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The effect of fresh seaweed and a formulated diet supplemented with seaweed on the growth and gonad quality of the collector sea urchin, *Tripneustes gratilla*, under farm conditions

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Abstract

This gonad enhancement study investigates the effect of different fresh and formulated feeds and feeding regimes on the growth and gonad quality of wild-collected adult sea urchin, *Tripneustes gratilla*, under farm conditions for over 18 weeks. In the first 12 weeks (phase 1), urchins were fed fresh *Ulva rigida* (U); a 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis* (UG); fresh *G. gracilis* (G) and a formulated diet 20U (containing 20% *U. rigida*), and in the final 6 weeks (phase 2) of the study, diet was changed to a formulated feed (20U diet). By the end of phase 1, urchins fed the 20U diet produced gonads (50.72 ± 5.4 g) that were significantly heavier ($p < .001$) than the gonads of urchins fed the fresh seaweed diets (U, UG & G). By the end of phase 2, gonad weight of urchins in treatment groups UG-20U and G-20U were similar to those fed the 20U-20U diet. Gonad colour of urchins in the G-20U treatment became significantly lighter (ANOVA, $p = .029$) and poorer quality, compared with urchins in the U-20U group. This gonad enhancement study, conducted on wild-collected adult *T. gratilla*, has shown that a formulated feed (20U diet) can enhance gonad growth and produce commercially acceptable gonads. This farm-based study supports previous findings from aquarium-based studies by our group and indicates that short-term sea urchin gonad enhancement can be carried out under farm conditions in South Africa.

KEYWORDS
echinoculture, feeding regime, gonad enhancement, gonad maturity, *Gracilaria gracilis*, *Ulva rigida*

1 | INTRODUCTION

The gonads of several sea urchin species are used to prepare an expensive popular seafood delicacy called 'Uni' in Japan. The demand for Uni is steady and is unlikely to decline in the future (James et al., 2016; Rahman, Arshad, & Yusoff, 2014; Sun & Chiang, 2015). The high demand for Uni has, however, led to overexploitation and depletion of many urchin populations in their natural habitat (Bertocci, Blanco, Franco, Fernández-Boo, & Arenas, 2018). Consequently, the world sea urchin fishery is now in a state of decline (Stefánsson, Kristinsson, Ziemer, Hannon, & James, 2017). Current and future demand for urchin products cannot be met by the sea urchin fishery alone (Asia, Villamor, & Faylogna, 2012). The global decline of wild urchin populations, high demand that cannot be matched by current supply, as well as the high monetary value associated with urchins gonads have resulted in an increased interest in culturing urchins globally (Phillips et al., 2010).

Several attempts have been made to culture sea urchins to supplement the gonads obtained from wild-harvested animals, but with limited success. This is partially as the result of the poor quality of cultured gonad that is often associated with the use of sub-optimal feeds or culture conditions (McBride, 2004; McLaughlin & Kelly, 2001; Walker et al., 2015). The quality of the gonad plays a key role in the price and marketability of sea urchins and is particularly affected by the type and quality of feed consumed by the animal (Pearce, Daggett, & Robinson, 2002a). Some of the factors that determine gonad quality are size, colour, texture, firmness and flavour (Pearce et al., 2002a; Prato et al., 2018).

One of the main constraint to urchin aquaculture is the availability of cost-effective diets that can boost both somatic and gonadal growth and produce gonads with the desired colour and taste (Eddy, Brown, Kling, Watts, & Lawrence, 2012). Numerous studies have tested a variety of formulated feeds for culturing sea urchins to improve both somatic growth and gonad quality (Barker, Keogh, Lawrence, & Lawrence, 1998; Cyrus, Bolton, De Wet, & Macey, 2013; Grosjean, Spirlet, & Jangoux, 1996; Prato et al., 2018; Volpe et al., 2018; Watts, Boettger, McClintock, & Lawrence, 1998). However, many formulated diets have been reported to produce large gonads with a poor, whitish colour (McLaughlin & Kelly, 2001; Robinson & Colborne, 1997; Shpigel, McBride, Marciano, Ron, & Ben-Amotz, 2005; Shpigel, Shauli, Odintsov, Ashkenazi, & Ben-Ezra, 2018; Watts et al., 1998). Conversely, other studies have shown that certain seaweed diets can produce gonads of marketable quality, but these are often small in size (Cyrus, Bolton, & Macey, 2015; Shpigel et al., 2005). The different types of feed administered to the urchin, the timing, manner in which the urchin is fed and the feeding regime, plays a significant role in not only determining the somatic and gonadal growth of cultured animals, but also the potential profitability of the overall echinoculture operation (Cyrus, Bolton, & Macey, 2015). Diet also affects the flavour of the gonad and is influenced by the amino acid composition (James, 2007; Phillips et al., 2010). Amino acids are not only the building blocks for proteins but also play a vital role in the taste and sensory

characteristics of many foods, including sea urchin gonads (Dincer & Cakli, 2007; Osako et al., 2006). Glycine and alanine contribute towards the sweet flavour of urchin gonads (Phillips et al., 2010; Takagi et al., 2017), whereas valine, leucine and isoleucine contribute to bitterness while glutamine contributes to the umami taste (Osako et al., 2007; Phillips et al., 2010; Takagi et al., 2017; Verachia et al., 2012).

The collector sea urchin, *Tripneustes gratilla*, has been identified as an indigenous species suitable for culture in South Africa (Cyrus et al., 2013). It is one of the most sought after species in the markets of the Philippines and Japan (Kato, 1972; Lawrence & Bazhin, 1998; Rahman, Tsuchiya, & Uehara, 2009). Even though there is no wild urchin fishery in South Africa and the existing urchin populations are not actively targeted by the recreational fishery and coastal communities, the abundance of *T. gratilla* is patchy and is therefore unlikely to sustain an economically viable industry/wild fishery. As a consequence, extensive laboratory-based research has been conducted over the last few years to develop culture technology for *T. gratilla* (Cyrus et al., 2013; Cyrus, Bolton, & Macey, 2015; Cyrus, Bolton, Scholtz, & Macey, 2015). Cyrus, Bolton, Scholtz, et al. (2015) have developed formulated feeds supplemented with varying amounts of the green seaweed *Ulva rigida* and have demonstrated the benefits of seaweed supplementation on urchin growth, gonad development (growth and gametogenic state) and the marketable properties (colour, texture, firmness) of the gonads in both gonad enhancement and full life cycle grow-out of *T. gratilla*. These authors have also demonstrated that fresh as well as dried *U. rigida* incorporated into formulated feed enhances the chemosensory properties of the feed, significantly improving feed conversion ratio (FCR), consumption and the digestible protein and energy intake by urchins (Cyrus et al., 2013). Furthermore, *U. rigida* in formulated feeds has also been shown to be actively incorporated into the gonad themselves, contributing to tissue composition (Cyrus, Bolton, & Macey, 2019). Cyrus, Bolton, and Macey (2015) divided the grow-out phase of *T. gratilla* for full life cycle cultivation into a somatic phase and gonadal development phase. Their study demonstrated that the use of seaweed (*U. rigida*) during the somatic growth-phase leads to large test growth, whereas formulated diet with high protein and supplemented with *U. rigida* produces high gonad during the gonadal phase. However, all of this research and technology has been developed and tested at a research/laboratory scale and needs to be further validated under farm conditions in South Africa. *Gracilaria gracilis* is a red alga (Family: *Gracilariaceae*), which is one of the main global seaweed aquaculture products, used for agar extraction, feed in aquaculture and food for humans (Buschmann et al., 2017). *G. gracilis* is produced on at least one South African abalone farm as a feed for abalone, but this seaweed has never been tested as a feed or supplementary feed for *T. gratilla*. A change of diet from fresh cultured macroalgae to a formulated diet containing 200 g of dried *U. rigida* per kg of feed (20U) was used in the current study based on the study of Cyrus, Bolton, and Macey (2015), which showed that urchins require different diets during different developmental

stages, where certain diets preferentially enhance somatic growth instead of gonadal growth or vice versa.

The aim of this study was therefore to assess the effects of fresh seaweed diets and previously developed feeds and feeding regimes on the somatic growth and gonad quality of adult wild-collected *T. gratilla* under commercial farming conditions.

2 | MATERIALS AND METHODS

2.1 | Collection, acclimatization and maintenance of urchins

The experiment was conducted at Wild Coast Abalone farm near Haga-Haga, in the Eastern Cape Province of South Africa (32°45′4.23″S, 28°16′41.30″E). Adult *T. gratilla* (50–85 mm test diameter) were collected in August 2017 during low tide from shallow rock pools in the vicinity of the farm (within 2 km). The urchins were acclimatized in the holding tanks for 10 days before the start of the experiments. During this period, urchins were fed with farm-grown *U. rigida* to allow animals to recover from the stress of collection, transportation and to acclimate to their new environment. The urchins were then starved for 7 weeks after which 10 urchins were dissected to confirm the significant reduction in gonad size which served to standardize the dietary state of each urchin before commencing with the gonad enhancement trial.

