Waste to profit in urchin fisheries: developing business opportunities to ensure fishery sustainability and safeguard reef dependent fisheries from destructive urchin grazing

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Executive Summary

This report examines two potential applications of Longspined Sea Urchin (*Centrostephanus rodgersii*) via pitot scale trials; processing waste as an agricultural fertiliser and use as Southern Rock Lobster bait. The biochemical composition of Longspined Sea Urchin waste products was analysed, and the project extended to include growth trials of tomato plants (*Solanum lycopersicum*) using dried urchin shell waste as fertiliser. Positive results from the greenhouse growth trial indicate high bioavailability and uptake of macro and micro-nutrients that warrant the further investigation and commercial trials. Southern Rock Lobster preference trials utilising Longspined Sea Urchin as potential bait were less promising, with trials showing limited application and strong preferences by lobsters to alternate species including traditional fish bait.

Solid waste from the Longspined Sea Urchin fishery was found to comprise 71% of the landed biomass wet weight, translating to 33% of the landed biomass when dried. Subsequently, we estimate that the Tasmanian 2019 wild harvest of Longspined Sea Urchin produced 398 tonnes of wet waste (185 tonnes dried) from the 560 tonnes landed. Forward projections by industry indicate approximately 250 t wet waste will be produced annually. Biochemical analysis of the sea urchin waste revealed the mineralised components of (test, spines and jaw) contained high levels of the macronutrients Calcium (Ca: 40.4%) and Magnesium (Mg: 1.8%), and high levels of the micronutrients Boron (38 ppm) and Iron (Fe: 19.4 ppm). The dried and ground waste had an alkaline pH 8.06 in water and electrical conductivity value of 7.64 dS/m. Nitrogen (N), phosphorus (P), potassium (K) were found to be 0.5, 0.03, 0.26 %, respectively.

The urchin waste powder was added to nutrient deprived soil at seven concentrations between 0.3% and 5% to examine nutrient uptake and growth of K1 tomato plants. Tomatoes were grown using urchin waste fertiliser for a period of 12 weeks and were compared to control plants fertilised with industry standard Hoagland solution. Plant growth, yield, mineral content and quality attributes of tomato were assessed. Results show that the urchin waste had an influence on tomato growth and productivity proportional to the quantity applied, with vegetative growth (e.g. plant height, branches, stem thickness, number of flowers) in tomatoes grown with higher concentrations of urchin fertiliser matching or exceeding those grown with the Hoagland solution. However, the Hoagland solution control had a significantly greater fruit yield. The soil pH was increased from 6.8 to 7 and higher available phosphorus was also detected in soils with the higher rates. No phytotoxic effects were detected despite the high concentration of calcium in soil and higher EC values detected at the highest rate. While, there is some inconsistence in the results of the nutritional compositions of tomato fruits, high dosages of urchin waste fertiliser closely followed the control presenting good quality nutritional values.

Overall, the Longspined Sea Urchin processing waste had a beneficial effect as mineral fertiliser providing Ca and microelements with some of them, such as boron, particularly deficient in Tasmania's basaltic and granitic soil. As there is a strong push for sustainable nutrient sources, Sea Urchin waste may provide a useful alternative for organic or biodynamic farming systems. As such further commercial development is warranted. Moreover, gaining economic value in the Longspined Sea Urchin fishery by reducing the costs of disposal and through by-product

development will incentivise harvest that ultimately will help reduce the pressure of this kelp over-grazer on coastal ecosystems.

Laboratory bait preference trials resulted in forage events by Southern Rock Lobsters originating from kelp habitat on Longspined Sea Urchin baits occurring in 32% of trials, compared to 84% on Jack Mackerel baits and 76% on Shortspined Sea Urchin baits. When Longspined Sea Urchin and Jack Mackerel baits were trialled simultaneously, forage events by the kelp lobsters on the Longspined Sea Urchin baits fell to 14%, while forage events on Jack Mackerel baits were five times higher at 76%. Similarly, forage events occurred on 76% of Shortspined Sea Urchin baits compared to 18% of Longspined Sea Urchin baits when supplied simultaneously. Subsequent to these results, a second set of trials were performed the following season utilising Southern Rock Lobsters originating from urchin barren habitat. The percentage of forage events by these lobsters on Longspined Sea Urchin baits was 70%, compared to 90% on Jack Mackerel baits. When Longspined Sea Urchin and Jack Mackerel baits were supplied simultaneously, forage events by barren lobsters was 44% on Longspined Sea Urchin and 72% on Jack Mackerel. Lobster size significantly affected bait preference by Southern Rock Lobsters originating from urchin barrens, with large lobsters (> 140 mm) foraging on Longspined Sea Urchin baits at higher rate than medium sized lobsters (110 – 140 mm). The trials indicate that Longspined Sea Urchin is unlikely to be as effective as other bait types for Southern Rock Lobster, but may have limited application in harvesting large rock lobsters from areas of high Longspined Sea Urchin abundance.

This project has highlighted the potential opportunities of Longspined Sea Urchin waste as an agricultural fertiliser product and warrants further research and development. The use of Longspined Sea Urchin as a bait product for the Southern Rock Lobster industry is less feasible given its low attractability.

Keywords
Longspined Sea Urchin, Shortspined Sea Urchin, Southern Rock Lobster, fisheries waste, byproduct, fertiliser, feed preference.

Introduction

Globally, the commercial Sea Urchin fishing industry harvests considerable volumes annually, with the 2017 catch estimated to be 75 thousand tonnes valued at 445 million USD (Stefánsson et al., 2017; FAO, 2017). The Japanese market is the biggest consumer and importer of Sea Urchins, consuming about 80-90% of the total current global supply. Mediterranean countries such as Italy, France and Spain are the biggest consumers in Europe (Andrew et al 2002; Stefánsson et al., 2017). Urchins are typically processed and are prized for their edible parts; the gonads or roe. The yield of gonads is variable among different species and ranges between 5 and 15% of total body weight. The remaining part of the Sea Urchin, like test, spines and guts, are considered waste. Typically, this waste ends up in landfill at a considerable expense to the processor, decreasing the overall profitability and viability of Sea Urchin fisheries.

Limited information is available on sea urchin waste disposal or usage generated by the international fishery. Brown (1995) noted that some USA processors composted byproducts while the others hauled the shells to dump at sea. Sea Urchin waste has also been delivered to farms to enable fertilisation of fields at minimal cost to both processor and farmer. Wyatt (1991) showed that a 30% increase in production of grasses could be obtained by adding up to 32 tons of urchin waste per acre. In Italy, Sea Urchin waste has shown value as a soil amendment to ameliorate chemical and biological properties of acidic Mediterranean soils (Garau *et al*., 2012). In Korea, sea urchin shell powder as a feed additive was shown to increase growth performance in chickens while reducing negative environmental impacts of litter. (Chung 2014, Kim et al., 2005a., Kim et al., 2005b, Kim et al., 2015).

Increasing the viability of urchin fisheries is particularly important in south eastern Australia for control of the range-extending Longspined Sea Urchin, *Centrostephanus rodgersii*, which is threatening the productivity and profitability of the lucrative Blacklip Abalone and Southern Rock Lobster fishing grounds as far south as the Tasman Peninsula (south-eastern Tasmania). Commercial harvesting is one of the key urchin control mechanisms but currently operates under subsidy (Cresswell et al., 2019). Waste products from the roe fishery account for majority of the harvested biomass and are currently dumped. Developing saleable by-products from the waste will increase the viability and profitability of urchin fisheries by 1) reducing waste disposal and transport costs, 2) enabling increased harvest levels temporally (longer seasons), spatially (further from port) and in lower density areas, and 3) creating new business opportunities. Creating additional opportunities would also add value to ‘take-all’ harvest activities where urchins not suitable for roe processing are also removed. ‘Take-all’ removals are the paid harvest of all urchins from a region, including both the commercially viable Sea Urchins as well as those that are not.

Cost effective control of Longspined Sea Urchins is a key priority of the Tasmanian Government as well as the Tasmanian Abalone Council and the Tasmanian Rock Lobster Fishery Association. Similarly, the State Government of Victoria and the Eastern Zone

Abalone Industry Association have highlighted the need to control the destructive overgrazing of urchins. A profitable Longspined Sea Urchin fishery is seen as an effective way to achieve this. However, profitability in the Centrostephanus fishery is currently low given the perceived lower roe quality of the species and market acceptance, coupled with the high cost of production resulting from labour intensive processing. Methods to reduce costs and increase profitability provides incentive for fishery expansion and a method of control for destructive urchin grazing.

Discussions with urchin processors in Tasmania and NSW during FRDC 2013/026 (Can commercial harvest of Longspined Sea Urchins reduce the impact of urchin grazing on abalone and lobster fisheries?) have separately identified the disposal of urchin waste as a key logistical issue and financial burden in the processing operation. To confront the waste issue, some processors have been informally trialling uses for waste including as a raw fertiliser, both within compost and worm farms, as well as commissioning some rudimentary biochemical analyses. The disclosing of the significance of this issue by industry and their initial attempts to address it has led to the development of this project. Given the significant mineral composition of sea urchins (Drozdov et al., 2016), utilization of the waste may result in a saleable product that mitigates the cost of disposal.

The potential of using Longspined Sea Urchin as Southern Rock Lobster bait has been raised by industry for the past decade given past research showed rock lobsters predate on Longspined Sea Urchin (Ling *et al*., 2009; Redd *et al*., 2014). If feasible, Sea Urchin baits could both increase bait security for the lobster fishery and help manage the threat of Longspined Sea Urchins on coastal reef ecosystems (Ling *et al*., 2009; Rizzari and Gardner, 2019). Fresh Longspined Sea Urchin for Rock Lobster bait can be sourced from either dedicated harvesting or through the utilisation of processing waste. Baits derived from dedicated harvesting of Sea Urchin contain gonad, a key component consumed by lobsters during natural predation and thus likely to have higher attractability (Eurich et al., 2014). Dedicated harvesting of Longspined Sea Urchins is currently required to be conducted by licenced commercial divers under fisheries management legislation. Processing waste from the commercial harvest industry could be utilised as a Rock Lobster bait source.

The aim of this study was to investigate options to increase the Longspined Sea Urchin profitability, both by assessing the use of waste as an organic fertiliser and utilisation of urchin as lobster bait. A third objective to identify bioactive molecules and assess anti-thrombotic activity in sea urchin gut and gonad extracts for nutraceutical applications was reported separately as FRDC 2017-050.

Objectives

1 To determine the biochemical composition and volume of Longspined Sea Urchin waste and identify applications for the Agricultural sector.
2 To assess the potential for using Longspined Sea Urchin as Southern Rock Lobster bait.

Methods

Longspined Sea Urchin waste volumes and biochemical composition

Data on annual Longspined Sea Urchin catch was obtained from NSW, Victoria and Tasmania via the relevant state regulatory bodies (NSW DPI, Vic DPI, and DPIPWE Tasmania). Data is not presented when there are fewer than five licence holders in any state in any given year (policy requirement to protect commercial confidentiality of data).

Longspined Sea Urchins were collected from 3 locations between central NSW and Southern Tasmania, namely Port Stephens, St Helens and Tasman Peninsula (Figure 1). Ten urchins of commercially harvestable size (> 85 mm) were harvested from 3 sites several kilometres apart at each location and processed the same day. Harvest sites were within incipient kelp/barren regions (labelled 'incipient barren') representative of typical commercial fishing operations. At St Helens three additional sites were sampled in extensive urchin barren regions (labelled 'extensive barren'). Weight measurements for live whole, drained, gonad, test (including spines), jaw, and gut were recorded for each urchin, with waste components were retrained and frozen.

Mineral components (tests, spines and jaws) were rinsed with tap water to eliminate salt residue and oven dried for 24 hrs at 105°C and reweighed. The proportion of gonad, waste materials and fluids were calculated as a percentage of whole wet weight. Dried material was finely ground using a grinding mill (A11 analytical mill, IKA, Staufen, Germany) (Figure 2) and sent to SWEP Pty Ltd Analytical Laboratories (Victoria, Australia) for nutrient analysis to determine elemental composition and physico-chemical parameters. Specifically, P, K, S, Ca, Mg, Na, Fe, Mn, Zn, Cu, Co, B, Mo were determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES) after acid digestion. Nitrogen was determined by the Dumas method (Dumas 1831). The pH of the powder in water (ratio 1:5) was measured with a pH reader (Rayment & Higginson 1992) and electrical conductivity (EC) was determined in a water extract and organic carbon with LECO carbon analyser following Rayment and Lyons (2011). Mean values for each location with 95% confidence intervals are presented.

Figure 1. Location of Longspined Sea Urchin collected for waste analysis.

Figure 2. Preparation of Sea Urchin waste for biochemical analysis.

Tomato growth trials

Urchin waste powder was constructed by homogenising total mineral components (tests, spines and jaws) across locations to be representative of a generalised industry fertiliser, and following no significant differences between locations (see results). Mineral characterization of the urchin waste powder provided insights for determining the rate additions for the experimental pot trial. A potting mix was prepared comprising of 90% composted pine bark, 5% sand, 5% cocopeat, plus 3 kg dolomite/0.5m^3 to produce a consistent growing medium with sufficient structure for plant growth. Seven treatment rates (0.3%; 0.5%; 0.8%; 1%; 2%; 3%; 5% by weight) of urchin waste powder were added to 4 kg of the potting mix at the commencement of the trial with ten replicate pots per treatment (Table 1). The potting mix used in the pot trial was very low in macronutrients N, P, K and Ca. Conversely, the potting mix had a higher content of Zn, Fe and Mn compared to urchin waste powder , but was low in B. The initial EC and pH were 0.470 dS/m and 7.30 respectively. An additional treatment with a standard Hoagland solution with ten pot replicates were used as the control of which 400 ml was applied twice a week for 12 weeks. One week after the preparation of the pot treatments, three tomato (*Solanum lycopersicum*) seedlings (variety K1) were added to each pot and after two weeks the strongest plant was retained, and the others discarded. pH and EC measurements of the growing medium for the eight treatments were recorded three days post-planting and before the addition of Hoagland solution in the control treatment and at the conclusion of the trial. Pots were randomly allocated to benches in a greenhouse at the Horticulture Centre of the University of Tasmania where they were maintained for 12 weeks. The experiment underwent a natural photoperiod, in an uncontrolled temperature environment and pots received automatic irrigation for two minutes, six times over 24 hr.

