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Effect of basket height and stocking density on production of the sea urchin *Tripneustes gratilla*: insights and recommendations

Bas C. De Vos[1] · **Mark D. Cyrus**[1,2] · **John J. Bolton**[1] · **Brett M. Macey**[1,3]

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Abstract

Basket height and stocking density are crucial and related factors for successful commercial sea urchin aquaculture, but these factors have not been definitively determined for production of *Tripneustes gratilla*. This study investigated the effects of varying basket heights (deep 30 cm vs. shallow 15 cm) and stocking densities (4, 6 and 8 $kg.m^{-2}$ or 13, 19 and 24% coverage of available basket surface area) on aquacultural production of *T. gratilla*. Contrary to previous suggestions, reduced consumption was identified as the cause of decreased production in deeper baskets. Consumption was significantly higher in shallow baskets than deep baskets for both the fresh seaweed, *Ulva lacinulata,* ($W = 38$, $p = 0.026$) and formulated feed ($W = 76.5$, $p = 0.007$). Consequently, baskets of approximately 15 cm deep are recommended to enhance production of *T. gratilla* and possibly other urchin species. Two subsequent trials assessed production of *T. gratilla* at different stocking densities. Trial 1 aimed to maximize urchin size over a three-month grow-out period using fresh *U. lacinulata*, while trial 2 focused on enhancing gonad production over two months using formulated feed. Although greater stocking density significantly reduced the specific growth rates of individual urchin mass in both trials ($p < 0.044$), there was no significant impact on mortality, net production or gonad size and quality. The variations in growth rates were attributed to spine loss resulting from negative behavioural interactions ($F_{2,\,9} = 9.551$; $p = 0.005$). Based on the objectives of both grow-out and gonad-enhancement phases, we recommend a stocking density of approximately 20% coverage.

Keywords *Tripneustes gratilla* · Stocking density · Echinoculture · Sea urchin · Grow out · Gonad enhancement

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✉ Bas C. De Vos
DVSSEB002@myuct.ac.za

[1] Department of Biological Sciences, University of Cape Town, Rondebosch, South Africa

[2] Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering, James Cook University, Townsville, QLD, Australia

[3] Department of Forestry, Fisheries and the Environment, Cape Town, South Africa

Introduction

Sea urchin gonads are a highly valued seafood, for which there is an increasing demand (Stefansson et al. 2017). Products from the sea urchin species *Tripneustes gratilla* of high quality are well accepted by the market and there has been considerable research recently into its commercial production (Cyrus et al. 2014, 2015; Shpigel et al. 2018). The primary motive of most commercial aquaculture farms of *T. gratilla* is financial, thus optimising stocking densities is crucial for maximizing yield, consistency and quality of urchin gonads, while maintaining animal welfare standards. However, the optimal stocking density for *T. gratilla* had not been definitively determined. Height of urchin basket has been observed to influence the production of urchins (Devin 2002; Pearce et al. 2002; Daggett et al. 2006; Christiansen and Siikavuopio 2007; Siikavuopio et al. 2007; Siikavuopio 2009) and therefore will likely affect the assessment of urchin stocking density optimization. Therefore, before establishing the optimal stocking density for *T. gratilla*, it is necessary to first understand the influence of basket height on urchin production and welfare. Baskets are frequently used as the attachment surface and management unit for urchins in aquaculture facilities, predominantly suspended in tanks, raceways or in the ocean. Hence, for practicality and simplicity, the term "basket" encompasses all forms of urchin holding units, representing the container in which the urchin is confined.

To clearly illustrate the impact of basket height on urchin production, Siikavuopio (2009) describe contrasting mortality rates between two very similar studies on *Strongylocentrotus droebachiensis* maintained under similar conditions, except for basket heights. Siikavuopio et al. (2007) observed high mortality (up to 80%) over 54 days with deeper baskets (69 cm), while Pearce et al. (2002) reported lower mortality (less than 6.5%) over 84 days with shallower baskets (28 cm). Siikavuopio (2009) suggested the relation between mortality and basket height is caused by a behavioural trait, where urchins of various species, including S. *droebachiensis* and *T. gratilla (*personal observation, de Vos), prefer attaching to the upper sidewalls of baskets. Urchins need to travel to the bottom of the basket to feed on pellets, resulting in collisions and spine breakage. Higher stocking densities exacerbate collisions during vertical movements. Echinoderms, including urchins, can regenerate organs and appendages, including spines, however regeneration affects resource allocation and therefore reduces urchin production (Hyman 1955; Ebert 1967; Edwards and Ebert 1991; Haag et al. 2016). Essentially, Siikavuopio (2009) hypothesise that deep baskets cause more collisions between urchins, resulting in spine loss which reduces urchin production. However, this had not been investigated empirically prior to this study.

Other studies have also noted links between urchin production and basket height, but they have different potential explanations. Daggett et al. (2006) hypothesized that the reduced production they observed in deeper baskets was linked to basket handling methods. When tanks containing urchins are cleaned, the baskets holding the animals are removed from the tanks, as common practice, and are therefore in open air. Urchins are unable to remain attached to the side walls for long and fall to the bottom of the baskets, resulting in damage and/or loss of tube feet, broken spines, and cracked tests. The length of the fall is suggested to increase the impact and the extent of damage, thus reducing urchin production.

Deeper baskets have also been suggested to reduce feed accessibility, as observed by farmers of *S. droebachiensis* in the Northeast Atlantic (Devin 2002). Urchins tend to cluster on the upper sides and corners of baskets, resulting in inefficient space utilization and limited feed access. *Tripneustes gratilla*, similar to other urchin species, exhibits low mobility (Koike et al. 1987; Stimson et al. 2007). Consequently, if food is not delivered directly to them, their consumption is likely to decrease (Devin 2002). When feed settles at the bottom of the basket, while urchins congregate at the top, the likelihood of feed consumption decreases. Moreover, accessing the feed necessitates movement, expending energy and resources that could otherwise be allocated to growth. Therefore, it is suggested that shallow and narrow baskets would provide better feed accessibility, leading to increased consumption and improved production. Again, this hypothesis had not been explicitly tested.

