposure**	**Time 0 (n=6)**	**Time 1 (n=30)**	**Time 2 (n=30)**	**Time 3 (n=30)**
Elapsed time		Start	4Hrs	16Hrs	28Hrs
WA12 - Control Tank	Water	12°C	/	/	/
NW4	Air - No Wind	/	4°C	4°C	4°C
W15	Air - Wind	/	15°C	4°C	4°C
NW15	Air - No Wind	/	15°C	4°C	4°C
W25	Air - Wind	/	25°C	4°C	4°C
NW25	Air - No Wind	/	25°C	4°C	4°C

(WA12) animals collected from the aquaria tank with water temperature at 12°C and immediately processed (Start- Time 0) to record a baseline of unstressed urchin physical parameters; (NW4) animals collected from the aquaria tank and immediately stored inside the fridge at 4°C (Start – Time 0) held in air inside fish bins protected from wind exposure and processed after 4hrs, 16hrs and 28hrs of storage (Time 1), (Time 2) and (Time 3) respectively. (W15), (NW15), (W25), (NW25), animals collected from the aquaria tank and immediately exposed (Start – Time 0) to atmospheric temperature (15°C and 25°C) and wind (W) or no wind (NW) effect. After 4hrs of exposure animals were processed (Time 1), remaining animals were stored inside the fridge at 4°C and processed after 16hrs (Time 2) and 28hrs (Time 3).

4.3.2 Data collection

At each of T0 – T3, a series of biological and physicochemical measurements of sea urchins were collected. The total wet weight of the urchins was recorded on a digital scale to the nearest 0.5 gram and the test diameter measured with a Vernier calliper in millimetres to one decimal place. The coelomic fluid (C.F.) was collected making an incision with a scalpel around the Aristoteles's lantern; the quantity of fluid in ml

was recorded, saved in Falcon tubes and stored inside the fridge at 4°C for further data collection, the drained weight of the animals was also recorded.

The test was then split in a half, the interiors were scoped with a spoon, gonad were separated from the guts and the gonad wet weight recorded. The gonad somatic index (GSI) was calculated as the gonad wet weight (gonad WW) divided by the whole wet weight of the animal (total WW) and multiplied by 100: GSI = (gonad WW / total WW) *100. The gonad sex was visually assigned, and gonad colour intensity was recorded with a colour meter (Konica Minolta Chroma Meter CR-400) with three replicate measurement on each gonad lobe and values averaged. The system used to record the colour was the international standard CIE L* a* b* that expresses colour as three values: L* for the lightness from black (0) to white (100), a* from green (−) to red (+), and b* from blue (−) to yellow (+). Hue and Chroma were then calculated from each measurement using the following formulas:

$$Hue = \arctan\left(\frac{b}{a}\right)$$

$$Chroma = (a^2 + b^2)^{0.5}$$

4.3.3 Coelomic fluid assessment

The coelomic fluid collected from the urchins was quantified by the total volume in mL and tested for pH, temperature, salinity and density, turbidity, colour, and L-lactate concentration. Temperature and pH were measured with a pH meter glass probe (Hanna Instrument, AU) On each occasion the instrument was calibrated, and the probe rinsed with distilled water and dried with blotting paper after each reading. Salinity and density were collected with a hand refractometer (Starr instrument, NZ). In order to measure the turbidity level and the colour of the coelomic fluid, a sub-sample (2mL) was centrifuged (14500 rpm for 10 min) in an Eppendorf 5424r centrifuge (Eppendorf, Germany), the fluid was placed in a glass cuvette and the absorbance of the supernatant read at 600nm in triplicate. The colour measurements of L* (whiteness), a* (redness) and b* (yellowness) for each coelomic fluid sub-sample were read between 375-780nm in three replicate with a UV-Vis spectrophotometer (Mettler Toledo, United States). The Lactic acid concentration of coelomic fluid was measured as L-lactate with a Lactate assay kit test (Cayman chemicals, United States) and values presented in µM/ml.

4.3.4 Statistical analysis

The dataset was screened through a scatter plot matrix (SPLOM) to monitor high correlation between variables. If two variables were found to be highly correlated one was excluded from the dataset. Urchin biological measurements and physicochemical parameters of the coelomic fluids were analysed separately. A one-way ANOVA was initially used to test for differences in animal biological measurements and coelomic fluid parameters such as pH, lactic acid, percentage solids, turbidity and colour coordinates.

Given the complex incomplete factorial design (the factorial design was unbalanced given one processing time for the tank control at time zero, one processing time at room exposure for each treatment, two processing time per each exposure treatment after fridge storage, three processing time for the fridge control treatment), the statistical analysis was broken into two phases. The first phase looked at the effects of the temperature/wind treatments at Time 1 relative to the control at T0 and the fridge control (T1_NW4) to determine if the post-harvest treatments created a detectable level of stress. This gave a one-way design with six treatment levels (T0, T1_NW4, T1_W15, T1_W25, T1_NW15, T1_NW25). This design was examined with a multivariate approach (nMDS, PERMANOVA), with the coelomic fluid parameters as dependent variables. If the main effect in this analysis was significant, then a second phase statistical design was implemented to compare the effect of varying fridge storage times on coelomic fluid parameters. This structured approach was predicated on the assumption that if there was no significant effect of the simulated fishing condition treatments, there was little value in examining the effect of post-harvest storage times on coelomic fluid parameters.

In the second phase analysis, we explored the effect of processing delay on coelomic fluid variables, specifically as to whether storage at 4 degrees stabilised the coelomic fluid parameters, or whether changes were accelerated under some handling treatment combinations. This analysis was conducted on normalised data using a Two-Way factorial permutational multivariate analysis of variance (PERMANOVA, $\alpha \leq 0.05$), with 9999 permutations and Type III sums of squares, mixed factors, and Euclidian distance with factors as Exposure and Temperature (NW4, W15, W25, NW15, NW25) and Time (T1, T2, T3). Group structure was

examined again with nMDS. In this second phase analysis of the T0 treatment was not included.

The similarity percentage (SIMPER) analysis was calculated to identify the coelomic fluid parameters contributing to differences between treatments. Based on the SIMPER analysis results, vectors representing the coelomic fluid parameters were then mapped onto a subset of the nMDS graphics to visualise relationships between treatments at Exposure x Temperature effect and processing delay. The L-lactate could only be measured in a reduced number of samples, and replication was inadequate to include in the multivariate matrix. Instead, the L-lactate results were analysed with 2-way ANOVA for factors Time and Exposure.

Univariate analyses were performed using the statistical package IBM SPSS Statistics Version26 (SPSS Inc, Chicago, IL, USA), for multivariate routines was used PRIMER 7 Version 7.0.13 (Plymouth Routines In Multivariate Ecological Research), (Clarke & Gorley 2015) with the PERMANOVA+1 add-on (Anderson et al 2008).

4.4 Results

4.4.1 Biological measurements

Sea urchin biological measurements were inconsistent between the three processing times and can be attributed to differences in size and weight of urchins randomly assigned to the treatments rather than to the effect of exposure and time (Table 4.13, data in appendix). No clear differences were observed nor expected for test diameter and gonad wet weight. However, the urchin total weight decreased over time and is attributed to loss of coelomic fluid during the exposure and through dehydration in the low humidity fridge environment. Statistical differences in total weight are observed only at the second sampling period T2 (Table 4.13_B, $F_{(4,25)} = 3.13$; $p = 0.032$) with the treatment W25 having the lightest mean weight (220gr) and test drain weight (212gr), and the lowest C.F. volume (7.83 mL, Table 4.14) indicating that most of the internal fluid was already lost. Treatments NW15 and the fridge control at T2 were able to preserve more coelomic fluid volume weighing (319gr and 295gr) respectively. Irrespective of statistical significance urchins at the NW treatments and the fridge control maintained a higher internal fluid volume over time compared to urchins exposed to wind. The GSI shows an apparent increasing trend from T1 to T3 also due to the decrease in total weight for loss of internal water over time (Table 4.13).

4.4.2 Assessment of stress induced by 4hrs air exposure on C. rodgersii coelomic fluid physical parameters.

In the first multivariate analysis, a simple test was run to determine if the post-harvest treatments created a detectable level of stress. The main effect was statistically significant for the coelomic fluid physical measurements (Table 4.2, PERMANOVA Main Effect Test, $F_{(5,30)}=3.86$; $p = 0.001$)

Table 4.2. PERMANOVA analysis of urchin coelomic fluid physical variables between the water control at time zero and the four exposure/temperature treatments and fridge control at Time 1.

Source	df	MS	Pseudo-F	P-value	Unique perms
Exposure	5	27.398	3.8586	**0.001**	997
Res	30	7.1004			
Total	35				

Factor Exposure/Temperature (Ex). Analysis done on a resemblance matrix of Euclidean distance of normalised data. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($p < 0.05$) is indicated in bold.

The PERMANOVA Pair-Wise test identified the treatments that differed from each other (Table 4.3); all experimental treatments (temperature, wind) at T1 and the fridge control (NW4) were statistically different to the Time Zero control - WA12 (Table 4.3, $p < 0.05$). Thus, we conclude that 4hrs after removal from water, metabolic stress in coelomic fluid measurements is detectable in all post-harvest treatments. Moreover, the treatment NW15 was significantly different from the treatments W15, W25, NW4 (Table 4.3, $p < 0.05$), suggesting that both wind effect and low temperature cause metabolic changes to happen more rapidly, the treatment NW25 was also significantly different from W15 (Table 4.3, $p < 0.05$). Representation of treatments in two dimensions (nMDS bubble plot, Figure 4.1) indicates within-group variation for the control WA12 and the NW15 group is smaller and tighter, with these two groups along with the fridge control NW4 separated from all the other samples. The vectors indicate group separation is aligned with high volume and high luminosity of the coelomic fluid. The separation of the NW15 can be attributed to a slight decrease in luminosity and an increase in colour parameters a* and b*. The treatments W25 and W15 scatter away from the NW15 treatment and control treatments with higher variability among the replicates within the groups, and with lower coelomic fluid volume. The treatments NW15 and NW4 partially overlap but in the latter, some samples drift away along the vector indicating an increase in turbidity.

Table 4.3. Results of PERMANOVA pair-wise test. Comparisons between the water control at Time zero, and the five exposure/temperature treatments at Time 1.

Groups	t	P-value	Perms	Groups	t	P-value	perms	Groups	t	P-value	perms
WA12, W15	3.30	**0.004**	407	**W15, NW15**	3.00	**0.002**	393	NW15, NW25	1.65	0.071	404
WA12, NW15	2.78	**0.004**	412	W15, W25	1.56	0.054	407	**NW15, NW4**	1.74	**0.046**	413
WA12, W25	1.69	**0.019**	415	**W15, NW25**	2.01	**0.014**	403	W25, NW25	0.94	0.478	409
WA12, NW25	1.88	**0.006**	415	W15, NW4	1.50	0.116	408	W25, NW4	1.33	0.147	408
WA12, NW4	2.31	**0.004**	396	**NW15, W25**	2.17	**0.002**	412	NW25, NW4	1.19	0.246	416

Water control time zero 12°C (WA12), wind exposure at 15°C (W15), air exposure no wind at 15°C (15NW), wind exposure at 25°C (25W), air exposure no wind 25°C (25NW), fridge air exposure no wind at 4°C (NW4). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($p < 0.05$) is indicated in bold, (n=6).

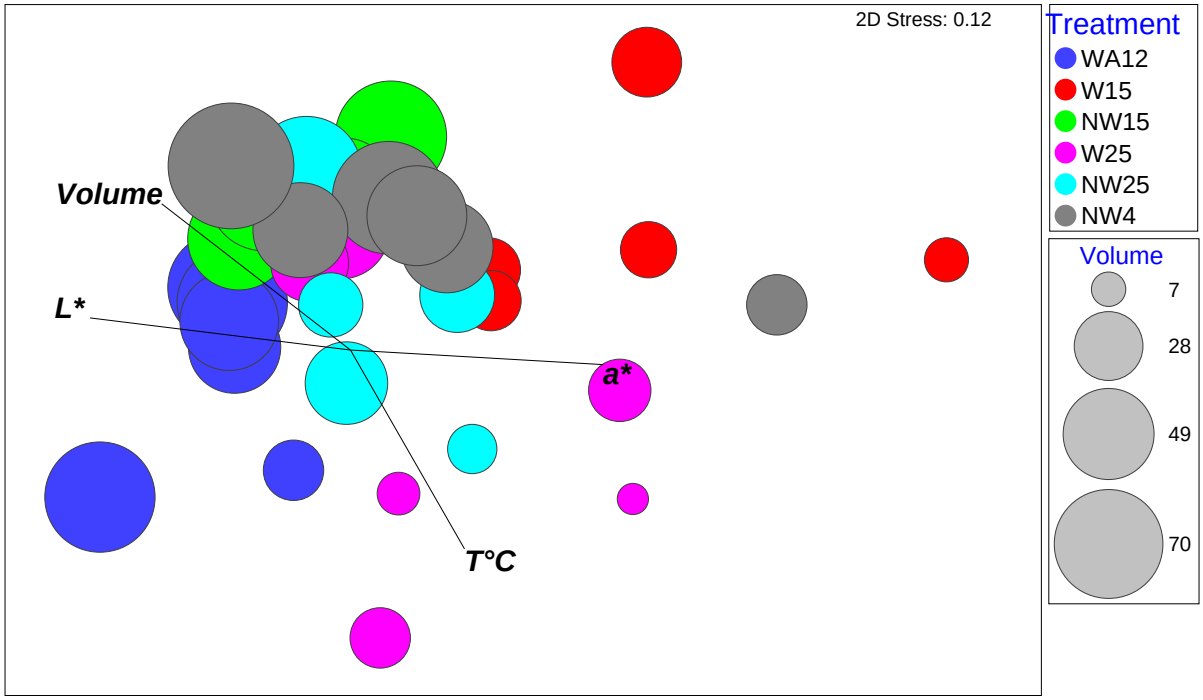

Figure 4.1. Non-metric multidimensional scaling (nMDS) bubble plot of urchin coelomic fluid physical measurements. Samples defined by Factor Treatment. The size of the bubbles indicates the Volume in mL of samples coelomic fluid. Vectors define the direction to which the represented variables increase their counts. The mapped variables were chosen among the four major contributors to the dissimilarity among the groups identified by the SIMPER analysis.

Visualising the same nMDS as Figure 1, this time with the coelomic fluid variable Turbidity represented by bubble size, the wind is clearly evident as a dominant factor affecting coelomic fluid parameters (Figure 4.2). Treatments with no effect of wind (NW) also form a tighter group compared to the wind-exposed treatments, as well as clustering in between the control and the wind treatments. Although some variability occurs and few samples in the NW present signs of stress, most replicates in this group show similarity with the water control WA12. Conversely, the W

group spread more across the plot, shows high variability, an increase in turbidity, density and colour parameters (Figure 4.2).

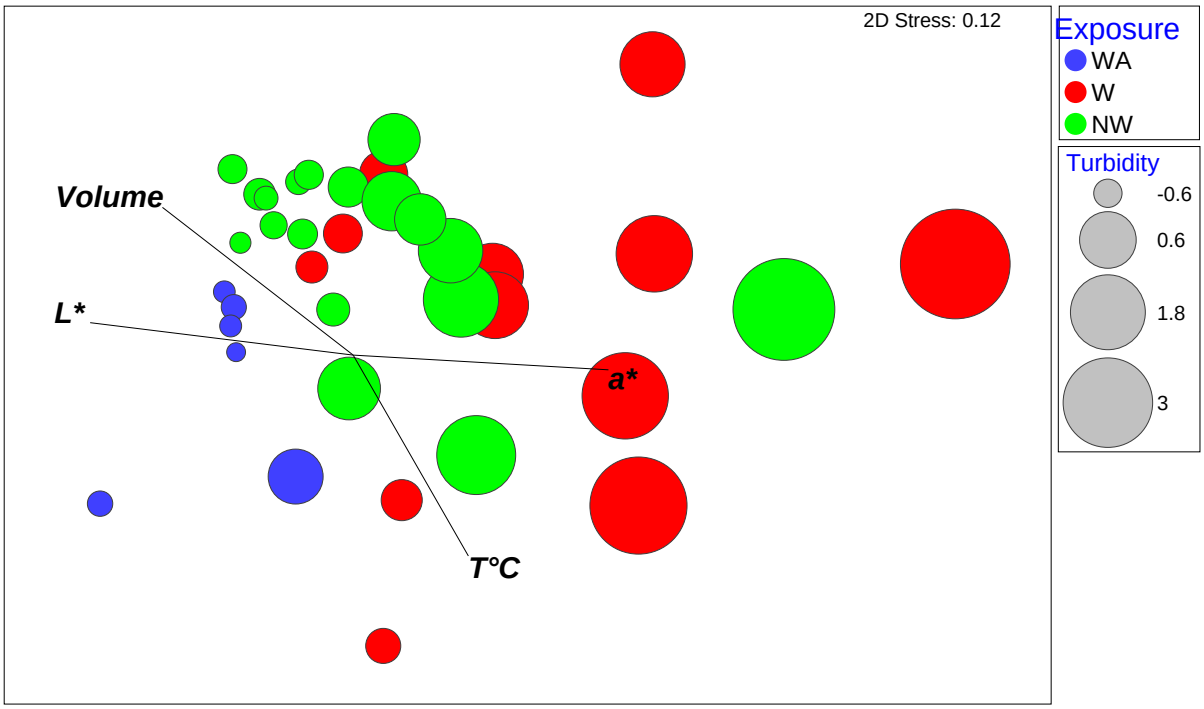

Figure 4.2. Non-metric multidimensional scaling (nMDS) bubble plot of urchin coelomic fluid measurements. Samples are defined by factor Exposure. The size of the bubbles indicates the level of turbidity of coelomic fluid samples. Vectors define the direction in which the represented variables increase their counts. The mapped variables were chosen among the four major contributors to the dissimilarity among the groups identified by the SIMPER analysis.

SIMPER analysis identified the primary variables that contributed the most to the separation between treatments (Table 4.4). By way of review, the volume of urchin's internal fluid (C.F.) is expressed in mL, with higher values indicating lower dehydration. High luminosity (L*) values indicate transparency of the coelomic fluid, and lower L* values denote an increase in turbidity, finally an increase in a* values indicate the presence of a reddish/brownish colouration of coelomic fluid. The water control treatment W12 shows a high average volume of internal fluid (40ml), the highest L* value (98.2) and the lowest a* value (1.03), indicated the animals are in a low-stress state as the coelomic fluid is clear and no colour is detectable. The experimental treatment NW15 shows a slight decrease in L* (97.5) and increase in a* (2.04), an indication that after 4hrs of exposure animals began to manifest metabolic stress, although urchins in this treatment maintained a high volume of internal water (45ml). The treatment NW25 was represented by a lower L*(92.7) and high a* (4.53) compared to NW15 and with a loss of at least one-third

of the coelomic fluid (28.3ml). The treatments W25 and W15 appear to be the most affected during the 4hrs of exposure with W15 presenting the lowest value of luminosity (83.9) and the highest value of a* (10.9) and W25 the lowest volume of C.F. (17.9ml). The fridge control NW4 shows values of L* (88.7) and a* (8.53) similar to treatment W25. This result was unexpected but indicates that storage at low temperature also triggers stress, although the fridge control urchins were able to maintain a higher volume of C.F. (40.8ml) than the wind treatments (Table 4.4). Urchins exposed at the NW15 although statistically different from the W12 showed the least sign of stress between the post-harvest treatment at T1.

Table 4.4. Results of Simper analysis of coelomic fluid variables between treatments at T1 and water control (n=6).

Treatment	Measure	Av.Value	Contrib%	Treatment	Measure	Av.Value	Contrib%
WA12	Volume	40	91.3	W25	Volume	17.9	46.96
	L*	98.2	4.39		L*	89.5	29.8
	a*	1.03	4.14		a*	8.13	22.67
W15	Volume	20.5	58.45	NW25	Volume	28.3	80.08
	L*	83.9	24.64		L*	92.7	13.66
	a*	10.9	16.61		a*	4.53	5.92
NW15	Volume	45	62.26	NW4	Volume	40.8	61.38
	a*	2.04	20.92		L*	88.7	23.84
	L*	97.5	16.55		a*	8.53	14.58

The top three coelomic fluid variables (measure) contributing to the difference between treatments are shown along with the mean value (Av.Value) and their contribution to the similarity within treatment (Cont %).

4.4.3 Comparative analysis of the stress post-harvest occurring over time: exposure time and storage time before processing.

The second phase analysis investigated whether there was differential deterioration in coelomic fluid parameters over time, based on the initial exposure treatment (temperature, wind). This was determined with a 2-way PERMANOVA analysis with two main effects of time (T1, T2, T3) and treatments (NW4, W15, W25, NW15, NW25) and the Time x Treatment interaction. The remaining urchins of all temperature and wind air exposure treatments were placed in the fridge at 4°C at the end of the T1 processing. The five treatments were processed a further two times after 12hrs and 24hrs of storage. The second test aimed at investigating the effect of increasing storage time prior to processing, to evaluate whether storage at low temperature was effective in slowing down urchin metabolic changes caused by the emersion and exposure to different environmental conditions.

The multivariate PERMANOVA analysis of urchins coelomic fluid physical parameters showed that the Time effect was the strongest factor and contained most of the variability. The test revealed a statistically significant interaction between the factors Time and Exposure (Table 4.5, $p < 0.01$). Given the significant interaction, the dataset was tested separately at each processing time with a one-way PERMANOVA for the factor Exposure. The multivariate analysis showed statistical differences in urchins coelomic fluid physical parameters among treatments at T1 and T2 ($p < 0.01$), but not at Time 3 (Table 4.6, $p = 0.422$).

Ordination (nMDS) plot on the full matrix with replicates labelled by Time reveal a pattern of differences between Time levels, and an increasing distance between members of each Time treatment from T1 to T3 (Figure 4.3). The group treatments at T1 are visualized mostly on the left of the plot but dispersed on the vertical axis; at this temporal sampling parameters of pH and Temperature drives most of the variability. Treatments at T2 spread left to right across the plot; in this case changes in colour variables, Salinity and C.F. volume are responsible for this trend. Group treatments at T3 appear less spread, there is less variation of internal parameters which explain the lack of statistical differences at this timing.

A second ordination plot on the same matrix with replicates labelled by Temperature (Figure 4.4), shows considerable overlapping between treatments at T1, however, a trend can be discerned. Members at the 4°C are grouped tighter and follow a horizontal dispersion determined by changes in C.F. volume and turbidity; the 15°C treatments begin to spread on the vertical axis indicating an effect of T°C and pH on the C.F. but not an important effect on C.F volume and L*; the 25°C treatments are even more dispersed on the vertical axis with few outliers already at T1. At T2 and T3 groups of the 15°C and 25°C scatter towards the right of the plot. Overall, appear that animals exposed to higher temperature once emerged develop more stress and an increase in C.F. turbidity, this stress is caused in the first 4hrs after emersion and exposure and is not slowed down with storage at 4°C. The treatments stored at 4°C since the start of the experiment show less variability and a clear trend over time driven by an increase in turbidity and density and a decrease in C.F. volume and luminosity. This treatment was effective in keeping internal

parameters such as pH and temperature for the first period T1. There was evidence however of stress at T2 and T3 (Figure 4.4).