The dynamics of plant growth was recorded with weekly measurements of a range of plant growth and reproductive characteristics. Individual plant height and width (cm) were obtained using a ruler, and stem cross section area (CSA) (mm) using Vernier callipers. The number of fully-grown branches, flowers and fruit were recorded for each plant weekly.

Table 1. Nutrient composition of the potting mix with powdered sea urchin waste applied at different rates and the total applied in the Hoagland control.

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

Values in parenthesis represent standard error of the mean.

After 12 weeks, fruit from each plant were counted and weighed, and the total yield per plant calculated. Harvested fruit were analysed for quality attributes (fruit colour, weight, diameter), and other physico-chemical traits such as total soluble solids (°Brix), acidity, pH, firmness, dry matter content and nutrient composition. To assess fruit quality attributes, fruit of similar ripe stage (maturity) were selected for comparison. Colour intensity through the coordinate L*, a* and b* was recorded with a colour meter (Chroma Meter CR-400, Konica Minolta, Tokyo, Japan) in three spots around the pericarp and values averaged. Values of a* are negative in green tomato and become positive when red colour starts to develop. Negative values represent unripe fruit while higher hue angle shows fruit in different ripening stages. Red is better represented by the hue angle which explains the colour change associated with the enzymatic degradation of chlorophylls and the appearance of lycopene (Su et al 2015). A minimum positive hue angle represents fully ripe and shows an intense red colour. Fruit firmness was measured with a compression meter (Güss fruit texture analyser, Strand, South Africa) which expresses deformation of the pericarp in millimetres in response to the applied load of 50 g for 0.4 s on the surface of the fruit using a 2 mm cylindrical probe at 4 mm depth. Each fruit was also dissected transversely to count the number of locules and to measure the pericarp thickness in mm at two locations on each fruit with a Vernier calliper and values were averaged. Following physical assessments, a random sub sample of the fruit were sliced, weighed and placed in an aluminium tray then oven dried at 60°C for four days. After drying, samples were weighed, and dry matter content and moisture calculated. Dried fruit samples from the same replicate were pooled together and sent to CSBP Soil and Plant Analysis Lab for nutrient composition analysis. Remaining fruit were pureed through a thin mesh, centrifuged and the extracted juice used to estimate total soluble solids, pH and titratable acidity. Total soluble solids (˚brix) were determined with a hand refractometer (Atago 3810 pal-1, Fukaya, Saitama, Japan). The refractometer was washed with distilled water after each assessment use and dried with blotting paper. Fruit acidity and pH was determined using a titrator (HI84532 Hanna Instruments, Melbourne, Australia).

At the end of the trial, the vegetative and reproductive weights of all tomato plants were calculated for each of the eight treatments. Each plant was cut at the base (soil surface) and the fresh weight recorded, then plants were oven dried for 48 hrs and dry weight and moisture content calculated. Five branches per plant from each treatment were cut and sent for nutrient analysis. Three replicates of standard potting mix and three replicates of soil from each treatment (10 g per sample) were collected at the end of the trial, sieved through a 2 mm mesh and air dried for approximately two weeks in aluminium foil trays. Dried samples from each treatment were pooled to make a composite sample and sent for analysis to determine changes in soil chemical parameters after the application of the urchin waste powder and the uptake of nutrients by the plants.

Macronutrients are presented as percentage of weight by weight (%w/w) and micronutrients are presented in part per million (ppm). One-way ANOVA was performed to compare the treatment effects on tomato plant growth, yield, and fruit quality parameters. Homogeneity of variances was verified with Levene's test. Two-way ANOVA with repeated measures was used on stem height, branch number, stem CSA, flower number and fruit number to analyse the interaction between fertilizer treatments and weekly measurements. Differences at the 5% significance level were compared using Tukey's Honestly Significant Difference (HSD) test. Permanova tests were performed on Euclidean distance matrix for leaf and tomato fruit nutrient content and fruit characteristics between each treatment and control to indicate significance of tested factors. Statistical analysis of One-Way ANOVA and Two-Way ANOVA with repeated measures were performed with SPSS (IBM SPSS Statistics for Windows, version 26.0. Armonk, NY: IBM Corp.). Permanova test and nMDS plots were performed using PRIMER 7 (Plymouth Routines In Multivariate Ecological Research) (Clarke & Gorley 2015).

Longspined Sea Urchin bait assessment.

Thirty Southern Rock Lobsters of legal size (>110 mm carapace length) were collected from kelp habitat in southern Tasmania by the means of baited pots set overnight. Lobsters were held in a 5000L tank supplied with raw flow through ambient (~18°C) seawater when not undergoing experimental treatment. These Lobsters were starved for three days before the commencement of each bait feeding trial. Trials were conducted with individual lobsters held in 1m diameter (150 L) tank supplied with same raw flow through seawater as the holding tank at a rate of ca. 2.5L per minute. Trials were conducted between January and March.

Three bait species were selected for the feeding trials: Longspined Sea Urchin - the test species; Jack Mackerel - a commercial bait species; and Shortspined Sea Urchin - a key natural prey species of Southern Rock Lobster. Live Sea Urchin of test diameter 80-90 mm were collected by divers and baits were prepared by breaking fresh urchin into segments (a segment containing at least one gonad), while Jack Mackerel baits consisted of 4 * 2cm fillet segment (similar size to the urchin gonad). Fresh Sea Urchin with gonad in was used for the trials to provide maximum activeness to Rock Lobster and representing the scenario of harvesting directly for bait.

For each bait feeding trial 5 large (>140 mm carapace length) and 5 medium (110 -140 mm) lobsters were randomly selected from the holding tank and placed in the test tank. Trials were conducted by placing baits on the opposite side of the tank to the lobster. Lobsters were observed and forage events recorded every 30 mins for two hours. A forage event was classified if there was clear evidence of lobster feeding interaction with the bait segment. This was typically observed by lobsters actively feeding on the bait, or the bait segment was torn at, shredded, or consumed. Only the first feeding interaction on a bait was classified as a forage event in any two-hour trial. Unconsumed bait was removed at the end of the two-hour period. This sequence was repeated daily for 5 days. Both individual bait trials, with baits of Longspined Sea Urchin, Jack Mackerel and Shortspined Sea Urchin, and paired bait trials, with combinations of Longspined Sea Urchin & Jack Mackerel, as well as Longspined Sea Urchin & Shortspined Sea Urchin, were conducted. Data were analysed using 2-factor analysis of variance (ANOVA) testing the effects of 'Bait Species' and "Lobster Size, as well as 'Bait Species' and 'Time', and the relative interaction terms, using R (R Development Core Team 2018).

Following the bait preference trials 5 large lobsters (> 140 mm) were held in individual aquaria for 10 days with two live Longspined Sea Urchin and two live Shortspined Sea Urchin, all of ~80-90 mm test diameter. Aquaria were checked daily with predation events recoded and predated urchins replaced.

Following the analysis of results from feeding trials on Southern Rock Lobsters originating from kelp habitat, additional Southern Rock Lobsters were obtained from urchin barrens habitat in North Eastern Tasmania. Individual and pair bait trials were repeated as above utilising Longspined Sea Urchin & Jack Mackerel as bait.

Results

Waste production and biochemical composition

Longspined Sea Urchin catch has fluctuated since 1999 and has totalled > 100 tonnes nationally in every year since 2011 (Figure 3). Fisheries wet waste, comprising of urchin test (including spines), jaws and guts, was estimated to comprise 71% of the landed biomass (Figure 4). Dried waste biomass was estimated to be of 33% of the whole urchin weight. The peak state landings of 560 tonne in Tasmania in 2019 resulting in an estimated 398 t of wet weight waste (185 t dried).

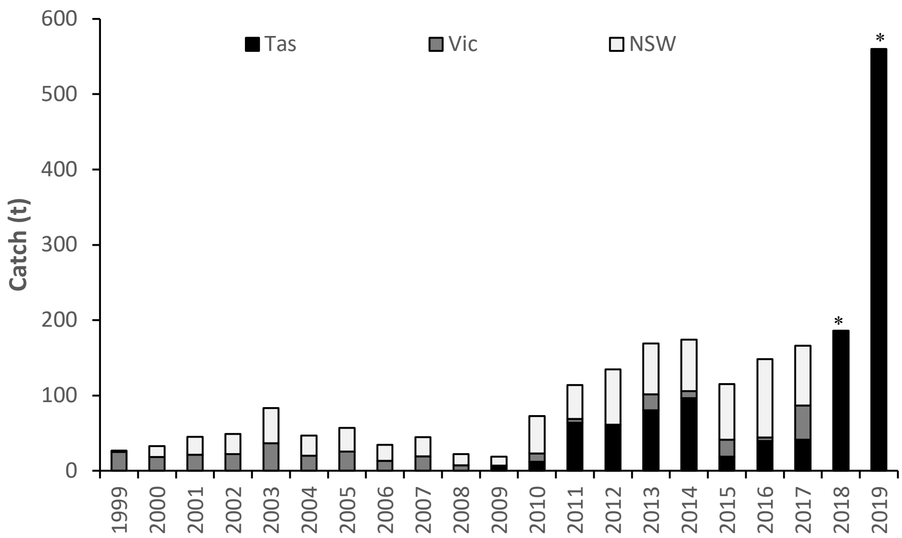

Figure 3. Total catch of Longspined Urchin by state since 1999. *Tasmanian data only.

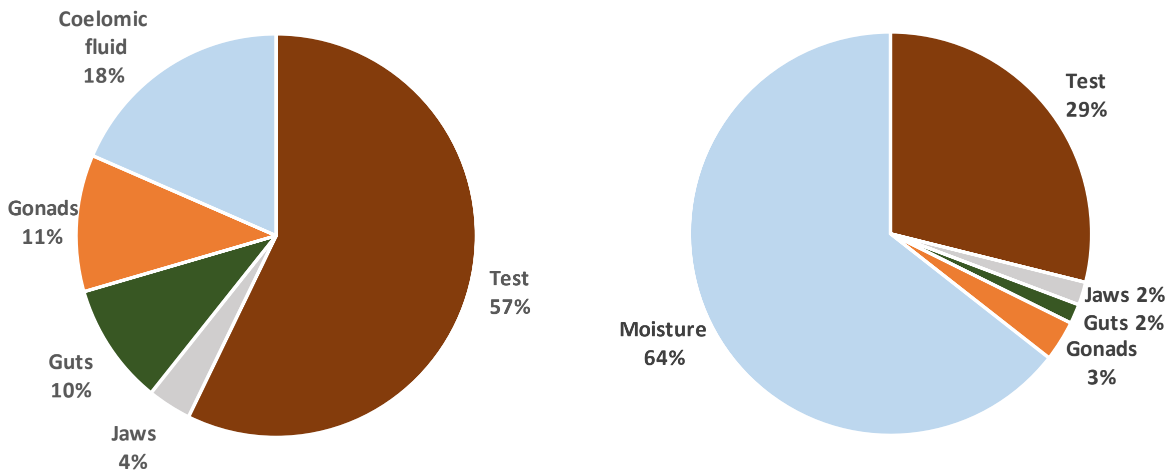

Figure 4. Breakdown of wet (left) and dried (right) Longspined Sea Urchin components expressed as a percentage of total wet weight pooled across all locations.

Results of biochemical analyses on Longspined Sea Urchin mineralised waste (test, spines jaws) showed significant difference in nutrient concentrations and general properties between components, but not between sites and habitat (Table 2). The major components of the waste were calcium (40.4: range 36.6 – 41.9%), Magnesium (1.8: 1.4 – 2.6%), Nitrogen (0.5: 0.2 - 0.9%) and Potassium (0.3: 0.2 – 0.5%). Among micronutrients, Boron showed the higher concentration with (38.1: 31.9 – 41.2 ppm), followed by Iron with (19.3: 13.3 – 22.1 ppm) and Zinc with (6.4: 5.8 – 8.5 ppm) (Table 2).

Table 2. Nutrients and general properties (means ±95% CI) of Longspined Sea Urchin mineal waste from Port Stephens, NSW, St Helens, Tas, and Tasman Peninsula, Tas.

