Optimizing sea urchin gonad enhancement and gastrointestinal parameters with newly formulated feeds at different temperatures with green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins in British Columbia, Canada

by

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We acknowledge and respect the lək^{w}əŋən peoples on whose traditional territory the university stands, and the Songhees, Esquimalt and W̱SÁNEĆ peoples whose historical relationships with the land continue to this day.

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Abstract

Sea urchins are an ecologically important species that can drastically alter marine communities due to their consumption and destruction of macroalgal beds (*e.g.* kelp forests). These beds form highly productive ecosystems that provide shelter and nursery habitat for many benthic and pelagic species. When their populations explode, due to a lack of predators and/or various environmental conditions, sea urchins can overgraze and decimate macroalgal beds. This creates areas called sea urchin barrens, which is a problem seen around the world. Sea urchin aquaculture is a method to remove these over-populated sea urchins from the environment, feed them either a prepared or macroalgal diet for approximately 12-weeks to produce a marketable roe product in a process termed roe or gonad enhancement. Two feeding trials were conducted on two species of sea urchins that are native to the waters off Vancouver Island, British Columbia, Canada: the green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchin. There were nine treatments per feeding trial, where three diets (two prepared diets; V10.1.9 and V10.1.10, and one natural bull kelp (*Nereocystis luetkeana*) diet and three different temperatures (8, 12, and 16^{o}C; which are temperatures commonly found in the waters around Vancouver Island) were examined to assess the feasibility of a sea urchin gonad enhancement operation with these species and diets.

Overall, green sea urchins fed V10.1.9 at 8 and 12°C produced the highest gonad yields (mean ± SE: 29.4 ± 1.1% and 29.4 ± 1.5%, respectively) while V10.1.9 at 12°C also had the highest gonad yield increase per week (mean ± SE: 2.2 ± 0.2%) and the lowest FCR-G (mean ± SE: 1.0^{E-2} ± 9.0^{E-4} feed g gonad increase g^{-1}). Green sea urchins fed V10.1.10 at 12°C, however, produced the most preferred gonad taste, gonad yields still above market minimum (mean ± SE: 25.6 ± 1.5%), and the third lowest FCR (mean ± SE: 1.5^{E-2} ± 1.9^{E-3} feed g gonad increase g^{-1}),

while urchins fed V10.1.10 at 16°C had the best colour (mean degree of colour difference ± SE: 6.0 ± 0.9). Therefore, it can be suggested that optimal conditions moving forward for green sea urchins would be feeding V10.1.10 at 12°C. For red sea urchins, those fed V10.1.10 produced the highest gonad yields at 12°C (mean ± SE: 12.7 ± 1.5%) and the best colour at 16°C (mean degree of colour difference ± SE: 30.3 ± 3.1), while red sea urchins fed V10.1.9 at 16°C produced the second highest gonad yields (mean ± SE: 11.0 ± 0.4%), the lowest FCR-G ($1.9^{E-3} \pm 2.8^{E-4}$ feed g gonad increase g^{-1}), the most preferred gonad taste, and a low degree of colour difference (mean ± SE: 32.3 ± 2.1). Therefore, it can be suggested that optimal conditions moving forward for red sea urchins would be feeding V10.1.9 at 16°C.

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Chapter One:

General introduction to sea urchin aquaculture

1.1. Introduction to aquaculture

As the world's population continues to increase worldwide, reaching 7.8 billion people in 2020, the need for sustainably produced food and secure employment is crucial. Seafood is one of the healthiest animal-protein sources, due to high levels of good fatty acids (primarily omega-3), and provides an average of 20% of the protein consumed per person (FAO, 2020). The production of seafood is considered to be one of the least environmentally impactful of all farmed animal species (FAO, 2020). Currently, only 65.8% of the world's fish stocks are considered biologically stable, leaving 34.2% overfished and unhealthy (FAO, 2020). The consumption of seafood increased 122% between 1990 and 2018 and the ocean and inland waters around the world are unable to sustainably supply aquatic protein at the current demand while maintaining healthy fish stocks and environmental conditions (FAO, 2020). Aquaculture, which can be defined as the farming of aquatic animals (finfish, crustaceans, molluscs, echinoderms, aquatic plants, and macroalgae) in salt, fresh, or brackish waters, is a method that can help to relieve pressure on over-fished stocks and meet global aquatic food demand. In total, aquaculture production increased 527% from 1990 to 2018 and reached an all-time high in 2018 with a production of 114.5 million tonnes worth USD 263.6 billion (FAO, 2020). Aquaculture is a major source of employment, particularly in small rural communities, with about 20.5 million people involved in the activity worldwide (FAO, 2020). In 2018, 82.1 million tonnes of aquatic animals were produced, dominated by finfish (54.3 million tonnes worth USD 139.7 billion) and molluscs (17.7 million tonnes worth USD 34.6 billion), the latter of which was dominated by

bivalves and crustaceans (9.4 million tonnes worth USD 2 billion) (FAO, 2020). Other marine invertebrates, including echinoderms (such as sea cucumbers and sea urchins), were produced in smaller quantities, but with considerable value [435,400 tonnes worth USD 2 billion (FAO, 2020)]. Global sea urchin aquaculture presently contributes a relatively small amount to the market – with Asia and Europe producing 8,243 tonnes (worth USD 52 million) and 1,053 tonnes (worth USD 2 million), respectively, in 2019 of *Strongylocentrotus* species (FAO Global Aquaculture Production 1950–2019) – but there is increasing interest in echinoid aquaculture in many countries.

1.2. Biology and ecology of sea urchins

Sea urchins (class Echinoidea) belong to the phylum Echinodermata, which includes sea cucumbers (class Holothuroidea) and sea stars (class Asteroidea). Sea urchins are generally round animals that exhibit penta-radial body symmetry, although some species, like sand dollars, exhibit bilateral symmetry (Kozloff, 1996). Their shell, or test, is comprised of fused plates made of calcium carbonate that are covered in thin dermis and epidermis layers (Kozloff, 1996). The test is divided into five ambulacral grooves, each having two rows of plates (20 in total), which are covered in rounded tubercles that house ball and socket joints that form the base of the characteristic spines that cover the sea urchin body (Kozloff, 1996). Sea urchins use these spines for protection and to help with movement (Kozloff, 1996). The tube feet are also located in the five ambulacral grooves., which protrude from pores in the test and are powered by hydraulic pressure from the sea urchin's water vascular system (Kozloff, 1996). The tube feet aid the sea urchin in movement and collection of food, are sensitive to chemicals and touch, and can absorb oxygen (Kozloff, 1996). Similar to the tube feet, the sea urchins also have pedicellariae which

have the same general structure as the tube feet, but have pinchers at the end to aid in food collection and cleaning (Kozloff, 1996).

The sea urchin mouth is located on the bottom or oral surface of the test and is called the peristome, while the anus, madreporite (perforated plate by which entry of seawater into the vascular system of an echinoderm is controlled), and gonopores (opening through which the sperm and eggs are released) are found on the top or aboral surface (Kozloff, 1996). The jaw apparatus, termed Aristotle's lantern, is comprised of five sharp teeth that are strong enough to allow some species of sea urchins to dig cavities in rocks (Kozloff, 1996). The Aristotle's lantern is surrounded by softer tissue that houses five pairs of gills and five pairs of modified tube feet (Lawrence et al., 2007a). The top of Aristotle's lantern leads to the pharynx and then esophagus, which then leads to the small and large intestine, where digestion of food occurs (Lawrence et al., 2007a). Lastly, the large intestine connects to the anus where the undigested food is expelled out the aboral side of the sea urchin (Lawrence et al., 2007a).

While their nervous system is relatively simple with no true brain, sea urchins have a nerve ring that allows them to be able to react to touch, light, and chemicals. This allows the sea urchin to seek out shelter, food, and to escape predation (Kozloff, 1996). The nerve ring encircles the mouthpiece, branching out into five nerves that then branch into numerous finer nerves that connect to the spines, tube feet, and pedicellariae (Burke et al., 2006). There are also numerous nerves located in the thin outer dermis of the sea urchins on the test and spines that allow the sea urchin to react to light, touch, and chemicals (Burke et al., 2006). So, although the sea urchin does not have eyes or eye spots, it can sense a threat/light and retreat/hide as well as sense food and move towards it (Burke et al., 2006).

Sea urchins are dioecious, but monomorphic, meaning the two sexes (males/female) are externally indistinguishable. Due to their penta-radial symmetry, sea urchins have five strips of gonads located within their test. They are the main organs for both gametogenesis and nutrient storage, and are comprised of both germinal cells and somatic cells, the latter also being known as nutritive phagocytes (Walker et al., 2007). Therefore, what the sea urchin consumes directly relates to the size and quality of the gonads produced (Lawrence et al., 1966; Siikavuopio et al., 2006). There are five different stages in the sea urchin reproductive cycle: recovering, growing, premature, mature, and partially spent (Kelly, 2004; McBride et al., 2004; Walker et al., 2007). Pre-mature and mature gonads tend to be larger with good colour and resiliency, while other stages may produce darker softer gonads, which are less desirable (McBride et al., 2004). In the pre-mature and growing stages, the gonads are primarily composed of nutritive phagocytes when they are bulking up prior to spawning (Böttger et al., 2006).

Sea urchins are an ecologically important group that can drastically alter marine communities due to their consumption of macroalgal beds such as kelp forests (Mann, 1977; Sivertsen, 1997; Lawrence, 2001; Graham, 2004; Filbee-Dexter and Scheibling, 2014). When their populations explode, due to a lack of predators and/or various environmental conditions, the sea urchins can overgraze and decimate macroalgal beds, creating areas called sea urchin barrens, which is a problem seen around the world (Filbee-Dexter and Scheibling, 2014). Macroalgal beds are characterized as highly productive ecosystems that provide shelter and nursery habitat for many fish and invertebrate species, however, urchin barrens are areas of low-lying encrusting coralline algae and have very low productivity (Cowen et al., 1982; Tegner and Dayton, 2000; Eurich et al., 2014; Filbee-Dexter and Scheibling, 2014, 2018).

1.3. Sea urchin fishery

Sea urchins have been fished around the world for many decades, with landings beginning to be recorded in the 1950s by FAO (FAO Global Aquaculture Production 1950 – 2019). Their test and spines have also been found in great abundance in prehistoric middens and are a traditional food source of many First Nations (Kuhnlein and Humphries, n.d.; Lawrence, 2007b), and are now considered a delicacy in many nations. Over time the wild fisheries have declined in many countries due to overfishing and/or poor fishery management (Andrew et al., 2002). In 2019, the total global fishery for sea urchins was 66,341 tonnes, which was down from 69,040 tonnes in 2010 and 93,319 tonnes in 2000 (FAO Global Capture Production 1950–2019). The largest sea urchin fishery presently occurs in Chile with the Chilean sea urchin (*Loxechinus albus*), where in 2019 the total capture was 37,226 tonnes (FAO Global Capture Production 1950–2019). Sea urchins from the genus *Strongylocentrotus* are the second most fished group at 27,718 tonnes in 2019 (FAO Global Capture Production 1950–2019). Harvesting methods for sea urchins depends on location, cost/benefit analyses, and environmental conditions (Kramer and Nordin, 1979; James and Hannon, 2017). The main methods used are diving, towed gear, and recreational trapping (Kramer and Nordin, 1979; James and Hannon, 2017). Self-contained underwater breathing apparatus (SCUBA) is the predominant method used out of all harvesting techniques (Kramer and Nordin, 1979; James et al., 2016; James and Hannon, 2017). With SCUBA, divers can harvest at varying depths using a pronged rake or gloved hand to gently remove the sea urchins from the substrate. The divers will crack open a few sea urchins as they swim along the seabed to ensure they are harvesting in a good quality area (Andrew et al., 2002). They are then placed into a mesh bag or basket and brought to the surface where they are transferred to holding tanks on a fishing vessel (Kramer and Nordin, 1979; James and Hannon,

2017). They are then taken to local processing plants for extraction and packaging of the gonads. Towed gear, like a dredge or beam trawl, is not commonly used, due to the potential environmental impact (Andrew et al., 2002; James and Hannon, 2017), however, in places with hazardous weather conditions, such as Iceland, it is the sole method used for harvesting them (James and Hannon, 2017). Trapping sea urchins is done recreationally using traps baited with fish or macroalgae and a soak time of up to seven days (James and Hannon, 2017).

In Canada, three sea urchin species from the genus *Strongylocentrotus* have been fished in the past, with two species still being commercially harvested. The purple sea urchin (*Strongylocentrotus purpuratus*) has a range on the west coast of North America from Vancouver Island, British Columbia (BC), Canada to Baja California, Mexico (Kozloff, 1996). It is a relatively small sea urchin with a maximum test diameter of 10 cm, but is typically smaller, with spines reaching up to 2.5 cm long (Biodiversity of the Central Coast, 2016). In the past, an experimental permit fishery for the purple sea urchin existed in BC from 1989 to 1992. This species, however, prefers the harsh open coast of west Vancouver Island and shallow subtidal areas which are prone to intense wave action and was therefore too difficult and dangerous to fish (Kozloff, 1996). The purple urchin, however, is of large ecological importance along the west coast of the United States of America (USA), where it is over-populated and decimates kelp forests. This has given rise to a lucrative fishery in Washington, Oregon, and California, USA (Kozloff, 1996; Pearse, 2006). Presently, the green (*S. droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins have fisheries in Canada, the former on both the east and west coasts of the country and the latter only on the west coast. The green sea urchin has a maximum test diameter of 10 cm, although average diameter is between 5 and 6 cm (Fisheries and Oceans Canada, 2019b), a maximum spine length of ~2.5 cm (Biodiversity of the Central Coast, 2016),

and a circumpolar distribution (Fisheries and Oceans Canada, 2019). This species has commercial dive fisheries in Canada on the west coast in BC [~220 tonnes worth CAD ~1,100,000 in the 2016–2017 fishing season (Green Sea Urchin Integrated Fisheries Management Plan 2018–2021)] and on the east coast in the Atlantic provinces [(1,325 tonnes worth CAD 7,206,000) in 2019 (Fisheries and Oceans Canada, 2019)]. The much larger red sea urchin is the largest urchin species in the world with a test diameter up to 19 cm and a spine length of 5–8 cm (Kato and Schroeter, 1985), with populations in Japan and western North America, ranging from northern Alaska, USA to northern Mexico (Lamb and Hanby, 2005). The species forms the basis of lucrative dive fisheries in BC [~2,721 tonnes worth CAD ~4,000,000 in 2018 (Fisheries and Oceans Canada, 2020)], Washington [115 tonnes in the 2020–2021 season (Washington Department of Fish and Wildlife, 2021)], Oregon [~2,273 tonnes in 2014 (Oregon Department of Fish and Wildlife, 2021)], California [7,265 tonnes worth CAD 14,018,000 in 2005 (British Columbia Seafood Alliance and the Seafood Value Chain Roundtable, 2006)], and Alaska [307 tonnes in the 2016–2017 season (Alaska Department of Fish and Game, n.d.).

1.4. Sea urchin aquaculture

Global sea urchin aquaculture presently contributes a relatively small amount to the market – with Asia and Europe producing 8,243 tonnes (worth USD 52,000,000) and 1,053 tonnes (worth USD 2,000,000), respectively, in 2019 of *Strongylocentrotus* species (FAO Global Aquaculture Production 1950–2019). There is interest, however, in increasing echinoid aquaculture production in many countries due to overfishing and high market prices. Two types of sea urchin aquaculture exist: full life cycle grow-out and gonad enhancement of wild-caught, low-gonad-yield sea urchins (Andrew et al., 2002; Woods et al., 2008). The former would be a multi-year

process involving brood stock conditioning and spawning, rearing of sea urchin larvae, larval settlement, and juvenile grow-out to produce market-sized adults (James and Siikavuopio, 2012; Cárcamo, 2015). This method can be used to either replenish natural populations or to produce gonads for the market (Daggett et al., 2005). Conversely, gonad enhancement entails retrieving adult-sized sea urchins (with low gonad yields) from barren grounds and feeding them either a natural or prepared diet for 8–12 weeks in captivity (either land- or sea-based). There has been a plethora of research on optimizing gonad enhancement, focusing on holding systems (both land- and sea-based; *e.g.* Devin, 2002; Cárcamo, 2004; Kelly, 2004; Böttger et al., 2006; Daggett et al., 2006; James, 2006; Hagen and Siikavuopio, 2010; Brown and Eddy, 2015; James et al., 2017; Takagi et al., 2017) and natural/formulated diets [*S. droebachiensis* in eastern Canada (Pearce et al., 2002a, b, 2004; Robinson et al., 2002; Daggett et al., 2005, 2006), northeastern USA (Vadas Sr. et al., 2000), and Norway (Siikavuopio et al., 2006, 2008; James and Siikavuopio, 2012; James et al., 2017); *M. franciscanus* in western USA (McBride et al., 2004, 2007; McBride, 2005) and western Canada (Mooney and Bunnell, 2001); *Lytechinus variegatus* in southeastern USA (Hammer et al., 2012; Taylor et al., 2017); *Evechinus chloroticus* in New Zealand (James et al., 2007; Woods et al., 2008); *Centrostephanus rodgersii* and *Heliocidaris erythrogramma* in Australia (Blount et al., 2017; Warren-Myers et al., 2020); *Loxechinus albus* in Chile (Olave et al., 2001; Cárcamo, 2004, 2015; Lawrence et al., 2007b); *Mesocentrotus nudus* in Japan (Takagi et al., 2017, 2018); and *S. intermedius* in China (Chang et al., 2005; Lawrence et al., 2009, 2011; Luo et al., 2014; Zhang et al., 2014; Zhao et al., 2016; Wei et al., 2017)]. On the west coast of Canada, the two primary candidates for sea urchin aquaculture are the green and red sea urchins and, although no commercial-scale aquaculture of these two species is presently occurring in BC,

there is increasing interest from local and foreign investors and First Nations in the development of an industry.

Sea urchins are fished or cultivated for their gonads, which are rigorously judged based on size, colour, texture, firmness, and taste. What a sea urchin consumes is a critical factor in the quality of the gonads produced. The optimal product for marketing is firm, has good texture (two distinct gonad segment halves and smooth), is sweet and savory (also termed *"umami")*, meets a minimum yield of 10–15% (gonad wet weight to whole wet weight), and is bright orange/yellow in colour, to garner the top price in the Japanese markets. Carotenoids are the naturally occurring pigments that contribute to the production of red, orange, and yellow colouration. Carotenoids, however, cannot be synthesized directly by an urchin and need to be obtained from the diet (Griffiths and Perrot, 1976; Tsushima and Matsuno, 1990; Watts et al., 1998; Tsushima, 2007). In sea urchins, the major carotenoid that contributes to gonad colour is β-echinenone, which is synthesized from β-carotene (Griffiths and Perrot, 1976), with conversion occurring in the gut wall (Tsushima and Matsuno, 1990; Tsushima et al., 1993). A higher concentration of these pigments in the diet will lead to better gonad colour (Tsushima and Matsuno, 1990; Tsushima et al., 1993). Pigment concentrations also tend to be lower in males than in females, with the latter tending to have better gonad colour than the former (Phillips et al., 2009; Zhao et al., 2010), and varies seasonally with the reproductive cycle of sea urchins (Griffiths and Perrot, 1976). Carotenoids are important for not only gonad colour, but also for the sea urchin's health. Individuals fed diets lacking carotenoids will show increasing spine loss and disease (Robinson et al., 2002). Uptake of pigments is also reliant on ingestion rates of the urchins, which varies with season, reproductive state, diet, and sea urchin size (Leighton, 1968; Miller and Mann,

1973; Larson et al., 1980; Klinger et al., 1997; McBride, 1997), and can also vary greatly daily (Miller and Mann, 1973).

Much research has been done on the effect of different diets on gonad enhancement in many echinoid species. These diets can be divided into two major groups, macroalgae and formulated feeds. Feeding the former typically produces high-quality gonads in terms of colour and flavour, but typically results in much lower gonad yields (Pearce et al. 2002b, 2004, Chang et al. 2005, Böttger et al. 2006, Woods et al. 2008, Azad et al. 2011, James and Siikavuopio 2012, Cárcamo 2015). In addition, macroalgae may be difficult to obtain in winter months, vary nutritionally both temporally and spatially, can be costly to harvest and store long term, and may be highly regulated for harvesting. Prepared diets typically produce large gonads relatively quickly (Pearce et al. 2004, James and Siikavuopio 2012), are easily and cheaply stored, and are nutritionally constant across time. Prepared diets, however, may also produce gonads with less-than-optimum colour, texture, firmness, and flavour, many times producing a pale colour and bitter flavour (Pearce et al. 2002b, 2004, Böttger et al. 2006, Woods et al. 2008, James and Siikavuopio 2012, Cárcamo 2015). One of the latest formulated diets – produced by an urchin farming company (Urchinomics) specifically for *S. droebachiensis* in Norway – however, has shown great promise in producing market-quality gonads (Brian Tsuyoshi Takeda, Urchinomics, pers. comm.). Urchinomics is an aquaculture business venture based in Norway aiming to (i) remove overgrazing sea urchins from barren grounds, (ii) deliver a premium sea urchin product to global markets, and (iii) restore kelp ecosystems. To date, this new feed has not been tested on *S. droebachiensis* or *M. franciscanus* in western Canada.

Water temperature is another factor that is critically important in gonad enhancement, with many studies having compared the effect of temperature on gonad development of various sea

urchin species (*e.g.* Griffiths and Perrot, 1976; McBride et al., 1997, 2007; Siikavuopio et al., 2006, 2008; James et al., 2007; Lawrence et al., 2009; Azad et al., 2011; Zhao et al., 2016). Water temperature affects many metabolic and reproductive processes in sea urchins. For instance, higher water temperatures (up until a point where thermal tolerance is reached) produces increased ingestion rates and therefore greater uptake of protein and pigments, which ultimately affects gonad production. Water temperature is also strongly linked to the urchin reproductive cycle and therefore gonad state and quality. Both diet and water temperature may affect a variety of metabolic processes in sea urchins such as ingestion rates, absorption efficiencies, feed conversion rations, and faecal composition and production. All of these processes are important in understanding the potential environmental impacts that a sea-based commercial sea urchin gonad enhancement operation might have in terms of organic matter accumulation in the benthos. There have been no studies examining this issue, however.

1.5. Objectives

The objective of this study was to optimize sea urchin gonad enhancement in green (*S. droebachiensis*) and red (*M. franciscanus*) sea urchins using two newly formulated feeds (Urchinomics: V10.1.9 and V10.1.10) and a kelp diet (*Nereocystis luetkeana*) at three different temperatures (8, 12, and 16°C). The study also assessed the potential environmental impact of this new industry. The following questions were investigated:

i. Is there an optimal diet and temperature combination for both green and red sea urchins that will produce high gonad yields and market quality product in 12 weeks?
ii. Does gonad quality differ between male and female sea urchins?

iii. Do different diet and temperature combinations affect ingestion rates, absorption efficiencies, feed conversion ratios, and faecal production in the green and red sea urchins?

iv. Do different diet and temperature combinations affect faecal pellet size, shape, and settling velocity and thus have implications for potential environmental impact of a commercial sea urchin gonad enhancement operation?

This thesis presents two separate feeding trials for green and red sea urchins examining somatic parameters, gonad yield and quality, and gastrointestinal and faecal pellet parameters. Based on published literature of previous sea urchin gonad enhancement trials and this study, optimal conditions for both green and red sea urchin gonad enhancement are discussed.

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Chapter Two:

Effect of diet and temperature on gonad yield and quality in the green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins

2.1. Introduction

Sea urchins are an ecologically important group that can drastically alter marine communities due to their consumption of macroalgal beds such as kelp forests (Mann, 1977; Sivertsen, 1997; Lawrence, 2001; Graham, 2004; Filbee-Dexter and Scheibling, 2014). When their populations explode due to a lack of predators and/or various environmental conditions, sea urchins can overgraze and decimate macroalgal beds, creating areas called sea urchin barrens, which is a problem seen around the world (Filbee-Dexter and Scheibling, 2014). Macroalgal beds are characterized as highly productive ecosystems that provide shelter and nursery habitat for many fish and invertebrate species. Urchin barrens are areas of low-lying encrusting coralline algae and have very low productivity (Cowen et al., 1982; Tegner and Dayton, 2000; Eurich et al., 2014; Filbee-Dexter and Scheibling, 2014; Filbee-Dexter and Wegner, 2018).

Sea urchins are fished or cultivated for their gonads, also termed roe or uni, which are the main organ for nutrient storage (Lawrence et al., 1966; Siikavuopio et al., 2006). Each sea urchin has five strips of gonad, which are rigorously judged on their size, colour, firmness, texture, and flavour. The optimal product is bright orange/yellow in colour, firm, has good texture (two distinct segment halves and smooth), is sweet and savory (also termed “umami”), and meets a minimum yield of 10–15% to garner the top price in the Japanese markets. Since gonads are the main organ for nutrient storage, what urchins eat directly relates to the size and quality of their

gonads (Lawrence et al. 1966, Siikavuopio et al. 2006). When sea urchins remain in barrens for long periods of time with little or no food, the resulting gonads are small and of poor quality (Mann, 1977). This makes the sea urchins unsuitable for harvest by the dive fishery, with predators avoiding them as well (Tegner and Levin, 1983; Eurich et al., 2014). This has led some jurisdictions to manually remove sea urchins from these barrens to promote the re-growth of macroalgae in the area. For example, culling has been trialed in Haida Gwaii, British Columbia (BC), Canada (Lynn Lee, Parks Canada, pers. comm. 2018) and Tasmania, Australia (Tracey et al., 2014, 2015). Another approach is to create an aquaculture industry which can make "empty" sea urchin barrens commercially viable.

