A pilot study on gonad enhancement in kelp-fed Newfoundland green sea urchin, *Strongylocentrotus droebachiensis*

by

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ABSTRACT

Sea urchin gonads, also known as “roe” or “uni”, are a highly prized delicacy in Asian and European seafood markets. Green sea urchin, *Strongylocentrotus droebachiensis*, produces one of the finest and most widely marketed roe in Asia, including top markets in Japan and Korea. Green sea urchin is abundant throughout eastern Newfoundland and holds a large potential for sea urchin roe enhancement and aquaculture, however no such industry has been developed despite initial research attempts in the late 1990s. In order to examine the use of locally abundant kelp as a feed option for sea urchin gonad enhancement, a 34-week experiment was carried out during which we maintained groups of green sea urchins from southeastern Newfoundland in flow-through tanks at ambient sea temperature and fed ad libitum with three locally abundant kelp diets: (1) kelp combo (*Alaria esculenta* and *Laminaria digitata*); (2) *L. digitata*; and (3) *Agarum clathratum*. Gonadosomatic index (GSI), gonad colour and gonad texture were assessed after 12 and 34 wk of feeding, and gonad taste after 34 wk. Feeding sea urchins a kelp combo or *L. digitata* resulted in the highest GSI and best quality gonads at 34 wk, however only *L. digitata* yielded market-quality roe at 12 wk. Although GSI was higher after 34 wk, gonad quality did not improve with the longer feeding duration and therefore results suggest that 12 wk or fewer may be enough to produce market-quality roe, therefore potentially lowering production costs. Collectively, results suggest that green sea urchins fed locally abundant and easily accessible kelp species produce large volumes of high-quality roe, however, determining the best diet to further improve roe colour and texture requires further research.

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CO-AUTHORSHIP STATEMENT

The work described in the present thesis was conducted by Samantha C. Trueman with guidance from Patrick Gagnon, Paul Snelgrove, and Iain McGaw. Samantha C. Trueman was responsible for field and laboratory data collection and analysis (with assistance from Patrick Gagnon) and contributed to modifications brought to the original design by Patrick Gagnon. All chapters were written by Samantha C. Trueman with intellectual and editorial input by Patrick Gagnon. Any publication in the primary literature resulting from work in the present thesis and from complementary work not presented will be co-authored by Samantha C. Trueman and Patrick Gagnon.

CHAPTER I

GENERAL INTRODUCTION

As a country surrounded by three major oceans (Atlantic, Pacific, and Arctic), Canada's economy relies heavily on its surrounding marine resources. Canadian seafood production (commercial fishing and aquaculture combined) exceeds ~$4 billion in gross earnings annually (Government of Canada 2018) and employs up to ~76,000 Canadians. The top exported seafood items include American lobster (*Homarus americanus*), snow crab (*Chionoecetes opilio*), Atlantic salmon (*Salmo salar*), sockeye salmon (*Oncorhynchus nerka*), and northern shrimp (*Pandalus borealis*), with a combined annual value estimated at ~$7 billion (Government of Canada 2018).

Atlantic Canada (including New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador), dominate Canada's seafood industry, with ~$3 billion of annual earnings (Government of Canada 2018). Indeed, Atlantic Canada depends heavily on the commercial fishing and aquaculture industries, in that the rural coastal communities that dominate the region rely on this income for their livelihood (Neis & Ommer 2014). In Newfoundland and Labrador specifically, the commercial fishing industry remains the major source of employment and wealth generation for rural communities, despite increasing instability in the province's fishery sectors (Neis & Ommer 2014).

Over recent years, Newfoundland fisheries have steadily declined, but the collapse of the groundfish fishery in the early 1990s produced the largest economic impact in the history of the province (Neis & Ommer 2014). Historically, Atlantic cod (*Gadus morhua*) was the most important fish species to Newfoundland economically, however, overexploitation of wild stocks led to the collapse of the northern population of the species and resulted in a government-imposed moratorium on the fishery in 1992 (Milich 1999; García *et al.* 2018). The fishery sector subsequently shifted to macroinvertebrates, including northern shrimp and snow crab, which both provided hope for the provincial economy following the cod decline (Schrank 2005). Despite initial prosperity, northern shrimp and snow crab stocks have both declined steadily in recent years; northern shrimp populations decreased dramatically throughout the mid 2000s (Mather 2013; Le Corre *et al.* 2019), and the Newfoundland snow crab fishery, once the largest snow crab fishery in the world, now faces a potential downturn similar to the collapse of the cod fishery (Mullowney *et al.* 2014).

Despite an unsteady fishery sector, the aquaculture industry in Newfoundland has expanded significantly since 2003, with a primary focus on blue mussel, Atlantic salmon, steel head trout, and Atlantic cod; this industry has provided an alternative means of economic renewal for rural coastal communities (DFA 2015a 2015b; NAIA 2015; Surprenant 2010). Since the Newfoundland and Labrador Aquaculture Strategic Plan was first introduced in 2000, total production and value from the industry has increased by over 1000%, bringing in ~$197 million to the province in 2013 (DFA 2015b). Continued growth in aquaculture in Newfoundland, and throughout Canada, requires further diversification of species used in aquaculture (Chopin 2015). The success of aquaculture in Newfoundland offers tremendous potential for expansion of the industry into other species, while also expanding aquaculture production to new regions of the province beyond the current concentration of the industry in the southern Connaigre Peninsula, and northern Notre Dame Bay (DFA 2015b).

Sea urchins represent one type of alternate marine species that is presently underutilised and could increase diversity within Canada's aquaculture sector. Sea urchins are harvested globally for their gonads, more commonly referred to as "roe" or "uni". Some 90% of urchin roe is sold to Japan, however, several other cultures around the world have consumed sea urchin roe historically, including those in Polynesia, Europe, and Chile (Andrew *et al.* 2002). Sea urchin roe is most commonly consumed on top of sushi, and the Japanese market will pay up to ¥12,000 (~$150

CAD) for a single tray of high quality roe, compared to ¥3,000-4,000 (~$30-50 CAD) for a tray of lower quality roe used in lower end food products or as a food additive (Explorations Unlimited Inc 2006). Presently, sea urchin aquaculture accounts for <0.01% of total sea urchins sold around the globe, however, many species show significant potential for successful aquaculture (James *et al.* 2016; Stefánsson *et al.* 2017). Furthermore, Canada has yet to establish a commercial-scale sea urchin aquaculture industry (Pearce & Robinson 2010).

Canada has already demonstrated successful fisheries for two sea urchin species: (1) green sea urchin [*Strongylocentrotus droebachiensis*]; and (2) red sea urchin [*Strongylocentrotus francisacanus*] (Pearce & Robinson, 2010). Green sea urchin has been the primary focal species in Canada because it is endemic to six of the ten provinces, it can grow to market size in under two years, it is amenable to culture in high-density cages, and it grows well on natural (i.e. *Laminaria* spp.) or prepared diets (Pearce & Robinson 2010). Green sea urchin roe is one of the most valued on the Japanese and Korean markets, even though it is not locally available in either country; the market therefore depends completely on imports from other countries (Andrew *et al.* 2002; Stefánsson *et al.* 2017).

The majority of harvest for *S. droebachiensis* in Canada occurs in British Columbia and New Brunswick, with a smaller harvest in Newfoundland, although virtually all of Newfoundland's catch is either sent to New Brunswick or Maine, USA, for processing (Pisces Consulting Limited 2014; Stefánsson *et al.* 2017). Currently, the small fisheries established in Trinity, Bonavista, and Notre Dame Bays in Newfoundland are highly seasonal and labour intensive, like most sea urchin fisheries elsewhere in the world, because divers must harvest urchins by hand (Pisces Consulting Limited 2014). Total roe landings from the province peaked in 2004 but declined until 2011 when landings slowly began to rebound (Pisces Consulting Limited 2014).

The reproductive cycle of *S. droebachiensis* in Newfoundland largely drives the seasonality of the fishery (Himmelman 1978) but the general association of urchin populations with sparse barrens creates challenges in finding enough sea urchins with gonads to warrant harvesting on a commercial scale (Himmelman & Steele 1971; Pisces Consulting Limited 2014). Large sea urchin barrens, areas of rocky bottom with abundant sea urchins, but devoid of large macroalgae, characterize the Newfoundland coast (Himmelman 1984; Frey & Gagnon 2016), and the lack of nutritional food results in sea urchins found in these barrens to have poor gonad development (Himmelman & Steele 1971). Sea urchin divers must seek out areas with abundant kelp, and test sea urchins prior to undertaking a commercial harvest in a given area (Pisces Consulting Limited 2014). Even within kelp beds, market quality gonads usually only occur close to spawning periods (typically January to April; Himmelman 1978; Scheibling & Hatcher 2007), resulting in a brief harvest season. An economically feasible harvest in Newfoundland requires a minimum gonad yield (weight of roe as a percentage of total body weight) of 8% (Pisces Consulting Limited 2014), however, a yield of 16 – 18% viable roe results in top dollar on the Japanese market (Explorations Unlimited Inc 2006).

The inconsistency of roe yield and quality in wild populations of *S. droebachiensis* in Newfoundland limits the potential growth of the fishery beyond its current levels ($4-6.6 million per year; Pisces Consulting Limited 2014). This small-scale harvest has also decreased sea urchin size, specifically in Bonavista, Placentia, and Trinity Bays where the main harvest occurs (Pisces Consulting Limited 2014), pointing to a need for further research to better understand how to sustainably utilize this valuable natural resource in Newfoundland. Sea urchins from barrens hold a remarkable potential for gonad enhancement, which Pearce & Robinson (2010) define as the process by which licensed harvesters can collect wild-harvested sea urchins and feed them in captivity in order to bring gonads to market quality. This strategy differs from hatchery-based aquaculture in using wild caught individuals rather than growing sea urchins from embryo to market size (Pearce & Robinson 2010). A gonad enhancement approach would allow harvesters to exploit all potential wild sea urchin populations, rather than just sea urchins near kelp beds, while potentially enabling re-establishment of biodiverse kelp beds (FilbeeDexter & Scheibling 2014). It could also support year-round harvesting if gonad enhancement can be achieved regardless of season. Development of better gonad enhancement techniques would benefit the aquaculture sector by providing further insight into optimal diets for top sea urchin roe quality.

Research on gonad enhancement on multiple sea urchin species has increased in recent years with several countries trying to break into the lucrative Japanese market. For example, New Zealand currently harvests the endemic kina sea urchin (*Evechinus chloroticus*) strictly for domestic markets because the roe does not meet Japan's high market standards. Nevertheless, the potential to break into international markets has inspired several studies on gonad enhancement of the latter species to improve market quality attributes (Woods *et al.* 2008; James & Heath 2008a, b; Phillips *et al.* 2009). Europe has achieved successful gonad enhancement in the common European sea urchin (*Paracentrotus lividus*), the most harvested sea urchin species in the region, using both artificial and natural diets, as well as vegetable discards (Carboni *et al.* 2015; Vizzini *et al.* 2015; Prato *et al.* 2018). Additionally, several studies have investigated gonad enhancement in several *Strongylocentrotus* spp. primarily in China, British Columbia (Canada), and California (USA) (McBride *et al.* 2004; Azad *et al.* 2011; Luo *et al.* 2014; Cuesta-Gomez & Sánchez-Saavedra 2014; Foster *et al.* 2015).

Studies in several locations, particularly in Maine, USA, have demonstrated the success of *S. droebachiensis* gonad enhancement as a means to sustain demand despite a declining fishery (Johnson *et al.* 2013). Research in the late 1990s demonstrated improved gonad yield and colour in *S. droebachiensis* fed natural kelp diets when held within cages attached to the sea floor (Vadas Sr. *et al.* 2000). Around the same time, research in New Brunswick investigated mixtures of different prepared diets and their effects on gonad yield and quality (Pearce *et al.* 2002, 2003, 2004). Prepared diets were effective in gonad enhancement of *S. droebachiensis*, however, taste quality of the gonads in urchins fed artificial diets was inferior to gonads from urchins fed natural kelp diets (Pearce *et al.* 2004).

Feeding trials conducted in western Newfoundland throughout the 1990s with *S. droebachiensis* indicated successful gonad enhancement when fed kelp of *Laminaria* spp. whether in land-based aquaria or in underwater cages; gonad yields of >20% were achieved in 25 weeks (Cuthbert *et al.* 1995; Hooper *et al.* 1996, 1997). However, since these trials, no further studies have addressed potential gonad enhancement of *S. droebachiensis* in Newfoundland, despite the initial success (Pearce & Robinson 2010).

The potential for gonad enhancement in green sea urchins of Newfoundland requires further feeding trials to determine the optimal protocols for future gonad enhancement and aquaculture work in the province. This thesis explores the relationship between natural kelp diets and gonad enhancement in *S. droebachiensis* from southeastern Newfoundland in order to identify a suitable natural kelp diet to produce market quality gonads from *S. droebachiensis* over different times of the reproductive cycle. Chapter II presents a 34-week experimental study on green sea urchins fed three different natural kelp diets and an assessment of the effects of each diet on gonadosomatic index (GSI), gonad colour, gonad structural segmentation and textural appearance, and gonad taste. Chapter III summarizes the study's main findings and their contribution to advancing knowledge about *S. droebachiensis* gonad enhancement in eastern Newfoundland, while identifying future research directions.

CHAPTER II

Suitability of natural kelp feeds for production of marketable gonads of green sea urchins (*Strongylocentrotus droebachiensis*) from eastern Newfoundland

2.1 INTRODUCTION

Sea urchin gonads, also known as “roe” or “uni”, are a highly prized delicacy in Asian, and more recently, European sea food markets, with global demand peaking at ~120,000 tonnes of urchins in 1995 (Andrew *et al.* 2002; Botsford *et al.* 2004). Since 1995, however, sea urchin populations around the world have declined, in part because of the high demand for roe, with annual landings in recent years averaging ~75,000 tonnes (Stefánsson *et al.* 2017). Without appropriate management strategies, urchin stocks will likely continue to decline, which could force the closure of multiple sea urchin fisheries around the world (Andrew *et al.* 2002; Anderson *et al.* 2011; Johnson *et al.* 2013). The spectre of a global collapse in wild sea urchin stocks has further motivated the development of approaches to optimize the use of natural sea urchin resources, including ways to produce high volumes of high-quality roe to limit sea urchin extraction from natural habitats (Foster *et al.* 2015; Takagi *et al.* 2017).

The green sea urchin, *Strongylocentrotus droebachiensis*, produces one of the finest and most widely marketed roe in Asia, including top markets in Japan and Korea (Andrew *et al.* 2002; Stefánsson *et al.* 2017). Because the species is not locally available, Japan imports live or processed green sea urchins from countries where they abound, typically Canada and the USA (Pisces Consulting Limited 2014). Despite the world economic crisis, the aquaculture sector in Newfoundland and Labrador (NL, eastern Canada) has grown steadily since 2003, with production focussed primarily on blue mussel, Atlantic salmon, steelhead trout, and Atlantic cod (DFA 2015a, 2015b; NAIA 2015). The province’s aquaculture industry is eager to explore opportunities to diversify the range of seafood products it produces and distributes on regional, national, and international markets (NAIA 2015). With densities of up to 300 individuals m^{-2}, green sea urchin is the dominant macroinvertebrate in shallow rocky habitats along the extensive coast of NL

(Himmelman 1984; Adey & Hayek 2011; Frey & Gagnon 2015), and therefore offers a large potential for the province from a fishery and aquaculture perspective. A green sea urchin fishery entirely dependent on wild harvest (manual collection) expanded in NL during the 1990s (Hooper *et al.* 1997; Pisces Consulting Limited 2014). However, success was, and remains limited by variable roe yield and quality, the only method of collection (by hand) imposed by government regulation, and the various logistical challenges of collecting sea urchins when acceptable gonad content and favourable sea conditions coincide. The provincial sea urchin fishery operates from November to April (depending on fishing zones), when roe content is high but sea conditions typically deteriorate (Hooper *et al.* 1993; Hooper *et al.* 1997; Pisces Consulting Limited 2014). More practical approaches to quick and consistent production of high-quality green sea urchin roe must be developed to help the province capitalize sustainably on an abundant marine resource.

Feeding trials in the 1990s suggested acceptable gonad yield and quality in green sea urchins from western Newfoundland fed during 25 weeks with two locally available kelp species: *Laminaria saccharina* and *L. digitata* (Cuthbert *et al.* 1995; Hooper *et al.* 1996). Both kelps produced large amounts of high quality roe, however, testing of other kelp (*Alaria esculenta* and *Agarum clathratum*) and non-kelp (*Ascophyllum nodosum* and *Fucus vesiculosus*) species yielded insufficient amounts of roe, or roe with improper or inconsistent quality (Cuthbert *et al.* 1995; Hooper *et al.* 1996). Lemire & Himmelman (1996) further demonstrated higher somatic and gonadic growth in green sea urchins from the northern Gulf of St. Lawrence fed during 18 weeks with *L. digitata*, compared to sea urchins fed over the same duration with *A. esculenta* and *A. clathratum*. Sea urchins fed *A. clathratum* consumed very low amounts and developed small, dark gonads (Cuthbert *et al.* 1995; Hooper *et al.* 1996; Lemire & Himmelman 1996). Together, these studies indicate which kelp species might produce the best roe in green sea urchins from eastern Canada. However, they were not specifically designed to achieve the procedural and temporal resolution needed to determine, and eventually refine, gonad suitability based on established market quality standards. Previous work addressed critical sea urchin gonad quality parameters such as colour, segmentation, texture, and taste (Explorations Unlimited Inc 2006; Pisces Consulting Limited 2014) in green sea urchins and other sea urchin species fed various kelp and non-kelp diets (Pearce *et al.* 2002, 2003, 2004; Robinson *et al.* 2002), but not specifically in *S. droebachiensis* from southeastern Newfoundland fed with local kelps. The abundance of this species in the region (Himmelman 1984; Blain & Gagnon 2014; Frey & Gagnon 2015), together with the proximity of an international airport in St. John's suggests the potential to quickly export sea urchin roe outside of the province, therefore reducing some of the risks of gonad quality loss associated with long transportation times from remote communities (Explorations Unlimited Inc 2006; Pisces Consulting Limited 2014).

Our study is the first of a series aimed at examining the potential for a sustainable green sea urchin roe industry in NL. Our multi-month experiment investigated gonad yield and quality in green sea urchins from NL's eastern Avalon Peninsula fed at ambient seawater temperature with three locally abundant kelp species: *A. esculenta*, *L. digitata*, and *A. clathratum*. We designed the 34-week experiment to test the overall prediction that *L. digitata* promotes the highest gonad yield and quality. The timing of the experiment (June to February), frequency of sampling (12 and 34 weeks), and exposure of sea urchins to natural variation in light and seawater temperature, allowed us to evaluate the suitability of different kelp diets for roe production under low operational requirements and at two points during the reproductive cycle of *S. droebachiensis*.

2.2 MATERIALS AND METHODS

2.2.1 Collection, maintenance, and acclimation of organisms

We carried out the present study with green sea urchins (*Strongylocentrotus droebachiensis*) collected on 5 June, 2015, at 3 to 6 m depths from a gently sloping bedrock platform (urchin barrens) in Bread and Cheese Cove (BCC, 47°18′30.8″ N, 52°47′19.1″ W), a semi-protected cove in Bay Bulls, Newfoundland (Canada). Sea urchins were transported to the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland in large containers filled with seawater. Upon arrival (~5 h after collection) they were transferred to five 330-L holding tanks (~200 individuals per tank) supplied with ambient flow-through seawater (~1 L min^{-1}) pumped from a depth of ~5 m in adjacent Logy Bay. Sea urchins with a test diameter (t.d.) of 45 to 55 mm were kept for use in the gonad enhancement experiment (see below). We chose sea urchins of this size because they are normally sexually mature (Scheibling & Hatcher 2007) and dominated numerically at the time of collection. To standardize hunger levels, we starved sea urchins for two weeks prior to the start of the experiment. Water temperature in the holding tanks during the 2-week acclimation phase averaged 4.3 (±1.3 [SE]) °C, and we removed feces and other debris daily.