			Ca	Mg	Na	N	P	K	S	Cu	Zn	Fe	Mn	Co	Mo	B	EC	pH	OC
Component	**Location**	**Habitat**	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	ppm w/w	ppm w/w	ppm w/w	ppm w/w	ppm w/w	ppm w/w	ppm w/w	µS/cm	1:5 Water	% w/w
Jaws	**NSW**	**Incipient**	35.87 ±2.74	2.40 ±0.19	1.08 ±0.1	1.69 ±0.22	0.08 ±0.01	0.52 ±0.02	0.84 ±0.05	0.66 ±0.06	8.37 ±1.14	12.24 ±10.09	7.69 ±5.62	0.03 ±0.05	0.11 ±0.06	32.83 ±3.72	6763.33 ±472.2	7.55 ±0.12	9.18 ±1.37
	St Helens	**Extensive**	37.37 ±0.94	2.44 ±0.03	1.10 ±0.13	1.39 ±0.05	0.07 ±0	0.41 ±0.09	0.64 ±0.03	0.63 ±0.41	7.19 ±0.39	12.23 ±3.35	2.69 ±0.91	0.07 ±0.05	0.15 ±0.11	35.73 ±5.11	6656.67 ±1826.53	7.55 ±0.4	8.68 ±0.65
		Incipient	36.40 ±1.24	2.44 ±0.16	1.07 ±0.2	1.57 ±0.38	0.07 ±0.02	0.41 ±0.15	0.54 ±0.44	0.54 ±0.43	8.13 ±2.31	17.03 ±19.84	3.25 ±0.94	0.04 ±0.06	0.14 ±0.12	34.53 ±2.96	6496.67 ±1713.39	7.60 ±0.11	9.64 ±4
	Tasman	**Incipient**	36.93 ±0.38	2.39 ±0.13	1.11 ±0	1.61 ±0.3	0.08 ±0.02	0.52 ±0.08	0.75 ±0.19	0.62 ±0.26	10.09 ±4.68	11.63 ±7.94	5.30 ±5.76	0.07 ±0.1	0.12 ±0.05	34.13 ±2.63	6876.67 ±447.37	7.71 ±0.23	9.15 ±1.37
Jaws mean			36.64 ±0.51	2.42 ±0.03	1.09 ±0.03	1.56 ±0.09	0.07 ±0	0.46 ±0.04	0.69 ±0.09	0.61 ±0.08	8.45 ±0.91	13.28 ±2.98	4.73 ±1.58	0.05 ±0.02	0.13 ±0.02	34.31 ±1.06	6698.33 ±297.1	7.60 ±0.07	9.16 ±0.54
Spines	**NSW**	**Incipient**	41.17 ±2.01	1.37 ±0.06	1.32 ±0.06	0.22 ±0.07	0.02 ±0.01	0.21 ±0.04	0.37 ±0.06	0.71 ±0.24	5.83 ±12.3	26.87 ±30.45	0.71 ±0.91	0.06 ±0.01	0.08 ±0.08	38.50 ±3.01	8253.33 ±720.97	8.52 ±0.33	7.42 ±2.27
	St Helens	**Extensive**	42.23 ±0.52	1.36 ±0.01	1.40 ±0.48	0.22 ±0.04	0.02 ±0	0.23 ±0.08	0.50 ±0.61	0.52 ±0.27	5.31 ±4.41	18.33 ±21.25	0.31 ±0.16	0.04 ±0.02	0.12 ±0.13	43.10 ±5.84	8690.00 ±3253.84	8.33 ±0.23	9.06 ±3.36
		Incipient	42.60 ±1.14	1.34 ±0.05	1.25 ±0.25	0.22 ±0.06	0.02 ±0	0.21 ±0.09	0.34 ±0.04	0.54 ±0.31	6.06 ±7.14	17.71 ±26.88	0.39 ±0.27	0.04 ±0.11	0.10 ±0.08	41.47 ±2.75	7420.00 ±2502.33	8.45 ±0.47	8.44 ±1.07
	Tasman	**Incipient**	41.47 ±1	1.38 ±0.02	1.78 ±0.21	0.22 ±0.04	0.02 ±0	0.24 ±0.05	0.38 ±0.03	0.74 ±0.44	5.79 ±6.86	25.53 ±21.6	0.82 ±1.44	0.07 ±0.02	0.12 ±0.11	41.83 ±1.62	11966.67 ±2068.46	8.41 ±0.39	7.94 ±1.93
Spines mean			41.87 ±0.47	1.37 ±0.01	1.44 ±0.15	0.22 ±0.01	0.02 ±0	0.22 ±0.02	0.40 ±0.08	0.63 ±0.1	5.75 ±1.8	22.11 ±6.17	0.56 ±0.24	0.05 ±0.02	0.10 ±0.02	41.23 ±1.37	9082.50 ±1253.11	8.43 ±0.09	8.22 ±0.64
Test	**NSW**	**Incipient**	37.07 ±1.25	2.58 ±0.13	1.21 ±0.12	0.85 ±0.11	0.04 ±0.01	0.29 ±0.02	0.59 ±0.03	0.63 ±0.13	5.49 ±1.58	19.27 ±27.56	7.04 ±4.14	0.10 ±0.06	0.13 ±0.08	29.33 ±1.27	7266.67 ±1043.44	8.19 ±0.17	6.71 ±1.87
	St Helens	**Extensive**	37.53 ±2.31	2.56 ±0.16	1.30 ±0.69	1.06 ±0.57	0.05 ±0.02	0.30 ±0.2	0.60 ±0.14	0.52 ±0.23	9.04 ±7.64	16.90 ±6.83	2.37 ±0.86	0.05 ±0.06	0.17 ±0.02	33.63 ±2.83	7993.33 ±6979.78	8.13 ±0.57	7.84 ±0.93
		Incipient	38.20 ±3.01	2.53 ±0.06	1.19 ±0.41	1.03 ±0.75	0.05 ±0.03	0.31 ±0.23	0.59 ±0.16	0.46 ±0.27	8.94 ±8.38	11.81 ±7.29	2.43 ±1.24	0.08 ±0.03	0.11 ±0.07	31.80 ±2.73	7263.33 ±4784.94	8.15 ±0.76	8.36 ±8.91
	Tasman	**Incipient**	38.77 ±0.38	2.58 ±0.04	1.11 ±0.04	0.70 ±0.18	0.05 ±0.01	0.28 ±0.07	0.54 ±0.03	0.57 ±0.09	5.74 ±2.77	9.51 ±1.72	5.02 ±5.92	0.06 ±0.04	0.13 ±0.07	32.93 ±4.02	6006.67 ±403.88	8.22 ±0.27	6.01 ±0.57
Test mean			37.89 ±0.61	2.56 ±0.03	1.20 ±0.1	0.91 ±0.14	0.05 ±0.01	0.29 ±0.03	0.58 ±0.03	0.54 ±0.06	7.30 ±1.71	14.37 ±4.11	4.22 ±1.52	0.07 ±0.01	0.14 ±0.02	31.93 ±1.25	7132.50 ±1044.83	8.17 ±0.11	7.23 ±1.17

Tomato growth and productivity

Effect of urchin powder supplement on weekly plant growth

All plant growth parameters increased with greater urchin waste powder volumes applied, with plant performance in some variables equivalent to that observed in the Hoagland solution control treatment (Figure 5, Figure 6, Table 4). Weekly measurements of plant growth parameters (height, branches and stem CSA) showed overall statistically significant differences in group means for the interaction between treatments and sampling time (Figure 6, Table 3). At the end of the experiment, shoot length and stem CSA of tomato plants receiving the highest urchin waste powder (Treatment 7) were not significantly different (Table 4, Tukey's test, α=0.05) to the standard Hoagland's solution. Shoot length, stem CSA and plant width had a moderate increase in the lowest three urchin waste powder treatments, with no significant difference (Tukey's test, α=0.05) in these plant parameters (Figure 6, Table 4). Mean plant width (canopy area) for T7 (5% urchin waste powder) was significantly higher than all other treatments including the Hoagland's control (Figure 6, Tukey's test, α=0.05). Branches of tomato plants receiving the highest urchin waste powder (T6, T7) were not significantly different but had significantly fewer branches than the Hoagland's control (T8) (Figure 6, Table 4, Tukey's test, α=0.05). Tomato plants receiving the Hoagland's control had the greatest total dry matter mass which significantly decreased with each urchin waste powder rate increase from T7 to T4 (Figure 6, Tukey's test, α=0.05). Dry matter content was greatest for the Hoagland's control but not significantly different to the highest urchin waste powder treatments (Table 4, Tukey's test, α=0.05).

Figure 5. Growth trials of tomato plants (*Solanum lycopersicum*) using Longspined Sea Urchin waste powder as fertiliser.

Table 3 Two-way Anova with repeated measure of weekly tomato plant growth parameters.

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

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

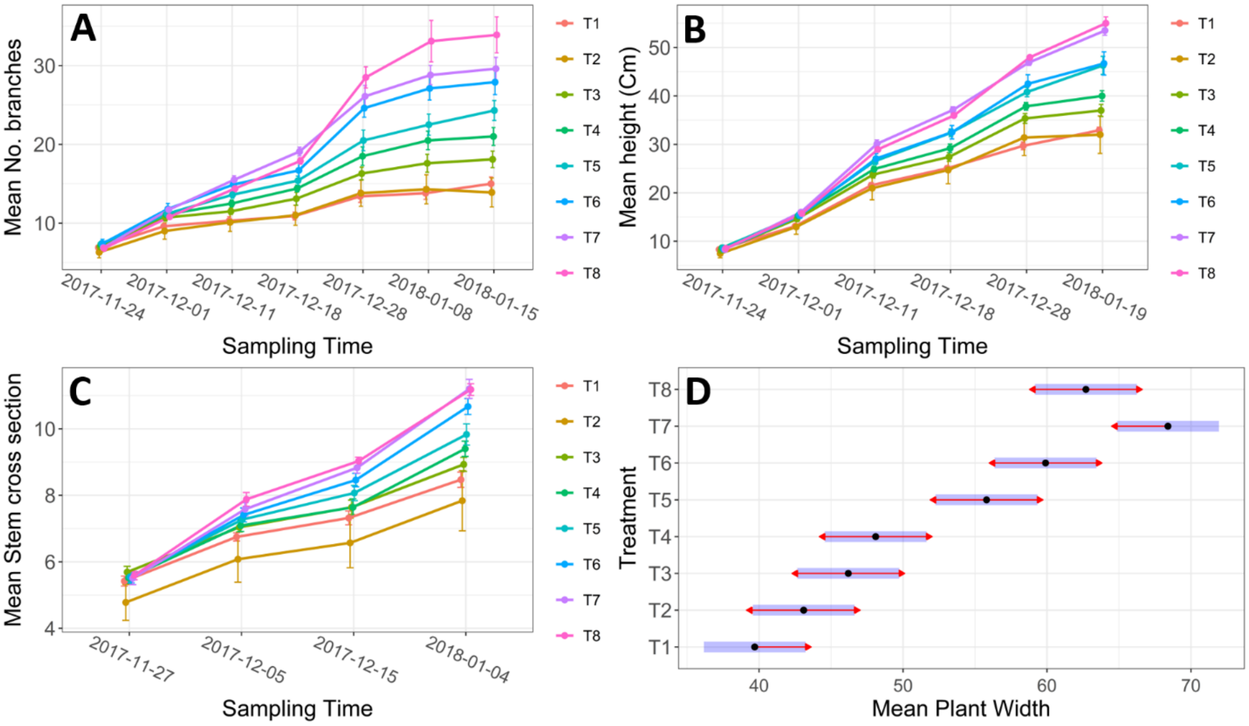

Figure 6. Plant growth measurements including number of branches (A), stem height (B), stem cross sectional area (C) and leaf width at the completion of the trial (D) for increasing sea urchin powder supplement rates (T1 – T7), and Hoagland's solution (T8). X-axis represents sampling event in weeks after planting. Error bars denote standard error (A, B, C). Plant width (D) represent a single sampling event. When arrows overlap among treatments, then the treatments are not significantly different.

Table 4. Vegetative growth parameters of tomato plants in greenhouse pot trial with sea urchin waste powder treatments.

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

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

Final nutrient composition of plant vegetative parts.

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

Nutrient (Total N, P, K and Ca and Mg) concentrations in the vegetative (combined shoots and leaves) parts of the plant showed an increasing response to higher urchin waste powder treatments (Table 5). All macronutrients increased significantly between T6, T7 and T8 (Table 5A, Tukey HSD, α=0.05). A similar trend of increasing micronutrient levels with increasing urchin waste powder was also observed (Table 5B). Plants grown in the Hoagland's solution (T8) contained higher nutrient concentrations than plants in the highest urchin waste powder treatment (T7) for all nutrients including Ca, Mg and micronutrients such as B, Zn, Fe and Mn (Table 5).

Table 5 Nutrient concentration in vegetative parts (combined shoot and leaf) of tomato plant in greenhouse pot trial with sea urchin waste powder treatments

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

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

The multivariate analysis on the proportion of leaf nutrient content showed overall statistical significant difference among treatment groups (Table 6). The nMDS plot reveal three clusters (Figure 7) of plant nutrient proportions where the four lowest urchin waste powder addition rates (T1-T4) form a tight grouping while two separate clusters are evident for T7 and T8, highlighting improved vegetative production and nutrient uptake in plants receiving the highest urchin waste powder addition (T7) but improved overall performance of plants receiving the Hoagland's solution (T8).

Table 6 Permanova analysis of proportion of leaf nutrient content for sea urchin waste treatments (T1-T7) and the Hoagland's control (T8).