Table 4.5. Results of 2-way PERMANOVA analysis with factors Time and Exposure/Temperature and the interaction between factors.

Source	df	MS	Pseudo-F	P-value	Unique perms
Time	2	142.43	26.497	**0.001**	998
Exposure	4	20.96	3.8993	**0.001**	999
TixEx	8	12.54	2.3328	**0.001**	999
Res	70	5.3754			
Total	84				

Time (Ti), exposure/temperature (Ex), interaction between time and exposure/temperature (TixEx/Te). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference (P<0.05) is indicated in bold.

Table 4.6. Results of PERMANOVA analysis of urchin coelomic fluid physical variables between exposure/temperature treatments at Time 1, Time 2 and Time 3.

T1	Df	MS	Pseudo-F	P-value	Unique perms
Exposure	4	22.933	3.019	**0.003**	999
Res	25	7.597			
Total	29				
T2	**Df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Exposure	4	29.434	4.272	**0.001**	997
Res	25	6.891			
Total	29				
T3	**Df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Exposure	4	10.287	1.035	0.422	999
Res	19	9.9396			
Total	23				

Factor Exposure/Temperature (Ex). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($p < 0.05$) is indicated in bold.

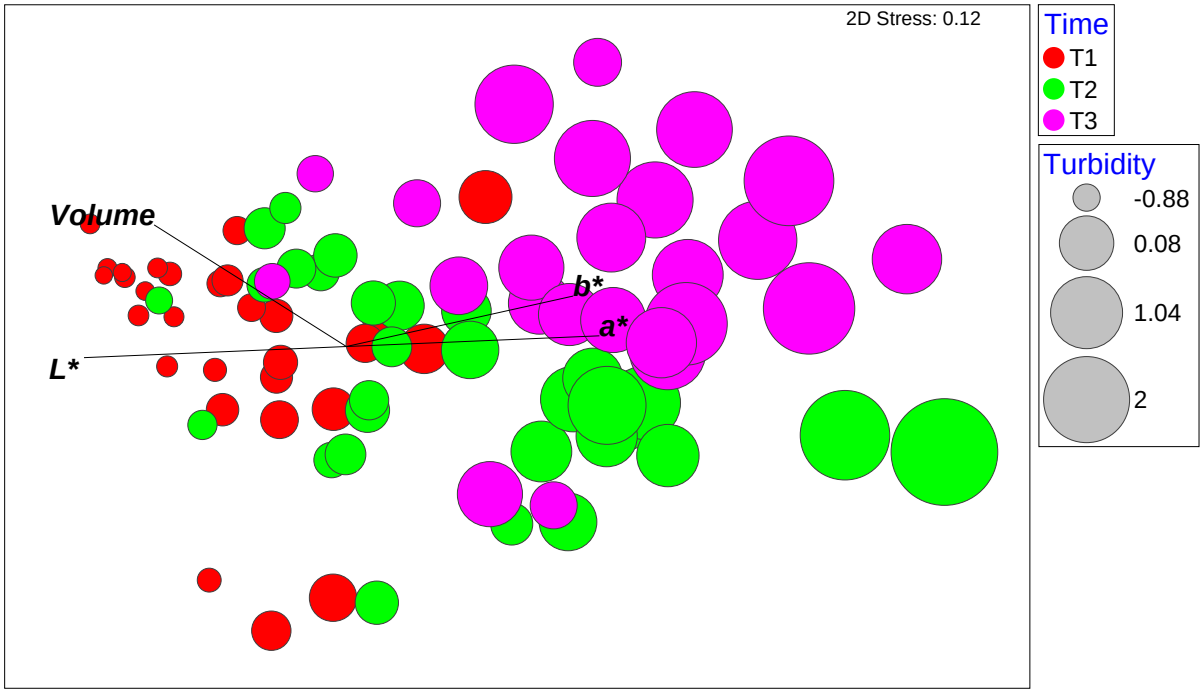

Figure 4.3. Non-metric multidimensional scaling (nMDS) bubble plot of urchin coelomic fluid physical measurements. Samples are defined by factor Time. T1 urchin processed after 4hrs of air exposure; T2 urchins processed after 16hrs (4hrs exposure and 12hrs of storage at 4°C); T3 urchins processed after 28hrs (4hrs exposure and 24hrs of storage at 4°C). The size of the bubbles indicates the level of turbidity of coelomic fluid samples. Vectors define the direction in which the represented variables increase their counts. The mapped variables were chosen among the four major contributors to the dissimilarity among the groups identified by the SIMPER analysis.

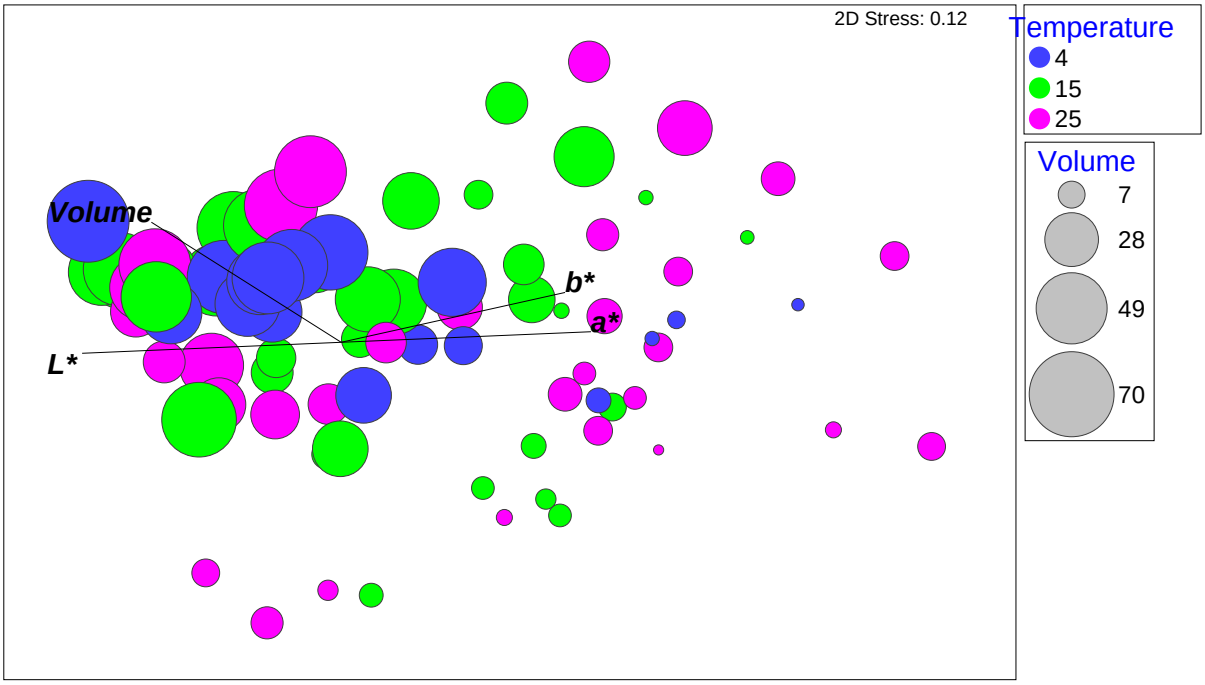

Figure 4.4. Non-metric multidimensional scaling (nMDS) bubble plot of urchin coelomic fluid physical measurements. Samples are defined by factor Temperature. Fridge storage at 4°C (4); samples exposed at 15°C room temperature for the first 4hrs (15); samples exposed at 25°C room temperature for the first 4hrs (25). The size of the bubbles indicates the volume of coelomic fluid samples in mL. Vectors define the direction in which the represented variables increase their counts. The mapped variables were chosen among the four major contributors to the dissimilarity among the groups identified by the SIMPER analysis.

At Time 1, the PERMANOVA pair-wise comparison between treatments (Table 4.7) found that NW15 was significantly different from each of the other treatments. There was also a difference observed between W15 and NW25 (Table 4.7_A). Time 2 shows an increase in significant results for the comparison between the W and NW treatments and absence of statistical differences resulted in NW4 compared to NW15 and NW25 exposure and between W15 and NW25 (Table 4.7_B). At Time 3 there were no multivariate significant differences between treatments, except between W15 and W25 (Table 4.7_C, P=0.047).

Table 4.7. Results of PERMANOVA pair-wise analysis. Comparison between exposure/temperature treatments at each processing time.

(A) T1	t	P-value	perms	(B) T2	t	P-value	perms	(C) T3	t	P-value	perms
W15, NW15	3.00	**0.001**	402	**W15, NW15**	2.60	**0.003**	411	W15, NW15	0.87	0.537	126
W15, W25	1.56	0.051	408	**W15, W25**	2.05	**0.015**	410	**W15, W25**	1.65	**0.047**	126
W15, NW25	2.01	**0.014**	402	W15, NW25	1.22	0.205	407	W15, NW25	0.70	0.822	126
W15, NW4	1.50	0.124	410	**W15, NW4**	1.80	**0.038**	406	W15, NW4	0.74	0.755	126
NW15, W25	2.17	**0.003**	408	**NW15, W25**	3.52	**0.001**	409	NW15, W25	1.43	0.130	126
NW15, NW25	1.65	**0.049**	406	**NW15, NW25**	1.77	**0.040**	413	NW15, NW25	0.62	0.788	126
NW15, NW4	1.74	**0.048**	403	NW15, NW4	1.17	0.296	409	NW15, NW4	0.76	0.605	126
W25, NW25	0.94	0.433	415	**W25, NW25**	1.71	**0.035**	414	W25, NW25	1.02	0.458	126
W25, NW4	1.33	0.129	402	**W25, NW4**	2.59	**0.012**	414	W25, NW4	1.43	0.102	126
NW25, NW4	1.19	0.237	403	NW25, NW4	1.12	0.300	413	NW25, NW4	0.79	0.525	126

Wind exposure at 15°C (W15), air exposure no wind at 15°C (15NW), wind exposure at 25°C (25W), air exposure no wind 25°C (25NW), fridge control air exposure no wind at 4°C (NW4), processing time after 4hrs, 16hrs 28hrs (T1, T2, T3). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($P<0.05$) is indicated in bold.

As for the Phase 1 analysis, Coelomic fluid volume and C.F. colour coordinates L* a* and b* were the variables identified by SIMPER analysis that mostly accounted for the dissimilarity between treatments groups at each processing time in phase 2 (Table 4.8). The trend of urchins' internal fluid volume in each treatment and overtime is represented in relation to the total wet weight of the animals (Figure 4.5). A greater fluid volume was present in animals of WA12 at T0 and in NW15 and NW4 at both T1 and T2, the lowest fluid content was always observed in W15, W25 and NW25 at T1 and T2. A constant decreasing trend can be observed in each treatment from T1 to T2 but at T3 all treatments showed a similar and low fluid content between 10 ml and 20 ml. The NW15 held more C.F. until T2 (C.F. > 40 ml) but the volume decreased drastically at T3 (C.F. < 20 ml). The NW4 kept a high fluid volume until T1 (C.F. > 40 ml) but then slightly decreased to 33ml at T2 and dropped again to roughly 20ml at T3 (Figure 4.5). To a decrease in C.F. volume correspond a decrease in the value of fluid luminosity (L*) and an increase in the values (a*) and (b*) which indicate appearance and intensification of fluid colour over time (Table 4.8).

Table 4.8. Results of SIMPER analysis of coelomic fluid variables at each processing time.

T1	Av.Value	Contrib%	T2	Av.Value	Contrib%	T3	Av.Value	Contrib%
Volume	31.1	39.7	b*	45.4	35.79	Volume	16.2	36.93
b*	16.6	35.84	Volume	22	29.03	L*	54.5	31.96
L*	90.3	14.43	L*	68.4	24.96	b*	59.8	19.09
a*	7.07	9.39	a*	21.5	9.43	a*	28.6	9.49

The top four coelomic fluid variables contributing to the dissimilarity between processing times are shown along with the mean value (Av.Value) and their contribution to the similarity within groups (Cont %). Coelomic fluid content (Volume mL), Luminosity (L*), tones of red (a*), tones of yellow (b*).

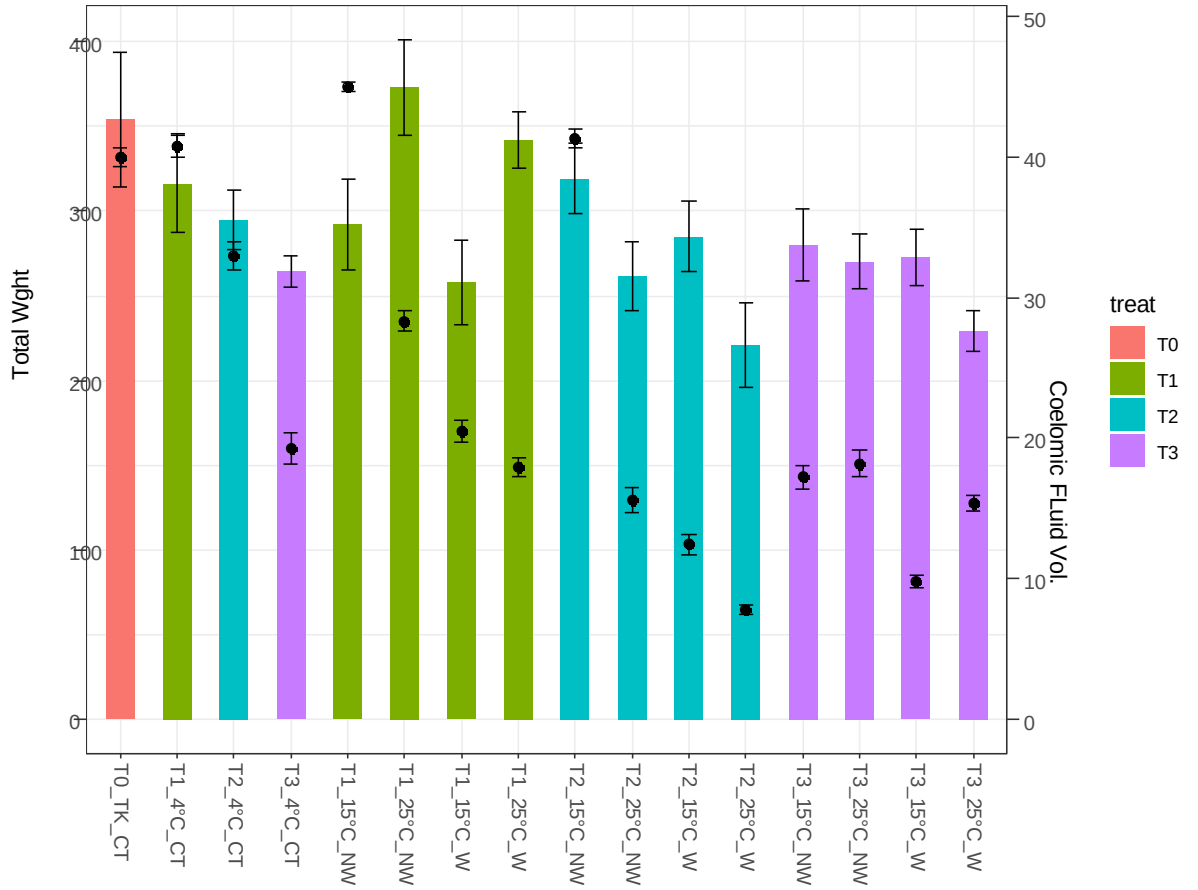

Figure 4.5. Bar chart of urchin total weight (gr.) and coelomic fluid content in mL (indicated by the black dots) of control at T0 and treatments at T1, T2 and T3. All treatments at T1 and T2 (n=6), at T3 W15, NW15, NW25 (n=5), W25 (n=4), NW4 (n=6).

For the measurement of lactate in urchins coelomic fluid the 2-way ANOVA was statistically significant for the factors Time and Exposure (Table 4.15, ANOVA test, $p < 0.01$, data in appendix). A constant and significant increase in lactate was recorded between processing time in urchin's coelomic fluid (Figure 4.6_A, Tukey's test, $\alpha=0.05$), the increase was mild from T0 to T1 (70.0 µM/ml and 84 µM/ml) respectively, lactate content roughly doubled at T2 (154.5 µM/ml) and was three times higher than T0 in T3 (225.0 µM/ml) (see appendix Table 4.16_A).

Statistical differences in lactate values between treatments are also recorded (Figure 4.6_B, Tukey's test, α=0.05), the NW15 showed the lowest lactate increase (95.3 µM/ml), followed by W15 (120.0 µM/ml), NW4 and NW25 shows similar values (144.0 µM/ml and 150.0 µM/ml) respectively, W25 presented the highest increase in lactate and was significantly different only from NW15(223.0 µM/ml) (Table 4.16_B, data in appendix). No statistical differences in lactate content were recorded for the interaction between processing time and exposure treatments (Table 4.16, ANOVA test, $p = 0.443$, data in appendix).

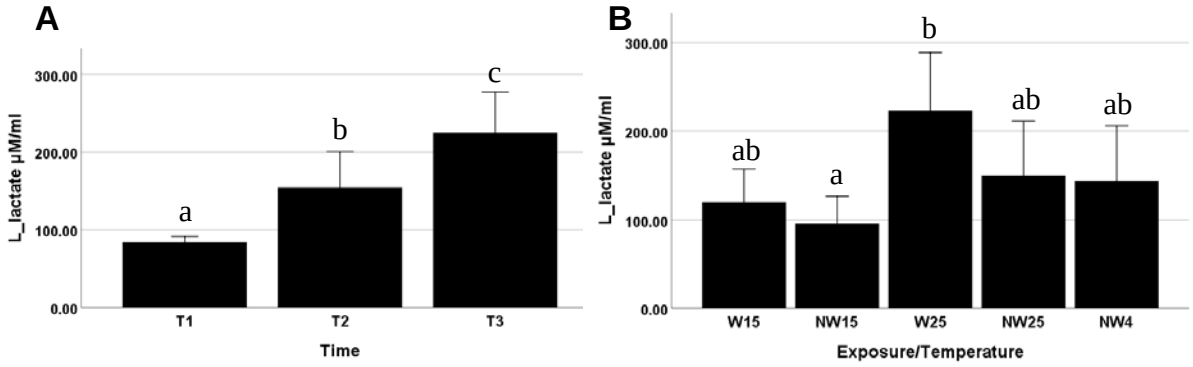

Figure 4.6. Bar charts represent L-lactate detected in urchins coelomic fluid at each processing time (A), and in each Exposure/Temperature treatment (B). Results are mean values ± standard error and expressed in µM/ml. Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and $p < 0.05$.

The variability of coelomic fluid physical parameters at each exposure/temperature treatment and handling time is illustrated with line charts (Figure 4.7). All urchins were collected from aquaria tank at 12°C water temperature, after 4hrs of air exposure (T1) each treatment increased the internal temperature which was found higher in W15 and W25 (15.5°C), and lower in NW25 (14.5°C) and NW15 (13.5°C) respectively. The internal temperature of each treatment kept increasing after 12hrs at 4°C (T2) but dropped after 24hrs of storage (T3) recording 12°C in W15 and 7.2°C in W25 despite the fact that both were measured at 18°C in T2 (Figure 4.7_A). The C.F. pH measurement shows variability between treatments and an unclear trend over time, at T1 the treatments W15 and NW15 show the lowest values (pH 6.5 and pH 6.6) and the NW25 and W25 (pH 6.9 and pH 7), respectively. At T2 the W15 increased sharply to pH 7.1 while all the other treatments decreased from their initial recording at around pH 6.5, at T3 W15 pH slightly decreased again (pH 6.9), W25 and NW25 increased to pH 6.7 and 6.8

respectively, and NW15 kept the decreasing trend recording pH 6.5 (Figure 4.7_B). The line chart of C.F. volumes confirms the outcome of multivariate analysis (PERMANOVA and nMDS), NW15 was able to hold more internal fluid at T1 and T2 while the other treatments at T1 had already lost most of the fluid. Values tend to converge at T3 with a rapid decrease in NW15 suggesting that after 16hrs out of the water urchins lose the ability to keep internal fluid, the apparent increase in fluid volume in NW25 and especially W25 is due to a reduced number of samples in these treatments at T3, few animals had lost all the internal fluid hence was not possible to record measurements (Figure 4.7_C). The level of turbidity increased in all treatments from T1 to T2 however, this increase was dramatic in W25 and while in the other treatments the turbidity kept increasing at T3 in W25 decreased, the turbidity level converged in all treatment at T3 (Figure 4.7_D). The luminosity of C.F. shows an inverse trend compared to the turbidity, in fact, samples presented higher luminosity at T1 and decreased converging at the same value in T3 (Figure 4.7_E). Hue shows changes in the tone of colour of C.F., at T1 the effect of different exposures resulted in variability in hue values, but the values converged at T2 and plateaued to T3 (Figure 4.7_F).