Divers hand collected the three kelp species given to sea urchins in the experiment, *A. esculenta*, *L. digitata*, and *A. clathratum*, at depths of 1 to 5 m (*A. esculenta* and *L. digitata*) or 5 to 10 m (*A. clathratum*) at BCC, Logy Bay (47°37′31.1″ N, 52°39′48.7″ W) or Flat Rock Cove (47°42′08.8″ N, 52°42′28.8″ W) depending on availability. They were transported in large containers filled with seawater to the OSC and held in 330-L holding tanks supplied with ambient flow-through seawater (~1 L min^{-1}). The first batch of kelp was collected following the 2-week acclimation period, and subsequent new batches of kelp were collected when supplies were low or when kelp blade stiffness or colouration degraded, typically once every two (*A. esculenta* and *L. digitata*) or three (*A. clathratum*) weeks.

2.2.2 Experimental approach

The experiment was carried out in 24, 75-L glass tanks (L x W x H: 62 x 31 x 43 cm) supplied with flow-through seawater (1 L min^{-1}). Tanks were spatially grouped in eight blocks of three tanks each. We randomly assigned each tank in each group one of the three diets, yielding eight replicates per diet. Tanks were exposed to indirect, natural light passing through three large (1 m across) circular windows on a wall opposite to tank locations. We initiated the experiment (summarized in Figure 2.1) on 19 June, 2015 by introducing 30 sea urchins to each tank (720 urchins in total) after measuring body size (t.d.) and whole body wet weight of each sea urchin with calipers (precision=0.1 mm) and a balance (precision=0.1 g; PB3002-S/FACT; Mettler Toldeo, respectively). We also measured t.d., whole body wet weight, gonad wet weight, and sex of an additional 65 sea urchins. The aquaculture industry commonly uses gonadosomatic index (GSI), the gonadal proportion of a sea urchin's weight, to standardize and compare gonad yield and suitability for markets. We calculated GSI for each of these 65 urchins with the following, commonly used relationship:

$$\text{GSI (\%)} = \left(\frac{\text{gonad wet weight (g)}}{\text{whole body wet weight (g)}} \right) \times 100$$

Throughout the experiment, we ensured that sea urchins received more kelp than they could consume by adding fresh kelp blades (no stipes) to each tank three times a week from June to August, twice a week from September to November, and once or twice a week from December to February. We adjusted kelp delivery rate to accommodate seasonal differences in sea urchin kelp consumption, which gradually declined from the onset to the end of the experiment. We cut all

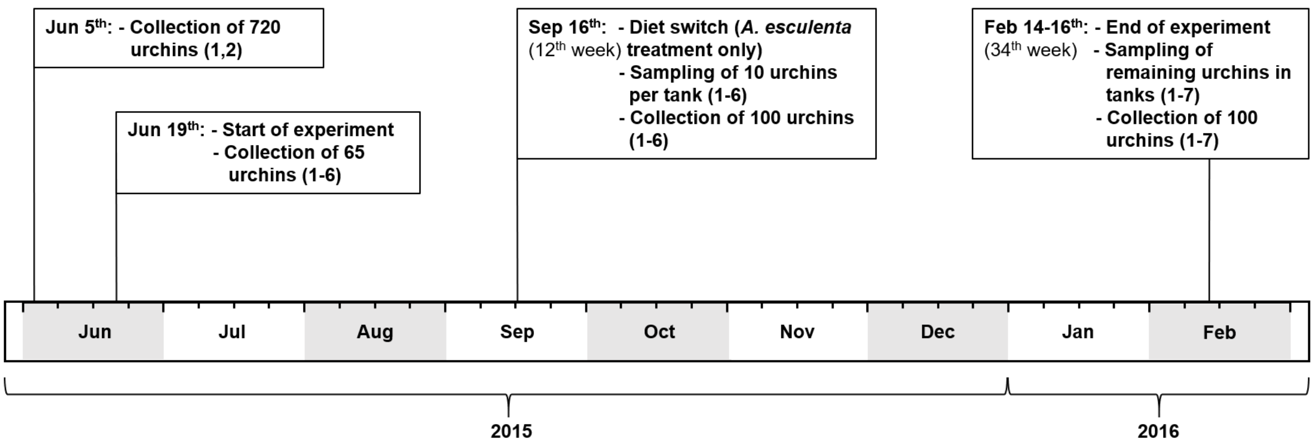


Figure 2.1 Timeline for the 34-week roe enhancement experiment. Numbers in parentheses indicate characteristics measured: 1: Sea urchin body size (test diameter); 2: Sea urchin whole body wet weight; 3: Gonad wet weight; 4: Gonadosomatic index (GSI); 5: Gonad colour; 6: Gonad segmentation and textural appearance; 7: Gonad taste.

kelp into small (~15 x 15 cm) pieces prior to adding them to the tanks to facilitate access, and we removed sea urchin feces and uneaten kelp from the tanks prior to adding new kelp. Occlusion in early August of water intake in one tank with *A. esculenta*, and one tank with *L. digitata*, caused rapid increase in water temperature in both tanks and rapid mortality (all sea urchins in the *A. esculenta* tank and 11 in the *L. digitata* tank died the following week, likely from thermal shock). We therefore terminated the experiment in the tank with *A. esculenta* but continued with the 19 surviving sea urchins in the *L. digitata* tank since the remaining sea urchins showed no signs of stress and did not spawn after this isolated warming event. On 16 September 2015, i.e. 12 weeks into the experiment, we removed 10 sea urchins from each of the 22 tanks with 30 individuals, with no collection in the tank with 19 sea urchins in order to maintain similar densities for the remainder of the experiment. We determined t.d., whole body wet weight, gonad wet weight, sex, and GSI for each of these sea urchins, as described above, as well as in 100 sea urchins freshly collected from BCC. For the sea urchins remaining in the tanks, we switched those individuals previously fed *A. esculenta* to a diet of *L. digitata* for the remainder of the experiment (no diet change in urchins fed *L. digitata* or *Agarum clathratum*). This change was necessary to address natural senescence in *A. esculenta* in early fall in eastern Canada, including southeastern Newfoundland (Himmelman 1984; Frey & Gagnon 2015), making a steady supply of the seaweed for the remainder of the experiment impossible. Urchins initially fed exclusively with *A. esculenta* but switched permanently to *L. digitata* at the end of the 12^{th} week, therefore consumed the two species of kelp in alternation, with most of the time (i.e. the last 22 weeks) consuming *L. digitata*. This diet, hereafter termed “kelp combo”, differed from the two other monospecific diets of *L. digitata* and *A. clathratum* in that it contained two different kelp species, provided sequentially to the urchins. These three kelp-based diets were presumably sufficiently different to capture both expected smaller effects of an early switch from *A. esculenta* to *L. digitata* (when compared to a diet of only *L. digitata*) and larger differences between more distinct diets such as *L. digitata* and *A. clathratum*. Temperature loggers (HOBO Pendant; Onset Computer Corporation) recorded water temperature in two randomly selected tanks every 30 min throughout the experiment.

The experiment ended on 14 February, 2016, for a total duration of 34 weeks (244 days). Test diameter, whole body wet weight, gonad wet weight, sex, and GSI were determined in all remaining sea urchins (i.e. 18 to 20 individuals per tank because of mortalities), as well as in 100 urchins freshly collected from BCC. Sex determination in sea urchins was deemed highly accurate based on histological verification of gonads from 183 urchins at the end of the experiment. Measurements were completed on 16 February, 2016. The experimental timeframe (June to February) encompassed as much of the natural, yearly gonad development cycle in *S. droebachiensis* in eastern Canada without overlapping with the main spring spawning period (Himmelman 1978; Scheibling & Hatcher 2007). We assessed gonad quality characteristics, namely colour, segmentation, textural appearance, and taste as per the schedules and procedures described below.

2.2.3 Colour

We assessed gonad colour at the onset of the experiment in urchins from the field, as well as at the end of the 12th and 34th weeks of the experiment in sea urchins from both the tanks and field (Figure 2.1). Each gonad sample was placed on a plain sheet of white paper and photographed with a digital camera (Powershot D30; Canon) located above the sample at a distance of 10 cm. We photographed all samples with a flash at high resolution (4000 x 3000 pixels) to standardize light conditions and photographed a colour standard (ColorChecker; Passport Photo) under the same conditions to calibrate red, green, and blue (RGB) values in gonad images prior to colour measurement. We applied the procedure described below to measure gonad colour in Adobe Photoshop CC 2015 for each calibrated gonad image.

The same trained observer (S. Trueman) haphazardly chose 10 different areas across the gonad avoiding areas where glare from the camera flash or by sea urchin bodily fluids and tiny body parts left from the dissection artificially altered images. Within each area, we obtained one pixel's red (R), green (G), and blue (B) values, each ranging from 0 to 255, with Photoshop's eye dropper colour sampling tool. We then averaged the 10 values for each R, G, and B colour channels, yielding one mean for each and then converted means to the CIE *L*a*b** (CIELAB) colour space with the ColorMine library converter (see details in Appendix A). Finally, we calculated deviation of these CIELAB-converted values from CIELAB-converted ideal sea urchin gonad market colour standard (bright yellow/orange blades of the DSM Yolk Color Fan), termed ΔE^*_{ab} (Appendix A). A lower ΔE^*_{ab} indicated higher similarity between the colour of a gonad sample and the market standard (Woods *et al.* 2008), using the following threshold ranges (Adekunte *et al.* 2010): (1) very dissimilar colours if $\Delta E^*_{ab} > 3$; (2) dissimilar colours if $1.5 \leq \Delta E^*_{ab} \leq 3$; and (3) similar colours if $\Delta E^*_{ab} < 1.5$.

2.2.4 Segmentation and textural appearance

In normal green sea urchins, one gonoduct runs longitudinally along each of the five gonads, carrying sperms or eggs to the aboral gonopore for external release. The characteristic segmentation of a firm, well-developed gonad in two distinct halves arises from the peripheral placement of the duct onto the gonad. Depending on developmental stage and diet, fluid content in a sea urchin gonad may vary, affecting its textural appearance, defined here as human-perceived visual surface characteristics, including firmness and texture. Other studies used somewhat subjective, arbitrary, or confounded classification schemes to assess various sea urchin gonad quality parameters, including firmness and texture (Pearce *et al.* 2002; Woods *et al.* 2008). For example, Pearce *et al.* (2004) assessed gonad firmness and texture in green sea urchin with two distinct, 4-point ranking scales judged separately based on visual (not gustatory or tactile) perception, which may be subjective given that visual assessment of firmness inevitably influences texture (and vice-versa). Moreover, fluid from swollen gonads can partly or completely conceal the gonoduct. Top quality roe on green sea urchin markets exhibits clear segmentation from a discernible duct, as well as a glossy, firm textural appearance (McBride *et al.* 2004; Explorations Unlimited Inc 2006; Pisces Consulting Limited 2014). Because roe segmentation and textural appearance co-vary and simultaneously affect human perception of gonad quality, we used a semi-objective, image-based gonad quality assessment approach that assessed gonad segmentation and textural appearance separately but combined them in a stratified ranking scale for interpretation.

We assessed gonad segmentation and textural appearance at the onset of the experiment in urchins from the field, as well as at the end of the 12th and 34th weeks of the experiment in sea urchins from both the tanks and field (Figure 2.1). The same trained observer (S. Trueman) quantified both gonad quality metrics from each image used in gonad colour assessment (section 2.2.3). Gonad segmentation was ranked as either: (1) absent, when the gonad completely concealed the gonoduct; (2) partial, when only a portion of the gonoduct was visible; and (3) complete, when the fully exposed gonoduct was visible across the entire length of the gonad. We also ranked textural appearance as either: (1) watery, when fluids drenched the gonads external surface; (2) soft, when gonad external surface appeared moist and glossy; and (3) firm, when gonad exhibited a granular, glossy surface. This dual classification scheme and assignment of a distinct numerical rank to each level in each quality metric allowed us to interpret segmentation and textural appearance separately or in combination based on the sum of numerical ranks. Summing the numerical values across the two-metrics meant various combinations could yield the same overall quality ranking, which is consistent with overall perception. Accordingly, the higher the sum (combined rank weight), the higher the quality, with lowest and highest values of 2 (segmentation = "absent", textural appearance = "watery") and 6 (segmentation = "complete", textural appearance = "firm"), respectively (Table 2.1).

2.2.5 Taste

Gonad taste was assessed three days after the end of the experiment (34th week) in urchins from both the tanks and field (Figure 2.1) by a panel of 24 adult volunteers recruited and overseen by the Centre for Aquaculture and Seafood Development (C-ASD) of MUN. Half (12) of the panelists, hereafter termed "inexperienced", had never consumed sea urchin roe. The other half, termed "experienced", had consumed sea urchin gonad at least once. Experienced panelists therefore had better knowledge of quality gonad taste, enabling us to evaluate whether experience tasting sea urchin gonad before influenced the outcome. All sea urchins from all tanks of a given kelp diet were removed and dissected to determine sex. We randomly selected twenty-four (24) individuals from each diet's pool of females, gently removed their gonads and soaked them in clean seawater to remove any debris. The best-looking gonad (see sections 2.2.3 and 2.2.4) of each female (24 individuals per four treatments; 96 gonads in total) was refrigerated in a labelled ice cube tray at 1°C to maintain freshness. We sampled only female gonads because they were visually more appealing than male gonads, and are preferred on international markets (Pisces Consulting Limited 2014).

Table 2.1 Breakdown of levels for each of two green sea urchin (*Strongylocentrotus droebachiensis*) gonad quality metrics, gonad segmentation (absent, partial, and complete) and textural appearance (watery, soft, and firm), assessed from imagery of gonad acquired at the onset of the gonad enhancement experiment in urchins from the field, as well as at the end of the 12th and 34th weeks of the experiment in sea urchins from both the tanks and field (see details in section 2.2.4). Numbers in parentheses next to each level give corresponding ranks used in statistical analysis and to determine the combined weight (sum) of the quality metrics.

Segmentation	Textural appearance	Combined weight	Overall quality
Absent (1)	Watery (1)	2	Very low
Absent (1)	Soft (2)	3	Low
Absent (1)	Firm (3)	4	Intermediate
Partial (2)	Watery (1)	3	Low
Partial (2)	Soft (2)	4	Intermediate
Partial (2)	Firm (3)	5	Good
Complete (3)	Watery (1)	4	Intermediate
Complete (3)	Soft (2)	5	Good
Complete (3)	Firm (3)	6	Excellent

On the test day, gonad samples were transported in less than 15 min from the OSC to the C-ASD facility in coolers with ice (no direct contact between samples and ice). Samples were transferred to refrigerators upon arrival at the C-ASD. The test was administered over four consecutive sessions, each with six different panelists. Inexperienced and experienced panelists were mixed between sessions based on availability. Panelists were instructed to rank each gonad sample for gustatory perception. First, they ranked "overall preference" from 1 (dislike extremely) through 9 (like extremely) (Table 2.2). Food sensory analysis utilizes this classical hedonic test, which incorporates all dimensions of gustatory perception, i.e. taste, smell, and texture (Lawless & Heymann 2010). The second scale specifically addressed "taste", rating from 1 (very bitter) to 6 (perfectly sweet) (Table 2.2). Each panelist sat in a booth and was given 15 min to rank four randomized gonad samples presented side by side and used only once; opaque dividers prevented visual contact with other panelists. Red lighting concealed true gonad colour. Panelists did not know treatment identity. They could taste each sample only once, in the prescribed order (from left to right), rinsing their mouth with water between samples to ensure no influence of repeated tastings or sample identity.

2.2.6 Statistical Analysis

2.2.6.1 Gonadosomatic Index (GSI)

A 3-way ANOVA with Sex (male and female sea urchins), Diet (three kelp diets used in the experiment; kelp combo, *L. digitata*, and *A. clathratum* - as well as natural diet of sea urchins collected from the field), and feeding Duration (12 or 34 wk) as fixed factors tested for differences in GSI among male and female sea urchins fed different diets over different lengths of time. The analysis was applied to the raw data, with two mean GSI values per tank and field collection group;

Table 2.2 Breakdown of levels for each of two green sea urchin (Strongylocentrotus droebachiensis) roe gustatory perception scales, “overall preference” and “taste”, assessed by a panel of 24 individuals presented roe samples acquired at the end of the roe enhancement experiment in urchins from both the tanks and field (see details in section 2.2.5). Numbers in parentheses next to each level are corresponding ranks.

Overall preference	**Taste**
Dislike extremely (1)	Very bitter (1)
Dislike very much (2)	Bitter (2)
Dislike moderately (3)	Bland (not sweet, not bitter; 3)
Dislike slightly (4)	Sweet (4)
Neither like, nor dislike (5)	Very sweet (5)
Like slightly (6)	Perfectly sweet (6)
Like moderately (7)	
Like very much (8)	
Like extremely (9)	

one for males, one for females (N=118 [59 per sex]). We excluded the random factor Block (eight blocks of three experimental tanks per kelp diet) because sea urchins from the field were not spatially blocked (Zar 1999). Given the significant 3-way interaction term (Sex X Diet X Duration) (Table C.1), we ran separate 2-way ANOVAs for males and females, with Diet (three kelp diets and natural diet of urchins from the field) and feeding Duration (12 or 34 wk) as fixed factors (Quinn and Keough 2002) to test more accurately for GSI differences (N = 59 per sex). These analyses were not treated as repeated measures because different sea urchins were sampled at 12 and 34 wk. We analyzed the data with classical general linear models because our data met assumptions of homoscedasticity and normality (Quinn & Keough 2002).

We utilized the exact same analyses and logic to test for differences in GSI change (ΔGSI, see calculation details below) among sea urchins fed different diets over different lengths of time. The following equation was used to calculate ΔGSI:

$$\Delta\text{GSI (\%)} = \left[\frac{\text{GSI}_F - \text{GSI}_I}{\text{GSI}_I}\right] \times 100$$

Where ΔGSI describes the percent change in GSI, GSI_F denotes the mean GSI of sea urchins at a given time (12^{th} or 34^{th} week into the experiment), and GSI_I denotes the mean GSI of the 65 sea urchins from the field collected at the onset of the experiment: 3.4 ± 0.3%.

Since sea urchins from the field were not spatially blocked, the seven (7) GSI_F values used to calculate corresponding ΔGSI values were the mean GSI of 14 (five groups) or 15 (two groups) sea urchins chosen randomly among the 100 sea urchins collected in each of the 12^{th} and 34^{th} weeks. Number of sea urchins (10 to 65) within each comparison group, as well as number of comparison groups (7 or 8) among kelp and natural diet treatments were therefore similar, and hence sufficiently balanced to eliminate the need for special statistical treatment (Quinn & Keough 2002).

2.2.6.2 Colour

We utilized the exact same analyses and logic as above to test for differences in gonad colour (1) deviation from ideal gonad market colour standard [ΔE^*_{ab}]; (2) lightness [L^*]; (3) redness [a^*]; and (4) yellowness [b^*] among male and female sea urchins fed different diets over different lengths of time (N=118) (see details of these colour parameters in section 2.2.3 and Appendix A). The non-significant 3-way term for b^* ($F_{3,102}$=1.8, p=0.16), meant we could interpret results of the 3-way ANOVA for that variable only.