Source	df	MS	Pseudo-F	P(Anderson et al)	Unique perms
Tr	7	352.36	59.234	**0.001**	998
Res	16	5.9487			
Total	23				

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

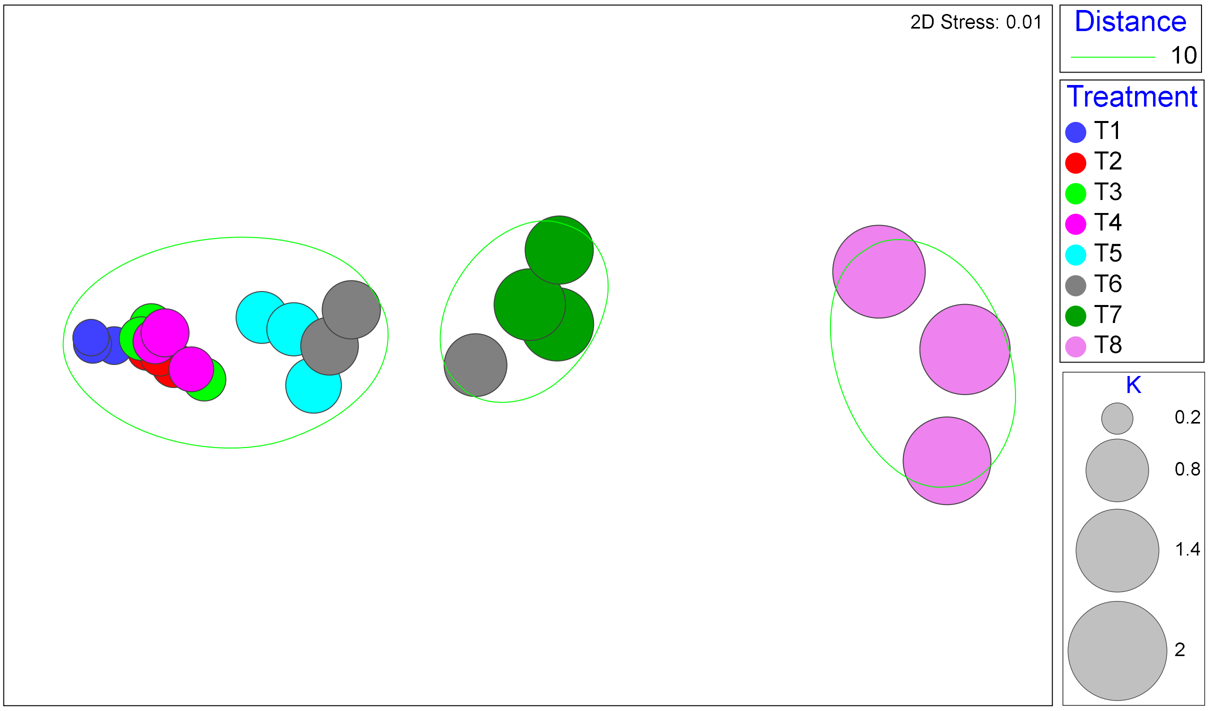

Figure 7 Non-metric multidimensional scaling (nMDS) bubble plot of Euclidian distances between the proportion of nutrients in tomato plant vegetative parts receiving sea urchin waste treatments (T1-T7) and the Hoagland control (T8). Bubble size indicate K content (w/w%).

Effect of urchin waste powder on flowering and fruit production

Overall statistically significant differences were observed in the weekly measurements between group means of flowers and fruit productivity for the interaction between treatments and sampling time (Table 7, Figure 8). Plant flowering and fruiting success measured at the end of the trial were variable across the treatments. A trend for increasing number of flower and fruit was observed with increasing application of urchin waste powder , however there were no clear statistical differences between the lowest four urchin waste powder treatments (Figure 8), (Table 8, Tukey's test, α=0.05). A main effect of urchin waste powder fertiliser was evident in T5, T6, and T7 (Figure 8) for these factors. Flower number receiving the highest urchin waste powder (T7) was not significantly different (Table 8, Tukey's test,

$\alpha=0.05$) to the standard Hoagland's solution.

Table 7 Two-way Anova with repeated measure of weekly tomato plant measurement of flower and fruit production.

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

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

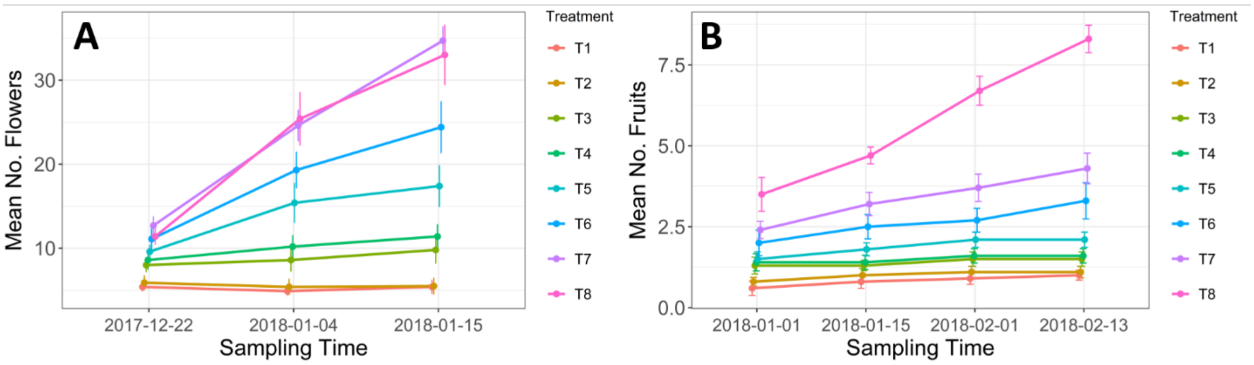

Figure 8 Flower (A) and fruit (B) production of tomato plants receiving sea urchin powder treatments (T1 – T7), and Hoagland's contol (T8). X-axis represents sampling event in weeks after planting.

Table 8 Average flower and fruit number, yield and fruit weight of tomato plants in greenhouse pot trial under sea urchin waste powder treatments (T1 – T7) and Hoagland's control (T8).

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

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

Average fruit number ranged from one fruit per plant in the lowest urchin waste powder treatment to eight fruit per plant in the Hoagland's control (Table 8) with significant differences in the mean values observed between the three highest rate treatments (T5 to T7) and the control (T8) ($F_{(7,72)}$=52.57, P=.000). Average fruit size and overall fruit weight (total yield) increased with increasing urchin waste powder ($F_{(7,176)}$=2.844, P=.008). Plants receiving the highest urchin waste powder (T7) yielded 238.1g fresh fruit which was almost half of the fresh fruit produced by plants in the Hoagland's treatment (448.1g) (Table 8, Tukey's test, α=0.05).

While there was a trend of decreasing percentage of fruit dry matter from T1 (7.3%) – T7 (6.7%) and T8 (6.5%), there were no significant differences in mean fruit dry matter per plant ($F_{(6,64)}$=.915, P=.501). Locule number was similar across all treatments varying from (6.00) to (8.00), ($F_{(7,72)}$=.489, P=.840). Pericarp thickness ranging from a minimum of 3.60 (T1) mm and a maximum of 5.75 (T8) mm and tended to significantly increase with higher urchin waste powder rates (Table 8) ($F_{(7,72)}$=2.951, P=.009).

Fruit nutrition and quality

Fruit nutrient levels as a measure of soil-nutrient uptake

Fruit nutrient concentrations increased in response to higher rates of urchin waste powder (Table 9). An increasing level of macronutrients (Total N, P, K and Ca and Mg) was observed with increasing rate of urchin waste powder applied. Both macro and micronutrients concentrations showed a mild increase in the first five treatments, no statistical differences observed (Table 9, Tukey HSD, α=0.05). In contrast, nutrient content significantly increased between T6, T7 and T8 (Table 9, Tukey HSD, α=0.05). Harvested fruit from tomato plants receiving the Hoagland's solution (T8) contained higher nutrient concentrations than fruit from plants receiving the highest urchin waste powder treatment (T7) for Ca, Mg and micronutrients including B, Zn, Fe and Mn (Table 9).

Table 9 Nutrient concentration in fruit of tomato plants receiving sea urchin waste powder (T1 – T7) treatments and the Hoagland's control (T8).

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

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

The multivariate analysis on the proportion of tomato fruit nutrient content showed overall statistical significant difference among treatment groups (Table 10). Again, from the nMDS plot three clusters appear (Figure 9), a closer group including proportion of fruit nutrient content of the five lowest urchin waste powder rates (T1-T5) with (T6) slightly separated from them but similarly part of the same cluster and a sharp distinction of T7 and T8 groups, resembling the outcomes of fruit production number and yield in these two treatments.

Table 10 Permanova analysis of proportion of fruit nutrient content for sea urchin waste treatments (T1-T7) and Hoagland control (T8).

Source	Df	MS	Pseudo-F	P(Anderson et al)	perms
Tr	7	1077.4	373.14	**0.001**	999
Res	16	2.8873			
Total	23				

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

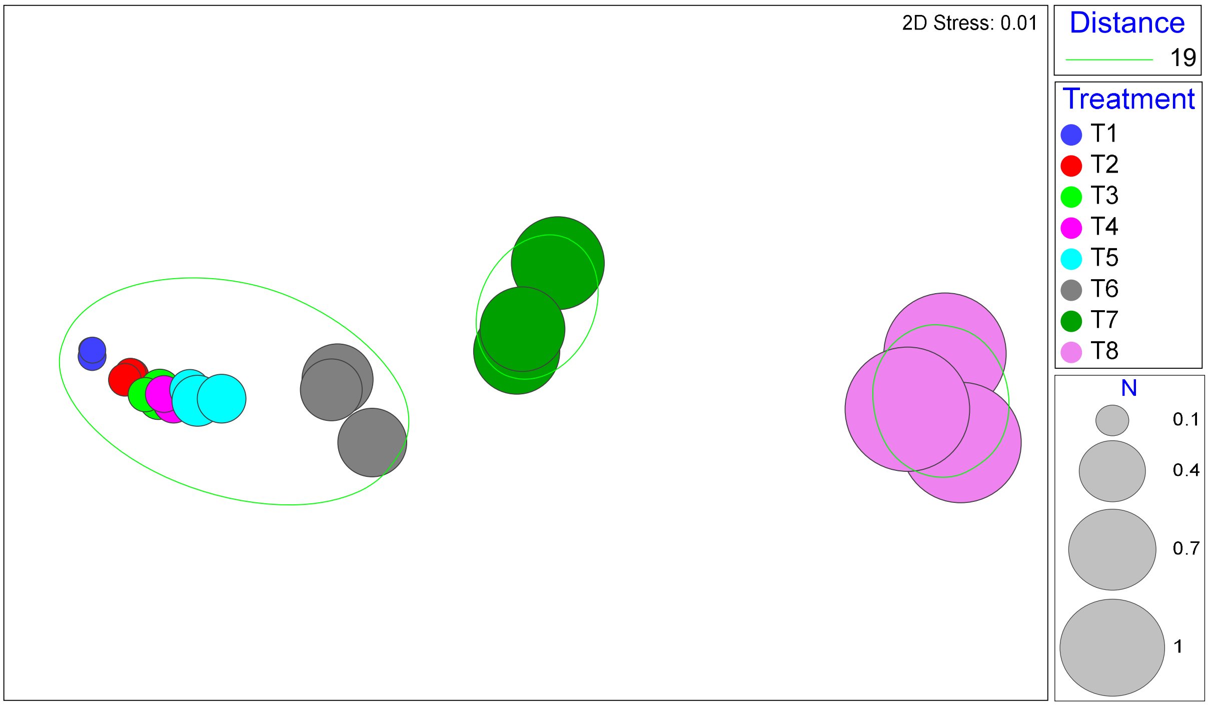

Figure 9 Non-metric multidimensional scaling (nMDS) bubble plot of Euclidian distances between the proportion of nutrients in tomato fruit of the seven urchin waste powder treatment (T1-T7) and Hoagland control (T8). The bubble size indicate N content (w/w%).

Fruit ripeness

Urchin waste powder treatments had a significant influence on the maturation of tomato fruit as measured by fruit firmness and colour. As fruit ripens over time, firmness values (as determined by resistance to the probe during the pressure test) decrease as the flesh gets softer and the red colour of fruit increases. Fruit firmness results were statistically higher in T1 compared to all other treatments (Table 11, Tukey's test, α=0.05) because fruit did not develop properly, remaining small and under ripe. Fruit harvested from plants receiving the Hoagland's solution were on average firmer than fruit from T7, but no statistical significance was observed (Table 11, Tukey's test α=0.05). Flesh firmness was inversely correlated to colour values and dry matter content in fruit with the lowest rate of urchin waste powder .

Table 11 Colour and firmness parameters in tomato fruit receiving sea urchin waste powder (T1 – T7) and Hoagland solution (T8) treatments.

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

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

Fruit colour analyses was consistent with other ripening variables and again followed a response curve to the treatments. L* values were higher in fruit treated with less urchin waste (48.6) in T1, reflecting a lower degree of ripeness - hence colour ranged from white green to pale red, while riper fruit showed an intense red colour that translate in lower L* values (35.18 and 36.81) as observed in T7 and T8 respectively (Table 11). Colour values of fruit from the first five treatments were more spread, indicating a range of ripening observed in the fruit harvested from these plants. Fruit from treatment T6 and T7 and the Hoagland's control had similar colour values. Fruit colour and firmness data explored with multivariate analysis (Anderson et al) were not significantly different (Table 12A, P=0.108)

There was no significant difference for pH ($F_{(7,37)}$=1.235, P=.309), with treatment means ranging from 4.06 to 4.24 while significant differences were observed for mean values of titratable acidity ($F_{(7,37)}$=12.23, P=.000) with 5.77 mg/100ml in T1, 3.51 mg/100ml in T7 and 3.43 mg/100ml in T8 (Figure 10a, Tukey's test α=0.05). The total soluble solids (°Brix) mean values ranged from 4.3 to 5.9 (Figure 10b) with the Hoagland's control and T7 showing significantly lower vales than all the other treatments ($F_{(7,37)}$=9.026, P=.000).

Fruit quality attributes pH, acidity, and °Brix were highly significant when analysed with Permanova (Table 12B, P=0.001), yet treatment groups did not appear clearly differentiated in nMDS ordination.