While these previous studies imply urchin farming is optimal in shallow baskets, it must be acknowledged that deeper baskets can offer practical advantages, such as supporting higher urchin densities (relative to farm surface area) and experiencing reduced temperature fluctuation (due to larger water bodies). Shallow baskets stacked in deep tanks can provide similar benefits, but are more intensive in terms of design, management, and capital (Grosjean et al. 1998; Devin 2002). While no research has been conducted on basket height of *T. gratilla*, observations suggest that, like other species, *T. gratilla* prefers the upper portion of baskets (personal observation, de Vos), potentially leading to production limitations. The combined effects of basket height and feed type need to be investigated, as the influence of basket height may vary depending on the rate at which the feed sinks to the bottom of the basket. It is also crucial to study the effects of removing urchins from the water on spine loss, as this is a necessary aspect of long-term maintenance (farming). Optimizing basket height can facilitate stocking density optimisation, which will ensure optimal production parameters and the feasibility of the industry. Furthermore, these experiments can contribute to system design for other urchin species with similar behaviour and help validate or refute existing theories.

Once the optimal basket height is determined, stocking density can be investigated. In animal aquaculture systems, stocking density is typically limited by water quality and behavioural interactions (MacIntyre et al. 2008). Mos et al. (2012) subjected *T. gratilla* stocked at various densities to different water exchange rates, providing valuable insight into how this species alters water chemistry and how this affects their somatic and reproductive growth. However, no study has clearly examined the relationship between behavioural interactions and stocking density. Examples of negative behavioural interactions between urchins include the aforementioned collisions that result in spine loss (Siikavuopio et al. 2007), feed competition/accessibility (Richardson et al. 2011) and cannibalism (Richardson et al. 2011; Sonnenholzner et al. 2011; Le Gault and Hunt 2016). The frequency and intensity of interactions among individuals in aquaculture systems likely increases with higher stocking density. To better understand the optimal stocking densities, it is crucial to separately investigate the impact of water quality and behavioural interactions, as they may interact to determine the ideal conditions for a given species.

Stocking density analysis of urchins faces challenges due to the lack of a standardized measurement. Most studies use the mass of the total urchins divided by the amount of surface area inside the basket (such as kg.m^{-2}). However, percentage cover is regarded as a superior metric (James et al. 2017). This, as defined in our study, is the surface area of all the urchins' tests (not including spines) in a basket occupied as a proportion of the total available surface area. This metric considers the surface area occupied by the urchins' tests in relation to the total available surface area, providing a more meaningful measure of density that accommodates different urchin sizes and allows for comparisons across size classes. In contrast, kg.m^{-2} does not account for the exponential mass-to-surface area ratio of urchins, leading to potential overcrowding of smaller individuals. By emphasizing percentage cover, researchers can accurately assess spatial distribution and the impact of stocking density on urchin growth and productivity in aquaculture systems.

While there is substantial literature on stocking density effects in other urchin species, it cannot be directly applied to *T. gratilla*, as it has been reported that different species have different stocking density requirements (Suckling et al. 2020). To the best of our knowledge, no study has been conducted to specifically investigate how behavioural interactions of *T. gratilla* limit stocking density. Literature does exist though providing some insight into appropriate stocking densities of *T. gratilla* (Table 1). These comparisons between stocking densities and production performance are not absolute because of differences in other culture parameters between the studies, however these results provided this study with estimations of which stocking density values to test. It is suggested that 15.47 $kg.m^{-2}$ or 43.74% cover is too high due to low survival (Juinio-Meñez et al. 2008). Contrary to this, Manuel et al. (2013), who used similar culture methods to Juinio-Meñez et al. (2008), reached similar stocking densities but found a considerably higher gonad somatic index (GSI) and specific growth rate (SGR; Table 1). While Mos et al. (2012) provides clear evidence attributing the differences in performance between treatments to carbonate chemistry and its influence on the availability of carbonate ions, they did find that the higher stocking densities of juveniles had a greater variance of size between individuals in a tank. This implied that certain individuals were outcompeting others for food, therefore growing larger (Qi et al. 2016). There are clear cases where urchins held at stocking densities of approximately 8 $kg.m^{-2}$ or 23% cover had similar performance (Shpigel et al. 2018) to the maximum performance observed for *T. gratilla* (Cyrus 2013). This suggests that a stocking density where the behavioural interactions may begin to limit production is near 8 $kg.m^{-2}$ or 23% cover.

There are two separate growth phases in aquaculture once juvenile *T. gratilla* shift from their benthic biofilm diet (Cyrus et al. 2015). The first phase is the grow-out phase, where the objective is to maximise somatic growth, specifically test diameter and height. There is evidence that feeding fresh *Ulva* is most efficient for this phase (Cyrus et al. 2015). The objective of the second growth phase is gonad enhancement where a high protein (ca. 26%), formulated feed with a 20% dried *Ulva* inclusion and a small portion of fresh *Ulva* was shown to be optimal during this growth phase (Cyrus et al. 2015). The different feed types and objectives may be influenced by behavioural interactions differently and therefore varied optimal stocking densities may apply. For this reason, separate stocking density experiments needed to be conducted for both phases. Therefore, experiments investigating specific behavioural interactions that might influence production at different stocking densities were undertaken in the current study.