2.2.6.3 Gonad segmentation, textural appearance, and overall quality

We used multiple ordinal logistic regression (McCullagh 1980), hereafter termed “MOLR”, to analyze gonad segmentation and overall quality ranks because this analysis is specifically designed for response variables on a scale where only relative ordering matters (McCullagh 1980; Kuzon *et al.* 1996; Harrell 2001; Kleinbaum & Klein 2010). We identified the model fit from several stepwise MOLRs, as judged by comparing variation (Δ) in Akaike’s information criterion (AIC) from one model to the next: larger ΔAIC between two models indicated more dissimilar models, with the lowest AIC defining the best model (Burnham & Anderson 2004). As per Singer & Willett (2003), we considered a ΔAIC of ≥4 between two models sufficiently large to declare both models different in their respective explanatory powers. When applicable, we chose the model with the lowest number of terms for similar AICs (Harrell 2001; Quinn & Keough 2002). McFadden’s pseudo-R^2 (R^2_{MF}), which assesses improvement in model fit compared to a null model, characterized degree of data fit of selected models (Hemmert, *et al.* 2018); R^2_{MF} is minimally affected by the number of categories of the dependent variable (v), and more suitable for sample sizes (N) < 200 (Veall & Zimmermann 1996; Hemmert *et al.* 2018). R^2_{MF} between 0.19 and 0.26, or > 0.26, indicates respectively good and excellent model fit (Hemmert *et al.* 2018).

We used MOLR to examine the relationship between gonad segmentation rank (1 [absent], 2 [partial], and 3 [complete]) and the fixed factors Sex (male and female sea urchins), Diet (three kelp diets used in the experiment; kelp combo, *L. digitata*, and *A. clathratum* – as well as natural diet of sea urchins collected from the field), and feeding Duration (12 and 34 wk). We excluded the random factor Block (eight blocks of three experimental tanks per kelp diet) because sea urchins from the field were not spatially blocked. We applied the best fitting MOLR to the raw data, with two modal segmentation rank values per tank and field collection group; one for males, one for females (N=118 [59 per sex]). We used mode rather than mean (and associated standard deviation) because the latter is inadequate for ordinal data (Allen & Seaman, 2007; Jamieson, 2004; Kuzon *et al.*, 1996).

We used the same logic as described above to test for differences in gonad textural appearance ranks of male and female sea urchins fed different diets over different lengths of time (N=118). However, because of the highly skewed textural appearance data, with predominately soft, sometimes firm, and never watery tissue (see results), we chose multiple logistic regression "MLR" (Kleinbaum & Klein 2010) analysis instead of MOLR. The textural appearance data violated MOLR's assumption of proportional odds among levels of the response variable, and offered a better fit to MLR's specific purpose of modeling a nominal, binary dependant response variable (Brant 1990; Quinn & Keough 2002).

Following the same logic and analysis described above for the gonad segmentation data, we used MOLR to examine the relationship between gonad overall quality rank (combined weight [sum] of ranks from both gonad segmentation and textural appearance metrics: 2 [very low], 3 [low], 4 [intermediate], 5 [good], and 6 [excellent]) on male and female sea urchins fed different diets over different lengths of time (N=118).

2.2.6.4 Taste

We also used MOLR to examine the relationship between gonad overall preference rank (nine values from Dislike extremely [1] to Like extremely [9]) and the fixed factors Diet (three kelp diets used in the experiment; kelp combo, *L. digitata,* and *A. clathratum* - as well as natural diet of sea urchins collected from the field) and panelist Experience (inexperienced and experienced). The same analysis was utilized for gonad taste rank (six values from Very bitter [1] to Perfectly sweet [6]). In both cases, we applied analysis of best-fitting MOLR to the raw data, with one ranking of overall preference and one ranking of taste for each of the four (female) gonad samples (one sample per diet) tested by each of the 24 panelists (N=96 for each MOLR).

In all regressions (MOLRs, MLRs) and ANOVAs, we verified homogeneity of variance and normality of residuals by examining the distribution of the residuals and the normal probability plot of the residuals, respectively (Snedecor & Cochran 1994). All MOLR met the proportional odds assumption and no factors showed scalar or nominal effects (McCullagh 1980; Brant 1990). In all analyses, we applied Bonferroni correction or probabilities to pairwise comparisons based on least-square means (Quinn & Keough 2002) to detect differences among levels within a factor. We used a significance level of 0.05 in all analyses, which were carried out in R 3.3.3 (R Core Team 2017), and present all means with standard error (mean ± SE) unless stated otherwise.

2.3 RESULTS

2.3.1 Thermal environment, growth, and gonadosomatic index

Water temperature in the experimental tanks varied between a minimum of 0.8 °C on 3 February, 2016, and a maximum of 14.8 °C on 2 September, 2015 (Figure 2.2). We observed two distinct seasonal phases: (1) a relatively rapid, ~3-mo warming period from June (onset of the experiment) to early September 2015, followed by; (2) a comparatively slower, ~6-month cooling period until February 2016 (end of the experiment). Test diameter and wet weight of sea urchins fed with a kelp combo (i.e. with *A. esculenta* during the first 12 weeks and *L. digitata* during the remainder of the experiment) or *L. digitata* increased by ~5-6% and ~20-23% respectively over 34 weeks, with no significant differences in augmentation between males and females (Tables B.1 and B.2). However, sea urchins fed with *A. clathratum* grew by <1% in both diameter and weight, though less in females than in males (Tables B.1-B.4). Intermediate growth of ~3% (size) and 13% (weight) characterized sea urchins from the field (Tables B.1-B.4).

After 12 weeks, the gonadosomatic index (GSI) of sea urchins fed *L. digitata*, ~14%, was nearly double that of sea urchins fed a kelp combo, and three to six times as high as that of sea urchins fed *A. clathratum* or from the field, with no marked differences between males and females in any diets (Tables B.1 and 2.3; Figure 2.3). Tripling the feeding duration to 34 weeks further increased GSI of *L. digitata*-fed sea urchins to ~23% (Table B.1, Figure 2.3). Interestingly, we observed a similarly high GSI at the end of the experiment in sea urchins fed the kelp combo, ~21% averaged across males and females (Tables B.1 and 2.3, Figure 2.3).

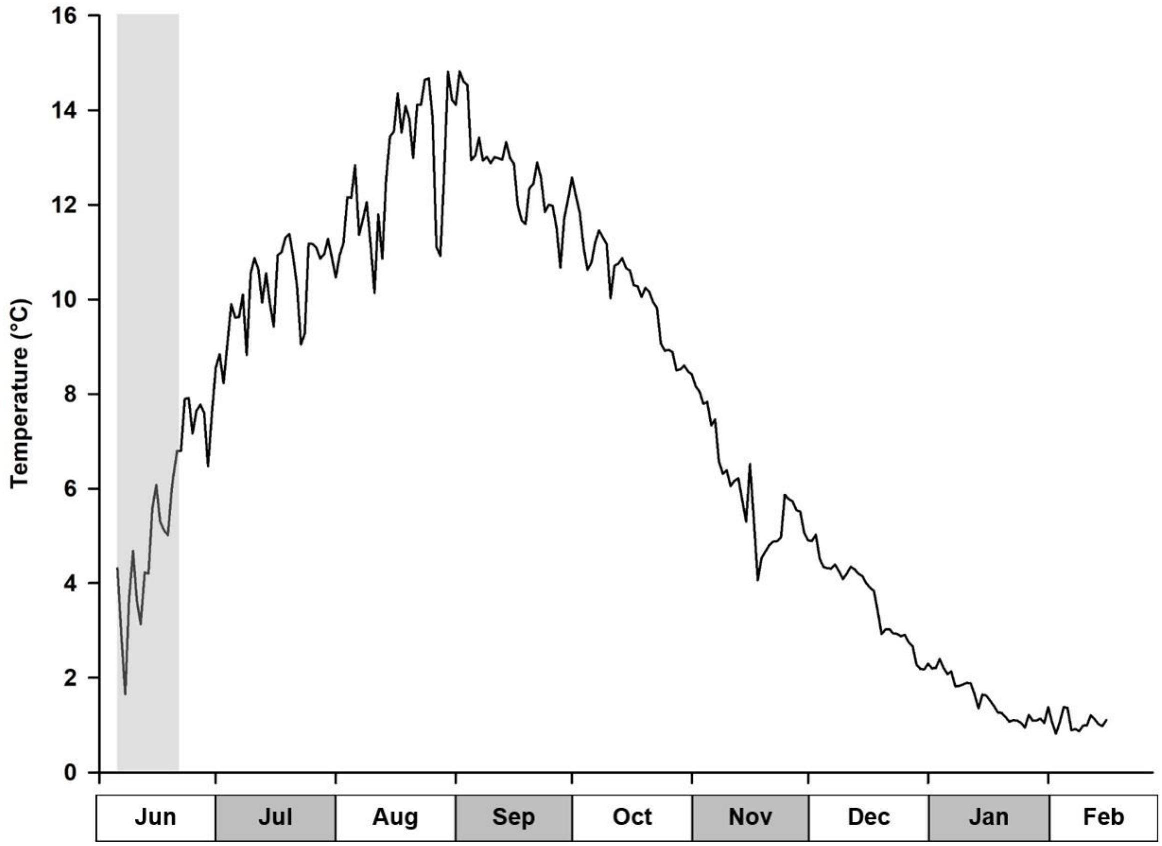


Figure 2.2 Mean sea water temperature from two of the five holding tanks during the 2-wk acclimation (vertical grey bar; 5 June to 18 June, 2015) of green sea urchins (*Strongylocentrotus droebachiensis*), and from two of the 24 experimental tanks during the gonad enhancement experiment (19 June, 2015 to 14 February, 2016).

Table 2.3 Summary of two-way ANOVAs (applied to raw data) examining the effect of Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and feeding Duration (12 and 34 wk) on gonadosomatic index (GSI) of male and female green sea urchins (*Strongylocentrotus droebachiensis*) in the gonad enhancement experiment (see section 2.2.7.1 and Table C.1 for background statistical analysis justifying the need to treat males and females separately).

Sex	**Source of variation**	***df***	**MS**	***F*-value**	***p***
Male	Diet	3	762.9	396.9	<0.001
	Duration	1	421.8	219.4	<0.001
	Diet x Duration	3	100.0	52.0	<0.001
	Error	51	1.9		
	Corrected total	58			
Female	Diet	3	995.1	485.1	<0.001
	Duration	1	1158.5	564.7	<0.001
	Diet x Duration	3	208.0	101.4	<0.001
	Error	51	2.1		
	Corrected total	58			

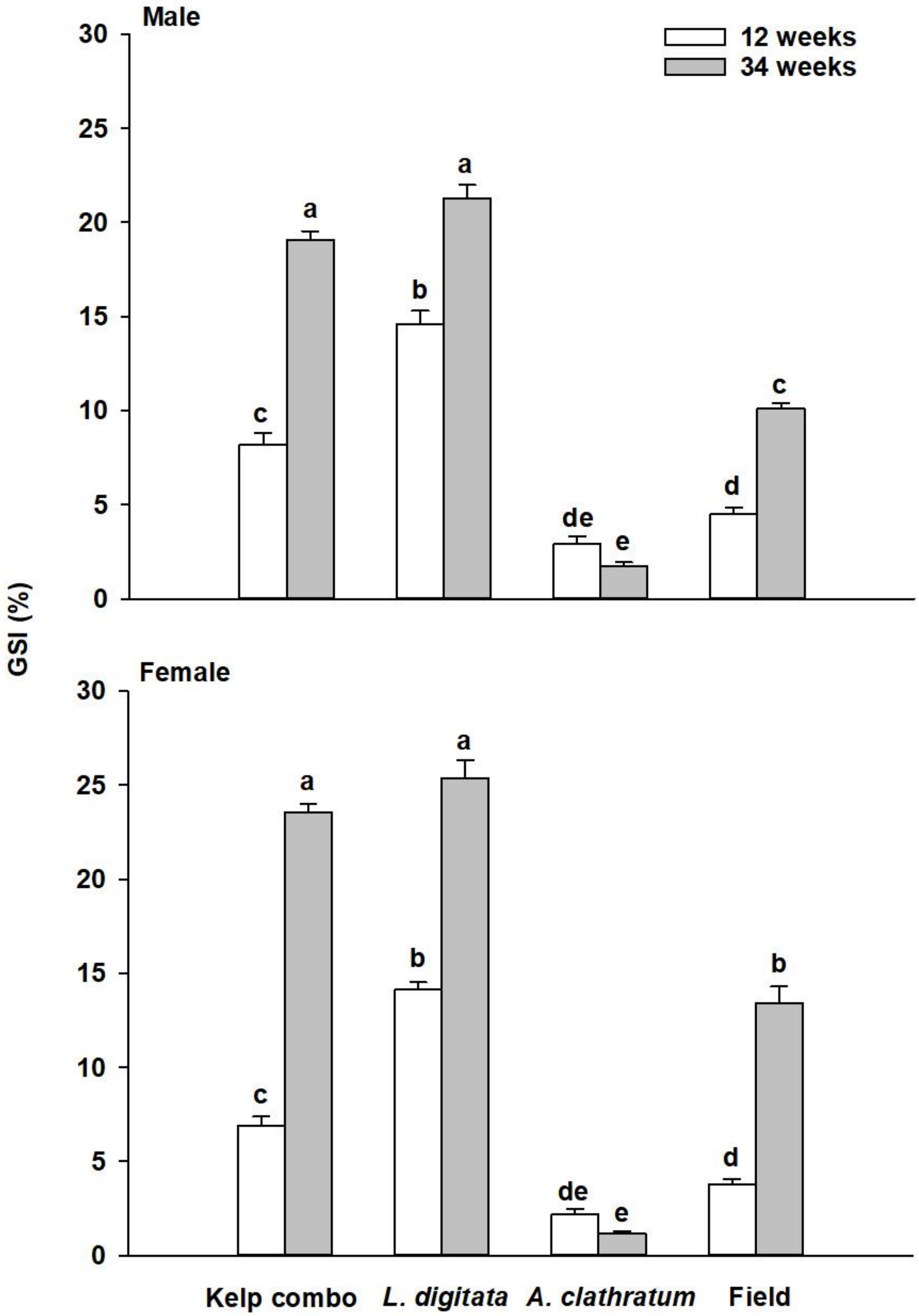

Male
12 weeks
34 weeks
30
25
20
15
10
5
0
a
a
b
c
c
d
de
e
GSI (%)
Female
30
25
20
15
10
5
0
a
a
b
b
c
d
de
e
Kelp combo
L. digitata
A. clathratum
Field

Figure 2.3 Mean (+SE) gonadosomatic index (GSI) of male and female green sea urchins (*Strongylocentrotus droebachiensis*) fed either of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*), or collected from the field, 12 and 34 wk after the onset of the gonad enhancement experiment. Bars not sharing the same letter differ statistically (LS means tests with Bonferroni correction of probabilities, p<0.05; for each sex: n = 7 and 7 [kelp combo], 7 and 8 [*L. digitata*], 8 and 8 [*A. clathratum*], and 7 and 7 [natural diet] for respectively the 12th and 34th weeks).

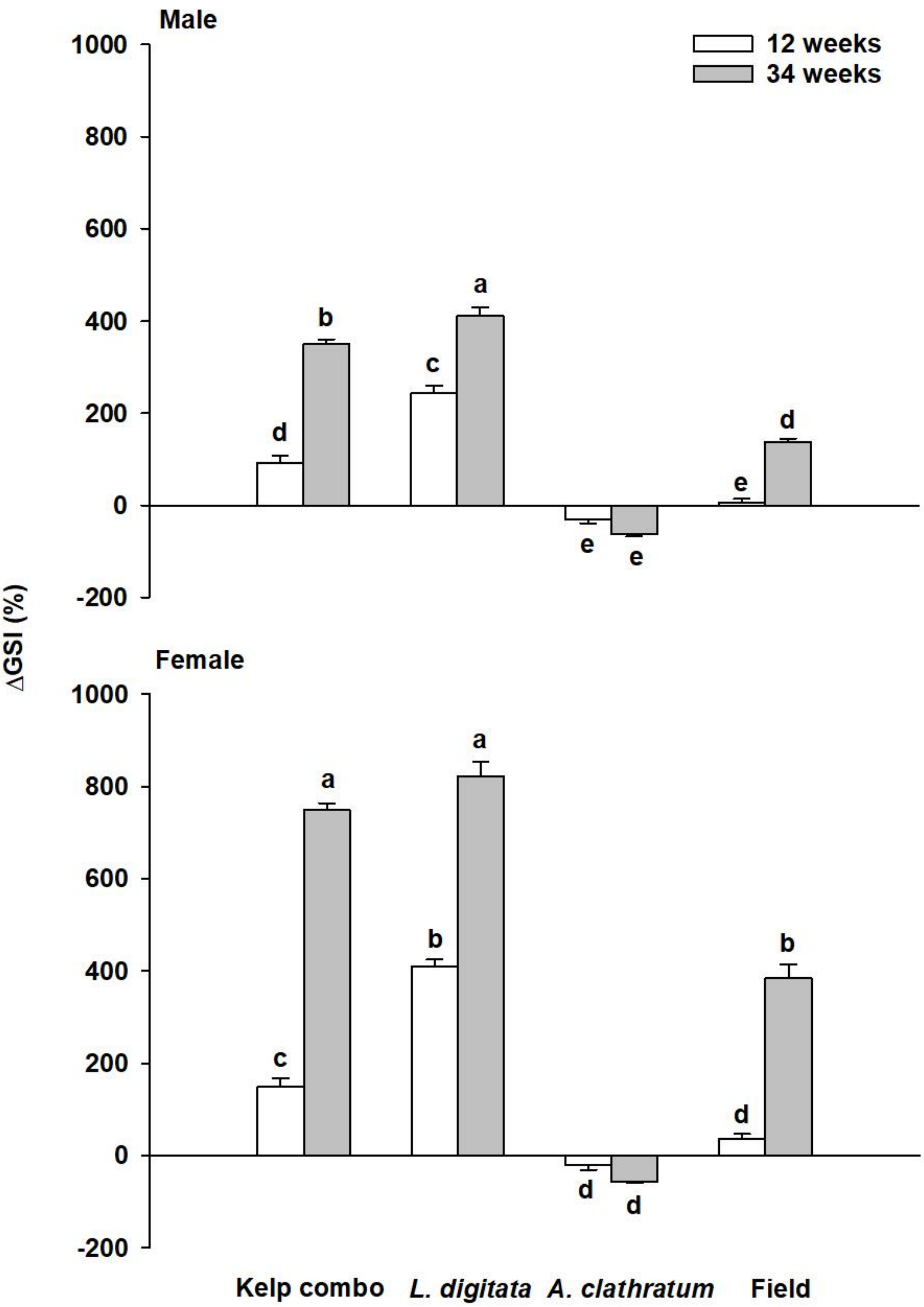
Consistent with their low growth, sea urchins fed *A. clathratum* consistently developed small gonads through the experiment, with GSI never exceeding ~3% at 12 weeks in males (Tables B.1 and 2.3, Figure 2.3). Sea urchins from the field exhibited intermediate GSI, increasing from ~4% after 12 weeks, to ~12% after 34 weeks, again with no perceptible differences between males and females (Tables B.1 and 2.3, Figure 2.3). We attribute the 595% and 527% increases in GSI after 34 weeks in sea urchins fed with *L. digitata* or the kelp combo respectively to a larger increase in females than males (Tables B.2 and 2.4, Figure 2.4). Gonads of sea urchins fed with *A. clathratum* atrophied through the experiment, as shown by negative changes in GSI of up to -3% (Tables B.2 and 2.4, Figure 2.4).