Table 12 Permanova analysis of fruit ripeness parameters (Colour coordinates and Firmness) and quality attributes (pH, Acidity and °Brix) for urchin waste powder and treatments (T1-T7) and Hoagland control (T8).

A Colour: Firmness

Source	**df**	**MS**	**Pseudo-F**	**P(Anderson et al)**	**perms**
Tr	7	392.22	1.515	**0.108**	998
Res	72	258.9			
Total	79				

B pH: Acidity: °Brix

Source	**df**	**MS**	**Pseudo-F**	**P(Anderson et al)**	**perms**
Tr	7	4.7608	10.754	**0.001**	998
Res	37	0.44269			
Total	44				

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

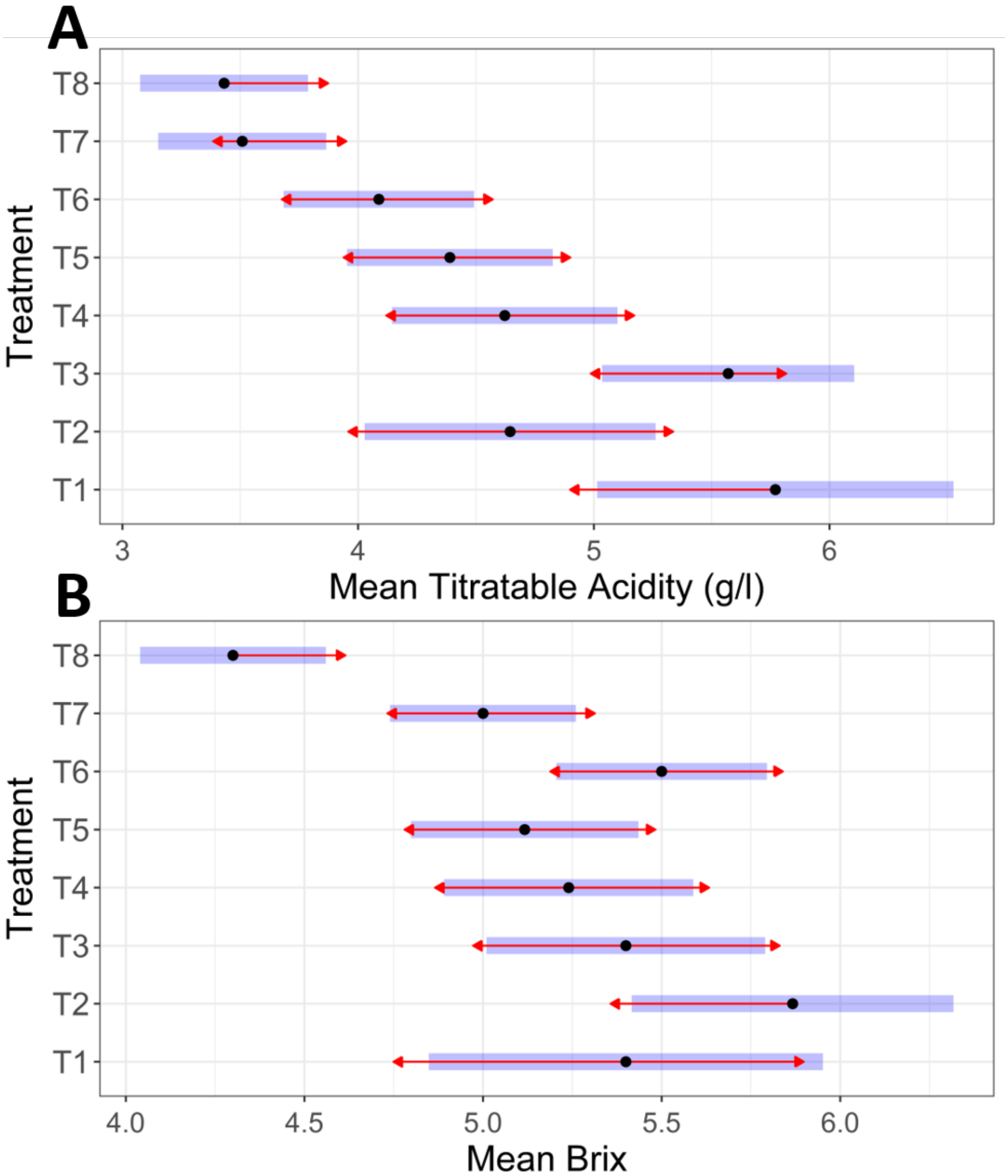

Figure 10 Fruit (TA) titratable acidity (A) and total soluble solids (°Brix) (B) across seven treatment rates of urchin powder (T1-T7) and Hoagland control (T8). Dots indicate means of fruit pooled together across the ten replicate pots in each treatment. Bars denote the confidence limit. When the red arrows overlap among treatments, then the treatments are not significantly different.

Soil nutrient content

The application of urchin waste powder increased the pH and EC of the growing medium (Figure 11a and b) with scale of response consistent with increasing rate of urchin waste powder application. The pH of the growing medium at end of trial shows an increasing trend in the three lower treatments from pH 6.7 to pH 6.9 and stabilizing slightly above pH 7 from T4 to T7 with significant statistical differences (Figure 11c, $F_{(6,14)}$=15.14, P=.000). The EC instead varied between 0.428 dS/m in T1, 0.563 dS/m in T6 and 0.693 dS/m T7 with no clear statistical differences among treatments (Figure 11d, $F_{(6,14)}$=1.618, P=.214).

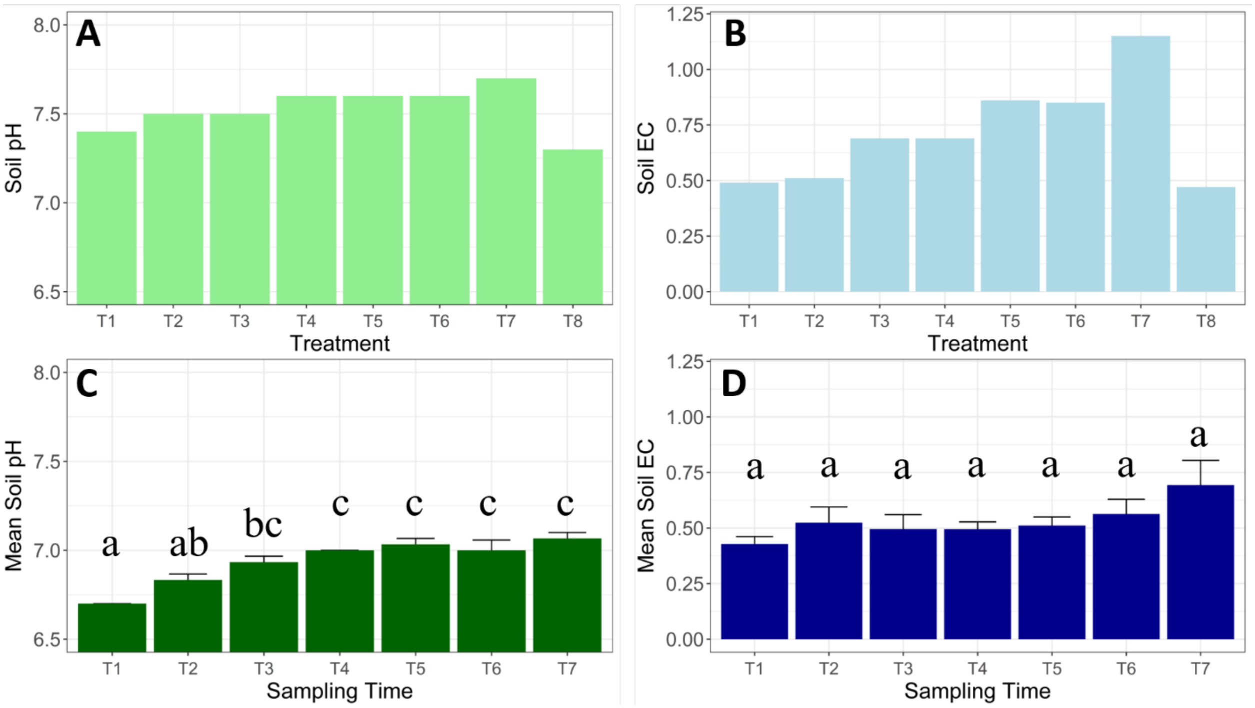

Figure 11 pH (A) and EC (B) of potting mix after the application of urchin waste powder at seven different rates at the start of the trial. T8 represent the potting mix before the Hoagland's addition. Values represent a single measurement per treatment from pooled sub-samples from the ten pot replicates three days post planting of tomato seedlings. pH (C) and EC (D) of potting mix with addition of the seven urchin waste powder treatments rate at the end of the trial. Error bars denote means of three replicates per each treatment. Means that do not share a letter indicate significant difference among treatments. Grouping Information Using the Tukey HSD Method and P < 0.05.

The nutrient content of the potting mix with the addition of urchin waste powder at seven different rates at the end of the trial is shown in Table 13. No statistical differences between the treatments were observed for N, P, Mg, Cu, Zn and B. The value of K in T7 was significantly lower than the first five treatments (Table 13, Tukey's test α=0.05), indicating that K was actively taken up by the plants in this treatment. The content of other elements in the pot appear more irregular. Ca increased from T1 to T5 then declined again to T7 with statistical difference between T1 and T5 (Table 13, Tukey's test α=0.05); S was significantly higher in T7 compared to the first five treatments and the same trend can be observed for Na (Table 13, Tukey's test α=0.05). Conversely, the remaining Fe and Mn was higher in the lower treatments with a decline towards T7.

Table 13 Nutrients concentration in potting mix with addition of urchin waste powder at seven rates at the end of the trial.

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

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

Bait Assessment: Lobsters from Kelp Habitat

Individual feeding trials

During individual feeding trials for Southern Rock Lobsters from kelp habitat, bait species significantly influenced the number of forage events observed (Table 14). The total forage events on Longspined Sea Urchin (32%) was less than half that on Shortspined Sea Urchin (76%) and Jack Mackerel (84%) (Table 15, Figure 12). Lobster size did not significantly affect forage events. Time of the forage event was significant as was the interaction between time and bait species (Table 14). Forage events on Shortspined Sea Urchin and Jack Mackerel were greatest within the first 30 min period, whereas forage events on Longspined Sea Urchin were at low levels throughout (Table 15, Figure 12). Although not quantified, lobsters were often observed to show near instantaneous positive attraction to Shortspined Sea Urchin and Jack Mackerel baits placed in the tanks. This was not observed with Longspined Sea Urchin. Additionally, test segments, as well as the roe, of Shortspined Sea Urchin baits were often partially or wholly consumed within the two-hour trial period. By comparison, there was no obvious consumption of Longspined Sea Urchin test, only the roe.

Table 14. Analysis of variance table for 2-factor ANOVA testing the effects of a) 'Lobster Size' (medium or large) and 'Bait Species' (Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Bait Species' and b) 'Time' (30 min period) and 'Bait Species' (Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Time', on Southern Rock Lobster (from kelp habitat) forage events when bait species was supplied individually.

a)

	Df	Mean Sq	F	P
Lobster Size	1	0.24	1.31	0.26
Baits Species	2	3.92	21.32	<0.0001
Bait Species * Lobster Size	2	<0.01	0.00	0.81
Residuals	144	0.8		

b)

	Df	Mean Sq	F	P
Time	3	1.06	8.51	<0.0001
Baits Species	2	0.98	7.85	<0.001
Bait Species * Time	6	0.36	2.85	<0.01
Residuals	588	0.12		

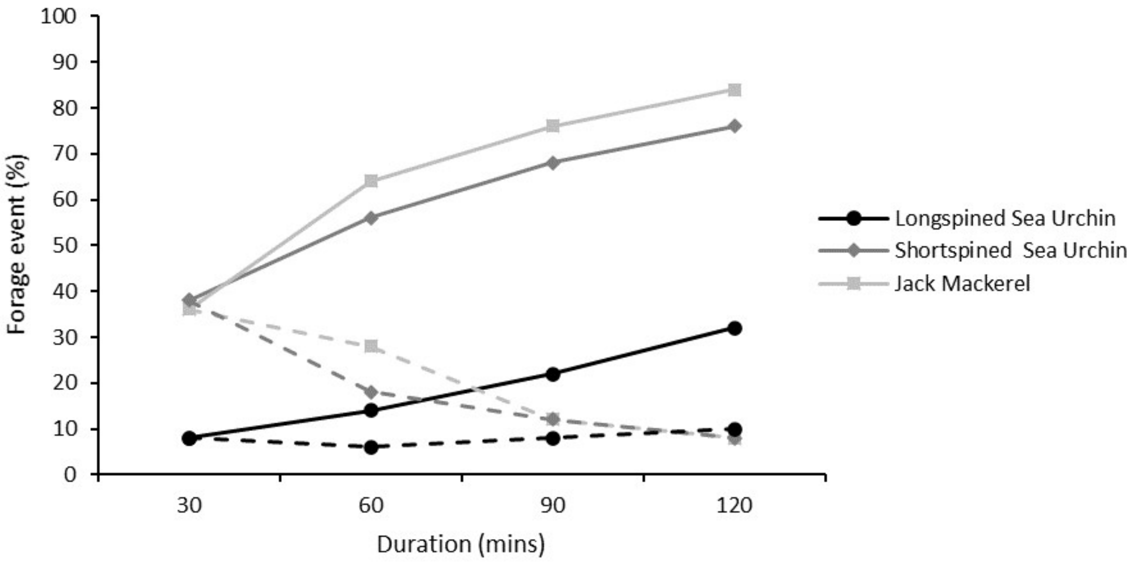

Figure 12. Cumulative (solid line) and period (dashed line) forage events (%) by Southern Rock Lobsters (from kelp habitat) on Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel bait recorded every 30 minute period over the two 2 hour trials.