2.2 | Experimental design

Three recirculatory tanks (L × W × H: 392 × 210 × 84 cm) each with 8 baskets (L × W × H: 84 × 56 × 42 cm) suspended in the tanks were utilized for the feeding trial (all phases of the experiment). Each tank had two pseudo-replicates of each treatment and baskets with different treatments were randomly distributed in each tank, resulting in a total of three true replicates and four treatments. A total of 23 urchins were placed in each of the 24 baskets at a stocking density of 115 urchins per m^2.

The recirculating aquaculture system (RAS) used for the trial was equipped with a drum filter, bio-filters, protein skimmer and a heat pump (Aquaheat TF130/3). A RAS system was used to enable better control of the water quality and to raise water temperature above ambient temperature. The RAS had a daily replacement of 22% new water, and the entire tank volume was replaced every 4 hr.

Each recirculating tank was fitted with an airline to ensure continual aeration. The seawater flowing into the tank was heated with an Aquaheat TF130/3 heat pump (South Africa) that was set to maintain seawater temperature as close as possible to 24°C, considered optimal for larvae, juvenile and adult *T. gratilla* (Dworjanyn & Pirozzi, 2008; Dworjanyn, Pirozzi, & Liu, 2007). Weekly mean water temperatures were mostly between 22 and 24°C except for

TABLE 1 Dietary ingredient (g/kg) and proximate composition of the 20U diet fed to *Tripneustes gratilla* during the period of the experiment

Ingredients (g/kg)	
Maize (extruded)	256.6
Wheat bran	256.6
Ulva	200
Fish meal	122.3
Soybean	122.3
Di-calcium phosphate	14.7
De-oiled lecithin	11.0
Vitamin and mineral premix	8.8
Oil-fish	7.7
Total	1,000
Proximate composition	
Protein (g/kg)	256.9
Fat (g/kg)	23.1
Moisture (g/kg)	96.1
Ash (g/kg)	138.9
Gross energy (MJ/kg)	15.49
Fibre (g/kg)	47.5
Carbohydrate (g/kg)	437.5

February where cooler ambient sea temperatures caused experimental temperatures to be between 20 and 21°C.

Temperature was measured throughout the experiment (once every 1 hr) with a Starmon mini temperature recorder, dissolved oxygen (6.8–7 mg/L) and pH (7.5–8.1) was measured with an Oxy-Guard Handy Polaris probe once every 2 weeks at 8:00 a.m. Ammonia (<0.01 mg/L), nitrate (<0 to 0.1 mg/L) and nitrite (0.1–0.25 mg/L) were all measured with a Palin test photometer 7100 once every 2 weeks. Uneaten feeds were removed every second day from the baskets of urchins fed algal diets, whereas uneaten feed was removed daily for those fed the 20U diet to maintain optimal water quality. Dead urchins were recorded and removed daily, and baskets were cleaned twice weekly. No animals were replaced during the experiment.

2.3 | Diet and feeding trial

Four dietary treatments were investigated in this study. Three of these treatments were seaweed-based: fresh *U. rigida* (U), a 50:50 mixture of fresh *G. gracilis* and fresh *U. rigida* (UG) and fresh *G. gracilis* (G); all cultured at the Wild Coast Abalone farm. The final dietary treatment consisted of a formulated diet incorporating 200 g of dried *U. rigida*/kg feed, designated as the 20U diet (developed by Cyrus et al., 2013; Table 1). Urchins were all fed ad libitum to ensure that feed was not a limiting factor in the trials. The urchins fed the three fresh cultured macroalgae diets were fed once every second day with more than enough feed to last 2 days, whereas urchins fed with the formulated diet (20U) were

fed once daily due to poor stability of the feed. The four diets (U, UG, G and 20U) were fed to the urchins over 12 weeks, after which all diets were switched to the formulated diet (20U) for a further 6 weeks. The diet regimes used were as follows: fresh *U. rigida* to 20U (U–20U), that is urchins previously fed fresh *U. rigida* were subsequently fed a 20U diet; likewise, fresh *U. rigida* mixed with *G. gracilis* to 20U (UG-20U); fresh *G. gracilis* to 20U (G-20U); and formulated diet throughout (20U-20U).

2.4 | Survival rates

Survival rate was calculated as the percentage of urchins that remained in each treatment at the end of the experimental period.

2.5 | Somatic growth measurements

Weight (W) was measured in grams with the aid of an electronic balance, whereas test diameter (TD) and test height (TH) were measured in millimetres (mm) using a Vernier calliper. Somatic growth parameters (test diameter, test height and weight) were measured before the start of the experiments, and every 3 weeks during the experiment, on all urchins.

2.6 | Gonad quantity and quality

Ten randomly selected urchins were sacrificed for gonad quantity measurements (gonad wet weight, gonad somatic index) at the start of the experiment, whereas 6 randomly selected urchins per treatment group were sacrificed for gonad quantity and gonad quality measurement (colour, texture and firmness) at weeks 6, 12 and 18.

For assessment of gonad weight, the gonads of the urchins were carefully removed from the test, while all other visceral tissues attached to the gonads were cleaned off.

2.6.1 | Gonad somatic index

Gonad somatic index was calculated using the formula used by Pearce et al. (2002a).

$$GSI(\%) = (wet\ gonad\ weight/whole\ urchin\ weight) \times 100$$

$$GSI\ increase\ per\ week = [(GSI_{(end)}) - (GSI_{(start)})/(No\ of\ \#days)] \times 7$$

where $GSI_{(end)}$ is the GSI at the end of the study, $GSI_{(start)}$ is the GSI at the beginning of the study and No of #days is the number of days from the start of the study to the end of the study.

2.6.2 | Gonad colour

Gonad colour was assessed by visually rating a randomly selected gonad from each sampled urchin within each treatment ($n = 6$; Table 2). Colour was visually rated according to Pearce et al. (2002a) and spectrophotometrically rated with a hand-held spectrocolorimeter (Lovibond® LC 100 spectrocolorimeter). For the spectrophotometer rated gonad colour, three replicate measurements of L^* (intensity or lightness), a^* (hue or redness) and b^* (chroma or yellowness) were taken from each sampled gonad.

A gonad from the *U. rigida* treatment that was deemed to have the most sought after colour, was used as a reference for 'grade A' gonad as per Cyrus et al. (2013). The total difference in gonad colour from the 'grade A' gonad was estimated using the formula of McBride (2004).

$$\Delta Eab* = [(L*Ulva - L*Sample)^2 + (a*Ulva - a*Sample)^2 + (b*Ulva - b*Sample)^2]^{1/2}$$

2.6.3 | Gonad texture and firmness

Gonad texture and firmness were rated according to methods of Pearce, Daggett, and Robinson (2002b; Table 2).

Category	Gonad colour	Gonad texture	Gonad firmness
1	Bright yellow-orange gonads (excellent quality)	Two discrete gonad segment halves that are very smooth	Very firm
2	Yellow-orange gonads (acceptable quality)	Two discrete gonad segment halves, that are smooth with discreteness and smoothness < number 1	Firm
3	Pale yellow-orange or dark yellow-orange gonads (low quality)	Discreteness of gonad segment halves possible, but are rough/granular, with granularity < number 2	Soft
4	White or brown gonads (unacceptable)	Discreteness of gonad segment halves not possible and are rough/granular	Very soft

TABLE 2 Rating of urchin gonad colour, texture and firmness (as described by Pearce et al., 2002a)

2.6.4 | Gonad maturity

Before the start of the experiment, a single gonad was removed from 10 sea urchins and fixed in Davidson's fixative to ascertain the gametogenic state of the urchins at the start of the feeding trial. Thereafter, six gonads obtained from six animals in each dietary treatment were fixed in Davidson's fixative and used to determine the maturity stage of each gonad at all other sampling dates. After 48 hr of immersion in the fixative, the samples were transferred to 70% ethanol for storage before paraffin histology (Bucke, 1989).

Gonad maturity was analysed according to the method described by Cyrus, Bolton, and Macey (2015). Gonads were categorized into one of 6 different maturity stages of echinoid gametogenesis, namely (a) recovery, (b) growing, (c) premature, (d) mature, (e) partly spawned, and (f) spent (Vaïtilingon, Rasolofonirina, & Jangoux, 2005). Gonads having little or no gametogenic activity were considered as high-quality, that is gonads in the growing or premature stages.