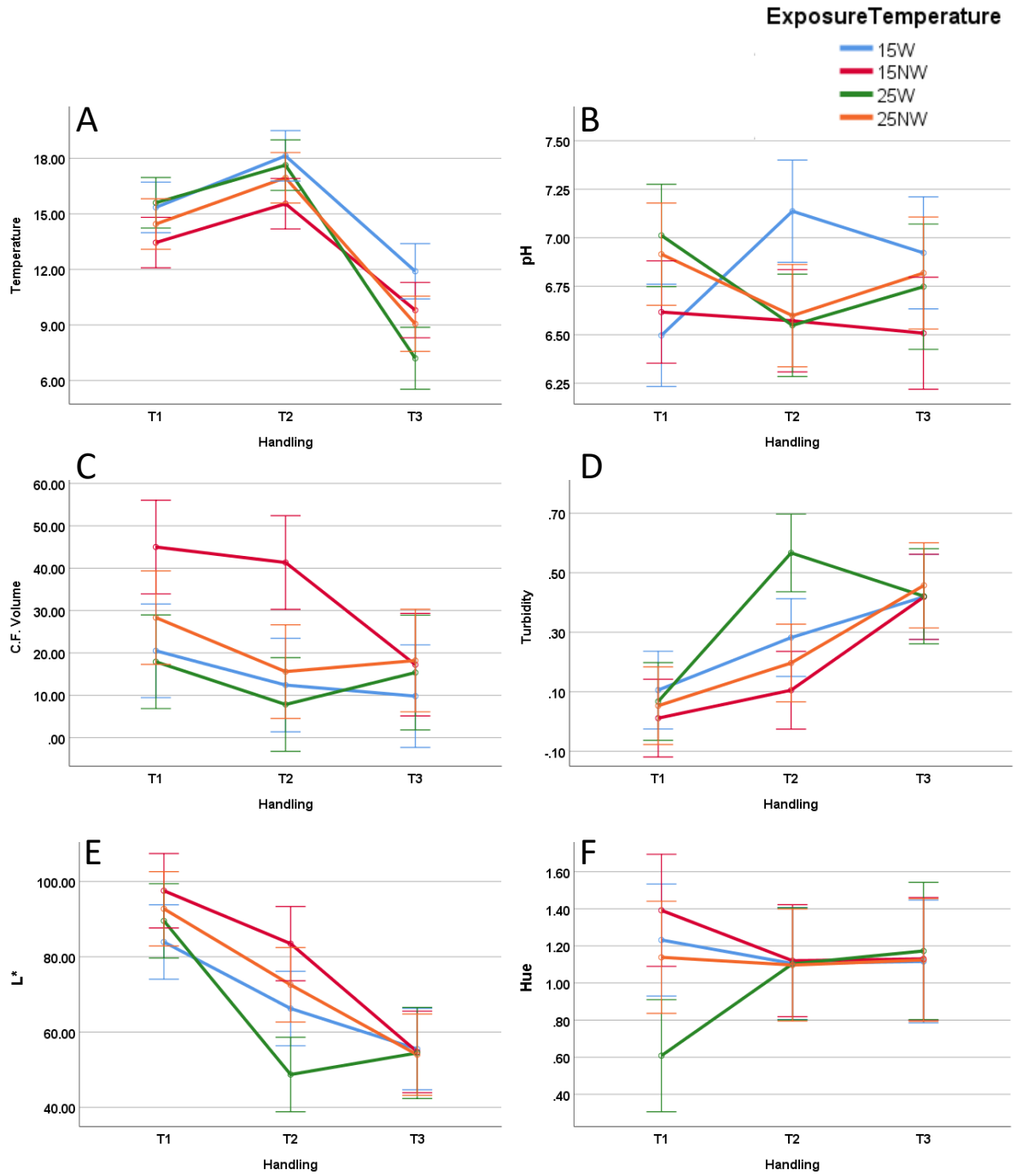

Figure 4.7. Line charts of coelomic fluid physical parameters on Exposure/Temperature treatments at three processing times. X-axis indicates processing time after the emersion of sea urchins, T1 (4hrs of environmental exposure), T2 (16hrs, 4hrs exposure and 12hrs storage at 4°C), T3 (28hrs, 4hrs exposure and 24hrs storage at 4°C). (**A**) Temperature of coelomic fluid (C.F) expressed in (°C). (**B**) C.F. pH (**C**) C.F. internal volume expressed in mL. (**D**) C.F. turbidity read in absorbance at 600 nm, high values indicate higher turbidity. (**E**) C.F. luminosity, high values indicate limpidity and absence of colour, lower values indicate colour appearance. (**F**) C.F. hue distinguish the tone of colours, higher values indicate appearance of light colour, lower values indicate an increase in the tone of colour.

4.4.4 Effect of handling and exposure on C. rodgersii gonad colour.

To evaluate the effect of exposure and processing delay on "roe" visual characteristics, changes in urchin gonad colour were investigated. The multivariate analysis of urchins' gonad colour displayed significant interaction between factors Time and Exposure (Table 4.9, $p < 0.01$). Given the significant interaction, the dataset was further analysed with a one-way PERMANOVA at each processing time to explain the Main effects. The treatments analysed for the gonad colour measurements were statistically different in multivariate space at each processing time (Table 4.10, $p < 0.01$).

Table 4.9. Results of 2-way PERMANOVA analysis on gonad colour coordinates with factors Time and Exposure/Temperature and the interaction between factors.

Source	df	MS	Pseudo-F	P-value	Unique perms
Time	2	39.43	7.359	**0.001**	999
Exposure	4	16.68	3.114	**0.003**	999
TixEx**	8	17.95	3.351	**0.001**	999
Res	80	5.36			
Total	95				

Time (Ti), exposure/temperature (Ex), interaction between time and exposure/temperature (TixEx/Te). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($p < 0.05$) is indicated in bold.

Table 4.10. Results of PERMANOVA analysis of urchin gonad colour measurements between exposure/temperature treatments at Time 1, Time 2 and Time 3.

T1	df	MS	Pseudo-F	P-value	Unique perms
Exposure	5	17.711	3.701	**0.001**	997
Res	30	4.785			
Total	35				
T2	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Exposure	4	15.259	3.557	**0.004**	999
Res	25	4.290			
Total	29				
T3	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Exposure	4	20.058	2.8208	**0.008**	999
Res	25	7.1109			
Total	29				

Factor Exposure/Temperature (Ex). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($p < 0.05$) is indicated in bold.

The pairwise PERMANOVA analysis revealed inconsistent results for the comparison between treatments at each processing time (Table 4.11). At T1 most of the differences result from the comparison of NW4 and W25 between other treatments, while at T2 the treatments W15 and W25 command deviation of colour parameters, at T3 only NW25 show statistical differences from each of the other treatments.

Table 4.11. Results of PERMANOVA pair-wise analysis. Comparison between exposure/temperature treatments at each processing time.

(A) T1	**t**	**P**	**perms**	**(B) T2**	**t**	**P**	**perms**	**(C) T3**	**t**	**P**	**perms**
W15, NW15	0.72	0.665	409	W15, NW15	0.91	0.429	404	W15, NW15	0.54	0.796	407
W15, W25	2.44	**0.018**	406	**W15, W25**	2.67	**0.005**	409	W15, W25	1.33	0.202	398
W15, NW25	0.79	0.55	409	**W15, NW25**	2.04	**0.019**	406	**W15, NW25**	2.21	**0.037**	408
W15, NW4	2.82	**0.005**	410	W15, NW4	1.14	0.272	410	W15, NW4	1.16	0.265	415
NW15, W25	1.44	0.149	415	**NW15, W25**	2.97	**0.002**	422	NW15, W25	0.79	0.584	413
NW15, NW25	0.76	0.593	410	NW15, NW25	1.76	0.062	400	**NW15, NW25**	2.27	**0.023**	414
NW15, NW4	2.13	**0.011**	392	NW15, NW4	0.52	0.815	96	NW15, NW4	0.72	0.628	417
W25, NW25	1.92	**0.045**	421	**W25, NW25**	1.90	**0.015**	415	**W25, NW25**	2.90	**0.016**	407
W25, NW4	2.79	**0.018**	403	**W25, NW4**	2.27	**0.014**	417	W25, NW4	0.65	0.659	404
NW25, NW4	1.62	0.112	417	NW25, NW4	1.10	0.292	411	**NW25, NW4**	2.22	**0.026**	399

Wind exposure at 15°C (W15), air exposure no wind at 15°C (15NW), wind exposure at 25°C (25W), air exposure no wind 25°C (25NW), fridge control air exposure no wind at 4°C (NW4), processing time after 4hrs, 16hrs 28hrs (T1, T2, T3). Data are normalised and analysed on a resemblance matrix of Euclidean distance. Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference ($p < 0.05$) is indicated in bold.

The nMDS plot on gonad colour variables visualizes groups distribution for the factor Time (Figure 4.8), samples processed at T1 overlap with samples at T0, indicating that 4hrs of exposure did not command gonad colour changes, however, samples at T1 are mostly distributed on a vertical axis where the ones on top are characterized by more yellowness and the ones at the bottom by more whiteness, this distribution is attributed to a range of variability in gonad colour influenced by urchins sex and diet. At T2 and T3 samples drift towards the right of the plot separated from T1 and tend to converge where the vectors indicate increase in turbidity and decrease in luminosity and chromaticity.

Five variables identified by SIMPER analysis contributed to more than 90% of the dissimilarity between Time factors (Table 4.12). Low luminosity index can be determined by decrease in moisture on the gonad surface. Values of L* are higher at T0 and steadily decrease to T3. Similarly, a gradual decrease in chromaticity results

in colour being less bright or dull. The decrease in b* values denote a reduced tone of yellowness determined by the increase in turbidity associated with the degranulation of the gut wall and release of red spherule coelomocytes as response of stress. The red spherule contains the pigment echinochrome which gives the coelomic fluid a reddish/brownish colour. Results suggest that the gonad colour of sea urchins held in air was affected over time by the contact with a turbid coelomic fluid.

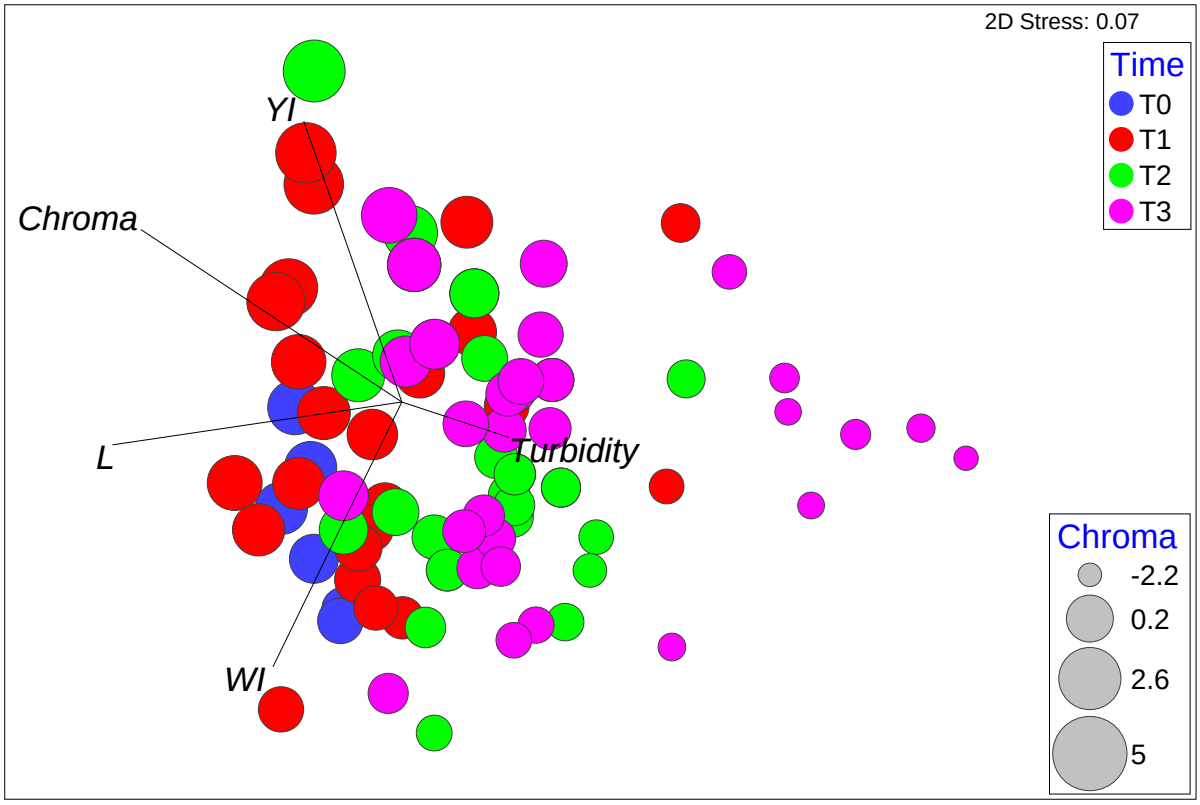

Figure 4.8. Non-metric multidimensional scaling (nMDS) bubble plot of urchin gonad colour coordinates. Samples are defined by factor Time. Urchins processed at the start of the trial (T0); urchins processed after 4hrs of air exposure (T1); urchins processed after 16hrs (4hrs exposure and 12hrs of storage at 4°C), (T2); urchins processed after 28hrs (4hrs exposure and 24hrs of storage at 4°C), (T3). The size of the bubbles indicates the value of chromaticity. Vectors define the direction in which the represented variables increase their counts. The mapped variables were chosen from the top five major contributors to the dissimilarity among the groups identified by the SIMPER analysis.

Table 4.12. Results of Simper analysis of urchin gonad colour coordinates at each processing time.

T0	Av.Val	Contr%	T1	Av.Val	Contr%	T2	Av.Val	Contr%	T3	Av.Val	Contr%
YI	105	63.66	YI	115	55.35	YI	112	65.2	YI	111	54.55
Ch	43.9	14.54	L*	52.2	13.5	b*	38.3	12.19	b*	35.3	15.69
b*	43.2	14.27	b*	42	12.63	Ch	39.3	12.01	Ch	36.7	13.67
WI	39.5	5.26	Ch	42.9	12.23	L*	48.6	5.87	L*	45.4	11.9
L*	58.5	1.7	WI	35.3	5.31	WI	35	3.93	WI	33.7	3.26

The top five gonad colour coordinates contributing to the dissimilarity between processing times are shown along with the mean value (Av.Value) and their contribution to the similarity within groups (Cont %). Yellowness (YI), Chromaticity (Ch), Whiteness (WI), Luminosity (L*), tones of yellow (b*).

4.5 Discussion

The coelomic fluid of sea urchins held in air showed traits that indicated ongoing metabolic stress whose degree of severity was indicative of the environmental conditions to which the animals were exposed. After 4hrs all experimental treatments presented a detectable level of stress compared to the water control. Wind exposure and high temperature showed to be the main factors in determining stress and decay of physiological conditions of homeostasis. Urchins held in air at 15 and 4°C and protected from the wind effect performed better and showed less sign of stress after 16hrs. At the 28hrs all animals were spoiled or dead and was not possible to detect significative differences between treatments. Volume, colour measurements and turbidity of the coelomic fluid were the main parameters affected by handling, exposure and temperature. Sea urchin gonad showed a change in colour over time correlated with the level of turbidity of coelomic fluid suggesting that an increase in metabolic stress and processing delay can lead to significant deterioration in gonad quality and consequent decrease of economic value.

Harvesting, handling and transport is known to provoke stress in sea urchins (Dale et al 2005) with many animals showing obvious damaged, abrasions, cracking or perforation of the test as well damage of the oral membrane which results in coelomic fluid loss and determine bacterial infections (Maes & Jangoux 1985). This study did not investigate survival rate after collection however results of previous trials suggest that *C. rodgersii* is not a robust species compared to others (Hill & Lawrence 2006, Lawrence 2013a, Sherman 2015). For example, rough methods of collection did not influence mortality rate, external urchin condition or gonad index in the purple sea urchin *Heliocidaris erythrogramma* (Warren-Myers et al 2019). Similarly, less than 2% mortality was reported by (Pert et al 2018) for *H. erythrogramma* collected and roe enhanced in ambient seawater. Arafa et al (2006) reported zero mortality in a 36-day trial with *Paracentrotus lividus* but observed a 50% decrease in gonad index in the first five days of live storage and a constant decrease in the following 26 days in both fed and starved animals and attributed the loss of gonadal indices to the stress induced by handling, transport and new rearing condition.

4.5.1 Determination of stress levels induced by handling and exposure on C. rodgersii.

A detectable level of stress was found in the coelomic fluid of all post-harvest treatments. All (Exposure x Temperature) treatments at T1 were significantly different (Table 4.3, PERMANOVA Pair-wise test, $P<0.05$) from the initial control at T0 (WA12). Urchins stored inside the fridge at 4°C maintained a higher level of coelomic fluid volume over time, most likely because they were subjected to less handling and protected from dehydration. Urchins protected from wind at lower temperatures (NW15) performed better and preserved more coelomic fluid than at higher temperatures (NW25). Animals exposed to the effect of wind had less coelomic fluid volume at both 15 and 25°C than other treatments but interestingly the W15 treatment had less coelomic fluid than the harsher W25 treatment. Once urchins were transferred to the fridge and held at 4°C the W25 treatment required more time to lower the temperature and had already lost most of the body wall turgidity.

4.5.2 Changes in Coelomic fluid parameters as an indicator of stress

After emersion, urchin metabolism was strongly affected by the level of exposure and processing delay, with animals showing a progressive decline of internal parameters based on whether they were processed after 4hrs, 16hrs or 28hrs (NMDS and PERMANOVA refer to the results section). Burnett et al (2002) reported that upon air exposure (emersion) urchins *Strongylocentrotus purpuratus* release a significant amount of internal fluid. While a loss of internal fluid over time can occur naturally, mechanical damages and exposure to adverse environmental conditions out of the water can accelerate the loss of fluid. A general decrease in animal total wet weight over time was observed and attributed to an inevitable loss of internal water through fracture of the coelomic membrane as well as general dehydration of urchins exposed to air, mainly through the epidermis, the membrane of the peristome area and spines Animals kept at 15°C or 4°C and protected from the effect of wind retained more coelomic fluid and for longer than those exposed to the wind or at 25°C. Observations during the acclimation phase identified significant and rapid loss of internal fluid and mortality occurred in animals that were found to have cracks in the shell or holes in the peristome membrane,

potentially caused by splitting during transfer to/from the vessel or punched by spines of other urchins during transport. Urchins exposed to wind and high temperature showed also much greater variation in their coelomic fluid parameters, represented by a wider scatter of replicates in the multidimensional space. The prompt storage at 4°C proved effective in stabilizing the general coelomic fluid values however, an increase in turbidity of coelomic fluid in animals of NW4 compared to the NW15 treatment was observed already at T1. Urchins at 4°C experience a metabolic change, consuming more energy and potentially becoming exhausted. At the final sampling period, T3 no major differences in coelomic fluid values were observed between treatments; the volume of internal fluid was very low in most of the animals and several urchins had no fluid at all. All animals showed high values of turbidity and density and an increase in L-lactate that indicate anaerobic metabolism and suggesting that between the 16 and 28hrs from the moment of emersion all individuals began to die or were no longer able to control the internal metabolism.

The increase in L-lactate detected over time although statistically significant was relatively minor. Results obtained by (Spicer et al 1988) on stress trials with the sea urchins *P. miliaris* and *E. esculentus* suggests that the glycolytic pathway of anaerobiosis occur in other end-product in addition to L-lactate, for instance, succinate and fumarate. However, L-lactate appears to be the major end-product of anaerobic metabolism. Burnett et al (2002) suggest that during emersion the aerobic metabolism remains active and gas exchange continues to occur through the oesophagus, which acts as a facultative lung, hence limiting lactate production. The aerobic metabolism may still be active during refrigeration since the storage temperature of 4°C did not determine a rapid death of the animals and the levels of L-lactate in NW4 was statistically similar to the other treatments except W25 which showed significantly higher values of L-lactate.

In sea urchins the pH of the coelomic fluid is generally reported to be lower (0.5–1.5 U) than seawater due to the limited ability of these animals to control gas exchange leading to a slight accumulation of CO2 and other acidic metabolites (Farmanfarmaian 1966, Shick 1983). After emersion and exposure to air a condition of acidosis of the internal fluids seemed more obvious in animals exposed to higher

temperatures, however after 4hrs of exposure the treatments at 15°C showed the lowest pH values. Studies on echinoderms revealed a very high buffer capacity of the coelomic fluid (Catarino et al 2012). Buffer capacity is primarily due to the bicarbonate buffer system of seawater and is also partly due to proteins (immune cells coelomocytes) or other nitrogen products present in the coelomic fluid (Burnett et al 2002, Collard et al 2013, Gellhorn 1927). Exposure to 25°C may have caused greater metabolic stress that triggered a defence reaction resulting in the release of numerous coelomocytes in the coelomic fluid which also acted as a buffer agent. The sharp pH increase in W15 at T2 doesn't have a clear explanation, although we can hypothesize that the low storage temperature, in this case, could have increased the energetic costs of maintenance functions and anaerobiosis which was buffered by the release of coelomocytes; however, this did not occur in the NW15 treatment.

Sea urchin gonad possess relative high levels of FAA which in addition to influencing aroma and taste (De La Cruz-García et al 2000, Liyana-Pathirana et al 2002b, Phillips et al 2010) are known to participate in osmoregulation and in counteracting the dehydration effect during emersion or hyper salinity (Arafa et al 2006, Liyana-Pathirana et al 2002b). In this study free amino acids were not detected but (Arafa et al 2006) report a decrease in the gonad and coelomic fluids nitrogen metabolites with active deamination of free amino acids in gonad and ammonia production after urchin's collection and acclimation to new rearing conditions.

In our trial the sea urchins were exposed to considerable stress which over time led to loss of coelomic fluid and an increase in salinity, this may have triggered the release of free amino acids from the lumen of the gonad into the coelomic fluid in order to counteract dehydration. The loss of FAA from the gonad would determine a further decrease in quality which in addition to affecting the aroma and flavour reduces the shelf life of the product. Further studies are needed to confirm this theory.

4.5.3 Consequences of delay in processing on roe colour

Characteristics of roe quality such as shape and colour are exceptionally important in marketing and determination of price (Sonu 2003). Bright yellow or orange gonad, firm, unbroken can be packed and presented neatly in trays and can be sold at highest prices (Sonu 2003). Signs of roes' quality deterioration are irregular shape of the lobes, melting and dull or brown colour.

In our study we observed a high variation of colour parameters between treatments at T1 but not a general decrease of colour quality, in fact, there were no significant differences with the control at T0. The initial colour variation is attributed to a sex effect; female gonad hold a more intense yellow colour, while males are brighter. The experiment was completed in May when gonad are mature and in gametogenesis. During this phase, the difference in gonad colour between sex is enhanced by the presence of male gametes in the lumen of the phagocytes which results in the detection of a higher White Index (WI) (Chapter 2- Section 2.4.2).

Signs of colour deterioration appeared at T2 and T3, resulting in less variation within treatments, a decrease in luminosity and an increase of redness (a*) on the surface of the gonad. The increasing of the red colour is attributed to the presence of the red spherule coelomocytes that release the red pigment Echinochrome A in the coelomic fluid as immune defence systems (Matranga et al 2005, Matranga et al 2000). The increase in red colour is correlated with the increase of coelomic fluid turbidity determined by the processing delay and exposure to air.

A highly turbid coelomic fluid led to staining and darkening of the gonad and the effect seemed to increase with processing delay. Moreover, when then the coelomic fluid is lost over time for dehydration or damage of the test, the guts lie on the gonad resulting in bleeding of pigments from the guts into the gonad; the staining contributes to a reduction in the roe classification and price. Overall, we observed a loss of colour quality at T2 and T3 in all treatments compared to T1 and at least one-third of the gonad at T3 was visually spoiled and deemed non-marketable.

4.5.4 Conclusions

This study demonstrated that wind, temperature, and prolonged storage prior to processing creates metabolic stress that trigger an anti-inflammatory reaction. Sea

urchins exposed to the wind effect and high temperature lost most of their internal fluid faster than urchins protected from the wind and held at 15°C moreover, the remaining fluid showed high turbidity and salinity which led to gonad dehydration and staining. The prompt storage at 4°C was effective in keeping control of internal parameters like pH, temperature and fluid volume but increased the energetic metabolic costs which determined stress and turbidity. Urchins held at 15°C and protected from the wind showed the best performances retaining most of the internal fluid for a longer period and presenting fewer signs of metabolic stress. After 28hrs all urchins were visibly stressed with few differences in the characteristics of the coelomic fluid, this reflected in the loss of colour quality of the gonad. The dense reddish/brownish colour of the coelomic fluid caused the staining of gonad diminishing the bright yellow appearance that is typical when urchins are fresh and still alive. Outcomes of exposure to harsh conditions after urchin collection and a long processing delay are diminished colour quality characteristics of the gonad, however other factors not investigated here can determine loss of quality for instance dehydration may determine leaking and deamination of free amino acids important for the aroma and flavour and deamination products may confer the gonad an unpleasant aroma.