Gonad enhancement of sea urchins entails retrieving adult-sized sea urchins from barren grounds (i.e. low gonad yields) and feeding them either a natural or prepared diet for 8–12 weeks in captivity, either land or sea-based. There has been a plethora of research on gonad enhancement diets (*i.e.* Vadas Sr. et al., 2000; Mooney and Bunnell, 2001; Pearce et al., 2002a, b, 2004; Taylor et al., 2017; Wei et al., 2017; Warren-Myers et al., 2020) and holding systems (*i.e.* Devin, 2002; Cárcamo, 2004; Kelly, 2004; Böttger et al., 2006; Hagen and Siikavuopio, 2010; and Takagi et al., 2017). The ideal cultivation system, whether land or sea-based or with a natural or prepared diet, should aim to increase surface area for rearing, decrease water usage (land-based), increase waste removal, lower mortality rates, and improve gonad yield and quality (Devin, 2002; Kelly, 2004; Böttger et al., 2006; Daggett et al., 2006; James, 2006; Brown and Eddy, 2015). Research examining gonad enhancement with natural macroalgae and prepared feeds has typically shown that the former produce better quality gonads (in terms of colour and flavour) than the latter, although natural feeds usually produce lower gonad yields (Pearce et al., 2002b, 2004; Chang et al., 2005; Böttger et al., 2006; Woods et al., 2008; Azad et al., 2011;

James and Siikavuopio, 2012; Cárcamo, 2015). Macroalgal diets, however, are difficult to obtain in winter months, vary nutritionally throughout the year, and are costly to harvest and store long term (Pearce et al., 2002b, 2004; Chang et al., 2005; Böttger et al., 2006; Woods et al., 2008; Azad et al., 2011; James and Siikavuopio, 2012; Cárcamo, 2015). In addition, unless the macroalgae are harvested from cultured crops or the wild harvests are well managed, macroalgal beds can be further decimated. This has led to many studies being conducted on the development of prepared diets to optimize gonad yield and quality. Numerous studies have shown that prepared feeds which are typically higher in animal protein, produce large yields quickly, but the gonads often have an undesirable pale colour and a bitter flavour (McBride et al., 1997; Pearce et al., 2002a, 2004; Robinson et al., 2002; Shpigel et al., 2005; Woods et al., 2008). Therefore, a critical issue in developing a prepared feed is identifying the type and concentration of pigments and protein needed to obtain the bright orange/yellow colour and sweet and savory flavour so as to garner top prices in the market (McBride et al., 1997; Pearce et al., 2002a, 2004; Robinson et al., 2002; Shpigel et al., 2005; Woods et al., 2008). Recently, a private company (Urchinomics) has produced a commercial sea urchin feed that has shown great promise in producing market-quality colour and flavour with the green sea urchin (*Strongylocentrotus droebachiensis*) in Norway (Brian Tsuyoshi Takeda, Urchinomics, pers. comm.).

Water temperature is another critical factor in optimizing a sea urchin gonad enhancement operation. Temperature, primarily linked to seasonal changes, has been shown to influence feed intake and metabolism, which ultimately affects the allocation of nutrients and pigments to the gut or gonads (Lawrence et al., 1966). Water temperature is also strongly linked to reproductive cycle and therefore gonad state and quality. Both green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins, which are native to the west

coast of North America, experience temperature ranges of -1 – 22ºC (Scheibling and Hatcher, 2001), however, optimum temperature is around 10-12ºC for green urchins (Siikavuopio et al., 2006, 2008) and 16ºC for red urchins (Kato and Schroeter, 1985; Leighton, 1971; McBride et al., 1997).This study compared three different rearing temperatures, 8, 12 and, 16ºC, which are temperatures commonly found in the waters surrounding British Columbia, Canada and fall within both species' normal temperature range.

The two primary sea urchin aquaculture candidates on the west coast of Canada are the green and red sea urchins. Green sea urchins have a maximum test diameter of 10 cm, although average diameter is between 5 and 6 cm (Fisheries and Oceans Canada, 2019), a maximum spine length of approximately 2.5 cm (Biodiversity of the Central Coast, 2016), and a circumpolar distribution (Fisheries and Oceans Canada, 2019). Red sea urchins are the largest species of echinoid with a maximum test diameter of 19 cm and spine length of 5–8 cm (Kato and Schroeter, 1985), with populations in Japan and western North America, ranging from northern Alaska, USA to northern Mexico (Lamb and Hanby, 2005). Both species form the basis of lucrative dive fisheries in British Columbia (BC), Canada with 2,909 metric tonnes live weight of the two species, worth CAD 7.4 million, being landed in 2018 (DFO Statistics). The red sea urchin is also fished in Mexico, Oregon, Washington, California, and Alaska (USA) and the green sea urchin in eastern Canada, Maine (USA), Iceland, and Norway (British Columbia Seafood Alliance and the Seafood Value Chain Roundtable, 2006; Fisheries and Oceans Canada, 2019). Presently, global sea urchin aquaculture contributes a relatively small amount to the market with Asia and Europe producing 8,243 tonnes (worth USD 52 million) and 1,053 tonnes (worth USD 2 million), respectively, in 2019 of *Strongylocentrotus* species (FAO Global Aquaculture Production 1950–2019) – but there is interest in increasing

echinoid aquaculture production in many countries, including Canada. Commercial sea urchin gonad enhancement trials are ongoing in Norway and Eastern Canada with green sea urchin, in California, USA with purple sea urchins (*S. purpuratus*), and in Japan with *Mesocentrotus nudus* (Lindsey, 2019; Loew, 2019; Simke, 2020; Stranden, 2020). Currently, no land-based gonad enhancement research, however, has been conducted on green or red sea urchins in western Canada.

The objective of the present study was to assess the interactive effect of diet [two newly formulated Urchinomics prepared feeds (V10.1.9 and V10.1.10) and bull kelp (*Nereocystis luetkeana*)] and temperature (8, 12, and 16°C) on gonad yield and quality (colour, texture, firmness, and taste) in the green and red sea urchin held in a land-based system.

2.2. Methodology

2.2.1. Sea urchin collection and holding

Adult green sea urchins (GSU) with a mean ± SD test diameter of 59.9 ± 3.1 mm were hand collected by SCUBA divers at Snake Island, BC (49º 12' 57.1" N, 123º 53' 26.4" W) on October 15, 2018. Adult red sea urchins (RSU) with a mean ± SD test diameter of 115.5 ± 8.1 mm were collected by SCUBA divers off the northern tip of Kendrick Island, BC (49º 07' 52.9" N, 123º 41' 47.2" W) on November 13, 2018. The urchins were transported on a boat in large tanks to the Pacific Biological Station (PBS), Nanaimo, BC. Transport time was ~1 hour for the GSU and ~2 hours for the RSU. At PBS, the sea urchins were placed into fiberglass tanks (L x W x H: 122.0 x 91.5 x 29.3 cm) and supplied with flow-through ambient (~8–10°C), sand-filtered and UV-treated, seawater. The sea urchins were left at this temperature and fed previously frozen (at -20°C) bull kelp (*N. luetkeana*) *ad libitum* once a week for 2 weeks, to

allow for any mortality from handling or transport stress to occur. Due to this being a gonad enhancement study, starting gonad yields of 8% or less are ideal, so spawning was induced for both species using an injection of 0.53-M potassium chloride (KCl) through the peristomial membrane and into the urchin test (Pearce and Scheibling, 1990). The amount of KCl used per individual urchin was determined by 0.05 ml of 0.53-M KCl per 1 mm of sea urchin test diameter (Pearce and Scheibling, 1990).

2.2.2. Experimental setup and design

These experiments were conducted for 12 weeks – between June 3 and September 3, 2019 for GSU, and between September 24 and December 10, 2019 for RSU. They examined the interactive effects of three diets [two prepared feeds (V10.1.9 and V10.1.10) and one macroalgae (bull kelp, *N. luetkeana*) as a control] and three temperatures (8, 12, and 16ºC) in a totally crossed, nine-treatment experiment. The two prepared diets were the latest formulations of Urchinomics [versions 10.1.9 and 10.1.10 (proximate analysis: 12.5% protein, 1.6% fats, and 10.0% water)]. Both feeds were dry, extruded, pellets based on offcuts of Kombu kelp (various species from the family *Laminariaceae*) from sustainable production for human consumption. The two feeds differ slightly from each other in the kelp blend used (Brian Tsuyoshi Takeda, Urchinomics, pers. comm.). Both feeds have recently shown promise in producing market-quality gonad colour and flavour with the GSU in Norway and *M. nudus* in Japan (Loew, 2019; Stranden, 2020). The bull kelp was previously collected in August 2016 from False Narrows, BC (49° 08' 04.7"N, 123° 47' 03.2"W) and frozen at -20°C, as fresh kelp was not readily available during the experiments. The three temperature treatments were chosen to reflect a range of temperatures typically found in BC (Sea Temperature, 2020).

Ninety urchins were randomly chosen from the stock tanks and placed into three of the fiberglass tanks described above (*i.e.* 30 sea urchins per tank) with the water in each tank being raised or lowered in temperature from ambient (~10.6ºC) at ~1°C per day to allow urchins to gradually acclimate to the respective treatment temperatures of 8, 12, and 16°C. Food was withheld during and 1 week post-temperature acclimation to standardize the hunger levels for all individuals. Eighteen of the 30 individuals in each of three tanks were randomly selected for the experiment, producing six replicates for each feed/temperature combination. Each urchin was placed into a PVC bucket (H x D: 30 x 30.5 cm, volume: 19 L), that had a mesh-covered hole (H x D: 20 x 20 mm, mesh size: 0.7 x 0.7 mm) at the top. This allowed water outflow but also prevented uneaten food waste and faeces from leaving the bucket. The buckets were then randomly placed into nine fiberglass tanks described above. Each bucket was individually supplied with filtered seawater at a flow rate of ~2.5 L min^{-1}, which was either 8, 12, or 16ºC depending on the treatment in which the sea urchin was allocated. The sea urchins were fed *ad libitum* once a week. Their respective diets were 0.7% of their body weight if fed the prepared diets, and 5% of their body weight if fed the macroalgae diet (Pearce et al. 2002a, b, 2004). Uneaten food and faeces were siphoned out from each bucket prior to the weekly feeding. Temperature (ºC), salinity (ppt) (YSI Pro30, YSI Incorporated, Yellow Springs, Ohip, USA), and dissolved oxygen (mg/L) (OxyGuard Handy Polaris, OxyGuard International A/S, Farum, Denmark) were recorded for each tank daily, as were mortalities. Temperature was also recorded in each tank every hour using HOBO® Tidbit® v2 temperature loggers (Onset, Bourne, Massachusetts, USA). Any unhealthy-looking or dead urchins (*e.g.* spine loss, spine drooping, unextended/loss of tube feet, and lesions) were removed from the experiment and taken for a

disease screen in the PBS Shellfish Pathology Laboratory, and were not replaced. See results section for further information on survivorship.

2.2.3. Gonad yield and quality

At the start of week 0 (June 3, 2019 for GSU and September 24, 2019 for RSU) and the end of week 9 (September 3, 2019 for GSU; trial had to end early due to a spawning event) and the end of week 12 (December 10, 2019 for RSU), the somatic parameters and gonad yield and quality were assessed. For the initial sampling at week 0, five randomly selected sea urchins from each temperature treatment that were not part of the experiment, and which were also at the end of the starvation period, were sampled to give baseline data. At the end of the experiment (week 9 for GSU and week 12 for RSU), all the experimental sea urchins were sampled. Each sea urchin was blotted dry with a paper towel for one minute and then weighed (± 2–5 g) to obtain total wet weight (g). Test diameter (mm), spine diameter (mm), and test height (mm) were measured using digital calipers (Mitutoyo ABSOLUTE Coolant Proof Caliper IP67, Mitutoyo Canada, Toronto, Ontario, Canada). The sea urchins were then cracked in half on the oral side and the five gonads scooped out, gently blotted dry, and placed onto a pre-weighed aluminum weigh boat to be weighed. The sea urchins were visually assessed for sex by observation of the presence of eggs or sperm. Gonad yield was calculated as a percentage of the total wet weight of the urchins, using the following equation:

$$\textit{Gonad yield (\%)} = \textit{(gonad wet weight (g) / sea urchin wet weight (g))} \times 100$$

Gonad yield increase per week was obtained for each species by calculating the percentage that the yield increased in total between week 0 and the end sampling date, and then dividing the value by the number of weeks in the trial using the equation below. This was done

to account for any significant differences in starting gonad yields between the temperature treatments at week 0.

Gonad yield increase per week (%) = (final gonad yield (%) – initial gonad yield (%)) / length of trial (weeks)

The colour of the gonads was assessed objectively using a reflected light fibre-optic Minolta Chroma Meter CR-100 (Konica Minolta Sensing Americas, Inc., Ramsey, New Jersey, USA) and the *Commission Internationale de l'Eclairage* (*CIE*) *1976* scale that measures lightness (L*), redness (a*), and yellowness (b*) (McLaren, 1976; Robinson et al., 2002; James, 2006). The chroma meter was calibrated under illuminant C (6774K) conditions and placed at a 90^{o} angle against the gonad sample surface. Two L*, a*, and b* measurements were obtained from each gonad sample and an average of the two calculated for use in statistical analysis. Using the *CIE* rating, degree of colour difference was calculated by obtaining the L*, a*, and b* values of a target gonad colour [bright orange/yellow; L*: 74.5, a*: 8.3, b*: 65.7 (this value was obtained from unpublished work conducted by the author)] and then comparing each other *CIE* rating to the target colour using the following equation (McBride et al., 2004):

$$\textit{Degree of colour difference } (\Delta E_{ab*}) = [(L*_{0} - L*_{sample})^2 + (a*_{0} - a*_{sample})^2 + (b*_{0} - b*_{sample})^2]^{1/2}$$

Gonad texture, firmness, and colour were assessed by eye using a subjective 1 – 4 rating scale, where 1 is the best and 4 the worst [see Table 2.1 for rating scale (Pearce et al., 2002a)]. To assess gonad taste in both species, separate tasting panels were done using the rating scale provided in Table 2.1, although neither were statistically analyzed. For GSU, eight urchins (not part of the experiment) were fed either V10.1.9 or V10.1.10 (four urchins per diet) for seven weeks at ambient temperature (mean ± SD: 12.5 ± 1.1°C, N = 12). The gonads were rinsed in freshwater to clean them and then tasted by three people with knowledge of preferred market

flavour; two of which are in the commercial roe enhancement industry and one in research with many years of experience in the industry. It is important to note, however, that rinsing in freshwater is not the preferred method for sampling gonads which could have contributed to the results. The three tasters were blind folded to remove any influence of colour and given a gonad sample of one GSU fed V10.1.9 and one fed V10.1.10. The gonad samples were ranked based on the scale provided in Table 2.1. For RSU, one replicate gonad from each treatment (*i.e.* kelp, V10.1.9, or V10.1.10 at 8, 12, and 16°C; excluding V10.1.9 at 8°C) were rinsed in filtered seawater and then tasted and ranked by two of same three tasters from the GSU trial using the same blindfolded method and ranking scale (Table 2.1).

2.2.4. Statistical analysis

Statistical analyses were conducted using JMP® 15 software (SAS, Marlow, Buckinghamshire, UK). For all analyses, statistical significance was assumed at $\alpha \leq 0.05$. The Shapiro-Wilks test and Levene's test were used for all variables to confirm that the assumptions of a normal distribution and homogeneous variances were met. If the data did not meet these assumptions, they were transformed [*i.e.* RSU: gonad yield week 0 and 12 (arcsine transformed), lightness and yellowness at week 0 (log transformed), spine diameter and yellowness at week 12 (log transformed). If after trying various transformations the data still did not meet the assumptions, a non-parametric Kruskal-Wallis test was used, with Steel-Dwass All Pairs tests being used for post-hoc multiple pair-wise comparisons (*i.e.* GSU: test diameter and redness values at week 9). For somatic variables (sea urchin wet weight, test height, test diameter, and spine diameter) at week 0, a two-way ANOVA was used to compare the effects of diet (D), temperature (T), and the interaction (D x T). For gonadal variables (wet gonad weight, gonad

yield, gonad lightness, redness, yellowness, and degree of colour difference) at week 0, a one-way ANOVA was used to assess any significant differences between the 15 sea urchins used for baseline data in the three starting temperature treatments. For all variables at the end of the experiment, if the assumptions were met, a two-way ANOVA was used to compare the effects of diet (D), temperature (T), and the interaction (D x T) on the variables, followed by Tukey's HSD for pair-wise comparisons among the treatments.

A one-way ANOVA was used to compare the effect of sex on lightness, redness, yellowness, and degree of colour difference for GSU at the end of the experiment. For texture, firmness, and colour using the rating scale provide in Table 2.1, the data were analyzed using a contingency analysis to compare the effects of diet, temperature, and the interaction on the variables. If the effects were significant, a correspondence analysis was used to assess any pair-wise comparisons. For any missing values due to sea urchin death, the average of the treatment from which the sea urchin was taken was used as a best estimate with which to replace them (Quinn & Keough 2002).

2.3. Results

2.3.1. Green sea urchins

2.3.1.1. Water quality and survivorship

GSU experienced a mean ± SD temperature of 7.8 ± 0.1°C, 12.4 ± 0.1°C, or 15.9 ± 0.1°C (N = 12,374), depending on the temperature treatment in which they were allocated. Mean ± SD salinity was 30.4 ± 0.5 ppt (N = 459) and dissolved oxygen was 8.5 ± 0.6 mg L^{-1} (N = 459). Survivorship was 100% during the 9-week feeding trial.

2.3.1.2. Somatic parameters

Mean ± SE initial and final somatic parameters are provided in Tables 2.2, 2.4, and 2.5, respectively. At week 0, the initial GSU wet gonad weights were significantly different (Table 2.4, Figure 2.3A), where wet gonad weight was significantly lower in the 12°C temperature treatment than the GSU at 8°C, but neither treatment significantly differed from the GSU in the 16°C treatment (Fig. 2.3A). The initial treatment groups at week 0 also significantly differed by temperature for sea urchin wet weight, test diameter, and spine diameter (Table 2.3, Fig. 2.2A, C, D). For both test diameter (ANOVA, $P < 0.0006$, Table 2.3, Fig. 2.2C) and spine diameter (ANOVA, $P < 0.0076$, Table 2.3, Fig. 2.2D), the GSU starting in the 12°C treatment were larger than individuals in the 8 or 16°C treatment. For urchin wet weight, individuals in the 12°C treatment weighed significantly more than those in the 8°C treatment, but neither differed from the 16°C treatment (ANOVA, $P < 0.0247$, Table 2.3, Fig. 2.2A). Neither diet, temperature, nor the interaction were significant for test height at week 0.

At week 9, the effect of temperature was solely significant for GSU wet weight (ANOVA, $P < 0.0018$, Table 2.7, Fig. 2.4A), test height (ANOVA, $P < 0.0193$, Table 2.7, Fig. 2.4B), and spine diameter (ANOVA, $P < 0.0060$, Table 2.7, Fig. 2.4D). The GSU held at 16°C had significantly lower wet weights compared to the GSU held at 8 and 12°C (Fig. 2.4A). For both tests (height and spine diameter) the GSU at 16°C had significantly smaller values than the GSU held at 12°C, but neither significantly differed from 8°C (Fig. 2.4B, D). For test diameter, both temperature (Kruskal-Wallis, $P < 0.0005$, Table 2.8, Fig. 2.4C) and the interaction of diet and temperature (Kruskal-Wallis, $P < 0.0094$, Table 2.8, Fig. 2.4C) were significant, where GSU at 16ºC had significantly smaller test diameters than 8 or 12ºC. Both diet (ANOVA, $P < 0.0001$, Table 2.7, Fig. 2.4E) and temperature (ANOVA, $P < 0.0001$, Table 2.7, Fig. 2.4E) were

significant for wet gonad weight. All three diets significantly differed from each other, where GSU fed V10.1.9 had the highest wet gonad weights for all three temperature treatments, reaching a maximum mean ± SE wet weight of 24.2 ± 1.6 g at 12°C (Table 2.5), and GSU fed kelp had the lowest wet gonad weights. For temperature, GSU held at 16°C had the lowest wet gonad weights compared to both the 8 and 12°C treatments (Fig. 2.4E).

2.3.1.3. Gonad yield and percent gonad increase

Mean ± SE initial and final gonad yields are provided in Tables 2.4 and 2.5, respectively. At week 0, there was no significant difference in gonad yield between the starting temperature treatments (Table 2.4). However, the GSU in the 12°C did have an overall lower mean ± SE starting yield of 9.3 ± 1.4%, compared to 8°C (14.4 ± 1.6%) and 16°C (12.3 ± 1.1%). At week 9, both diet (ANOVA, $P < 0.0001$, Table 2.6, Fig. 2.4F) and temperature (ANOVA, $P < 0.0325$, Table 2.6, Fig. 2.4F) were significant, but the interaction was not. All three diets significantly differed from each other, where V10.1.9 consistently produced the highest yields at all three temperature treatments, and the GSU fed kelp produced the lowest yields (Table 2.5, 2.6, Fig. 2.4F). The GSU at 16°C also produced significantly lower yields than the GSU in the 8 and 12°C treatments, which did not significantly differ from each other (Table 2.5, 2.6, Fig. 2.4F). Although, GSU at week 0 had initially high and significantly different gonad yields (Table 2.4) so it could not be compared week 12 values. Therefore, percent gonad increase per week was analyzed**.** For the percent increase in gonad yield per week over the nine-week trial, both diet (ANOVA, $P < 0.0001$, Table 2.6, Fig. 2.4G) and temperature (ANOVA, $P < 0.0001$, Table 2.6, Fig. 2.4G) were significant. As seen in gonad yield, all three diets significantly differed from each other, where V10.1.9 produced the largest percent increase in gonad at all three

temperatures per week (with the largest increase achieved with V10.1.9 at 12°C, mean ± SE: 2.2 ± 0.2 % per week), and GSU fed kelp produced the lowest increase per week (Table 2.6, Fig. 2.4G). For temperature, GSU at 12°C had a significantly larger increase in gonad yield per week compared to GSU at 8 and 16°C which did not significantly differ from each other (Table 2.6, Fig. 2.4G). Overall, the highest gonad yields, and percent gonad increase per week was obtained by the GSU fed V10.1.9 at 12°C.

2.3.1.4. Gonad colour (L, a*, b*, degree of colour difference, and colour by sex)*

Mean initial and final colour variables are provided in Tables 4 and 5, respectively. At week 0, lightness (L*), and redness (a*) did not significantly differ between the starting temperature treatments (Table 4). Yellowness (b*) at week 0 significantly differed between the starting temperature treatments (ANOVA, $P < 0.0027$, Table 2.4, Fig. 2.3E), where the GSU at 12°C had significantly lower b* ratings than the GSU at the 8 and 16°C treatments. Colour difference also significantly differed between the initial temperature groups where GSU at 12°C had a significantly worse or less desirable colour than sea urchins in the 8 and 16°C treatments, which did not significantly differ from each other (ANOVA, $P < 0.0047$, Table 2.4, Fig. 2.3F).

At week 9, diet and temperature were significant for L*, a*, b*, and degree of colour difference, however, the interaction was only significant for a* (Table 2.6, 2.7). The GSU fed kelp had a significantly higher L* rating than sea urchins fed V10.1.9 and V10.1.10. The two prepared diets, however, did not significantly differ from each other (ANOVA, $P < 0.0003$, Table 2.6, Fig. 2.4H). GSU at 16°C also had a significantly lower L* rating than GSU at 8 and 12°C (ANOVA, $P < 0.0001$, Table 2.6, Fig. 2.4H). For a*, the GSU fed kelp had the lowest a* ratings, and GSU fed V10.1.9 or V10.1.10 did not significantly differ from each other (Kruskal-

Wallis, $P < 0.0001$, Table 2.7, Fig. 2.5B). Redness was also significantly higher in the GSU at 16°C compared to GSU at 8°C, and neither significantly differed from 12°C (Kruskal-Wallis, $P < 0.0226$, Table 2.7, Fig. 2.4I). For b*, both V10.1.9 and V10.1.10 produced significantly higher b* ratings than GSU fed kelp (ANOVA, $P < 0.0001$, Table 2.6, Fig. 2.4J), and GSU at 8 and 12°C produced significantly lower b* ratings than GSU at 16°C (ANOVA, $P < 0.0010$, Table 2.6, Fig. 2.4J). The degree of colour difference was significantly lower (i.e. closer to the target colour of bright orange/yellow) for GSU fed V10.1.9 and V10.1.10 compared to GSU fed kelp (ANOVA, $P < 0.0001$, Table 2.6, Fig. 2.4K). It was also significantly lower for the GSU at 16°C, compared to GSU at the 8 and 12°C treatments, which did not significantly differ from each other (ANOVA, $P < 0.0004$, Table 2.6, Fig. 2.4K). Overall, the best colour (i.e. lowest degree of colour difference) was achieved by the GSU fed V10.1.10 at 16°C (Table 2.5, 2.6).

All colour measurements, L*, a*, b*, and degree of colour difference, were significantly different when split by the sex of the sea urchins. Lightness ratings were significantly higher (ANOVA, $P < 0.0001$, Table 2.8, Fig. 2.5A), and a* (ANOVA, $P < 0.0156$, Table 2.8, Fig. 2.5B) and b* (ANOVA, $P < 0.0004$, Table 2.8, Fig. 2.5C) ratings were significantly lower for males than females. Females also had a significantly lower degree of colour difference compared to males, and therefore were more often closer to the target colour of a bright orange/yellow (ANOVA, $P < 0.0003$, Table 2.8, Fig. 2.5D).

2.3.1.5. Gonad texture, firmness, colour, and taste

Using the rating scale provided in Table 2.1 (Pearce et al., 2002a), gonad texture, firmness, and colour were ranked on a scale of 1-4, where 1 is the best and 4 the worst. Neither diet, temperature, nor the interaction were significant for gonad texture or firmness at week 0 or

week 9. Gonad colour had a significant diet effect (Likelihood ratio, $P < 0.0002$, Table 2.7) and interaction (Likelihood ratio, $P < 0.0229$, Table 2.7) effect at week 9, where GSU fed V10.1.10 had overall better ranked colour than both GSU fed V10.1.9 or kelp. For the interaction effect, The GSU fed V10.1.10 at all three temperatures had significantly better colour than the other treatments (Table 2.7). Although not statistically analyzed, a replicate of GSU gonad either fed V10.1.9 and V10.1.10 for 7 weeks at 12.5 ± 1.1°C was tasted by three blindfolded individuals (rating scale provided in Table 2.1). The average overall preference for the V10.1.9 gonad samples was 4.7, which is between "dislike slightly", and "neither like nor dislike." Average sweetness, bitterness, and umami was a rating of 2 (mild sweetness), 3 (mild bitterness), and 2 (mild umami), respectively. The average overall preference for the V10.1.10 gonad samples was 7.3, which is between "like moderately" and "like very much". Average sweetness, bitterness, and umami was a rating of 2.7 (between "mild" and "medium" sweetness), 3.7 (between "mild" and "none" for bitterness), and 3.3 (between "medium" and "very" for umami), respectively. It is important to note however, that the gonad samples were rinsed in freshwater (as opposed to saltwater) prior to tasting, which would have affected the overall flavour profile.

2.3.2. Red sea urchins

2.3.2.1. Water quality and survivorship

Red sea urchins experienced a mean ± SD temperature of 7.7 ± 0.4°C, 12.4 ± 0.6°C, or 16.3 ± 0.7°C (N = 12.374), depending on the treatment in which the RSU was allocated and mean ± SD salinity was 30.4 ± 0.5 ppt (N = 459), and dissolved oxygen was 8.5 ± 0.6 mg L^{-1} (N = 459) throughout the twelve week trial. Survivorship was 90.7% for the initial 54 RSU used in this feeding trial. Three RSU died early on in the experiment [(i) sea urchin fed V10.1.9 at 12°C,

(ii) sea urchin fed V10.1.10 at 16℃, and (iii) sea urchin fed kelp at 16℃] and were replaced. Two others died later in the experiment and were not replaced [(i) sea urchin fed kelp at 12℃ and (ii) sea urchin fed V10.1.9 at 12℃].