2.6.5 | Amino acid analysis

Gonad samples were analysed for amino acid content and quantity before (one gonad from 6 separate urchins) and at the end of the feeding experiment (week 18, n = 6 urchins per treatment). Each gonad sample was frozen separately at −20°C and thawed, before being homogenized and freeze-dried. A 5 ml aliquot of 6 M HCl was added to a 100 mg aliquot of freeze-dried gonad before being hydrolysed for 24 hr at 110°C, cooled down to room temperature and diluted (1:9) with 70% methanol (v/v). A 100 µl volume was then transferred into a 2-ml microcentrifuge tube and dried completely under a gentle stream of nitrogen. The samples were reconstituted and derivatized with 30 µl N-nethyl-N-(Tert-Butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) and 100 µl acetonitrile at 100°C for 1 hr, cooled down to room temperature and injected into the GC-MS instrument. Separation was performed on a gas chromatograph (6890N, Agilent Technologies Network) coupled to an Agilent Technologies inert XL EI/CI Mass Selective Detector (MSD; 5975, Agilent Technologies Inc.). The GC-MS system was coupled to a CTC Analytics PAL autosampler. Separation of amino acids was performed on a ZB-Semi-volatile (30 m, 0.25 mm ID, 0.25 µm film thickness) Zebron 7HG-G027-11-GGA capillary column. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 250°C, while 1 µl of the sample was injected in splitless mode. The oven temperature was programmed as follows: 100°C for 5 min and ramped up to 325°C at a rate of 20°C/min for 4 min and held for 0.25 min. The MSD was operated in scan/sim mode, and the source and quad temperatures were maintained at 240°C and 150°C respectively. The transfer line temperature was maintained at 250°C. The mass spectrometer was operated under electron impact mode at ionization energy of 70 eV, scanning from 40 to 650 m/z.

2.7 | Statistical analysis

Prior to statistical analysis, the data were tested for homogeneity of variance (Levene's test) and normality of distribution (Kolmogorov–Smirnov test). Wilcoxon's test was used to test for differences in survival between treatment groups. A one-way analysis of variance was used to test for the effect of feed, before and after the switch in diets, on somatic growth (weight, diameter and height), gonad texture, firmness, gonad colour ($L^*a^*b^*$) and eye-rated colour of gonads. Where homogeneity of variance or the normality of distribution test failed, a Kruskal–Wallis one-way analysis of variance on ranks test was used. Where a significant result was found ($p < .05$), Tukey's multiple range test was used for all post hoc multiple comparisons to identify significant differences among treatments.

2.8 | Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (Certificate reference number: VIN011SJOH01).

## 3 | RESULTS

3.1 | Urchin survival rate

The survival rate for urchins fed *G. gracilis* (G), *U. rigida* (U), *U. rigida* and *G. gracilis* (UG; combined), and the formulated feed 20U was 94%, 93%, 97% and 87% respectively. The average survival rate of *T. gratilla* over the course of the study was 93% and was not significantly different among the various treatment groups (Wilcoxon's test, $p = .328$).

3.2 | Somatic growth

There was no significant difference in weight, test diameter and test height of urchins over the entire study. Overall, urchins grew in weight from 177.7 ± 5.2 to 188.2 ± 6.5 g before the diet change, with a mean weight of 197.7 ± 5.3 g (mean ± *SE*) being achieved at the end of the trial (18 weeks; Figure 1).

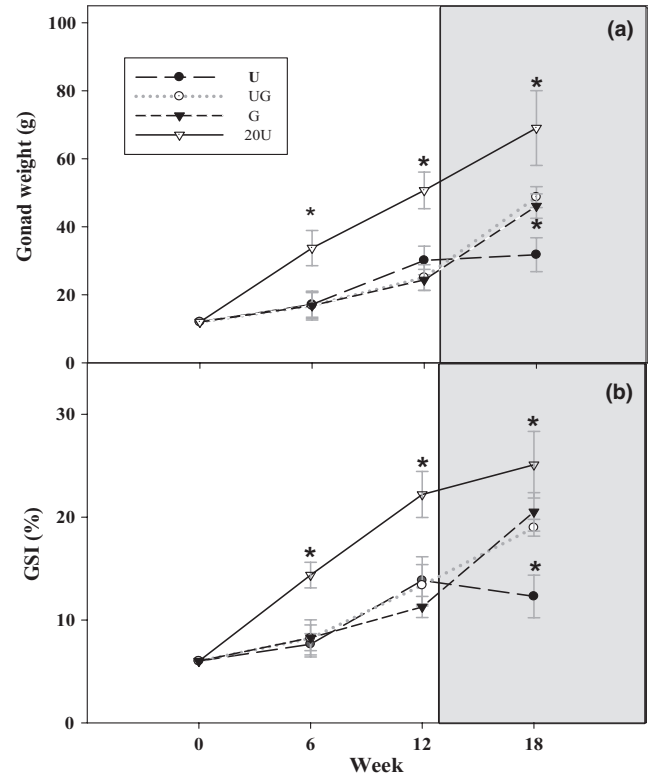### 3.3 | Gonad weight and gonad somatic index

By week 6 and for the remainder of phase 1 of the feeding trial, the mean gonad weight of urchins fed the 20U diet was significantly greater (one-way ANOVA, $p < .001$; $F = 8.696$) than the gonad weight of urchins fed any of the other diets (Figure 2a). During phase 2 of the trial (week 12–18), the gonad weight of urchins in treatment groups UG-20U and G-20U had increased from 25.1 ± 3.8 to 48.7 ± 3.1 g
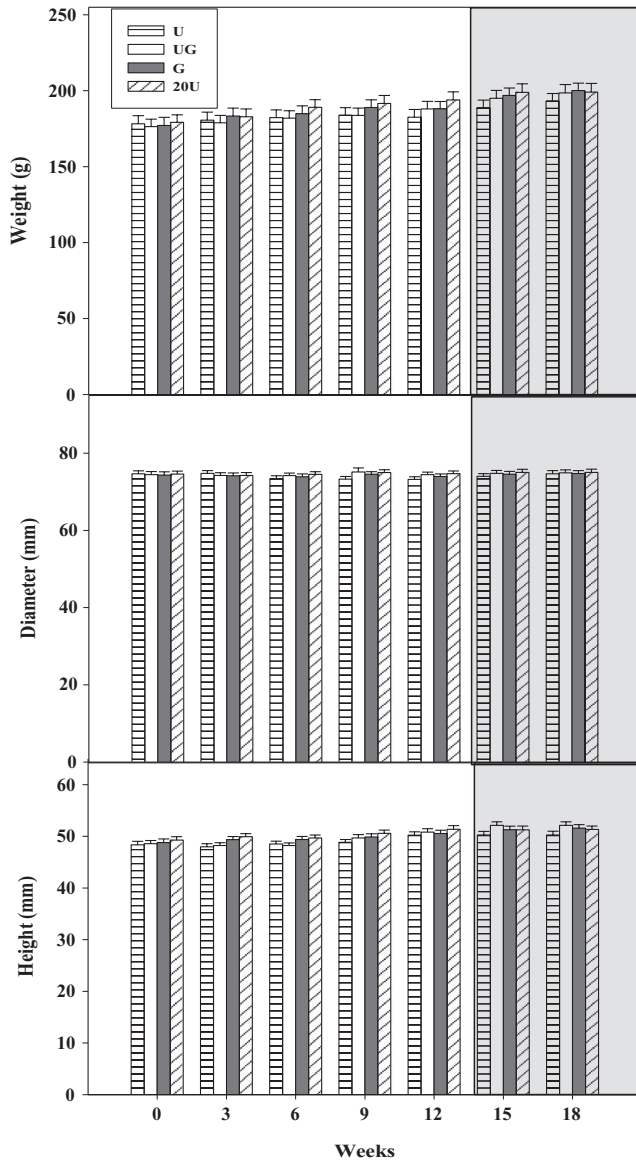
FIGURE 1 Mean wet weight (a), diameter (b) and height (c) of *Tripneustes gratilla* fed four experimental diets for 18 weeks. Data are presented as mean ± *SE*. The shaded part of the graph represents the date when diets were switched to 20U. Treatment: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis*; G = fresh *G. gracilis*; 20U = 20% *U. rigida* in diet

and 24.4 ± 3.1 to 46.10 ± 3.6 g (mean ± *SE*) respectively. Also, gonad weight of urchins in treatment groups U-20U and 20U increased from 30.1 ± 4.2 to 31.8 ± 5.0 g and 50.7 ± 5.4 to 69.0 ± 11.0 g respectively (Figure 2a). By week 18, there was no longer a significant difference in the gonad weight of urchins between any of the treatment groups (Kruskal–Wallis ANOVA, *p* = .002) except for urchins in the 20U treatment group that remained significantly greater than those previously fed *U. rigida* (U-20U).

At the end of phase 1 (week 12), urchins fed the 20U diet achieved a gonad yield (GSI) of 22.2 ± 2.2%, while those fed *U. rigida*, *U. rigida* mixed with *G. gracilis* and *G. gracilis* only achieved a GSI of 13.8 ± 2.3%; 13.4 ± 2.0% and 11.7 ± 1.0% respectively (Figure 2b).