2.3.2 Colour

Deviation from ideal gonad market color (ΔE^*_{ab}) was highest in males and females fed with *A. clathratum*, peaking in females at 34 weeks (Tables C.3 and 2.5; Figure 2.5). In males, all other diets yielded significantly lower and similar ΔE^*_{ab}; between 37 at 34 weeks when fed *L. digitata*, and 40 at 12 weeks when collected from the field (Table 2.5, Figure 2.5). We also found significantly lower ΔE^*_{ab} in female sea urchins fed a kelp combo, *L. digitata*, or collected from the field than *A. clathratum*-fed sea urchins, between 30 at 34 weeks when fed with a kelp combo, and 47 at 12 weeks when collected from the field (Table 2.5, Figure 2.5). Statistically significant decreases in ΔE^*_{ab} from 12 to 34 weeks of 30% and 34%, demonstrate that resemblance between actual gonad color and ideal gonad market color improved over time in female urchins fed a kelp combo or when collected from the field, respectively (Figure 2.5). In contrast, resemblance

Table 2.4 Summary of two-way ANOVAs (applied to raw data) examining the effect of Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of urchins collected from the field) and feeding Duration (12 and 34 wk) on change in gonadosomatic index (ΔGSI) of male and female green sea urchins (*Strongylocentrotus droebachiensis*) in the gonad enhancement experiment (see section 2.2.7.1 and Table C.2 for background statistical analysis justifying the need to treat males and females separately).

Sex	**Source of variation**	***df***	**MS**	***F*-value**	***p***
Male	Diet	3	424046	396.9	<0.001
	Duration	1	234420	219.4	<0.001
	Diet x Duration	3	55558	52.0	<0.001
	Error	51	1068		
	Corrected total	58			
Female	Diet	3	1294781	485.1	<0.001
	Duration	1	1507314	564.7	<0.001
	Diet x Duration	3	270576	101.4	<0.001
	Error	51	2669		
	Corrected total	58			

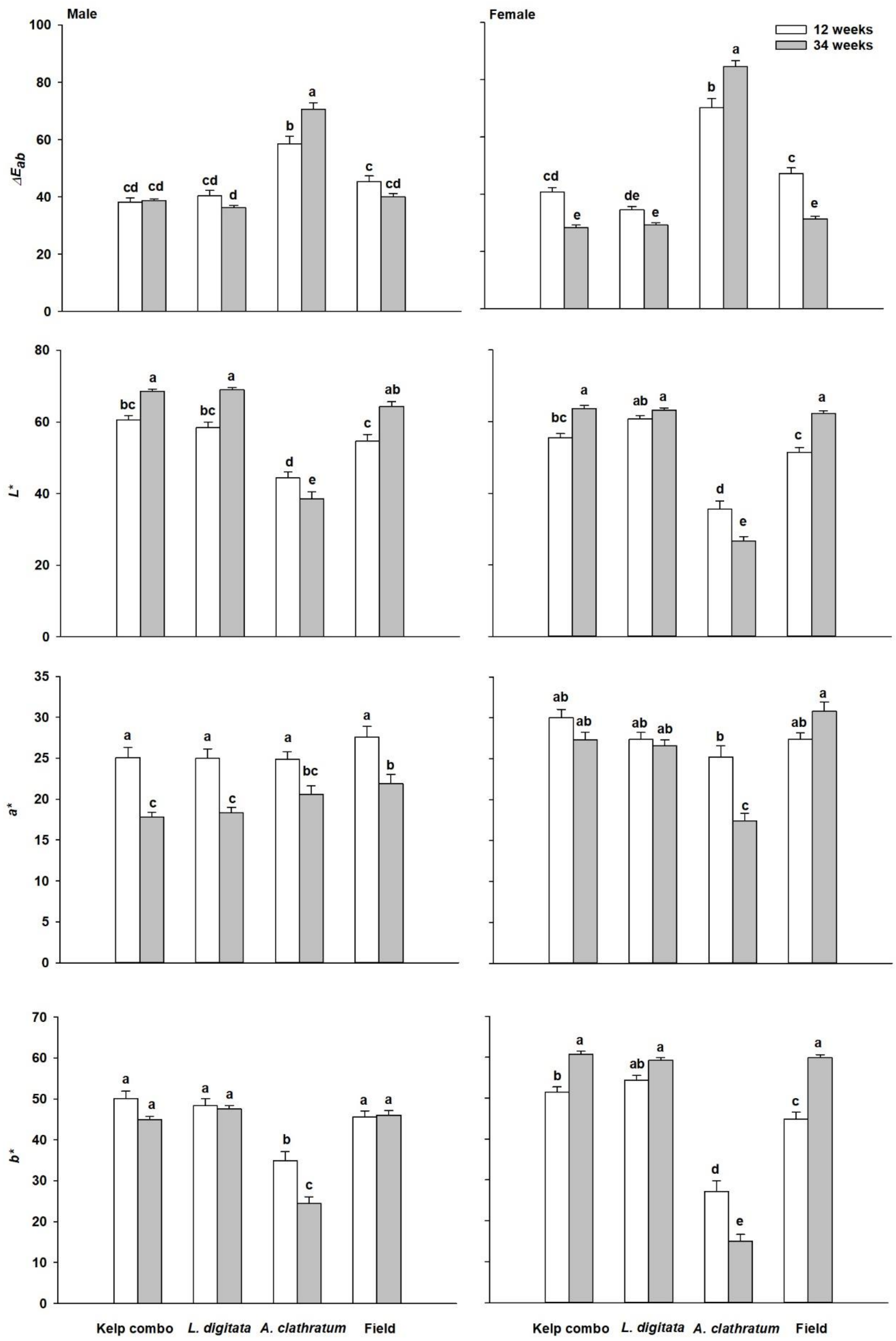


Male
12 weeks
34 weeks
1000
800
600
400
200
0
-200
ΔGSI (%)
Female
1000
800
600
400
200
0
-200
Kelp combo
L. digitata
A. clathratum
Field

Figure 2.4 Mean (+SE) percent change in gonadosomatic index (ΔGSI) of male and female green sea urchins (*Strongylocentrotus droebachiensis*) fed either of the three kelp (kelp combo, *Laminaria digitata*, *Agarum clathratum*) diets, or collected from the field, 12 and 34 weeks after the onset of the gonad enhancement experiment. Benchmark GSI used to calculate ΔGSI was the mean GSI of urchins from the field collected at the onset of the experiment (see details in section 2.2.7.1). Bars not sharing the same letter differ statistically (LS means tests with Bonferroni correction of probabilities, $p<0.05$; for each sex: n = 7 and 7 [kelp combo], 7 and 8 [*L. digitata*], 8 and 8 [*A. clathratum*], and 7 and 7 [natural diet] for respectively the 12th and 34th weeks).

Table 2.5 Summary of two-way ANOVAs (applied to raw data) examining the effect of Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of urchins collected from the field), and feeding Duration (12 and 34 wk) on green sea urchin (*Strongylocentrotus droebachiensis*) roe colour's: deviation from ideal roe market colour standard (ΔE^{*}_{ab}), lightness (L^*), and redness (a^*) in the roe enhancement experiment (see section 2.2.7.2 and Table C.3 for background statistical analysis justifying the separate analysis of males and females).

Sex	**Parameter**	**Source**	***df***	**MS**	***F*-value**	***p***
Male	ΔE^*_{ab}	Diet	3	2362.4	54.8	< 0.001
		Duration	1	51.1	1.1	0.28
		Diet x Duration	3	399.7	9.3	< 0.001
		Error	51	43.1		
		Corrected total	58			
	L^*	Diet	3	1830.7	56.0	< 0.001
		Duration	1	314.2	9.6	0.003
		Diet x Duration	3	329.6	10.1	< 0.001
		Error	51	32.7		
		Corrected total	58			
	a^*	Diet	3	563.2	65.3	< 0.001
		Duration	1	3.0	0.3	0.56
		Diet x Duration	3	593.7	68.7	< 0.001
		Error	51	10.2		
		Corrected total	58			
Female	ΔE^*_{ab}	Diet	3	7104.2	231.5	< 0.001
		Duration	1	239.7	7.8	0.007
		Diet x Duration	3	643.0	20.9	<0.001
		Error	51	30.7		
		Corrected total	58			
	L^*	Diet	3	3275.4	156.8	< 0.001
		Duration	1	106.1	5.1	0.02
		Diet x Duration	3	261.6	12.5	< 0.001
		Error	51	20.9		
		Corrected total	58			
	a^*	Diet	3	205.5	34.2	< 0.001
		Duration	1	69.7	11.6	0.0013
		Diet x Duration	3	70.1	11.7	< 0.001
		Error	51	6.0		
		Corrected total	58			



Male
Female
12 weeks
34 weeks
ΔE_ab
L*
a*
b*
Kelp combo
L. digitata
A. clathratum
Field

Figure 2.5 Mean (+SE) roe colour deviation from ideal roe market colour standard (ΔE^*_{ab}; describes very dissimilar colours if $\Delta E^*_{ab} > 3$; dissimilar colours if $1.5 \leq \Delta E^*_{ab} \leq 3$; similar colours if $\Delta E^*_{ab} < 1.5$), lightness (L^*; 0 [black] to 100 [white]; standard: 78.3), redness (a^*; -120 [green] to 120 [magenta]; standard: 16.3), and yellowness (b^*; -120 [blue] to 120 [yellow]; standard: 81.1) in male and female green sea urchins (*Strongylocentrotus droebachiensis*) fed either of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*), or collected from the field, 12 and 34 wk after the start of the roe enhancement experiment (see Appendix A for details of colour parameters and conversion). Bars not sharing the same letter differ statistically (LS means tests with Bonferroni correction of probabilities, $p<0.05$; for each sex: n = 7 and 7 [kelp combo], 7 and 8 [*L. digitata*], 8 and 8 [*A. clathratum*], and 7 and 7 [natural diet] for the 12th and 34th weeks respectively).

significantly decreased over time, by ~22% in both males and females fed with *A. clathratum* (Figure 2.5).

In both males and females, gonad colour lightness (L^*) was higher (only significant for males), by 3 to 22%, at 34 than 12 weeks when fed a kelp combo or *L. digitata*, or when collected from the field, indicating improvement of L^* over time (Table 2.5, Figure 2.5). L^* at 12 weeks in males and females fed with *A. clathratum* was significantly lower than in all other diets, with the smallest difference, 22%, in males from the field (Figure 2.5). Prolonged feeding on *A. clathratum* to 34 weeks decreased L^* in both males and females by 14% and 25%, respectively (Figure 2.5).

Patterns of gonad redness (a^*) differed between males and females (Table 2.5). In males, we observed similar values for a^* at 12 weeks across the three kelp diets and sea urchins from the field (Figure 2.5). We also observed significantly higher values for a^* at 12 than 34 weeks in any kelp diet as well as in individuals from the field, with the largest difference, 29%, in individuals fed with a kelp combo (Table 2.5; Figure 2.5). In females, we also observed no difference in a^* at 12 weeks among the three kelp diets and sea urchins from the field, and values were comparable to those in males at 12 weeks (Figure 2.5). Only the females fed with *A. clathratum* differed statistically in a^* between 12 and 34 weeks, declining by ~32% (i.e. no temporal change in the other kelp diets and females from the field; Figure 2.5). The highest value in the *A. clathratum* treatment was about 81% of that (31) in females from the field at 34 weeks.

Patterns of gonad yellowness (b^*) also differed between males and females, with more pronounced temporal differences in females. In males, b^* was relatively consistent across individuals fed a kelp combo, *L. digitata*, or from the field, with no significant differences between 12 and 34 weeks (Table 2.6, Figure 2.5). The only significant change from 12 to 34 weeks was a drop of 31%, from 35 to 24 in *A. clathratum* treatments (Figure 2.5). In females, b^* was also

Table 2.6 Summary of three-way ANOVA (applied to raw data) examining the effect of Sex (male and female), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and feeding Duration (12 and 34 wk) on green sea urchin (*Strongylocentrotus droebachiensis*) gonad yellowness (b^*) in the gonad enhancement experiment (see section 2.2.7.2 and Table C.3 for background statistical analysis justifying the need to treat b^*differently than ΔE^*_{ab}, L^*, and a^*).

Source of variation	***df***	**MS**	***F*-value**	***p***
Sex	1	131.1	5.8	0.017
Diet	3	3967.1	177.1	< 0.001
Duration	1	154.8	6.9	0.001
Sex x Diet	3	1058.5	47.2	< 0.001
Sex x Duration	1	976.9	43.6	< 0.001
Diet x Duration	3	1046.3	46.7	< 0.001
Sex x Diet x Duration	3	39.5	1.8	0.16
Error	102	22.4		
Corrected total	117			

significantly lowest when fed *A. clathratum*, dropping 25%, from 36 at 12 weeks to 27 at 34 weeks. b^* significantly increased by up to 21% over time in females fed a kelp combo or from the field, yet it remained consistent from 12 and 34 weeks when fed *L. digitata* (Figure 2.5).

2.3.3 Gonad segmentation, textural appearance, and overall quality

As determined with stepwise multiple ordinal logistic regression analysis (Tables D.1 and 2.7), the best-fitting model to gonad segmentation rank data was:

$$\text{Segmentation rank} = S + Di + Du$$

where S denotes Sex, Di is Diet, and Du describes feeding Duration. At 12 weeks, over 85% of the gonad in males fed either a kelp combo, *L. digitata*, or from the field exhibited partial or complete segmentation, whereas nearly 50% of the gonads completely lacked segmentation when fed *A. clathratum* (Figure. 2.6). Proportions (>43%) of gonads with complete segmentation were conserved at 34 weeks, relative to 12 weeks, in males fed a kelp combo or from the field. However, gonad of males fed *L. digitata* lost segmentation between 12 and 34 weeks, as shown by an increase of partially segmented gonads from 43 to 75%. Complete absence of segmentation in gonads from males fed *A. clathratum* increased over time, peaking at 75% after 34 weeks (Figure 2.6). Patterns in females were more dichotomous than in males, with individuals largely restricted to partially segmented gonads or gonads without any segmentation at both 12 and 34 weeks, regardless of diet (Figure 2.6). The only noticeable exception was in females fed a kelp combo, where 71% of the gonads was completely segmented at 12 weeks yet decreased to only 25% at 34 weeks. As seen in males, we observed little gonad segmentation in females fed with *A. clathratum*, with 100% showing no segmentation at 34 weeks (Figure 2.6).

Table 2.7 Summary of best-fitting multiple ordinal logistic regression (applied to modes of rank data) examining the effect of Sex (male and female urchins), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field), and feeding Duration (12 and 34 wk) on green sea urchin (*Strongylocentrotus droebachiensis*) gonad segmentation (R^2_{MF}=0.28; see Table D.1 for results of stepwise model selection procedure).

Source of variation	***df***	**χ^2**	***p***
Sex	1	5.9	0.015
Diet	3	62.9	<0.001
Duration	1	5.4	0.021

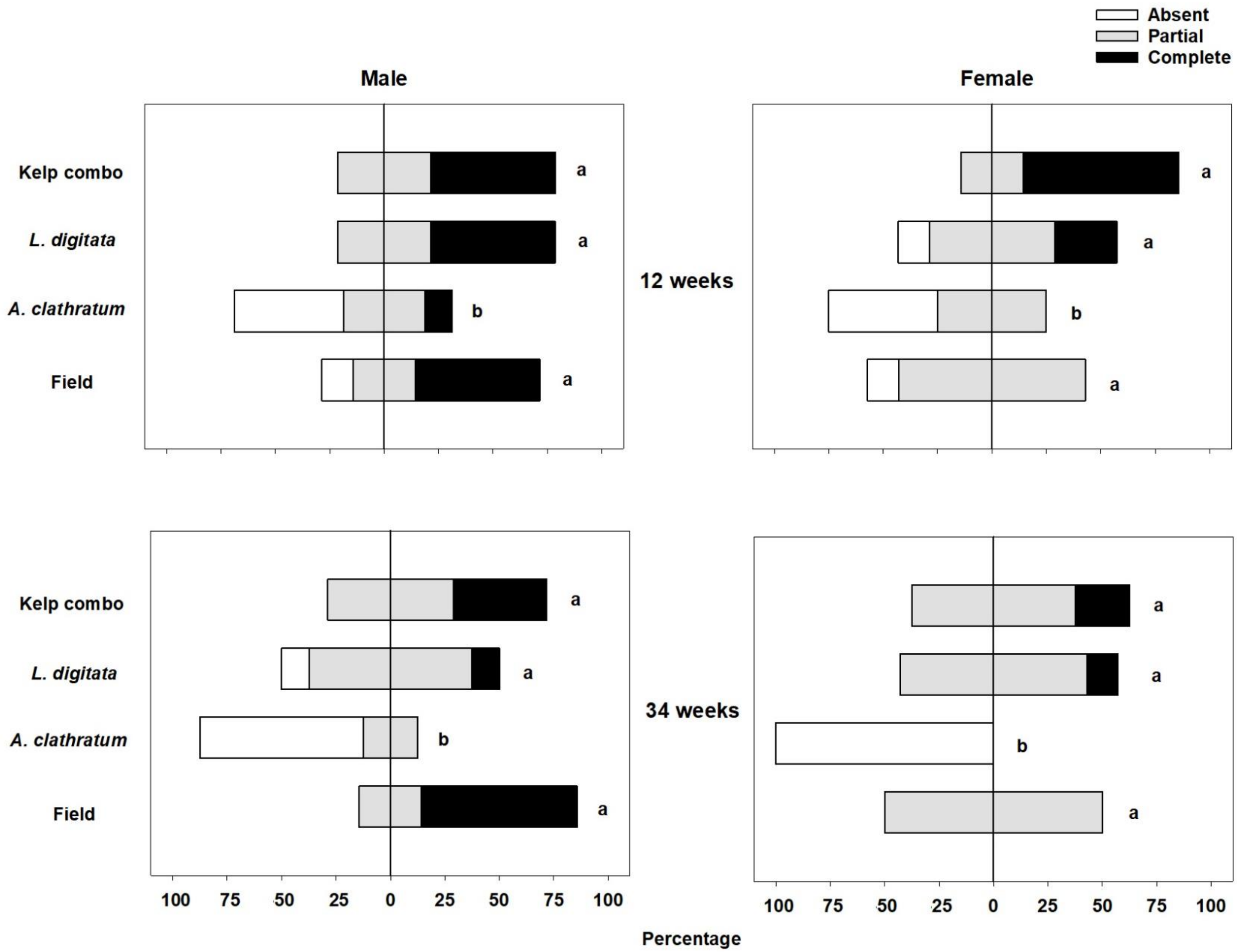

Absent
Partial
Complete
Male
Female
Kelp combo
L. digitata
A. clathratum
Field
12 weeks
34 weeks
a
b
100 75 50 25 0 25 50 75 100
Percentage

Figure 2.6 Percentage of gonads exhibiting no (absent), partial, or complete segmentation in male and female green sea urchins (*Strongylocentrotus droebachiensis*) fed either of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*), or collected from the field, 12 and 34 wk after the start of the gonad enhancement experiment. Bars not sharing the same letter, within each panel, differ statistically (LS means tests with Bonferroni correction of probabilities, $p<0.05$; for each sex: n = 7 and 7 [kelp combo], 7 and 8 [*L. digitata*], 8 and 8 [*A. clathratum*], and 7 and 7 [natural diet] for respectively the 12th and 34th weeks).

As determined with stepwise multiple logistic regression (Tables D.2 and 2.8), the best-fitting model to textural appearance rank data was:

Textural appearance rank = S + Di

where S denotes Sex and Di is Diet (no effect of feeding duration). At least 93% of the gonads appeared soft in both males and females fed any of the three kelp diets (Figure 2.7). Individuals fed with a kelp combo (males and females) or *L. digitata* (females) occasionally (<7%) produced firm-looking gonads, somewhat more frequently (29%) in females from the field. None of the gonads looked watery (Figure 2.7).