2.3.2.2. Somatic parameters

Mean ± SE initial and final somatic variables are provided in Table 2.3, 2.4, and 2.6, respectively. At week 0, RSU wet weight was significantly different for both the diet (ANOVA, $P < 0.0156$, Table 2.3, Fig. 2.6A) and temperature (ANOVA, $P < 0.0175$, Table 2.3, Fig. 2.6A) effect, where RSU starting in the V10.1.9 treatment were significantly larger than RSU in the V10.1.10 treatment and did not differ from the kelp treatment, and RSU in the 8℃ treatment were significantly larger than RSU in the 12 or 16℃ temperature treatments (Fig. 2.6A). No effects at week 0 were significant for test height, but temperature was significant for test diameter and spine diameter (Table 2.3). Test diameter (ANOVA, $P < 0.0113$, Table 2.3, Fig. 2.6C) and spine diameter (ANOVA, $P < 0.0006$, Table 2.3, Fig. 2.6D) were significantly smaller for RSU starting in the 8℃ treatment, compared to the 12 and 16℃ treatment. The initial wet gonad weights for the starting RSU did not significantly differ between the temperature treatments (Table 2.4).

At week twelve, the diet and temperature effect were significant for both RSU wet weight and test diameter, diet and the interaction were significant for wet gonad weight, and no effects were significant for test height and spine diameter (Table 2.9). RSU wet weight (ANOVA, $P < 0.0100$, Table 2.9, Fig. 2.8A) and test diameter (ANOVA, $P < 0.0074$, Table 2.9, Fig. 2.8C) were both significantly larger for RSU fed V10.1.9 than RSU fed V10.1.10. Neither differed from those fed kelp. For temperature, RSU had significantly larger wet weights (ANOVA, $P <$

0.0145, Table 2.9, Fig. 2.8A) and test diameters (ANOVA, $P < 0.0002$, Table 2.9, Fig. 2.8C) at 8ºC compared to those in the 12 and 16ºC treatments. For wet gonad weight, RSU fed V10.1.10 at 12ºC and V10.1.9 at 16ºC, had significantly larger wet gonad weights than RSU fed kelp at all three temperature treatments (ANOVA, $P < 0.0442$, Table 2.9, Fig. 2.8E).

2.3.2.3. Gonad yield and percent gonad increase

Mean ± SE initial and final yields are provided in Tables 2.4 and 2.6, respectively. At week 0, gonad yield did not significantly differ between the starting temperature treatment groups (Table 2.4, Fig. 2.7B). At week twelve, both diet (ANOVA, $P < 0.0002$, Table 2.9, Fig. 2.8F) and temperature (ANOVA, $P < 0.0272$, Table 2.9, Fig. 2.8F) were significant, where RSU fed V10.1.9. and V10.1.10. had significantly higher gonad yields than RSU fed kelp, and RSU at 12°C had significantly larger yields than RSU at 8°C, and neither significantly differed from 16°C (Fig. 2.8F). For percent increase in gonad yield per week over the twelve week trial, only diet was significant, where RSU fed V10.1.9 or V10.1.10 had a larger percent increase per week for gonad yield than RSU fed kelp (ANOVA, $P < 0.0003$, Table 2.9, Fig. 2.8G). Overall, the highest gonad yields, and percent gonad increase per week was obtained with RSU fed V10.1.10 at 12°C (Table 2.6).

2.3.2.4. Gonad quality (L, a*, b*, and degree of colour difference)*

Mean ± SE initial and final colour variables are provided in Table 2.4 and Table 2.6, respectively. At week 0, there were no significant differences between the starting temperature treatments for L*, a*, b*, and degree of colour difference (Table 2.4, Fig. 2.7). At week twelve, temperature was significant for L* (ANOVA, $P < 0.0469$, Table 2.9, Fig. 2.8H) and a*

(ANOVA, $P < 0.0151$, Table 2.9, Fig. 2.8I), where the L* ratings were significantly lower for RSU in the 8°C treatment compared to the 12 and 16°C treatment (Fig. 2.8H), and a* ratings were significantly higher for RSU in the 16°C treatment compared to the 8 or 12°C treatment (Fig. 2.8I). Diet was significant for both b* (ANOVA, $P < 0.0005$, Table 2.9, Fig. 2.8J) and degree of colour difference (ANOVA, $P < 0.0046$, Table 2.9, Fig. 2.8K), where RSU fed V10.1.10 had significantly higher b* ratings and a significantly lower degree of colour difference (*i.e.* closer to target colour of bright orange/yellow) than RSU fed V10.1.9. or kelp (Fig. 2.8J, K). RSU were not producing enough gametes at the time of sampling for an easy identification of sex to be done by eye, therefore there are no results for colour by sex. Overall, the best colour (i.e. lowest degree of colour difference) was obtained with V10.1.10 at 16ºC, which was also seen with the GSU (Table 2.6).

2.3.3.5. Gonad texture, firmness, colour, and taste

Using the rating scale provided in Table 2.1 (Pearce et al., 2002a), gonad texture, firmness, and colour were ranked on a scale of 1-4, where 1 is the best and 4 the worst. None of the effects were significant for either gonad texture, firmness, or colour at either week 0 or week 12. Although not statistically analyzed, a replicate gonad of each treatment (i.e. RSU fed either kelp, V10.1.9, or V10.1.10, at 8, 12, and 16°C; excluding V10.1.9 at 8°C) was tasted and scored using the rating scale provided in Table 2.1. The average overall preference for the kelp gonad samples was 6.6, which is between "like slightly" and "like moderately." Average sweetness, bitterness, and umami was 2.6 (between "mild" and "medium" sweetness), 3.2 (between "mild" and "none" for bitterness), and 3.2 (between "medium" and "very" for umami), respectively. Average overall preference for the V10.1.9 gonad samples was 7 ("like moderately"), and

average sweetness, bitterness, and umami ratings were 2.5 (between “mild” and “medium” sweetness), 3.3 (between “mild” and “none” for bitterness), and 3.3 (between “medium” and “very” for umami), respectively. Average overall preference for the V10.1.10 gonad samples was 5.5 (between “neither like nor dislike” and “like slightly”), and average sweetness, bitterness, and umami ratings were 2.6 (between “mild” and “medium” sweetness), 2.6 (between “medium” and “mild” bitterness), and 2.6 (between “mild” and “medium” for umami), respectively. However, the overall preference of all three diets ranked highest at 16°C, compared to the 8 and 12°C treatments.

2.4. Discussion

Diet and temperature are arguably the two most important factors when considering optimizing a sea urchin gonad enhancement operation. Many studies have shown that prepared diets made with animal protein produce higher yields in a shorter period of time compared to feeding a macroalgal diet (Pearce et al., 2002b, 2004; Chang et al., 2005; Böttger et al., 2006; Woods et al., 2008; Azad et al., 2011; James and Siikavuopio, 2012; Cárcamo, 2015). However, macroalgal diets typically produce gonads with better colour and taste compared to feeding a prepared diet, which has been shown to produce pale coloured gonads, and a bitter taste (Pearce et al., 2002b, 2004; Chang et al., 2005; Böttger et al., 2006; Woods et al., 2008; Azad et al., 2011; James and Siikavuopio, 2012; Cárcamo, 2015). The two prepared diets tested in this study (V10.1.9 and V10.1.10) are newly formulated and are primarily kelp based, made from offcuts of Kombu kelp (various species from the family *Laminariaceae*). The two diets differ slightly from each other in the kelp blend used (Brian Tsuyoshi Takeda, Urchinomics, pers. comm.). The GSU in this study produced the largest wet gonad weights and gonad yields when fed V10.1.9 (mean ±

SE: 28.7 ± 0.7%) compared to V10.1.10 (mean ± SE: 24.8 ± 0.7%). Kelp overall produced the lowest yields (mean ± SE: 17.8 ± 0.7%), although the initial starting yields were already high. V10.1.9 was also able to produce the largest increase in gonad yield per week (mean ± SE: 1.8 ± 0.1%) and kelp the lowest (mean ± SE: 0.6 ± 0.1%). A study by Pearce et al. (2002c) had a 1.3% gonad yield increase per week with GSU fed a prepared diet. Both V10.1.9 and V10.1.10 in this study exceeded this amount on a weekly basis with 1.8% and 1.4% per week, respectively. The RSU at week 0 were more representative of what a barren urchin would be like, starting with an initial mean ± SE gonad yield of 1.7 ± 0.3%. At week twelve, RSU fed V10.1.9 or V10.1.10 had higher gonad wet weights, and gonad yields (mean ± SE: 9.2 ± 0.5% and 10.8 ± 0.8%, respectively) compared to RSU fed kelp (mean ± SE: 7.2 ± 0.5%). Both V10.1.9 and V10.1.10 were also able to produce a larger increase in gonad yield per week (mean ± SE: 0.6 ± 0.04% and 0.7 ± 0.07%, respectively) than the RSU fed kelp (mean ± SE: 0.4 ± 0.04%).

Market minimum gonad yield is between 10-15%. The GSU in all treatments were able to far exceed this minimum in nine weeks, although initial starting yields were already between ~9-14%. The RSU, which are a much larger species, started with very low yields (more representative of a barren sea urchin) and were able to reach the market minimum with V10.1.9 at 16°C and with V10.1.10 at 12°C and 16°C (Table 2.6). The other treatments were not far below the minimum, and with the gonad yield increase per week obtained with the prepared diets (Table 2.5, 2.6), it would only take a couple more weeks to reach the market minimum. These results are consistent with numerous gonad enhancement studies that have examined the effect of prepared diets producing higher gonad yields than sea urchins fed a macroalgae diet (*S. droebachiensis*: de Jong-Westman et al., 1995; Pearce et al., 2002a, b, c; Pearce et al., 2004; and James, 2017; *S. purpuratus*: Azad et al., 2011; *M. franciscanus*: McBride et al., 1997, 2004; *S.*

intermedius: Chang et al., 2005; Zhao et al., 2016; and Lawrence et al., 2009; *Evenichus chloroticus*: Woods et al., 2008; and *Lytechinus variegatus*: Hammer et al., 2006, 2012). However, it should be noted that the kelp (*N. luetkeana*) used in this study was previously collected in 2016 and frozen at -20°C until use in this study, and partially thawed before feeding. Thus, it may have been less nutritious or palatable than fresh kelp. Daggett et al. (2010) found that kelp (*Saccharina latissima*) that was frozen long term at -15°C (>2 months) produced the lowest growth rates in juvenile GSU compared to the GSU fed *S. latissima* frozen short term at -15°C (1 wk), air dried (~20°C), or presented fresh. Thawing also changed the texture of the kelp, causing it to become slimy and for tissue layers to separate which could affect the palatability for urchins (Daggett et al., 2010).

The quality of gonads produced is also reliant on the diets fed to the sea urchins. In the GSU, lightness (L*), redness (a*), yellowness (b*), and degree of colour difference were all significantly affected by diet. GSU fed either V10.1.9 or V10.1.10 had lower L* ratings, higher a* and b* ratings, and a lower degree of colour difference (i.e. closer to target colour of bright orange/yellow) than GSU fed kelp. Overall, the best gonad colour for GSU was obtained with V10.1.10 at 16ºC, which was shown both objectively (lower degree of colour difference) and subjectively (by eye using the rating scale provided in Table 2.1). RSU fed V10.1.10 had significantly higher b* ratings and a lower degree of colour difference than either V10.1.9 or kelp, and the best colour was obtained with V10.1.10 at 16ºC which is the same result seen with the GSU. The majority of the existing literature shows that as protein increases in a diet, gonad colour may become less desirable, with high-protein/animal-protein diets producing a pale/cream colour which is not commercially acceptable (McBride et al., 1997; Pearce et al., 2002a, 2004; Robinson et al., 2002; Shpigel et al., 2005; Woods et al., 2008). However, Pearce et al. (2004)

had contrasting results, where better gonad colour was obtained on a prepared diet compared to kelp, and both diets produced better colour than wild sea urchins. In that study, the authors used beta-carotene, from a microalga (*Dunaliella salina*), at a concentration of 200 mg kg^{-1} (Pearce et al., 2004). Echinenone, which is synthesized from beta-carotene, has been identified as a particularly effective pigment for producing proper colouration in sea urchin gonads (Griffiths and Perrott, 1976; Tsushima and Matsuno, 1990; Tsushima et al., 1993; Robinson et al., 2002). Pigments in general have also been shown to have a positive effect on sea urchin health (Kawakami et al., 1998; Robinson et al., 2002). Protein concentration is another important consideration in developing a prepared diet. Previous studies have shown that protein levels need to be between 10 and 19% in order to maximize gonad enhancement and quality (de Jong-Westman et al., 1995; McBride, 1998; Akiyama et al., 2001; Kennedy et al., 2001; Pearce et al., 2002c).

Another critical factor to consider when trying to optimize gonad enhancement is water temperature. This study compared three different rearing temperatures, 8, 12 and, 16^{o}C, which are temperatures commonly found in the waters surrounding British Columbia, Canada. Temperature, primarily linked to seasonal changes, has been shown to influence feed intake and metabolism, which ultimately affects the allocation of nutrients and pigments to the gut or gonads (Lawrence et al., 1966). The GSU achieved the largest wet gonad weights and highest yields at week twelve in the 8°C (mean ± SE: 24.6 ± 1.2%) and 12°C (mean ± SE: 24.5 ± 1.3%) temperature treatment and were significantly higher than GSU at 16°C (22.2 ± 1.3%), although all three of these far exceed the market minimum of 10-15%. However, the GSU in the 12°C temperature treatment had a significantly higher gonad yield increase per week (mean ± SE: 1.7 ± 0.1%), than either the 8 or 16°C treatments. Several studies have shown that optimal gonad

production in GSU occurs between 10-12°C (Siikavuopio et al., 2006, 2008), although similar temperatures were also deemed optimal for both *S. purpuratus* and *S. intermedius*, which agrees with the results of this study for GSU. Azad et al. (2011) found that *S. purpuratus* produced the largest wet gonad weights when fed a prepared diet in the 12 or 16ºC treatment compared to kelp, and the two diets did not significantly differ at 8ºC. For *S. intermedius*, Zhao et al. (2016) had the largest gonad yields and wet gonad weights at temperatures ranging from 10.6–18.8ºC, which was significantly higher than sea urchins held at 22ºC. Lawrence et al. (2009) also produced the highest yields in *S. intermedius* at 12ºC and the lowest yields at 22ºC. In this study, the highest yields were consistently obtained in the 8 and 12ºC treatments, and the lowest yields at 16ºC for GSU (Table 2.5, Fig. 2.4F).

In general, higher temperatures increase feed intake and metabolism, however, at a certain point, high temperatures become detrimental (Siikavuopio et al., 2006). These results indicate that temperatures of 16ºC and greater would be less optimal for producing high gonad yields in the GSU. The RSU in the 12ºC (mean ± SE: 9.9 ± 0.7%) treatment had significantly larger yields than the 8ºC (mean ± SE: 7.8 ± 0.5%), although neither significantly differed from the 16ºC treatment (mean ± SE: 9.5 ± 0.7%). Temperature alone did not significantly affect wet gonad weight in RSU. However, the largest wet gonad weights were obtained with V10.1.10 at 12ºC and V10.1.9 at 16ºC (Table 2.6). McBride et al. (1997) showed that optimal gonad production in RSU can be maintained at 12.1–16.9ºC in Northern California, which is similarly shown in the results of this study. However, a study by Leighton (1971), suggested that the optimal feeding range for RSU in Southern California is 15.7–17.1ºC, so these optimal gonad production ranges are likely area specific.

The influence of temperature on feed intake and metabolism also affects the quality of the gonads produced due allocation of pigments to the gut or gonads (Lawrence et al., 1966). The GSU in this study had significantly lower L* ratings at 16ºC, than 8ºC or 12ºC, and significantly higher a* ratings at 16ºC than 8ºC, and higher b* ratings at 16ºC than the other temperature treatments (Fig. 2.4H, I, J). Overall, a significantly lower degree of colour difference was achieved at 16ºC than the other two temperature treatments, which did not significantly differ from each other (Fig. 2.4K). RSU had significantly higher L* ratings at 12ºC and 16ºC than at 8ºC, and significantly higher a* ratings at 16ºC compared to 12ºC, but neither significantly differed from 8ºC (Fig. 2.8H, I). The temperature effect alone did not significantly affect b* or the degree of colour difference in RSU. Temperature is also related to season, and McBride et al. (2004) found that in RSU, the gonad L* and b* increased in the spring (while a* did not change) and in the summer and fall the a* and b* decreased. Season also has implications on the reproductive quality of the gonads, and peak condition of gonads occurs in the post-spawning recovery stages (Bernard, 1977). GSU begin to develop their gonads in the winter, to prepare for spawning in late winter or spring (Himmelman, 1978), and at the advanced stages of gonad maturation, the quality beings to decrease due to gamete leakage and softness (Bernard, 1977). The presence of gametes in the GSU could have contributed to our colour results. The best colour, however, was obtained with V10.1.10 at 16ºC. Temperature may not be as significant for RSU, due to the lack of any significant effect on b* or degree of colour difference. Although, as mentioned by McBride et al. (2004), RSU consumed less in the fall compared to the spring, and feed intake determines how much protein and pigment is available for gonad production. The RSU are also the largest species of sea urchin, and these diets are primarily

formulated for use in the smaller green and purple sea urchins. Therefore, it may take longer than the recommended 8–12 weeks to see the full effects of temperature on gonad production in RSU.

Colour of the GSU gonads was also significantly affected by the sex of the sea urchin. GSU at the time of sampling were in advanced stages of gonad maturation [their spawning is thought to be triggered by the spring phytoplankton boom in BC, Canada (Himmelman, 1978)] and were leaking gametes which made it relatively easy to sex the sea urchins. The RSU, however, have a spawning season later in the year [late spring and summer (Bernard, 1977)], and were not leaking gametes during the sampling date, therefore they could not be easily sexed. Overall, the female GSU at week twelve had lower L* ratings, higher a* and b* ratings, and a lower degree of colour difference (i.e. better colour) (Fig. 2.5A, B, C, D), compared to the males. This is shown in other studies, where female sea urchins were more likely to have bright orange/yellow gonads compared to males. Zhao et al. (2010) had the same results with *S. intermedius*, where the females had significantly lower L* ratings, and significantly higher a*, and b* ratings compared to the males. Phillips et al. (2009), also had significantly better colour of female *Evechinus chloroticus* gonads, compared to males. This is likely due to the presence of sperm in the male gonads which would cause the gonad surface to be lighter and whiter than the females.

Factors such as texture, firmness, and taste of the gonads are critical in obtaining a market quality product. Overall, no effects were significant for texture and firmness for both GSU and RSU. However, standard industry practice is to firm the gonads by soaking them in a saltwater solution containing anhydrous potassium alum [$KAl(SO_4)_2$] (Kato and Schroeter, 1985), which was not done in this study. Pearce et al. (2004) found that GSU fed a kelp diet had firmer gonads compared to the sea urchins fed a prepared diet. They hypothesized that the

percentage of water in the gonads increases with a prepared diet, as well as faster production of gonad tissue leads to it being softer (Pearce et al., 2004). McBride et al. (2004) also showed that mature gonads with a higher water content were ultimately softer. Although water content was not measured in this study, the GSU gonads were in the mature stages of their reproductive cycle and were leaking gametes. However, another study by Pearce et al. (2002b) resulted in the GSU that were fed the prepared diet having firmer gonads than the GSU fed kelp. Azad et al. (2011) conducted a similar study but with purple sea urchins (*S. purpuratus*), but found no significant effects for diet, temperature, or the interaction on texture and firmness. Taste, however, was significantly affected by diet and interaction. Azad et al. (2011) found that *S. purpuratus* fed the prepared diets had better taste (i.e. sweet/savory, and not bitter), compared to the sea urchins fed kelp. In this present study, the GSU fed V10.1.10 in the 12ºC treatment, had overall better tasting gonads that were liked moderately – liked very much, than the sea urchins fed V10.1.9. However, the GSU were only fed for seven weeks and the gonads were rinsed in freshwater prior to tasting (standard practice is to rinse gonads in saltwater), so these factors could have contributed to these results. RSU fed V10.1.9 had the highest overall preference for taste, and all three diets rated the same for average sweetness, bitterness, and umami. There was also a preference for the gonad samples that were reared at 16°C, compared to the ones at 8 and 12°C. This could be due to that the optimal rearing temperature for RSU is between 12-17°C (Leighton, 1971; McBride et al., 1997), compared to 10-12°C for GSU (Siikavuopio et al., 2006, 2008). The RSU gonads were rinsed in filtered seawater prior to tasting, unlike the GSU. However, the majority of existing literature shows that prepared diets made with animal protein consistently produces a bitter/bland taste compared to sea urchins fed a kelp diet (Pearce et al., 2004) and taste of the gonads is strongly linked to the amino acid profile. Studies have shown

that the desired sweet flavour is caused by the amino acids glycine and alanine, while an undesirable bitterness is caused by the presence of valine, and a savory flavour (also termed "umami") is caused by the presence of glutamine (Lee and Haard, 1982; De la Cruz-Garcia et al., 2000; Liyana-Pathirana et al., 2002). Further research is needed to assess whether these diets are able to produce the appropriate amino acid profile to produce market quality flavour.

2.5. Conclusion

The results from this study indicate that these two newly formulated prepared diets (V10.1.9 and V10.1.10) can provide sufficient protein and pigment to achieve market yields and quality in both the GSU and RSU in a land-based system. The highest gonad yields were achieved with GSU fed V10.1.9 at 8 and 12°, although all yields were well above the market minimum of 10-15%. The GSU had the best gonad colour with V10.1.10 at 16°C, although, the colour produced with either prepared diet was better than all kelp treatments (except V10.1.9 at 8°C). V10.1.10 also produced the highest overall preference for taste at 12°C. This diet and temperature combination may be optimal for the GSU. The RSU achieved the highest yields with V10.1.10 at all 3 temperatures and V10.1.9 at 16°C, and the same treatments also achieved the best colour results. V10.1.9 also had the preferred taste in RSU, and therefore this diet fed at 16°C may be optimal in a gonad enhancement operation. Further research is needed to ensure the appropriate amino acid profile is achieved with these diets. More research would also be needed on scaling gonad enhancement up so as to make it a new commercial industry in British Columbia, Canada.

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Table 2.1. Criteria for subjective assessment of gonad texture, firmness, colour, and flavour for the sea urchins *Strongylocentrotus droebachiensis* and *Mesocentrotus franciscanus*. Rating scale of texture, firmness, and colour taken from Pearce et al. (2002a).

Criterion	Scale
Gonad texture (by eye, ratings 1-4)	1 = two distinct gonad segment halves, very smooth 2 = two distinct gonad segment halves, smooth 3 = distinction of gonad segment halves possible but <2, rough/granular 4 = distinction of gonad halves not possible, rough/granular
Gonad firmness (by eye, ratings 1-4)	1 = very firm 2 = firm 3 = soft 4 = very soft
Gonad colour (by eye, ratings 1-4)	1 = bright yellow or orange 2 = paler yellow or orange 3 = yellow-brown, orange-brown, red-brown, cream 4 = anything other than above (e.g. dark brown, grey)
Gonad Sweetness (ratings 1-4)	1 = none 2 = mild 3 = medium 4 = very
Gonad Bitterness (ratings 1-4)	1 = very 2 = medium 3 = mild 4 = none
Gonad Umami (ratings 1-4)	1 = none 2 = mild 3 = medium 4 = very
Gonad Flavour Overall Preference (ratings 1-9)	1 = dislike extremely 2 = dislike very much 3 = dislike moderately 4 = dislike slightly 5 = neither like, nor dislike 6 = like slightly 7 = like moderately 8 = like very much 9 = extremely like

Table 2.2. Mean ± SE initial somatic attributes of the experimental green sea urchins (*Strongylocentrotus droebachiensis,* GSU) and red sea urchins (*Mesocentrotus franciscanus*, RSU) at week 0 in the different diet [Bull kelp (*Nereocystis luetkeana*), and two prepared diets (V10.1.9 and V10.1.10)] and temperature treatments (8, 12, and 16ºC). n = 6.

	GSU Sea urchin wet weight (g)			GSU Test height (mm)		
Temperature (°C) ± SD, n = 12,374	*Nereocystis luetkeana*	V10.1.9	V.10.1.10	*Nereocystis luetkeana*	V10.1.9	V.10.1.10
7.8 ± 0.9	64.7 ± 3.7	62.3 ± 3.5	55.8 ± 2.5	32.4 ± 0.5	32.8 ± 1.0	33.0 ± 1.1
12.5 ± 0.5	69.8 ± 3.4	69.8 ± 4.0	68.0 ± 6.3	35.0 ± 0.9	34.2 ± 0.8	34.6 ± 1.9
15.5 ± 1.7	67.2 ± 2.7	62.8 ± 2.3	62.5 ± 2.2	35.1 ± 0.7	33.5 ± 1.4	35.0 ± 1.0
	GSU Test diameter (mm)			GSU Spine diameter (mm)		
Temperature (°C) ± SD, n = 12,374	*Nereocystis luetkeana*	V10.1.9	V.10.1.10	*Nereocystis luetkeana*	V10.1.9	V.10.1.10
7.8 ± 0.1	57.3 ± 1.2	58.7 ± 0.3	58.4 ± 1.2	78.7 ± 1.6	78.6 ± 0.8	76.7 ± 2.7
12.4 ± 0.1	62.3 ± 1.0	59.9 ± 1.4	63.4 ± 1.5	83.6 ± 1.5	82.3 ± 1.8	80.6 ± 1.1
15.9 ± 0.1	60.9 ± 0.7	58.6 ± 1.0	59.3 ± 1.1	81.4 ± 1.0	78.1 ± 1.1	76.9 ± 2.1
	RSU Sea urchin wet weight (g)			RSU Test height (mm)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	573.3 ± 52.0	586.8 ± 35.2	467.2 v 39.4	55.5 ± 2.7	58.3 ± 2.2	54.3 ± 3.4
12.4 ± 0.6	410.3 ± 24.6	538.4 ± 22.0	462.0 ± 31.0	53.2 ± 1.7	58.6 ± 1.2	52.8 ± 1.6
16.3 ± 0.7	466.2 ± 44.8	495.0 ± 33.1	424.6 ± 36.1	57.8 ± 1.4	55.2 ± 1.1	54.6 ± 2.9
	RSU Test diameter (mm)			RSU Spine diameter (mm)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	121.4 ± 3.5	121.0 ± 1.4	117.1 ± 5.4	205.7 ± 1.8	208.7 ± 0.1	203.2 ± 3.4
12.4 ± 0.6	112.1 ± 4.2	117.2 ± 2.3	110.4 ± 1.9	192.6 ± 5.0	192.2 ± 5.4	195.9 ± 5.1
16.3 ± 0.7	110.5 ± 3.3	116.3 ± 2.6	111.3 ± 3.0	197.8 ± 4.7	200.1 ± 5.8	192.5 ± 3.9

Table 2.3. Results of separate two-way ANOVAs for the initial somatic attributes of the experimental green sea urchins (*Strongylocentrotus droebachiensis*, GSU) and red sea urchins (*Mesocentrotus franciscanus*, RSU) at week 0, comparing the different diet and temperature treatments. Sources of variation are diet (D), temperature (T), the interaction (D x T), and error.