FIGURE 2 Mean gonad wet weight (a) and gonad somatic index (GSI) (b) of *Tripneustes gratilla* fed four experimental diets for 18 weeks. Data are presented as mean ± *SE*. *(*p* < .05) symbolises a significant difference in the mean of urchins fed with the various diets at specific sampling date. The shaded area after week 12 represents the period after the switch in diets to 20U. Treatment: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis*; G = fresh *G. gracilis*; 20U = 20% *U. rigida* in formulated diet

The GSI increase per week in phase 1 of the trial was 0.4%, 0.6%, 0.7% and 1.35% for urchins fed *U. rigida* mixed with *G. gracilis* (UG), *G. gracilis* (G), *U. rigida* (U) and the formulated diet (20U) respectively.

Six weeks after the change of diet, the GSI of urchins in treatment groups UG-20U and G-20U increased from 13.4 ± 2.0% to 19.0 ± 0.8% and 11.7 ± 1.0% to 20.51 ± 1.9% (mean ± *SE*) respectively; and there was no longer a significant difference in GSI between the latter treatments and the 20U treatment (Figure 2b). Conversely, urchins in treatment groups U-20U exhibited a decrease in GSI (from 13.8 ± 2.3% to 12.29 ± 2.1%). The GSI of urchins in the 20U treatment group increased marginally from 22.0 ± 2.2% to 25.0 ± 3.2% during phase 2 of the trial (Figure 2b). During phase 2 of the trial (week 12–18), the change in GSI per week was −0.26; 0.48; 0.93 and 1.54 for the U-20U; G-20U; UG-20U and 20U treatments respectively.

3.4 | Gonad colour

Mean eye-rated gonad colour in the various treatment groups ranged from yellow-orange (acceptable quality) and pale/dark yellow-orange

(low quality) over the course of the feeding trial (Figure 3). There was no significant difference in the eye-rated gonad colour of urchins between treatment groups at any of the sampling dates. A one-way ANOVA at week 12 showed that the mean lightness (L^*) of gonads obtained from urchins fed the 20U and *G. gracilis* diets were significantly different (one-way ANOVA, $p = .023$; $F = 3.967$) from each other, with the 20U diet producing paler gonads compared to the *G. gracilis* diet (Figure 4a). However, the gonad lightness of urchins fed the latter two diets was not significantly different from urchins fed *U. rigida* and a mixture of *U. rigida* and *G. gracilis*. At the end of the feeding trial (week 18), urchin gonads in the G-20U treatment were significantly paler than gonads examined from urchins in treatment U-20U (one-way ANOVA, $p = .029$; $F = 3.71$).

Gonad redness (a^*) and yellowness (b^*) did not vary significantly between treatments at any of the sampling dates (Figure 4b,c), even though both the redness (a^*) and yellowness (b^*) of urchin gonads in all treatment groups appeared to decrease over the course of the trial. Similarly, the total colour difference from 'grade A' roe revealed that the gonad colour of urchins fed the various diets (*U. rigida*; *U. rigida* mixed with *G. gracilis*; *G. gracilis* and the 20U diet) did not differ significantly from each other at weeks 6, 12 and 18 (Figure 5).

3.5 | Gonad texture and firmness

Gonad firmness and texture did not differ significantly between any of the dietary treatments at weeks 6, 12 and 18 (Figures 6 and 7). The values of gonad texture and firmness for urchins fed with the various diets ranged from 2 and 3, which is regarded as acceptable market quality. All dietary treatments produced gonads with two discrete gonad halves, with some apparent follicles (Figure 6), and all the gonads ranged from being firm (2) to firm-soft (3).

3.6 | Gonad maturity

At the start of the experiment, 80% of the gonads were recorded to be in a partly spawned stage and 20% in a recovery stage (Figure 8b). By week 6, gonad sampled from urchins fed the UG and 20U diets were in a growing (>60%) or premature stage, whereas all urchins fed *U. rigida* (U) and *G. gracilis* (G) were in the growing stage (Figure 8b). By week 12, 33% of urchins in the *U. rigida* (U) and 20U treatment groups were in a premature stage while 67% of urchins in the *G. gracilis* (G) treatment group and all (100%) urchins sampled in the *U. rigida* mixed with *G. gracilis* (UG) treatment group were in the premature stage (Figure 8c).

Following the change in diets (after week 12), 67% of the urchins examined from the U-20U and UG-20U treatment groups and 40% from the G-20U treatment had progressed beyond the mature stage and were in a partly spawned stage (Figure 9). Conversely, urchins in the 20U-20U treatment had progressed further, 33%, 17% and 33% of the examined animals found to be in a partly spawned, spent and recovery stage respectively. There were, however, no significant differences in the levels of gonad maturity between the four dietary treatments.

3.7 | Amino acids

Alanine increased significantly from the start of the experiment to week 18 for all treatment groups (one-way ANOVA; $p < .001$; $F = 7.99$). However, there was no significant difference in the alanine content of urchin gonads between the four treatments. Serine increased significantly in the UG-20U, G-20U and 20U treatments by week 18, when compared to values recorded at the start of the experiment (one-way ANOVA; $p = .004$; $F = 5.01$), but the content of serine was not significantly different between treatments.

FIGURE 3 Mean eye-rated gonad colour of *Tripneustes gratilla* fed four experimental diets for 18 weeks. Data are presented as mean ± *SE*. Gonad colour was categorized by visual inspection ranging from excellent quality to unacceptable quality. The shaded area represents the switch in diets to 20U. Treatment: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis*; G = fresh *G. gracilis*; 20U = 20% *U. rigida* in formulated diet
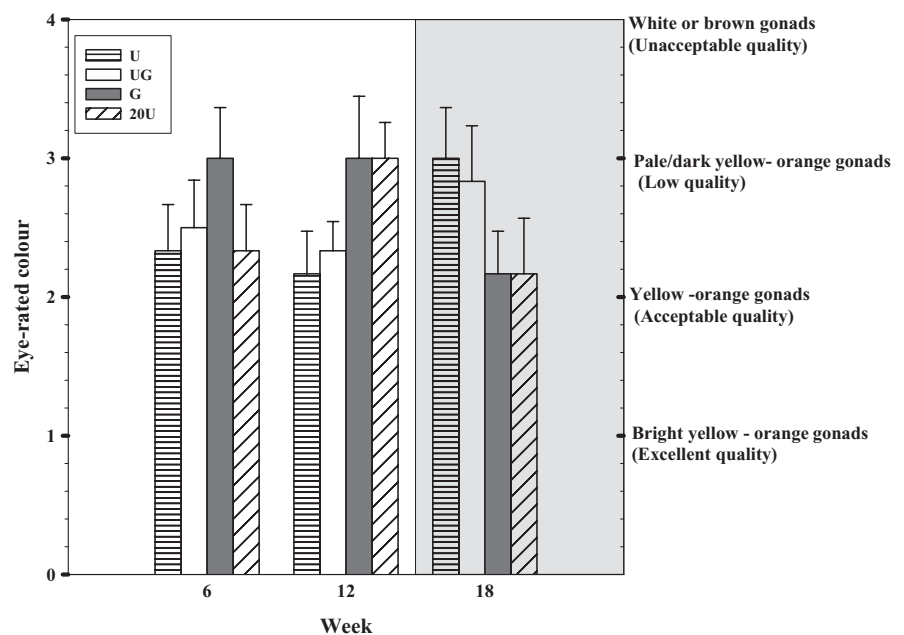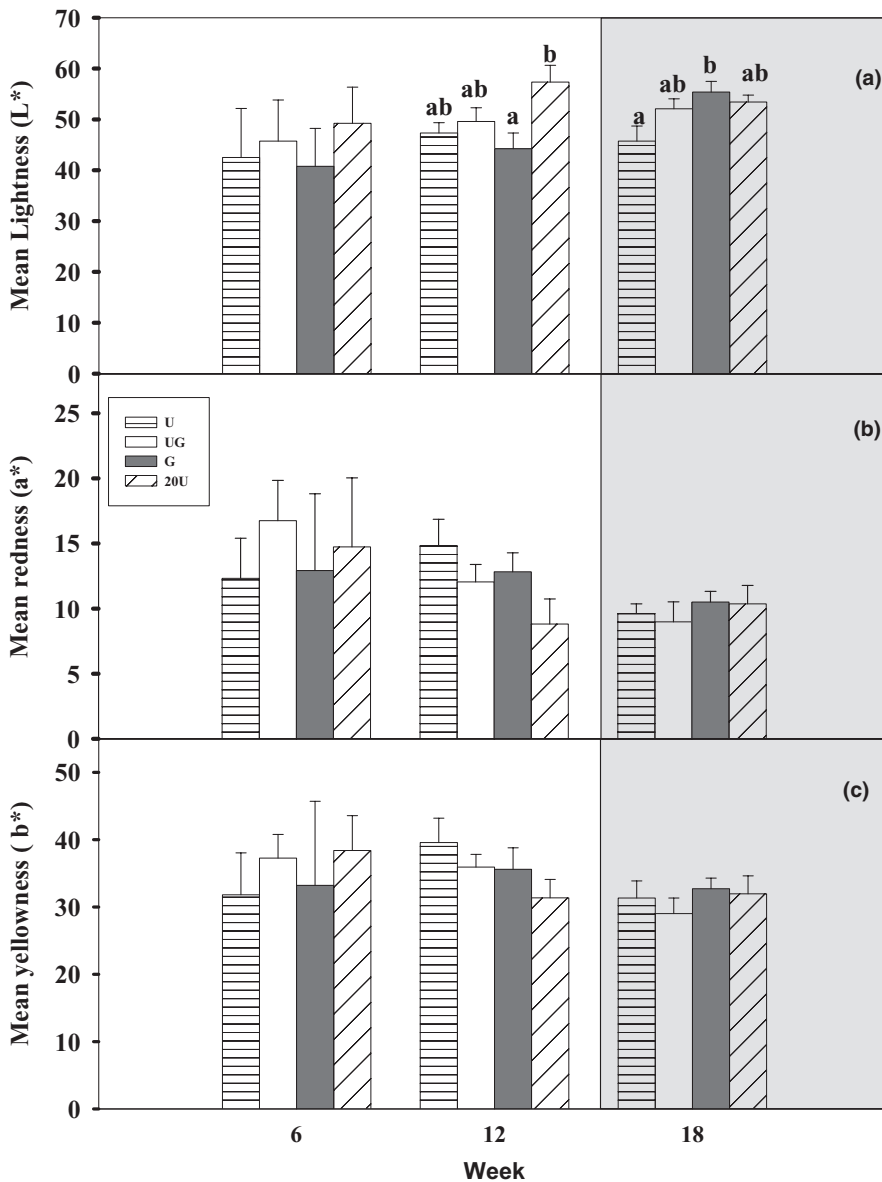
FIGURE 4 Mean gonad lightness (*L**; a), gonad redness (*a**; b) and gonad yellowness (*b**; c) of *Tripneustes gratilla* fed four experimental diets for 18 weeks. Data are presented as mean ± *SE*. Different letters above each bar indicate a significant difference between treatments (*p* < .05) at each sampling date. The shaded area after week 12 represents the period postswitch in diets to 20U. Treatment: U = fresh *U. rigida*; UG = 50:50 mixture of fresh *Ulva rigida* and *Gracilaria gracilis*; G = fresh *G. gracilis*; 20U = 20% *U. rigida* in formulated diet