Best practices would be recommended limiting mechanical damages of urchins' test during harvesting and transport in order to prevent sudden loss of internal fluids. The boating time should be reduced to the minimum to limit the aerial exposure especially during hot days and urchins should be protected from the wind effect and kept humid all the time. Transport and storage temperatures near the ambient water temperature of harvest allow urchins to keep stable internal parameters without increasing the metabolic rate, keeping urchins alive for a longer period. While results here show that with 4°C pre-processing storage, processing should take place within 12hrs to optimise gonad quality, pre-processing storage temperatures nearer to ambient water temperature may extend the processing opportunity time to maximise quality.

4.6 Appendix

Table 4.13. Results of one-way anova of urchin biological measurements between Exposure/Temperature treatments at three processing times.

A

Time 1	T0_12°C_WA	T1_15°C_W	T1_15°C_NW	T1_25°C_W	T1_25°C_NW	T1_4°C_NW	F(5,30) (P)
Total Wt.	354.26±39.54	257.7±25.03	292.06±27	341.6±16.67	373.3±28.32	316.05±28.42	F =2.28; p =0.072;
Dia.	87.83±4.54	87.33±2.57	87.66±3.14	88.33±1.87	97.33±2.55	90.16±3.50	F =1.48; p =0.225;
Drain Wt.	314.26±38.86	237.2±20.29	247.06±25.29	323.68±17.98	344.96±26.82	275.21±23.92	F =2.75; p =0.037;
Gonad Wt.	42.28±4.48	36.90±3.46	40.25±5.88	43.05±2.81	47.00±2.21	36.30±1.44	F =1.19; p =0.335;
GSI	12.18±0.93	14.51±1.26	13.51±1.22	12.71±0.93	12.93±1.02	11.91±1.02	F =0.77; p =0.576;
L*	58.52±0.50	49.28±3.16	56.97±0.45	47.49±1.29	50.98±2.77	56.45±1.95	F =5.37; p =0.001;
a*	7.75±0.22	8.12±0.49	8.92±0.60	9.15±0.34	9.15±0.52	7.17±0.27	F =3.66; p =0.011;
b*	43.22±1.47	39.12±1.54	49.46±1.93	38.11±1.94	41.80±2.82	41.66±1.35	F =4.40; p =0.004;
Chroma	43.91±1.48	39.98±1.50	50.26±1.99	39.20±1.93	42.85±2.67	42.28±1.37	F =4.38; p =0.004;
Hue	1.39±0.00	1.36±0.01	1.39±0.00	1.33±0.00	1.34±0.02	1.39±0.00	F =3.10; p =0.007;

B

Time 2	T2_15°C_W	T2_15°C_NW	T2_25°C_W	T2_25°C_NW	T2_4°C_NW	F	(P)
Total Wt.	285.01±20.84	319.23±20.56	220.8±25.04	261.40±20.45	294.86±17.97	$F_{(4,25)}$ =3.13;	p =0.032;
Dia.	95.63±2.94	100.00±2.97	90.33±3.17	91.83±3.54	94.08±2.99	$F_{(4,25)}$ =1.43;	p =0.254;
Drain Wt.	272.60±20.53	277.90±19.30	212.96±25.01	245.81±15.09	261.86±15.39	$F_{(4,25)}$ =1.81;	p =0.158;
Gonad Wt.	44.05±4.07	39.50±2.69	29.70±3.52	43.48±1.83	40.88±3.92	$F_{(4,25)}$ =3.05;	p =0.036;
GSI	15.40±0.53	12.43±0.64	13.78±1.44	16.93±0.91	13.93±1.27	$F_{(4,25)}$ =2.81;	p =0.047;
L*	45.94±1.05	51.31±1.83	51.29±1.98	47.16±1.12	47.49±1.29	$F_{(4,25)}$ =2.75;	p =0.050;
a*	8.39±0.28	7.75±0.46	9.86±0.51	9.38±0.24	9.15±0.34	$F_{(4,25)}$ =4.75;	p =0.005;
b*	35.27±1.25	35.22±1.97	43.65±3.31	39.26±2.01	38.11±1.94	$F_{(4,25)}$ =2.48;	p =0.070;
Chroma	36.27±1.24	36.09±1.92	44.80±3.20	40.37±1.99	39.20±1.93	$F_{(4,25)}$ =2.73;	p =0.051;
Hue	1.33±0.00	1.35±0.01	1.34±0.02	1.33±0.01	1.33±0.00	$F_{(4,25)}$ =0.23;	p =0.918;

C

Time 3	T3_15°C_W	T3_15°C_NW	T3_25°C_W	T3_25°C_NW	T3_4°C_NW	F	(P)
Total Wt.	277.91±14.39	269.08±20.37	230.93±12.23	261.06±15.94	264.11±9.11	$F_{(4,25)}$ =1.42;	p =0.255;
Dia.	95.83±2.25	94.00±1.57	90.16±1.81	91.66±1.49	88.66±0.66	$F_{(4,25)}$ =3.07;	p =0.035;
Drain Wt.	269.75±12.81	254.73±15.56	220.68±12.44	245.9±11.65	244.86±4.06	$F_{(4,25)}$ =2.23;	p =0.094;
Gonad Wt.	46.91±3.96	34.91±6.14	40.33±2.74	33.58±2.29	41.33±2.88	$F_{(4,25)}$ =1.93;	p =0.137;
GSI	17.06±1.61	12.76±2.11	17.43±0.47	13.11±1.19	15.60±0.80	$F_{(4,25)}$ =2.52;	p =0.067;
L*	41.07±1.63	49.11±1.34	42.95±3.40	45.11±2.60	48.69±1.37	$F_{(4,25)}$ =2.49;	p =0.069;
a*	11.25±0.61	9.03±0.60	8.22±0.61	8.71±0.58	9.50±0.45	$F_{(4,25)}$ =4.05;	p =0.011;
b*	28.48±2.66	39.50±1.67	33.20±2.73	35.54±2.14	37.18±2.94	$F_{(4,25)}$ =2.88;	p =0.043;
Chroma	30.80±2.31	40.54±1.71	34.29±2.57	36.61±2.14	38.39±2.91	$F_{(4,25)}$ =2.52;	p =0.066;
Hue	1.17±0.04	1.34±0.01	1.32±0.03	1.33±0.01	1.31±0.01	$F_{(4,25)}$ =5.94;	p =0.002;

Weight (Wt.) values are given in (gr.), test diameter (Dia.) values are given in (mm), gonad somatic index (GSI) values are given in (%) of the total urchin weight. L*, a*, b*, Chroma and Hue are gonad colour coordinates. Values are presented as mean and standard error of the mean (n = 6).

Table 4.14. Results of one-way anova of urchin coelomic fluid measurements between Exposure/Temperature treatments at three processing times.

A

Time 1	T0_12°C_WA	T1_15°C_W	T1_15°C_NW	T1_25°C_W	T1_25°C_NW	T1_4°C_NW	F(5,30) (P)
Volume	40.0±5.62	20.50±6.12	45.0±2.75	17.91±5.32	28.33±6.18	40.83±6.89	F =4.12; p =0.01;
pH	7.06±0.05	6.49±0.12	6.61±0.06	7.01±0.15	6.91±0.13	6.58±0.04	F =5.15; p =0.00;
T	15.86±0.24	15.35±0.41	13.45±0.17	15.60±0.56	14.45±0.38	14.53±0.38	F =5.57; p =0.00;
Salinity	36.16±0.3	41.16±0.79	37.66±0.55	38.0±0.73	38.33±0.71	38.83±0.40	F =7.25; p =0.00;
Density	1.02±0.0	1.02±0.0	1.02±0.0	1.02±0.0	1.02±0.0	1.02±0.0	F =7.18; p =0.00;
Turbidity	0.0±0.0	0.1±0.03	0.01±0.0	0.06±0.03	0.05±0.02	0.06±0.03	F =2.29; p =0.07;
L*	98.22±1.23	83.92±3.97	97.53±1.42	89.53±4.24	92.74±2.55	88.69±4.29	F =2.92; p =0.03;
a*	1.02±1.19	10.93±3.26	2.04±1.59	8.13±3.70	4.52±1.68	8.53±3.35	F =2.20; p =0.08;
b*	5.33±1.23	32.25±9.20	6.72±1.73	14.21±3.4	7.90±1.55	21.51±6.28	F =4.64; p =0.00;
Chroma	5.80±1.46	34.12±9.71	7.26±2.2	17.20±4.44	9.40±2.03	23.23±7.06	F =4.15; p =0.01;
Hue	0.86±0.45	1.23±0.03	1.39±0.08	0.60±0.44	1.13±0.12	1.25±0.05	F =1.17; p =0.35;

B

Time 2	T2_15°C_W	T2_15°C_NW	T2_25°C_W	T2_25°C_NW	T2_4°C_NW	F	(P)
Volume	12.41±5.94	41.33±5.3	7.83±2.83	15.58±7.52	33.0±8.10	F(4,25) =5.34;	p =0.003;
pH	7.13±0.17	6.57±0.08	6.54±0.10	6.59±0.10	6.67±0.03	F(4,25) =4.93;	p =0.005;
T	18.13±0.10	15.55±0.24	17.63±0.43	16.95±0.82	15.73±0.49	F(4,25) =5.51;	p =0.003;
Salinity	42.33±0.84	38.83±0.54	47.83±1.13	43.33±1.22	39.83±0.47	F(4,25) =15.4;	p =0.000;
Density	1.03±0.0	1.02±0.0	1.03±0.0	1.03±0.0	1.02±0.0	F(4,25) =6.10;	p =0.001;
Turbidity	0.28±0.07	0.10±0.02	0.56±0.13	0.19±0.04	0.22±0.07	F(4,25) =4.72;	p =0.006;
L*	66.25±6.22	83.48±3.34	48.72±7.29	72.55±4.71	71.2±6.41	F(4,25) =4.83;	p =0.005;
a*	23.9±3.98	12.77±3.45	29.15±3.34	21.77±2.83	20.08±3.99	F(4,25) =2.83;	p =0.046;
b*	47.3±6.76	29.22±8.18	60.09±5.92	44.63±7.47	45.88±5.93	F(4,25) =2.52;	p =0.066;
Chroma	53.1±7.70	32.67±8.29	67.73±4.57	49.87±7.73	50.6±6.39	F(4,25) =3.11;	p =0.033;
Hue	1.1±0.03	1.12±0.01	1.1±0.08	1.09±0.04	1.16±0.07	F(4,25) =0.12;	p =0.973;

C

Time 3	T3_15°C_W	T3_15°C_NW	T3_25°C_W	T3_25°C_NW	T3_4°C_NW	F	(P)
Volume	9.80±3.83	17.22±6.95	15.37±4.96	18.20±7.97	19.25±9.15	F(4,20) =0.27;	p =0.894;
pH	6.92±0.23	6.50±0.09	6.74±0.14	6.81±0.15	6.60±0.11	F(4,20) =1.14;	p =0.367;
T	11.90±1.03	9.80±1.0	7.20±1.72	9.06±1.17	13.86±0.40	F(4,20) =6.09;	p =0.002;
Salinity	41.4±2.40	43±1.14	49.25±2.28	44.4±1.02	43.16±1.35	F(4,20) =2.80;	p =0.057;
Density	1.03±0.0	1.03±0.0	1.03±0.0	1.03±0.0	1.03±0.0	F(4,20) =2.60;	p =0.067;
Turbidity	0.41±0.07	0.41±0.07	0.42±0.07	0.45±0.11	0.40±0.12	F(4,20) =0.04;	p =0.996;
L*	55.49±5.54	54.73±5.62	54.44±5.81	53.99±8.14	59.80±9.50	F(4,20) =0.11;	p =0.977;
a*	27.72±3.38	29.67±3.48	29.58±2.95	28.07±3.68	23.06±6.70	F(4,20) =0.37;	p =0.830;
b*	56.39±5.55	62.62±3.88	69.34±4.20	58.47±7.32	51.69±4.86	F(4,20) =1.46;	p =0.252;
Chroma	62.98±6.13	69.59±4.13	75.46±4.74	65.02±7.87	57.89±6.25	F(4,20) =1.14;	p =0.366;
Hue	1.11±0.03	1.13±0.04	1.17±0.02	1.12±0.03	0.67±0.43	F(4,20) =0.85;	p =0.513;

Volume is given in mL. L*, a*, b*, Chroma and Hue are coelomic fluid colour coordinates. Values are presented as mean and standard error of the mean (n = 6).

Table 4.15. Results of 2-way ANOVA for L-lactate detected in urchins coelomic fluid at different processing time and exposure treatments.

Source	Type III Sum of Squares	df	Mean Square	F	P-value
Corrected Model	179077.824a	14	12791.27	7.25	0.001
Intercept	754190.48	1	754190.48	427.50	0.001
Time	100883.40	2	50441.70	28.59	**0.001**
ExpTemp	49945.61	4	12486.40	7.08	**0.001**
Time * ExpTemp	14842.46	8	1855.31	1.05	0.433
Error	35283.54	20	1764.18		
Total	964842.93	35			
Corrected Total	214361.36	34			

Table 4.16. Results of L-lactate measurements in urchins coelomic fluid.

A	Time	T0	T1	T2	T3
	L-lactate	70.09 ± 0.59	84.03 ± 3.51	154.50 ± 20.61	224.91 ± 23.22
B	**ExpTemp**	**W15**	**NW15**	**W25**	**NW25**	**NW4**
	L-lactate	119.96 ± 18.6	95.31 ± 15.56	223.34 ± 32.64	149.79 ± 30.76	143.74 ± 31.02

Processing time T0 n=6; T1-T3 n=30 (A) and Exposure/Temperature treatment n=18 (B). Results are mean values ± standard error and expressed in µM/ml.

Chapter 5 Tomato growth and productivity using dried powdered processing waste from sea urchin (*Centrostephanus rodgersii*) fishery

5.1 Abstract

In this study, a greenhouse experiment with tomato plants was conducted to evaluate the influence of the long-spined sea urchin *Centrostephanus rodgersii* processing waste on growth and productivity of tomato. The long-spined sea urchin mineral parts, spines, test and jaws were comprised of Ca 40%, Mg 1.7%, Fe 19.34 ppm, B 38 ppm, had an alkaline pH 8.06 in water and electrical conductivity value of 7.64 dS/m. Dried and finely ground urchin waste powder (UWP) at seven different rates (0.3%; 0.5%; 0.8%; 1%; 2%; 3%; 5%) was applied to 4 kg potting mix. The pots were planted with tomato seedlings variety K1 and the effects compared against control pots receiving Hoagland solution. Plant growth, yield, mineral content and quality attributes of tomato were assessed. Results showed that UWP had an influence on tomato growth and productivity proportional to the quantity applied, however, the Hoagland solution control had a significantly greater yield. The soil pH increased from 6.8 to 7 and higher available phosphorus was also detected in soils receiving higher rates of UWP. No phytotoxic effects were detected despite the high concentration of calcium in soil and higher EC values detected at the highest rate UWP. Although, there is some inconsistency in the results of the nutritional compositions of tomato fruits, the highest UWP treatment matched the Hoagland control presenting good quality fruit and nutritional values. In conclusion, *C. rodgersii* UWP has strong potential as a mineral fertiliser providing plant-available Ca and some microelements such as Boron. As there is a strong push for sustainable nutrient sources, sea urchin waste may provide a useful alternative for organic or biodynamic farming systems.

5.2 Introduction

Globally, the commercial sea urchin fishing industry harvest fluctuates between 60,000 and 75,000 tons annually (Fishery & Statistics 2016, Stefansson et al 2017). The Japanese market is the biggest consumer and importer of sea urchins, followed by France and Korea while in other countries such as Chile, New Zealand and Italy there is a smaller domestic market (Andrew et al 2002). Urchins are sold live or processed and are prized for their edible parts, the gonad or "roe". The yield of gonad is variable among different species and ranges between 5 and 15% of total body weight. The remaining parts of the sea urchin (guts, test, spines and jaws) are considered waste whose global amount is around 64,000 tons.

Processing of seafood from industrial fisheries results in considerable waste, posing logistical disposal and environmental problems (Ravindran & Jaiswal 2016). Animal processing leftovers are considered to be a potential resource and has recently been termed "rest raw materials" (Rustad et al 2011). Seafood by-product waste material cannot be sold as a value-adding product such as fillet, round, eviscerated or beheaded fish, but rather as biological materials that can be recycled after treatment. When treatment and reuse are not possible, the seafood waste must be destroyed through incineration, added to landfill or composted adding significant expense to the fishery (Rustad 2002). In Australia, the seafood industry produces more than 50,080 tons of fish waste at the manufacturing stage (Verghese & Lockrey 2019) and on average processors pay more than $200/t for removal to landfill (Knuckey et al 2004). The reuse and repurposing of waste are of critical importance as the world's population and the amount of waste produced continues to increase (Lehmann 2011).

Several studies have investigated the potential uses and application of fishery by-products as sources of proteins and lipids in feed (Datta 2013), extraction of bioactive compounds useful in pharmaceutical and cosmetic (Sen et al 2016) and as fertilizers in agricultural crops (López-Mosquera et al 2011). In an agricultural context, the application of products made from animal excreta, animal processing wastes or food processing waste can be used to improve the structure and stability of the soil (Edmeades 2003, Ge et al 2010, Reardon & Wuest 2016) in addition to enhancing the yield and quality of the crop plants (Golabi et al 2007, Hammed et al

2019, Mahmoud et al 2009). Food waste products provide an organic, more sustainable nutrient source alternative to synthetic fertilisers. Extensive use of inorganic fertilizers is associated with downstream environmental pollution (Good & Beatty 2011) and alteration of the physical properties of the soil (Mulvaney et al 2009, Savci 2012) with subsequent effects on the nutritional value of the crop (Geng et al 2019). As there is increasing sensitivity to environmental issues associated with agriculture and greater awareness from consumers who are looking for sustainably produced crops, investigation into the agronomic benefits of utilising processing waste as an alternative to synthetic fertilisers provides an opportunity to decrease costs while increasing environmental sustainability.

Centrostephanus rodgersii is a large echinoid found in southeastern Australian coastal waters and in the past four years, approximately 830 tons of urchins have been harvested resulting in an estimated 705 ton of waste. The elemental content of *C. rodgersii* endoskeleton is still unknown but different studies have characterized the mineral composition of various species of sea urchins including *Strongylocentrotus intermedius*, *Mesocentrotus nudus*, *Scaphechinus mirabilis*, and *Echinocardium cordatum* from the Japan sea (Drozdov et al 2016), the red (*Strongylocentrotus franciscanus*) and green (*Strongylocentrotus droebachiensis*) sea urchins from the West and East coasts of Canada, respectively (Amarowicz et al 2012) and *Paracentrotus lividus* from the coasts of Sardinia in the Mediterranean sea (Garau et al 2012). High calcium and relatively high magnesium content were found in all species with N, P, K in minor quantity and among the micronutrients identified were Fe, Zn, Mn, Cu, Cd, Pd etc. In urchins from the Japan Sea, Zr and Sr were always present while Sn, Sb, Rb and Ba were alternatively present and attributed to the place of collection of the animals (Drozdov et al 2016).

Several studies have demonstrated the potential use of calciferous waste generated by bivalve farming. Lee et al (2008) tested the effect of oyster shell powder on soil cultivated for cabbage and demonstrated an increase in soil pH and generally improved soil chemical and biological properties resulting in increased cabbage productivity. Oyster powder was also used to extend the shelf life of tofu and shredded cabbage (Choi et al 2006, Kim et al 2007). Mussel shells (grounded and calcinated) and lime were compared as amendment in soil with a low pH (Álvarez

et al 2012), resulting in comparable increases to soil pH, exchangeable Ca and decreased exchangeable Al. The amendment also had a positive effect on dry matter yield and concentration of Ca in the plants. Sea urchin (*Paracentrotus lividus)* waste was assessed by (Garau et al 2012) as an amendant in acidic soil proving to significantly increase soil pH and electrical conductivity, available phosphorus, active carbonate as well as microbial abundance and activity (Garau et al 2012).

This study aims to test the use of waste produced by the fishery for longspined sea urchin *C. rodgersii* as potential mineral fertilizer. We selected Tomato as our model species due to its salt tolerance (Bergmann 1992) and the expectation that sea urchin powder is likely to increase the electrical conductivity (EC) of the soil. Using a base soil medium with known properties, we investigated whether tomato plants take up nutrients derived from sea urchin powder. Specifically, we tested productivity and fruit quality of tomato using powdered dry sea urchin waste at increasing rates against a standard nutrient fertiliser regime.

5.3 Materials and methods

5.3.1 Processing of sea urchin waste material.

Waste material from the sea urchin *C.* rodgersii collected along the east coast of Tasmania was stored after processing to extract roe for human consumption. The processing waste including tests (endoskeletons), spines and jaws were rinsed with tap water to eliminate salts residue and oven-dried for 24 hr at 105 °C. Dried material was finely ground using a grinding mill (A11 analytical mill, IKA, Staufen, Germany) and samples were sent to SWEP Laboratory (Victoria, Australia) for nutrient analysis to determine elemental composition and physico-chemical parameters of urchin powder. Specifically, P, K, S, Ca, Mg, Na, Fe, Mn, Zn, Cu, Co, B, Mo were determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES) after acid digestion, N was determined by the Dumas method (Dumas 1831), pH of the powder in water (ratio 1:5) was measured with a pH reader (Rayment & Higginson 1992), electrical conductivity (EC) was determined in a water extract and organic carbon with LECO carbon analyser following Rayment and Lyons (2011). Urchin waste powder mineral characterization provided insights for determining the rate of sea urchin waste additions for the experimental pot trial.

5.3.2 Pot trial establishment.

A potting mix was prepared comprising 90% composted pine bark, 5% sand, 5% cocopeat, plus 3 kg dolomite/0.5m^3 to produce a consistent growing medium with sufficient structure for plant growth. Urchin waste powder (UWP) was added at seven different treatment rates (0.3%; 0.5%; 0.8%; 1%; 2%; 3%; 5% by weight) into 4 kg of the potting mix at the commencement of the trial with ten replicate pots per treatment (Table 5.1). The potting mix used in the pot trial was very low in macronutrients N, P, K and Ca. Conversely, the potting mix had a higher content of Zn, Fe and Mn compared to urchin powder, but was low in B. The initial EC and pH were 0.470 dS/m and 7.30 respectively (Table 5.1). Additional treatment with a standard Hoagland solution with ten pot replicates was used as the control of which 400 ml was applied twice a week for 12 weeks. The Hoagland solution is a composition which formula/recipe provides every essential nutrient required by

green plants to support growth. The nutrient profile of the growing medium over the life of the experiment varied between the potting mix plus Hoagland solution and potting mix plus urchin supplement (Table 5.1), and this was intentional as the objective was to compare urchin powder supplement against a standard control medium. The pot treatments were set up the 3rd of November 2017 and left with irrigation for a week to allow the UWP to stabilize with the potting mix. One week after the preparation of the pot treatments, the 10th of November 2017, three tomato (*Solanum lycopersicum*) seedlings (variety K1) were added to each pot and after two weeks the strongest plant was retained, and the others discarded. pH and EC measurements of growing medium for the eight treatments were recorded three days post-planting and before the addition of Hoagland solution in the control treatment and at the conclusion of the trial. Plants were maintained for 12 weeks in a greenhouse at the Horticulture Centre of the University of Tasmania. The experiment underwent a natural photoperiod, in an uncontrolled temperature environment and pots received automatic irrigation for two minutes, six times over 24 hr.