As determined with stepwise multiple ordinal logistic regression (Tables D.3 and 2.9), the best-fitting model to gonad overall quality rank data was:

Overall quality rank = Di + Du + Di*Du

where Di denotes Diet and Du is feeding Duration (no effect of sex). At 12 weeks, we observed the best-quality gonads in urchins fed a kelp combo, with 64% of the gonads considered good or excellent (Figure 2.8). Gonads of urchins fed with *L. digitata* or from the field exhibited slightly lower quality, with 79% and 93% considered as intermediate or good respectively (Figure 2.8). We observed lowest gonad quality in sea urchins fed *A. clathratum*, with 94% ranking as low or intermediate (Figure 2.8). Gonad quality improved over time in sea urchins fed *L. digitata* as shown by similarly high proportions, ~50%, of intermediate quality gonads at 12 and 34 weeks, increasing from 14 to 31% of good-quality gonads during that period. Quality improved most in sea urchins from the field, good and excellent gonads increasing from 21% at 12 weeks to 64% at 34 weeks (Figure 2.8). In contrast, gonad quality decreased over time in sea urchins fed a kelp combo, deteriorating from 64% good and excellent gonads at 12 weeks to only 21% at 34 weeks

Table 2.8 Summary of best-fitting multiple logistic regression (applied to modes of rank data) examining the effect of Sex (male and female urchins) and Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of urchins collected from the field) on green sea urchin (*Strongylocentrotus droebachiensis*) gonad textural appearance (R^2_{MF}=0.21; see Table D.2 for results of stepwise model selection procedure).

Source of variation	***df***	**χ^2**	***p***
Sex	1	3.1	0.077
Diet	3	2.2	0.524

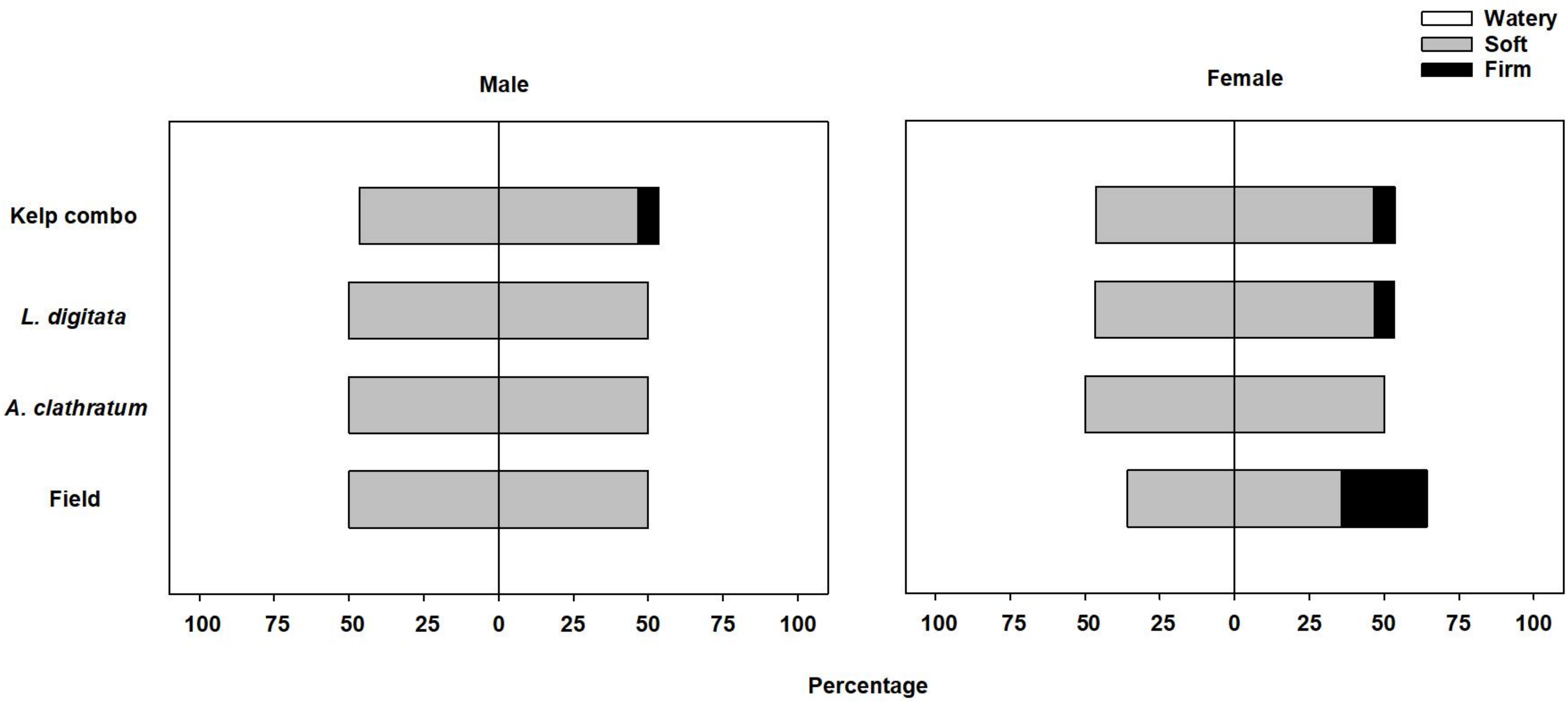
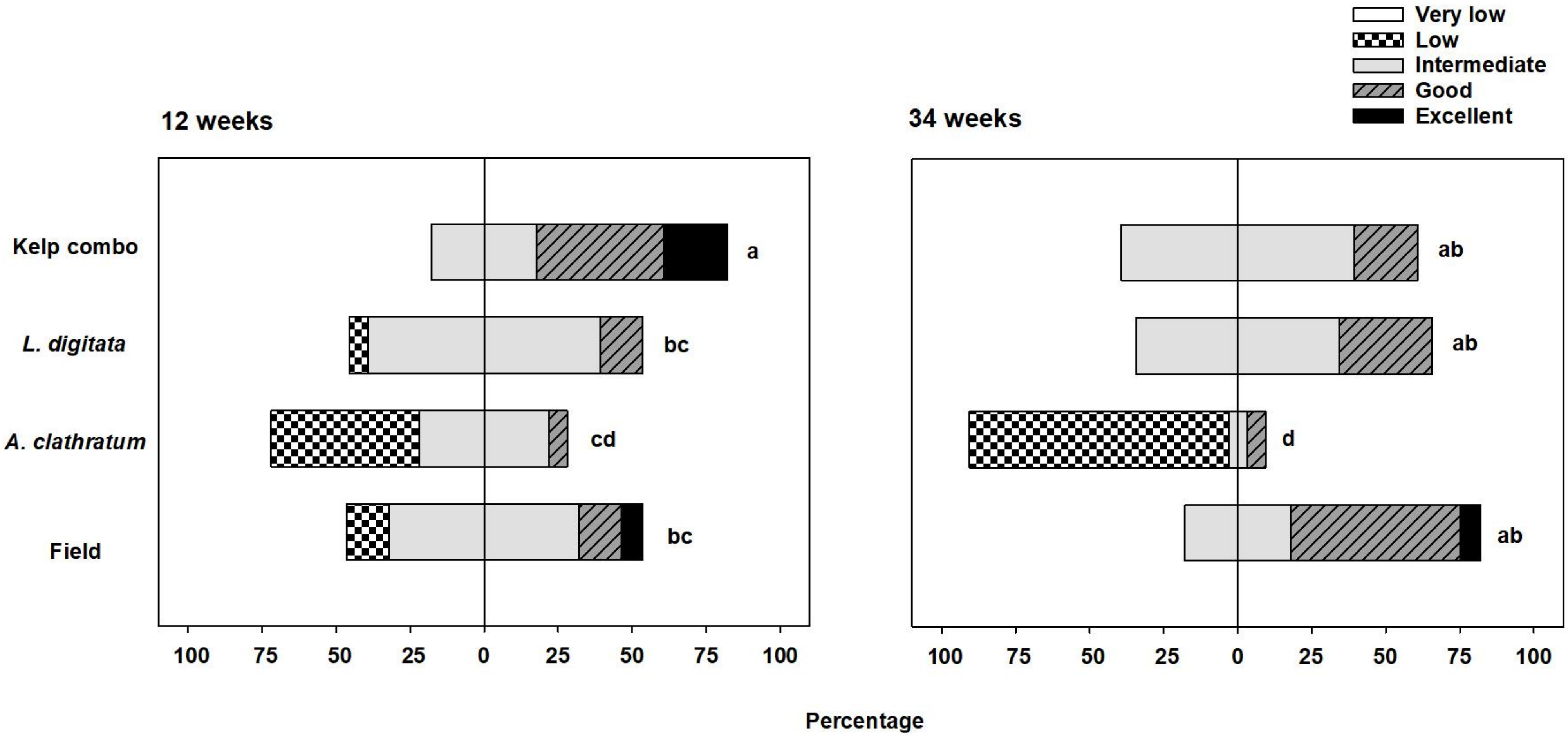

Watery
Soft
Firm
Male
Female
Kelp combo
L. digitata
A. clathratum
Field
100
75
50
25
0
25
50
75
100
Percentage

Figure 2.7 Percentage of gonads exhibiting a watery, soft, or firm texture in male and female green sea urchins (*Strongylocentrotus droebachiensis*) fed either one of the three kelp diets (kelp combo, *Laminaria digitata*, and *Agarum clathratum*), or collected from the field, 12 and 34 wk after the start of the gonad enhancement experiment (for each sex: n = 14 [kelp combo], 15 [*L. digitata*], 16 [*A. clathratum*], and 14 [natural diet]; data pooled across feeding durations [12 and 34 wk] because best-fitting multiple logistic regression model did not include feeding Duration [see Table 2.8]).

Table 2.9 Summary of best-fitting multiple ordinal logistic regression (applied to modes of raw data) examining the effect of Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and feeding Duration (12 and 34 wk) on green sea urchin (*Strongylocentrotus droebachiensis*) overall gonad quality (R^2_{MF}=0.28; see Table D.3 for results of stepwise model selection procedure).

Source of variation	***df***	χ^2	***p***
Diet	3	57.5	<0.001
Duration	1	0.3	0.570
Diet*Duration	3	19.0	<0.001



Very low
Low
Intermediate
Good
Excellent
12 weeks
34 weeks
Kelp combo
L. digitata
A. clathratum
Field
a
bc
cd
bc
ab
ab
d
ab
100
75
50
25
0
25
50
75
100
Percentage

Figure 2.8 Percentage of gonads exhibiting very low, low, intermediate, good, or excellent overall quality (segmentation and textural appearance combined) in green sea urchins (*Strongylocentrotus droebachiensis*) fed one of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*), or collected from the field, 12 and 34 wk after the start of the gonad enhancement experiment. Bars not sharing the same letter, across both panels, differ statistically (LS means tests with Bonferroni correction of probabilities, p<0.05: n = 14 and 14 [kelp combo], 14 and 16 [*L. digitata*], 16 and 16 [*A. clathratum*], and 14 and 14 [natural diet] for respectively the 12th and 34th weeks; data pooled across male and female sea urchins because best-fitting multiple logistic regression model did not include urchin Sex [See Table 2.9]).

(Figure 2.8). Gonad texture quality decreased considerably after 34 weeks in sea urchins fed *A. clathratum*, with 88% ranked as low (Figure 2.8).

2.3.4 Taste

As determined with stepwise multiple ordinal logistic regression, the best-fitting models to gonad overall preference rank data (Tables D.4 and 2.10) and to gonad taste rank data (Tables D.5 and 2.10) were:

$$\text{Overall preference rank} = \text{Di}$$

$$\text{Taste rank} = \text{Di}$$

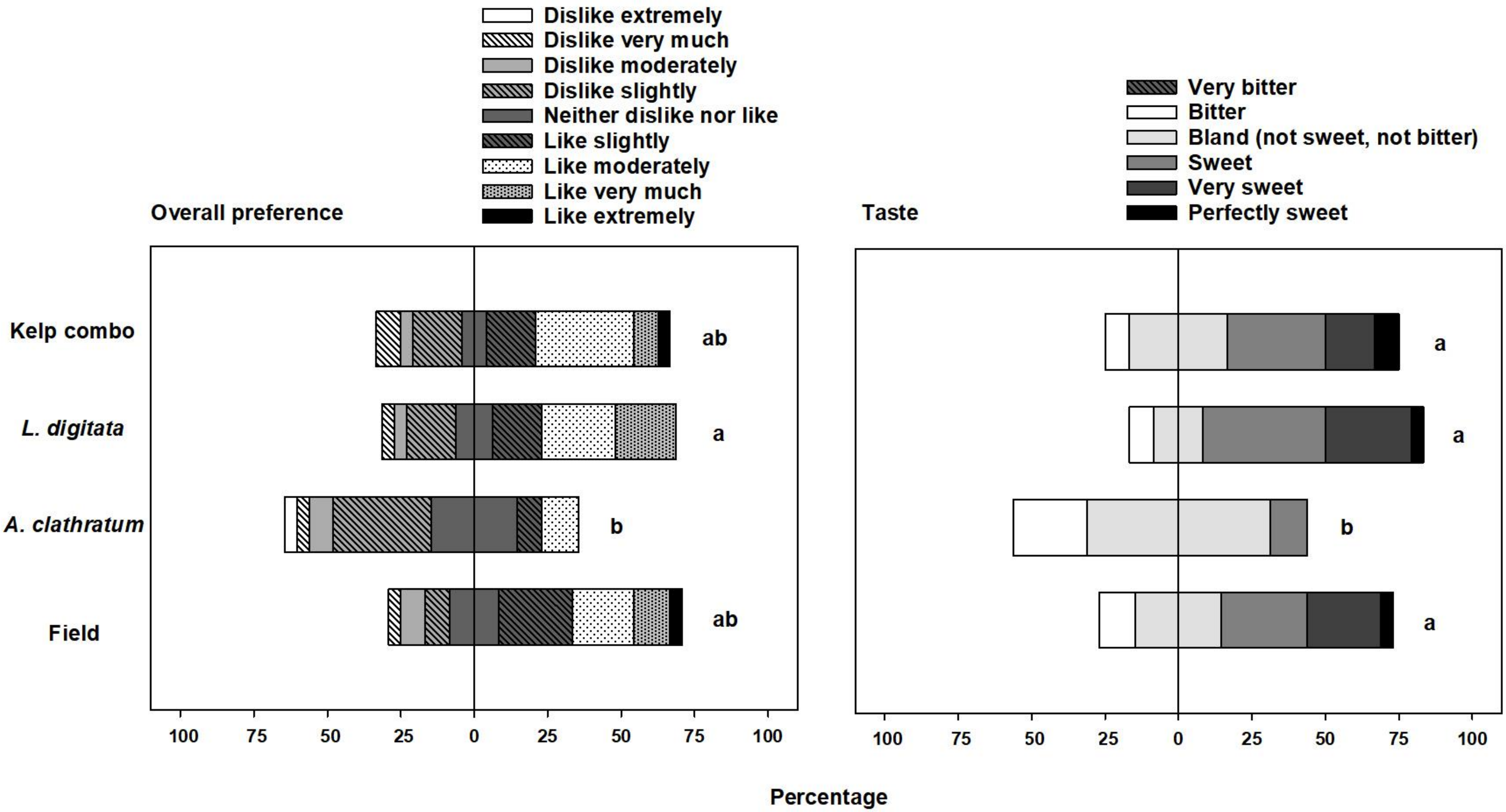
where Di denotes Diet (no effect of panelist Experience). Panelists largely disliked gonads of urchins fed *A. clathratum*, but equally liked sea urchins fed a kelp combo, *L. digitata*, or from the field, as shown by the same high proportions (~63%) of gonads liked slightly to extremely (Figure 2.9). We observed similar patterns of appreciation for gonad taste, with a majority (88%) of panelists evaluating gonads of *A. clathratum*-fed sea urchins as bland or bitter, whereas gonads of sea urchins fed either a kelp combo, *L. digitata*, or collected from the field was ranked as predominantly (>58%) sweet to perfectly sweet (Figure 2.9).

2.4 DISCUSSION

Our study demonstrates the potential to increase GSI and gonad quality of *S. droebachiensis* through laboratory feeding of natural kelp diets. After 12 weeks of feeding, only sea urchins fed *L. digitata* achieved market standard yield and quality; after 34 weeks, sea urchins fed a kelp combo, *L. digitata*, or from the field, all achieved market standard yield and quality,

Table 2.10 Summary of best-fitting multiple ordinal logistic regressions (applied to raw data) examining the effect of Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) on green sea urchin (*Strongylocentrotus droebachiensis*) gonad overall preference (R^2_{MF}=0.03; see Table D.4 for results of stepwise model selection procedure) and taste (R^2_{MF}=0.07; see Table D.5 for results of stepwise model selection procedure).

Parameter	**Source of variation**	***df***	**χ^2**	***p***
Overall preference	Diet	3	10.1	0.018
Taste	Diet	3	19.9	<0.001



Dislike extremely
Dislike very much
Dislike moderately
Dislike slightly
Neither dislike nor like
Like slightly
Like moderately
Like very much
Like extremely
Overall preference
Kelp combo
L. digitata
A. clathratum
Field
ab
a
b
ab
100 75 50 25 0 25 50 75 100
Very bitter
Bitter
Bland (not sweet, not bitter)
Sweet
Very sweet
Perfectly sweet
Taste
a
a
b
a
100 75 50 25 0 25 50 75 100
Percentage

Figure 2.9 Percentage of gonads exhibiting overall preference ranks from Dislike extremely (1) to Like extremely (9) (left panel) and taste ranks from Very bitter (1) to Perfectly sweet (6) (right panel) in green sea urchins (*Strongylocentrotus droebachiensis*) fed either of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*), or collected from the field, 34 wk after the start of the gonad enhancement experiment. Bars not sharing the same letter differ statistically (LS means tests with Bonferroni correction of probabilities, $p<0.05$; $n=24$ per diet [96 total]; data were pooled across panelists Experience because the best-fitting ordinal logistic regression model did not include panelist Experience [see Table 2.10]).

although colour and texture were not top quality for all diets (Table 2.11). Sea urchins fed *A. clathratum* did not meet market standard yield or quality after 12 or 34 weeks feeding (Table 2.11).

Overall, *L. digitata* has the highest potential as a natural kelp diet for increased gonad production, and market quality roe, thus offering the best candidate species among locally abundant kelps for future gonad enhancement and aquaculture practices of urchins from eastern Newfoundland. However, high GSI did not always coincide with high quality gonads, with respect to colour, texture, and taste. Successful enhancement or culturing of any sea urchin species requires high GSI, however, high GSI will not help product quality if the sea urchin roe tastes or looks inferior. Although 12 and 34 weeks of feeding increased GSI, 34 weeks did not improve all quality parameters, suggesting that 12 weeks may be enough to produce market quality roe from *S. droebachiensis*, which, from a production standpoint, would offer greater potential at lower costs.

2.4.1 Gonadosomatic Index

After 12 weeks, sea urchins fed *L. digitata* had the highest GSI at 14.4 ± 0.4 %, exceeding the market minimum for Newfoundland (≥8%), and sea urchins fed a kelp combo the second highest at 7.7 ± 0.3 %, just below the provincial minimum (Explorations Unlimited Inc 2006; Pisces Consulting Limited 2014). In contrast, sea urchins fed *A. clathratum*, and sea urchins collected from the field produced an average GSI <3%, far below the province minimum. Therefore, laboratory feeding of *L. digitata* enhanced sea urchin GSI during the rapid warming period (see section 2.3.1), and directly following the natural spawning period of *S. droebachiensis* in Newfoundland, when gonads from wild sea urchins is not viable for harvest (Himmelman 1978; Pisces Consulting Limited 2014). Other studies reported similar results for *S. droebachiensis* from Nova Scotia (Canada) and Maine (USA), where short-term feeding (8-12 weeks) of natural kelp

Table 2.11 Summary of results of the effect of each diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, or sea urchins from the natural diet sampled from the field) on gonadosomatic index (GSI), gonad colour, texture, and taste, for the roe enhancement experiment, after 12 and 34 weeks of feeding. Letters represent quality based on results (see section 2.3 for details): H = high quality; I = intermediate quality; and L= low quality.