	GSU Sea urchin wet weight (g)				GSU Test height (mm)			
Source	df	SS	*F* ratio	*P* value	df	SS	*F* ratio	*P* value
D	2	236.4	1.5	0.2303	2	5.8	0.4	0.6793
T	2	626.8	4.0	**0.0247**	2	41.5	2.8	0.0723
D x T	4	109.8	0.3	0.8411	4	6.8	0.2	0.9212
Error	45	3506.3			45	335.0		
	GSU Test diameter (mm)				GSU Spine diameter (mm)			
Source	df	SS	*F* ratio	*P* value	df	SS	*F* ratio	*P* value
D	2	16.8	1.2	0.3177	2	91.4	2.9	0.0676
T	2	126.5	8.8	**0.0006**	2	173.9	5.4	**0.0076**
D x T	4	44.1	1.5	0.2073	4	17.9	0.3	0.8888
Error	45	322.5			45	718.5		
	RSU Sea urchin wet weight (g)				RSU Test height (mm)			
Source	df	SS	*F* ratio	*P* value	df	SS	*F* ratio	*P* value
D	2	72836.8	4.6	**0.0156**	2	110.3	2.3	0.1150
T	2	70645.8	4.4	**0.0175**	2	14.3	0.3	0.7467
D x T	4	43539.0	1.4	0.2611	4	103.1	1.1	0.3865
Error	45	358830.1			45	1092.8		
	RSU Test diameter (mm)				RSU Spine diameter (mm)			
Source	df	SS	*F* ratio	*P* value	df	SS	*F* ratio	*P* value
D	2	255.9	2.2	0.1172	2	87.1	0.5	0.5939
T	2	564.1	4.9	**0.0113**	2	1464.9	8.9	**0.0006**
D x T	4	81.8	0.4	0.8361	4	235.5	0.7	0.5875
Error	45	2560.1			45	3716.7		

Values in bold are significant at $P \leq 0.05$. $n = 6$

Table 2.4. Mean ± SE initial gonadal attributes of the green sea urchin (*Strongylocentrotus droebachiensis,* GSU) and red sea urchin (*Mesocentrotus franciscanus*, RSU) in the starting temperature treatments and ANOVA results for each attribute comparing the three temperatures at week 0. *CIE* = Commission Internationale de l'Eclairage (L* = lightness, a* = redness, and b* = yellowness).

Temperature (°C) ± SD, *n* = 12,374	GSU Wet gonad weight (g)	GSU Gonad yield (%)	GSU *CIE* L* value	GSU *CIE* a* value	GSU *CIE* b* value	GSU Degree of colour difference (ΔE_{ab*})
7.8 ± 0.1	9.3 ± 1.4	14.4 ± 1.6	64.7 ± 1.3	8.5 ± 0.5	43.9 ± 1.7	23.9 ± 2.1
12.4 ± 0.1	4.8 ± 0.8	9.3 ± 1.4	60.1 ± 0.7	6.8 ± 1.1	36.1 ± 1.5	33.0 ± 1.6
15.9 ± 0.1	7.4 ± 0.9	12.3 ± 1.1	62.1 ± 1.7	8.6 ± 0.7	42.9 ± 0.4	26.1 ± 0.9
SS Temperature	49.1	65.3	52.7	9.7	179.9	224.5
SS Error	70.4	117.5	97.9	40.7	107.0	155.7
F value	4.2	3.3	3.2	1.4	10.1	8.6
P value	**0.0419**	0.0704	0.0756	0.2772	**0.0027**	**0.0047**
df=2,12						
Temperature (°C) ± SD, *n* = 17,674	RSU Wet gonad weight (g)	RSU Gonad yield (%)	RSU *CIE* L* value	RSU *CIE* a* value	RSU *CIE* b* value	RSU Degree of colour difference (ΔE_{ab*})
7.7 ± 0.4	5.9 ± 2.1	1.1 ± 0.3	43.8 ± 3.6	5.5 ± 1.6	14.7 ± 3.0	59.7 ± 4.3
12.4 ± 0.6	8.8 ± 1.8	2.4 ± 0.7	58.1 ± 6.3	2.9 ± 0.7	25.2 ± 6.2	44.9 ± 7.7
16.3 ± 0.7	5.3 ± 1.0	1.7 ± 0.4	55.1 ± 7.5	2.2 ± 1.3	25.5 ± 5.9	46.4 ± 7.9
SS Temperature	36.4	0.0	0.0	30.6	0.1	662.4
SS Error	177.0	0.0	0.1	99.0	0.8	2795.9
F value	1.2	1.9	1.6	1.8	1.1	1.4
P value	0.3256	0.1817	0.2376	0.1987	0.3622	0.2792
df=2,12						

Values in bold are significant at $P \leq 0.05$. $n = 5$

Table 2.5. Mean ± SE somatic and gonadal attributes of the green sea urchin, *Strongylocentrotus droebachiensis,* in the different diet and temperature treatments at the end of the experiment (week 9). *CIE* = *Commission Internationale de l'Eclairage* (L* = lightness, a* = redness, and b* = yellowness). *n* = 54.

	Sea urchin wet weight (g)			Test height (mm)		
Temperature (°C) ± SD, *n* = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	76.2 ± 4.0	78.7 ± 2.3	71.4 ± 4.3	35.9 ± 1.0	34.3 ± 1.0	33.1 ± 2.1
12.4 ± 0.1	81.9 ± 4.3	82.4 ± 3.4	79.5 ± 6.9	36.2 ± 1.2	36.8 ± 1.0	36.0 ± 2.0
15.9 ± 0.1	67.7 ± 3.0	68.6 ± 2.6	69.1 ± 4.4	34.1 ± 0.8	32.2 ± 0.4	33.7 ± 0.9
	Test diameter (mm)			Spine diameter (mm)		
Temperature (°C) ± SD, *n* = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	66.8 ± 3.9	64.1 ± 0.8	60.9 ± 1.8	84.9 ± 1.5	85.6 ± 1.2	81.9 ± 2.3
12.4 ± 0.1	64.8 ± 0.5	63.7 ± 0.6	63.0 ± 2.2	87.8 ± 1.8	87.0 ± 1.6	83.4 ± 2.7
15.9 ± 0.1	58.7 ± 0.7	60.0 ± 0.6	63.4 ± 5.5	81.7 ± 1.5	81.2 ± 1.5	79.9 ± 2.3
	Wet gonad weight (g)			Gonad yield (%)		
Temperature (°C) ± SD, *n* = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	14.9 ± 1.4	23.0 ± 0.4	17.7 ± 1.0	19.4 ± 1.0	29.4 ± 1.1	25.0 ± 1.2
12.4 ± 0.1	15.2 ± 1.3	24.2 ± 1.6	20.4 ± 2.1	18.5 ± 1.2	29.4 ± 1.5	25.6 ± 1.5
15.9 ± 0.1	10.6 ± 1.1	18.7 ± 0.6	16.4 ± 0.8	15.5 ± 1.2	27.4 ± 0.9	23.8 ± 0.9
	Gonad yield increase per week (%)			*CIE* L* values		
Temperature (°C) ± SD, *n* = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	0.6 ± 0.1	1.7 ± 0.1	1.2 ± 0.1	83.9 ± 1.5	80.4 ± 1.3	79.7 ± 1.6
12.4 ± 0.1	1.0 ± 0.1	2.2 ± 0.2	1.8 ± 0.2	84.2 ± 1.1	81.7 ± 0.9	79.0 ± 1.2
15.9 ± 0.1	0.4 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	79.4 ± 0.8	74.8 ± 1.4	76.5 ± 1.0
	CIE a* values			*CIE* b* values		
Temperature (°C) ± SD, *n* = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	-0.2 ± 0.5	2.0 ± 0.8	3.3 ± 1.1	34.0 ± 2.8	44.5 ± 2.2	50.4 ± 3.3
12.4 ± 0.1	-0.2 ± 0.7	3.3 ± 0.7	4.6 ± 0.8	38.1 ± 3.4	51.2 ± 2.6	49.5 ± 3.3
15.9 ± 0.1	0.4 ± 0.5	5.9 ± 0.8	8.2 ± 0.5	42.2 ± 1.4	51.1 ± 2.1	61.1 ± 1.4
	Degree of colour difference (ΔE_{ab*})					
Temperature (°C) ± SD, *n* = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10			
7.8 ± 0.1	34.3 ± 2.9	23.1 ± 2.4	17.2 ± 3.5			
12.4 ± 0.1	30.6 ± 3.5	17.2 ± 2.4	17.2 ± 3.6			
15.9 ± 0.1	25.4 ± 1.4	15.1 ± 2.2	6.0 ± 0.9			

Table 2.6. Mean ± SE somatic and gonadal attributes of the red sea urchin, *Mesocentrotus franciscanus,* in the different diet and temperature treatments at the end of the experiment (week 12). *CIE* = Commission Internationale de l'Eclairage (L* = lightness, a* = redness, and b* = yellowness). n = 6.

	Sea urchin wet weight (g)			Test height (mm)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	588.9 ± 52.9	604.1 ± 35.7	490.1 ± 39.8	57.1 ± 2.8	57.6 ± 0.8	52.8 ± 3.3
12.4 ± 0.6	425.2 ± 20.6	568.0 ± 21.5	484.5 ± 31.5	52.1 ± 1.9	54.6 ± 2.3	58.2 ± 2.6
16.3 ± 0.7	476.8 ± 45.1	514.5 ± 33.8	437.1 ± 32.5	53.4 ± 3.4	56.4 ± 2.3	52.9 ± 2.0
	Test diameter (mm)			Spine diameter (mm)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	122.5 ± 4.2	122.9 ± 2.3	117.9 ± 1.9	202.0 ± 3.9	205.8 ± 2.5	200.4 ± 3.2
12.4 ± 0.6	109.8 ± 1.3	119.0 ± 0.8	110.9 ± 2.1	195.9 ± 3.4	203.7 ± 2.0	201.2 ± 3.2
16.3 ± 0.7	113.1 ± 3.7	116.4 ± 1.7	109.8 ± 2.3	198.2 ± 3.2	200.9 ± 3.3	193.6 ± 5.3
	Wet gonad weight (g)			Gonad yield (%)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	34.9 ± 3.8	46.5 ± 4.2	45.3 ± 2.0	6.0 ± 0.6	7.8 ± 0.8	9.5 ± 0.9
12.4 ± 0.6	33.1 ± 1.7	50.4 ± 3.0	59.8 ± 4.0	8.0 ± 0.8	8.9 ± 0.6	12.7 ± 1.5
16.3 ± 0.7	34.6 ± 3.9	56.8 ± 4.7	42.8 ± 7.0	7.5 ± 1.1	11.0 ± 0.4	10.1 ± 1.7
	Gonad yield increase per week (%)			*CIE* L* values		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	0.4 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	50.2 ± 2.7	49.5 ± 1.4	54.8 ± 2.2
12.4 ± 0.6	0.5 ± 0.1	0.5 ± 0.0	0.9 ± 0.1	56.5 ± 2.3	51.7 ± 2.0	56.2 ± 1.1
16.3 ± 0.7	0.5 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	55.0 ± 1.4	54.4 ± 1.7	56.2 ± 2.2
	CIE a* values			*CIE* b* values		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	8.5 ± 1.7	8.4 ± 1.0	7.4 ± 0.2	31.9 ± 2.2	33.5 ± 1.9	41.2 ± 2.7
12.4 ± 0.6	7.1 ± 0.8	8.3 ± 1.0	7.9 ± 0.7	35.3 ± 1.9	34.8 ± 1.7	39.6 ± 1.9
16.3 ± 0.7	8.7 ± 0.7	10.4 ± 1.0	10.7 ± 1.2	34.5 ± 2.0	40.7 ± 1.7	42.0 ± 2.4
	Degree of colour difference (ΔE_{ab*})					
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10			
7.7 ± 0.4	42.0 ± 3.1	40.9 ± 2.3	31.5 ± 3.4			
12.4 ± 0.6	35.8 ± 2.3	38.5 ± 2.5	32.0 ± 2.0			
16.3 ± 0.7	36.9 ± 2.4	32.3 ± 2.1	30.3 ± 3.1			

Table 2.7. Results of separate two-way ANOVAs for the somatic and gonadal attributes of the green sea urchin, *Strongylocentrotus droebachiensis,* comparing the different diet and temperature treatments at the end of the experiment (week 9). Sources of variation are diet (D), temperature (T), the interaction (D x T), and error. *CIE* = Commission Internationale de l'Eclairage (L* = lightness, a* = redness, and b* = yellowness).

	Sea urchin wet weight (g)				Test height (mm)			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	93.7	0.5	0.6323	2	12.7	0.7	0.5216
T	2	1470.4	7.3	**0.0018**	2	83.3	4.3	**0.0193**
D x T	4	104.9	0.3	0.9026	4	24.9	0.6	0.6330
Error	45	4555.2			45	434.5		
	Spine diameter (mm)				Wet gonad weight (g)			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	105.9	2.5	0.0912	2	646.1	33.9	**<0.0001**
T	2	241.3	5.7	**0.0060**	2	211.6	11.1	**0.0001**
D x T	4	17.5	0.2	0.9323	4	19.7	0.5	0.7231
Error	45	943.4			45	429.2		
	Gonad yield (%)				Gonad yield increase per week (%)			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	1105.3	63.2	**<0.0001**	2	13.6	63.2	**<0.0001**
T	2	64.7	3.7	**0.0325**	2	3.8	17.8	**<0.0001**
D x T	4	11.2	0.3	0.8624	4	0.1	0.3	0.8624
Error	45	393.5			45	4.8		
	CIE L* values				*CIE* b* values			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	176.6	9.6	**0.0003**	2	2296.6	27.9	**<0.0001**
T	2	256.0	13.9	**<0.0001**	2	665.7	8.1	**0.0010**
D x T	4	28.7	0.8	0.5442	4	216.8	1.3	0.2785
Error	45	414.2			45	1853.2		
	Degree of colour difference (ΔE_{ab*})							
Source	df	SS	*F* value	*P* value				
D	2	2601.6	30.0	**<0.0001**				
T	2	813.5	9.4	**0.0004**				
D x T	4	132.2	0.8	0.5556				
Error	45	1951.5						

Values in bold are significant at $P \leq 0.05$. $n = 6$.

Table 2.8. Results of non-parametric Kruskal-Wallis test for test diameter and gonad *CIE* redness (a*) and results of the contingency analysis for gonad colour of the green sea urchins, *Strongylocentrotus droebachiensis,* comparing the different diet and temperature treatments at the end of the experiment (week 9). Gonad colour is based on the 1-4 rating scale where 1 is the best and 4 the worst (see Table 2.1). Sources of variation are diet (D), temperature (T), and the interaction (D x T).

	Test diameter (mm)			*CIE* a* values		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	1.7	2	0.4285	28.4	2	**<0.0001**
T	15.2	2	**0.0005**	7.6	2	**0.0226**
D x T	20.2	8	**0.0094**	38.2	8	**<0.0001**

	Gonad colour rating		
Source	ChiSquare	df	*P* > ChiSq
D	22.1	4	**0.0002**
T	5.1	4	0.2740
D x T	29.2	16	**0.0229**

Values in bold are significant at $P \leq 0.05$. $n = 6$.

Table 2.9. Mean ± SE gonadal attributes of the green sea urchin, *Strongylocentrotus droebachiensis,* for males, females, and unknown, and ANOVA results for each attribute at the end of the experiment (week 9). *CIE* = Commission Internationale de l'Eclairage (L* = lightness, a* = redness, and b* = yellowness).

Source	*CIE* L* values	*CIE* a* values	*CIE* b* values	Degree of colour difference (ΔE_{ab*})
Male (N = 22)	82.9 ± 0.6	1.6 ± 0.5	41.5 ± 1.8	26.6 ± 1.9
Female (N = 20)	77.6 ± 0.7	4.2 ± 0.7	52.9 ± 2.0	14.5 ± 2.0
Unknown (N = 12)	78.4 ± 1.2	3.9 ± 1.1	46.9 ± 2.3	20.2 ± 2.5
SS Sex	321.6	83.5	1338.8	1517.1
SS Error	553.9	471.3	3693.4	3981.7
F value	14.8	4.5	9.2	9.7
P value	**<0.0001**	**0.0156**	**0.0004**	**0.0003**
df=2,51				

Values in bold are significant at $P \leq 0.05$. N = 54.

Table 2.10. Results of separate two-way ANOVAs for the somatic and gonadal attributes of the red sea urchins, *Mesocentrotus franciscanus,* comparing the different diet and temperature treatments at the end of the experiment (week 12). Sources of variation are diet (D), temperature (T), the interaction (D x T). *CIE* = Commission Internationale de l'Eclairage (L* = lightness, a* = redness, and b* = yellowness).

	Sea urchin wet weight (g)				Test height (mm)			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	80128.3	5.1	**0.0100**	2	41.3	0.5	0.5728
T	2	73005.5	4.6	**0.0145**	2	23.9	0.3	0.7268
D x T	4	45623.8	1.4	0.2316	4	23.9	7.3	0.2708
Error	45	352680.7			45	1676.9		
	Test diameter (mm)				Spine diameter (mm)			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	401.9	5.5	**0.0074**	2	291.7	2.0	0.1415
T	2	754.6	10.3	**0.0002**	2	237.9	1.7	0.2004
D x T	4	127.2	0.9	0.4912	4	155.0	0.5	0.7051
Error	45	1650.3			45	3213.2		
	Wet gonad weight (g)				Gonad yield (%)			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	3128.9	15.6	**<0.0001**	2	0.0	10.2	**0.0002**
T	2	276.8	1.4	0.2613	2	0.0	3.9	**0.0272**
D x T	4	1068.2	2.7	**0.0442**	4	0.0	1.4	0.2403
Error	45	4503.9			45	0.1		
	Gonad yield increase per week (%)				*CIE* L*values			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	0.8	9.9	**0.0003**	2	134.7	3.0	0.0593
T	2	0.1	0.9	0.3925	2	146.7	3.3	**0.0469**
D x T	4	0.2	1.4	0.2459	4	62.2	0.7	0.5994
Error	45	1.9			45	1007.2		
	CIE a* values				*CIE* b* values			
Source	df	SS	*F* value	*P* value	df	SS	*F* value	*P* value
D	2	0.0	1.0	0.3748	2	461.2	9.0	**0.0005**
T	2	0.1	4.6	**0.0151**	2	118.9	2.3	0.1099
D x T	4	0.0	0.4	0.8374	4	112.9	1.1	0.3674
Error	45	0.6			45	1153.1		
	Degree of colour difference (ΔE_{ab*})							
Source	df	SS	*F* value	*P* value				
D	2	507.3	6.1	**0.0046**				
T	2	221.7	2.6	0.0812				
D x T	4	152.3	0.9	0.4651				
Error	45	1878.1						

Values in bold are significant at $P \leq 0.05$. $n = 6$.

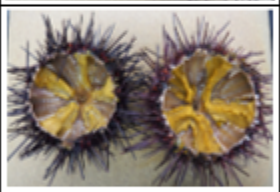
	7.7 ± 0.4 °C	12.4 ± 0.6 °C	16.3 ± 0.7 °C
Nereocystis luetkeana			
V10.1.9			
V10.1.10			

Figure 2.1. Gonads of the red sea urchins, *Mesocentrotus franciscanus,* from each diet and temperature treatment after feeding for 12 weeks. Individuals were randomly selected to be photographed at the end of the experiment. Temperatures are mean ± SD.

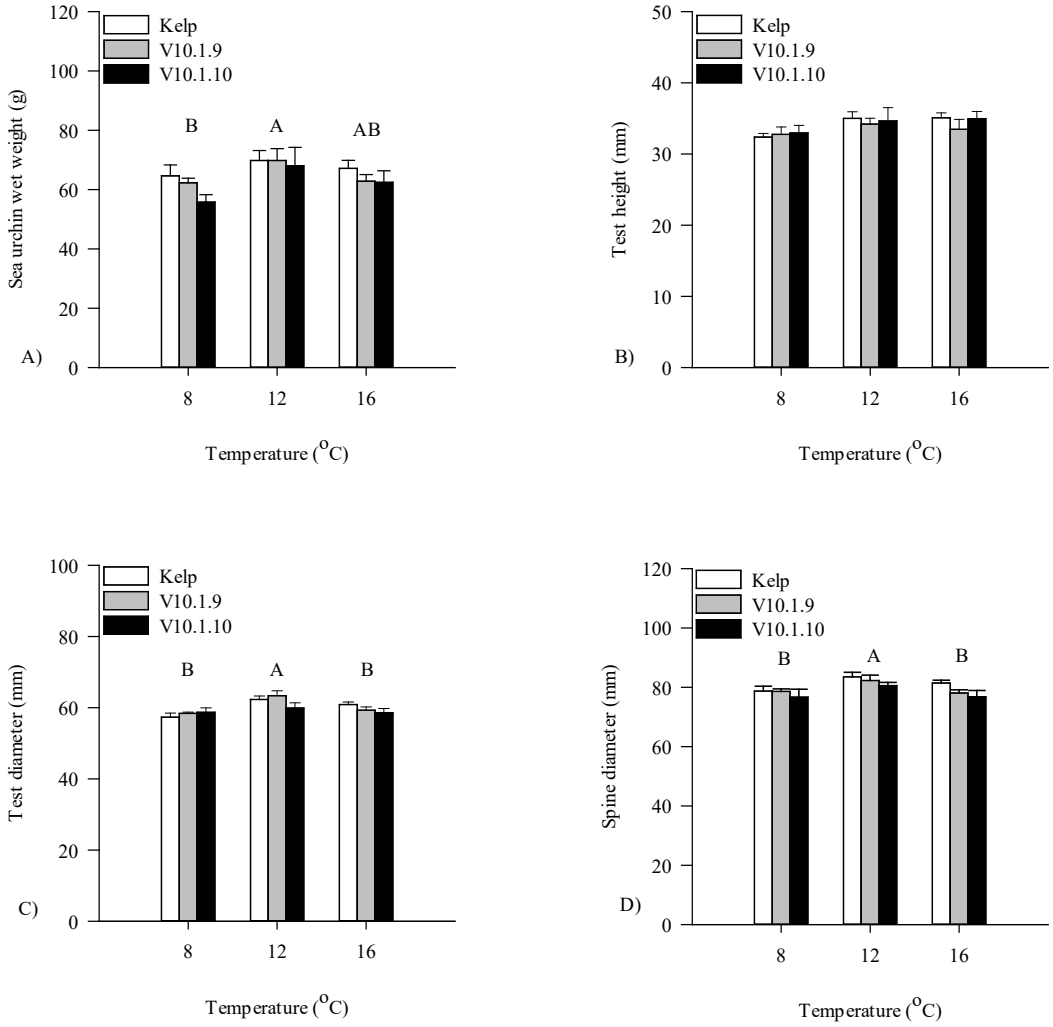


Figure 2.2. Mean ± SE initial somatic variables for green sea urchins (*Strongylocentrotus droebachiensis*) at week 0 for the dietary and temperature treatments. A two-way ANOVA and pairwise comparison were done with Tukey's HSD for all variables. Different numbers beside diet, and capital letters above bars indicate a significantly different diet and temperature effect ($P \leq 0.05$). Lower-case letters above the bars indicates a significantly different interaction effect ($P \leq 0.05$). $n = 6$.