Table 5.1. Nutrient composition of the potting mix with powdered sea urchin waste applied at different rates and the total applied in the Hoagland control

Element	Unit	Potting mix	Hoagland Solution mg/Pot/ Week	Total addition of Hoagland solution mg/Pot	Urchin Waste Powder	Urchin waste powder application rates (g./Pot)						
						0.30%	0.50%	0.80%	1%	2%	3%	5%
						12gr	20gr	32gr	40gr	80gr	120gr	200gr
Ca	% w/w	0.383 (0.01)	173	2076	40.40 (0.67)	4.85	8.08	12.93	16.16	32.32	48.48	80.80
Mg	% w/w	0.057 (0.001)	29.4	352.8	1.77 (0.02)	0.21	0.35	0.57	0.71	1.42	2.12	3.54
Na	% w/w	0.012 (0.001)	0.0034	0.0408	1.35 (0.16)	0.16	0.27	0.43	0.54	1.08	1.62	2.70
K	% w/w	0.164 (0.005)	140.4	1684.8	0.26 (0.03)	0.031	0.052	0.083	0.104	0.208	0.312	0.52
S	% w/w	0.0057 (0.0013)	38.4	460.8	0.47 (0.08)	0.056	0.094	0.150	0.188	0.376	0.564	0.94
N	% w/w	0.0007 (0.0001)	162.5	1950	0.50 (0.07)	0.060	0.100	0.160	0.200	0.400	0.600	1.00
P	% w/w	0.0029 (0.0002	18.6	223.2	0.03 (0.003)	0.004	0.006	0.010	0.012	0.024	0.036	0.06
Cu	ppm w/w	0.87 (0.19)	0.02	0.24	0.60 (0.12)	0.072	0.12	0.19	0.24	0.48	0.72	1.20
Zn	ppm w/w	18.06 (1.21)	0.05	0.6	6.36 (2.24)	0.76	1.27	2.04	2.54	5.09	7.63	12.72
Fe	ppm w/w	63.26 (7.34)	0.3	3.6	19.34 (5.60)	2.32	3.87	6.19	7.74	15.47	23.21	38.68
Mn	ppm w/w	38.30 (2.63)	0.47	5.64	1.87 (0.96)	0.22	0.37	0.60	0.75	1.50	2.24	3.74
Mo	ppm w/w	n/d	0.072	0.864	0.114(0.027)	0.014	0.023	0.036	0.046	0.091	0.14	0.23
B	ppm w/w	0.68 (0.006)	0.31	3.72	38.11 (1.86)	4.57	7.62	12.20	15.24	30.49	45.73	76.22
EC	dS/m	0.470 (0.06)	1.70	1.70	7.64 (0.974)							
pH Level	1:5 Water	7.30 (0.10)	5.8	5.8	8.06 (0.10)							

Values in parenthesis represent standard error of the mean (n = 3).

5.3.3 Plant and soil assessments

The dynamics of plant growth was recorded with weekly measurements of a range of plant growth and reproductive characteristics. Individual plant height and width (cm) were obtained using a ruler, and stem cross-section area (mm) using Vernier callipers. The number of fully-grown branches, flowers and fruits was recorded for each plant weekly. At the end of the trial, the vegetative and reproductive weights of all tomato plants were calculated for each of the eight treatments. Each plant was cut at the base and the fresh weight recorded, then plants were oven-dried for 48 hrs and dry weight and moisture content calculated. Five branches per plant from each treatment were cut and sent for analysis of nutrients. Three replicates of standard potting mix and three replicates of soil from each treatment (10 g per sample) were collected at the end of the trial, sieved through a 2 mm mesh and air-dried for approximately two weeks in aluminium foil trays. Dried samples from each treatment were pooled to make a composite sample and sent for analysis to determine changes in soil chemical parameters after the application of the fertilizer (sea urchin waste) and the use of nutrients by the plants.

At the conclusion of the trial fruits from each plant were counted and weighed, and the total yield per plant calculated. Harvested fruits were analysed for their fruit quality attributes (fruit colour, weight, diameter), and other physico-chemical traits such as Degrees of Brix (°Brix is a measure of the amount of dissolved solids in the fruit juice), acidity, pH, firmness, dry matter content and nutrient composition. To assess fruit quality attributes, fruits of similar ripe stage (maturity) were selected for comparison. Colour intensity through the coordinate L*, a* and b* was recorded with a colour meter (Chroma Meter CR-400, Konica Minolta, Tokyo, Japan) in three spots around the pericarp and values averaged. Values of a* are negative in green tomato and become positive when fruits start to develop red colour. Negative values represent unripe fruits while higher hue angle shows fruits in different ripening stages. Red is better represented by the hue angle which explains the colour change associated with the enzymatic degradation of chlorophylls and the appearance of lycopene, a red carotenoid pigment (Su et al 2015). Fruits with minimum positive hue angle are fully ripe and show an intense red colour. Fruit firmness was measured with a compression meter (Güss fruit texture analyser,

Strand, South Africa) which expressed deformation of the pericarp in millimetres in response to the applied load of 50 g for 0.4 s on the surface of the fruit using a 2 mm cylindrical probe at 4 mm depth. Each fruit was also dissected transversely to count the number of locules and to measure the pericarp thickness in mm at two locations on each fruit with a Vernier calliper and averaged. Following physical assessments, a random subsample of the fruits was sliced, weighed and placed in an aluminium tray then oven-dried at 60 °C for 4 days. After drying, samples were weighed, and dry matter content and moisture calculated. Dried fruit samples from the same replicate were pooled together and sent to CSBP Laboratories for nutrient composition analysis. Remaining fruits were pureed through a thin mesh, centrifuged and the extracted juice used to estimate total soluble solids, pH and titratable acidity. Total soluble solids (TSS) (˚brix) were determined with a hand refractometer (Atago 3810 pal-1, Fukaya, Saitama, Japan). The refractometer was washed with distilled water after each assessment use and dried with blotting paper. Fruit acidity and pH was determined using a titrator (HI84532 fruit titratable acidity Hanna Instruments, Melbourne, Australia).

At the end of the trial, the total amount of each nutrient that was added with the Hoagland solution during the 12 weeks was compared with the amount of each nutrient provided with the mineral fertilizer (urchin powder) for the seven treatments.

5.3.4 Statistical analysis

The data are presented as the mean values with standard errors. Mineral macronutrients are presented in percentage of weight by weight (%w/w) equivalent of g/100g and micronutrients are presented in part per million (ppm) equivalent of mg/Kg. One-way ANOVA was performed to compare the effects of UWP treatments and Hoagland control on tomato plants growth at the end of the experiment and tomato yield and fruit quality parameters. Homogeneity of variances was verified with Levene's test. Two-way ANOVA with repeated measures was used on stem height, branches number, stem CSA, flower number and fruit number to analyse the interaction between fertilizer treatments and weekly measurements. Differences at the 5% significance level were compared using Tukey's Honestly Significant Difference (HSD) test. PERMANOVA tests were performed on

Euclidean distance matrix for leaf and tomato fruit nutrients content and fruit characteristics between each treatment and control to indicate significance of tested factors. Statistical analysis of One-Way ANOVA and Two-Way ANOVA with repeated measure were performed with SPSS (IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp.). PERMANOVA test and nMDS plots were performed using PRIMER 7 (Plymouth Routines In Multivariate Ecological Research) (Clarke & Gorley 2015).

5.4 Results

5.4.1 Tomato growth and productivity

5.4.1.1 Effect of urchin powder supplement on weekly plant growth

All plant growth variables increased with increasing dried UWP treatments, with plant performance in some variables equivalent to that observed in the Hoagland solution control treatment (Figure 5.1, Table 5.3). Weekly measurements of plant growth parameters (height, branches and CSA) showed overall statistically significant differences in group means for the interaction between treatments and sampling time as determined by two-way ANOVA with repeated measures (Figure 5.1, Table 5.2). At the end of the experiment, shoot length and stem CSA of tomato plants receiving the highest sea urchin treatment (Treatment 7) were not significantly different (Table 5.3, Tukey's test, α=0.05) from the standard Hoagland's solution. Shoot length, stem CSA and plant width had a moderate increase in the lowest three UWP treatments, with no significant difference (Tukey's test, α=0.05) in these plant parameters (Figure 5.1, Table 5.3). Mean plant width (leaf area) for T7 (5% urchin supplement) was significantly higher than all other treatments including Hoagland's control (Figure 5.1, Tukey's test, α=0.05). Branches of tomato plants receiving the highest UWP (T6, T7) were not significantly different but had statistically fewer branches than the Hoagland's control (T8), (Figure 5.1, Table 5.3, Tukey's test, α=0.05). Tomato plants receiving the Hoagland's control had the greatest total dry matter mass which significantly decreased with each rate of sea urchin powder from T7 to T4 (Figure 5.1, Tukey's test, α=0.05). Dry matter content expressed as a % of the total wet weight was greatest for Hoagland's control but not significantly different to the highest UPW treatments (Table 5.3, Tukey's test, α=0.05).

Table 5.2. Two-way ANOVA with repeated measure of weekly tomato plant growth parameters.

	Height	Branches	CSA
Tr	df=7; F=14.67; P<.0001	df=7; F=21.8; P<.0001	df=7; F=13.4; P<.0001
St	df=2; F=2609; P<.0001	df=2; F=575.2; P<.0001	df=2.7; F=1157.7; P<.0001
Tr*St	df=12; F=20; P<.0001	df=13; F=17.2; P<.0001	df=18.8; F=9.65; P<.0001

Tr: treatments; St: sampling time; df: degrees of freedom.

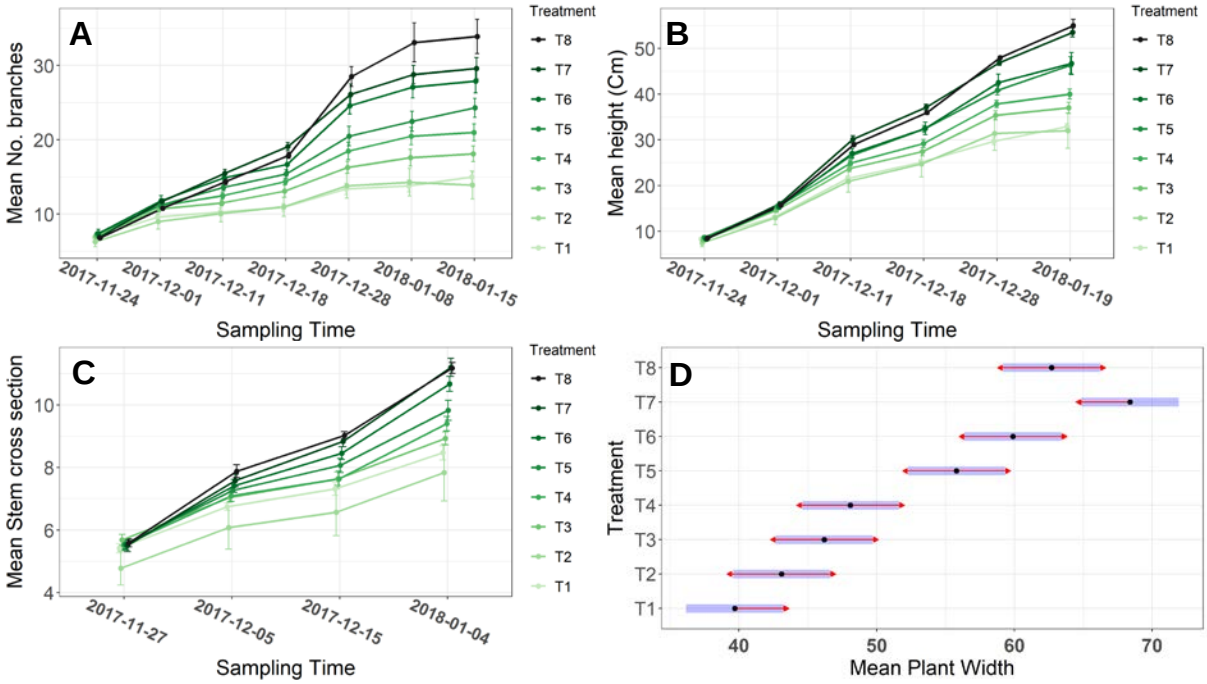

Figure 5.1. Line graphs of plant growth measurements including number of branches (A), stem height (B), stem cross sectional area (C) and leaf width at the completion of the trial (D) for increasing sea urchin powder supplement rates (T1 = 0.3%; T2 = 0.5%; T3 = 0.8%; T4 = 1%; T5 = 2%; T6 = 3%; T7 = 5%), and Hoagland's solution (T8). X-axis represents sampling event in weeks after planting. Error bars denote standard error and letters above the bars represent result of Tukey (HSD) post-hoc test.

Table 5.3. Vegetative growth parameters of tomato plants in greenhouse pot trial under sea urchin waste powder treatments.

	Shoot length (cm)	Stem CSA (mm)	Plant width (cm)	Branches (n◦)	Plant dw. (gr.)	Plant dmc (%)
T1	32.9 (2.08)a	8.47 (0.73)a	39.7 (4.16)a	15.0 (2.71)a	5.61 (1.29)a	17.66 (1.54)abc
T2	32.0 (12.3)a	8.66 (0.80)ab	43.1 (5.53)a	15.4 (3.40)ab	6.96 (2.19)ab	16.65 (1.29)a
T3	37.0 (3.94)a	8.93 (0.66)abc	46.2 (6.30)ab	18.1 (3.31)abc	8.39 (2.74)ab	16.94 (1.21)ab
T4	40.0 (3.40)ab	9.40 (0.72)abc	48.1 (4.38)ab	21.0 (3.59)bc	9.58 (2.84)b	17.48 (2.14)abc
T5	46.3 (5.93)bc	9.83 (1.01)bcd	55.8 (7.30)bc	24.3 (4.00)c	15.39 (4.96)c	17.82 (0.68)bc
T6	46.7 (7.57)bc	10.67 (0.76)cd	59.9 (6.72)cd	27.9 (5.02)d	21.48 (7.16)d	18.00 (0.86)cd
T7	53.5 (3.10)d	11.20 (0.90)e	68.4 (5.02)e	29.6 (4.55)d	32.14 (4.73)e	18.19 (0.58)d
T8	55.0 (4.19)d	11.18 (0.55)e	62.7 (4.60)cd	33.9 (7.23)e	38.63 (4.34)f	19.00 (0.47)d

Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05. Values in parenthesis represent standard error of the mean (n = 10).

5.4.2 Final nutrient composition of plant vegetative parts.

5.4.2.1 Plant nutrient levels as a measure of soil-nutrient uptake.

Nutrient (Total N, P, K and Ca and Mg) concentrations in the vegetative (combined shoots and leaves) parts of the plant showed an increasing response to higher UWP treatments (Table 5.4). All macronutrients increased significantly between T6, T7 and T8 (Table 5.4_A, Tukey HSD, α=0.05). A similar trend of increasing

micronutrient levels with increasing urchin powder was also observed (Table 5.4_B). Plants grown in the Hoagland's solution (T8) contained higher nutrient concentrations than plants in the highest sea urchin treatment (T7) for all nutrients including Ca, Mg and micronutrients such as B, Zn, Fe and Mn (Table 5.4).

Table 5.4. Nutrient concentration in vegetative parts (combined shoot and leaf) of tomato plant in greenhouse pot trial with sea urchin waste powder treatments.

A	Tot. N (% w/w)	P (% w/w)	K (% w/w)	Ca (% w/w)	Mg (% w/w)	Na (% w/w)
T1	0.08 (0.001)a	0.018 (0.001)a	0.17 (0.005)a	0.16 (0.009)a	0.031 (0.002)a	0.002 (0.0004)a
T2	0.09 (0.006)a	0.018 (0.002)a	0.21 (0.021)a	0.26 (0.026)a	0.042 (0.002)a	0.003 (0.0001)a
T3	0.11 (0.003)a	0.018 (0.001)a	0.23 (0.001)a	0.31 (0.025)ab	0.050 (0.004)ab	0.003 (0.0003)a
T4	0.13 (0.008)a	0.018 (0.001)a	0.26 (0.012)ab	0.31 (0.011)ab	0.053 (0.002)ab	0.003 (0.0004)a
T5	0.22 (0.010)b	0.027 (0.001)a	0.36 (0.015)bc	0.50 (0.020)bc	0.076 (0.006)bc	0.004 (0.0006)a
T6	0.31 (0.011)c	0.029 (0.003)ab	0.44 (0.025)c	0.68 (0.073)cd	0.098 (0.012)c	0.008 (0.0008)b
T7	0.45 (0.014)d	0.040 (0.003)b	0.63 (0.027)d	0.80 (0.026)de	0.135 (0.002)d	0.014 (0.0001)c
T8	0.76 (0.019)e	0.076 (0.005)c	1.02 (0.033)e	0.94 (0.068)e	0.182 (0.013)e	0.010 (0.0014)b
B	S (% w/w)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	B (mg/kg)
T1	0.023 (0.001)a	0.17 (0.06)a	3.25 (0.30)a	1.67 (0.11)a	0.95 (0.06)a	2.16 (0.09)a
T2	0.032 (0.001)ab	0.16 (0.05)a	3.80 (0.29)a	2.89 (016)ab	1.25 (0.12)a	2.87 (0.14)ab
T3	0.036 (0.003)ab	0.12 (0.03)a	4.06 (0.33)a	3.34 (0.42)bc	1.34 (0.11)ab	3.17 (0.19)ab
T4	0.035 (0.002)ab	0.10 (0.001)a	3.81 (0.07)a	3.53 (0.23)bc	1.41 (0.18)ab	3.29 (0.09)ab
T5	0.053 (0.002)bc	0.24 (0.09)a	6.77 (0.55)ab	4.86 (0.27)cd	2.26 (0.27)bc	4.93 (0.11)bc
T6	0.066 (0.007)c	0.35 (0.04)a	8.55 (0.70)b	6.40 (0.49)d	2.78 (0.38)c	6.83 (0.74)c
T7	0.076 (0.003)c	0.30 (0.03)a	12.41 (0.94)c	9.86 (0.34)e	4.11 (0.20)d	9.73 (0.66)d
T8	0.155 (0.013)d	1.07 (0.17)b	17.61 (1.52)d	13.31 (0.46)f	5.13 (0.12)e	15.93 (0.85)e

Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and $P < 0.05$. Values in parenthesis represent standard error of the mean ($n = 3$).

The multivariate analysis on the proportion of leaf nutrient content showed overall statistically significant difference among treatment groups (Table 5.5). The nMDS plot reveals three clusters (Figure 5.2) of plant nutrient proportions where the four lowest UWP addition rates (T1-T4) form a tight grouping while two separate clusters are evident for T7 and T8, highlighting improved vegetative production and nutrient uptake in plants receiving the highest UWP addition (T7) but further still, the better overall performance of plants receiving the Hoagland's solution (T8).

Table 5.5. Results of PERMANOVA analysis of proportion of leaf nutrient content for UWP treatments (T1-T7) and Hoagland control (T8).

Source	df	MS	Pseudo-F	P-value	Unique perms
Tr	7	352.36	59.234	**0.001**	998
Res	16	5.9487			
Total	23				

Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference (P<0.05) is indicated in bold.

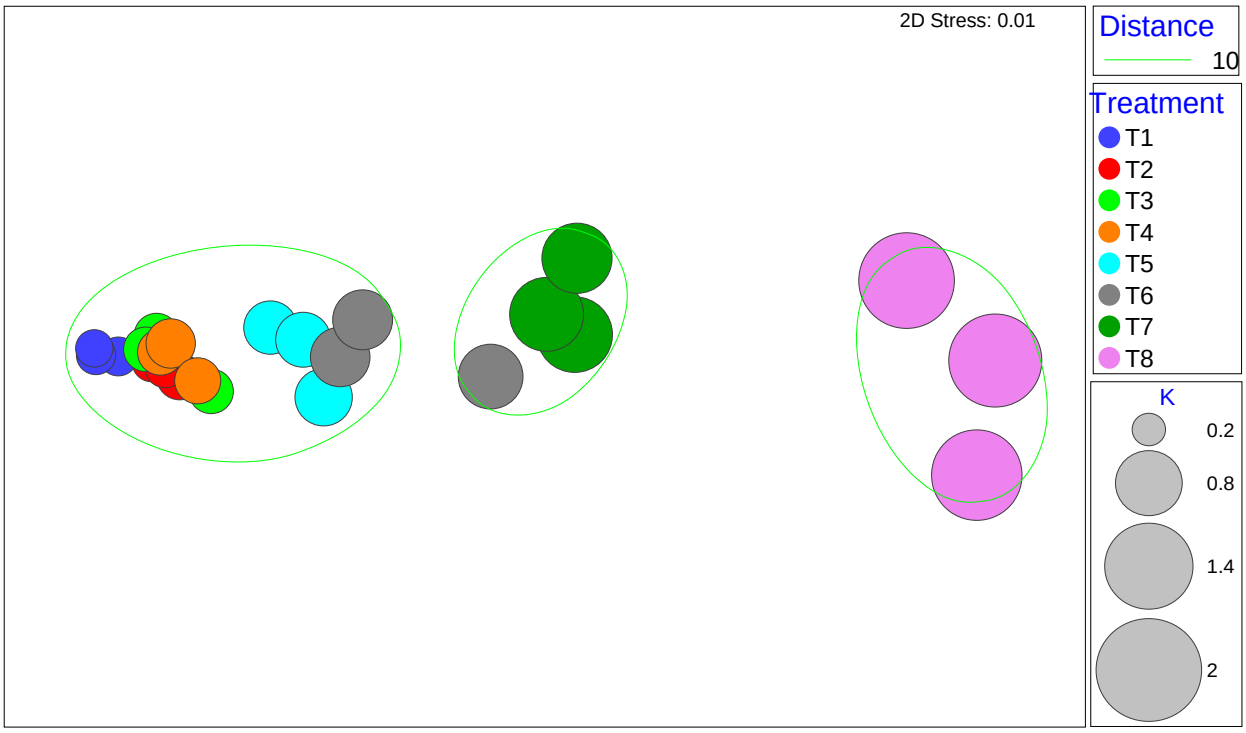

Figure 5.2. Non-metric multidimensional scaling (nMDS) bubble plot of Euclidian distances between the proportion of nutrients in tomato plant vegetative parts receiving sea urchin waste powder treatments (T1 = 0.3%; T2 = 0.5%; T3 = 0.8%; T4 = 1%; T5 = 2%; T6 = 3%; T7 = 5%) and the Hoagland control (T8). Bubble sizes indicate K content (w/w%).

5.4.3 Effect of UWP on flowering and fruit production

Overall statistically significant differences were observed in the weekly measurements between group means of flowers and fruits productivity for the interaction between treatments and sampling time as determined by two-way ANOVA with repeated measures (Table 5.6, Figure 5.3). Plant flowering and fruiting success measured at the end of the trial was variable across the treatments. A trend of increasing number of flower and fruits was observed with increasing application of UWP, however there were no clear statistical differences between the lowest four UWP treatments (Figure 5.3, Table 5.7, Tukey's test, α=0.05). Yet, a

main effect of UWP fertiliser was evident in T5, T6, and T7 (Figure 5.3) for these factors. Flower number receiving the highest UWP (T7) was not significantly different (Table 5.7, Tukey's test, α=0.05) from the standard Hoagland's solution.