	GSI		**Colour**		**Texture**		**Taste**	
Diet	12 wk	34 wk	12 wk	34 wk	12 wk	34 wk	12 wk	34 wk
Kelp combo	I	H	I	I	H	I	-	H
Laminaria digitata	H	H	I	I	I	I	-	H
Agarum clathratum	L	L	L	L	L	L	-	L
Field	L	H	I	I	I	I	-	H

diets (*L longicruris* and *L. saccharina*, *A. esculenta*, *Ulva lactuca* and *Palmaria palmata* respectively) increased GSI to >10% in post-spawned sea urchins (Minor & Scheibling 1997; Vadas Sr. *et al.* 2000).

After 34 weeks, GSI in sea urchins from kelp combo, *L. digitata*, or from the field, all exceeded the 8% market standard of the Newfoundland sea urchin fishery (Pisces Consulting Limited 2014), but we observed a higher GSI in females than males from the same treatments. These results echo Siikavuopio *et al.* (2014), who reported higher GSI in female green sea urchins than males by the end of their experiment, despite similar growth capacity in both sexes. GSI increased from 12 to 34 weeks for all diets, except *A. clathratum*, and, of the three diets, GSI was highest in sea urchins from the kelp combo and *L. digitata* diets. Previous studies documented that long-term feeding of natural and artificial diets can significantly increase GSI of many sea urchin species (Jong-Westman *et al.* 1995; Carrier *et al.* 2017; Prato *et al.* 2018). Feeding trials previously conducted in western Newfoundland demonstrated high GSI and market quality gonad in *S. droebachiensis* fed natural diets of *Laminaria* spp., but did not find market standard GSI or quality in urchins fed *A. esculenta* or *A. clathratum* (Cuthbert *et al.* 1995; Hooper *et al.* 1996).

Our study documents higher GSI in sea urchins fed *L. digitata* for 12 weeks than in sea urchins from the field at 34 weeks, the peak harvest time for wild sea urchins in Newfoundland; (Pisces Consulting Limited 2014). This finding suggests significant potential for future gonad enhancement of *S. droebachiensis* in eastern Newfoundland if laboratory fed sea urchins can produce a high GSI year round, rather than depending on the seasonal reproductive cycle of *S. droebachiensis*; this would also allow sea urchin divers to expand out of harvesting only in kelp beds and allow them to also harvest in low yielding sea urchin barrens. The smaller feeding duration could also reduce production costs if market quality gonads can be achieved in less time.

Our study assessed GSI at the onset of the experiment and at 12 and 34 weeks into it. *S. droebachiensis* fed *L. digitata* could potentially yield a GSI of 8% or more in less than 12 weeks. Further testing with more frequent measurement of GSI in the initial phases (e.g. at 4 and 8 weeks) would help determine the minimum feeding duration to achieve market-standard gonad yields.

2.4.2 Colour

Quality of gonad colour in *S. droebachiensis* improved from 12 to 34 weeks in sea urchins fed a kelp combo, *L. digitata*, and from sea urchins collected from the field, though colour improved more in females than males. Poor colour quality characterized male and female sea urchins fed *A. clathratum* at both feeding durations, with worse colour quality in the longer feeding duration than at 12 weeks. Quality improved significantly in female sea urchins fed a kelp combo and from the field. Deviation from ideal gonad market color (ΔE_{ab}^{*}) was highest in male and female sea urchins fed *A. clathratum* and smallest in female urchins fed a kelp combo, *L. digitata*, and from the field, after 34 weeks. However, given a $\Delta E_{ab}^{*} > 3$ in all diets, no diet produced the exact same colour as the market standard (Adekunte *et al.* 2010).

High gonad lightness (L^*) characterized male and female sea urchins fed a kelp combo, *L. digitata*, and from the field, after 12 weeks, and improved in sea urchins fed a kelp combo and from the field after 34 weeks. Feeding duration had no effect on gonad redness (a^*) in female sea urchins fed a kelp combo, *L. digitata*, and from the field, however, a^* decreased in all sea urchins from all diets from 12 to 34 weeks. The decrease in gonad redness of male and not female sea urchins corresponds with typical colour differences; yellower gonads generally characterize males in contrast to more orange female gonads (Lee & Haard 1982; Jong-Westman *et al.* 1995; Siikavuopio *et al.* 2014). Lastly, gonad yellowness (b^*) was highest in female sea urchins fed a kelp combo, *L. digitata*, and from the field, and increased from 12 to 34 weeks in those fed a kelp combo, and sea urchins from the field. Feeding duration did not significantly affect b^* of males fed a kelp combo, *L. digitata*, or from the field. The changes in colour observed in our study from 12 to 34 weeks highlights the seasonal changes in sea urchin gonad colour, as previously demonstrated in *S. francisacanus*, with best quality colour occurring in the fall (McBride *et al.* 2004).

Importantly, our study demonstrated consistency in colour composition of female sea urchins fed *L. digitata*, where feeding duration had no significant effect on any of the colour parameters and gonad colour remained consistent between the two feeding durations. Although feeding duration affected male sea urchins fed *L. digitata* (increased lightness and decreased redness at 34 weeks), the colour values for males at 12 weeks corresponded to intermediate market quality. Therefore, colour improvement in sea urchins fed *L. digitata* may not require the longer feeding duration.

The composition of *L. digitata* in comparison with the other diets likely explains the consistency in gonad colour of sea urchins fed *L. digitata* because diet composition strongly influences sea urchin gonad colour (Pearce *et al.* 2002, 2003; Robinson *et al.* 2002; Shpigel *et al.* 2005; Symonds *et al.* 2007; Woods *et al.* 2008). In particular, Pearce *et al.* (2002) demonstrated no effect of protein source (i.e. rockweed, soybean, fish meal, etc.) on gonad colour but reported a negative effect of increased dietary protein concentration on gonad colour. Additionally, they demonstrated improved colour with increased concentrations of the pigment β-carotene. High levels of β-carotene characterize all brown seaweeds (Burtin 2003), however, species differ in carbohydrate and protein levels. Despite similar carbohydrate levels in *A. esculenta* and *L. digitata* (72 and 71% respectively; Schiener *et al.* 2015) protein content differs (~9-12% and 5-8%

respectively, varying with season; Schiener *et al.* 2015). Therefore, given the chemical composition of *L. digitata* and the results from our study, we propose *L. digitata* as the best candidate for enhancing gonad colour in *S. droebachiensis* from eastern Newfoundland.

2.4.3 Gonad segmentation, textural appearance, and overall quality

The number of sea urchin gonads with complete structural segmentation decreased in males and females fed a kelp combo and *L. digitata* from 12 to 34 weeks; male and female sea urchins fed *A. clathratum* had the most gonads with no evident segmentation and the lowest overall quality compared to gonads from urchins fed the other three diets. Longer feeding duration did not improve textural appearance in either sex, or any diet; most sea urchin gonads ranked as soft for textural appearance, and few ranked as firm. After 12 weeks, sea urchins fed *A. esculenta* had the best texture quality, although sea urchins fed a kelp combo, *L digitata* or from the field after 34 weeks were statistically similar in overall quality ranks, even though the former two diets resulted in gonads with primarily intermediate ranks.

Previous work demonstrated differential seasonal quality in sea urchin roe texture; *S. francisacanus* gonads had higher texture quality in the fall when gonads were still developing, and lower quality in the spring when gonads were more mature and the sea urchins were closer to spawning (McBride *et al.* 2004). Additionally, we found no difference in texture quality between laboratory sea urchins fed a kelp combo or *L. digitata*, and sea urchins sampled from the field. In contrast, Bureau *et al.* (1997), reported better texture quality in laboratory kelp fed *S. francisacanus* than sea urchins of the same size class (47-56 mm) sampled from the field.

Texture quality in sea urchins depends more on seasonality/stage of gametogenesis (Lawrence 2001; McBride *et al*. 2004; Unuma 2002) than diet composition (Pearce *et al.* 2002; Woods *et al.* 2008; Phillips *et al.* 2009). Gonads in later stages of gametogenesis become very delicate, and eggs or sperm may leak from the gonoduct with the slightest touch, subsequently diminishing gonad textural consistency (Unuma 2002). Future studies investigating texture quality in sea urchins should evaluate the relationship between gametogenesis and diet to further understand the links to overall gonad texture quality in *S. droebachiensis*. Additionally, although our study aimed for greater objectivity than previous studies, our ranking scales nonetheless added some subjectivity; ideally a more quantitative method could be used to assess texture in sea urchin gonads. McBride *et al*. (2004) eliminated the subjectivity of texture analysis by quantitatively analyzing hardness and resilience of *S. francisacanus* gonads, and future work on *S. droebachiensis* should consider using similar methods.

2.4.4 Taste

Taste panel responses spanned all categories for overall preference and taste, however, gonads from sea urchins fed either a kelp combo, *L. digitata*, or sampled from the field yielded positive responses (i.e. preferred and sweet flavour), and gonads from sea urchins fed *A. clathratum* primarily ranked negatively (i.e. not preferred and bitter). Our results concur with previous research that reported sweet tasting gonads from urchins fed kelp in a laboratory setting (Phillips *et al.* 2009; Luo *et al.* 2014). Diet composition strongly influences taste in sea urchins, so much so that the composition of artificial diets can alter the taste of gonads negatively, resulting in a more bitter flavour comparted to sweet flavoured gonads from urchins fed kelp diets (Pearce *et al.* 2002, 2003, 2004; Woods *et al.* 2008).

2.4.5 Conclusion

In conclusion, our study demonstrates the production of marketable gonads from *S. droebachiensis* in eastern Newfoundland, while addressing short- and long-term effects of laboratory feeding on GSI and gonad quality. We demonstrated that the use of locally abundant natural kelp diets, can enhance gonads in *S. droebachiensis* by increasing GSI and gonad quality to be comparable to, or better than, that of wild sea urchins of eastern Newfoundland. Our study identified *L. digitata* as the most promising kelp diet to promote urchin gonad enhancement in eastern Newfoundland. Our results also demonstrate the potential for high quality roe from *S. droebachiensis* fed *L. digitata* for as little as 12 weeks. Future work in sea urchin gonad enhancement of *S. droebachiensis* in eastern Newfoundland should therefore focus on shorter feeding durations, in order to determine the shortest feeding duration necessary to achieve market quality roe, thus decreasing production costs.

CHAPTER III

Summary

3.1. Overall objective of the study

Green sea urchin, *Strongylocentrotus droebachiensis*, is harvested for its gonads, more commonly referred to as "roe" or "uni", producing one of the most valued types of urchin roe on the Japanese and Korean seafood markets. Global demand for sea urchin roe has increased, most prominently in Europe, however, this increased demand has led to the depletion of wild sea urchin populations in many countries through overexploitation and poor resource management (Andrew *et al.* 2002). Green sea urchins occur in shallow rocky habitats along the coast of Newfoundland but due to unreliable wild populations that limit the current fishery to harvesting in select areas (i.e. kelp beds and not the extensive urchin barrens), the province needs more practical and consistent production methods to use this abundant marine resource sustainably as a means of supplementing unsteady fishery and rapidly growing aquaculture sectors (Pisces Consulting Limited 2014; DFA 2015b). Following feeding trials in Newfoundland in the 1990s (Cuthbert *et al.* 1995, Hooper *et al.* 1997), no further research has occurred in the province since, despite promising results.

Our study was the first of a planned series aimed at examining the potential for a sustainable green sea urchin roe industry in eastern Newfoundland. Specifically, we used a multi-month experiment to examine the effects of different locally abundant kelp species (*Alaria esculenta*, *Laminaria digitata*, and *Agarum clathratum*) on gonadosomatic index (GSI) and gonad quality (colour, texture, and taste) of green sea urchins from eastern Newfoundland. The 34-week gonad enhancement experiment evaluated GSI and gonad quality at two sampling points (12 and 34 weeks) in order to test the effect of feeding duration and allowing comparison of gonad production at different times of the reproductive cycle of *S. droebachiensis* in eastern Newfoundland. The experiment was carried out at the Ocean Sciences Centre of Memorial University of Newfoundland with green sea urchins collected from Bread and Cheese Cove (BCC) in Bay Bulls, and kelp collected from BCC, Logy Bay, or Flat Rock Cove.

3.2 Summary of results

Chapter II examined the effects of three natural kelp diets on GSI and gonad quality in *S. droebachiensis* from eastern Newfoundland, during two segments of the reproductive cycle. We demonstrated that feeding urchins with locally abundant natural kelp diets can significantly enhance GSI and gonad quality over 12 and 34 weeks, however, not all kelps yielded positive results. The use of a kelp combo (*A. esculenta* and *L. digitata*), and *L. digitata* yielded positive results for most gonad quality criteria, whereas urchins fed *A. clathratum* lacked market quality gonad in any of the criteria. Our study demonstrates that GSI in *S. droebachiensis* from eastern Newfoundland increases most when fed *L. digitata* over short (12-weeks) and long (34-weeks) periods relative to other kelps.

3.2.1 Kelp combo (*Alaria esculenta* and *Laminaria digitata*)

Sea urchins fed a kelp combo were fed *A. esculenta* for the first 12 weeks of the study, and then switched to *L. digitata* for the remainder of the experiment. *Alaria esculenta* was not used for the full duration of the experiment given its natural senescence in early fall in eastern Canada. After 12-weeks of feeding, GSI in sea urchins fed a kelp combo averaged ~8%, a >100% percent change from onset; GSI continued to improve significantly after 34-weeks, with average GSI exceeding 20%, a >300% increase from onset. Overall, GSI of sea urchins fed a kelp combo was intermediate quality after 12 weeks, and high quality at 34 weeks.

Despite high colour quality for L^*, a^*, and b^*, ΔE^*_{ab} >3 indicated colour below market standard. Thirty-four (34) weeks of feeding improved gonad colour quality in female sea urchins, however ΔE^*_{ab} remained >3 and colour therefore remained below top market quality. Male gonad colour only improved after 34 weeks with respect to L^*; all other colour parameters either remained consistent (ΔE^*_{ab} and b^*) or decreased (a^*). Overall, we observed intermediate colour quality for both male and female sea urchins at 12 and 34 weeks.

For gonad texture quality, of the laboratory-fed sea urchins, only those fed a kelp combo yielded gonads with excellent overall quality rankings and the best texture quality at 12 weeks of all the four diets tested. Texture did not remain stable after 34 weeks with an intermediate overall quality ranking for the majority of gonads at longer feeding durations. Overall, we observed high quality texture in urchins fed a kelp combo at 12 weeks, but intermediate quality at 34 weeks.

Lastly, we found high quality in the taste of gonads from urchins fed a kelp combo at 34 weeks, with the sweet flavour favoured by the market in much of the roe. Of the four diets, the kelp combo was the second-best diet for potential gonad enhancement. Although this diet produced the best texture at 12 weeks, GSI was not sufficiently high to be considered an option for gonad enhancement on a short-term scale, however, a combination of *A. esculenta* and *L. digitata* over a shorter duration could produce higher GSI, and better texture quality in gonads.

3.2.2 *Laminaria digitata*

Past studies document the effectiveness of *Laminaria* spp. in enhancing sea urchin gonad development (Lemire & Himmelman 1996; Hooper *et al.* 1997; Vadas Sr. *et al.* 2000) and results of our study confirm these findings. A natural kelp diet of *L. digitata* increased GSI in *S.*

droebachiensis at 12 (>10%) and 34 weeks (>20%). Consistently intermediate colour quality in gonads for both feeding durations, deviated slightly only in male sea urchins at 34 weeks, however, ΔE^*_{ab} was consistently >3 and resulted in intermediate quality ranking for both durations. Texture quality also remained intermediate for both feeding durations; despite high structural segmentation at 12 weeks, the proportion of gonads with complete segmentation lowered after 34 weeks, whereas feeding duration had no effect on textural appearance and gonads were consistently ranked as soft. Consequently, overall texture quality remained intermediate for both feeding durations. Taste panelists ranked gonads from urchins fed *L. digitata* highly and gonads achieved market quality sweet flavour with the longer feeding duration.

Overall, sea urchins fed *L. digitata* yielded the highest quality gonads at 12 and 34 weeks, despite only intermediate colour and texture. Additionally, the GSI of *S. droebachiensis* fed *L. digitata* for 12 weeks, exceeded the GSI of sea urchins from the natural diet at 34 weeks, corresponding to the normal peak time for sea urchin fishing. The results of our study highlight the potential to achieve market quality GSI in *S. droebachiensis* from eastern Newfoundland in as little as 12 weeks, noting that *L. digitata* was the only diet to successfully yield viable market quality gonads during this point of the reproductive cycle.

3.2.3 *Agarum clathratum*

Feeding green sea urchins *A. clathratum* did not yield positive results, with negative ∆GSI for both *A. clathratum* feeding durations. The highest ΔE^*_{ab} of all the diets characterized the gonads from these sea urchins, as well as low values for all other colour parameters. Low texture and taste quality, and insufficient market quality characterized sea urchins fed *A. clathratum* for either parameter. Overall, *A. clathratum* shows poor potential as a natural kelp diet to explore for future gonad enhancement work in *S. droebachiensis* from eastern Newfoundland.

3.2.4 Natural diet (sampled from the field)

The gonads in sea urchins sampled from the field at each feeding duration yielded GSI, colour, texture, and taste expected of sea urchins from the region, given the timing of the reproductive cycle at 12 and 34 weeks. At 12 weeks, low GSI in field sea urchins further highlights the seasonality of the sea urchin fishery in Newfoundland given that GSI standards alone exclude the roe from current international markets. However, after 34 weeks GSI increased significantly in field sea urchins, with intermediate colour and texture quality and high taste quality. Again, these results reflect expectations for sea urchins from this region given that the 34-week point coincided with the typical start of the sea urchin fishery season in Newfoundland. However, at 34 weeks, higher GSI characterized sea urchins from the kelp combo and *L. digitata* diets, with quality parameters comparable to field sea urchins.

3.3 Importance of study

Declining wild stocks of several economically important marine species, including the Atlantic cod, snow crab, and northern shrimp, in a once prosperous fishery in Newfoundland, leaves the province in search of new sources of economic growth. Some of this growth should come in the form of aquaculture, given that successes in several species offers the province new hope for economic renewal while continuing to utilize the substantial marine resources available to the province. However, continued success in the aquaculture sector requires further diversification of species; the green sea urchin represents a promising candidate.

Our study is the first to investigate gonad enhancement of *S. droebachiensis* from eastern Newfoundland and we have demonstrated potential utilization of this species in the province with positive gonad enhancement results. Additionally, our study adds to previous work in western Newfoundland that also demonstrated successful gonad enhancement in *S. droebachiensis* on locally abundant natural kelp diets (Cuthbert *et al*. 1995, Hooper *et al*. 1996). Sea urchin enhancement holds clear market potential for Newfoundland in the future and could offer one possible solution to help rural regions of the province establish a new, potentially prosperous industry.

3.4 Future Directions

Our study provides a framework for further research into gonad enhancement of *S. droebachiensis* in eastern Newfoundland, as well as other areas of the province since the species occurs in coastal regions throughout the province. However, we used only natural kelp diets, which although these diets provided positive results, large-scale production using natural diets may not prove maintainable if kelp populations are not also managed sustainably. Therefore, future research should investigate artificial diets in tandem with natural diets to determine whether combining both types of diets can yield positive GSI and gonad quality results.