The initial aim of this study was to investigate why basket height influences the production of *Tripneustes gratilla* based on theories presented in the literature. To achieve this, the four objectives were to: determine the relationship between basket height and spine loss, investigate the effect of removing baskets of varying height from the culture system on spine loss, find whether consumption is influenced by basket height and examine if basket height can affect the mortality rate. Once a recommended basket height was established, the second aim was to determine the optimal stocking density of *T. gratilla* at the recommended basket height, at both the grow-out (*Ulva*-fed) and gonad-enhancement (pellet-fed) phases of the urchin production cycle. Lastly, we investigate possible reasons for potentially reduced production at higher stocking densities.

Methods

System

The experiments were conducted at the Department of Forestry, Fisheries and Environment (DFFE) Marine Research Aquarium in Sea Point, Cape Town, South Africa. All trials were conducted in three 1 386 L plastic tanks (140×110×90 cm, length×width×height). Each

Table 1 The most relevant data extracted or calculated from papers which provide insight into stocking density of *Tripneustes gratilla*. Some data are approximate as they were not explicitly provided and therefore is read off graphs and/or deduced by calculation. To determine mass from diameter or vice versa, the exponential relationship from Balisco (2015) was applied [a]. GSI (gonad somatic index) is the urchin's gonad mass relative to total wet mass of the urchin (Eq. 5). SGR (specific growth rate) is rate of urchin growth relative to time (Eq. 6)

Reference	Water exchange rate	Feed	Initial average size	Experiment period	End average size	Initial stocking density		End stocking density		SGR	GSI	Survival
	Exchanges per hour		Test diameter (mm)	Days	Test diameter (mm)	$Kg.m^{-2}$	% cover	$Kg.m^{-2}$	% cover	Test diameter (mm)	%	%
Juinio-Menez et al. 2008	NA[d]	*Sargassum*	26.77	390	74.94	0.56	3.06	7.67	21.21	0.35	5.86	92.31
					72.45	1.50	8.40	15.47	43.74	0.34	3.68	67.91
Asia et al. 2009	ND[e]	*Sargassum*	45.5	120	93.8	1.92	8.19	4.35	13.57	0.11	5.77	ND[e]
Mos et al. 2012 (juvenile)	3	*Sargassum*	21.7	70	67.66	0.15	1.37	3.72	13.32	1.62	NA	> 90
						0.30	2.74	5.93	21.65	1.48	NA	> 90
						0.45	4.11	7.78	23.60	1.25	NA	> 90
Mos et al. 2012 (adult)[b]	3	*Sargassum*	74	42	97	0.81	2.93	1.67	4.84	0.59	5.2	100
						2.44	8.77	4.21	13.78	0.54	5.1	100
						4.06	15.19	6.91	21.78	0.43	3.4	100
Manuel et al. 2013	NA[d]	*Sargassum*	38.67	150	86.69	1.74	8.39	10.77	42.16	0.54	9.79	ND
Cyrus 2013	ND[e]	Fresh *Ulva* then artificial feed	33	224	78	0.11	0.57	1.32	3.19	0.38	17.5	100
Shpigel et al. 2018[c]	2.5	Pellets	24.98	251	44.44	0.3	2.09	2.09	6.62	0.22	14.4	78
		U. lactuca			73.28			7.73	18.00	0.43	13.7	88

[a] $Mass = 0.733x(Diameter)^{2.673}$

[b] For the sake of this comparison focusing on behavioural interactions, only the data points of the high exchange rate (three per hour) were considered

[c] Lower survival was due to a system malfunction

[d] Not applicable as the experiments were conducted in the ocean

[e] No data provided in the paper

tank was supplied with 75 µM filtered seawater, which was recirculated through a sand- and bio-filter, with a daily replacement rate of 84%, and maintained at a constant temperature of 25°C using a heat pump, 25°C was used as it was the nearest value to the optimal temperature range of 26–28°C for *T. gratilla* (Mos et al. 2012) that could be consistently achieved at this facility. Each tank had an hourly water exchange rate of 0.5 (full water exchange—turnovers per hour). The incoming water entered each tank parallel to the water surface at a depth of ca. 15 cm and at a high velocity (ca. 700 l/hr) to ensure that there was a high level of circulation in each tank. All tanks were constantly aerated using an air stone to further promote the circulation/mixing of water. Urchins were housed in baskets of varying dimensions (details provided below) within each tank and were made from HDPE plastic mesh (3 mm mesh size) supported by PVC conduit. The bottom of each tank was siphoned clean of debris and faecal matter once every second day to ensure optimal water quality.

Feeds

Ulva lacinulata was sourced from the Buffeljags abalone farm in the Western Cape of South Africa, where it is cultivated in raceways. The nutrient analysis (Table 2) was conducted at a private laboratory, Microchem Lab Services (Pty) Ltd, using protocols described by (AOAC International 2002). The crude protein content was determined by multiplying the nitrogen content by a factor of 5.45 (Angell et al. 2017). The artificial feed used in this experiment was the same formula as described in Cyrus et al. (2015) with 20% dried *Ulva* inclusion in extruded chips with approximate dimensions of 40 × 10 × 5 mm, L x W x H. Nutrient analyses (Table 2) were conducted by the same laboratory using the same methods as described above. The stability of the formulated feed was determined by recording the percentage dry matter lost after the feed was submerged in the same tank and the same type of basket the urchins were cultured in (thus with the same conditions) for 24 h.

The kelp (*Ecklonia maxima*) used in this study was wild kelp collected directly in front of the Marine Research Aquarium. Only the fronds, cleaned of epiphytes and other debris, were used. A nutrient analysis was not conducted for the kelp as it was only used for a single experiment that did not measure growth rates.

Basket height experimental set-up

Each of the three large plastic tanks described above was equipped with six baskets, including three deep baskets and three shallow baskets (Fig. 1). The arrangement of baskets in this trial, and all those to follow, were randomised to reduce positional bias.