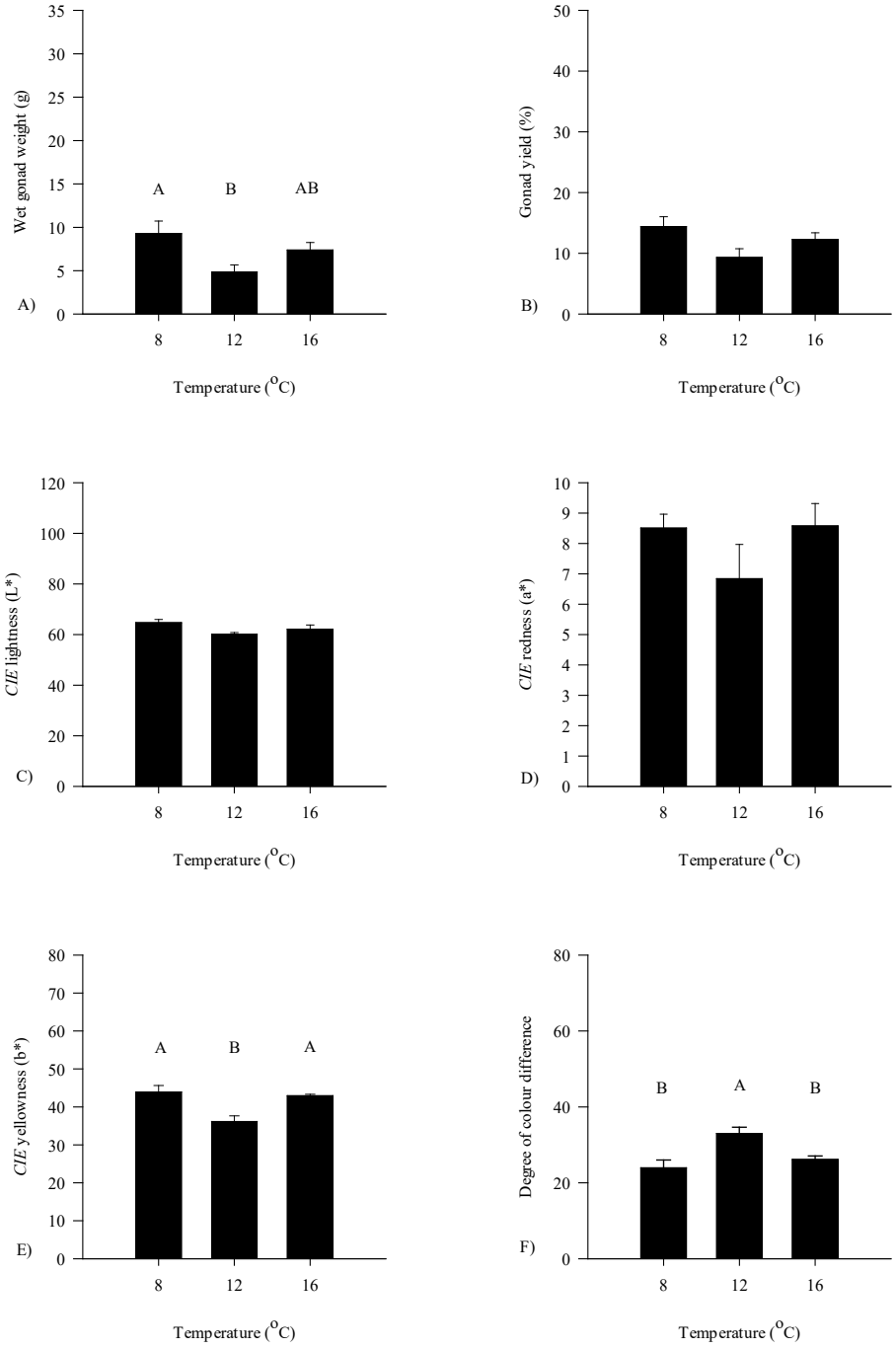
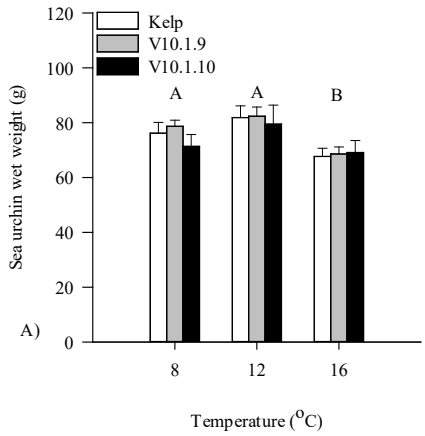
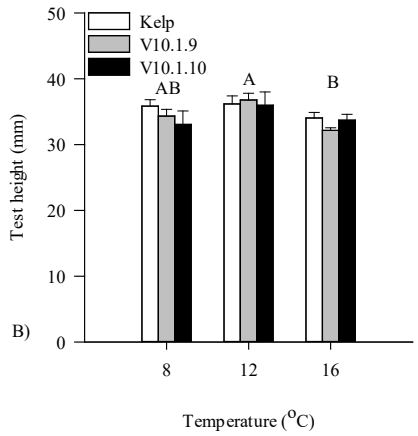
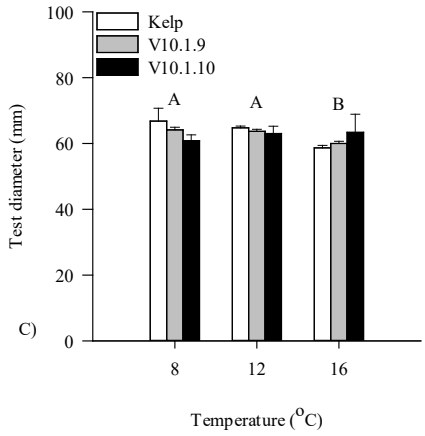
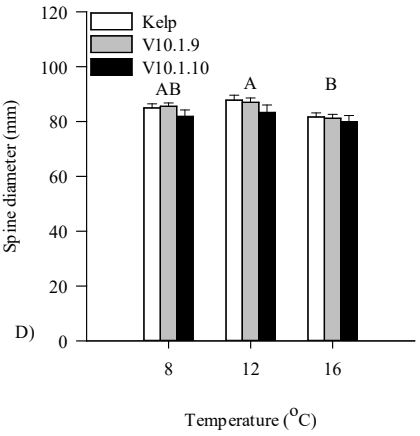


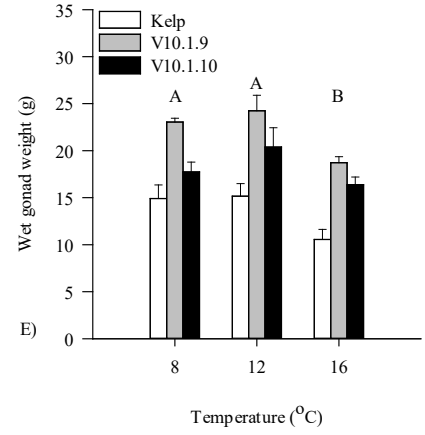
Figure 2.3. Mean ± SE initial gonadal variables for green sea urchins, *Strongylocentrotus droebachiensis*, at week 0 for the temperature treatments. A one-way ANOVA and pairwise comparison were done with Tukey's HSD for all variables. Different capital letters above bars indicate a significantly different temperature effect ($P \leq 0.05$). $n = 5$.

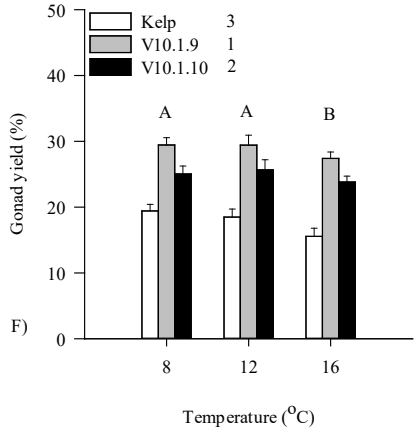

Kelp
V10.1.9
V10.1.10
Sea urchin wet weight (g)
120
100
80
60
40
20
0
A
A
B
A)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
Test height (mm)
50
40
30
20
10
0
AB
A
B
B)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
Test diameter (mm)
100
80
60
40
20
0
A
A
B
C)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
Spine diameter (mm)
120
100
80
60
40
20
0
AB
A
B
D)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
Wet gonad weight (g)
35
30
25
20
15
10
5
0
A
A
B
E)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
3
1
2
Gonad yield (%)
50
40
30
20
10
0
A
A
B
F)
8
12
16
Temperature (°C)

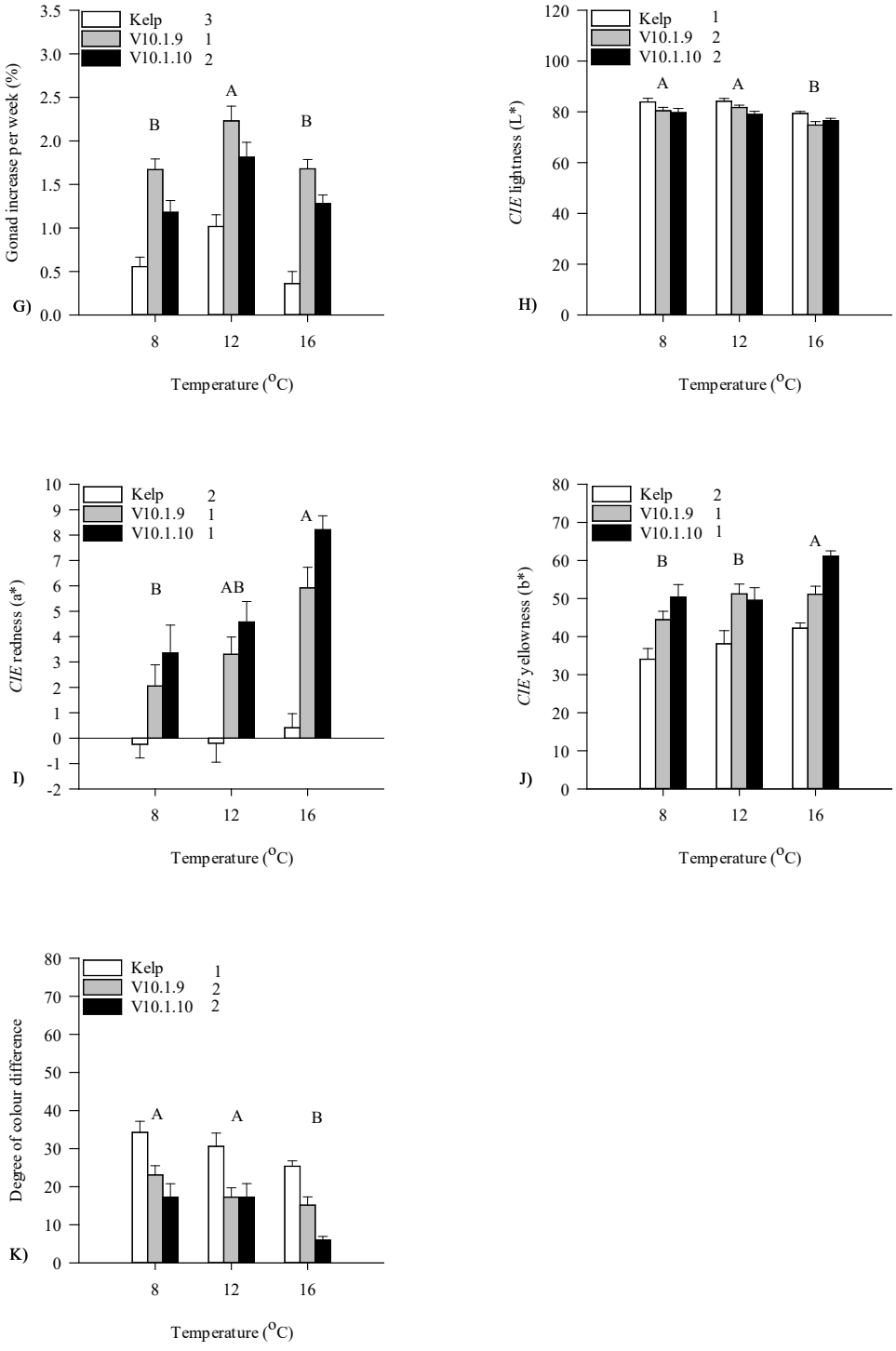


Figure 2.4. Mean ± SE somatic and gonadal variables for GSU (*Strongylocentrotus droebachiensis*) at the end of the experiment (week 9) for the dietary and temperature treatments. A two-way ANOVA and pairwise comparison were done with Tukey's HSD

for all variables (except test diameter and *CIE* redness where a Kruskal-Wallis test and Steel-Dwass All Pairs test was used). Different numbers beside diet, and capital letters above bars indicate a significantly different diet and temperature effect ($P \leq 0.05$). Lower case letters above the bars indicates a significantly different interaction of the diet and temperature effect ($P \leq 0.05$). $n = 6$.

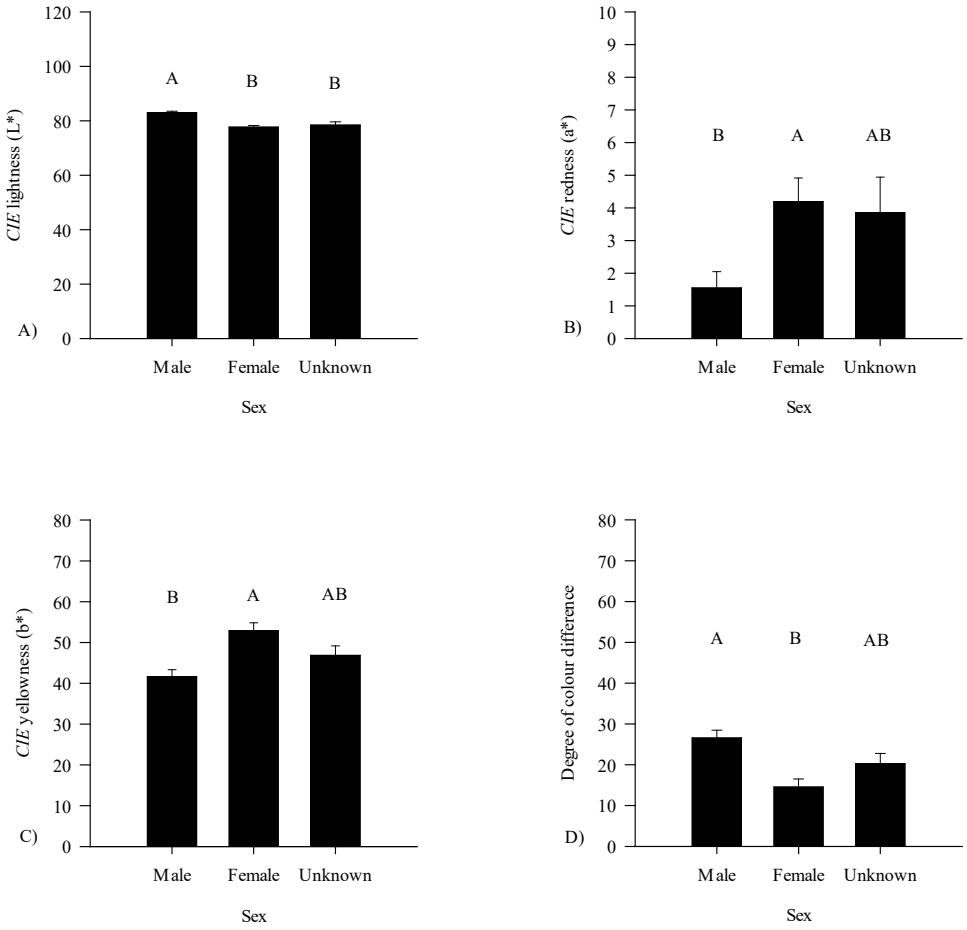


Figure 2.5. Mean ± SE gonadal variables for green sea urchins, *Strongylocentrotus droebachiensis*, at the end of the experiment (week 9) comparing the gonad colour (*CIE* lightness, redness, yellowness, and degree of colour difference) between the sexes. A one-way ANOVA and pairwise comparison were done with Tukey's HSD for all variables. Different capital letters above bars indicate a significant difference between the sexes ($P \leq 0.05$). N = 54.

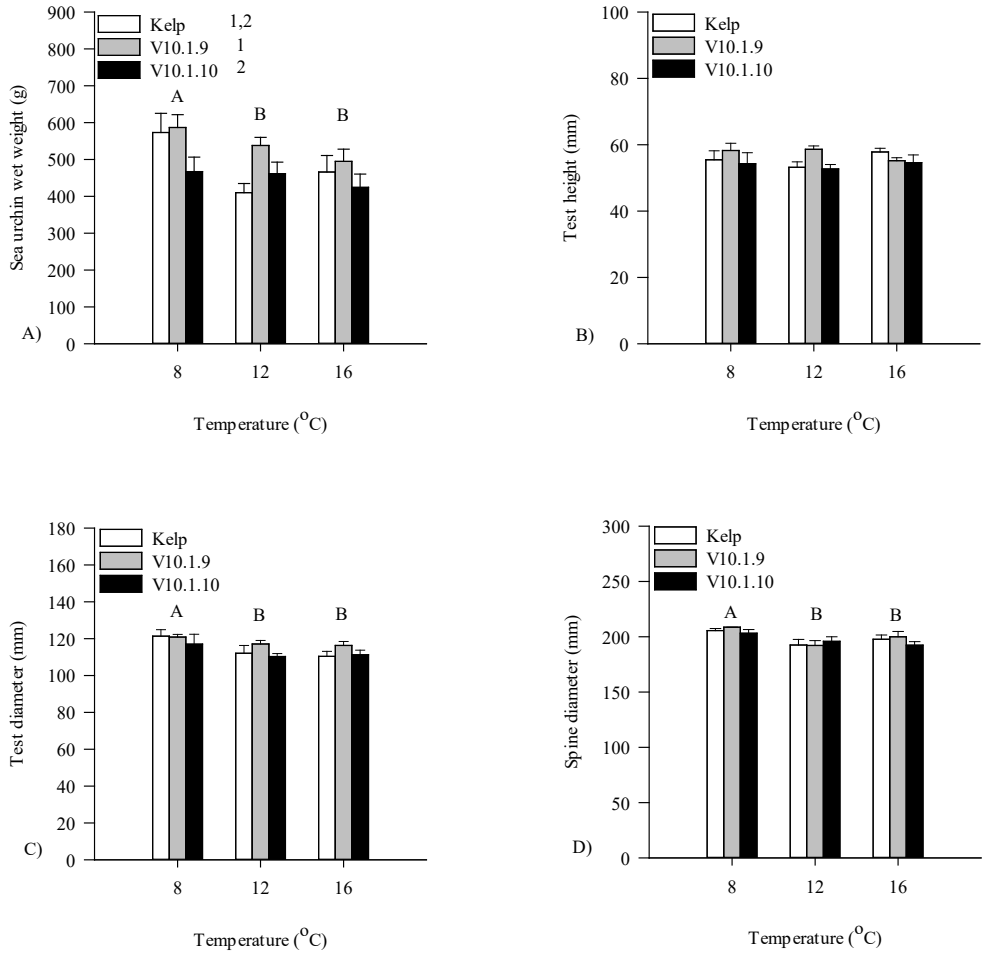


Figure 2.6. Mean ± SE initial somatic variables for red sea urchins, *Mesocentrotus franciscanus,* at week 0 for the dietary and temperature treatments. A two-way ANOVA and pairwise comparison were done with Tukey's HSD for all variables. Different numbers beside diet, and capital letters above bars indicate a significantly different diet and temperature effect ($P \leq 0.05$). Lower case letters above the bars indicates a significantly different interaction of the diet and temperature effect ($P \leq 0.05$). $n = 6$.

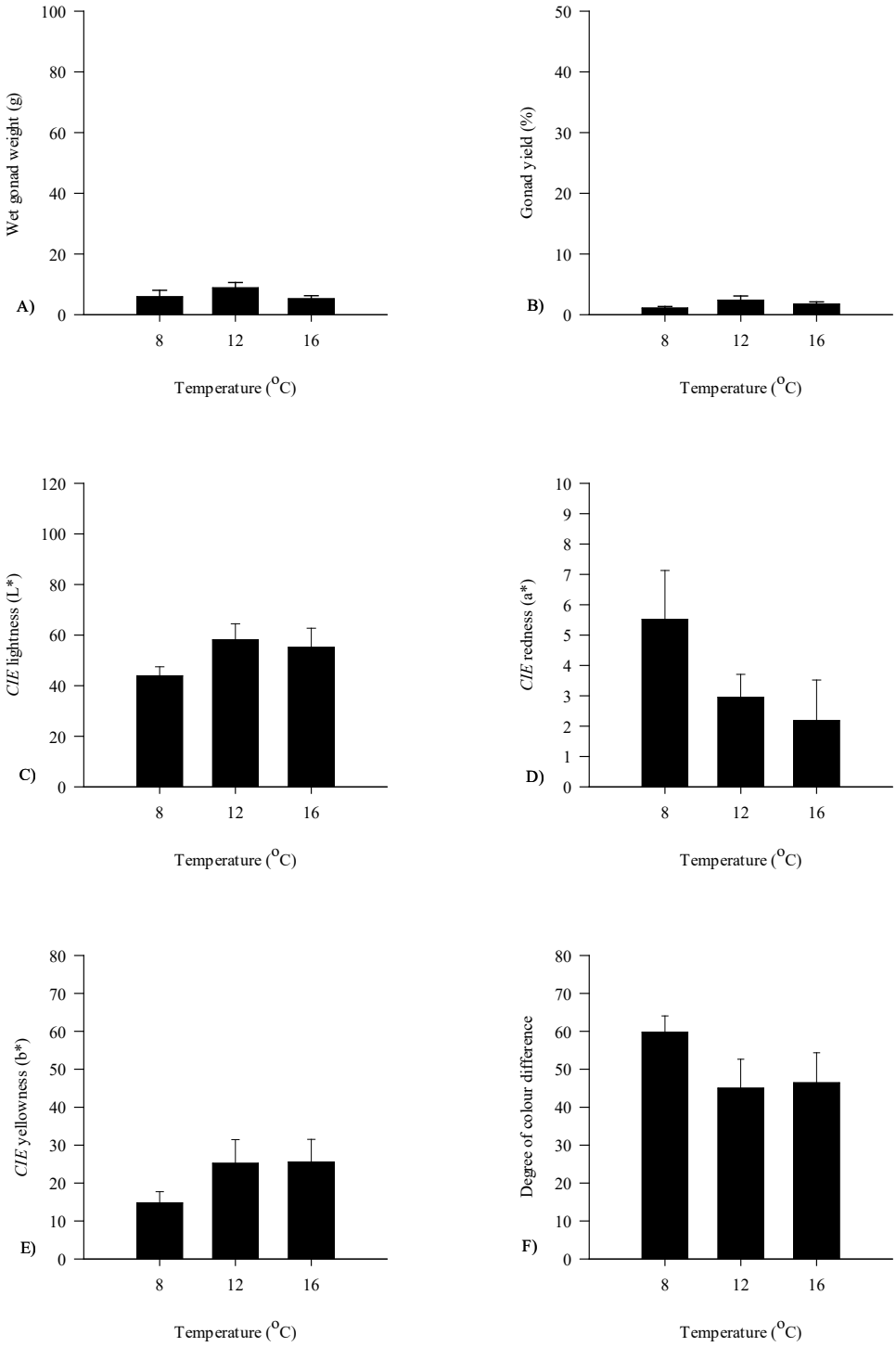
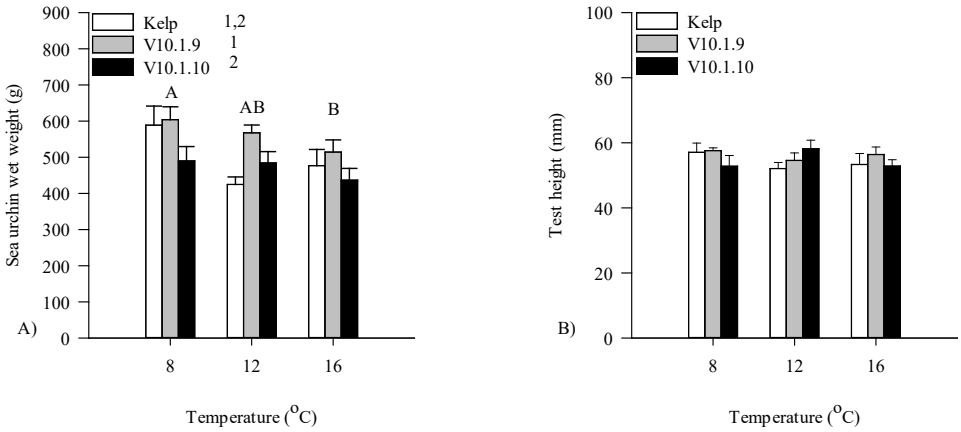
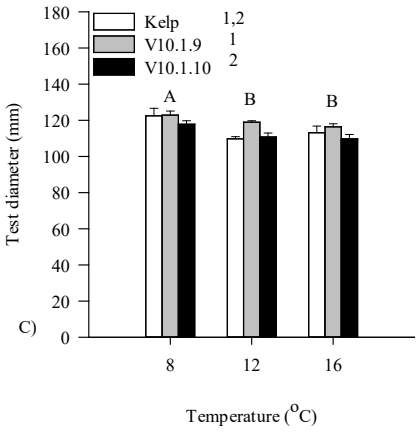


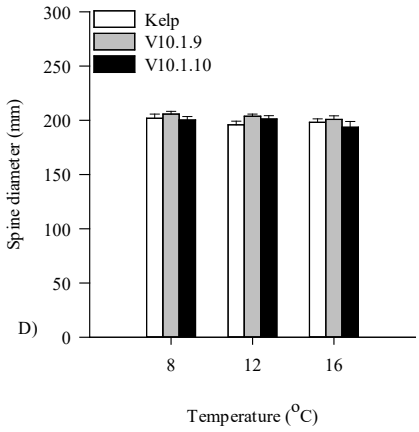
Figure 2.7. Mean ± SE initial gonadal variables for red sea urchins, *Mesocentrotus franciscanus*, at week 0 for the temperature treatments. A one-way ANOVA and pairwise comparison

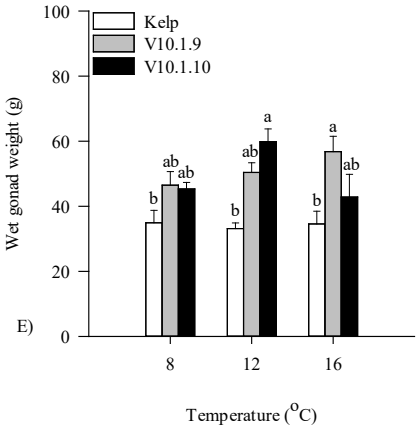
were done with Tukey's HSD for all variables. Different capital letters above bars indicate a significantly different temperature effect ($P \leq 0.05$). $n = 5$.

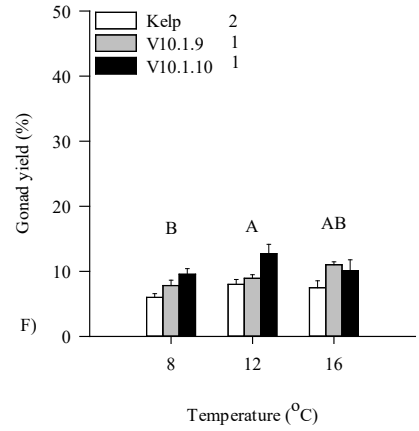

Kelp
V10.1.9
V10.1.10
1,2
1
2
A
AB
B
Sea urchin wet weight (g)
A)
Temperature (°C)

Kelp
V10.1.9
V10.1.10
Test height (mm)
B)
Temperature (°C)


Kelp
V10.1.9
V10.1.10
1,2
1
2
A
B
B
Test diameter (mm)
C)
Temperature (°C)


Kelp
V10.1.9
V10.1.10
Spine diameter (mm)
D)
Temperature (°C)


Kelp
V10.1.9
V10.1.10
b
ab
ab
b
ab
a
b
a
ab
Wet gonad weight (g)
E)
Temperature (°C)


Kelp
V10.1.9
V10.1.10
2
1
1
B
A
AB
Gonad yield (%)
F)
Temperature (°C)

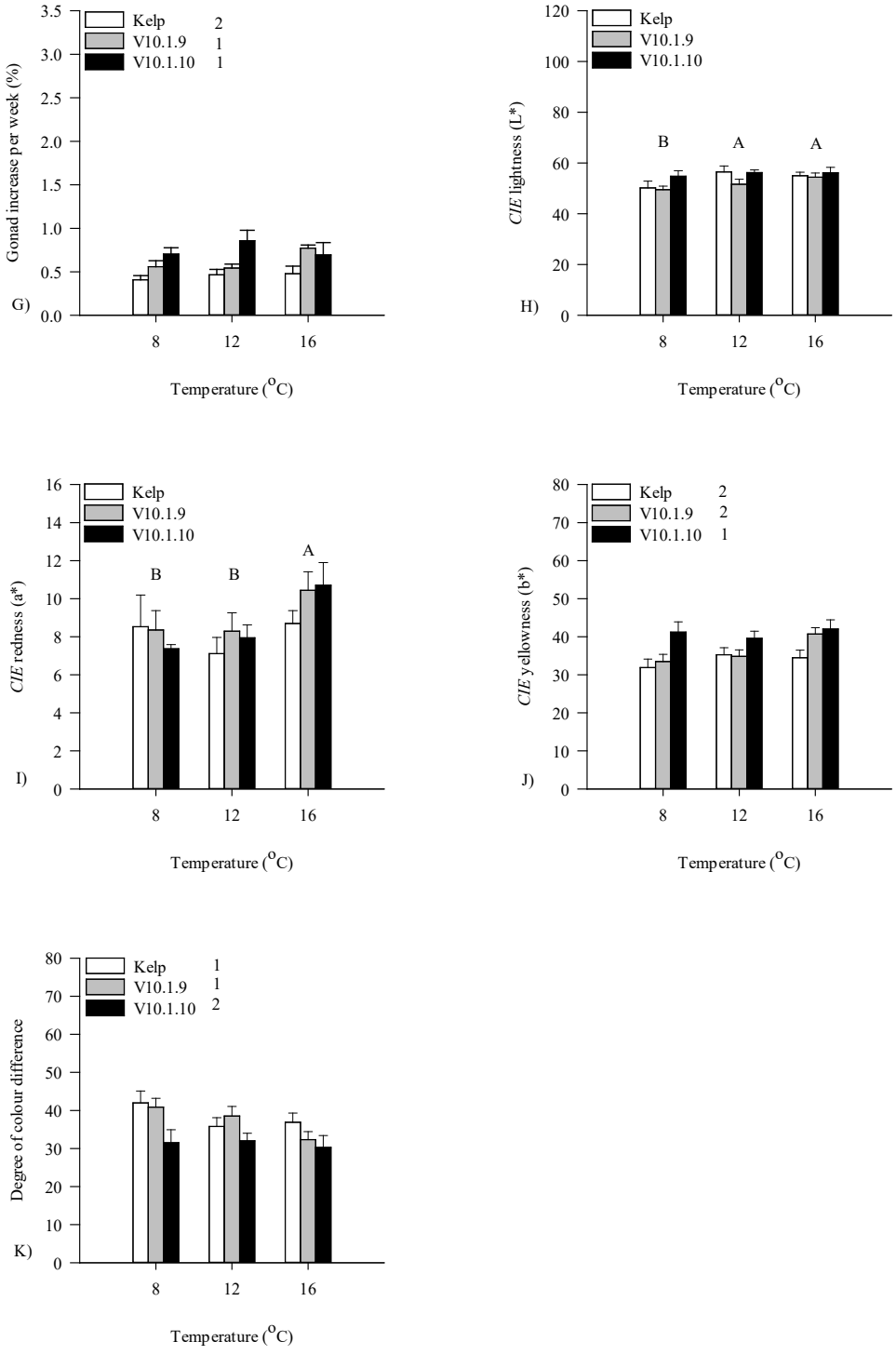


Figure 2.8. Mean ± SE somatic and gonadal variables for red sea urchins, *Mesocentrotus franciscanus*, at the end of the experiment (week 12) for the dietary and temperature

treatments. A two-way ANOVA and pairwise comparison were done with Tukey's HSD for all variables. Different numbers beside diet, and capital letters above bars indicate a significantly different diet and temperature effect ($P \leq 0.05$). Lower case letters above the bars indicates a significantly different interaction of the diet and temperature effect ($P \leq 0.05$). $n = 6$.