Conversely, the serine content recorded from gonads obtained from urchins fed the U-20U diets did not differ significantly from initial serine levels at week 18 (Table 3). Threonine increased significantly in gonad tissue analysed from urchins fed G-20U, but did not change in urchin gonads fed the U-20U, UG-20U and 20U diets (one-way ANOVA; *p* = .019; *F* = 3.56).

There was no significant difference between the initial and week 18 levels in seven of the 'bitter' amino acids (isoleucine, phenylalanine, methionine, lysine, histidine, tyrosine and cysteine) and the 'umami' amino acid, aspartic acid, for all treatment groups, nor was there a significant difference in the levels of each of these amino acids between treatments. Conversely, the amount of valine, a bitter AA, recorded from the gonads of urchins fed the UG-20U diet was significantly higher at the end of the experiment compared to the initial value (one-way ANOVA; *p* = .03; *F* = 3.16). However, gonads analysed from urchins in the UG-20U treatment did not differ significantly in valine content compared with animals in the U-20; G-20U and 20U-20U treatments.

4 | DISCUSSION

4.1 | Survival rate

The overall survival rate recorded for urchins in this study (92.7 ± 2.1) is similar to those reported by (Cyrus et al., 2013) and Barker et al. (1998) who worked with *T. gratilla* and *Evechinus chloroticus* respectively. In both Cyrus et al. (2013) and Barker et al. (1998), urchins were fed a fresh macroalgae diet: *U. rigida* (as *U. armoricana*) or *Macrocystis pyrifera* and a formulated feed, and survival ranged between 92.5%–97.5% and 95%–96% respectively. It should, however, be noted that both of these studies were conducted in laboratories under controlled environment conditions, while this study was run under farm conditions. Similarly, Juinio-Meñez, Bangi, and Malay (2008) reared *T. gratilla* in the sea and achieved a survival rate of 98%, which did not differ between urchins fed either a mixture of seaweeds *Sargassum crassifolium* and *Sargassum cristaefolium* or the seagrass *Thalassia hemprichii*.
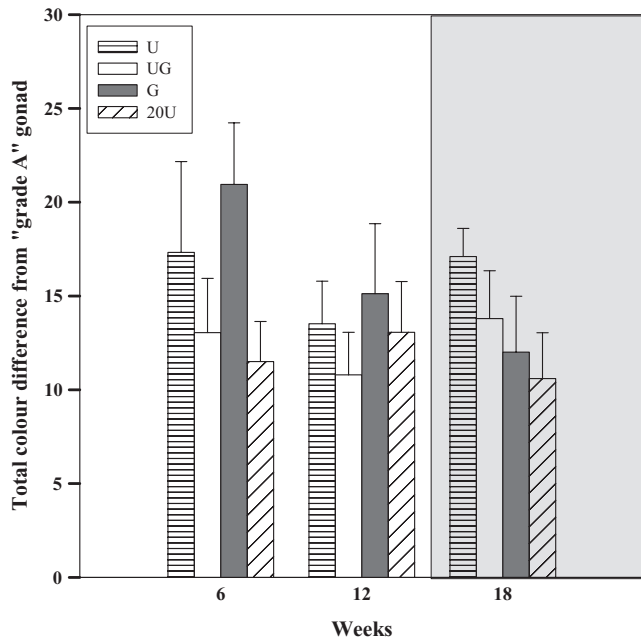
FIGURE 5 The total difference in the spectrophotometer rated gonad colour of *Tripneustes gratilla* fed four experimental diets for 18 weeks. Data represent mean ± *SE*. The shaded area after week 12 represents the switch in diets to 20U. Treatment: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis*; G-20U = fresh *G. gracilis*; 20U = 20% *U. rigida* in formulated diet

4.2 | Effect of feed and change of diet on somatic growth (weight, diameter and height)

Urchins fed various fresh cultured macroalgae diets (*U. rigida*; *U. rigida* mixed with *G. gracilis* and *G. gracilis* alone) in this study produced similar somatic growth (weight, diameter and height) to urchins fed the formulated diet (20U). This result is similar to findings of Asia et al. (2012), who demonstrated that the somatic growth

of juvenile *T. gratilla* (36–55 mm) fed a fresh diet of *Sargassum* macroalgae is comparable to those fed a dry formulated diet containing *Sargassum*. Although somatic growth did not differ significantly between treatments in this study, this is most likely due to the use of large adult urchins which are already reproductively mature (75 mm mean test diameter). It is hypothesized that this resulted in reduced somatic growth of the test due to resource allocation shifting to gonad development and gamete production (Harris & Eddy, 2015).

Cyrus et al. (2013) also reported no difference in the somatic growth (test diameter) of adult *T. gratilla* fed a fresh diet of *U. rigida* compared with urchins fed formulated diets supplemented with a varying amount of dried *U. rigida* in a gonad enhancement study. Cyrus, Bolton, and Macey (2015), however, showed that when feeding juvenile *T. gratilla*, in a life cycle grow-out study, with a formulated feed not containing seaweed, that test height was significantly affected by its absence when compared to feeding fresh *U. rigida* or a formulated feed containing seaweed (20% *U. rigida*). This was not the case in the current study even though the formulated feed used also contained 20% *U. rigida*.