The most promising result from our study is the possibility of achieving market quality gonad in *S. droebachiensis* in as little as 12-weeks. From a production standpoint, this potential is far more desirable than achieving market quality only after 6 months or longer. Our study did not assess GSI and gonad quality between the onset of, and 12 weeks into the experiment. It may be possible to achieve market quality roe in less than 12 weeks, suggesting a need to test multiple feeding durations to achieve maximum market quality in the least amount of time.

Even though kelp combo and *L. digitata* yielded high GSI, gonad colour and texture quality were consistently intermediate. Texture quality in sea urchins depends more on seasonality and stage of gametogenesis (Lawrence 2001; McBride et al. 2004; Unuma 2002) than diet composition (Pearce *et al.* 2002; Woods *et al.* 2008; Phillips *et al.* 2009). More mature gonads in later stage of gametogenesis become very delicate, and eggs or sperm may leak from the gonoduct with the slightest touch, subsequently diminishing gonad texture consistency (Unuma 2002). Additionally, most international markets prefer urchin gonads with more nutritive phagocytes (nutritional cells that support the development of reproductive cells) than reproductive cells because a high proportion of reproductive cells in the gonads reduce quality of texture (Lawrence 2001; Unuma, 2002). Therefore, manipulating gametogenesis rather than diet may offer the solution to achieving more consistent texture in sea urchin gonads (Walker *et al*. 2007). Photoperiod (James & Siikavuopio 2011), water temperature (Bronstein & Loya 2015), and food availability (Garrido & Barber, 2001) all influence reproduction in multiple sea urchin species, and experimental manipulation of these factors can alter natural reproductive cycles of sea urchins (Bronstein & Loya 2015; Kelly 2001; Kirchhoff *et al*. 2010; Walker & Lesser 1998). Additionally, manipulating gametogenesis in *S. droebachiensis* may also improve colour quality. In *S. francisacanus*, seasonality significantly impacted sea urchin gonad colour, with brighter and better colors in the fall, coinciding with the later stages of gametogenesis (McBride *et al.* 2004). Future research on *S. droebachiensis* in eastern Newfoundland should therefore consider manipulation of gametogenesis to achieve better gonad quality in combination with high GSI.

LITERATURE CITED

Adekunte AO, Tiwari BK, Cullen PJ, Scannell AGM & O'Donnell CP (2010) Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. Food Chem 122(3):500–507.

Adey WH & Hayek L-AC (2011) Elucidating marine biogeography with macrophytes: quantitative analysis of the North Atlantic supports the thermogeographic model and demonstrates a distinct subarctic region in the northwestern Atlantic. Northeast Nat 18(8):1–128.

Allen EI & Seaman CA (2007) Likert scales and data analyses. Qual Prog 40:64–65.

Anderson SC, Mills Flemming J, Watson R & Lotze HK (2011) Rapid global expansion of invertebrate fisheries: trends, drivers, and ecosystem effects. PLoS ONE 6(3):e14735.

Andrew NL, Agatsuma Y, Ballesteros E, Bazhin AG, Creaser EP, Barnes DKA, Botsford LW, Bradbury A, Campbell A, Dixon JD, Einarsson S, Gerring PK, Hebert K, Hunter M, Hur SB, Johnson CR, Juinio-Menez MA, Kalvass P, Miller RJ, Moreno CA, Palleiro JS, Rivas D, Robinson SML, Schroeter SC, Steneck RS, Vadas RL, Woodby DA & Xiaoqi Z (2002) Status and management of world sea urchin fisheries. Oceanogr Mar Biol Annu Rev 40:343–425.

Azad AK, Pearce CM & McKinley RS (2011) Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*). Aquaculture 317:187–196.

Blain C & Gagnon P (2014) Canopy-forming seaweeds in urchin-dominated systems in eastern Canada: structuring forces or simple prey for keystone grazers? PLOS ONE 9(5):e98204.

Botsford LW, Campbell A & Miller R (2004) Biological reference points in the management of North American sea urchin fisheries. Can J Fish Aquat Sci 61:1325–1337.

Brant R (1990) Assessing proportionality in the proportional odds model for ordinal logistic regression. Biometrics 46:1171–1178.

Bureau D, Campbell A & Hartwick EB (1997) Roe enhancement in the red sea urchin, *Strongylocentrotus franciscanus*, fed the bull kelp, *Nereocystis leutkeana*. Bull Aquacul Assoc Canada:26–30.

Burtin P (2003) Nutritional value of seaweeds. Electron J Environmental Agric Food Chem 2:498-503.

Carboni S, Hughes AD, Atack T, Tocher DR & Migaud H (2015) Influence of broodstock diet on somatic growth, fecundity, gonad carotenoids and larval survival of sea urchin. Aquac Res 46(4):969–976.

Carrier TJ, Eddy SD & Redmond S (2017) Solar-dried kelp as potential feed in sea urchin aquaculture. Aquac Int 25:355–366.

Chopin T (2015) Marine aquaculture in Canada: well-established monocultures of finfish and shellfish and an emerging integrated multi-trophic aquaculture (IMTA) approach including seaweeds, other invertebrates, and microbial communities. Fisheries 40:28–31.

Cuesta-Gomez DM & Sánchez-Saavedra MP (2014) Increased gonad growth of the purple sea urchin (*Strongylocentrotus purpuratus*) fed the giant kelp (*Macrocystis pyrifera*) and the sea lettuce (*Ulva lactuca*) enriched with nutrients. Aquac Res 47(7):2150–2163.

Cuthbert FM, Hooper R & McKeever TJ (1995) Sea urchin feeding and ranching experiments. St. John’s Nfld: Canadian Centre for Fisheries Innovation.

Department of Fisheries and Aquaculture (DFA) (2015a) Annual Report 2014-15.

Department of Fisheries and Aquaculture (DFA) (2015b) Newfoundland and Labrador sustainable aquaculture strategy 2014.

Explorations Unlimited Inc (2006) Sea urchin fishery profiles.

Foster MC, Byrnes JEK & Reed DC (2015) Effects of five southern California macroalgal diets on consumption, growth, and gonad weight, in the purple sea urchin *Strongylocentrotus purpuratus*. PeerJ 3:e719.

Frey DL & Gagnon P (2016) Spatial dynamics of the green sea urchin *Strongylocentrotus droebachiensis* in food-depleted habitats. Mar Ecol Prog Ser 552:223–240.

Frey DL & Gagnon P (2015) Thermal and hydrodynamic environments mediate individual and aggregative feeding of a functionally important omnivore in reef communities. PLoS ONE 10(3):e0118583.

García SM, Yimin Y, Rice J & Charles A (2018) Rebuilding of marine fisheries. FAO Fish Aquac Tech Pap FAO Eng No 6302.

Government of Canada (2018) Canada’s Fisheries Fast Facts 2017. Available at: http://www.dfo-mpo.gc.ca/stats/facts-Info-17-eng.htm.

Harrell F (2001) Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. Springer-Verlag: New York.

Hemmert GAJ, Schons LM, Wieseke J & Schimmelpfennig H (2018) Log-likelihood-based pseudo-R^2 in logistic regression: deriving sample-sensitive benchmarks. Sociol Methods Res 47(3):507–531.

Himmelman JH (1978) Reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*. Can J Zool 56:1828–1836.

Himmelman JH (1984) Urchin feeding and macroalgal distribution in Newfoundland, eastern Canada. Nat Can 111:337–348.

Himmelman JH & Steele DH (1971) Foods and predators of the green sea urchin *Strongylocentrotus droebachiensis* in Newfoundland waters. Mar Biol 9:315–322.

Hooper R G, Cuthbert F M & McKeever T (1997) Feasibility trials for sea urchin aquaculture using natural feeds. Bull Aquacult Assoc Can 97:5–7.

Hooper RC, Cuthbert F & McKeever TJ (1996) Sea urchin aquaculture phase II: ration size, seasonal growth rates, mixed kelp diets, fish diet, old urchin growth and baiting confinement. St. John’s Nfld: Canadian Centre for Fisheries Innovation.

Hooper RG, Cuthbert FM & Patey D (1993) Newfoundland sea urchins: studies on roe quality, yield and maturation in relation to specific habitats around the coasts of Newfoundland. Newfoundland Department of Fisheries. St. John's NL.

James P, Noble C, Hannon C, Stefánsson G, Þórarinsdóttir G, Ziemer N & Lochead J (2016) Sea urchin fisheries, management and policy review. Nofima.

James PJ & Heath PL (2008a) Long term roe enhancement of *Evechinus chloroticus*. Aquaculture 278:89–96.

James PJ & Heath PL (2008b) The effects of season, temperature and photoperiod on the gonad development of *Evechinus chloroticus*. Aquaculture 285:67–77.

Jamieson S (2004) Likert scales: how to (ab)use them. Med Educ 38:1217–1218.

Johnson TR, Wilson JA, Cleaver C, Morehead G & Vadas R (2013) Modeling fine scale urchin and kelp dynamics: Implications for management of the Maine sea urchin fishery. Fish Res 141:107–117.

Jong-Westman M, March BE & Carefoot TH (1995) The effect of different nutrient formulations in artificial diets on gonad growth in the sea urchin *Strongylocentrotus droebachiensis*. Can J Zool 73:1495–1502.

Kleinbaum DG & Klein M (2010) Logistic regression: a self-learning text, 3rd ed. Springer-Verlag: New York.

Kuzon WM, Urbanchek MG & McCabe S (1996) The seven deadly sins of statistical analysis. Ann Plast Surg 37:265–272.

Larrauri García JA & Saura Calixto F (2000) Evaluation of CIE-lab colour parameters during the clarification of a sugar syrup from Mesquite pods (*Prosopis Pallida* L.). Int J Food Sci Technol 35:385–389.

Lawerence JM (2001) Edible sea urchins. Developments in Aquaculture and Fisheries Science 32:1–4.

Lawless HT & Heymann H (2010) Sensory evaluation of food: principles and practices. Springer Science & Business Media: New York.

Le Corre N, Pepin P, Han G, Ma Z & Snelgrove PVR (2019) Assessing connectivity patterns among management units of the Newfoundland and Labrador shrimp population. Fish Oceanogr 28:183–202.

Lee YZ & Haard NF (1982) Evaluation of the green sea urchin gonads as a food source. Can Inst Food Sci Technol J 15:233–235.

Lemire M & Himmelman JH (1996) Relation of food preference to fitness for the green sea urchin, *Strongylocentrotus droebachiensis*. Mar Biol 127:73–88.

Luo S, Zhao C, Chang Y, Feng W & Tian X (2014) Banana peel provides a new insight into improving gonad flavor in the sea urchin *Strongylocentrotus intermedius*. Aquac Int 22:833–841.

Mather C (2013) From cod to shellfish and back again? The new resource geography and Newfoundland's fish economy. Appl Geogr 45:402–409.

McBride SC, Price RJ, Tom PD, Lawerence JM & Lawerence AL (2004) Comparison of gonad quality factors: color, hardness and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. Aquaculture 233:405–422.

McCullagh P (1980) Regression models for ordinal data. J R Stat Soc Ser B Methodol 42:109–142.

Milich L (1999) Resource mismanagement versus sustainable livelihoods: the collapse of the Newfoundland cod fishery. Soc Nat Resour 12:625–642.

Minor MA & Scheibling RE (1997) Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. Mar Biol 129:159–167.

Mullowney DRJ, Dawe EG, Colbourne EB & Rose GA (2014) A review of factors contributing to the decline of Newfoundland and Labrador snow crab (*Chionoecetes opilio*). Rev Fish Biol Fish 24:639–657.

Neis B & Ommer R (2014) Moving forward: building economically, socially and ecologically resilient fisheries and coastal communities. Community-University Research for Recovery Alliance.

Newfoundland Aquaculture Industry Association (NAIA) (2015) Newfoundland and Labrador aquaculture production statistics. retrieved from http://naia.ca/nl-aquaculture/nl-production-stats/

Pathare PB, Opara UL & Al-Said FA-J (2013) Colour measurement and analysis in fresh and processed foods: a review. Food Bioprocess Technol 6:36–60.

Pearce CM, Daggett TL & Robinson SMC (2002) Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 214:307–332.

Pearce CM, Daggett TL & Robinson SMC (2004) Effect of urchin size and diet on gonad yield and quality in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 233:337–367.

Pearce CM, Daggett TL & Robinson SMC (2003) Effects of starch type, macroalgal meal source, and β-carotene on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis* (Müller), fed prepared diets. J Shellfish Res 22:505–519.

Pearce CM & Robinson SMC (2010) Recent advances in sea-urchin aquaculture and enhancement in Canada. Bull Aquac Assoc Can 108:38–48.

Phillips K, Bremer P, Silcock P, Hamid N, Delahunty C, Barker M & Kissick J (2009) Effect of gender, diet and storage time on the physical properties and sensory quality of sea urchin (*Evechinus chloroticus*) gonads. Aquaculture 288:205–215.

Pisces Consulting Limited (2014) Sea urchin fishery review. Department of Fisheries and Aquaculture.

Prato E, Fanelli G, Angioni A, Biandolino F, Parlapiano I, Papa L, Denti G, Secci M, Chiantore M, Kelly MS, Ferranti MP & Addis P (2018) Influence of a prepared diet and a macroalga (*Ulva* sp.) on the growth, nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus*. Aquaculture 493:240–250.

Quinn GP & Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press. New York.

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing: Vienna.

Robinson SMC, Castell JD & Kennedy EJ (2002) Developing suitable colour in the gonads of cultured green sea urchins (*Strongylocentrotus droebachiensis*). Aquaculture 206:289–303.

Scheibling RE & Hatcher BG (2007) Ecology of *Strongylocentrotus droebachiensis*. In: Edible sea urchins: biology and ecology. J.M. Lawerence (ed). Elsevier, Amsterdam. pp. 353–392.

Schiener P, Black KD, Stanley MS & Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. J Appl Phycol 27:363–373.

Schrank WE (2005) The Newfoundland fishery: ten years after the moratorium. Mar Policy 29:407–420.

Shpigel M, McBride SC, Marciano S, Ron S & Ben-Amotz A (2005) Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*. Aquaculture 245:101–109.

Siikavuopio SI, James P & Sæther B-S (2014) Long-term growth study of male and female green sea urchins, *Strongylocentrotus droebachiensis*, under constant light and temperature regime. J World Aquac Soc 45:481–486.

Singer J & Willett J (2003) Applied longitudinal data analysis: modeling change and event occurrence. Oxford University Press: Oxford; Toronto.

Snedecor G & Cochran W (1994) Statistical methods, 8th ed (DH Jones, Ed.). Iowa State University Press, Ames.

Stefánsson G, Kristinsson H, Ziemer N, Hannon C & James P (2017) Markets for Sea Urchins: A Review of Global Supply and Markets. Available at: http://urchinproject.com/2017/11/02/markets-sea-urchins-review-global-supply-markets/.

Surprenant D (2010) Aquaculture in eastern Canada. Library of Parliament.

Symonds RC, Kelly MS, Caris-Veyrat C & Young AJ (2007) Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9′-cis-echinenone as the dominant carotenoid in gonad colour determination. Comp Biochem Physiol B Biochem Mol Biol 148:432–444.

Takagi S, Murata Y, Inomata E, Endo H, Aoki MN & Agatsuma Y (2017) Improvement of gonad quality of the sea urchin *Mesocentrotus nudus* fed the kelp *Saccharina japonica* during offshore cage culture. Aquaculture 477:50–61.

Unuma T (2002) Gonadal growth and its relationship to aquaculture in sea urchins. In: The sea urchin: from basic biology to aquaculture. CRC Press. pp. 115–127.

Vadas Sr. RL, Beal B, Dowling T & Fegley JC (2000) Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. Aquaculture 182:115–135.

Veall MR & Zimmermann KF (1996) Pseudo-R^2 measures for some common limited dependent variable models. J Econ Surv 10:241–259.

Vizzini S, Miccichè L, Vaccaro A & Mazzola A (2015) Use of fresh vegetable discards as sea urchin diet: effect on gonad index and quality. Aquac Int 23:127–139.

Vuilleumier JP (1969) The 'Roche Yolk Colour Fan'—an instrument for measuring yolk colour. Poult Sci 48:767–779.

Woods CMC, James PJ, Moss GA, Wright J & Siikavuopio S (2008) A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus* Valenciennes. Aquac Int 16:49–68.

Zar JH (1999) Biostatistical analysis, Fourth. Prentice Hall: Upper Saddle River, NJ.

Appendix A

Conversion of RGB values of gonad samples and deviation from gonad market standard colour

Mean R, mean G, and mean B values for each gonad image were converted to the CIE $L^*a^*b^*$ (CIELAB) colour space, which is a standard colour space adopted by the International Commission on Illumination that is widely used in food sciences to compare colours of food products to colour market references (Pathare *et al.* 2013). The three parameters of the CIELAB represent (1) the lightness of the colour, L^*, which ranges from 0 [black] to 100 [white]; (2) the position of the colour between red/magenta and green, a^*, with negative [-120] and positive [120] values indicating more green or magenta hues, respectively; and (3) the position of the colour between yellow and blue, b^*, with negative [-120] and positive [120] values indicating more blue or yellow chroma, respectively (Larrauri García & Saura Calixto 2000; Robinson *et al.* 2002). The free, open-source, MIT-licensed ColorMine library (www.colormine.org) was used to carry out this conversion.

Deviation of each CIELAB-converted mean R, mean G, and mean B gonad sample values from CIELAB-converted R, G, and B gonad market standard values was calculated with the following equation:

$$\Delta E_{ab}^* = \left[\left(L_0^* - L_{sample}^*\right)^2 + \left(a_0^* - a_{sample}^*\right)^2 + \left(b_0^* - b_{sample}^*\right)^2\right]^{1/2}$$

where $\Delta \mathrm{E}_{\mathrm{ab}}^*$ (total colour difference) is the Euclidian distance difference between the gonad sample colour and gonad market standard colour; L_0^*, a_0^*, and b_0^* are respectively the lightness of the colour, position of the colour between red/magenta and green, and position of the colour between yellow and blue of the gonad market standard; and L_{sample}^*, a_{sample}^*, and b_{sample}^* are respectively the lightness of the colour, position of the colour between red/magenta and green, and position of the colour between yellow and blue of the gonad sample (McBride *et al.* 2004). Although the market standard for sea urchin gonad colour varies with demand and geographically, pale (whitish) or dark gonads is generally unacceptable (Robinson *et al.* 2002). Desirable gonad colour is typically bright yellow/orange, corresponding broadly to the higher blades of the 15-blade DSM Yolk Color Fan (Vuilleumier 1969). Other studies of urchin gonad have used the higher blades of the DSM Yolk Color Fan as gonad colour standards (e.g. Siikavuopio *et al.* 2014). Accordingly, the gonad colour standard in the present study was obtained by first converting the R, G, and B values of blades 8 to 13 of the DSM Yolk Color Fan to the CIE $L^*a^*b^*$ colour space. This conversion yielded six L^*, six a^*, and six b^* values (one $L^*a^*b^*$ set for each of the six blades), which were then averaged into unique values defining L_0^* (78.3), a_0^* (16.3), and b_0^* (81.1) in the equation above (Table A.1).

Table A.1. Red (R), Green (G), and Blue (B) values of the six blades (8 to 13) of the DSM Yolk Color Fan used to calculate corresponding L*, a*, and b* values, and overall L_0^* (78.3), a_0^* (16.3), and b_0^* (81.1) values.