Chapter Three:

Effect of diet and temperature on ingestion rate, absorption efficiency, feed conversion ratios, and faecal production for the green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins.

3.1. Introduction

Aquaculture is a fast-growing industry worldwide as market demands for seafood continue to grow. With this growth, however, come concerns about the impacts the industry has on the environment and therefore means to regulate and manage the impacts are required. For any new industry, it is critical to know the environmental impacts and this knowledge can actually be required for regulation and management purposes (Cromey et al., 2002; Chamberlain et al., 2005). In Canada, management of aquaculture is shared among federal, provincial, and territorial governments, and varies depending on the province in which the aquaculture is occurring (Environment and Climate Change Canada 2020). In 2010, the federal government (Fisheries and Oceans Canada) assumed the primary role in the management of aquaculture in British Columbia (B.C.), and created new Pacific Aquaculture Regulations under the Fisheries Act to ensure sustainable operations (Fisheries and Oceans Canada 2011). These new regulations were intended to conserve marine ecosystems and minimize the environmental impacts of any aquaculture operations, whether marine finfish, shellfish, land-based, or enhancement facilities (Fisheries and Oceans Canada 2011). One of the many concerns with aquaculture is the impact of nutrient loading with faeces and uneaten feed that enter the water column and ultimately settle on the seabed (Cromey et al., 1998; Corner et al., 2006). Nutrient loading can increase water-column/sediment nitrogen/phosphorous concentrations, bacteria populations, biological oxygen

demand, and can ultimately be harmful to benthic communities (Panchang et al., 1997; Callier et al., 2006; McIver et al., 2018). In land-based flow-through or recirculation systems, solid waste can be easily removed with settling tanks and filtration and (potentially re-purposed as fertilizer) and the water bio-remediated through wetlands or wild/cultured plants/algae (Cripps and Bergheim, 2000; Chávez-Crooker and Obreque-Contreras, 2010; Snow et al., 2012; Lawton et al., 2020). In an open sea-based system, however, waste deposition to the benthos is inevitable as there are no means of collecting it easily. The level of organic benthic deposition will be highly variable and dependent on cultured species, diet formulation, feeding ration, and various environmental conditions such as temperature, hydrodynamics, bathymetry, weather, and benthic community structure.

Waste-deposition models, such as DEPOMOD (developed by Cromey et al., 2002), which is the most widely used model for aquaculture, can be used for regulation, licensing, environmental monitoring, prediction of production numbers, holding capacities, disease transfer, nutrient loading, and site selection (Silvert and Sowles, 1996; Dudley et al., 2000; Nath et al., 2000; Henderson et al., 2001; Callier et al., 2006; Corner et al., 2006). DEPOMOD is a particle-tracking model that requires information on various farm site parameters as well as erosion, transport, and deposition of waste materials to predict solids accumulation on the seabed underneath a farm (Cromey et al., 2002). Many studies have used DEPOMOD to examine the environmental impact of finfish farms with various species (Cromey et al., 2002; Chamberlain et al., 2005; Chamberlain and Stucchi, 2007; Page et al., 2007; Cromey et al., 2009, 2012; Keeley et al., 2013; Chang et al., 2014). Few, however, have examined impacts from shellfish (but see Weise et al., 2009) and none have determined impacts from echinoderms. Although DEPOMOD

was not used in the present study, the data collected in this research will be valuable for the model once potential commercial farm sites are selected.

Sea urchin aquaculture, where urchins are cultivated for their gonads (also termed roe or uni), is a newer industry and there is a limited understanding of its environmental impact. Sea urchin aquaculture contributes a relatively small amount to the global uni market – with Asia and Europe producing 8,243 tonnes (worth USD 52 million) and 1,053 tonnes (worth USD 2 million), respectively, in 2019 of cultured *Strongylocentrotus* species (FAO Global Aquaculture Production 1950–2019) – but there is interest in increasing echinoid aquaculture production in many countries, including Canada. The green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins are the primary candidates for aquaculture in North America and these are already the basis of rather lucrative wild fisheries in British Columbia (BC) where 3,168 tonnes worth CAD 8,288,000 were fished in 2019 (Fisheries and Oceans Canada, 2019). Two main types of aquaculture facilities exist to rear and enhance sea urchins – land-based and sea-based (Daggett et al., 2006; James, 2006; Woods et al., 2008; James and Siikavuopio, 2012) – with both types being under consideration for further commercial development in various countries. Currently, however, there is no commercial industry for either species on the west coast of Canada, although there is increasing interest from various industry stakeholders and First Nations. To make this new industry feasible, it is critical to understand ingestion rates, absorption efficiency, feed conversion ratios, as well as faecal production and characteristics of the urchins, to not only optimize production, but to also estimate benthic loading in order to mitigate any potential environmental impact of sea-based culture.

In the wild, sea urchins often aggregate in high densities and form grazing fronts that slowly turn flourishing macroalgal beds into urchin barrens, which are characterized by low-

lying encrusting coralline algae and low productivity (Breen and Mann, 1976; Hagen, 1995; Scheibling et al., 1999; Konar and Estes, 2003; Gagnon et al., 2004; Vasquez et al., 2006; Lauzon-Guay and Scheibling, 2007; Marzloff et al., 2013; Filbee-Dexter and Scheibling, 2014). These grazing fronts produce large quantities of faeces that can travel far distances due to waves and currents (Sauchyn and Scheibling, 2009a; Sauchyn et al., 2011). These faeces are considered an important food source for many suspension and deposit feeders (Frankenberg and Smith, 1967; Taghon et al., 1984; Sauchyn and Scheibling, 2009a, b; Dethier et al., 2019) and various studies have examined the composition and degradation of sea urchin faecal pellets in the wild [Koike, 1987 (S. *droebachiensis*); Mamelona and Pelletier, 2005 (S. *droebachiensis*); Sauchyn and Scheibling, 2009a,b (S. *droebachiensis*); Sauchyn et al., 2011 (S. *droebachiensis*); Dethier et al., 2019 (S. *droebachiensis* and *M. franciscanus*); Filbee-Dexter et al., 2020 (S. *droebachiensis* and *Echinus esculentus*)]. Little work has been done, however, to assess faecal pellet characteristics of sea urchins fed non-macroalgal diets (*e.g.* formulated feeds) under aquaculture conditions (but see Orr et al., 2014) or to examine the effect of temperature on these characteristics. Yet information on the impacts of these factors on ingestion rates, absorption efficiency, feed conversion ratios, and faecal pellet shape, size, and settling velocity will be important for determining benthic organic enrichment of sea-based urchin farms through models such as DEPOMOD.

The objective of the present study was to assess the effect of two newly formulated feeds (Urchinomics, V10.1.9 and V10.1.10) and a natural macroalgae [bull kelp (*Nereocystis luetkeana*)] at three temperatures (8, 12, and 16°C; temperatures commonly found in BC waters) on dry-weight ingestion rate (IR), absorption efficiency (AE), feed conversion ratio in terms of gonad production (FCR-G), and faecal pellet size, shape, and settling velocity in the green and

red sea urchins held in a land-based culture system. This information will be useful in future waste-deposition modeling to help predict and mitigate the potential environmental impacts of commercial-scale, sea-based sea urchin farms.

3.2. Methodology

3.2.1. Sea urchin collection, holding, and experimental setup

Adult green sea urchins (GSU) with a mean ± SD test diameter (TD) of 59.9 ± 3.1 mm were hand collected by SCUBA divers at Snake Island, BC (49º 12' 57.1" N, 123º 53' 26.4" W) on October 15, 2018. Adult red sea urchins (RSU) with a mean ± SD TD of 115.5 ± 8.1 mm were collected by SCUBA divers off the northern tip of Kendrick Island, BC (49º 07' 52.9" N, 123º 41' 47.2" W) on November 13, 2018. The urchins were transported on a boat in large tanks to the Pacific Biological Station (PBS), Nanaimo, BC. Transport time was ~1 hour for the GSU and ~2 hours for the RSU. At PBS, the sea urchins were placed into fibreglass tray tanks (L x W x H: 122.0 x 91.5 x 29.3 cm) and supplied with flow-through ambient (~8–10°C), sand-filtered and UV-treated, seawater. The sea urchins were left at this temperature and fed frozen (-20°C) bull kelp (*N. luetkeana*) *ad libitum* once a week for two weeks, to allow for any mortality from handling or transport stress to occur.

The experiments were conducted for nine weeks (June 3 to September 3, 2019) for GSU (trial had to end early for GSU due to a spawning event) and for 12 weeks (September 24 to December 10, 2019) for RSU. It examined the interactive effects of three diets [two prepared feeds and one macroalgae (bull kelp, *Nereocystis luetkeana*) as a control] and three temperatures (8, 12, and 16ºC) in a totally crossed, nine-treatment experiment. The two prepared diets were the latest sea urchin feed formulations of Urchinomics [versions 10.1.9 and 10.1.10 (proximate

analysis: 12.5% protein, 1.6% fats, and 10.0% water)] and were dry, extruded, pelleted feeds based on offcuts of Kombu kelp (various species from the family *Laminariaceae*) from sustainable production for human consumption, with the two feeds differing slightly in the kelp blend used (Brian Tsuyoshi Takeda, Urchinomics, pers. comm.). The bull kelp was previously collected in August 2016 from False Narrows, BC (49° 08' 04.7"N, 123° 47' 03.2"W) and frozen at -20°C, as fresh kelp was not readily available during the experiments. The three temperature treatments were chosen to reflect a range of temperatures found in BC waters (Sea Temperature, 2020).

Ninety urchins were randomly chosen from the stock tanks and placed into three of the fibreglass tray tanks described above (*i.e.* 30 sea urchins per tank) with the water in each tank being raised or lowered in temperature from ambient (~10.6ºC) ~1°C per day to allow urchins to gradually acclimate to the respective treatment temperatures of 8, 12, and 16°C. Food was withheld during and 1 week post-temperature acclimation to standardize hunger levels for all individuals. Eighteen of the 30 individuals in each of the three tanks were randomly selected for the experiment, producing six replicates for each feed/temperature combination. Each urchin was placed individually into a PVC bucket (H x D: 30 x 30.5 cm, volume: 19 L), that had a mesh-covered hole (H x D: 20 x 20 mm, mesh size: 0.7 x 0.7 mm) at the top. This allowed water outflow, and also prevented uneaten food waste and faeces from leaving the bucket. The buckets were then randomly placed into the nine fibreglass tanks described above. Each bucket was individually supplied with sand-filtered and UV-treated seawater at a flow rate of ~2.5 L min^{-1}, which was either 8, 12, or 16ºC depending on the treatment to which the sea urchin was allocated. The sea urchins were fed *ad libitum* once a week with their respective diet at 0.7% of their body weight if fed the prepared diets and 5% of their body weight if fed the macroalgae diet

(Pearce et al. 2002a, b, 2004). Uneaten food and faeces were siphoned out from each bucket prior to feeding. Temperature (ºC), salinity (ppt) (YSI Pro30, YSI Incorporated, Yellow Springs, Ohio, USA), and dissolved oxygen (mg/L) (OxyGuard Handy Polaris, OxyGuard International A/S, Farum, Denmark) were recorded in each tank daily, as were mortalities. Temperature was also recorded in each tank every hour using HOBO® Tidbit® v2 temperature loggers (Onset, Bourne, Massachusetts, USA). Any unhealthy-looking urchins (*e.g.* spine loss, spine drooping, unextended/lost tube feet, or lesions) were removed and taken for a disease screen in the PBS Shellfish Pathology Laboratory. Any unhealthy/dead sea urchins were counted and removed from the experiment, but not replaced.

3.2.3. Uneaten feed and faeces collection and analysis

Any uneaten food, faeces, and broken spines were removed from each bucket 24 hr after feeding using a siphon on weeks 0, 4, 8, and 9 for GSU (the GSU trial had to end early at week 9 due to a spawning event) and weeks 0, 4, 8, and 12 for RSU. The uneaten feed was separated from the faeces and spines using a sieve and tweezers and frozen at -80°C until processing. The faecal pellets were then collected 24 hours after the buckets were cleaned (48 hours after the urchins had been fed), separating them from any spines using and tweezers. The faecal pellets were then frozen at -80°C until processing. Both the uneaten food and faecal samples were thawed, rinsed with fresh water to remove any salts, and placed onto separate filter papers where the water was allowed to drain (Orr et al., 2014). They were then placed individually into pre-weighed, ashed crucibles to obtain the wet weight (g), dried at 70ºC for approximately 48 hr, or until a constant weight was obtained, and then re-weighed (Orr et al., 2014). The ash-free dry weight was obtained by putting the samples in a muffle furnace for approximately 4 hr at 450ºC

(Orr et al., 2014). Dry-weight ingestion rate (IR) was then calculated based off the adjusted start weight of the feed (percent change of feed wet weight to dry weight) by determining the average moisture content of each diet, using the formula below, where S_a is adjusted start weight of the feed (g), E is the end dry weight of feed (g), U is the urchin live wet weight (g) (weighed at the time of feeding), and D is the length of the trial in days (Daggett et al., 2005):

$$\textit{Dry-weight IR (feed g urchin}^{-1}\textit{ g day}^{-1}\textit{)} = [(Sa\text{-}E)/U/D]$$

Absorption efficiency (AE) [the percentage of organic material absorbed by the sea urchin as material passes through the digestive system (Reid et al., 2010)] and feed conversion ratio per gonad weight (gonads were weighed at the end of the trial during sampling) (FCR-G; the amount of feed needed to produce 1 g of gonad tissue) were calculated as follows:

$$\textit{AE (\%) = ((dry weight ingested (g) – dry weight egested (g)) / (dry weight ingested (g))) x 100}$$

$$\textit{FCR-G (feed g gonad increase g}^{-1}\textit{) = feed ingested g / g change in gonad wet weight}$$

3.2.4. Faecal pellet size, shape, and settling rates

Three sea urchins per treatment were randomly selected to have their faecal pellets collected to examine settling rates on weeks 0, 3, 6, 9, and 12 (excluding week 12 for GSU). The sea urchins were fed their respective treatment diet and left to feed for 24 hours. Following this, all uneaten food and faeces were removed gently with a siphon and then separated using a sieve and tweezers. The faecal pellets from each urchin were then placed into individual 50-mL centrifuge tubes and held on ice (Orr et al., 2014). Three randomly selected faecal pellets from each sea urchin were placed under a dissecting microscope where pellet length and width were measured using i-Solution Total Imaging Software™ (IMT i-Solution Inc., Burlington, Ontario, Canada). The shape of the pellets was calculated as the Corey Shape Factor (CSF), where a value

equal to 1 means the pellets are spherical and a value less than 1 is ellipsoidal (Sauchyn and Scheibling, 2009b). Size was calculated as the equivalent circular diameter (Sauchyn and Scheibling, 2009b) using the equations below:

$$Corey\ Shape\ Factor = CSF = pellet\ width / (\sqrt{pellet\ length \times pellet\ width})$$

$$Size = \sqrt{pellet\ length \times pellet\ width}$$

For faecal pellet settling velocity (cm s^{-1}), a 50-mL graduated cylinder was set up with two marks on it that were located 10 cm apart on the side of the cylinder. The upper mark was located 7 cm below the top of the cylinder (*i.e.* 7 cm below the water level) (Callier et al., 2006). The time it took for the pellet to descend between the two marks was recorded. The mean pellet length, width, size, shape, and settling velocity was then calculated from the three pellets from each sea urchin for use in statistical analyses.

3.2.3. Statistical Analysis

Statistical analyses were conducted using JMP® 15 software (SAS, Marlow, Buckinghamshire, UK). All variables were averaged across all experimental sampling points and these mean values then used in the subsequent statistical analyses, as the temporal effect was not likely to be significant and I was not interested in examining the effect of time. The Shapiro-Wilks test and Levene's test were used for all variables to confirm normal distribution and homogeneity of variances, respectively. If the data did not meet these assumptions, they were transformed [*i.e.* GSU: AE (arcsine transformed), GSU: FCR-G (Ln transformed); RSU: AE (arcsine transformed); GSU: faecal pellet length (log transformed)]. If after trying various transformations the data still did not meet the assumptions, non-parametric Kruskal-Wallis tests were used on the separate effects of diet and temperature, with Steel-Dwass All Pairs tests being

used for post-hoc multiple pair-wise comparisons (*i.e*. all variables except RSU faecal pellet length, width, and size). If the data met the assumptions, a two-way ANOVA was used to compare the effects of diet, temperature, and their interaction on the variables, followed by Tukey's HSD for pair-wise comparisons among the treatments. For any missing values, the data were replaced with the average of the treatment for which the sea urchin was in for the particular sampling week.

3.3. Results

3.3.1. Green sea urchins

3.3.1.1. Water quality and survivorship

GSU experienced a mean ± SD temperature of 7.8 ± 0.1ºC, 12.4 ± 0.1ºC or 15.9 ± 0.1ºC (N = 12,374), depending on the temperature treatment in which the sea urchin was allocated. Mean ± SD salinity was 30.4 ± 0.5 ppt (N = 459) and dissolved oxygen was 8.5 ± 0.6 mg L^{-1} (N = 459). Survivorship was 100% in all nine treatments for the GSU during this 9-week feeding trial.

3.3.1.2. Dry-weight ingestion rate (IR), absorption efficiency (AE), and feed conversion ratio-gonads (FCR-G)

Mean ± SE dry-weight IR, AE, and FCR-G are provided in Table 3.1. Diet, temperature, and their interaction were all significant for dry-weight IR for GSU (Table 3.3). While the GSU fed kelp had higher dry-weight IR than the GSU fed V10.1.9 or V10.1.10 at all three temperatures, there was only one significant pairwise difference among the nine treatments (8°C/V10.1.9 < 16°C/kelp) (Fig. 3.2A).

Diet and its interaction with temperature were both significant for AE, but not the main effect of temperature (Table 3.3). GSU fed kelp had higher AEs than those fed the prepared diets at all three temperatures, with all kelp vs V10.1.9/V10.1.10 pairwise comparisons being significant except for 8°C/kelp, 12°C/kelp, 16°C/kelp, and 16°C/V10.1.10 (Fig. 3.2B).

Diet, temperature, and their interaction were all significant for FCR-G (Table 3.3). GSU fed kelp had higher FCR-G values than those fed the two prepared diets, although not all kelp vs V10.1.9/ V10.1.10 pair-wise comparisons were significant (one at 8°C, one at 12°C, and two at 16°C, Fig. 3.2C).

3.3.1.3. Faecal pellet size, shape, and settling rates

Mean ± SE faecal pellet length, width, shape, and settling velocity are provided in Table 3.1. The diet and interaction effect, but not temperature, were significant for all variables (Table 3.3). GSU fed kelp had significantly larger faecal pellet lengths and size than those fed V10.1.9 or V10.1.10, at all three temperature treatments (Fig. 3.2D, F). The same trend was evident for faecal pellet width, although not all of the kelp vs V10.1.9/ V10.1.10 pair-wise comparisons were significant (Fig. 3.2E). GSU fed V10.1.9 and V10.1.10 feeds had significantly more spherical faecal pellets (Fig. 3.2G) and faster settling velocities (Fig. 3.2H) than GSU fed kelp, at all three temperature treatments, but did not significantly differ from each other.

3.3.2. Red sea urchins

3.3.2.1. Water quality and survivorship

The RSU experienced a mean ± SD temperature of 7.7 ± 0.4ºC, 12.4 ± 0.6ºC, or 16.3 ± 0.7ºC (N = 12,374), depending on the treatment in which the RSU was allocated, mean ± SD salinity was 30.4 ± 0.5 ppt (N = 459), and dissolved oxygen was 8.5 ± 0.6 mg L^{-1} (N = 459) throughout the twelve week trial. Survivorship was 90.7% for the initial 54 RSU used in this feeding trial. Three RSU died early on in the experiment (one each in V10.1.9 at 12℃, V10.1.10 at 16℃, and kelp at 16℃) and were replaced. Two others died (one each in kelp at 12℃ and V10.1.9 at 12℃) later in the experiment and were not replaced.

3.3.2.2. Dry-weight ingestion rate (IR), absorption efficiency (AE), and feed conversion ratio-gonads (FCR-G)

Mean ± SE dry-weight IR, AE, and FCR-G are provided in Table 3.2. Both diet and the interaction effect, but not temperature, were significant for IR (Table 3.3). The RSU fed kelp had significantly higher dry-weight IR than those fed V10.1.9 or V10.1.10, with all of the kelp vs V10.1.9/ V10.1.10 pair-wise comparisons being significant (Fig. 3.3A).

Only the temperature effect was significant for AE, but not diet or the interaction effect (Table 3.3). RSU held at 12ºC had significantly higher AE than RSU at 16ºC, and neither significantly differed from 8ºC (Table 3.3, Fig. 3.3B).

For FCR-G, both the diet effect and the interaction of diet and temperature were significant, but not temperature alone (Table 3.3). RSU fed kelp at all three temperatures had significantly higher FCR-G than those fed V10.1.9 or V10.1.10, which did not significantly differ from each other (Fig. 3C).

3.3.2.3. Faecal pellet size, shape, and settling rates

Mean ± SE faecal pellet length, width, shape, and settling velocity are provided in Table 3.2. The main temperature effect was not significant for any of the faecal pellet variables, while the main diet effect was significant for all of them (Tables 3.3, 3.4). Both diet and the interaction were significant for pellet shape and settling velocity (Table 3.3). RSU fed kelp had significantly larger faecal pellet lengths, widths, and size compared to those fed V10.1.9 or V10.1.10 (Fig. 3.2D, E, F). RSU fed kelp had less spherical pellet shapes than those fed V10.1.9 or V10.1.10 at all three temperature treatments, although the kelp vs V10.1.9/ V10.1.10 pair-wise comparisons only being significant at 12 and 16°C (Fig. 3.3G). For faecal pellet settling velocity, RSU fed kelp at all three temperature treatments had significantly slower settling velocities than those fed V10.1.9 or V10.1.10 (Fig. 3H).

3.4. Discussion

3.4.1. Dry-weight ingestion rate (IR)

Diet, temperature, and their interaction were all significant for dry-weight IR for GSU (Table 3.3), where GSU fed kelp had higher dry-weight IR than GSU fed V10.1.9 or V10.1.10 at all three temperatures, however, there was only one significant pairwise difference among the nine treatments (8°C/V10.1.9 < 16°C/kelp) (Fig. 3.2A). Both diet and the interaction effect, but not temperature, were significant for dry-weight IR for RSU (Table 3.3), where RSU fed kelp had significantly higher dry-weight IR than those fed V10.1.9 or V10.1.10, with all of the kelp vs V10.1.9/ V10.1.10 pair-wise comparisons being significant (Fig. 3.3A). There is a plethora of studies examining IRs in various species of sea urchins fed both prepared and macroalgal diets (*e.g.* McBride et al., 1997; Fernandez and Boudouresque, 2000; Lawrence et al., 2003; Daggett et al., 2005; Hammer et al., 2006, 2012; Azad et al., 2011; Orr

et al., 2014; Taylor et al., 2017). The approach to measuring and analyzing IR data, however, is not consistent throughout the literature. Different studies use different feeds that all have unique nutritional profiles, and some studies do not standardize IR for individual urchin weight or per unit time (as was done in the present study). Therefore, while general trends can be examined, it is difficult to make direct comparisons to most other studies. Nonetheless, the present research produced results that were consistent with the general trends of similar echinoid research on IRs.