These findings are also in agreement with results from Daggett, Pearce, Tingley, Robinson, and Chopin (2005) and Barker et al. (1998), who reported similar somatic growth for juvenile *Strongylocentrotus. droebachiensis* and *Evechinus chloroticus* fed with either fresh seaweed or a formulated diet. Conversely, Kennedy, Robinson, Parsons, and Castell (2005) reported that juvenile *S. droebachiensis* fed with brown kelp (*Saccharina longissima*) had significantly higher somatic growth compared with animals fed formulated diets. This could be because the formulated diets of Kennedy et al. (2005) lacked seaweed even though they were high in protein content. Other studies have demonstrated that formulated feeds outperform macroalgae diets in terms of somatic growth, for example, in *Pseudocentrotus depressus* (Akiyama, Unuma, & Yamamoto, 2001), *Psammechinus miliaris* (Otero-Villanueva, Kelly, & Burnell, 2004) and *S. droebachiensis*

FIGURE 6 Mean texture of *Tripneustes gratilla* gonads fed four experimental diets for 18 weeks. Data represent mean ± *SE*. The shaded area after week 12 represents the switch in diets to 20U. Gonad texture was categorized by visual inspection ranging from very smooth to rough. The gonads were ranked as follows: (a) two discrete gonad halves (very smooth); (b) two discrete gonad halves (smoothness < 1); (c) two discrete gonad halves possible (granularity < 2); (d) discreteness and gonad halves not possible (rough/granular). Treatment: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and; G = fresh *Gracilaria gracilis*; 20U = 20% *U. rigida* in formulated diet
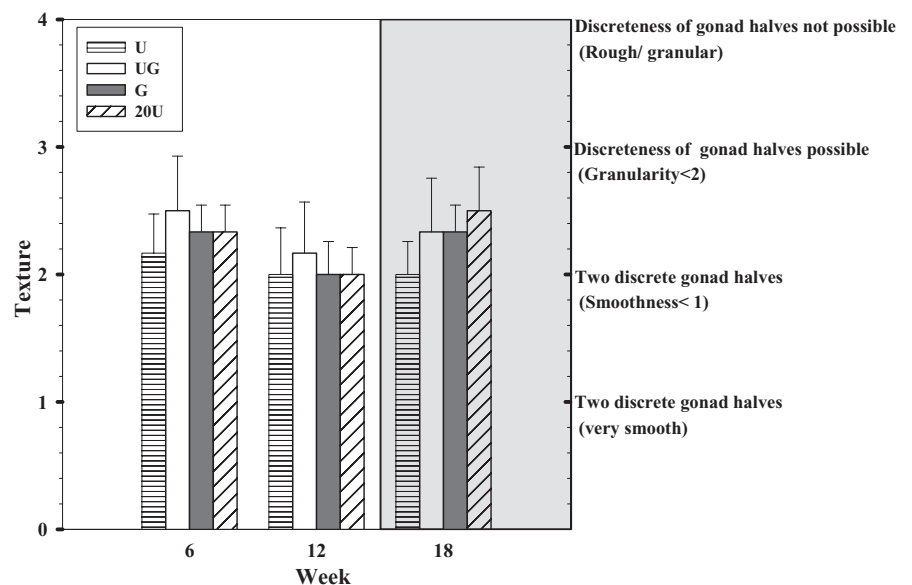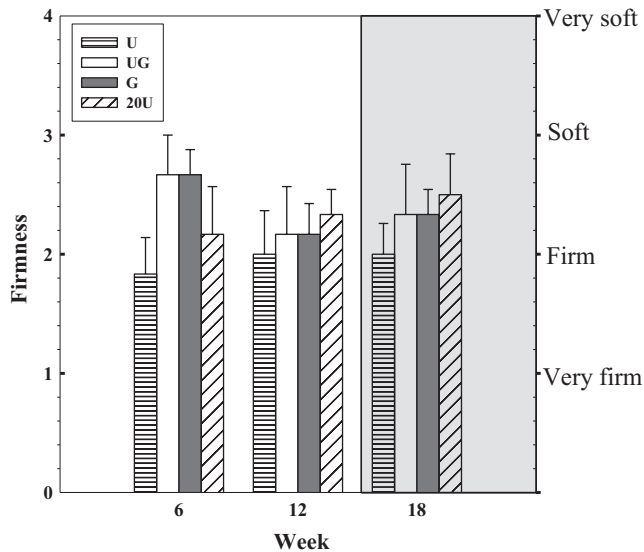
FIGURE 7 Mean gonad firmness of *Tripneustes gratilla* fed four experimental diets for 18 weeks. Data represent mean ± *SE*. The area after week 12 represents the switch in diets to 20U. Gonad firmness was categorized by visual inspection ranging from very firm to very soft. The gonads were ranked as follows: (a) very firm; (b) firm; (c) soft; (d) very soft. Treatment: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis*; G = fresh *G. gracilis*; 20U = 20% *U. rigida* in formulated diet

(Eddy et al., 2012). It should, however, be noted that the formulated diets used by these researchers are different in terms of composition (protein content and source and seaweed content) which would have different qualities for growth enhancement. Whether formulated diets may perform better than macroalgae diets for somatic growth of small *T. gratilla* will depend on the quality of the formulated diet and the nutritional value of the macroalgae diet. As observed in the study of Cyrus, Bolton, and Macey (2015), test height can be affected by the presence of *U. rigida* in the diets, where all other ingredients and nutrient levels were kept equivalent.

After the switch of diet to a formulated feed (20U), no improvement was observed in somatic growth. This supports the study by Cyrus, Bolton, Scholtz, et al. (2015) who reported no difference in the weight, diameter and height of *T. gratilla* after a switch in diet from a fresh macroalgae diet (*U. rigida*) to a formulated diet (with or without *U. rigida*) during the gonad enhancement phase.

4.2.1 | Effect of feed/change of diet on GSI and gonad weight

Difference between the GSI of *T. gratilla* fed a formulated diet, and those fed the fresh macroalgae diets was noticed from the 6th week of the study. Urchins fed the formulated diet had a higher GSI which was almost double (14.37 ± 1.3%) compared to urchins fed the fresh macroalgae diets (U = 7.64 ± 1.0%; UG = 8.21 ± 1.8%; G = 8.27 ± 1.3%). This trend continued until the end of the experiment. The GSI of *T. gratilla* fed the formulated diet increased at the rate of 1.35% weekly

(table 5.1) and had attained a GSI of 22.2% by the end of the study. This study confirms the effectiveness of the 20U diet, specifically formulated for *T. gratilla* (Cyrus et al., 2013) to increase GSI and gonad growth compared to fresh macroalgal diets. The high gonad yield observed in urchins fed formulated diets compared to macroalgae diets has been attributed to the increased protein content of the formulated diets (Fabbrocini & D'Adamo, 2011; Hammer, Hammer, Watts, Lawrence, & Lawrence, 2006; Jacquin et al., 2006). The formulated diet used in this study has a crude protein content of 26%, while *U. rigida* grown in aquaculture systems in South Africa has been shown to have an average crude protein content of 18% (Cyrus et al., 2013). Most formulated diets for sea urchins contain crude protein levels that are higher than those found in macroalgae diets alone.

In the present study, the switch in diet to 20U after week 12 was shown to increase both gonad weight and GSI of urchins previously fed the *U. rigida/G. gracilis* mixture (UG-20U) and *G. gracilis* (G-20U). However, gonad weight and GSI of urchins previously fed *U. rigida* (U-20U treatment) did not increase following the switch to 20U. The gonad weight and GSI of urchins in the 20U-20U treatment group continued to increase after week 12. The increase in gonad weight and GSI of urchins previously fed seaweed diets, except for the *U. rigida* treatment in this study, supports the findings of Cyrus et al. (2013), Cyrus, Bolton, and Macey (2015) and further demonstrates that a formulated diet can improve the gonad yield of urchin. Spawning is known to lead to a reduction in gonad size (Phillips et al., 2010; Rocha et al., 2019) and this could have impacted urchins in the U-20U and 20U-20U treatments, where urchins were seen to release their gametes due to temperature drop during the feeding trial over the period when the diets were switched. The GSI and gonad histology results, following the change of diet, support this notion and revealed a decrease in GSI and a more advanced state of gametogenesis. Most (66%) of urchins in the U-20U group were in a partly spawned state, while 33% of gonads in the 20U-20U group were in a partly spawned stage, with 17% in a spent stage and 33% of the sampled urchin gonads having already reached the end of the reproductive cycle and shown to be in the recovery stage.

Variation in temperature, especially seawater temperature is one of the cues that influence spawning (Garrido & Barber, 2001; James & Siikavuopio, 2018; Vadas, Beal, Dudgeon, & Wright, 2015). Variations in temperature occurred in the current study due to the tanks being situated outside and exposed to natural environmental conditions and fluctuations in incoming seawater temperatures. Fluctuations in temperature, especially the decrease in water temperature experienced in February, could have been the cause of the spawning event at the end of the trial that compromised the quality of urchin gonad (especially animals in the U-20U and 20U-20U groups which seemed to be more reproductively advanced; though not statistically significant).