Table 2 The nutrient content of the feeds used (per dry weight)

Feed	*Ulva lacinulata*	Artificial feed
Protein (%)	20.71	32.30
Fat (%)	1.36	4.22
Moisture (%)	15.30	8.30
Ash (%)	27.34	13.50
Calcium (%)	0.34	1.26
Magnesium (%)	2.61	< 0.01
Gross energy (MJ/kg)	No Data	12.26

Both the shallow and deep baskets had an available internal surface area (ISA) of 0.4 m^2 (Eq. 1). To maintain a constant available area for the urchins while varying the depth of the baskets, the width was adjusted accordingly. This was the only approach viable and there are no conceivable ways varying widths of the baskets would influence the urchins. The basket widths were always substantially greater than twice the maximum height of the urchins used in this study, meaning there was sufficient space for the urchins to move past each other when positioned oppositely on the sides of all baskets without their arboreal spines interacting. The calculation of ISA will vary depending on the design of the urchin enclosure, where all surfaces that urchins can attach to should be considered (such as baffles and lids), however the following calculation was used for the baskets used throughout this study (Eq. 1).

$$\mathrm{ISA} = \mathrm{lw} + 2(\mathrm{hw}) + 2(\mathrm{hl}) \tag{1}$$

where:

ISA	internal surface area
l	length of basket
h	height of basket
w	width of basket

Fig. 1 Photograph of deep (40 × 15 × 30.9 cm, Length × Width × Height) and shallow baskets (40 × 40 × 15 cm) used for the basket height experiments. The dimensions of the baskets differ but the surface area within the baskets is the same

Tripneustes gratilla used in this experiment were produced and reared from larvae at this facility. For four months prior to this experiment, urchins were held at similar stocking densities, in baskets of homogenous design and fed a mixture of *Ulva* and kelp. At the beginning of the experiments detailed below, each basket was stocked with 22 individual sea urchins with an average mass of 86.36 g and a test diameter of 65.71 mm. This resulted in a biomass of 1.9 ± 0.03 kg (Mean ± SD) that equates to a stocking density of approximately 4.75 kg.m^2 in each basket (Eq. 2).

$$\text{Stocking density by mass} = \frac{\text{Total urchin mass}}{\text{ISA}} \tag{2}$$

All the urchins were haphazardly placed in their respective baskets and left for five days without feed to acclimatize before the experimentation began.

Effects of basket height and feed type on spine loss

A complete 2 × 3 factorial experimental design with two-treatment levels, basket height and food type, was used for this experiment, resulting in six treatments: (1) deep baskets fed with *Ulva*, (2) deep baskets fed with pellets, (3) deep baskets fed with kelp, (4) shallow baskets fed with *Ulva*, (5) shallow baskets fed with pellets, and (6) shallow baskets fed with kelp. There was one treatment per tank (experimental unit), thus three replications per treatment. As limited resources (space and tanks) only allowed three replications per treatment and variance was expected to be high, the entire experiment was repeated on three separate occasions. Each experiment was treated in an identical fashion and ran for three days. Prior to the start of each experiment, all urchins were starved for five days to stimulate higher consumption and increase the likelihood that the effect could be detected. To collect the lost spines, 200-micron mesh bags were placed around each of the baskets before the urchins were fed their respective diets. All excess pellets and kelp were removed daily prior to the following feeding (as they decompose, unlike *Ulva* that was subsequently not removed) and the urchins were fed ad libitum. After 72 h, the mesh bags were carefully removed, and the faeces were separated from the broken spines by filtration and panning. The spines were dried to a constant weight in an oven at 60 °C and weighed to the nearest 0.001 g.

Effects of basket removal and height on spine loss

The experiment ran for a period for seven days and there were three shallow baskets and three deep baskets per tank, as previously described. Urchins were not fed throughout the experiments, and 200-micron mesh bags were placed around each of the baskets to capture spines. During the experiment, one set of deep and shallow baskets from each tank were not removed, whereas the second set of baskets was removed for five seconds once every day and the third set of baskets was removed for five minutes once every day. At the end of the week, the mesh bags around the baskets were removed and spines were separated from the other material in the mesh bags, before drying spines to a constant weight in an oven at 60 °C overnight and weighing to the nearest 0.001 g.

Effects of basket height on consumption

The experimental set-up was as described above, with the exception that the mesh bags were not used as spines or faeces were not collected. Treatments were also similar, with the exception that kelp was not used due to it being found to be an inappropriate feed in commercial applications (as detailed later). Prior to the initiation of the experiment, urchins were starved for five days. The experiment started when each basket received a known mass of allocated feed. The mass of fresh *Ulva* was recorded after rotating the *Ulva* in a salad spinner 20 times before being weighed, which brought it to a constant weight (Morgan J Brand, University of Cape Town, unpublished). All baskets were fed approximately 120 g of fresh *Ulva* or 25 g of pellets. This quantity was administered to ensure that surplus food always remained after 24 h and that food was not a limiting factor in the experiment. The feed was supplied daily at midday and removed the following day at the same time. The *Ulva* was removed both by hand and siphon and then spun and weighed as described above. The uneaten pellets were carefully siphoned through a 200-micron filter and dried in an oven at 60 °C until a constant weight, before being weighed to the nearest 0.001 g. To account for any change in feed mass not caused by the urchins, a feed stability test was conducted. This was done by adding a portion of each feed type in a basket without urchins and measuring the weight of the feed before and after being in the water for 24 h. The process of determining the difference in mass of the feed over 24 h was repeated six times (over six days) for the pellet-fed baskets and four times for the *Ulva*-fed baskets. The values of these replicates were averaged for each basket to meet the assumption of independence. The rate of consumption was calculated via Eq. 3:

$$\mathrm{IR} = \mathrm{F_p} - \mathrm{F_R} \tag{3}$$

where:

IR	ingestion rate
Fp	weight of feed provided
$\mathrm{F_R}$	weight of feed not eaten – weight loss/gain in control