Table 15. Forage events on Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel baits by Southern Rock Lobsters (from kelp habitat; n = 10) fed individually. Individual feeding trial duration was 120 min and repeated daily for 5 days for each bait species.

Bait species	Duration (min)				Forage total
	30	60	90	120	
Longspined Sea Urchin					
Day 1	1	1	1	1	4
Day 2	1	0	0	1	2
Day 3	1	1	1	0	3
Day 4	1	1	1	1	4
Day 5	0	0	1	2	3
Cumulative forage (%)	8	14	22	32	
Shortspined Sea Urchin					
Day 1	6	0	2	1	9
Day 2	3	5	1	0	9
Day 3	3	1	0	1	5
Day 4	4	2	1	1	8
Day 5	3	1	2	1	7
Cumulative forage (%)	38	56	68	76	
Jack Mackerel					
Day 1	3	4	1	0	9
Day 2	4	4	1	1	7
Day 3	4	2	1	1	9
Day 4	3	3	1	1	7
Day 5	4	1	2	1	6
Cumulative forage (%)	36	64	76	84	

Paired feeding trials

During paired bait feeding trials for Southern Rock Lobsters from kelp habitat, a significant preference for Jack Mackerel bait over Longspined Sea Urchin bait was observed (Table 17, Figure 13). Forage events on Jack Mackerel occurred on 76% of trials, compared to 14% on Longspined Sea Urchin. Lobster size was not significant in influencing bait choice. Across the trial. First feeding (30 minute observations) was on Jack Mackerel 76%, Longspined Sea Urchin 10% and no feeding 14 % of trials (Table 17).

Table 16. Analysis of variance table for 2-factor ANOVA testing the effects of a) 'Lobster Size' (medium or large) and 'Bait Species' (Longspined Sea Urchin & and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Bait Species' and b) 'Time' (30 min period) and 'Bait Species' (Longspined Sea Urchin and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Time', on Southern Rock Lobster forage when the two bait species supplied together.

a)

	Df	Mean Sq	F	P
Lobster Size	1	0.01	0.07	0.798442
Baits Species	1	9.61	63.02	<0.0001
Bait Species * Lobster Size	1	0.49	3.21	0.0762
Residuals	96	0.15		

b)

	Df	Mean Sq	F	P
Time	3	0.08	0.89	0.45
Baits Species	1	2.40	25.92	<0.0001
Bait Species * Time	3	0.32	3.41	0.02
Residuals	392	0.09		

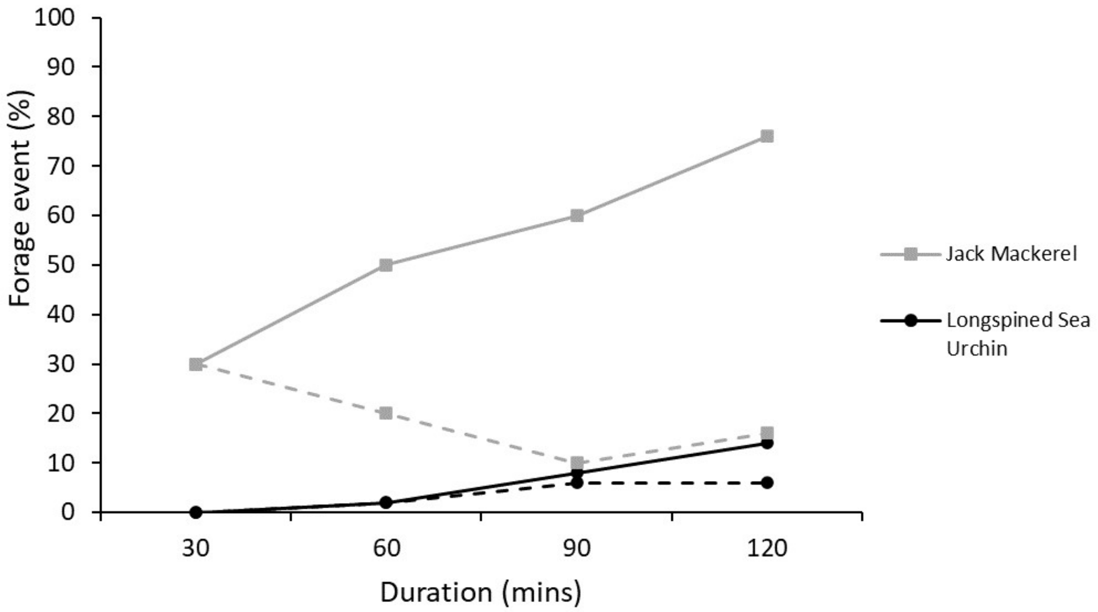

Figure 13. Cumulative (solid lines) and period (dashed lines) forage events (%) by Southern Rock Lobsters (from kelp habitat) on Jack Mackerel and Longspined Sea Urchin during 120 minute paired forage trials.

Table 17. Forage totals of Longspined Sea Urchin and Jack Mackerel segments by Southern Rock Lobsters (from kelp habitat; n = 10) over 120 min feeding trials repeated daily for 5 days.

Bait species	Duration (min)				Forage total (n)
	30	60	90	120	
Longspined Sea Urchin					
Day 1	0	0	0	0	0
Day 2	0	0	2	1	3
Day 3	0	0	0	1	1
Day 4	0	0	1	0	1
Day 5	0	1	0	1	2
Cumulative forage (%)	0	2	8	14	
Jack Mackerel					
Day 1	4	2	0	3	9
Day 2	3	1	1	2	7
Day 3	4	3	1	1	9
Day 4	2	4	1	0	7
Day 5	2	0	2	2	6
Cumulative forage (%)	30	50	60	76	

During paired bait feeding trials with Longspined Sea Urchin and Short Spined Sea Urchin, Southern Rock Lobsters from kelp habitat showed a significant feeding preference for Shortspined Sea Urchin bait over Longspined Sea Urchin bait (Table 18, Figure 14). Forage events on Shortspined Sea Urchin occurred on 76% of trials, compared to 18% on Longspined Sea Urchin. No significant difference of Lobster size on bait preference was observed. Across the trial, first feeding (30 minute observations) was on Shortspined Sea Urchins 70%, Longspined Sea Urchins 12%, both species 6% and no feeding 12 % (Table 19).

Table 18. Analysis of variance table for 2-factor ANOVA testing the effects of a) 'Lobster Size' (medium or large) and 'Bait Species' (Longspined Sea Urchin and Shortspined Sea Urchin), plus the interactive term of 'Lobster Size' * 'Bait Species' and b) 'Time' (30 min period) and 'Bait Species' (Longspined Sea Urchin and Shortspined Sea Urchin), plus the interactive term of 'Lobster Size' * 'Time', on Southern Rock Lobster forage events when the two bait species supplied together.

a)

	Df	Mean Sq	F	P
Lobster Size	1	0.01	0.06	0.81
Baits Species	1	8.41	48.99	<0.0001
Bait Species * Lobster Size	1	0.01	0.06	0.81
Residuals	96	0.17		

	Df	Mean Sq	F	P
Time	3	0.16	1.66	0.18
Baits Species	1	2.10	21.45	<0.0001
Bait Species * Time	3	0.16	1.59	0.19
Residuals	392	0.10		

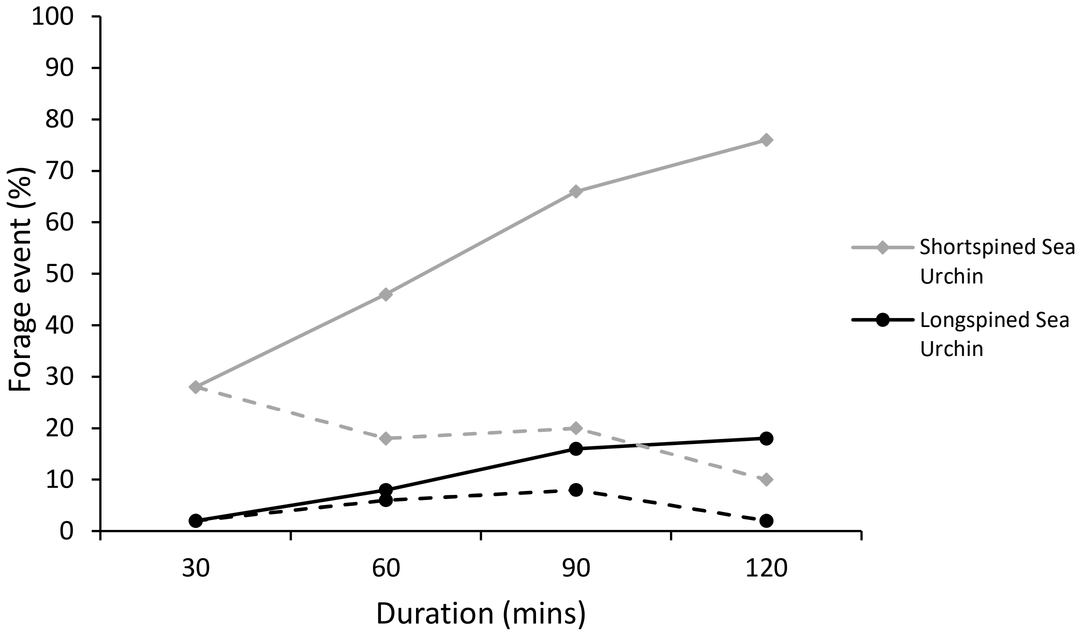

Figure 14. Cumulative (solid lines) and period (dashed lines) forage events (%) by Southern Rock Lobsters (from kelp habitat) on Longspined Sea Urchin and Shortspined Sea Urchin baits during 120 minute paired forage trials.

Table 19. Forage events of Longspined and Shortspined Sea Urchin baits Southern Rock Lobsters (from kelp habitat; n = 10)over 120 min feeding trials repeated daily for 5 days.

Bait species	Duration (min)				Forage total (n)
	30	60	90	120	
Longspined Sea Urchin					
Day 1	0	1	1	0	2
Day 2	0	0	0	0	0
Day 3	0	0	1	1	2
Day 4	0	1	1	0	2
Day 5	1	1	1	0	2
Cumulative forage (%)	2	8	16	18	
Shortspined Sea Urchin					
Day 1	4	2	1	2	9
Day 2	2	2	3	1	8
Day 3	2	1	3	1	7
Day 4	3	2	2	0	7
Day 5	3	2	1	1	7
Cumulative forage (%)	28	46	66	76	

Live predation trial

During live predation trials, predation by large Southern Rock Lobsters from kelp habitat was observed only on live Shortspined Sea Urchin, with no predation on live Longspined Sea Urchins occurring during the 10 day trial. Predation rates averaged 0.98 Shortspined Sea Urchins/Rock Lobster/day. It was observed that Southern Rock Lobsters often consumed the Shortspined Sea Urchins test, particularly in the first few days of the trial, and in some instances few remnants of tests were evidence at the end of the 24 hr observation period.

Bait Assessment: Lobsters from Barrens Habitat

Individual feeding trials

During individual feeding trials for Southern Rock Lobsters from barrens habitat, both bait species and lobster size significantly influenced the number of forage events (Table 20). The total forage events on Longspined Sea Urchin (70%) were less than that on Jack Mackerel (90%) (Table 20, Figure 15). The influence of lobster size was evidenced by forage events on Longspined Sea Urchin; large lobsters foraged on 88% of urchin baits, compared to 52% by medium lobsters (Figure 16). Time of the forage event was significant, as was the interaction between time and bait species (Table 20). Forage events on Longspined Sea Urchin and Jack Mackerel were greatest within the first 30 min period (Table 21, Figure 15). Although not quantified, lobsters were often observed to show near instantaneous positive attraction to both Longspined Sea Urchin and Jack Mackerel baits placed in the tanks. Additionally, test segments, as well as the roe, of Longspined Sea Urchin baits were often partially consumed within the two-hour trial period.

Table 20. Analysis of variance table for 2-factor ANOVA testing the effects of a) 'Lobster Size' (medium or large) and 'Bait Species' (Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Bait Species' and b) 'Time' (30 min period) and 'Bait Species' (Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Time', on Southern Rock Lobster (from barrens habitat) forage when bait species was supplied individually.

a)

	Df	Mean Sq	F	P
Lobster Size	1	1.44	10.47	>0.01
Baits Species	1	1	7.27	>0.01
Bait Species * Lobster Size	1	0.36	2.62	0.11
Residuals	96	0.1375		

b)

	Df	Mean Sq	F	P
Time	3	19.3	<0.0001	0.21
Baits Species	1	0.25	0.12	0.61
Bait Species * Time	3	4.09	<0.0001	0.74
Residuals	392	40.36		

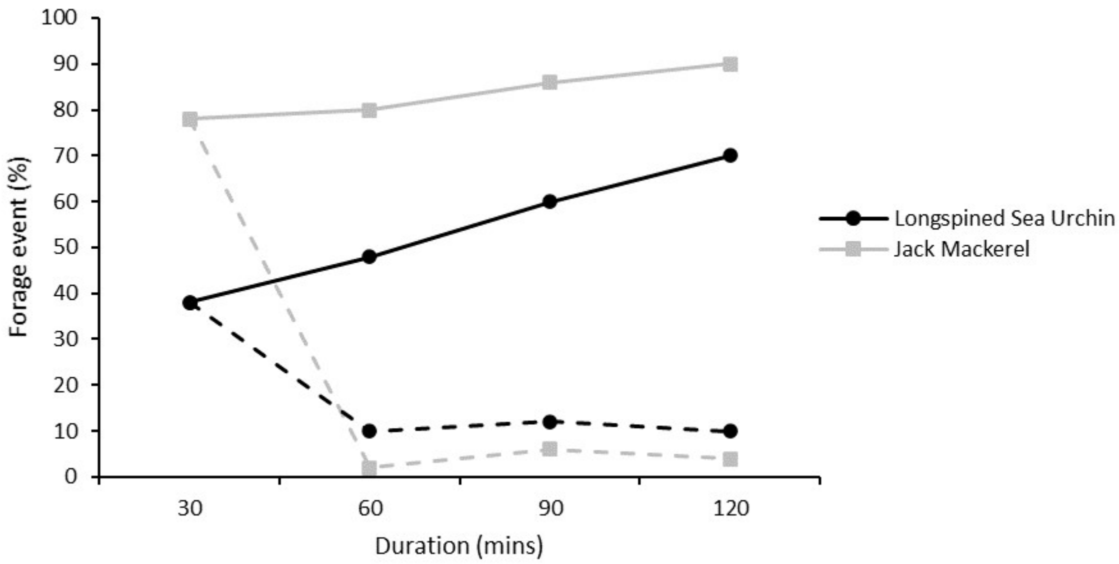

Figure 15. Cumulative (solid line) and period (dashed line) forage events (%) by Southern Rock Lobsters (from barren habitat) on Longspined Sea Urchin and Jack Mackerel bait recorded every 30 minute period over the two 2 hour trials.