Blade	**R**	**G**	**B**	***L****	***a****	***b****
8	254	201	0	83.4	4.8	84.6
9	254	194	0	81.6	8.4	83.4
10	255	185	1	79.6	13.5	82.0
11	255	176	1	77.5	18.2	80.5
12	255	162	0	74.3	25.5	78.4
13	255	158	0	73.4	27.6	77.9
			Mean	78.3 (L_0^*)	16.3 (a_0^*)	81.1 (b_0^*)

Appendix B

Summary of bodily characteristics of urchins used in the gonad enhancement experiment

Table B.1 Mean (±SE; n) test diameter, whole body wet weight, gonad wet weight, and gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) at the onset and end of the 12^{th} and 34^{th} weeks of the gonad enhancement experiment in sea urchins fed either of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*) and from the field. Assessment of gonad wet weight and GSI necessitated killing sea urchins. Both bodily characteristics at the onset of the experiment were therefore assessed in sea urchins from the field only for use as indicators of overall gonad quantity in kelp-fed sea urchins

		Onset			12 weeks			34 weeks		
Parameter	**Diet**	**Overall**	**Male**	**Female**	**Overall**	**Male**	**Female**	**Overall**	**Male**	**Female**
Test Diameter (mm)	Kelp combo	47.5 (0.1; 240)	-	-	48.8 (0.3; 70)	49.1 (0.4; 31)	48.7 (0.4; 39)	49.9 (0.3; 140)	49.5 (0.3; 96)	50.8 (0.5; 44)
	L. digitata	47.3 (0.2; 240)	-	-	48.4 (0.4; 70)	48.5 (0.5; 31)	48.4 (0.4; 39)	50.3 (0.2; 157)	50.2 (0.3; 92)	50.4 (0.4; 65)
	A. clathratum	47.2 (0.2; 240)	-	-	47.7 (0.3; 80)	47.5 (0.4; 37)	47.8 (0.4; 43)	47.9 (0.2; 152)	47.9 (0.3; 63)	47.9 (0.3; 89)
	Field	47.5 (0.3; 65*)	47.1 (0.3; 33)	48.1 (0.4; 29)	47.6 (0.2; 100)	47.1 (0.3; 32)	47.8 (0.2; 68)	48.9 (0.3; 100)	48.9 (0.4; 56)	49.0 (0.5; 44)
Body wet weight (g)	Kelp combo	51.6 (0.5; 240)	-	-	57.0 (1.0; 70)	57.9 (1.5; 31)	56.3 (1.3; 39)	61.5 (0.7; 140)	60.8 (0.8; 96)	62.9 (1.2; 44)
	L. digitata	50.6 (0.5; 240)	-	-	57.2 (1.0; 70)	58.3 (1.4; 31)	56.3 (1.4; 39)	62.6 (0.8; 157)	61.9 (1.0; 92)	63.5 (1.1; 65)
	A. clathratum	50.7 (0.5; 240)	-	-	51.1 (0.8; 80)	49.7 (1.2; 37)	52.3 (1.1; 43)	51.6 (0.7; 152)	51.5 (0.9; 63)	51.7 (1.0; 89)
	Field	51.2 (0.9; 65*)	49.8 (1.1; 33)	52.4 (0.6; 29)	52.4 (0.7; 100)	50.1 (1.0; 32)	53.5 (0.9; 68)	57.8 (0.9; 100)	57.8 (1.2; 56)	57.7 (1.5; 44)
Gonad wet weight (g)	Kelp combo	-	-	-	4.4 (0.5; 70)	5.0 (0.3; 31)	4.0 (0.2; 39)	12.6 (0.3; 140)	11.6 (0.3; 96)	14.9 (0.5; 44)
	L. digitata	-	-	-	8.2 (0.3; 70)	8.5 (0.4; 31)	8.0 (0.3; 39)	14.5 (0.3; 157)	13.8 (0.3; 92)	16.2 (0.5; 65)
	A. clathratum	-	-	-	1.2 (0.1; 80)	1.4 (0.1; 37)	1.1 (0.1; 43)	0.8 (0.1; 152)	0.9 (0.1; 63)	0.6 (0.1; 89)
	Field	1.7 (0.2; 65*)	2.1 (0.2; 33)	1.5 (0.2; 29)	1.9 (0.2; 100)	2.1 (0.1; 32)	1.8 (0.1; 68)	6.7 (0.3; 100)	5.8 (0.3; 56)	7.9 (0.5; 44)
GSI (%)	Kelp combo	-	-	-	7.7 (0.3;70)	8.2 (0.6; 31)	6.9 (0.4; 39)	20.5 (0.4; 140)	19.1 (0.5; 96)	23.5 (0.4; 44)
	L. digitata	-	-	-	14.4 (0.4;70)	14.6 (0.7; 31)	14.1 (0.7; 39)	23.3 (0.4; 157)	21.3 (0.4; 92)	25.4 (1.0; 65)
	A. clathratum	-	-	-	2.4 (0.2;80)	2.9 (0.4; 37)	2.2 (0.2; 43)	1.5 (0.1; 152)	1.7 (0.3; 63)	1.2 (0.1; 89)
	Field	3.4 (0.4;65*)	2.8 (0.3; 33)	4.2 (0.4; 29)	4.1 (0.2;100)	4.5 (0.4; 32)	3.8 (0.3; 68)	11.6 (0.4;100)	10.1 (0.3; 56)	13.4 (0.9; 44)

* Sex could not be determined in three urchins with empty gonads.

Table B.2 Mean (±SE; n) percent increase (%, relative to the onset of the experiment) in body size (test diameter), whole body wet weight, gonad wet weight, and gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) at the end of the 12^{th} and 34^{th} weeks of the gonad enhancement experiment in sea urchins fed either of the three kelp diets (kelp combo, *Laminaria digitata*, *Agarum clathratum*) and from the field.

		12 weeks			34 weeks		
Parameter	**Diet**	**Overall**	**Male**	**Female**	**Overall**	**Male**	**Female**
Test Diameter (mm)	Kelp combo	2.7 (0.8; 14)	4.2 (0.8; 7)	0.8 (1.4; 7)	5.3 (0.8; 14)	5.0 (0.4; 7)	5.2 (1.4; 7)
	L. digitata	2.0 (0.6; 14)	2.8 (0.8; 7)	0.8 (1.1; 7)	6.0 (0.6; 16)	6.5 (0.6; 8)	5.1 (1.0; 8)
	A. clathratum	0.3 (0.7; 16)	0.7 (1.1; 8)	-0.6 (0.9; 8)	1.0 (0.6; 16)	1.6 (0.5; 8)	-0.0 (1.1; 8)
	Field	-0.1 (0.4; 14)	0.4 (0.5; 7)	-0.9 (0.7; 7)	3.1 (0.7; 14)	3.8 (0.7; 7)	1.9 (1.2; 7)
Body wet weight (g)	Kelp combo	11.2 (2.2; 14)	15.9 (3.3; 7)	7.1 (2.8; 7)	20.4 (1.5; 14)	22.0 (1.4; 7)	19.3 (3.2; 7)
	L. digitata	12.1 (1.9; 14)	16.5 (3.0; 7)	8.4 (2.7; 7)	22.5 (1.8; 16)	24.7 (2.5; 8)	20.8 (2.5; 8)
	A. clathratum	-0.6 (2.0; 16)	-0.6 (3.3; 8)	-0.3 (2.1; 8)	1.0 (1.5; 16)	3.5 (2.3; 8)	-0.9 (2.2; 8)
	Field	0.9 (1.6; 14)	1.0 (2.5; 7)	1.2 (1.4; 7)	13.0 (1.8; 14)	16.1 (2.8; 7)	10.5 (2.6; 7)
Gonad wet weight (g)	Kelp combo	154.2 (16.0; 14)	126.1 (17.2; 7)	159.9 (36.7; 7)	674.7 (30.3; 14)	451.5 (16.5; 7)	883.7 (29.2; 7)
	L. digitata	385.1 (11.7; 14)	303.4 (15.3; 7)	434.8 (14.9; 7)	768.1 (26.8; 16)	540.0 (21.7; 8)	971.7 (29.0; 8)
	A. clathratum	-23.7 (6.9; 16)	-30.1 (9.2; 8)	-24.9 (7.8; 8)	-55.4 (3.6; 16)	-58.3 (4.5; 8)	-57.3 (3.9; 8)
	Field	15.6 (6.7; 14)	1.0 (0.2; 7)	21.0 (9.4; 7)	301.7 (24.8; 14)	176.8 (7.9; 7)	423.0 (42.1; 7)
GSI (%)	Kelp combo	122.0 (17.4; 16)	93.1 (14.8; 7)	149.2 (17.2; 7)	526.7 (20.3; 14)	349.8 (10.6; 7)	749 (14.9; 7)
	L. digitata	322.2 (11.8; 14)	244.0 (16.8; 7)	409.2 (15.1; 7)	594.8 (21.8; 16)	412.0 (17.9; 8)	821.1 (32.9; 8)
	A. clathratum	-24.3 (10.4; 14)	-29.8 (8.4; 8)	-21.8 (9.4; 8)	-58.7 (3.7; 16)	-62.1 (5.1; 8)	-56.6 (3.6; 8)
	Field	21.9 (7.0; 14)	5.9 (8.6; 7)	37.0 (9.3; 7)	245.9 (18.8; 14)	138.2 (7.0; 7)	384.1 (29.4; 7)

Table B.3 Summary of three-way ANOVA (applied to raw data) examining the effect of Sex (male and female urchins), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of urchins collected from the field), and feeding Duration (12 and 34 wk) on test diameter (mm) of green sea urchins (*Strongylocentrotus droebachiensis*) in the gonad enhancement experiment.

Source of variation	***df***	**MS**	**F-value**	***p***
Sex	1	2.2	1.5	0.221
Diet	3	64.1	14.5	< 0.001
Duration	1	44.1	29.9	< 0.001
Sex x Diet	3	0.0	0.0	0.999
Sex x Duration	1	1.0	0.7	0.402
Diet x Duration	3	10.4	2.3	0.078
Sex x Diet x Duration	3	4.2	0.9	0.423
Error	102	1.5		
Corrected total	117			

Table B.4 Summary of three-way ANOVA (applied to raw data) examining the effect of Sex (male and female urchins), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of urchins collected from the field), and feeding Duration (12 and 34 wk) on body wet weight (g) of green sea urchins (*Strongylocentrotus droebachiensis*) in the gonad enhancement experiment.

Source of variation	***df***	**MS**	**F-value**	***p***
Sex	1	18.9	1.5	0.227
Diet	3	525.7	41.2	< 0.001
Duration	1	511.2	40.0	< 0.001
Sex x Diet	3	4.8	0.4	0.769
Sex x Duration	1	0.2	0.0	0.910
Diet x Duration	3	42.9	3.4	0.022
Sex x Diet x Duration	3	18.1	1.4	0.241
Error	102	12.8		
Corrected total	117			

Appendix C

Summary of full model ANOVAs

Table C.1 Summary of three-way ANOVA (applied to raw data) examining the effect of Sex (male and female), Diet (kelp combo, *Laminaria digitata, Agarum clathratum*, and natural diet of sea urchins collected from the field), and feeding Duration (12 and 34 wk) on gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) in the gonad enhancement experiment.

Source of variation	***df***	**MS**	**F-value**	***p***
Sex	1	28.7	14.4	< 0.001
Diet	3	1748.4	880.0	< 0.001
Duration	1	1489.1	749.5	< 0.001
Sex x Diet	3	9.59	4.8	0.004
Sex x Duration	1	91.12	45.9	< 0.001
Diet x Duration	3	298.04	150.0	< 0.001
Sex x Diet x Duration	3	9.87	5.0	0.003
Error	102	1.99		
Corrected total	117			

Table C.2 Summary of three-way ANOVA (applied to raw data) examining the effect of Sex (male and female), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and feeding Duration (12 and 34 wk) on change in gonadosomatic index (ΔGSI) of green sea urchins (*Strongylocentrotus droebachiensis*) in the gonad enhancement experiment.

Source of variation	***df***	**MS**	**F-value**	***p***
Sex	1	791709	423.6	< 0.001
Diet	3	1598788	855.5	< 0.001
Duration	1	1465295	784.1	< 0.001
Sex x Diet	3	120039	64.2	< 0.001
Sex x Duration	1	276439	147.9	< 0.001
Diet x Duration	3	285599	152.8	< 0.001
Sex x Diet x Duration	3	40535	21.7	< 0.001
Error	102	1869		
Corrected total	117			

Table C.3 Summary of three-way ANOVAs (applied to raw data) examining the effect of Sex (male and female), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and feeding Duration (12 and 34 wk) on green sea urchin (*Strongylocentrotus droebachiensis*) gonad colour's: deviation from ideal gonad market colour standard (ΔE^*_{ab}), lightness (L^*), and redness (a^*) in the gonad enhancement experiment.

Parameter	**Source**	***df***	**MS**	**F-value**	***p***
ΔE^*_{ab}	Sex	1	177.3	5.3	0.023
	Diet	3	6847.8	206.2	<0.001
	Duration	1	36.7	1.1	0.29
	Sex x Diet	3	1342.8	40.4	<0.001
	Sex x Duration	1	781.4	23.5	<0.001
	Diet x Duration	3	1821.3	54.8	<0.001
	Sex x Diet x Duration	3	96.9	2.9	0.038
	Error	102	33.2		
	Corrected total	117			
L^*	Sex	1	1646.0	65.9	<0.001
	Diet	3	3662.9	146.7	<0.001
	Duration	1	81.1	3.3	0.074
	Sex x Diet	3	446.4	17.9	<0.001
	Sex x Duration	1	30.9	1.2	0.27
	Diet x Duration	3	1159.3	46.4	<0.001
	Sex x Diet x Duration	3	162.5	6.5	<0.001
	Error	102	25.0		
	Corrected total	117			
a^*	Sex	1	18.6	2.3	0.133
	Diet	3	540.3	66.6	<0.001
	Duration	1	21.7	2.7	0.103
	Sex x Diet	3	685.3	28.2	<0.001
	Sex x Duration	1	50.8	6.3	0.014
	Diet x Duration	3	305.8	37.7	<0.001
	Sex x Diet x Duration	3	358.0	44.1	<0.001
	Error	102	8.1		
	Corrected total	117			

Appendix D

Stepwise multiple ordinal logistic regression and multiple logistic regression analysis

Table D.1 Results of stepwise multiple ordinal logistic regression analysis of green sea urchin (*Strongylocentrotus droebachiensis*) gonad segmentation rank examining the Akaike Information Criterion (AIC) and variation in AIC (ΔAIC) from one model to the next, in backward model terms selection mode, based on Sex (male and female urchins), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field), and feeding Duration (12 and 34 wk). Best-fitting model (selected according to model selection procedure outlined in section 2.2.7.3) is bolded. S = Sex; Di = Diet; Du = Duration.

Model	AIC	ΔAIC
1. S + Di + Du + S*Di + Di*Du	186.9	0.0
2. S + Di + Du + Di*Du	187.4	0.5
3. S + Di + Du + S*Di	187.5	0.6
4. S + Di + Du	**188.2**	**1.3**
5. S + Di + Du + S*Di + S*Du + Di*Du + S*Di*Du	188.7	1.8
6. S + Di + Du + S*Di + S*Du + Di*Du	188.8	1.9
7. S + Di + Du + S*Du + Di*Du	189.3	2.4
8. S + Di + Du + S*Di + S*Du	189.4	2.5
9. S + Di + Du + S*Du	190.1	3.2
10. S + Di + S* Di	191.5	4.6
11. S + Di	191.5	4.6
12. Di+ Du + Di*Du	191.8	4.9
13. Di + Du	192.1	5.2
14. Di	195.3	8.4
15. S + Du	245.1	58.2
16. S	245.7	58.8
17. Du	246.7	59.8

Table D.2 Results of stepwise multiple logistic regression analysis of green sea urchin (*Strongylocentrotus droebachiensis*) gonad textural appearance rank examining the Akaike Information Criterion (AIC) and variation in AIC (ΔAIC) from one model to the next, in backward model terms selection mode, based on the Sex (male and female urchins), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field), and feeding Duration (12 and 34 wk). Best-fitting model (selected according to model selection procedure outlined in section 2.2.7.3) is bolded. S = Sex; Di = Diet; Du = Duration.

Model	**AIC**	**ΔAIC**
1. S + Di + Du + S*Du + Di*Du	47.9	0.0
2. S + Di + Du + Di*Du	48.5	0.6
3. S + Di + Du + S*Di + Di*Du	50.8	2.9
4. Di + Du + Di*Du	51.4	3.5
5. S + Di	**51.8**	**3.9**
6. S + Di + Du + S*Di + S*Du + Di*Du	52.8	4.9
7. S	52.9	5.0
8. S + Di + Du + S*Du	53.5	5.6
9. S + Di + Du	53.6	5.7
10. Di	54.1	6.2
11. S + Di + S* Di	54.5	6.6
12. S + Du	54.8	6.9
13. Di + Du	56.0	8.1
14. S + Di + Du + S*Di + S*Du	56.2	8.3
15. S + Di + Du + S*Di	56.4	8.5
16. Du	57.0	9.1
17. S + Di + Du + S*Di + S*Du + Di*Du + S*Di*Du	58.8	10.9

Table D.3 Results of stepwise multiple ordinal logistic regression analysis of green sea urchin (*Strongylocentrotus droebachiensis*) gonad overall quality rank examining the Akaike Information Criterion (AIC) and variation in AIC (ΔAIC) from one model to the next, in backward model terms selection mode, based on Sex (male and female urchins), Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field), and feeding Duration (12 and 34 wk). Best-fitting model (selected according to model selection procedure outlined in section 2.2.7.3) is bolded. S = Sex; Di = Diet; Du = Duration.

Model	**AIC**	**ΔAIC**
1. S + Di + Du + Di*Du	212.2	0.0
2. S + Di + Du + S*DU + Di*Du	214.1	1.9
3. Di + Du + Di*Du	**214.4**	**2.2**
4. S + Di + Du + S*Di + Di*Du	217.9	5.7
5. S + Di + Du + S*Di + S*Du + Di*Du	219.8	7.6
6. S + Di + Du + S*Di + S*Du + Di*Du + S*Di*Du	220.9	8.7
7. S + Di	223.5	11.3
8. S + Di + Du	225.2	13.0
9. Di	225.7	13.5
10. S + Di + Du + S*Du	227.1	14.9
11. Di + Du	227.4	15.2
12. S + Di + S* Di	229.2	17.0
13. S + Di + Du + S*Di	230.9	18.7
14. S + Di + Du + S*Di + S*Du	232.8	20.6
15. S	275.8	63.6
16. S + Du	277.8	65.6
17. Du	278.9	66.7

Table D.4 Results of stepwise multiple ordinal logistic regression analysis of female green sea urchin (*Strongylocentrotus droebachiensis*) gonad overall preference rank examining the Akaike Information Criterion (AIC) and variation in AIC (ΔAIC) from one model to the next, in backward model terms selection mode, based on Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and panelist Experience (inexperienced and experienced). Best-fitting model (selected according to model selection procedure outlined in section 2.2.7.3) is bolded. Di = Diet; E = panelist Experience.

Model	**AIC**	**ΔAIC**
1. Di	**384.4**	**0.0**
2. Di + E	384.4	0.0
3. Di + E + Di*E	385.4	1.0
4. E	390.1	5.7

Table D.5 Results of stepwise multiple ordinal logistic regression analysis of female green sea urchin (*Strongylocentrotus droebachiensis*) gonad taste rank examining the Akaike Information Criterion (AIC) and variation in AIC (ΔAIC**)** from one model to the next, in backward model terms selection mode, based on Diet (kelp combo, *Laminaria digitata*, *Agarum clathratum*, and natural diet of sea urchins collected from the field) and panelist Experience (inexperienced and experienced). Best-fitting model (selected according to model selection procedure outlined in section 2.2.7.3) is bolded. Di = Diet; E = panelist Experience.

Model	AIC	ΔAIC
1. Di	**269.9**	**0.0**
2. Di + E	271.9	2.0
3. Di + E + Di*E	274.3	4.4
4. E	285.9	16