Stocking density experiments

Effects of stocking density on the grow-out phase

The trial compared growth and gonad development of adult *T. gratilla* held at various stocking densities when fed fresh *Ulva* ad libitum over three months. Urchins were fed a mixed diet of *Ulva* and kelp for one month and then starved for five days before the experiment began. The baskets for this trial measured 40 × 40 × 15 cm (L × W × D, ISA = 0.4 $\mathrm{m^2}$). These dimensions were chosen based on the results of the basket dimension trials described above and because they needed to be as near to commercial scale as logistics would allow. The diameter, height and mass of all urchins from the holding tank were recorded (using vernier callipers and a scale) and only animals with a test diameter of 62–70 mm were used, as these were the only size class available at the time. The average mass of the urchins was 109.73 g. Each of the three baskets in each tank was stocked at a different density, providing three treatments and three replicates across the three tanks. The initial stocking densities were 4, 6 and 8 kg.m^{-2} (Eq. 2), or 13, 19 and 24% coverage of the available basket surface area (Eq. 4), made up of 15, 22 and 29 individuals, respectively.

$$\text{Percent coverage} = \frac{\sum_{i=0}^{n} \pi r^2}{\text{ISA}} \tag{4}$$

where:

$\sum_{i=0}^{n} \pi r^2$	the total area occupied by all urchins in the basket.
ISA	internal surface area of basket.

At the end of the three-month experiment, diameter, height and mass were recorded for each urchin from every basket. Two individuals from each basket were then haphazardly selected, dissected and their gonads weighed. A hand-held spectrocolorimeter (Lovibond LC100) was used to record the lightness (L*), redness (a*) and yellowness (b*) of the gonads. Three repeated measurements of these light values were taken of each gonad, which were then averaged. To ascertain initial colour values for the gonads, 10 urchins of the same size class and cohort were dissected at the beginning of the experiment prior to treatment. Histological analysis of gonads was conducted to determine whether the reproductive phase of the urchins was affected by stocking density. To do this, a single gonad from each urchin was placed into Davidson's Fixative (three-parts 95% ethyl alcohol, two-parts 100% formalin, one-part glycerol, one-part glacial acetic acid and three-parts distilled water) and fixed for 48 h, before being transferred into 70% ethanol and processed for routine hematoxylin and eosin histology (Bucke, 1989). The reproductive phase (stage of gonad maturity) of each gonad was then determined as described by Cyrus et al. (2015).

Effect of stocking density on the gonad-enhancement phase

This trial compared gonad growth of *T. gratilla* held at various stocking densities while fed the previously described formulated feed over a two-month period. The initial intention was to run this experiment as a continuation of the previous experiment, but this was interrupted by COVID and the ensuing lockdown. Consequently, the urchins from the above experiment were mixed and maintained for a period of three-months at a stocking density of ca. 6 kg.m^{-2} and fed ad libitum on a diet of kelp. Once access to the facility was regained, the experiment began.

For this experiment, an additional basket of the same material was included for each treatment in each tank to increase the sample size and help reduce unexplained variability. Consequently, the baskets used were slightly smaller than those used previously and were 25×40×15 cm (L×W×D, ISA = 0. 295m^2). Urchins with diameters between 68 and 86 mm and an average mass of 170.1 g were randomly selected for each treatment and a subsample (n = 10) was dissected to get initial GSI values (Eq. 5).

$$\text{GSI} = \frac{M_G}{M_U} * 100 \tag{5}$$

where:

GSI	gonad stomatic index
M_U	urchin mass
M_G	gonad mass

The stocking densities were approximated to those from the previous experiment; 4 kg.m^{-2}, 6 kg.m^{-2} and 8 kg.m^{-2} or 10.49%, 15.24% and 20.75% cover, holding 7,10 and 14 individuals, respectively. The experiment also included a treatment where urchins were held individually so that they could not physically interact with one another. This was done by segregating the baskets into units measuring 13.3 × 20 × 15 cm (L × W × D) with the resulting stocking densities ranging from 1.04 to 1.9 kg.m^{-2} (1.46 to 2.13% cover). All treatments were fed the formulated feed at a rate of approximately 1.5% of the total urchin mass in each basket. Animals were fed on a Tuesday, Thursday and Sunday. Based on preliminary experiments (unpublished), the 1.5% feed ration was deemed to be optimal, since all feed was consumed within 24 h, thereby avoiding surplus feed leaching into the water column and adversely affecting water quality. On Fridays, the urchins were fed fresh *Ulva* at a rate of 6% of total body mass in each basket. All Ulva would be consumed by the following week. *Ulva* was fed once a week to improve the colour of the gonads, as suggested by Cyrus et al. (2015), and to improve the quality of the water. At the end of the two-month experimental period the body mass, test diameter and height for each individual was assessed. Five individuals from each basket were randomly selected and dissected for gonad analysis (GSI and histology), as previously described.

The effect of stocking density on urchin behaviour (consumption, faecal production and spine loss)

To investigate why stocking density affects urchin production, when water quality remains the same between treatments, urchin behaviours were quantified, specifically consumption (measured via both feed reduction and faeces production) and spine loss. An identical experimental design as described above for the gonad enhancement experiment was used. There were six replicates for each of the stocking densities (4, 6 and 8 kg.m^{-2}) as well as three baskets, one in each tank, each containing six individually housed *T. gratilla*. A 200-micron mesh bag was placed around each basket and animals were fed their respective feeds. All urchins were fed with 100 g, 150 g and 200 g (± 0.05 g) of fresh *Ulva* for the 4, 6 and 8 kg.m^{-2} stocking density treatment groups, respectively. The individually housed animals were each provided with 10 g of *Ulva*. Feed amounts were determined from preliminary experiments to ensure that there was always feed available during the feeding periods. The feed remained in the baskets for 24 h, after which uneaten food was removed by siphoning, with care to minimise spine loss. The remaining feed was weighed and subtracted from the starting mass to determine consumption (Eq. 3). The stability of the feed was factored in, as described previously. The following day, the mesh bags were carefully removed without taking the baskets out of the water. The spines and faecal matter were removed from the bags and separated using panning. The matter was then dried separately over 48 h to a constant weight (at 60 °C) and weighed to the nearest 0.001 g. The mesh bags were reattached to the baskets, before administering new feed. The experiment was repeated three times. The experiment was not conducted using the formulated feed because a preliminary trial revealed the water flow was reduced by the mesh bags, resulting in high dissolved nutrient levels (such as ammonium) that would negatively affect the urchins and skew the experiment.