Table 5.6. Two-way ANOVA with repeated measure of weekly tomato plant measurement of flower and fruit production.

	Flower	Fruit
Tr	df=7; F=27.14; P<.0001	df=7; F=47.00; P<.0001
St	df=2; F=114.91; P<.0001	df=2; F=49.26; P<.0001
Tr*St	df=12.6; F=17.00; P<.0001	df=15; F=10.67; P<.0001

Tr: treatments; St: sampling time; df: degrees of freedom.

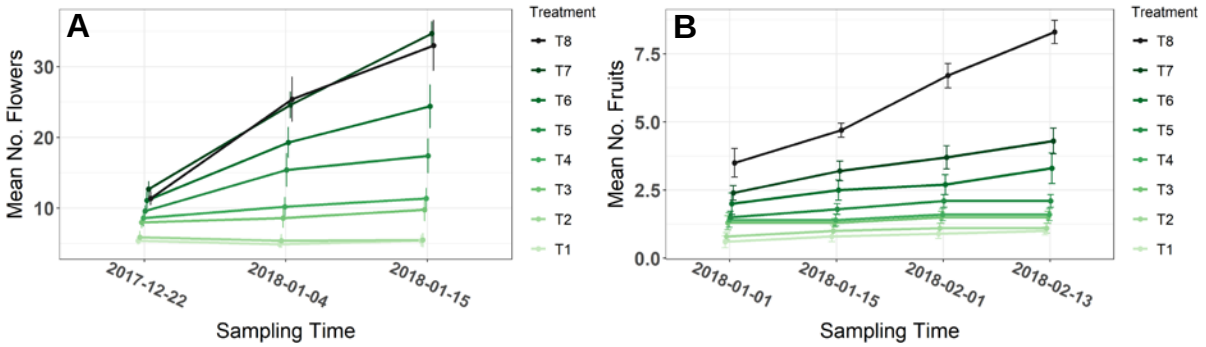

Figure 5.3. Line graphs of flower (A) and fruit (B) production for eight treatments representing increasing urchin powder supplement rates (T1 = 0.3%; T2 = 0.5%; T3 = 0.8%; T4 = 1%; T5 = 2%; T6 = 3%; T7 = 5%), and Hoagland's solution (T8). X-axis represents sampling event in weeks after planting.

Table 5.7. Average flower number, fruit number, yield and fruit weight of tomato plants in greenhouse pot trial under sea urchin waste powder treatments (T1 – T7) and Hoagland's control (T8).

	Flowers (n◦)	Fruits (n◦)	Fruits yield (gr.)	Fruit Wt. (gr.)	Pericarp (mm)
T1	5.4 (2.50)a	1.1 (0.47)a	23.64 (17.57)a	21.49 (4.53)a	4.64 (1.35)ab
T2	6.1 (2.42)a	1.2 (0.57)a	41.12 (20.99)ab	34.27 (5.11)ab	4.88 (0.74)a
T3	9.8 (4.96)ab	1.5 (0.71)ab	65.22 (22.59)bc	43.48 (6.46)ab	4.75 (0.93)ab
T4	11.4 (4.43)ab	1.6 (0.70)ab	69.19 (25.33)bc	43.24 (6.65)ab	4.00 (0.91)bc
T5	17.4 (7.69)bc	2.1 (0.74)b	84.95 (27.95)c	40.45 (6.70)ab	3.60 (0.78)c
T6	24.4 (9.59)cd	3.3 (1.77)c	144.3 (45.10)d	43.72 (6.77)ab	4.00 (0.94)bc
T7	34.7 (5.19)e	4.3 (1.49)d	238.1 (63.20)e	55.36 (9.70)b	5.00 (0.78)cd
T8	33.0 (11.16)e	8.3 (1.34)e	448.1 (74.00)f	53.99 (5.54)ab	5.75 (0.35)d

Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05. Values in parenthesis represent standard error of the mean (n = 10).

Average fruit number ranged from one fruit per plant in the lowest UWP treatment to eight fruits per plant in the Hoagland's control (Table 5.7) with significant differences in the mean values observed between the three highest rate treatments

(T5 to T7) and the control (T8) ($F_{(7,72)}$=52.57, P=.000). Average fruit size and overall fruit weight (total yield) increased with increasing UWP ($F_{(7,176)}$=2.844, P=.008). Plants receiving the highest UWP (T7) yielded 238.1g fresh fruit which was almost half of the fresh fruit produced by plants in the Hoagland's treatment (448.1g) (Table 5.7, Tukey's test, α=0.05).

While there was a trend of decreasing percentage of fruit dry matter from T1 (7.3%) – T7 (6.7%) and T8 (6.5%), there were no significant differences in mean fruit dry matter per plant ($F_{(6,64)}$=.915, P=.501). Locule number was similar across all treatments varying from (6.00) to (8.00), ($F_{(7,72)}$=.489, P=.840). Pericarp thickness ranging from a minimum of 3.60 (T1) mm and a maximum of 5.75 (T8) mm and tended to significantly increase with higher UWP rates (Table 5.7, $F_{(7,72)}$=2.951, P=.009).

5.4.4 Fruit nutrition and quality

5.4.4.1 Fruit nutrient levels as a measure of soil-nutrient uptake

Fruit nutrient concentrations increased in response to higher rates of UWP (Table 5.8). An increasing level of macronutrients (Total N, P, K and Ca and Mg) is noted as result of the increased rate of UWP. Both macro and micronutrients exhibit a mild increase in the first five treatments with no statistical differences observed (Table 5.8, Tukey HSD, α=0.05), in contrast, nutrients significantly increased between T6, T7 and T8 (Table 5.8, Tukey HSD, α=0.05). Tomato fruits grown in the Hoagland's solution (T8) contained higher concentration than fruits in the highest UWP treatment (T7) for all nutrients including Ca, Mg and micronutrients such as B, Zn, Fe and Mn (Table 5.8).

Table 5.8. Nutrient concentration in fruit of tomato plants receiving sea urchin waste powder treatments (T1 – T7) and Hoagland's control (T8).

A	Tot. N (% w/w)	P (% w/w)	K (% w/w)	Ca (% w/w)	Mg (% w/w)	Na (% w/w)
T1	0.039 (0.003)a	0.017 (0.001)a	0.146 (0.004)a	0.006 (0.0003)a	0.005 (0.0003)a	0.001 (0.0001)a
T2	0.062 (0.004)ab	0.029 (0.002)a	0.272 (0.023)ab	0.013 (0.002)ab	0.009 (0.001)ab	0.002 (0.0002)a
T3	0.084 (0.008)ab	0.034 (0.002)a	0.359 (0.024)b	0.021 (0.001)ab	0.012 (0.001)ab	0.003 (0.0005)ab
T4	0.091 (0.005)ab	0.035 (0.003)a	0.383 (0.021)b	0.024 (0.001)ab	0.012 (0.001)ab	0.002 (0.0003)ab
T5	0.128 (0.017)b	0.040 (0.003)ab	0.444 (0.019)b	0.025 (0.001)ab	0.014 (0.001)b	0.003 (0.0004)ab
T6	0.264 (0.022)c	0.077 (0.008)bc	0.851 (0.046)c	0.038 (0.003)b	0.027 (0.003)c	0.008 (0.001)ab
T7	0.451 (0.026)d	0.110 (0.016)c	1.28 (0.080)d	0.064 (0.00)c	0.041 (0.002)d	0.021 (0.011)b
T8	0.873 (0.016)e	0.246 (0.012)d	2.55 (0.038)e	0.116 (0.014)d	0.087 (0.002)e	0.016 (0.002)ab
B	S (% w/w)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	B (mg/kg)
T1	0.005 (0.0003)a	0.16 (0.034)a	1.72 (0.11)a	0.32 (0.020)a	0.70 (0.053)a	0.67 (0.028)a
T2	0.010 (0.0005)ab	0.21 (0.04)a	2.90 (0.02)a	0.57 (0.057)ab	1.26 (0.09)ab	1.26 (0.112)ab
T3	0.013 (0.0005)ab	0.24 (0.025)a	3.29 (0.24)a	0.72 (0.042)ab	1.52 (0.137)ab	1.77 (0.09)bc
T4	0.015 (0.0003)b	0.26 (0.005)a	3.86 (0.06)a	0.81 (0.081)ab	1.66 (0.048)ab	1.97 (0.052)bc
T5	0.018 (0.001)b	0.36 (0.05)a	5.08 (0.57)a	0.93 (0.076)b	2.26 (0.254)b	2.31(0.028)c
T6	0.034 (0.003)c	0.78 (0.014)ab	9.85 (1.24)b	1.71 (0.142)c	4.66 (0.365)c	3.91 (0.209)d
T7	0.053 (0.0035)d	1.14 (0.208)b	14.22 (1.34)c	2.86 (0.193)d	7.48 (0.57)d	6.35 (0.295)e
T8	0.100 (0.0022)e	2.57 (0.336)c	23.60 (1.06)d	5.66 (0.136)e	10.99 (0.496)e	11.98 (0.382)f

Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and $P < 0.05$. Values in parenthesis represent standard error of the mean (n = 3).

The multivariate analysis on the proportion of tomato fruit nutrient content showed overall statistically significant difference among treatment groups (Table 5.9).

Again, from the nMDS plot three clusters appear (Figure 5.4), a closer group including proportion of fruit nutrient content of the five lowest UWP rates (T1-T5) with (T6) slightly separated from them but similarly part of the same cluster and a sharp distinction of T7 and T8 groups, resembling the outcomes of fruit production number and yield in these two treatments.

Table 5.9. Results of PERMANOVA analysis of proportion of Fruit nutrient content for UWP treatments (T1-T7) and Hoagland control (T8).

Source	Df	MS	Pseudo-F	P-value	perms
Tr	7	1077.4	373.14	**0.001**	999
Res	16	2.8873			
Total	23				

Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference (P<0.05) is indicated in bold.

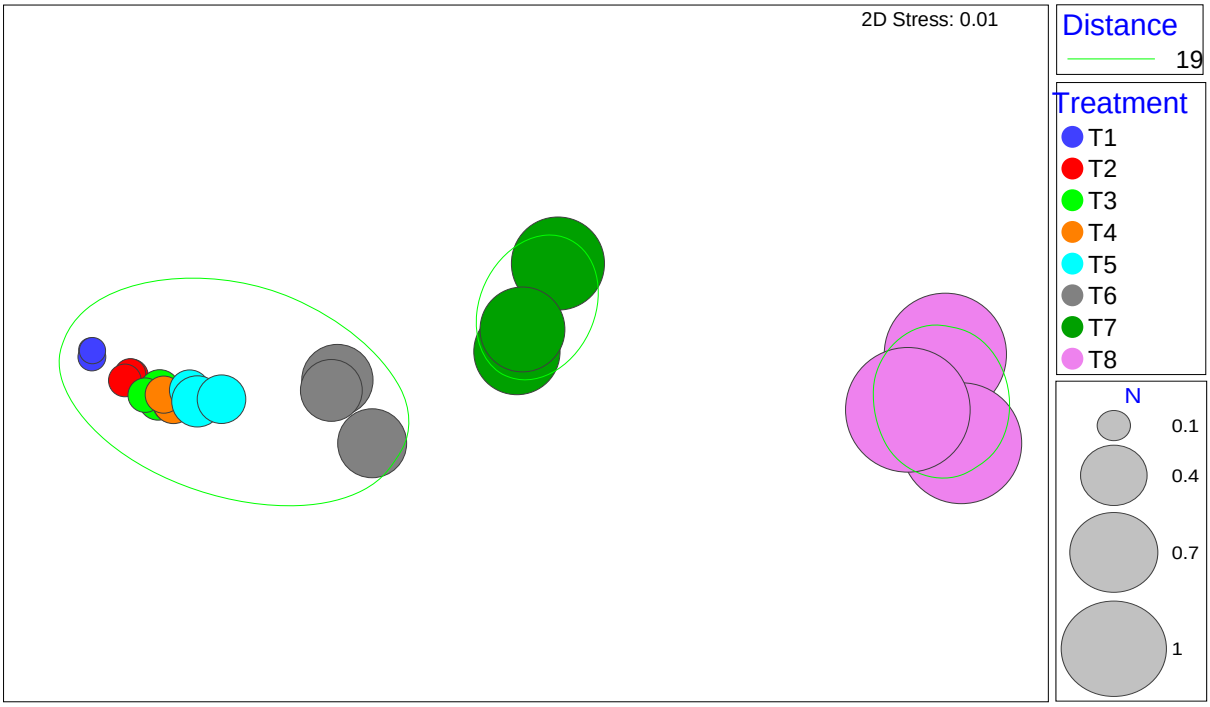

Figure 5.4. Non-metric multidimensional scaling (nMDS) bubble plot of Euclidian distances between the proportion of nutrients in tomato fruit of the seven UWP treatment (T1 = 0.3%; T2 = 0.5%; T3 = 0.8%; T4 = 1%; T5 = 2%; T6 = 3%; T7 = 5%) and Hoagland control (T8). The bubble size indicates N content (w/w%).

5.4.4.2 Fruit ripeness

UWP treatments had a significant influence on the maturation of tomato fruit as measured by fruit firmness and colour. As fruit ripens over time, firmness values (as determined by resistance to the probe during the pressure test) decrease as the flesh gets softer and the red colour of fruit increases. Fruit firmness results were statistically higher in T1 compared to all other treatments (Table 5.10, Tukey's test, α=0.05) because fruit did not develop properly hence remaining small and under-ripe. Fruits harvested from plants receiving the Hoagland's solution were on average firmer than fruit from T7, but no statistical significance was observed (Table 5.10, Tukey's test α=0.05). Flesh firmness was inversely correlated to colour values and dry matter content in fruits with the lowest rate of UWP.

Table 5.10. Colour and firmness parameters in tomato fruit receiving sea urchin waste powder (T1 – T7) and Hoagland solution (T8) treatments.

	L*	a*	b*	Hue_angle	Chroma	Firmness
T1	48.60 (2.98)a	18.25 (2.45)a	31.05 (2.98)a	1.02 (0.09)a	36.86 (2.15)a	0.67 (0.06)a
T2	37.37 (0.37)b	22.72 (1.08)abc	21.35 (0.63)bc	0.76 (0.01)b	31.20 (1.18)abc	0.49 (0.02)b
T3	40.10 (0.66)b	25.34 (1.25)bc	25.64 (0.89)ab	0.80 (0.03)b	36.16 (1.22)a	0.46 (0.01)b
T4	40.03 (0.79)b	27.00 (1.27)c	25.65 (1.29)ab	0.76 (0.02)b	37.31 (1.64)a	0.49 (0.02)b
T5	40.19 (1.79)b	22.62 (1.62)abc	24.33 (2.00)bc	0.81 (0.06)b	33.79 (1.55)ab	0.48 (0.03)b
T6	37.93 (0.97)b	22.48 (1.60)abc	21.78 (1.65)bc	0.77 (0.02)b	31.37 (2.20)abc	0.44 (0.02)b
T7	35.18 (0.32)b	19.58 (0.83)ab	17.66 (0.63)c	0.74 (0.01)b	26.39 (0.97)c	0.40 (0.02)b
T8	36.81 (1.19)b	18.72 (0.77)a	19.19 (1.30)bc	0.79 (0.04)b	27.03 (0.96)bc	0.49 (0.03)b

Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and $P < 0.05$. Values in parenthesis represent standard error of the mean ($n = 10$).

Fruit colour analyses were consistent with other ripening variables and again followed a response curve to the treatments. L* values were higher in fruits treated with less urchin waste (48.6) in T1, reflecting a lower degree of ripeness - hence colour ranged from white green to pale red, while riper fruits showed an intense red colour that translates in lower L* values (35.18 and 36.81) as observed in T7 and T8 respectively (Table 5.10). Colour values of fruit from the first five treatments were more spread, indicating a range of ripening observed in the fruit harvested from these plants. Fruit from treatment T6 and T7 and Hoagland's control had similar

colour values. Fruits colour and firmness data explored with multivariate analysis PERMANOVA do not show statistical differences (Table 5.11_A, P=0.108)

There was no significant difference for pH ($F_{(7,37)}$=1.235, P=.309), with treatment means ranging from 4.06 to 4.24 while significant differences were observed for mean values of titratable acidity ($F_{(7,37)}$=12.23, P=.000) with 5.77 mg/100ml in T1, 3.51 mg/100ml in T7 and 3.43 mg/100ml in T8 (Figure 5.5_A, Tukey's test α=0.05). The total soluble solids (TTS °Brix) mean values ranged from 4.3 to 5.9 (Figure 5.5_B) with Hoagland's control and T7 showing significantly lower vales than all the other treatments ($F_{(7,37)}$=9.026, P=.000).

Table 5.11. Results of PERMANOVA analysis of fruit ripeness parameters (Colour coordinates and Firmness) and quality attributes (pH, Acidity and Brix) for UWP and treatments (T1-T7) and Hoagland control (T8).

A Colour: Firmness

Source	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Tr	7	392.22	1.515	0.108	998
Res	72	258.9			
Total	79				

B pH: Acidity: °Brix

Source	**df**	**MS**	**Pseudo-F**	**P-value**	**Unique perms**
Tr	7	4.7608	10.754	**0.001**	998
Res	37	0.44269			
Total	44				

Analysis uses Fixed effect with Type III sum of square (partial) 999 permutation of data residual to determine significance. Significant difference (P<0.05) is indicated in bold.

Fruit quality attributes pH, acidity, and °Brix were highly significant when analysed with PERMANOVA (Table 5.11_B, P=0.001), yet treatment groups did not appear clearly differentiated in nMDS ordination.

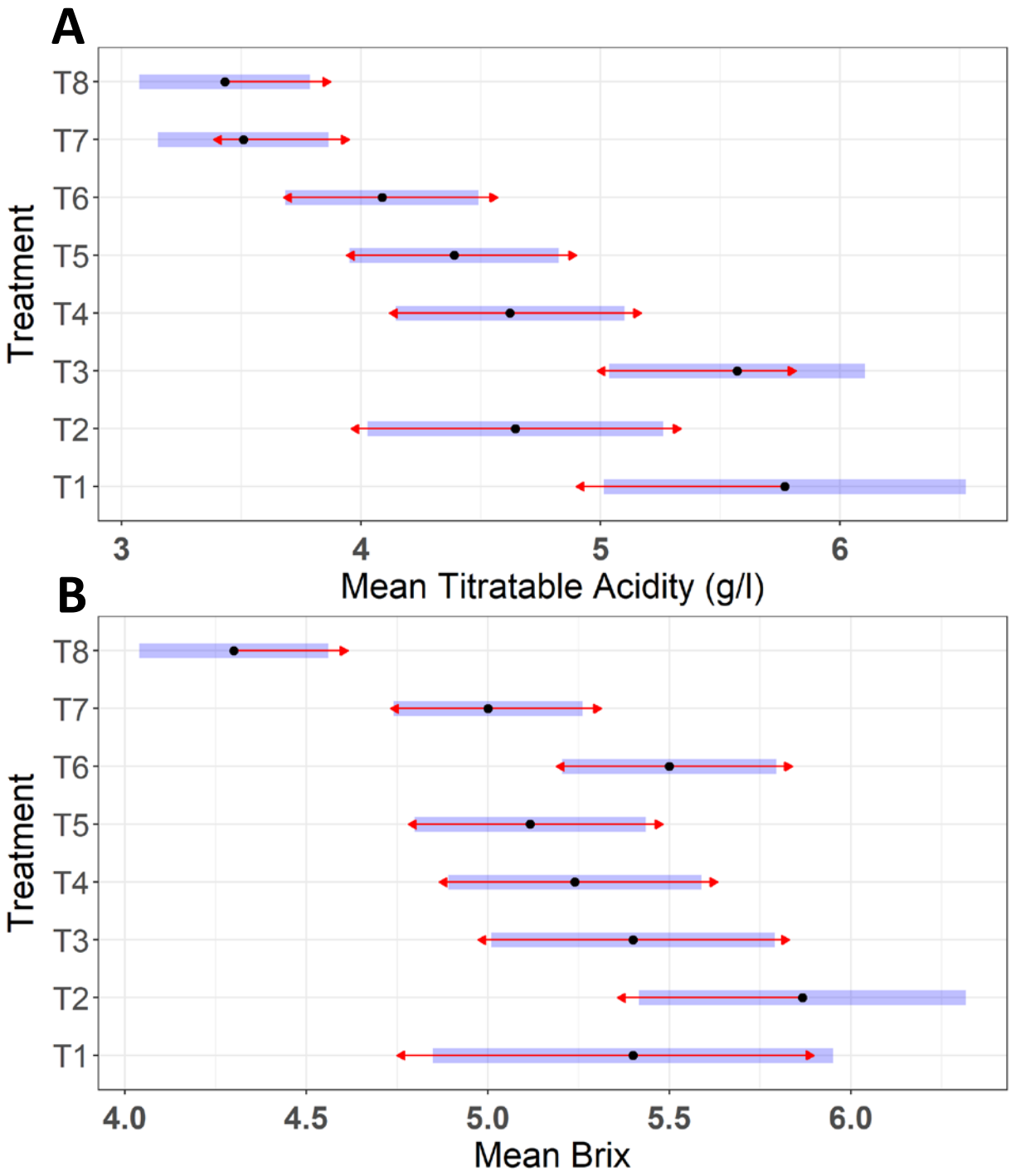

Figure 5.5. Fruit (TA) titratable acidity (**A**) and total soluble solids (°Brix) (**B**) across seven treatment rates of urchin powder (T1 = 0.3%; T2 = 0.5%; T3 = 0.8%; T4 = 1%; T5 = 2%; T6 = 3%; T7 = 5%) and Hoagland control (T8). Dots indicate means of fruit pooled together across the ten replicate pots in each treatment. Bars denote the confidence limit. When the red arrows overlap among treatments, then the treatments are not significantly different.

5.4.5 Soil nutrient content

The application of urchin powder increased the pH and EC of the growing medium (Figure 5.6_A and B) with scale of response consistent with increasing rate of UWP application. The pH of the growing medium at end of trial shows an increasing trend in the three lower treatments from pH 6.7 to pH 6.9 and stabilizing slightly above pH 7 from T4 to T7 with significant statistical differences (Figure 5.6_C, $F_{(6,14)}$=15.14, P=.000). The Electrical Conductivity instead varied between 0.428 dS/m in T1, 0.563 dS/m in T6 and 0.693 dS/m T7 with no clear statistical differences among treatments (Figure 5.6_D, $F_{(6,14)}$=1.618, P=.214).