For example, many studies have shown that sea urchins fed macroalgal diets have higher IRs than those fed prepared diets (McBride et al., 1997; Fernandez and Boudouresque, 2000; Lawrence et al., 2003; Daggett et al., 2005; Hammer et al., 2006, 2012; Azad et al., 2011; Orr et al., 2014; Taylor et al., 2017). This is thought to be linked to the protein content and quality of the feed provided, where lower quality and lower protein foods require more feed to be ingested by the sea urchin to meet the energy requirements for somatic and gonadal growth (Azad et al., 2011). For example, Fernandez and Boudouresque (2000) reported that IRs in *Paracentrotus lividus* were higher when they were fed a plant-protein-based food than when given other feed types and they found a significant correlation between ingestion rates and protein levels. Decreased sea urchin survival may also be linked to lower protein levels in the feed (Hammer et al., 2006). This trend is similar to that seen in the present study with both the GSUs and RSUs, where individuals fed the kelp had significantly higher dry-weight IRs than those fed either V10.1.9 or V10.1.10 prepared diets. Siikavuopio et al. (2007a) measured IR at day 14 and day 560 in small (30–40 mm TD), medium (45 mm TD), and large (50 mm TD) GSU fed a prepared diet of approximately 59.1% protein content at 10ºC. The "large" size group, the most similar to the size of GSU used in this study (mean TD: 59.8 mm),

produced a mean ± SD IR of 0.011 ± 0.002 g ingested g urchin^{-1} day^{-1} at day 14 and 0.004 ± 0.0001 g ingested g urchin^{-1} day^{-1} at day 560. The former is ~2 orders of magnitude greater than those measured for GSU in the present study (0.0029 ± 2.5^{E-4} and 0.0031 ± 2.2^{E-4} g ingested g urchin^{-1} day^{-1} for V10.1.9 and V10.1.10, respectively), but the latter is relatively similar. For RSU, few studies have examined IR (but see McBride et al., 1997, 1999) and the ones that exist do not factor in RSU wet weight and therefore direct values cannot be compared to the present work. However, McBride et al. (1997) fed bull kelp (*N. luetkeana*) and a prepared diet to RSU (mean wet weight: 248g) at 12.9ºC and 16.1ºC and the RSU fed kelp had an IR three times greater than the RSU fed a prepared diet, which is a similar trend as seen in the present study.

Temperature is another critical factor in determining IR and many studies have examined this relationship with various echinoid species (McBride et al., 1997; Fernandez and Boudouresque, 2000; Siikavuopio et al., 2006; Lawrence et al., 2009; Azad et al., 2011; Siikavuopio et al., 2012). Several studies indicate that IR tends to increase with temperature, until a thermal tolerance is exceeded for the particular species. For example, Azad et al. (2011), working with the purple urchin (*S. purpuratus*), found that both wet and dry IR increased with temperature (8, 12, 16°C), with the highest IR at 16ºC and the lowest at 8ºC. In addition, Siikavuopio et al. (2006) fed GSU a prepared diet in both the winter and summer months and showed significantly higher IRs at 14ºC and 12ºC compared to 8ºC. The authors suggested that when food supply is unlimited, temperature is likely the most important factor in determining IR. Siikavuopio et al. (2012) also reported higher feed intake at 12 and 14ºC compared to 6 and 10ºC in GSUs. Finally, Siikavuopio et al. (2007a) also found that feed intake varied strongly with season for all GSU size groups (small: 30–40 mm TD; medium: 45

mm TD; large: 50 mm TD), where those fed a prepared diet in the summer had significantly higher feed intakes (0.5–0.6 g/day) than in the winter (0.2–0.3 g/day). In the present study, GSU IRs for each feed tended to increase with temperature, although there was only one significant pairwise comparison among the nine treatments (8°C/V10.1.9 < 16°C/kelp). For RSUs, IR was significantly higher at 12 and 16°C than at 8°C, but this was only apparent for kelp, with no temperature effect for the two prepared diets. These results align with McBride et al. (1997), who found that RSU fed *N. luetkeana* at 16.1ºC had significantly higher IRs that those at 12.9ºC. It should be noted that not all species may respond the same to temperature differences. For example, Lawrence et al. (2009), working with *S. intermedius*, showed that sea urchins fed a prepared feed between February and March (7ºC) had higher IRs than those fed between March and April (12 and 17ºC). Ultimately, echinoid IR response to temperature is likely species and feed dependent.

3.4.2. Absorption efficiency (AE)

Diet and its interaction with temperature were both significant for AE, but not the main effect of temperature for GSU (Table 3.3), where GSU fed kelp had higher AEs than those fed the prepared diets at all three temperatures, with all kelp vs V10.1.9/V10.1.10 pairwise comparisons being significant except for 8°C/kelp, 12°C/kelp, 16°C/kelp, and 16°C/V10.1.10 (Fig. 3.2B). Only the temperature effect was significant for AE, but not diet or the interaction effect RSU (Table 3.3), where RSU held at 12ºC had significantly higher AE than RSU at 16ºC, and neither significantly differed from 8ºC (Table 3.3, Fig. 3.3B). Similarly, as with IR, AE can be highly influenced by diet and temperature, as seen in Lowe and Lawrence (1976), Larson et al (1980), Thompson (1983), McBride et al. (1999), Lawrence et al. (2009), Azad et

al. (2011), Orr et al. (2014), and Rubillar et al. (2016), where generally AE are higher with sea urchins fed kelp compared to a prepared diet and at higher temperatures.

A study by Azad et al. (2011) found that *S. purpuratus* had significantly higher AEs with individuals fed *N. luetkeana* (~75–85% AE) than ones fed a prepared diet (~65–80% AE). Lowe and Lawrence (1976) reported both relatively low and high AEs with *Lytechinus variegatus* fed various marine algae (excluding bull kelp), ranging between 34 and 71%. Orr et al. (2014) also had similar AEs between the two diets for GSU fed either sablefish (*Anoplopoma fimbria*) waste or giant kelp (*Macrocystis pyrifera*) at 10ºC, which did not significantly differ from each other. Larson et al (1980) found AEs ranging between 26 and 77% for GSUs fed various macroalgae species, Thompson (1983) reported a range of AE between 60 and 90% for GSUs fed a mixture of blue mussel (*Mytilus edulis*) tissue and kelp (*Alaria esculenta*), and Rubillar et al. (2016) found AEs between 80 and 90% in *Arbacia dufresni* fed a prepared diet. For RSU, the diet effect was not significant, however, temperature and the interaction were, although overall, RSU fed kelp produced a higher AE than either prepared diet.

Several studies show that temperature also greatly influences AE, however, the temperature effect was not significant with the GSUs in the present study, and RSU in the present study had higher AE at 16 than at 12ºC, but neither significantly differed from 8ºC. Azad et al. (2011) found that *S. purpuratus* had increasing AE with increasing temperature, AEs being significantly higher at 16ºC than at 8 or 12ºC. Lawrence et al. (2009) had similar results to the present study with *S. intermedius*, where temperature did not affect AE, however those fed a prepared diet at 17ºC had a much lower AE (32.2–47.3%) compared to the other diet/temperature treatments. The only comparable literature found for RSU was a study

conducted by McBride et al. (1999), where AE ranged between 64 and 70% for RSU fed a prepared diet at 15.7ºC. As shown above with IR, AE is quite variable in the literature, which makes direct comparison to other studies difficult. However, the AE achieved in the present study for GSU falls within the range of 63.9–88.4% for all diets and temperatures, which is within the range of the studies aforementioned. All the AE for RSU in the present study (74.4–92.2%) were higher than the ones obtained in McBride et al. (1999), which could be due to the different nutritional profiles of the prepared diets.

3.4.3. Feed conversion ratio - gonads (FCR-G)

For GSU, diet, temperature, and their interaction were all significant for FCR-G (Table 3.3), where GSU fed kelp had higher FCR-G values than those fed the two prepared diets, although not all kelp vs V10.1.9/ V10.1.10 pair-wise comparisons were significant (one at 8°C, one at 12°C, and two at 16°C (Fig. 3.2C). For RSU, both the diet effect and the interaction of diet and temperature were significant, but not temperature alone (Table 3.3), where RSU fed kelp at all three temperatures had significantly higher FCR-G than those fed V10.1.9 or V10.1.10, which did not significantly differ from each other (Fig. 3C). No literature found examined FCR-G in RSU, however, the results follow the same trends as the GSU, and studies described below.

FCR-G is important in determining optimal conditions in a sea urchin gonad enhancement operation, to be able to feed the least amount of food and still produce a market quality product. Therefore, in interpreting results, the lower the FCR-G the better, because it indicates a high feed conversion efficiency (Siikavuopio et al., 2006). The FCR for somatic growth was not examined in this study due to the slow growth of sea urchins and the short

time period of this feeding trial, the growth would be negligible (Christiansen and Siikavuopio, 2007). As well, prepared feeds are designed to promote gonad growth and not somatic growth (de Jong-Westman et al., 1995; Lawrence et al., 1997; Cook et al., 1998; Kelly et al., 1998; Pearce et al., 2002a, b; Mortensen et al., 2003; Siikavuopio et al., 2006). FCR-G is strongly influenced by diet and temperature and this trend is seen in other studies, however most of the literature examined FCR-G in relation to temperature and other factors such as, season, stocking density, and water quality. Cuesta-Gomez and Sánchez-Saavedra (2017) was one study that soley examined the effect of diet on FCR-G where *S. purpuratus* fed a prepared diet at 18.5ºC produced a high FCR-G of 10.3-14.3 feed g gonad increase^{-1} g, which is much higher than either GSU or RSU in this present study which produced a FCR-G range of 0.001 ± 9.0^{E-4} – 0.27 ± 2.0^{E-3} feed g gonad increase^{-1} g.

Temperature is an important factor in determining FCR-G and a majority of the studies found examined this factor's effect. Siikavuopio et al. (2006) examined the FCR-G produced by GSU fed fresh kelp (*Laminaria hyperborea*) in both the winter and summer at 8, 12, and 14ºC, and found that FCR-G was significantly affected by both season and temperature, where the range of FCR-G was between 3.3-9.9 feed g gonad increase^{-1} g for all treatments which were all higher than the FCR-G produced in this study. The authors concluded that in the summer, FCR-G in GSU did not significantly vary between 6 and 12ºC. However, the GSU at 14ºC produced the highest FCR-G and, therefore, the worst feed conversion efficiency. In winter, the FCR-G at 4, 6, and 8ºC did not significantly differ, but 14ºC still produced the worst FCR-G (Siikavuopio et al., 2006). The authors suggest that when food supply is unlimited, temperature is the most important factor in determining the FCR-G (Siikavuopio et al., 2006). The optimum growth for GSU may be in the summer at 12ºC (FCR-G of 3.3 feed g

gonad increase^{-1} g) and at 6ºC in the winter (FCR-G of 3.1 feed g gonad increase^{-1} g) (Siikavuopio et al., 2006). Similar results are seen in Siikavuopio et al. (2012) where the GSU fed a prepared diet at 6, 10, 12, and 14ºC produced the lowest FCR-G at 10ºC, followed by 6ºC, and produced a high feed intake and high FCR at 12 and 14ºC. However, FCR-G can be greatly influenced by many other factors that were not tested in this study such as stocking density, urchin size, and carbon dioxide concentrations in the water. Christiansen and Siikavuopio (2007), fed GSU a prepared diet at low (2.5 kg m^2), medium (3.7 kg m^2), and high (7.3 kg m^2) stocking densities, and found that low densities produced a better FCR-G (3.09 feed g gonad increase^{-1} g) compared to high densities (3.32 feed g gonad increase^{-1} g). Siikavuopio et al. (2007a) found that smaller GSU (30-40 mm TD) had a significantly higher FCR-G than medium (45 mm TD) and large (50 mm TD) sized urchins, however, all the FCR-G (11.2-14.1 feed g gonad increase^{-1} g) were much higher than the ones produced in this study ($0.001 \pm 9.0^{E-4}$ – $0.27 \pm 2.0^{E-3}$ feed g gonad increase^{-1} g). Lastly, Siikavuopio et al. (2007b) found that GSU are very sensitive to CO_2 increases, and urchins in the control group produced an FCR-G of 3.98 feed g gonad increase^{-1} g, compared to 11.97 feed g gonad increase^{-1} g for GSU held at high CO_2 concentrations. Although stocking density, urchin size, and carbon dioxide concentrations were not analyzed in the current study, they are important to consider in a commercial gonad enhancement operation.

3.4.4. Faecal pellet length, width, size, shape, and settling velocity

For GSU, the effect of diet and the interaction of diet and temperature, was significant for all variables (faecal pellet length, width, size, shape, and settling velocity), indicating that temperature alone does not have a significant effect on faecal pellet characteristics (Table 3.3).

For RSU, the main temperature effect was not significant for any of the faecal pellet variables, while the main diet effect was significant for all of them (Tables 3.3, 3.4), but both diet and the interaction were significant for pellet shape and settling velocity (Table 3.3). For both GSU and RSU, sea urchins fed the either V10.1.9 or V10.1.10 are significantly smaller, rounder, and faster settling velocities than sea urchins fed kelp. Few studies have examined the effect of diet or temperature on GSU faecal pellet size and settling velocity (Sauchyn and Scheibling, 2009a, b; Orr et al., 2014; Filbee-Dexter and Wernberg, 2018), and no studies for RSU. Furthermore, only Orr et al. (2014) examined a diet other than kelp and was conducted in a laboratory setting rather than in the wild. Therefore, there is limited literature to compare our results to, but despite this, general comparable trends are seen in the few similar studies. No other studies to our knowledge compared different temperatures, but rather they examine the effect of different diets and pellet sizes. Orr et al. (2014) compared feeding Sablefish (*Anoploploma fimbria*) waste and M*acrocystis pyrifera* to GSU, and found that the urchin faecal pellets were easily distinguishable (colour and shape) by which diet was fed to the GSU. This dietary characteristic was seen is the present study as well for both GSU and RSU (Fig. 1). Sea urchins that were fed the prepared diets consistently had brown/beige and spherical faecal pellets, whereas, sea urchins fed kelp had green, elongated pellets which contained noticeable fragments of the kelp (Fig. 1). GSU fed both the Sablefish waste and *M. pyrifera* in Orr et al. (2014), produced smaller faecal pellets [L x W (mean ± SE): Sablefish waste diet: 1.68 ± 0.05 mm x 1.46 ± 0.06 mm; *M. pyrifera*: 3.21 ± 0.33 mm x 1.78 ± 0.08 mm) than what was produced in the current study (Table 3.1). However, this difference is likely a result of the different sized GSU used (the mean ± SE GSU wet weights used in Orr et al. (2014) was 41.1 ± 3.8 g compared to the wet weight of the GSU used in the present study at

week 9 which was 75.1 ± 10.8 g) and larger urchins tend to produce larger faecal pellets [also evident in the fact that the RSU in the present study produced larger pellets than the GSU (Table 3.1 and 3.2, respectively). The results of the present study are more comparable to Sauchyn and Scheibling (2009b) which used larger GSU (mean TD: 45-60 mm) similar to the GSU used in the current study (mean TD ± SE: 62.8 ± 6.1 mm), however, the GSU in Sauchyn and Scheibling (2009b) still produced smaller faecal pellets than the ones in the present study. The GSU were fed either clean *Saccharina latissimi*, encrusted *S. latissimi*, or *Codium fragile*, and mean diameter of the faecal pellets from all three diets was 2.48 mm (Sauchyn and Scheibling, 2009b), which was smaller than either pellet length or width for the GSU fed *N. luetkeana* in the present study (Table 3.1).

The shape of the faecal pellets was calculated as the Corey Shape Factor (CSF), where a value of 1 means the pellet is spherical, and anything less than that indicates an ellipsoidal pellet. GSU fed either V10.1.9 or V10.1.10 had significantly rounder pellets (*i.e.* CSF = 1) than GSU fed *N. luetkeana* at all three temperature (Table 3.3, Fig 2G). This is similarly seen in Orr et al. (2014) where GSU fed the Sablefish waste had a CSF of 0.8-1, and GSU fed *M. pyrifera* had a CSF of ~0.6, and in Sauchyn and Scheibling (2009b), GSU fed the different kelp diets had an average CSF of 0.76. Overall, GSU fed the prepared diets, had significantly smaller sized and rounder faecal pellets than GSU fed *N. luetkeana* at all three temperatures. Faecal pellet settling velocity also varied across the literature (Sauchyn and Scheibling, 2009a, b), however, the general trend observed and in the present study is that sea urchins fed a prepared diet and larger pellets had significantly faster settling velocities than urchins fed kelp or had smaller pellets. This is likely a result of the prepared diets and larger pellets being denser than pellets that are smaller in size or consisted of kelp. As previously mentioned, no

literature was found that examined RSU faecal pellet length, width, size, shape, or settling velocity, therefore we cannot directly compare our results to any other studies. However, the same general trends that are described above for GSU were observed with the RSU.

3.5. Conclusion

The general trends seen in IR, AE, FCR-G, and faecal pellet characteristics in the present research are generally consistent with the literature. The results of the present study are an important step in determining optimum dietary/temperature conditions for GSU and RSU gonad enhancement. Knowing IR and AE with different diets and at different temperatures, and how they contribute to FCR-G, helps to determine the optimal enhancement conditions for both GSUs and RSUs. Overall, the lowest FCR-G for GSUs and RSUs was obtained with feeding V10.1.9 at 12°C and V10.1.9 at 16°C, respectively, and these would be the optimal diet and temperature combinations for these species in terms of producing the most gonad with the least feed. Various studies (Christiansen and Siikavuopio, 2007; Siikavuopio et al., 2007a, b) have shown that many factors, however, such as dissolved oxygen, carbon dioxide concentration, stocking density, urchin size, and many more, can affect various gastrointestinal parameters, so further research would be needed to ensure the values in the present work are representative of large-scale land- or sea-based operations. However, further research would be needed to determine faecal pellet composition, such as, organic matter content.

The information obtained in this study is also useful in determining the potential environmental impact of a commercial sea-based gonad-enhancement operation. The results, combined with a specific farm's site parameters (such as bathymetry, current, depth, etc.) in a waste-deposition model (*e.g.* DEPOMOD) could produce a footprint of potential benthic

organic enrichment. The resultant model information could be used to ensure proper site placement and mitigation of any environmental harm sea urchin aquaculture could impose.

3.6. Literature Cited

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Table 3.1. Mean ± SE gastrointestinal parameters of the green sea urchin, *Strongylocentrotus droebachiensis,* in the different diet and temperature treatments throughout the 9-week experiment. n = 54.

	Dry-weight ingestion rate (feed g individual^{-1}g day^{-1})			Absorption efficiency (%)		
Temperature (°C) ± SD, n = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	$3.5^{E-3} \pm 4.9^{E-4}$	$2.3^{E-3} \pm 3.1^{E-4}$	$2.6^{E-3} \pm 2.7^{E-4}$	87.9 ± 1.9	64.8 ± 4.9	63.9 ± 4.5
12.4 ± 0.1	$4.2^{E-3} \pm 4.4^{E-4}$	$2.9^{E-3} \pm 2.3^{E-4}$	$3.1^{E-3} \pm 2.1^{E-4}$	88.0 ± 1.5	68.8 ± 2.9	70.4 ± 3.9
15.9 ± 0.1	$4.7^{E-3} \pm 5.1^{E-4}$	$3.5^{E-3} \pm 2.2^{E-4}$	$3.7^{E-3} \pm 1.8^{E-4}$	88.4 ± 2.1	66.8 ± 5.5	74.1 ± 3.9
	FCR – Gonads (feed g gonad increase^{-1}g)			Faecal pellet length (mm)		
Temperature (°C) ± SD, n = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	$5.1^{E-2} \pm 9.6^{E-3}$	$1.1^{E-2} \pm 1.4^{E-3}$	$2.1^{E-2} \pm 2.7^{E-3}$	3.5 ± 0.2	2.2 ± 0.1	1.9 ± 0.1
12.4 ± 0.1	$2.8^{E-2} \pm 3.6^{E-3}$	$1.0^{E-2} \pm 9.0^{E-4}$	$1.5^{E-2} \pm 1.9^{E-3}$	3.6 ± 0.2	2.2 ± 0.1	2.1 ± 0.1
15.9 ± 0.1	$2.6^{E-2} \pm 8.3^{E-2}$	$2.0^{E-2} \pm 1.6^{E-3}$	$2.7^{E-1} \pm 2.0^{E-3}$	3.4 ± 0.2	2.0 ± 0.1	$2.1 \pm 4.8^{E-2}$
	Faecal pellet width (mm)			Faecal pellet size		
Temperature (°C) ± SD, n = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	2.6 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	3.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
12.4 ± 0.1	2.7 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	3.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
15.9 ± 0.1	2.5 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.9 ± 0.1	$2.0 \pm 4.9^{E-2}$	2.0 ± 0.1
	Faecal pellet shape			Faecal pellet settling velocity (cm sec^{-1})		
Temperature (°C) ± SD, n = 12,374	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.8 ± 0.1	$0.9 \pm 1.2^{E-2}$	$1.0 \pm 2.6^{E-3}$	$1.0 \pm 3.2^{E-3}$	4.0 ± 0.5	12.4 ± 0.7	11.6 ± 0.8
12.4 ± 0.1	$0.9 \pm 1.5^{E-2}$	$1.0 \pm 6.7^{E-3}$	$1.0 \pm 5.1^{E-3}$	4.9 ± 0.4	11.3 ± 0.4	12.1 ± 0.3
15.9 ± 0.1	$0.9 \pm 1.8^{E-2}$	$1.0 \pm 5.9^{E-3}$	$1.0 \pm 6.2^{E-3}$	3.0 ± 0.2	10.6 ± 0.7	11.2 ± 0.5

Table 3.2. Mean ± SE gastrointestinal parameters of the red sea urchin, *Mesocentrotus franciscanus,* in the different diet and temperature treatments throughout the 12-week experiment. $n = 54$.

	Dry-weight ingestion rate (feed g individual^{-1}g day^{-1})			Absorption efficiency (%)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	$3.5^{E-3} \pm 3.1^{E-4}$	$1.4^{E-3} \pm 1.2^{E-4}$	$1.4^{E-3} \pm 1.2^{E-4}$	90.3 ± 1.6	79.1 ± 4.8	74.4 ± 4.9
12.4 ± 0.6	$4.7^{E-3} \pm 9.1^{E-5}$	$1.2^{E-3} \pm 1.6^{E-4}$	$1.3^{E-3} \pm 9.7^{E-5}$	90.5 ± 1.0	80.9 ± 3.3	75.1 ± 5.3
16.3 ± 0.7	$4.7^{E-3} \pm 1.7^{E-4}$	$1.1^{E-3} \pm 1.6^{E-4}$	$1.1^{E-3} \pm 1.4^{E-4}$	89.6 ± 1.6	92.2 ± 2.5	83.5 ± 5.9
	FCR – Gonads (feed g gonad increase^{-1}g)			Faecal pellet length (mm)		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	$1.2^{E-2} \pm 1.7^{E-3}$	$3.2^{E-3} \pm 3.6^{E-4}$	$3.0^{E-3} \pm 2.5^{E-4}$	3.8 ± 0.2	2.8 ± 0.1	2.7 ± 0.1
12.4 ± 0.6	$1.7^{E-2} \pm 8.7^{E-4}$	$2.4^{E-3} \pm 3.7^{E-4}$	$2.3^{E-3} \pm 2.0^{E-4}$	3.4 ± 0.1	2.9 ± 0.1	2.7 ± 0.1
16.3 ± 0.7	$1.5^{E-2} \pm 1.1^{E-3}$	$1.9^{E-3} \pm 2.8^{E-4}$	$3.1^{E-3} \pm 6.2^{E-4}$	3.7 ± 0.2	2.8 ± 0.1	3.0 ± 0.1
	Faecal pellet width (mm)			Faecal pellet size		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	3.1 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	3.4 ± 0.5	2.7 ± 0.5	2.6 ± 0.4
12.4 ± 0.6	2.7 ± 0.2	2.8 ± 0.1	2.6 ± 0.1	3.0 ± 0.5	2.8 ± 0.3	2.7 ± 0.4
16.3 ± 0.7	2.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	3.3 ± 0.5	2.8 ± 0.4	2.9 ± 0.5
	Faecal pellet shape			RSU Faecal pellet settling velocity (cm sec^{-1})		
Temperature (°C) ± SD, n = 17,674	*Nereocystis luetkeana*	V10.1.9	V10.1.10	*Nereocystis luetkeana*	V10.1.9	V10.1.10
7.7 ± 0.4	$0.9 \pm 1.3^{E-2}$	$1.0 \pm 4.5^{E-3}$	$1.0 \pm 6.0^{E-3}$	3.3 ± 0.2	12.2 ± 0.8	12.4 ± 0.6
12.4 ± 0.6	$0.9 \pm 1.3^{E-2}$	$1.0 \pm 5.3^{E-3}$	$1.0 \pm 6.3^{E-3}$	3.2 ± 0.4	15.0 ± 0.7	14.0 ± 0.6
16.3 ± 0.7	$0.9 \pm 1.4^{E-2}$	$1.0 \pm 3.9^{E-3}$	$1.0 \pm 7.5^{E-3}$	4.1 ± 0.5	14.2 ± 0.5	13.5 ± 0.7

Table 3.3. Results of non-parametric Kruskal-Wallis tests for gastrointestinal parameters of the experimental green (GSU; *Strongylocentrotus droebachiensis*) and red (RSU; *Mesocentrotus franciscanus*) sea urchins, comparing the different diet and temperature treatments throughout the experiment. Sources of variation are diet (D, fixed factor), temperature (T, fixed factor), and the interaction (D x T).

	GSU Dry-weight ingestion rate (feed g urchin^{-1}g day^{-1})			GSU Arcsine absorption efficiency (%)		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	10.6	2	**0.0049**	58.2	2	**<0.0001**
T	18.5	2	**<0.0001**	1.7	2	0.4341
D x T	30.2	8	**0.0002**	61.0	8	**<0.0001**
	GSU Ln FCR – Gonads (feed g gonad increase^{-1}g)			GSU Faecal pellet length (mm)		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	60.9	2	**<0.0001**	62.5	2	**<0.0001**
T	28.1	2	**<0.0001**	0.76	2	0.6822
D x T	90.1	8	**<0.0001**	65.2	8	**<0.0001**
	GSU Faecal pellet width (mm)			GSU Faecal pellet size		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	43.9	2	**<0.0001**	57.2	2	**<0.0001**
T	1.9	2	0.3728	0.9	2	0.6424
D x T	47.2	8	**<0.0001**	59.7	8	**<0.0001**
	GSU Faecal pellet shape			GSU Faecal pellet settling velocity (cm sec^{-1})		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	64.0	2	**<0.0001**	68.9	2	**<0.0001**
T	0.7	2	0.7122	3.1	2	0.2072
D x T	67.6	8	**<0.0001**	74.5	8	**<0.0001**
	RSU Dry-weight ingestion rate (feed g urchin^{-1}g day^{-1})			RSU Arcsine absorption efficiency (%)		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	97.3	2	**<0.0001**	2.4	2	0.3057
T	0.1	2	0.9395	10.5	2	**0.0052**
D x T	102.7	8	**<0.0001**	19.9	8	0.0612
	RSU FCR – Gonads (feed g gonad increase^{-1}g)			RSU Faecal pellet shape		
Source	ChiSquare	df	*P* > ChiSq	ChiSquare	df	*P* > ChiSq
D	98.4	2	**<0.0001**	50.4	2	**<0.0001**
T	0.5	2	0.7807	0.2	2	0.9051
D x T	104.8	8	**<0.0001**	55.3	8	**<0.0001**
	RSU Faecal pellet settling velocity (cm sec^{-1})					
Source	ChiSquare	df	*P* > ChiSq			
D	71.4	2	**<0.0001**			
T	4.1	2	0.1311			
D x T	78.3	8	**<0.0001**			

Values in bold are significant at $P \leq 0.05$. $n = 6$.