Water quality monitoring

Water quality was assessed during both stocking density trials to support the assumption that only behavioural interactions and not water quality affected growth, behaviour and gonad development across treatments. Oxygen concentration and pH were assessed biweekly using a probe (DTK2017-SD, Lovibond Tintometer GmbH) which was calibrated before use each time. Samples were taken from inside each basket and from the inflowing water to determine ammonia levels. During the gonad enhancement trial when pellets were fed, the ammonia levels in the water were also determined in both sampling times an hour after the pellets were fed, which is when nitrogen levels were likely to be at a maximum (Piedecausa et al. 2010). The ammonia analysis was determined using Palintest® ammonium test kit (Palintest®, Gateshead, UK).

Statistics

The R statistical computing environment was used alongside Microsoft Excel for data organization. Through discussions concerning the experimental designs, we ensured the assumptions of independence and non-selectivity were met.

To explore the effect of basket height, feed type, and basket removal time on spine loss, we analysed average daily spine loss data. For processing multiple treatment factors and facilitating pairwise comparisons, we applied a linear regression model with least-squares means. Least-squares means predict from linear models, summarizing factor effects and testing linear contrasts in data analysis (Lenth 2016). The model's assumptions were duly met (Supplementary materials A and B). Pairwise T-tests were used for pairwise comparisons. Linear models were chosen over ANOVAs due to their superior ability to detect interactions.

For investigating basket height's influence on pellet and *Ulva* consumption, we carried out separate analyses for each, relying on average daily consumption over the experimental period. These datasets fulfilled assumptions of independence, normality, and homoscedasticity (Supplementary material C). Considering limited replication and the potential for a type II error, a Mann–Whitney U test was chosen over a one-tailed two-sample t-test.

To analyse the effects of basket height on mortality, a general linear model of the Poisson family was used to identify a factor that could correlate with mortality. Factors modelled included basket removals, feed type, blocks (tanks) and their interactions.

The stocking density experiments were randomised complete block designs (RCBD), where tanks were treated as blocks. The required assumptions were tested (Supplementary materials D and E) to allow for the application of two-way analysis of variance (ANOVA) and Tukey tests. The specific growth rate (SGR, Eq. 6) of mass and height was log-transformed to be normally distributed. The initial stocking density experiment, the *Ulva*-fed grow-out trial, is an RCBD with a single replicate (basket) per block (tank) which means the assumption of additivity is required (Wilk 1955). Boxplots with an indication of blocks demonstrated that GSI, colour a* and colour b* were non-additive (Supplementary material E), therefore statistical tests were not applied to these variables as there are currently no alternative tests available. The visualisation of the data provided sufficient insight into these parameters. The remaining parameters all met these assumptions without transformation.

$$\mathrm{SGR} = 100 \times \ln\left(\mathrm{L_T}/\mathrm{L_0}\right)/\mathrm{t} \quad (6)$$

where:

SGR	specific growth rate
L_0	initial length/mass
L_T	final length/mass
t	days of culture

Change in coefficient of variation (CV) was also calculated to assess the presence of adverse behavioural interactions between *T. gratilla* held at various stocking densities (Qi et al. 2016). This was done by subtracting the CV recorded between individual urchin mass within each basket at the end of the experimental period from the CV from the beginning of the experiment. To investigate the relationship between faecal production and consumption, Pearson's product-moment correlation was applied.

To assess whether the water quality between treatments significantly differed, we constructed a generalized linear model that encompassed additional factors (blocks, dates of sample collection, and feed type) potentially influencing water quality. The validation of assumptions involved assessing the spread of residuals, quantile–quantile (Q-Q) plots, and a frequency graph (Supplementary material F), all of which indicated the assumptions were satisfied.

Results

Effects of basket height and feed type on spine loss

Basket height had no significant main effect on spine loss ($R^2 = 0.925$, $F_{(5,\ 12)} = 22.84$, $p = 0.842$). Regardless of height, kelp resulted in significantly higher spine loss than *Ulva* (Fig. 2; $R^2 = 0.925$, $F_{(5,\ 12)} = 22.84$, $p < 0.001$) and pellets ($R^2 = 0.925$, $F_{(5,\ 12)} = 22.84$, $p < 0.001$). There was no significant difference in spine loss between urchins fed pellets and *Ulva* ($R^2 = 0.925$, $F_{(5,\ 12)} = 22.84$, $p = 0.995$) when maintained in deep or shallow baskets. None of the interactions within this analysis were found to be significant ($R^2 = 0.925$, $F_{(5,\ 12)} = 22.84$, $p > 0.407$).

Effects of basket removal and height on spine loss

Regardless of basket height, removing baskets containing *T. gratilla* from the water column for five minutes resulted in significantly higher spine loss (0.341 ± 006 g) than a short removal period of five seconds (0.089 ± 0.001 g; $F_{(5,\ 12)} = 31.7$, $p < 0.001$) or if not removed from the water at all (0.072 ± <0.001g; $F_{(5,\ 12)} = 31.7$, $p < 0.001$). There was no significant difference in spine loss between short removals and no removals ($F_{(5,\ 12)} = 31.7$, $p = 0.386$). While deep baskets removed from the water for five minutes resulted in higher spine loss than shallow baskets (Fig. 3), there was no significant difference ($F_{(5,\ 12)} = 31.7$, $p = 0.100$).