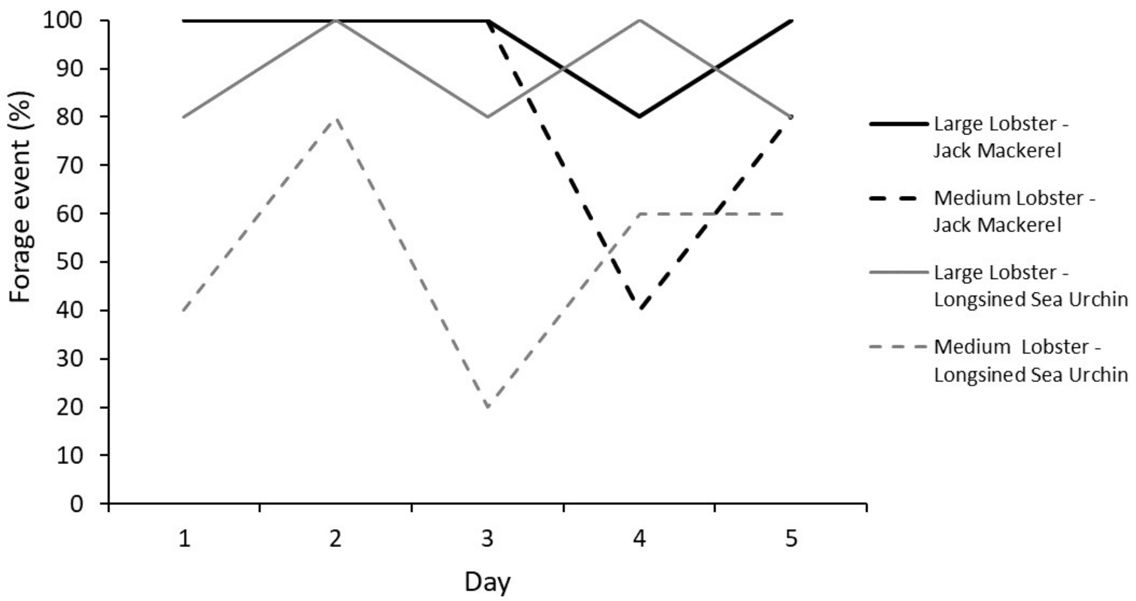

Figure 16. Forage events (%) by large and medium Southern Rock Lobsters originating from barrens habitat on Jack Mackerel and Longspined Sea Urchin bait during 120 minute individual forage trials.

Table 21. Forage events on Longspined Sea Urchin, Shortspined Sea Urchin and Jack Mackerel baits by Southern Rock Lobsters (from barren habitat; n = 10) fed individually. Individual feeding trial duration was 120 min and repeated daily for 5 days for each bait species.

Bait species	Duration (min)				Forage total
	30	60	90	120	
Longspined Sea Urchin					
Day 1	2	1	2	1	6
Day 2	5	1	1	2	9
Day 3	4	0	1	0	5
Day 4	5	1	1	1	8
Day 5	3	2	1	1	7
Cumulative forage (%)	39	1	3	2	
Jack Mackerel					
Day 1	10	0	0	0	10
Day 2	10	0	0	0	10
Day 3	8	1	0	1	10
Day 4	5	0	0	1	6
Day 5	6	0	3	0	9
Cumulative forage (%)	58	6	9	7	

Paired feeding trials

During paired bait feeding trials for Southern Rock Lobsters from barrens habitat, a significant preference for Jack Mackerel bait over Longspined Sea Urchin bait was observed (Table 22, Figure 17). Forage events on Jack Mackerel bait occurred on 72% of trials, compared to 44% on Longspined Sea Urchin. Lobster size was significant in influencing bait choice with large Southern Rock Lobsters foraging on Longspined Sea Urchin in 80% of trials, compared to 30% by medium Southern Rock Lobsters (Figure 18). Across the trial, first feeding (30 minute observations) was on Jack Mackerel 50%, Longspined Sea Urchin 24%, Both 10% and no feeding 16 % (Table 23).

Table 22. Analysis of variance table for 2-factor ANOVA testing the effects of a) 'Lobster Size' (medium or large) and 'Bait Species' (Longspined Sea Urchin & and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Bait Species' and b) 'Time' (30 min period) and 'Bait Species' (Longspined Sea Urchin and Jack Mackerel), plus the interactive term of 'Lobster Size' * 'Time', on Southern Rock Lobster (from barren habitat) forage when the two bait species supplied together.

a)

	Df	Mean Sq	F	P
Lobster Size	1	1.44	6.80	<0.05
Baits Species	1	1.96	9.26	<0.005
Bait Species * Lobster Size	1	0.64	3.02	0.085
Residuals	96	0.21		

b)

	Df	Mean Sq	F	P
Time	3	1.28	11.19	<0.0001
Baits Species	1	0.49	4.27	<0.05
Bait Species * Time	3	0.10	0.84	0.47
Residuals	392	0.11		

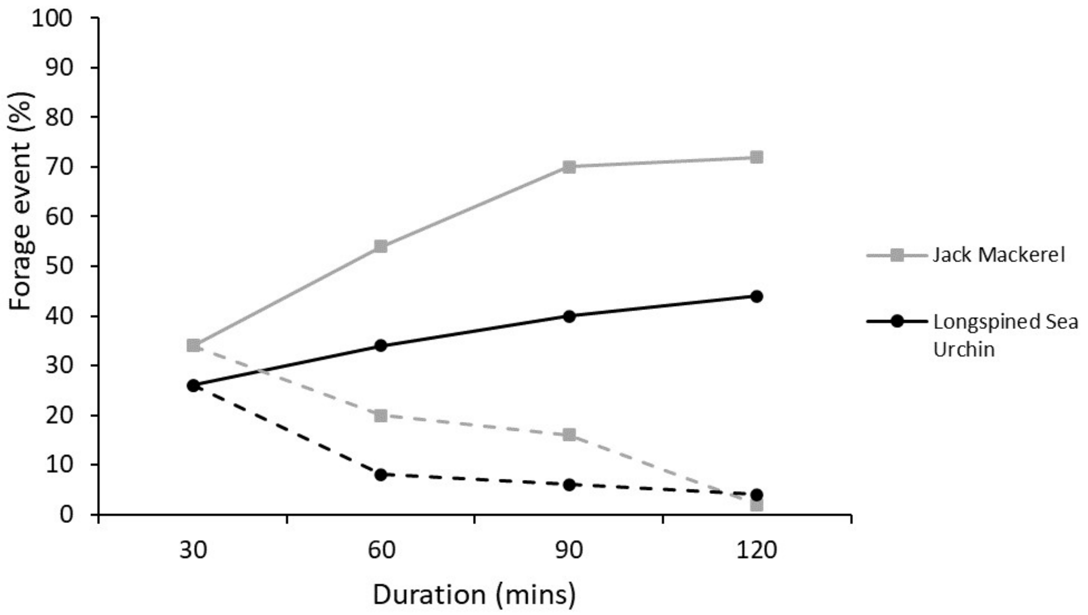

Figure 17. Cumulative (solid lines) and period (dashed lines) forage events (%) by Southern Rock Lobsters from barrens habitat on Jack Mackerel and Longspined Sea Urchin during 120 minute paired forage trials.

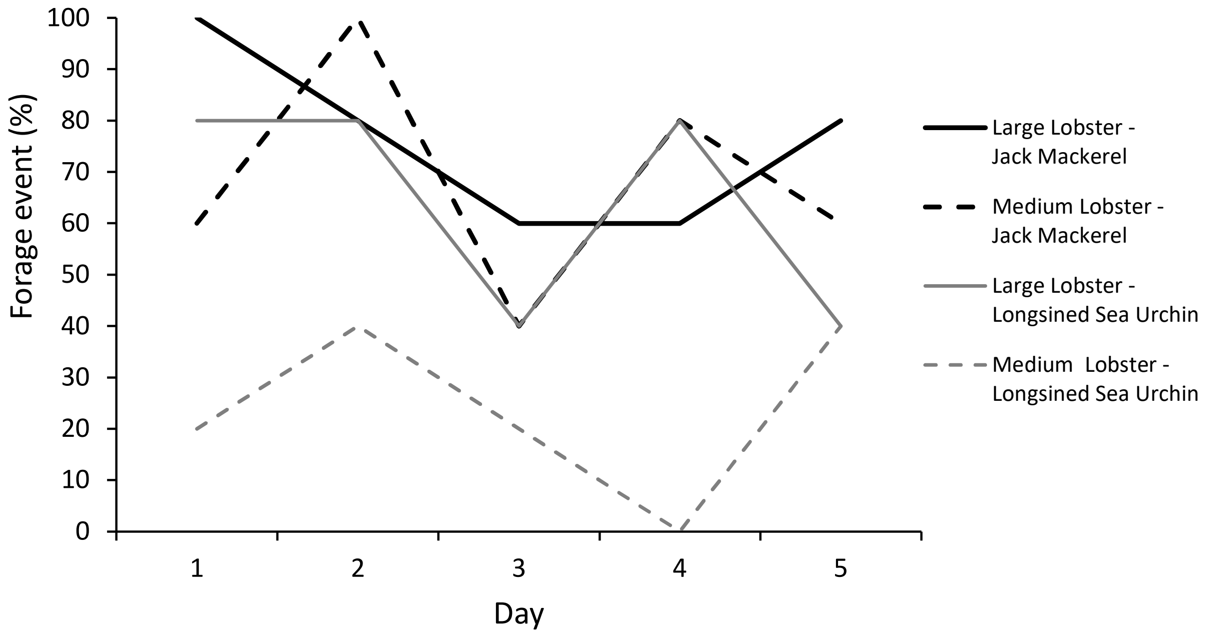

Figure 18. Forage events (%) by Large and Medium Southern Rock Lobsters from barrens habitat on Jack Mackerel and Longspined Sea Urchin baits during 120 minute paired forage trials.

Table 23. Forage totals of Longspined Sea Urchin and Jack Mackerel segments by Southern Rock Lobsters from barrens habitat (n = 10) over 120 min feeding trials repeated daily for 5 days.

Bait species	Duration (min)				Forage total (n)
	30	60	90	120	
Longspined Sea Urchin					
Day 1	3	1	0	1	5
Day 2	3	1	2	0	6
Day 3	2	0	0	1	3
Day 4	3	0	1	0	4
Day 5	2	2	0	0	4
Cumulative forage (%)	26	34	40	44	
Jack Mackerel					
Day 1	4	3	1	0	8
Day 2	5	1	2	1	9
Day 3	1	2	2	0	5
Day 4	3	3	1	0	7
Day 5	4	1	2	0	7
Cumulative forage (%)	34	54	70	72	

Discussion

Longspined Sea Urchin resources

Large biomasses of Longspined Sea Urchin are present off NSW, Victoria and Tasmania. It was estimated that 5,500 tonnes of the urchin were in Tasmanian waters in 2017 alone, with the biomass growing at a rate of 190 t per annum before accounting for fisheries removals (Ling and Keane, 2018; Cresswell et al., 2020). While commercial harvesting for roe occurs across all three states, the resource is largely underutilised. Of the landed biomass, we estimate that just ~ 11% is currently saleable product (i.e. the roe). This small usable percentage leaves vast biomasses of a potential resource being dumped.

In Tasmania, annual harvest levels have fluctuated greatly since the establishment of the fishery in 2009 resulting from processors entering / exiting the industry. A harvest subsidy was introduced in 2016 to accelerate the industry and promote large-scale removal as a tool for urchin control. The harvest peaked at 560 t in 2019, but it's projected that roe-fishery landings will average 300-400 t per annum given current processor constraints (Beth Mathison, *pers. comm*.). Opportunities for further processor expansion increasing supply are plausible but not foreseen. Thus, it is forecasted that ca. 250 t of wet waste, 120 dry waste, will be produced in Tasmania each year.

Product development from the roe-fishery waste is key to increasing the industries profitability and secure financial sustainability. Reducing current waste costs and transforming waste to a profitable commodity would reduce subsidy reliance and future subsidy uncertainty. However, product development from roe-processing waste needs to consider the supply limitations including seasonality (January to June), total volume (factory processing limitations) and short-term fluctuations (market and weather driven). Given the large volumes of waste produced and variable supply, product handling and storage processes may restrain product development for some applications. The production of any by-product would most likely have to be dried or frozen.