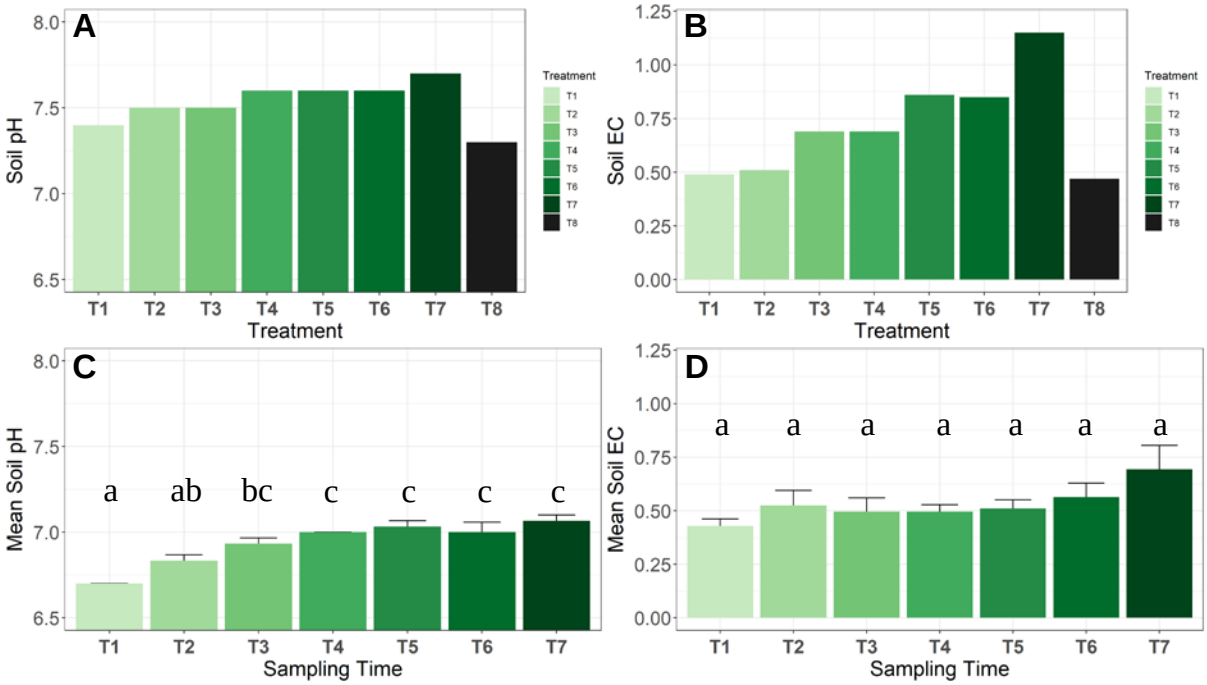

Figure 5.6. pH (A) and EC (B) of potting mix after the application of UWP at seven different rates at the start of the trial. T8 represent the potting mix before Hoagland's addition. Values represent a single measurement per treatment from pooled sub-samples from the ten pot replicates three days post-planting of tomato seedlings. pH (C) and EC (D) of potting mix with addition of the seven UWP treatments rate at the end of the trial. Error bars denote means of three replicates per each treatment. Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05.

The nutrient content of the potting mix with the addition of UWP at seven different rates at the end of the trial is shown in Table 5.12. No statistical differences between the treatments were observed for N, P, Mg, Cu, Zn and B. The value of K in T7 was significantly lower than the first five treatments (Table 5.12, Tukey's test α=0.05), a sign that K was actively taken up by the plants in this treatment. The residue of

other elements in the pot appear more irregular, Ca increase from T1 to T5 then decline again to T7 with statistical difference between T1 and T5 (Table 5.12, Tukey's test α=0.05); S shows statistical higher values in T7 compared to the first five treatments and same increasing trend can be observed for Na where T7 was statistically higher than T1 (Table 5.12, Tukey's test α=0.05); conversely, the residuals of Fe and Mn present higher values in the lower treatments and a decline towards T7. The present results do not show a consistent increasing response curve to higher rate of urchin powder for each element sign that the nutrients available were uptaken by the plants as highlighted in the previews tables.

Table 5.12. Nutrients concentration in potting mix with addition of urchin waste powder at seven rates at the end of the trial.

A	Tot. N (% w/w)	P (% w/w)	K (% w/w)	Ca (% w/w)	Mg (% w/w)	S (% w/w)
T1	0.0009 (0.0002)	0.0012 (0.00007)	0.066 (0.004)a	0.496 (0.019)a	0.050 (0.002)	0.003 (0.001)a
T2	0.0010 (0.0003)	0.0013 (0.00003)	0.058 (0.006)a	0.501 (0.012)a	0.044 (0.001)	0.004 (0.001)ab
T3	0.0007 (0.0001)	0.0011 (0.00003)	0.047 (0.005)ab	0.523(0.024)ab	0.041 (0.002)	0.004 (0.001)ab
T4	0.0009 (0.0002)	0.0012 (0.00003)	0.058 (0.010)a	0.527 (0.014)ab	0.043 (0.002)	0.004 (0.001)ab
T5	0.0008 (0.0003)	0.0014 (0.00007)	0.039 (0.004)abc	0.625 (0.040)b	0.048 (0.002)	0.004 (0.001)ab
T6	0.0011 (0.0001)	0.0014 (0.00023)	0.021 (0.005)cd	0.589 (0.018)ab	0.045 (0.002)	0.008 (0.001)bc
T7	0.0018 (0.0006)	0.0013 (0.00019)	0.015 (0.004)d	0.559 (0.018)ab	0.042 (0.003)	0.009 (0.002)c
B	Na (% w/w)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	B (mg/kg)
T1	0.010 (0.001)a	12.12 (0.98)	54.50 (1.86)a	47.70 (2.23)a	20.43 (1.66)	0.52 (0.009)
T2	0.017 (0.004)ab	7.70 (0.84)	43.47 (4.89)ab	41.39 (2.71)ab	13.53 (0.62)	0.52 (0.030)
T3	0.014 (0.001)ab	7.23 (1.14)	38.23 (1.16)b	39.88 (0.34)ab	14.04 (0.72)	0.54 (0.030)
T4	0.017 (0.002)ab	9.78 (0.44)	37.42 (1.49)b	39.76 (1.19)ab	15.01 (0.23)	0.53 (0.019)
T5	0.022 (0.004)ab	12.47 (0.43)	37.46 (2.00)b	41.82 (0.39)ab	16.89 (0.92)	0.55 (0.003)
T6	0.025 (0.003)ab	12.10 (1.92)	37.31 (1.75)b	41.72 (2.38)ab	17.32 (1.86)	0.52 (0.010)
T7	0.032 (0.009)b	11.11 (1.61)	30.27 (2.35)b	35.09 (2.31)b	18.97 (3.20)	0.57 (0.017)

Means that do not share a letter indicate significant different among treatments. Grouping Information Using the Tukey HSD Method and $P < 0.05$. Values in parenthesis represent standard error of the mean (n = 3).

5.5 Discussion

Powdered sea urchin waste improved the growth and productivity of tomato plants with increased performance at higher rates. For all vegetative and reproductive parameters measured, significant improvements were often observed with each increasing rate. The standard Hoagland's fertiliser regime (control treatment) produced tomato plants with similar vegetative size characteristics to the highest UWP treatment (T7 - 5% w/w) yet were substantially bigger and healthier plants than those receiving the lower UWP treatments. Consistent vegetative growth of the tomato seedlings was observed in the early stage of the trial with shoot growth of all treatments matching the control plants. Growth rate of plants in the low rate UWP treatments (T1 – to T4) significantly slowed after four weeks suggesting a nutrient depletion under these treatments. Plants from T5 kept growing very slowly until the eighth week while T6 and T7 had the best performance of all UWP treatments. Plants receiving the Hoagland's solution had superior yield and fruit quality attributes than all UWP treatments (T1 – T7). Tomato fruit yield in the control was double the yield of the highest rate UWP T7 even though plant size was similar, which reflects the higher and more readily absorbed soluble nutrient supply over the course of the trial.

5.5.1 Tomato fruit quality

Apart from superior yields, Hoagland's control solution produced significantly improved quality fruit in most parameters tested. For the fruit texture test which functioned as a perforation test, values show resistance/deformation of the pericarp to the probe. Higher values were generally recorded in the early stages of fruit ripening, where the pericarp was less elastic and prone to perforation regardless of treatment, reducing as fruit becomes less firm as they matured. However, fruits harvested from plants receiving the Hoagland's solution were firmer than the T7, even though they were of similar maturity which may be a consequence of greater water content (bigger fruit) in these plants. This increases the tautness of the flesh and is further evidence for the superior quality fruit harvested from plants receiving this treatment. Fruit firmness is also related to total soluble solid content and can positively influence fruit flavour and shelf life (Beckles 2012). Sugar content and its

ratio to organic acids are the main determinants of tomato taste (Zhao et al 2016), the higher acidity was recorded in fruits from the T7 and T8 treatments.

Increased DMC is generally associated with improved nutrition (specifically nitrogen) which result in greater vegetative growth and photosynthesis (Kaniszewski & Rumpel 1986). We observed this result for plant total DMC as well for the fruit where plants from T1 to T6 had significantly lower total TMC compared to plants receiving T7 and T8 UWP which is not unexpected given the increased nutrient supply with the higher rates applied.

Fruit colour parameters pointed towards increased ripeness in fruit harvested from the highest UWP and Hoagland's solution treatments. Decreasing values of L* from T1 to T8 were observed. Decreasing L* values indicate the darkening of the red colour (from pink to full red) due to the synthesis of red colour pigments associated with fruit ripening. The a* component showed a clear increase between ripening stages from green (not ripe) to light red (ripening). The changes of a* from negative (green colour) to positive (red colour) values are attributed to chlorophyll degradation and lycopene synthesis. The b* values were higher at the pink-light red stage, the pale-yellow colour is due to the ζ-carotenes that reach their highest concentration before full ripening, where lycopene (red colour) and β-carotene (orange colour) are predominant (Fraser et al., 1994; Choi et al., 1995). But the lower values of Chroma in T7 and T8 compared to the lesser rate UWP treatments may reflect the start of fruit senescence rather than a major accumulation of lycopene in those treatments.

5.5.2 Tomato nutrition

A comparison of the nutrient content provided by the UWP at different rates against the nutrients supplied with the Hoagland solution provides some explanation for the improvements in the vegetative and reproductive attributes of the tomato plants. Given the nutrient composition of the UWP, treatments were not expected to perform as well as Hoagland's control, however there was clear evidence of nutrient uptake by tomato plants derived from UWP treatments. Hoagland's solution provided 1950 mg N in T8, twice the amount supplied to the plants in T7. Almost four times the amount of P was provided in the Hoagland's solution (223 mg) compared to 65 mg in T7 and three times the amount of K was provided. In contrast, some macro elements like Ca, Mg and S and microelements like B, Cu, Zn and Fe were supplied in higher proportions through the UWP which may have benefited the vegetative growth of tomato plants observed in T7 relative to the Hoagland's control. Specifically, for Ca, less than half the amount was provided in Hoagland's control (2076 mg) compared to T1 (4850 mg), the lowest rate UWP treatment and much lower than treatment 7 (80800 mg). The application of Mg in Hoagland's control was 353 mg, higher than treatment 1 (210 mg) and similar to treatment 2 (360 mg) but lower than all the other treatments with T7 at 3550 mg. S in T8 reached 461 mg and in this case the number sets between T5 with 376 mg and T6 (564 mg) but half than T7 (940 mg).

Nitrogen and K play an important role in plant growth and development (Leghari et al 2016, Prajapati & Modi 2012). For tomatoes, N supply has been shown to significantly increase crop growth and N uptake (Tei et al 2000), plant yield and fruit quality when supplied adequately (Wang et al 2015), whereas insufficient N content in tomatoes can lead to limited vegetative growth, reduced shoot length and leaf area (Scholberg et al 2000), net photosynthetic rate decline associated to decreased chlorophyll content and leaf senescence (Guidi et al 1997), blossom drop with consequent low yield (Ozores-Hampton & McAvoy 2017). Symptoms of N deficiency were visible in plants of the lower treatments where the four-week leaves became chlorotic, had completely yellowed and subsequently fallen. Nitrogen provided with UWP treatments is in the form of amino acids and bound peptides which require the transporter activity to facilitate the transfer of N compounds

across cellular membranes (Tegeder & Rentsch 2010). This has been demonstrated by the identification of a specific proteinaceous transporter that enables the accumulation of the free amino acid Proline in tomato pollen during maturation (Schwacke et al 1999).

Potassium is important in plants for regulating the opening and closing of stomata and the activation of enzymes (Dorais et al 2010). Inadequate K nutrition in tomatoes has been shown to negatively affect growth, fruit set, dry matter distribution, and fruit quality (Besford & Maw 1975, Çolpan et al 2013). Physiological disorders such as blotchy ripening, greenback, yellow shoulder, decreased lycopene content, and irregular shaped and hollow tomato fruits are associated with K deficiency (Eshu et al 2014, Serio et al 2007). Fruit appearance in UWP treatments was not affected negatively by the low K content but this associated with the low application of N may have influenced the reduced fruit set in T6 and 7 as well as the poor plant performance in the lowest rate UWP treatments.

Phosphorus is a crucial element for plant growth and low P availability in soils is considered among the many causes that limit crop yields worldwide (Richardson et al 2011). Phosphorous deficiency in tomato plants reduces CO_2 assimilation (Biddinger et al 1998), leading to a decrease in biomass production (Fujita et al 2003). In this study, the biomass of tomato plants significantly increased with each higher rates of UWP and the highest rate (T7) resulted in comparable plant biomass to the T8 control, even though the P content of that T7 was four times lower than in the Hoagland control. Uchida (2000) showed that the mobilization of P from old parts of the plant to new tissue causes the appearance of dark to blue-green (purpling) colouration on older leaves. Symptoms of leaf purpling were clearly visible across the plants of the seven UWP treatments, however in T6 and T7 only the lower and oldest leaves were affected while the first five treatments showed more severe symptoms of P deficiency.

Magnesium is essential for the photosynthesis process being a component of the chlorophyll molecule (Marschner 2011) and when limited results in decreased biomass production and lower yield in greenhouse tomatoes (Hao & Papadopoulos

2004). The high content of Mg in T6 and 7 is likely to have promoted vegetative growth despite low levels of N and K.

Boron plays a key role in the growth of many fruit and vegetable plants and many studies have highlighted the importance of boron in tomato fruit quality (Huang & Snapp 2009, Naz et al 2012, Sathya et al 2010, Smit & Combrink 2004). Davis et al (2003) demonstrated that foliar and root application of B increased tomato growth promoted the uptake of N, Ca and K in plant tissue whilst improving fruit shelf life and firmness. Boron favours the uptake of Ca ions which form complexes with pectin and polyhydroxy polymers (Huang & Snapp 2004) giving stability and strength to cell wall membranes (Ahmad et al 2009). Boron deficiency in tomatoes is associated with damaged fruit through concentric and radial cracking (Davis et al 2003, Liebisch et al 2009), while blossom-end rot in tomato is a physiological fruit disorder caused by insufficient Ca availability (Saure 2001) and can reduce the marketability of the fruit (Taylor & Locascio 2004). In this study, both B and Ca were provided in the UWP at higher rates than Hoagland's control and evidence of uptake of these micronutrients can be seen in the leaf and fruit nutrient dry matter analyses.

Osmotic pressure in the roots area is important for plant health whilst low levels of EC affect both plant growth and yield, high EC limit water absorption (Li et al 2001). High levels of EC are determined by excess of salts and the threshold is plant-specific. The EC limit for tomato is indicated at 2.5 dS/m (Sonneveld & Welles 1988). Eltez et al (2000) reported a decrease in fruit yield of tomato plants when the EC of the treatment solution exceeded 2.0 dS/m. In this study the EC never reached level of toxicity even in the treatment with the highest application of UWP (T7 - 1.15 dS/m), the soil showed an immediate beneficial change soon after the application, but the EC level dropped in all treatments at the end of the trial.

Soil pH can have a strong influence on plant nutrient uptake. Kang et al (2011) found that at both pH 4 and pH 8 an unsuitable root zone limited the growth of tomato seedlings, reducing P content in the roots, Ca in the shoots and water absorption under high nutrient concentrations, and that dry and fresh weight and shoot and root areas were particularly affected by pH 8. The normal range of soil pH for optimum tomato growth is from 5.5 to 7.0 (Sainju et al 2003), low pH (4.5 –

5.5) was shown to improve micronutrient availability (Clark & Baligar 2000, Wang et al 2006), but a growing medium with very low pH (3.5 – 4.5) led to decreased tomato plant mineral nutrition, inhibited root elongation, branch formation and water absorption (Foy 1992, Wright 1989). The potting mix used in our study had a base pH of 7.2 which increased after the application of UWP in each treatment rate to pH 7.7 in T7. At the end of the trial the growing medium recorded a decrease in pH in each treatment and plateaued around pH 7.0 from T4 onwards. We did not observe a negative influence on tomato productivity and nutrition of the higher pH in T7 suggesting that EC and pH were still in an optimal range to facilitate cation exchange in the root area.

Tomato plants were shown to absorb the nutrients in the quantity provided relative to each increasing rate of UPW. In treatments with a lower rate of UWP, the plants stopped growing after four weeks, mainly due to a lack of N, P and K as evidenced by fewer leaves, less branching and reduced plant height. Where flowers and fruit were formed, most did not grow and ripen adequately. Plants receiving higher rate UWP treatments (T6 and T7) had much greater nutrient content at the end of the trial which facilitated improved vegetative growth, taller and thicker stems, greater leaf area and greater number of flowers and fruit. Further evidence for plant nutrient uptake is the relatively similar nutrient content of the potting mix at the end of the trial across all the treatments. The low content of K in T7 potting mix at the end of the trial is in line with the higher production of fruit in this treatment compared to the lower UWP additions. Clearly, the main limiting elements of the UWP were N and K as evidenced by the significantly reduced total yield and plant size relative to Hoagland's solution. The most readily absorbed micronutrients were B, Fe and Zn suggesting that UWP could act as useful micronutrient supplement.

5.5.3 Conclusion

The UWP used here as a mineral fertiliser increased productivity of tomato plants with better performance at higher rates. Plant growth was directly related to the rate of added urchin waste, T7 showed improved plants performance for all parameters measured in all replicates. Although vegetative growth for the highest sea urchin waste powder treatment (T7) compared well with Hoagland's solution, this did not result in comparable yield and fruit quality to plants treated with the Hoagland's

fertiliser. These results suggest that UWP requires supplementary addition of macronutrients to overcome deficiency in N and P as these were clearly exhausted during the vegetative growth of plants and flowers and were no longer available during fruiting. Given the high ratios of Ca, Mg and B in the UWP relative to N, P and K, there is clearly a risk of oversupply of micronutrients, which may limit the amount of UWP that can be applied as a fertiliser to avoid nutrient toxicity.

Gypsum and dolomite are often used as agricultural fertilisers, both containing high content of Ca, in addition gypsum contain SO_4^{2} and dolomite Mg. However, they lack an array of other macro and micronutrient that are alternatively found in the UWP including K, P, Fe, Zn and B. Boron, an essential micronutrient deficient in Australian soils is present in relatively high concentrations in the shells of sea urchins. After Zn, B deficiency in plants is the most widespread micronutrient deficiency around the world and causes large losses in crop production and crop quality (Alloway 2008). Results from this trial suggest that UWP could be used as an alternative to other relatively expensive soil supplements if it can be produced in sufficient quantity at a reasonable cost.

Fresh seafood waste requires prompt stabilisation to facilitate handling as the decomposition processes can lead to offensive odours and the loss of nutrients by leakage (MacLeod et al 2006). Excessive processing, on the other hand, require drying and finely crush waste material into a powder, which can increase the costs of an already low-value product, discouraging waste repurposing such as used in this study. Whilst UWP may not have the desirable high P and N content found in other seafood waste e.g. scale fish waste, (MacLeod et al 2006), it requires less pre-treatment costs and the high micronutrient content, especially Ca and B can add significant value as a nutrient supplement. The UWP used here comprised only the spines, jaws and tests of the urchin; adding urchin derived liquid gut waste may provide additional plant available nutrient to overcome some of the deficiencies identified.

5.5.4 Acknowledgements

The author would like to thank Mr Phil Andrews (Horticultural Research Centre) for his help and advice in setting up the trial. Mrs Caroline Claye (Field Horticulture

Laboratory) for help and advice in the analysis of tomato fruit. Ms Lisette Robertson Laboratory Coordinator IMAS (Fishery & Aquaculture) for help processing the urchin waste.

Funding: This work was supported by the FRDC (Fisheries Research and Development Corporation), grant number 2016-208.

Chapter 6 General discussion

This thesis examined pre- and post-harvest aspects of the sea urchin *Centrostephanus rodgersii* fishery in Tasmanian coastal waters. A holistic approach was used to expand our knowledge of fundamental biological characteristics of *C. rodgersii* and examine potential improvements in the utilisation of the target species as part of the fishery. This new information is presented in four data chapters, whose major findings were:

- Fishing pressure and feeding habitat influenced *C. rodgersii* size structure and gonad quality. Larger and older animals showed signs of poorer gonad quality related to age. Urchins collected in kelp were larger and heavier compared to barren habitat showing patterns of somatic growth and accumulation of nutrients in the gonad related to the availability of food.
- The biochemical composition and sensory quality characteristics such as colour and texture of the urchin gonad was influenced by seasonality linked to the reproductive cycle, sex and feeding habitat. Moisture, lipid, protein, carbohydrate and ash composition followed a trend of nutrient accumulation transferred from the feed source to the gonad during summer and autumn in preparation for the gonad's maturation (production of gametes and spawning).
- The lipid, fatty acid and amino acid composition followed a seasonal trend with clear sex-related differences. Feeding habitat influenced gonad lipid and fatty acid profiles.
- Urchins in captivity provided with a manufactured diet promoted gonad growth to a greater extent compared to monospecific natural diets. In general, captive urchins were able to retain or biosynthesize some fatty acids and amino acids with the potential to improve gonad sensory quality attributes in a relatively short time period.
- Monitoring of key stress parameters during post-harvest handling and storage showed that roe quality characteristics of *C. rodgersii* can be highly impacted by current commercial harvest practices. The longspined sea urchin appears

to have limited ability to endure even short periods of post-harvest stress compared to other urchin species.

- Trials of urchin fishery waste as a soil supplement showed that the macro and micronutrient composition of the waste product is suitable for use in agriculture.

6.1 Factors influencing urchin gonad quality and biochemical composition

The seasonal collection of urchins from different locations and habitats provided a better understanding of the biological and biochemical changes that held back/prevented the expansion of the fishery for *C. rodgersii* and addressed some misconceptions about *C. rodgersii* gonad quality and variability. The longspined sea urchins fishing history in Tasmanian coastal water is fairly recent starting in 2008 on the northeast coast of the island with relatively few catches per year. The species however was first recorded in Tasmania around 1978 (Edgar 1997) and is a relatively long-living echinoid (Ling & Johnson 2009, Pecorino et al 2012); it is reasonable then to assume that initial harvests were dominated by old specimens of *C. rodgersii*. We found that urchins collected in the Elephant Rock site, a low-intensity fishing area since 2009, had larger and heavier test than Sloop Rock site, a high-intensity fishing area located 3.5km apart. Several urchins at Elephant Rock presented very large gonad of irregular shape, coarse granularity and dark brown or pale colour. We attribute this variability and the presence of gonad with poor quality characteristics to animals of old age. These older animals have gonad quality attributes that are not preferred by the sea urchin industry and did not match with the standard required by the Japanese market. At the heavily fished Sloop Rock there was less variation in urchins' body size and greater consistency in gonad colour and granularity, moreover, the GSI of medium size urchins at Sloop Rock was greater than that of the very large animals. In the perspective of the fishery and export of the product for human consumption, consistency in roe's quality is paramount as sea urchins are not sold as a whole live product but as extracted roe displayed on a tray. Consequently, the presentation and the visual traits of the roe assume great importance, and preferably with uniform gonad size, shape, colour and texture.