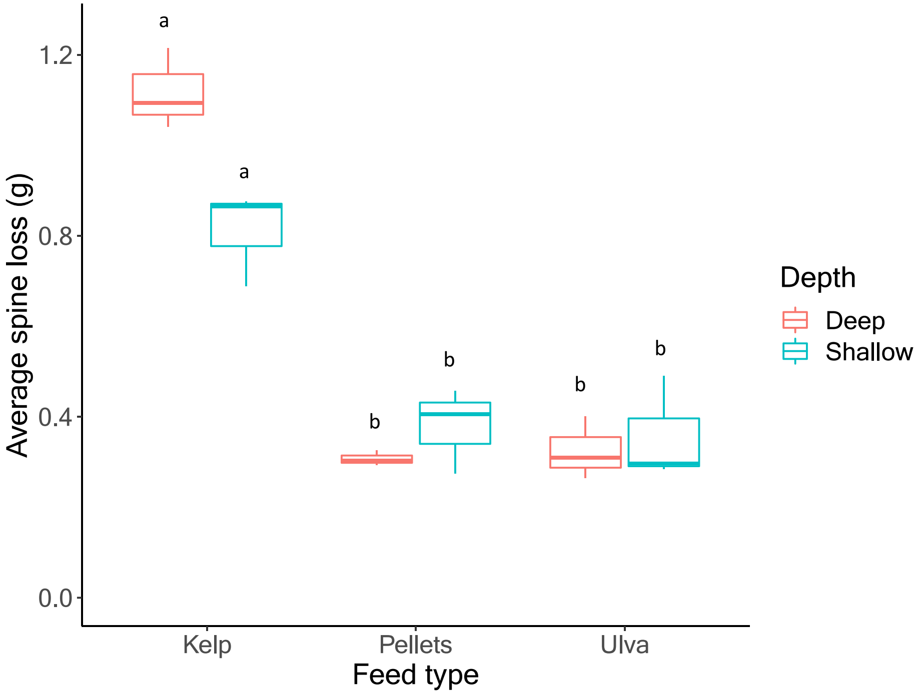

Fig. 2 The daily spine loss of *Tripneustes gratilla* over the experimental period, when fed different feed types and maintained in the deep (30 cm) or shallow (15 cm) baskets. Boxes with red lines represent spine loss for deep baskets, while blue is for shallow baskets. The corresponding letters denote significant differences between treatments. The boxplot indicates the spread of data in each treatment with the bottom and top-most reach of each line representing the maximum and minimum data of the treatment and the thick central line representing the median

There was also no significant difference in spine loss from urchins between deep or shallow baskets when baskets were not removed from the water ($F_{(5,\ 12)}=31.7$, $p=0.3528$). Shallow baskets resulted in higher spine loss than deep baskets when removed from the water for a short period ($F_{(5,\ 12)}=31.7$, $p=0.007$).

Effects of basket height on consumption

Daily pellet consumption was significantly ($W=76.5$, $p=0.007$) higher in shallow baskets (22.48 ± 1.176 g) than in deep baskets (19.26 ± 2.058 g; Fig. 4A). These values translate to a consumption rate of 1.23% and 1.05% dry pellets per wet urchin body mass per day, respectively.

The average daily consumption of fresh *Ulva* (Fig. 4B) in the shallow baskets (92.7 ± 61.15 *g* of fresh *Ulva* daily or 5.05% of BW.d^{-1}) was also significantly higher ($W=38$, $p=0.026$) than in deep baskets (70.94 ± 84.10 *g* of fresh *Ulva* or 3.99% of BW.d^{-1}). This translates to a dry *Ulva* consumption to body weight ratio of 0.83% and 0.63% for shallow and deep baskets respectively.

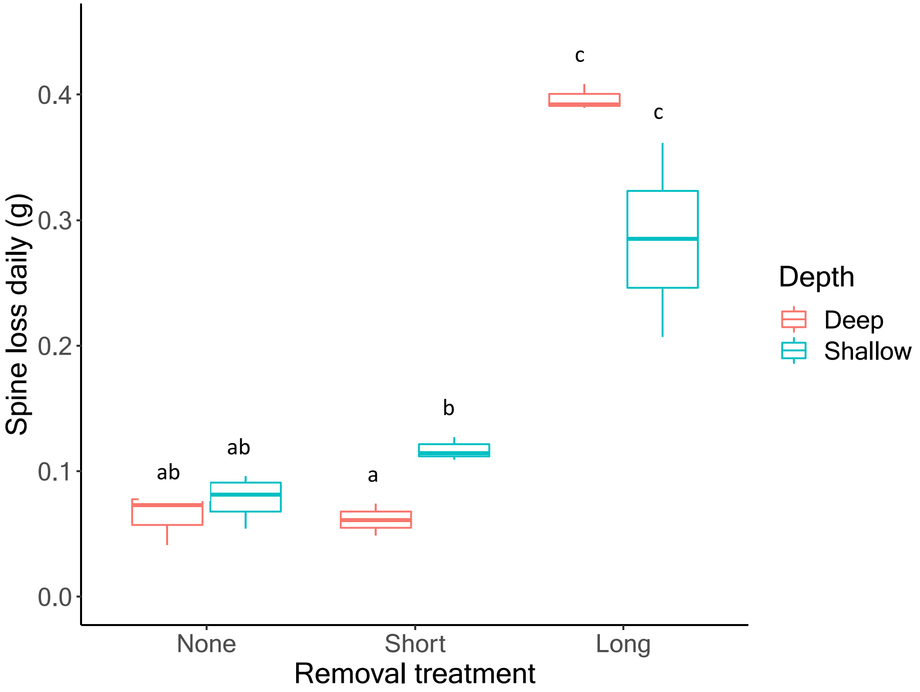

Fig. 3 Average daily spine loss (g) from *Tripneustes gratilla* maintained in deep (30 cm) and shallow (15 cm) baskets in response to three different removal treatments: none (not removed from the water), short (five-second removal daily) and long (five-minute removal daily). Different lowercase letters above box plots represent significant differences between the mean spine loss of urchins within each treatment