Table 3.4. Results of separate two-way ANOVAs for faecal pellet length, width, and size for the experimental red sea urchins, *Mesocentrotus franciscanus,* comparing the different diet and temperature treatments throughout the experiment. Sources of variation are diet (D), temperature (T), the interaction (D x T), and error.

	Log Faecal pellet length (mm)				Faecal pellet width (mm)			
Source	df	SS	*F* ratio	*P* value	df	SS	*F* ratio	*P* value
D	2	0.3	29.7	**<0.0001**	2	1.8	4.7	**0.0116**
T	2	5.9^{E-3}	0.6	0.5372	2	0.3	0.8	0.4277
D x T	4	2.1^{E-2}	1.1	0.3439	4	1.5	1.8	0.1223
Error	99	0.5			99	19.2		

	Faecal pellet size			
Source	df	SS	*F* ratio	*P* value
D	2	6.3	15.8	**<0.0001**
T	2	0.3	0.8	0.4248
D x T	4	1.3	1.6	0.1681
Error	99	19.8		

Values in bold are significant at $P \leq 0.05$. $n = 6$.

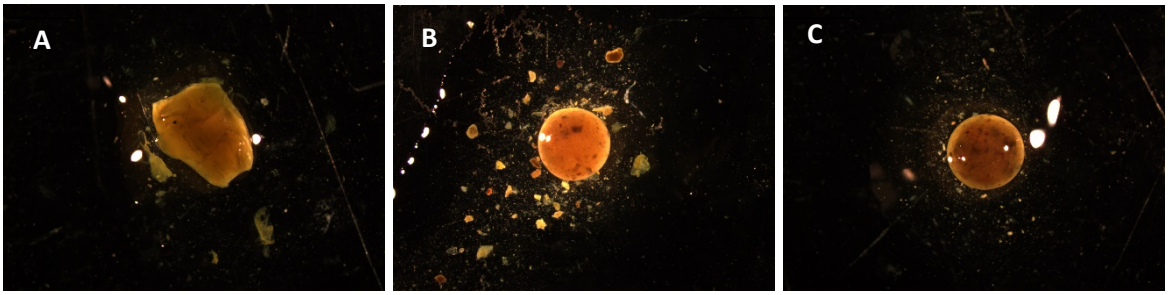
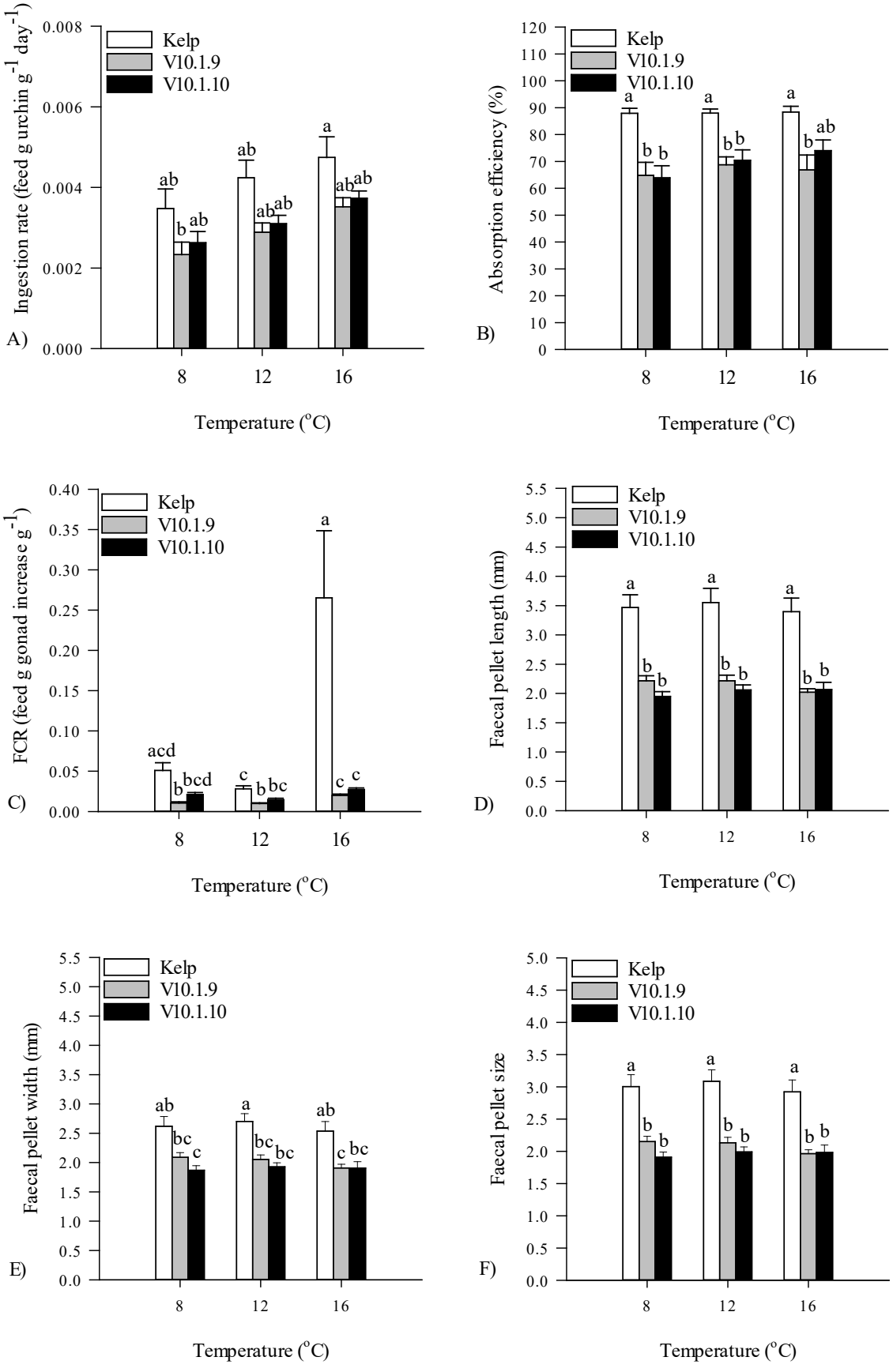


Figure 3.1. Example of faecal pellets produced by sea urchins fed (A) bull kelp (*Nereocystis luetkeana*), (B) prepared diet: V10.1.9, and (C) prepared diet: V10.1.10.


A)
Ingestion rate (feed g urchin g-1 day-1)
Kelp
V10.1.9
V10.1.10
Temperature (°C)
B)
Absorption efficiency (%)
C)
FCR (feed g gonad increase g-1)
D)
Faecal pellet length (mm)
E)
Faecal pellet width (mm)
F)
Faecal pellet size

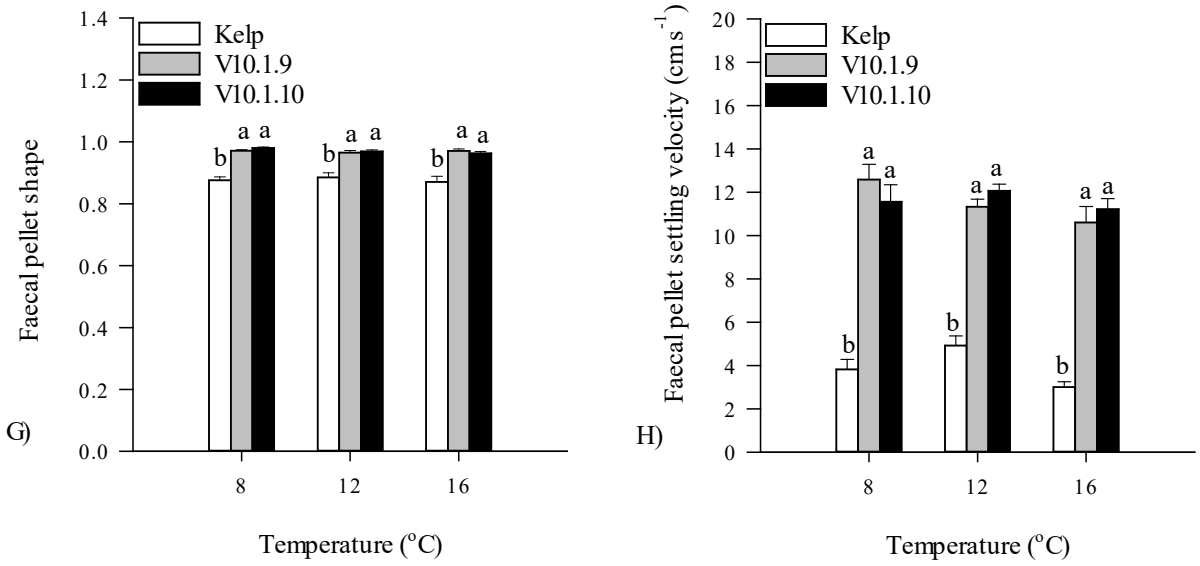
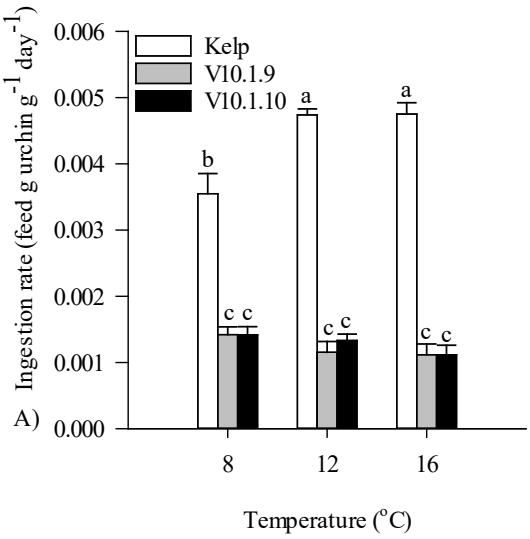
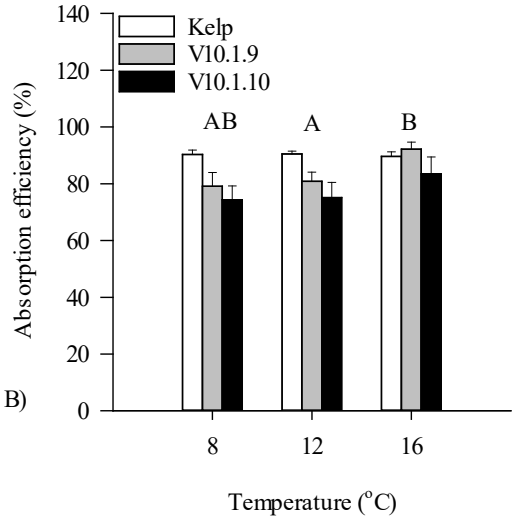
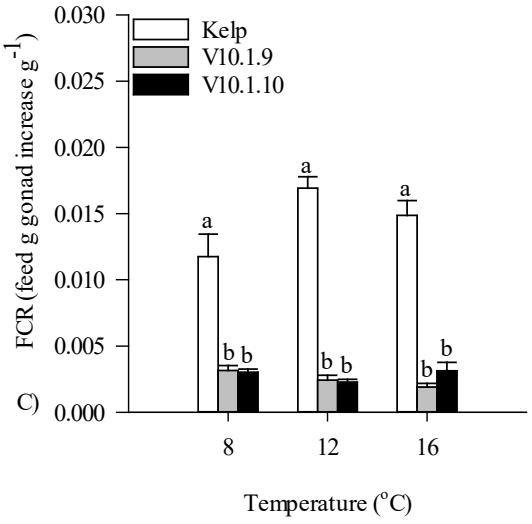
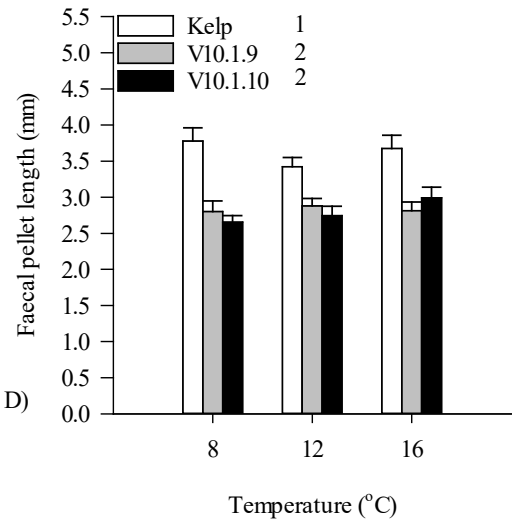


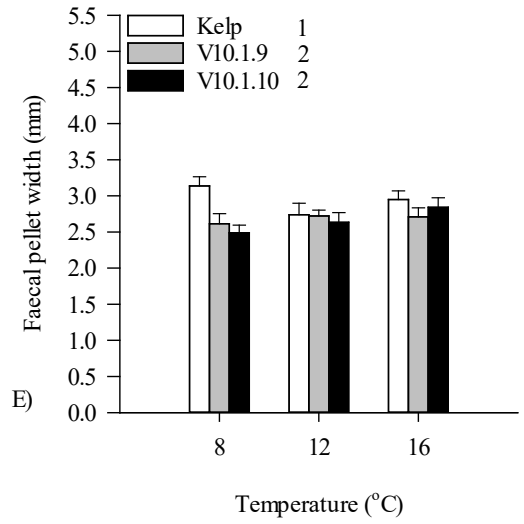
Figure 3.2. Mean ± SE gastrointestinal parameters for GSU (*Strongylocentrotus droebachiensis*) at the end of the experiment (week 9) for the dietary and temperature treatments. A non-parametric Kruskal-Wallis test and pairwise comparison done with Steel-Dwass All Pairs test was used for all variables. Different numbers beside diet, and capital letters above bars indicate a significantly different diet and temperature effect ($P \leq 0.05$). Lower case letters above the bars indicates a significantly different interaction of the diet and temperature effect ($P \leq 0.05$). $n = 6$.

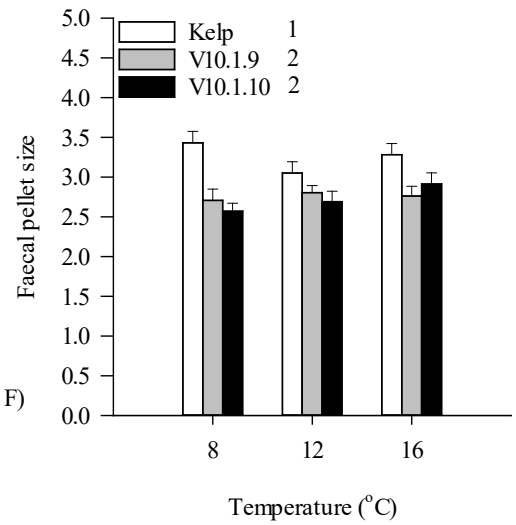

Kelp
V10.1.9
V10.1.10
Ingestion rate (feed g urchin g -1 day -1)
0.006
0.005
0.004
0.003
0.002
0.001
0.000
b
a
a
c c
c c
c c
A)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
Absorption efficiency (%)
140
120
100
80
60
40
20
0
AB
A
B
B)
8
12
16
Temperature (°C)


Kelp
V10.1.9
V10.1.10
FCR (feed g gonad increase g -1)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
a
a
a
b b
b b
b
C)
8
12
16
Temperature (°C)


Kelp 1
V10.1.9 2
V10.1.10 2
Faecal pellet length (mm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
D)
8
12
16
Temperature (°C)


Kelp 1
V10.1.9 2
V10.1.10 2
Faecal pellet width (mm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
E)
8
12
16
Temperature (°C)


Kelp 1
V10.1.9 2
V10.1.10 2
Faecal pellet size
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
F)
8
12
16
Temperature (°C)

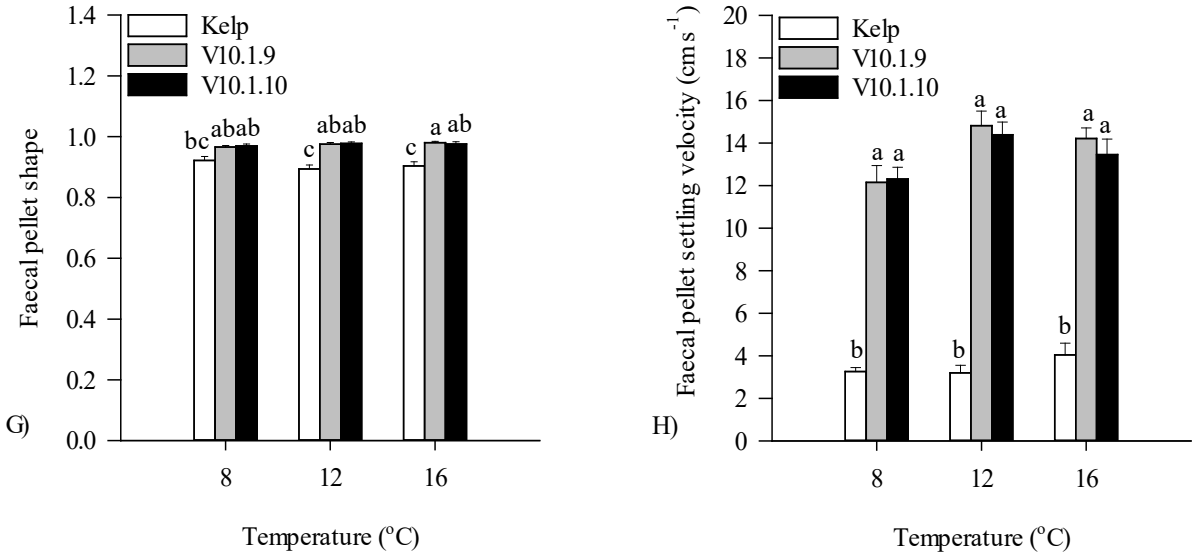


Figure 3.3. Mean ± SE gastrointestinal parameters for RSU (*Mesocentrotus franciscanus*) at the end of the experiment (week 12) for the dietary and temperature treatments. A non-parametric Kruskal-Wallis test and pairwise comparison done with Steel-Dwass All Pairs test was used for all variables (except for faecal pellet length, width, and size, where a two-way ANOVA was used and Tukey's HSD for all pair-wise comparisons). Different numbers beside diet, and capital letters above bars indicate a significantly different diet and temperature effect ($P \leq 0.05$). Lower case letters above the bars indicates a significantly different interaction of the diet and temperature effect ($P \leq 0.05$). $n = 6$.

Chapter Four:

Summary of results and implications for sea urchin aquaculture

The objective of this study was to examine the interactive effects of feed type and temperature on gonad enhancement in the green (*Strongylocentrotus droebachiensis*) and red (*Mesocentrotus franciscanus*) sea urchins, using two newly formulated feeds (Urchinomics, V10.1.9 and V10.1.10) and a kelp diet (*Nereocystis luetkeana*) at three different temperatures (8, 12, and 16°C) and to assess potential environmental impacts of this new industry. The following questions were investigated:

i. Is there an optimal diet and temperature combination for both green and red sea urchins that will produce high gonad yields and market quality product in 12 weeks?
ii. Does gonad quality differ between male and female sea urchins?
iii. Do different diet and temperature combinations effect ingestion rates, absorption efficiencies, feed conversion ratios, and faecal production in the green and red sea urchins?
iv. Do different diets and temperatures effect faecal pellet size, shape, and settling velocity and do they have implications for potential environmental impact of a commercial sea urchin gonad enhancement operation.

Using two separate feeding trials, one for green sea urchins (GSU) and one for red sea urchins (RSU), somatic parameters, gonad yield and quality, ingestion rate, absorption efficiency, feed conversion ratios, and faecal pellet parameters were examined. Based on the published literature of previous sea urchin gonad enhancement trials and results from this study, optimal conditions for both GSU and RSU gonad enhancement will be discussed.

Overall, the results from the present study indicate that the two newly formulated diets can provide sufficient nutrition and pigment to achieve market yields and quality in both urchin species in a land-based system. The highest gonad yields were achieved with GSU fed V10.1.9 at 8 and 12°C, although yields in all treatments were well above the market minimum of 10–15%. GSU had the best gonad colour with V10.1.10 at 16°C, although the colour with either prepared diet was better than all kelp treatments (except V10.1.9 at 8°C). V10.1.10 also produced the highest overall preference for taste at 12°C and this diet and temperature combination may be the best for GSU. RSU had the highest yields achieved with V10.1.10 at all temperatures and V10.1.9 at 16°C and the same treatments achieved the best colour results. V10.1.9 also produced the preferred flavour in RSU gonads and, therefore, RSU fed V10.1.9 at 16ºC may be the best for gonad enhancement in this species. Colour of the GSU gonads was also significantly affected by the sex of the sea urchin. At the time of sampling GSU were in advanced stages of gonad maturation and were leaking gametes, which made it relatively easy to sex the sea urchins. The RSU, however, have a spawning season later in the year [late spring and early summer (Bernard, 1977)] and were not leaking gametes during the sampling date, therefore they could not be easily sexed. Overall, the female GSU at week 9 had lower L* ratings, higher a* and b* ratings, and a lower degree of colour difference with a target colour (*i.e.* better colour) than GSU males.

The effect of diet and temperature was also examined in terms of dry ingestion rate (IR), absorption efficiency (AE), feed conversion ratio in terms of gonad production (FCR-G), and faecal pellet size, shape, and settling velocity. Overall, both GSU and RSU had significantly higher ingestion rates when fed kelp, compared to being fed either prepared diet. Ingestion rates also tended to increase with temperature until a thermal tolerance was reached for the particular species. In the present study, GSU at 16°C had significantly higher IR than GSU at 8°C, although

neither significantly differed from 12°C while RSU fed kelp at 12 or 16°C had significantly higher IR than those fed kelp at 8°C (temperature did not, however, affect the IR for the RSU fed either V10.1.9 or V10.1.10 diets). The same general trend presented for AE for GSU where diet, temperature, and the interaction effect were all significant. GSU fed kelp at all three temperature treatments had significantly higher AE than GSU fed either prepared diet, with the one exception of GSU fed V10.1.10 at 16°C, which did not significantly differ from any other treatments. For RSU, the diet effect was not significant, however, temperature and the interaction were, although overall, RSU fed kelp produced higher AE than either prepared diet. RSU in the present study had higher AE at 16°C than at 12°C, but interestingly neither significantly differed from 8°C. Ultimately this all contributes to the feed conversion ratio (FCR-G), to know how much feed will be needed to produce one gram of gonad tissue and to therefore have an efficient gonad enhancement operation. The lowest FCR-G was obtained with GSU fed V10.1.9 at 12°C and V10.1.9 at 16°C for RSU.

Determining optimal conditions for gonad enhancement would therefore be dependent on the variable and species of interest. GSU fed V10.1.9. at 8 and 12°C produced the highest gonad yields (mean ± SE: 29.4 ± 1.1% and 29.4 ± 1.5%, respectively) while V10.1.9 at 12°C also had the highest gonad yield increase per week (mean ± SE: 2.2 ± 0.2%) and the lowest FCR-G (mean ± SE: $1.0^{E-2} \pm 9.0^{E-4}$ feed g gonad increase g^{-1}). GSU fed V10.10 at 12°C, however, produced the most preferred gonad taste, gonad yields still above market minimum (mean ± SE: 25.6 ± 1.5%), and the third lowest FCR (mean ± SE: $1.5^{E-2} \pm 1.9^{E-3}$ feed g gonad increase g^{-1}), while GSU fed V10.1.10 at 16°C had the best colour (mean degree of colour difference ± SE: 6.0 ± 0.9). Therefore, it can be suggested that optimal conditions moving forward for GSU would be feeding V10.1.10 at 12°C. For RSU, those fed V10.1.10 produced the highest gonad yields at

12°C (mean ± SE: 12.7 ± 1.5%) and the best colour at 16°C (mean degree of colour difference ± SE: 30.3 ± 3.1), while RSU fed V10.1.9 at 16°C produced the second highest gonad yields (mean ± SE: 11.0 ± 0.4%), the lowest FCR-G (mean ± SE: $1.9^{E-3} \pm 2.8^{E-4}$ feed g gonad increase g^{-1}), the most preferred gonad taste, and a low degree of colour difference (mean ± SE: 32.3 ± 2.1). Therefore, it can be suggested that optimal conditions moving forward for RSU would be feeding V10.1.9 at 16°C.

Aquaculture is a fast-growing industry worldwide as market demands for seafood continue to grow with an increasing global population. With this growth, however, there are concerns about the impact this industry has on the environment and it is therefore critical to examine the potential environmental impacts of new aquaculture techniques/species – this can actually be required for regulation and management purposes (*e.g.* Cromey et al., 2002; Chamberlain et al., 2005). One of the many concerns with sea-based fed aquaculture is the potential impact of nutrient loading from the faeces and uneaten feed that enters the water column and ultimately settles on the seabed (Cromey et al., 1998; Corner et al., 2006). Nutrient loading increases ammonia concentrations, bacteria, biological oxygen demand, and nitrogen in the water column and sediment, all of which can be harmful to benthic communities (Panchang et al., 1997; Callier et al., 2006). In a sea-based system, waste deposition is highly variable and can depend on a number of environmental conditions such as hydrodynamics, bathymetry, weather, and benthic community structure. It is also important to understand faecal production and faecal pellet characteristics (size, shape, settling velocity) to better understand the benthic loading on a commercial scale.

For GSU, the effect of diet and the interaction of diet and temperature, was significant for all variables (faecal pellet length, width, size, shape, and settling velocity), indicating that

temperature alone does not have a significant effect on faecal pellet characteristics. For RSU, the main temperature effect was not significant for any of the faecal pellet variables, while the main diet effect was significant for all of them, but both diet and the interaction were significant for pellet shape and settling velocity. For both GSU and RSU, sea urchins fed the either V10.1.9 or V10.1.10 are significantly smaller, rounder, and faster settling velocities than sea urchins fed kelp. While a waste-deposition model was not specifically used in this study, the results obtained can be used when a specific farm location is selected to examine potential benthic organic loading. Faecal pellet size and settling velocity will allow for a waste deposition footprint to be produced and for the development of potential mitigative strategies to prevent negative impacts. While this information would be most useful for an ocean-based commercial farm, it would also be of benefit for a land-based operation to be able to have proper waste removal procedures.

Sea urchin aquaculture is a promising industry, however, some more research is needed to further the commercial viability. In terms of diet, while the both prepared diets produced desirable colour and taste, both could be tweaked further to produce higher market quality gonads. Optimal holding conditions for both GSU and RSU need to be examined further as well to determine optimal densities and water flow on gonad enhancement in both land- and sea-based systems, as well as tank designs that facilitate faecal material removal. Lastly, pilot scale trials should be initiated to be able to conduct an economic and market analysis, as well as allow for waste deposition research if occurring in a sea-based system.

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