In Tasmania, the interest in development of a fishery targeting *C. rodgersii* is twofold, an economically viable fishery and, a potential mediating mechanism for the destructive grazing activity of *C. rodgersii*. There are widespread concerns that destructive grazing by *C. rodgersii* has already transformed a large part of the once productive rocky reef into barrens. Several measures have been put in place to control the urchin density such as culling programs and subsidies to encourage and expand the fishery. However, it is unlikely that fishermen will harvest in areas where populations dominated by old urchins with poor quality gonad are present. We suggest that subsidised harvest and/or culling programs may be required in areas that do not provide an economic return in the short term but could be seen as an investment for the future of the fishery and/or reef productivity. This could be more relevant in certain areas of New South Wales. Alternatively, development of economically feasible urchin-based products other than for human consumption, such as fertiliser as demonstrated in the present study (Chapter 5) would increase economic and environmental sustainability. Further research exploring the possibility to transform the urchins into a stable product/ingredient that can be included in a formulated feed for aquafarming may also be warranted.

6.2 Nutritional aspects and comparisons

A survey conducted among Australian consumers revealed that the primary factors of seafood consumption are nutrition, taste, and convenience, while price, availability, and concern about quality represent the main limitations (Christenson et al 2017). Although human nutrition is a complex scientific area, there is a clear understanding that some foodstuff contains substances essential for life support (Lanham-New et al 2019). Marine organisms (fish and shellfish) are in general considered a good source of high-quality protein and lipids but low in calories, with a high proportion of Essential Amino Acids (EAA) and long-chain Omega 3 Polyunsaturated Fatty Acids (PUFAs), vitamins and minerals (Bhavan et al 2010, Chandravanshi et al , Venugopal & Gopakumar 2017).

Seafood is also considered a high energy density food source for the typically high protein and lipid content in tissues (Dempson et al 2004). Protein intake by humans is necessary to obtain various amino acids needed for body function. The EAAs that

are nutritionally required by humans are also key parameters in food quality assessment (Desai et al 2018, Tessari et al 2016). *Centrostephanus rodgersii* gonad showed a high nutritional profile similar to other commercially exploited urchin species (Archana & Babu 2016, De La Cruz-García et al 2000, Verachia et al 2012b).

The application of different methodologies across urchin studies for the determination of proteins may lead to results that are not always concordant. In this investigation, the protein content of urchin gonad (Chapter 2) was determined with a colourimetric method (Bradford) which usually gives an underestimation of the real amount of proteins, as opposed to the method of nitrogen determination which can lead to overestimation as it includes non-protein nitrogen (Maehre et al 2018, Mariotti et al 2008). Some studies that determined the protein content in urchin gonad with the total nitrogen determination method and a protein conversion factor of (6.25) report a variable protein content between 14-18% (Chen et al 2010, Mamelona et al 2010). Based on the amino acid determination and the determination of the protein content through nitrogen quantitation (Chapter 3, data non reported) with conversion factor (5.60), we can estimate that the real proteins content in the gonad of *C. rodgersii* range between 12-16% by wet weight. The Australian dietary guideline recommends a daily protein intake of 64 gr (NHMRC 2013) hence the consumption of 100 gr of fresh *C. rodgersii* gonad would provide around 14 gr of protein which fulfil 22% of the daily requirement. Additionally, the protein composition of the gonad during the harvest season was high in EAAs accounting for 43.58% of total amino acids and the ratio EAA to NEAA was (0.88). Marine foods with EAA: NEAA ratios greater than 0.5 are considered important source of dietary proteins (Mamelona et al 2010). Other studies report lower values compared to ours, for example, the percentage of EAAs in the urchins gonad of *S. variolaris* and *S. nudus* was 32.1% (Archana & Babu 2016, Xu et al 2009). The EAA to NEAA ratio was 0.50 in *S. variolaris* and 0.58 *P. lividus* (Mol et al 2008). In the Japanese abalone muscle *Haliotis discus hannai* the EAAs accounted for 34.86% of total AAs with a ratio EAA to NEAA of 0.54.

The gonad lipid content reported in the present study (6.4% - 8.0%) was higher than values presented for other commercial urchin species (Chen et al 2013, Liyana-

Pathirana et al 2002c, Mol et al 2008). The metabolism of lipids assumed through the diet allows efficient production of energy for the body. They are a source of essential fatty acids and carry fat-soluble vitamins and carotenoids. In addition, phospholipids are the structural constituent of the cellular and sub-cellular membranes (Clandinin et al 1991, Luvizotto-santos et al 2003). In the present study, the level of total lipids was found to be higher in female gonad when compared to the male; this was expected as lipids were found in higher proportions in female gonad of several other urchin species (Rocha et al 2019, Unuma et al 2003, Verachia et al 2012b, Zárate et al 2016). Moreover, the levels of polyunsaturated fatty acids (PUFA), such as EPA, LA, GLA were found to be higher in female while docosahexaenoic (DHA), and ARA were higher in males. Fatty acids are essential in human nutrition (Khalili Tilami & Sampels 2018) and play a role in the prevention of coronary heart diseases, autoimmune disorders, arrhythmias, and they are also known to have an anti-inflammatory effect and lower blood pressure (Pal et al 2018). The National Health and Medical Research Council recommend daily consumption of EPA + DHA is between 250-500 mg in order to decrease the risk of cardiovascular diseases in humans (Capra 2006). Sea urchins are harvested and consumed between December and June, during this period the concentration of PUFA in fresh gonad is around (9.8 mg-g^{-1}), thus an intake of 50 g of *C. rodgersii* roe (the average gonad mass of a small adult urchin) can provide up to 490 mg of EPA + DHA. Thus, consumption of roe from a single small urchin can provide the full daily recommended dose of EPA + DHA to address cardiovascular risk. Sea urchins are usually eaten in small portions compared to fish fillets, but they can be regarded as a supplementary source of important nutritional components in the right proportion. For example, in the abalone muscle, the protein content per gram of tissue is twice that of *C. rodgersii* roe but the EAAs content is more balanced in *C. rodgersii*, which is also more lipid rich in ω-3.

6.3 Effect of diet on gonad quality

Previous research and development in echinoculture have focused on enhancing sea urchin' growth and gonad production. In this study, gonad wet weight and percent gonad yield at the end of the feeding experiment were greater for urchins fed a

commercial urchin diet compared to urchins fed with monospecific wild kelp. Over the 12 weeks feeding trial, the mean percent increase in gonad wet weight for the treatment fed formulated diet was 8.3% per week with a total of 126.35% and percent gonad yield was 7.9% per week or 95% in 12 weeks. The gonad yield of the formulated diet treatment at the end of the trial was, however, similar to the wild control.

In this study, the feeding trial demonstrated that amino acids conferring sweet taste like glycine, alanine, proline and threonine accumulate in the gonad when provided in higher proportion with the diet. One possible strategy for manipulating roe quality could be to focus on the improvement of taste by manipulating the AA profile. This would require less time than the usual 12-16 week period to enhance the yield.

Several urchin feeding trials with natural and formulated diets demonstrated that a fresh algae diet is not adequate to sustain gonad growth in aquafarming. Nutrients derived from algae are difficult to digest and absorb due to the thick cell wall of most algae, in particular, brown algae; sea urchins are reported to assimilate between 15-20% of what they eat, and the rest found in the aegesta remains almost intact. Seaweeds are high in carbohydrates enriched in dietary fibers (Stiger et al 2016). Sea urchins also have little capacity to digest insoluble structural carbohydrates (Lawrence et al 2013). This affects digestibility and capacity for production. An artificial feed instead can be formulated to the requirement of the target species; in this study, the feed pellets provided an adequate source of energy through extruded carbohydrates that can be more easily digested, allowing the urchins efficient assimilation of proteins and lipids in the gonad tissue. The quality of seaweed protein is however acceptable due to its high content of essential amino acids (Fleurence 1999). A feed suitable for the nutritional requirements of *C. rodgersii* could be formulated with local macroalgae with the highest protein content. Gonad proteins content post-spawning was low and dietary proteins are more rapidly accumulated at this stage when good nutritional quality food is available, for instance, gonad yield enhancement can target wild animals in a recovering phase between September and December. In the three-month experiment, the gonad yield was comparable to that of wild urchins, while none of

the natural monospecific diets guaranteed sufficient gonad growth. However, given that the natural algal diets often had specific effects on gonad composition, provision of a diet with specific proportions of natural algae may deliver a similar outcome to the formulated feed product.

Roe colour along with taste are the most critical parameters to determine roe acceptance by the consumer (Pearce et al 2004). Previous studies aimed at improving roe colour achieved a small non-significant enhancement when compared to control groups (Robinson et al 2002, Suckling et al 2011). The colour of the gonad is determined by a combination of carotenoids, with many of these pigments synthesised from precursors that are obtained through the diet, instead of direct incorporation of these pigments from the food source (Robinson et al 2002, Shpigel et al 2005, Shpigel et al 2006). However, colour determination in organic tissues with an instrumental determination can be difficult since other than pigments, parameters such as moisture and density of macro-nutrients can influence the colour (Symonds et al 2007, Symonds et al 2009).

In this study, change in colour of wild urchin gonad was primarily influenced by seasonality and sex, while location and feeding habitat had relatively little effect on the composition of carotenoid pigments in the gonad tissue. In the feeding trial experiment where urchins were supplied a specific diet, likewise, no significant differences in gonad colour were observed between the control groups and the specific diet groups. This was unusual given the range of diet types offered, with the most likely explanation being that the carotenoids pigments in adult urchins are retained in the tissue and the three months experiment was not sufficient to determine a turnover of pigments.

Historically, commercial fishers in the northern states of Victoria and New South Wales avoided harvesting sea urchins from barrens ground because the low food availability in these areas typically resulted in urchins with small, poor quality roe that is unsuitable for sale (Blount & Worthington 2002, Sanderson 1996, Worthington & Blount 2003). In contrast, we found that sea urchin inhabiting the barrens ground presented gonad weight and GSI in the range required for marketability, albeit lower than in neighbouring kelp beds. Furthermore, there were no substantive qualitative differences between urchin roe in barren and kelp such as

to discourage marketability. The isotopic analysis revealed strong similarities between urchin gonad in kelp and barrens which indicates that in both habitats urchins are feeding on kelp and mostly on brown algae. The difference being that availability is higher and or supply more regular in kelp beds, whereas in barrens urchins are reliant on drift algae. The most important difference found was the greater amount of lipids present in the gonad of sea urchins in kelp beds (28.6% d. wt.) compared to barrens (24.6% d. wt.). Nonetheless, the amount of lipids found in the barrens was abundant and proportionally higher than other reported for urchin species (Chen et al 2010, Verachia et al 2012b, Wang et al 2015).

Gonad texture and appearance is also an important sensory quality factor determining market acceptance and price. Gonad texture is determined by protein and carbohydrate content and by the percentage of moisture, with increasing moisture negatively affecting texture. Gonad moisture content varies seasonally as a function of the phase of gonad development (McBride et al 2004). Urchin gonad in the recovery phase have less water content and are firmer than urchin gonad in the growing phase, both of which maintain better texture characteristics than mature and spawning gonad (Takagi et al 2017). Firm gonad can be more easily handled, processed, and packaged for export. We found the lowest moisture content from September and December after spawning and during recovery. The moisture increased steadily during maturation while keeping a firm texture thanks to the accumulation of proteins and carbohydrates. The highest moisture content was recorded in July coincident with gonad in pre-spawning and spawning. At these stages gonad have extremely poor or no-texture due to the active hydrolysis of macro-nutrients and then release of gametes; handling and packaging during this phase becomes difficult with visual characteristics deeply affected. Thus, while gonad at the spawning stage may have a higher nutritional value and better flavour profile than during recovery and onset of gonad growth stages, the lack of firmness and the fluid leakage makes difficult the preservation and presentation of the product for the export market.

6.4 Post-harvest exposure determines urchins' metabolic changes and impact gonad visual quality

In wild fisheries and marine aquaculture production, losses due to poor post-harvest practices and management are a major problem (Akande & Diei-Ouadi 2010, Thilsted et al 2016). Post-harvest marine product losses occur when a product is either discarded during the processing phase or sold at a relatively low price because of quality deterioration (Getu et al 2015). Compared to fishes, sea urchin gonad are less prone to rapid spoilage after collection due to their ability to survive long periods when exposed to air given the low metabolism and ability to hold internal water. However, stress tolerance in sea urchins is highly species-specific and also depends on many external factors such as temperature and wind as well as harvesting and handling practices. *Centrostephanus rodgersii* appear to be less tolerant of harvest process than other commercially exploited species.

The collections of sea urchins for the different experiments of the present study (Chapter 3 and Chapter 4) demonstrated the limited ability of *C. rodgersii* to withstand and recover from events that generate structural damage and metabolic stress. In particular, rough harvesting methods and bad weather conditions resulted in around 44% mortality of sea urchins held in aquaria tanks during the 4-weeks that followed the collection. A second harvest carried with more gentle procedures and favourable weather conditions showed much reduced mortality (18%). Previous studies on urchins also reported high post-harvest mortality during acclimatization in aquaria tanks or during the early stages of the trial followed by low or no mortality (James 2007, Siikavuopio 2009).

In Chapter 4 we also demonstrated through the analysis of coelomic fluid that air exposure and long storage trigger a level of stress that ultimately affected the appearance of the gonad. The factors that most impacted the urchins upon emersion were high temperature and wind exposure while protecting the animals from wind and keeping them at a temperature of 15°C resulted in limited dehydration and minimised coelomic fluid turbidity. Our results suggest that during air exposure *C. rodgersii* should be kept at an environmental temperature close to that of the seawater at the time of collection, protected from direct sunlight and limiting

excessive shaking or rough handling that could coarse loss of internal fluid. Under these moderate conditions of low wind and ambient sea temperature, the animals have a higher likelihood to survive long hours in storage prior to processing. Nonetheless, irrespective of the treatment our trial showed that between 16hrs and 28hrs after the harvest all urchins showed signs of decay, with some fatalities in harsh treatments and with very low volume of highly turbid coelomic fluid. After 12hrs of storage urchin gonad in all treatments recorded low values of luminosity and chromaticity that translated in a diminished brightness and true colour tone appearance; the same values after 4hrs from the emersion and environmental exposure were not significantly different from the control animals for all treatments. These outcomes suggest that urchins impacted by harvesting, handling and air exposure may still preserve gonad quality attributes if processed not long after the 4hrs from the collection. Gonad of some urchins appeared heavily stained by the brown/purplish tissue of the gut. We presume this is a consequence of a ruptured gut membrane, allowing contact with or fluid transfer from around the gut to the gonad. It is reasonable to assume that heavily stained gonad are less marketable and in general gonad of urchin processed after 12hrs from the harvest are assigned to a lower grade just from the visual assessment (P. Campus personal observations).

6.5 Fertilising effect of urchin waste mineral composition

Fish waste management can be a major problem for many fisheries (Arvanitoyannis & Kassaveti 2008). The transformation of fish waste into marketable by-products is a strategy that can be adopted to repurpose waste and offset the costs of disposal. Aquatic waste (either from aquaculture or wild stock) is commonly utilised in the manufacture of fishmeal/oil, the production of silage or in the manufacture of organic fertiliser (Arvanitoyannis & Kassaveti 2008).

Sea urchin waste represents around 85% of the drained animal and mainly consists of inorganic material. The elemental analysis of *C. rodgersii* waste parts (test, spines and jaws) contain a high proportion of Calcium (40% w/w), in a form not dissimilar to agriculture lime. The urchin waste also contains a limited amount of several other essential macronutrients - Magnesium, Nitrogen, Potassium and Phosphorous. Waste material also contains an array of micronutrients such as

Boron, Zinc, Iron, Manganese and Copper that are not usually present in commercial Lime, Gypsum or Dolomite used for agricultural purposes.

In this study, using urchin waste in powdered form (UWP) improved the growth and productivity of tomato plants with performances directly related to the rate of added urchin waste (Chapter 5). The treatment with higher addition of UWP (T7) showed vegetative growth comparable to the standard fertilizer control (Hoaglands' solution), although resulted in only half of the fruit production. The quality of the fruit produced however was high and there were no qualitative differences between fruits in T7 and Hoagland's control. In addition to that, no toxic effects of nutrients added at the highest rate were detected.

Key limitations of major important nutrients (N, P, K) in the UWP suggest urchin waste can be used as a supplement to other traditional fertilisers or as a soil amendment/ameliorant in place of lime. The UWP used in this study did not include gut content which also represents waste and obviously did not include the gonad that represent the primary product of urchin fishery. Adding these components which are high in protein could significantly improve the macro-nutrient content of the UWP supplement, particularly Nitrogen.

In Tasmania, and elsewhere in southern Australia urchin culling programs as a control measure to limit the destructive grazing of *C. rodgersii* are still in place. Since a significant part of the urchin population is represented by old specimens whose gonad do not meet qualitative marketable standards, UWP supplement could provide a cost-effective alternative to the expensive procedure of urchin culling. A careful analysis of the harvesting costs (pay of diver per kg of urchin), related to the quantity of dehydrated product and marketing operations are necessary to make the product profitable and competitive. Several of the current fish waste and kelp fertiliser products are also lacking in several macronutrients, and more balanced versions modified by the addition of N, P, K macronutrients are commonly offered for sale. Ultimately, gaining economic value in the sea urchin by-product and hence reducing the costs of disposal, will benefit the fishery and provide incentive for a greater harvest that will ultimately help reduce the pressure of this grazer over the kelp system in coastal areas.

6.6 Future directions

This study highlighted a number of interesting patterns, anomalies, and potential opportunities to improve the economic viability of Long-spined Sea Urchin fisheries. In terms of roe quality, any improvement to processes (handling, storage, supplementary feeds) that increases the proportion of harvested roe classified as A-grade roe will improve the economic viability of this fishery. Similarly, establishing alternative uses for waste, including information on nutrient content and plant uptake, will assist with marketing urchin fishery waste as a premium product and potentially attract a high market price.

6.6.1 Maximising benefit of waste and/or alternate high-value forms of consumption

Urchin roe is a potentially rich source of antioxidants (carotenoids and vitamins), and bioactive dietary compounds associated with health benefits (Archana & Babu 2016, Mamelona & Pelletier 2010). Antioxidants prevent the formation of free radicals that damage cells of organisms (Lobo et al 2010), consumption of which is associated with anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities (Zhou et al 2011, Zhou et al 2012). Antioxidants are typically used in the food industry for preventing rancidity of foodstuffs. Evaluation of the potential content and market value of these bioactive components of longspined sea urchins could provide an alternative high-value market for the product at times when roe is unsuitable for traditional human consumption.

Additional investigation on the characterisation of the gonad Free Amino Acids profile is also required since the Total Amino Acids content resulted in important information on the nutritional aspect. On the other hand, the FAAs will be determinant in the evaluation of gonad taste which is the most important quality aspect of urchin roes along with colour. Moreover, to better understand the trophic relationships between sea urchins and habitats the present gonad isotopes signature could be integrated with more information from different food sources in both barrens and kelp area as well as from the gut and gut contents. This would help understand urchins' food preference and contribution to the gonad' biochemical composition, especially in barrens grounds.

6.6.2 Achieving a higher proportion of A-grade roe

From the perspective of aquaculture practices, the potential for urchin gonad conditioning to enhance sweet and umami taste in a short period (6 - 8 weeks) needs to be investigated. This would be far less expensive than programs to increase gonad yield which in Tasmania is not required as GSI is more than adequate and could potentially increase the grade of the gonad hence the price. An artificial feed for *C. rodgersii* could be formulated with the addition of local seaweeds at different ratio, for example 4:2:2 green, brown and red kelp. Green algae showed the highest proportion in sweet amino acids, while red kelp was proportionally higher in proteins. The brown kelp is preferred and source of important PUFAs, but as adult urchins showed to retain lipids a high ratio may not be necessary; however, different ratios should be trialled. Free amino acids analysis and a sensory panel test would be necessary to identify differences in taste between the enhanced gonad and wild ones collected in kelp and barrens.

The effect of post-harvest handling and exposure on the free amino acids content of gonad requires urgent research. Free amino acids are osmotic pressure-regulating molecules, the release of free amino acids from the lumen of gonad phagocytes to the coelomic fluid to counteract dehydration can result in a loss of flavour and in a diminished appearance which ultimately determines the gonad to be assigned a lower grade and price. Evidence-based recommendations for change in harvest and storage practices hold the greatest short-term possibilities for improvement in roe quality.

Moreover, the deamination of coelomic fluids nitrogenous compounds (free amino acids and nucleic acids) during emersion could lead to the formation of end-products conferring unpleasant flavours and negatively impact gonad shelf-life (Verachia et al 2013). Understanding and preventing this regressive process will help the industry achieve higher gonad quality and market value.

Finally, further trials are needed to investigate the potential of whole urchins collected from the non-fishing areas (old, non-commercial urchins) as agricultural fertiliser. A desktop study could explore different plants nutrient requirements and soil properties and match those specifications to the urchin fertiliser. Field studies could then investigate the use of urchin waste powder with the addition of nitrogen

or the multiple applications of urchin fertiliser in powder and liquid form during the plant's growth (powder at the start mixed with soil and liquid form during plant's growth).

6.7 Conclusions

In conclusion, this thesis expanded the fisheries ecological knowledge of the echinoid species *Centrostephanus rodgersii* in Tasmanian coastal waters. It provided insights into the biochemical composition of the gonad during various stages of the gametogenic cycle with emphasis on the gonad quality and nutritional aspects for human consumption. It also demonstrated that good quality gonad could be obtained from urchins collected in barren grounds, most likely due to a greater contribution of drift algae from nearby kelp beds as a prominent food source. This investigation also identified potential options for coastal zone managers by providing a rationale for when to create subsidised harvest and/or culling programs in areas where large sea urchin specimens with low-quality gonad are more abundant.

This work demonstrated the limited ability of *C. rodgersii* to overcome or recover from stress associated with harvesting, air exposure and pre-processing storage delays with direct implications on gonad quality. The information obtained from the post-harvest experiments were intended to signal possible best practices directed to fishermen and processors with the goal to preserve or increase the sensory quality characteristics of the gonad.

The high gonadal index of the sea urchins present in the barrens makes aquaculture feeding practices for increasing gonad mass unnecessary. However, the experiments described here, in addition to having demonstrated similarities with other urchins exploited commercially, suggests the possibility of obtaining in a short time the increase in the sweet and umami taste of the gonad through the administration of formulated feed.

Ultimately, the use of the inedible parts of sea urchins as fertilizer has provided evidence of a possible further commercial use of this resource which would promote the economic viability of the urchin fishery and maximise the economic return.

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