In addition to the roe-fishery, a 'take-all' harvests of Longspined Sea Urchin have been trialled in Tasmania to help control this range-extending species (Larby, 2020). This harvest strategy removes all urchins, including those un-processable for roe (e.g. too small), and has been proposed as an alternate method to culling as it has the potential to provide (at least partial) economic return. Estimates from a government funded 'take-all' trial in southern Tasmania indicates approximately one third of the landed biomass was not processable for roe and dumped (Keane, unpublished data). Adding value to such waste would help offset the costs of 'take-all' harvesting as an urchin control method.

Agricultural potential

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

Tomato nutrition

A comparison of the nutrient content provided by the urchin waste powder at different rates against the nutrients supplied with the Hoagland solution, provides some explanation for the improvements in the vegetative and reproductive attributes of the tomato plants. Given the nutrient composition of the urchin waste powder, treatments were not expected to perform as well as the Hoagland's control, however there was clear evidence of nutrient uptake by tomato plants receiving urchin waste powder. The Hoagland's solution provided 1950 mg N in T8, twice the amount supplied to the plants in T7. Almost four times the amount of P was provided in the Hoagland's solution (223 mg) compared to 65 mg in T7 and three times the amount of K was provided. In contrast some macro elements like Ca, Mg and S and microelements like B, Cu, Zn and Fe were supplied in higher proportions through the urchin waste powder in the higher rate treatments which may have supported the vegetative growth of tomato plants observed in T7 in the context of limited N supply.

Nitrogen and K play an important role in plant growth and development (Leghari et al 2016, Prajapati & Modi 2012). For tomatoes, N supply has been shown to significantly increase crop vegetative growth (Tei et al 2000), plant yield and fruit quality when supplied adequately (Wang et al 2015), whereas insufficient N content in tomatoes can lead to limited vegetative growth, reduced shoot length and leaf area (Scholberg et al 2000), net photosynthetic rate decline associated to decreased chlorophyll content and leaf senescence (Guidi et al 1997) and blossom drop with subsequent low yields (Ozores-Hampton & McAvoy 2017). Symptoms of N deficiency were visible in plants receiving the lower urchin waste powder treatments where the four-week-old leaves became chlorotic, had completely yellowed and subsequently dehisced. Nitrogen in the Hoagland solution was in form of ammonia and nitrate which are both readily available forms for plant uptake. In contrast, N provided in urchin waste powder treatments is in the form of amino acids and bound peptides which require proteinaceous transporters to facilitate the transfer of N compounds across cellular membranes (Tegeder & Rentsch 2010). In tomatoes, Schwake et al. (1999) showed that a specific proteinaceous transporter enabled the accumulation of the free amino acid Proline in tomato pollen during maturation (Schwacke et al 1999).

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

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

Magnesium is essential for the photosynthesis process being a component of the chlorophyll molecule (Marschner 2011) and when limited results in decreased biomass production and lower yield in greenhouse tomatoes (Hao & Papadopoulos 2004). The high content of Mg in T6 and 7 is likely to have promoted vegetative growth despite low levels of N and K.
Boron plays a key role in the growth of many fruit and vegetable plants and many studies have highlighted the importance of boron in tomato fruit quality (Huang & Snapp 2009, Naz et al 2012, Sathya et al 2010, Smit & Combrink 2004). Davis et al (2003) demonstrated that foliar and root application of B increased tomato growth promoted the uptake of N, Ca and K in plant tissue whilst improving fruit shelf life and firmness. Boron favours the uptake of Ca ions which form complexes with pectin and polyhydroxy polymers (Huang & Snapp 2004) giving stability and strength to cell wall membranes (Ahmad et al 2009). Boron deficiency in tomatoes is associated with damaged fruit through concentric and radial cracking (Davis et al 2003, Liebisch et al 2009), while blossom-end rot in tomato is a physiological fruit disorder caused by insufficient Ca availability (Saure 2001) and can reduce the marketability of the fruit (Taylor & Locascio 2004). In this study both B and Ca were provided in the urchin

waste powder at higher rates than the Hoagland's control and evidence of uptake of these micronutrients can be seen in the leaf and fruit nutrient dry matter analyses.

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

Soil pH can have a strong influence on plant nutrient uptake. Kang et al (2011) found that at both pH 4 and pH 8 an unsuitable root zone limited the growth of tomato seedlings, reducing P content in the roots, Ca in the shoots and water absorption under high nutrient concentrations, and that dry and fresh weight and shoot and root areas were particularly affected by pH 8. The normal range of soil pH for optimum tomato growth is from 5.5 to 7.0 (Sainju et al 2003), low pH (4.5 – 5.5) was shown to improve micronutrient availability (Clark & Baligar 2000, Wang et al 2006), but a growing medium with very low pH (3.5 – 4.5) led to decreased tomato plant mineral nutrition, inhibited root elongation, branch formation and water absorption (Foy 1992, Wright 1989). The potting mix used in our study had a base pH of 7.2 which increased after the application of urchin waste powder in each treatment rate to pH 7.7 in T7. At the end of the trial the growing medium recorded a decrease in pH in each treatment and plateaued around pH 7.0 from T4 onwards. We did not observe a negative influence on tomato productivity and nutrition of the higher pH in T7 suggesting that EC and pH were still in an optimal range to facilitate cation exchange in the root area.

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

Tomato fruit quality

Apart from superior yields, the Hoagland's control solution produced significantly improved quality fruit in most parameters tested. For the fruit texture test which functioned as a perforation test, values show resistance/deformation of the pericarp to the probe. Higher values were generally recorded in the early stages of fruit ripening, where the pericarp was less elastic and prone to perforation regardless of treatment, reducing as fruit become less firm as they matured. However, fruit harvested from plants receiving the Hoagland's solution were firmer than the T7, even though they were of similar maturity which may be a consequence of greater water content (bigger fruit) in these plants. This increases the tautness of the flesh and is further evidence for the superior quality fruit harvested from plants receiving this treatment. Fruit firmness is also related to total soluble solid content and can positively influence fruit flavour and shelf life (Beckles 2012). Sugar content and its ratio to organic acids are the main determinants of tomato taste (Zhao et al 2016), the higher acidity was recorded in fruits from the T7 and T8 treatments.

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

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

Rock Lobster bait potential

Longspined Sea Urchin as Southern Rock Lobster bait could be sourced from either dedicated harvesting or through the utilisation of processing waste. Baits containing gonad, i.e. sourced from dedicated harvesting, are likely to have higher attractability, as gonad tissue is a key component consumed by lobsters during natural predation. This is supported by evidence of Californian spiny lobsters showing preference to healthy urchins over those with impoverished gonads (Eurich et al., 2014). As such, the trials here representing dedicated harvesting for bait reflects the scenario where maximum attractiveness should be achieved.

Dedicated harvesting of Longspined Sea Urchin could supply fresh crushed urchins with gonad, and could follow the seasonal cycle of rock lobster bait demand. Catch rates in the commercial harvest fishery are typically 100-400 kg/hr with a beach price of ~$2 – 2.50/kg (Cresswell *et al.,* 2019). Celomic fluid loss when urchins are crushed for bait would result in a ~18% weight loss, resulting in approximately 1.22kg of whole live urchin required to produce 1 kg of crushed urchin bait. The cost of whole Longspined Sea Urchin bait would be ~$2.44 – 3.05 /kg. This is comparable to frozen fish bait (currently ~$3/kg; Tony Garth Seafoods, 2019). Dedicated harvesting of Longspined Sea Urchins is currently required to be conducted by licenced commercial divers under fisheries management legislation.

Despite Longspined Sea Urchin being highly abundant, and could be supplied at a comparative cost to traditional baits, both individual and paired bait preference trials showed that Southern Rock Lobster preferred traditional fish bait, in this case Jack Mackerel, and the native Shortspined Sea Urchin over Longspined Sea Urchin. The strong preferences indicate that Longspined Sea Urchin used as bait will unlikely provide catch rates equivalent to that of current and/or other bait sources. Results showed that there was increased attractability of large Southern Rock Lobsters originating from urchin barrens habitat to the Longspined Sea Urchin bait. As such, the use of urchin bait may be feasible to harvest large lobsters from urchin barrens habitat, but still may not be as efficient as fish bait. Nevertheless, the results show low scope of application given the majority of Southern Rock Lobster catch is outside the region of high Longspined Sea Urchin abundance.

High levels of attraction of Southern Rock Lobster to Shortspined Sea Urchin baits were reported. As such, Shortspined Sea Urchin may be a viable bait source should alternate bait sources be required to increase bait security in the Southern Rock Lobster industry. Furthermore, as Southern Rock Lobster often consumed the test of Shortspined Sea Urchins in addition to the roe, the utilisation of processing waste from the Shortspined Sea Urchin harvest as bait may be plausible.

Conclusion

The urchin waste powder used here as a mineral fertiliser increased productivity of tomato plants with better performance at higher rates. Plant growth was directly related to the rate of added urchin waste, with improved plant performance for all parameters measured. Although vegetative growth for the highest sea urchin powder treatment compared well with the Hoagland's solution, this did not result in comparable yield and fruit quality to plants treated with the Hoagland fertiliser. These results suggest that urchin waste powder requires supplementary addition of macronutrients to overcome deficiency in N and P as these were clearly exhausted during the vegetative growth of plants and flowers and were no longer available during fruiting. Given the high ratios of Ca, Mg and B in the urchin waste powder relative to N, P and K, there is clearly a risk of oversupply of micronutrients, which may limit the amount of urchin waste powder that can be applied as a fertiliser to avoid nutrient toxicity.

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

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

The project also concludes that the application of Longspined Sea Urchin as a bait product for the Southern Rock Lobster industry is limited given its low attractability compared to other bait products. Jack Mackerel, a current commercial fish bait species, stimulated 2.6 times more forage events than Longspined Sea Urchin. The forage event response time to Jack Mackerel was also significantly quicker than to Longspined Sea Urchin. The attractability of Shortspined Sea Urchin to Southern Rock Lobster was similar to that of Jack

Mackerel, indicating that the low attractability of Longspined Sea Urchin is species specific, as opposed to low attraction to sea urchins in general.

Increasing economic returns to the underutilised Longspined Sea Urchin resource is essential to the development cost-effective control. This includes both roe-fishery waste utilisation and/or alternate uses of whole urchin from dedicated ‘take-all’ harvests. Examples of recent costs for removals include a $250K roe-harvest subsidy for 560 tonnes landed in the 2018/19 season and $101K for a 35 tonne ‘take-all’ removal in 2020 (Creswell *et al*., 2019, Larby 2020). This project concludes from the current information the best opportunity for developing products to offset such costs is via the agricultural sector.

Implications, recommendations and further development

This project demonstrated Longspined Sea Urchin processing waste has potential application as an agricultural fertilizer and/or soil ameliorant. Results showed high uptake of macro and micro-nutrients which could have commercial applications. The research has led to the main urchin processor in Tasmania (RTS PauaCo), with state government grant funding, investing in commercial scale waste processing technology capable of drying and grinding up to 5000 kg/day. Further development is underway to test urchin waste applications on commercial crops in FRDC 2019-128: *Assessing the benefits of sea urchin processing waste as an agricultural fertiliser and soil ameliorant.* The project showed limited potential for Longspined Sea Urchin as Southern Rock Lobster Bait as lobsters exhibited low attraction to the urchin compared to fish. No further development in this space is recommended.

Extension and Adoption

Results of this project were presented at a Centrostephanus Forum at Blundstone Arena which was attended by over 60 people from government, industry, recreational and scientific sectors. The project has been directly discussed in an ongoing manner with the urchin processing industry and government. The industry with a government grant have invested in industrial waste processing equipment. The research is being extended to trials on commercial crops via FRDC 2019-128: *Assessing the benefits of sea urchin processing waste as an agricultural fertiliser and soil ameliorant.* Observed lobster behaviour during this research has led to a PhD project investing lobster diet and prey preference.

Project coverage

ABC Landline (TV - 2019) - https://www.abc.net.au/landline/spiky-success:-turning-invasive-sea-urchin-into/11402602

Tasmanian Country Hour (Radio - 2021) - https://www.abc.net.au/radio/programs/tas-country-hour/urchin-compost/13023570

'Urchin's Beware!' – IMAS Public Exhibition, IMAS exhibition space, Hobart. – Date TBA (Installed 2020 but delayed due to Covid-19).

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FRDC FINAL REPORT CHECKLIST

Project Title:			
Principal Investigators:	XXXX (include all recognised authors -)		
Project Number:	XXXX/XXX		
Description:	Brief one/two paragraph overview of what the project did and achieved.		
Published Date:	XX/XX/XXXX (if applicable)	Year:	XXXX
ISBN:	XXXXX (if applicable)	ISSN:	XXXXXXXXXXXXX (if applicable)
Key Words:	Needs to include key subject areas and species name (see www.fishnames.com.au)		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	Comments
Foreword (optional)		
Acknowledgments		
Abbreviations		
Executive Summary		
– What the report is about		
– Background – why project was undertaken		
– Aims/objectives – what you wanted to achieve at the beginning		
– Methodology – outline how you did the project		
– Results/key findings – this should outline what you found or key results		
– Implications for relevant stakeholders		
– Recommendations		
Introduction		
Objectives		
Methodology		
Results		
Discussion		

Conclusion		
Implications		
Recommendations		
Further development		
Extension and Adoption		
Project coverage		
Glossary		
Project materials developed		
Appendices		