4.2.2 | Effect of feed and change of diet on gonad colour

Various studies have demonstrated that gonad colour can be enhanced through dietary means (Cyrus et al., 2013; Cyrus, Bolton,

FIGURE 8 Percentage of *Tripneustes gratilla* in each maturity stage at (a) week 0; (b) week 6 and (c) week 12. Diets: U = fresh *Ulva rigida*; UG = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis*; G = fresh *G. gracilis*; 20U = 20% *U. rigida* in formulated diet
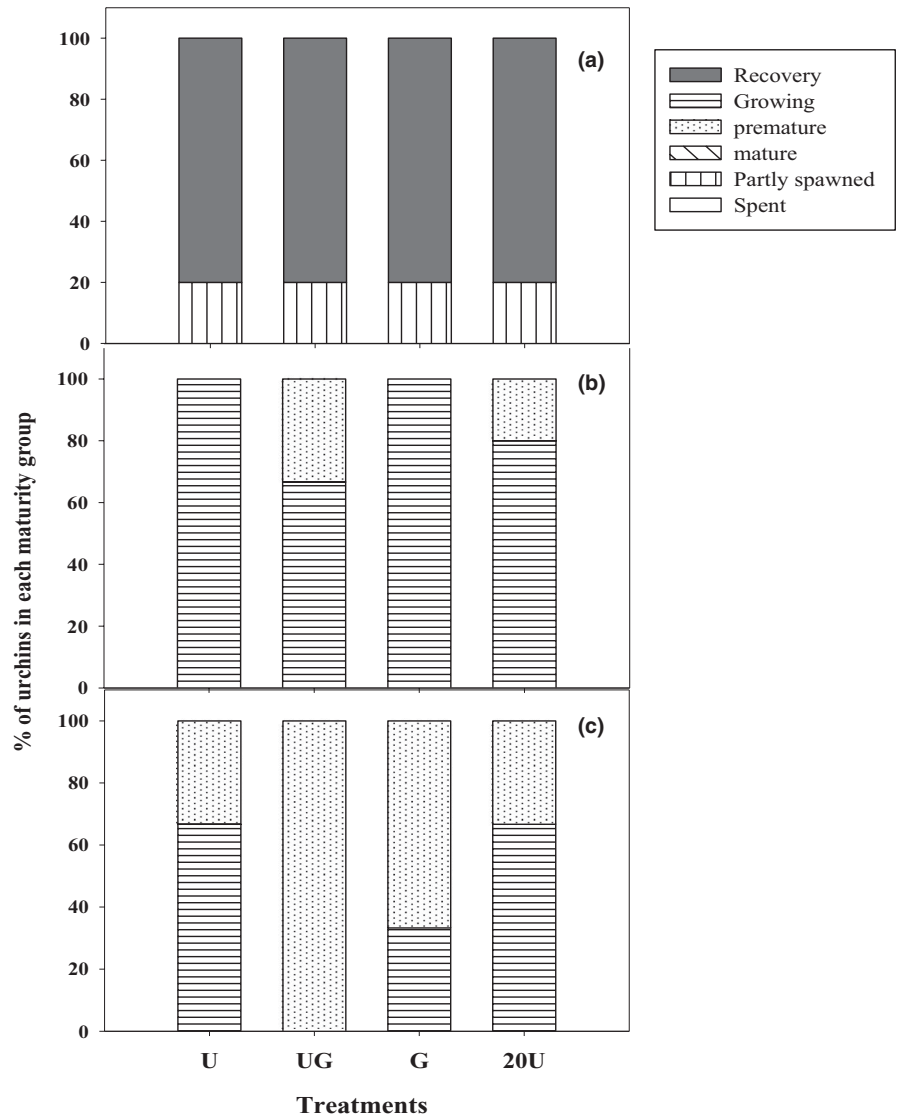
FIGURE 9 Percentage of *Tripneustes gratilla* after 18 weeks in each maturity stage. Diets: U-20U = fresh *Ulva rigida* to 20% *U. rigida* in formulated diet; UG-20U = 50:50 mixture of fresh *U. rigida* and *Gracilaria gracilis* to 20% *U. rigida* in formulated diet; G-20U = fresh *G. gracilis* to 20% *U. rigida* in formulated diet; 20U = 20% *U. rigida* in formulated diet
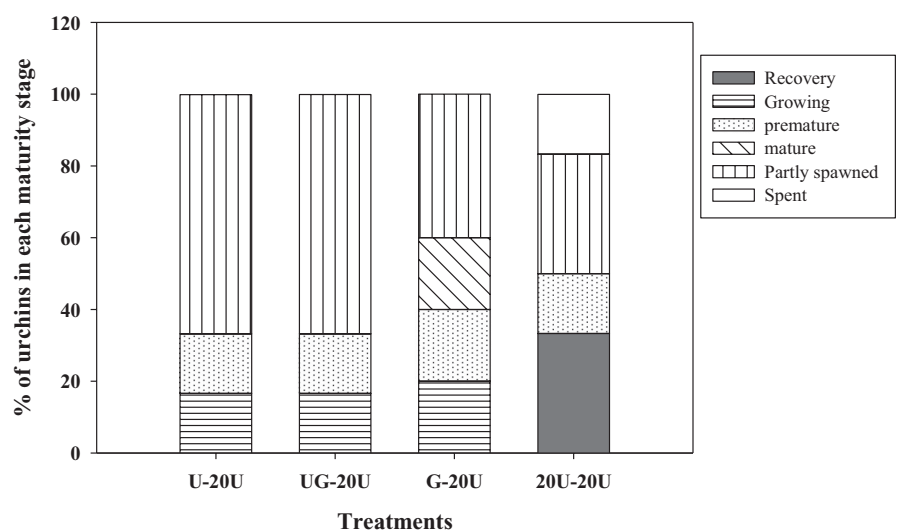
& Macey, 2015; Goebel & Barker, 1998; McLaughlin & Kelly, 2001; Robinson, Castell, & Kennedy, 2002; Watts et al., 1998). Seaweeds have been reported to yield better gonad colouration compared to most formulated diets (Asia et al., 2012; Shpigel et al., 2005). The mean eye-rated colour of urchins fed *U. rigida* and a mixture of *U. rigida* and *G. gracilis* in the present study was shown to improve over time,

TABLE 3 Amino acid composition (and associated taste characteristics) of *Tripneustes gratilla* gonads fed four experimental diets for 18 weeks. Results are mean values of three replicates ± *SE*. Different superscripts within a row denote significant differences (*p* > .05) between dietary treatments for a specific amino acid

Amino acid	Taste	Before treatment	U-20U	UG-20U	G-20U	20U-20U
Alanine	Sweet	61 ± 9.6[a]	106.6 ± 7.5[b]	120 ± 9.0[b]	125.5 ± 0.5[b]	112 ± 7.1[b]
Glycine	Sweet	154.4 ± 24.4[a]	218.4 ± 30.4[a]	221.2 ± 11.0[a]	252.5 ± 22.1[a]	190.6 ± 21.6[a]
Proline	Sweet	46.4 ± 9.0[a]	66.9 ± 3.8[a]	63.7 ± 6.4[a]	78.5 ± 8.1[a]	62.9 ± 4.2[a]
Serine	Sweet	90.4 ± 13.5[a]	135.5 ± 7.2[ab]	151.5 ± 9.4[b]	167.5 ± 27.7[b]	149.4 ± 10.3[b]
Threonine	Sweet	91.6 ± 18.1[a]	174.4 ± 16.0[ab]	178.5 ± 33.8[ab]	266.6 ± 82[b]	171.8 ± 20.7[ab]
Aspartic acid	Umami	167.1 ± 35.8[a]	226.2 ± 10.8[a]	217.4 ± 28.8[a]	234.2 ± 57.8[a]	215.7 ± 21.06[a]
Glutamic acid	Umami	178 ± 41[a]	271 ± 26.6[a]	201 ± 21.3[a]	256 ± 77.0[a]	256 ± 45.1[a]
Valine	Bitter	81 ± 14.5[a]	114.2 ± 4.8[ab]	133.4 ± 7.1[b]	124.7 ± 5.8[ab]	121.1 ± 8.6[ab]
Leucine	Bitter	109.1 ± 20.3[a]	157.2 ± 10.20[b]	169.6 ± 6.0[b]	175.7 ± 11.4[b]	162.1 ± 10.0[b]
Isoleucine	Bitter	71.1 ± 12.8[a]	102 ± 6.3[a]	110.8 ± 7.1[a]	110.3 ± 7.6[a]	103.8 ± 7.2[a]
Phenylalanine	Bitter	62 ± 15.2[a]	88.8 ± 3.1[a]	91.4 ± 10.4[a]	91.9 ± 17.6[a]	83.9 ± 8.2[a]
Methionine	Bitter	22 ± 8.1[a]	34.4 ± 6.6[a]	43.9 ± 19.6[a]	27.6 ± 5.7[a]	33.9 ± 5.3[a]
Lysine	Bitter	174.2 ± 31.4[a]	270.6 ± 37.5[a]	177.9 ± 34.3[a]	322 ± 116.8[a]	235 ± 31.4[a]
Histidine	Bitter	19.1 ± 5.2[a]	31.4 ± 3.8[a]	28 ± 4.0[a]	37.3 ± 15.3[a]	34.2 ± 5.3[a]
Tyrosine	Bitter	67.8 ± 28.0[a]	96.6 ± 11.1[a]	98.9 ± 13.8[a]	94.3 ± 27.1[a]	98.5 ± 17.2[a]
Cysteine	Bitter	153.4 ± 37.1[a]	230.1 ± 14.8[a]	222.3 ± 28.5[a]	255.4 ± 80.7[a]	211.4 ± 20.7[a]

while the gonads of urchins fed the formulated diet deteriorated and became pale with time. After 12 weeks of exposure to the various treatments, urchins fed with the *U. rigida* diet had more brightly coloured gonads of more acceptable market quality, followed by those fed a mixed diet of *U. rigida* and *G. gracilis*. Urchins fed *G. gracilis* and the formulated diet (20U) produced gonads of poorer quality (pale/dark yellow-orange gonads) when assessed visually. The spectrophotometer rated gonad lightness *L** values showed a similar trend to the mean eye-rated colour. The *L** value shows that the gonad of urchins fed *G. gracilis* was dark compared to those fed *U. rigida*, while the colour of those fed the formulated diet (20U diet) was pale compared to those fed *U. rigida*. Those fed either *U. rigida* or a mixture of *U. rigida* and *G. gracilis* were similar in lightness. These findings are in agreement with results of numerous other urchin gonad enhancement trials, where formulated diets have been reported to generally produce lightly coloured gonads when compared with animals fed macroalgae diets (Asia et al., 2012; McLaughlin & Kelly, 2001; Robinson & Colborne, 1997; Shpigel et al., 2005; Watts et al., 1998). Conversely, Pearce et al. (2002a) reported that the gonad colour of *S. droebachiensis* fed with their formulated diet did not differ significantly from the gonad colour of urchins fed kelp. The latter findings of Pearce et al. (2002a) may be the result of the formulated diet containing 0.3 mg/kg of synthetic β-carotene. Similarly, the formulated diet (20U) tested by Cyrus et al. (2013) produced gonad colour that was not significantly different from those produced by *U. rigida*, further demonstrating that inclusion of certain seaweeds in formulated feeds can improve gonad colour. The 20U diet in the present study, based on the formulation of Cyrus et al. (2013), did not produce bright coloured gonads in this study. The contrasting result could

be due to the difference in temperature between these two studies [22.5°C ± 0.2 (current study); 24–25°C in Cyrus et al. (2013) study] and the subsequent spawning event coupled with the fluctuations in temperature experienced in the current study. The bright colour produced by urchins fed the *U. rigida* diet has been shown to be the result of the presence of the β-carotene pigment found in *U. rigida* (Shpigel et al., 2005). The formulated diet contains less β-carotene pigment; therefore, it incorporates less pigment.

The dark gonad colouration of urchins fed *G. gracilis* could be due to the presence of phycoerythrin pigment which is largely responsible for the dark reddish colour of *Gracilaria*. This study demonstrates that not all macroalgae diets produce gonads with good colour. The acceptable colour obtained in the U and UG treatment groups may be caused by the large quantity of β-carotene pigment present in *Ulva*, as the colour of urchins in the G treatment group remained constant throughout the first phase of the experiment.

In their grow-out study, Cyrus, Bolton, and Macey (2015) reported that a change in feeding regime of *U. rigida* to formulated diet (20U) produced urchins with gonads of a similar colour to those fed fresh *U. rigida* alone. After the switch of diet in this current gonad enhancement study, gonads from urchins previously fed the *U. rigida* diet deteriorated and returned to the colour they were 6 weeks after feeding with fresh *U. rigida*. Besides, a switch in diet to 20U was seen to make the gonad colour of urchins previously fed the *G. gracilis* diet pale. These differences could be because Cyrus, Bolton, and Macey (2015) used a single cohort of laboratory spawned juvenile urchins fed solely on controlled diets, while the present study used adult urchins collected from the wild, most likely from varying cohorts that

had fed on unknown diets. The reproductive stage of urchins has not only been shown to affect the texture and firmness of gonads but also the gonad colour of urchins and it has been suggested that urchins should be harvested at a premature stage as this could increase the chances of harvesting urchins with acceptable gonad colour (Blount & Worthington, 2002; Phillips et al., 2010). The spawning event that took place during the course of the experiment had a major impact on the gonad colour of urchins fed the formulated diet.

4.2.3 | Effect of feed on texture and firmness

Pearce et al. (2002a) stated that the gonad texture and firmness of *S. droebachiensis* fed kelp was similar to those fed a formulated diet and (Cyrus et al., 2013) reported that there was no significant difference in both texture and firmness of *T. gratilla* fed either fresh *U. rigida* or formulated diet (20U). The texture and firmness of gonads in urchins fed the various experimental diets in this study were similar; they were generally firm and smooth.

At the end of the change of diet experiment, the gonad texture and firmness were seen to decline slightly with the consumption of the 20U diet. This, however, is most likely due to the advanced maturity stage of the urchins during the change of diet period and supported by the data that indicate that gonad texture and firmness of urchins in the dietary groups were not significantly different from each other.

4.2.4 | Gonad maturity

The reproductive stage of a sea urchin affects its market acceptability, with gonads in the growing or premature stages considered to be the best (Unuma, 2002). The 20U diet increased the rate of gonad maturation in this study. This is in accordance with the findings of Cyrus, Bolton, and Macey (2015) who showed that the 20U diet yields increase gonad maturity. However, Shpigel et al. (2018) reported no significant difference in the gonad maturity of *T. gratilla* fed either *Ulva lactuca*, *Gracilaria conferta* or a formulated diet containing 10% *U. lactuca*. Their formulated diet had higher protein content (29.75%) than that of Cyrus, Bolton, and Macey (2015; 25.69%), and the experiment was conducted for 251 days and the latter for 224 days.

4.2.5 | Amino acid

The characteristic tastes of each amino acid within a gonad are largely determined by their concentration, pH and coexisting substances (like inhibitors and enhancers) and may make the boundaries between their taste classification indistinct. In the current study, there was no difference in the amount of sweet, umami and bitter-tasting amino acid across the treatment groups. The gonads of urchins sampled at the beginning and end of the experiment in the various treatment groups all had lysine, an AA that contributes

towards a bitter taste, as the predominant amino acid, except for the UG-20U treatment group where cysteine (bitter) and glycine (sweet) were the predominant AAs. This result corresponds with a study by Liyana-Pathirana, Shahidi, Whittick, and Hooper (2002a) who showed that cultured *S. droebachiensis* had lysine as the dominant amino acid when fed formulated diet. However, Liyana-Pathirana et al. (2002a) reported that glycine was the predominant amino acid detected in the gonads of wild-collected *S. droebachiensis*. Conversely, Cruz-Garcia, Lopez-Hernandez, Gozalez-Castro, De Quiros, and Simal-Lozana (2000) and Archana and Babu (2016) reported that glycine was not the dominant amino acid in *P. lividus* and *Stomopneustes variolaris*, respectively, but contributed a relatively large amount to the quantity of TAA. The ten essential amino acids were all detected in the gonad of *T. gratilla* in the current study along with other amino acids including alanine, arginine, aspartic acid, glutamic acid, serine and proline. The amino acid of sea urchins, just like other nutrients, has been reported to vary with gonadal stage and diet (Liyana-Pathirana, Shahidi, & Whittick, 2002b). In the present study, there was no difference in the amino acid concentration of urchins fed the various experimental feeds. A similar result was reported by Volpe et al. (2018) when the sea urchin *P. lividus* were fed with various diets.

5 | CONCLUSIONS

The fresh seaweed (*U. rigida* and *G. gracilis*) and formulated diets used in this study had a similar effect on the somatic growth of adult *T. gratilla*. The formulated diet was more effective in yielding an increased gonad growth and GSI compared to the fresh macroalgae diets; however, the formulated diet yielded gonads pale in colour unlike yellow-orange gonads produced by the fresh macroalgal diets. Further research should focus on a full life cycle grow-out study with a single cohort of animals under farm conditions to assess each of these diets/feeding regimes. Also, the appropriate pigment levels within the added *U. rigida* and/or the quantity of *U. rigida* inclusion in formulated diets should be ascertained so as to lead to bright coloured urchin gonads grown under farm conditions. Alternatively, a fresh macroalgae diet should be fed in combination with formulated diet under farm conditions. This study has demonstrated that *T. gratilla* fed a diet of *U. rigida* or a 50:50 mixture of *U. rigida* and *G. gracilis* produced desirable gonad colour and urchins fed the 20U diet had the largest gonads. Also, growing urchins initially on seaweed-only diets does not affect somatic growth, and switch to diet of high-protein formulated feed containing seaweed (*U. rigida*) is capable of rapid production of high-quality urchin gonads. In addition, the study showed that *T. gratilla* can survive and produce acceptable gonads under farming conditions in South Africa.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The shared data used in this article are available. The authors present at each data collection took photographs of the raw data. After the data were compiled in spreadsheets, it was sent to all authors. All authors had full access to all the data in the study and therefore take responsibility for the integrity of the data and the accuracy of the data analysis.

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