Effects of basket height on mortality

Over the two-month study period of the basket height analysis, there were eight mortalities across all treatment groups, which accounted for a 2.02% overall mortality rate. Six of these mortalities occurred in the shallow baskets, whereas only two occurred in the deep baskets. The generalised linear models (GLMs) found that none of the possible explanatory variables (height, tank, removal, feed and their interactions) had a significant influence on mortality ($Z_{(17)} < 1.346$; $p > 0.179$). As such, using Akaike information criterion (AIC) comparison of various models was irrelevant and there was no statistical evidence mortalities were influenced by basket height or the other possible variables.

Effects of stocking density on the grow-out phase

The average starting mass of an individual urchin was 109.66 g. After three months of being fed *Ulva* ad libitum, the mean masses at the low (4 kg.m^{-2}), medium (6 kg.m^{-2}) and high (8 kg.m^{-2}) stocking densities were 147.61 g, 133.07 g and 130.3 g, respectively. A two-way ANOVA revealed stocking density had a significant effect on mass specific growth rate (SGR, $F_{2, 4} = 9.434$; $p = 0.031$). The low stocking density had an average SGR of 0.194g.d^{-1}, which was significantly higher (Fig. 5A) than the medium and high stocking

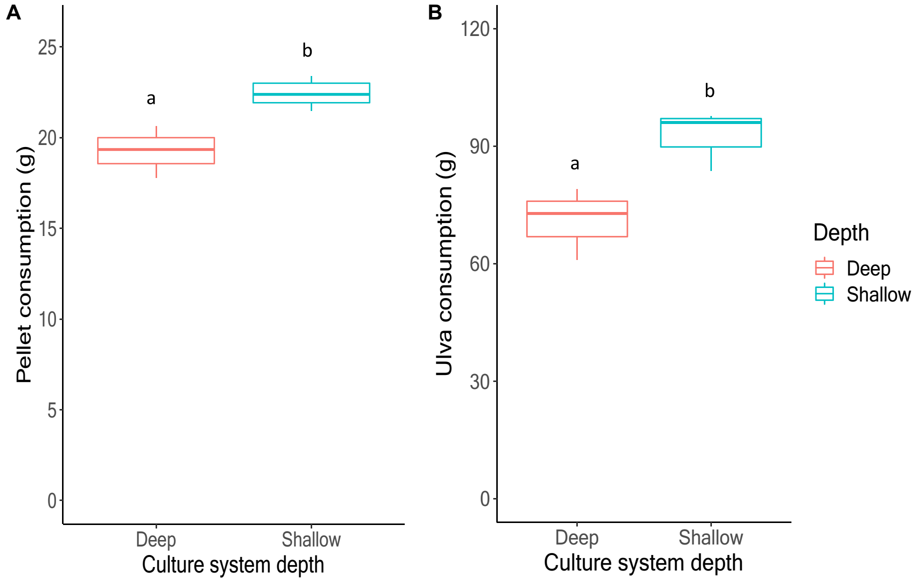

Fig. 4 Box plots comparing average daily consumption of dry pellets (**A**) and fresh *Ulva* lacinulata (**B**) by 22 *Tripneustes gratilla* housed in shallow (15 cm) and deep (30 cm) baskets. Different lowercase letters above box plots represent significant differences in consumption of pellets between treatments

densities ($p=0.044$ and $p=0.034$, respectively). There was no significant difference in mass SGR between medium and high densities ($p=0.869$). Stocking density also significantly influenced SGR of diameter (Fig. 5B), supported by the ANOVA ($F_{2,\ 4}=15.458$; $p=0.013$), and there was a significant difference between the low and high densities ($p=0.011$). This experiment provided no indication that the tested stocking densities had any effect on the SGR (ca. 0.05 mm.d^{-1}) of height of *T. gratilla* (Fig. 6C, F2, 4 = 0.936; $p=0.464$).

At the beginning of this experiment, the low, medium, and high stocking density baskets were stocked with a total average of 1609.88 g, 2502.49 g and 3129.58 g of urchins, respectively. At the end of the experiment, the baskets had a total mass of 2129.72 g, 3034.44 g and 3696.99 g, respectively (Fig. 6B). The percentage cover of the low, medium and high stocking density baskets increased from 13%, 19% and 24% to 15.25%, 22.77% and 29.87%, respectively. A two-way ANOVA revealed the greater the initial stocking density, the greater the total urchin yield at the end of the experiment ($F_{2,\ 4}=46.653$; $p=0.002$), with significant differences between each treatment (Fig. 7B, $p<0.033$). However, the total increase of urchin mass in each basket did not vary significantly across treatments over the three-month period ($F_{2,\ 4}=0.07$; $p=0.934$), where basket weight increased by approximately 0.5 kg regardless of stocking density (Fig. 6A). The coefficient of variance (CV) in mass of urchins maintained at a low stocking density decreased by an average of 1.98%, while the CV in the medium and high stocking densities increased by 0.10% and 0.66%, respectively, over the same period (Fig. 6C), however, there were no significant effects from treatments ($F_{2,\ 4}=1.029$; $p=0.436$).

The mean gonadal somatic index (GSI) of urchins across all treatments at the end of the experimental period was 13.11%. The GSI data did not meet the assumptions

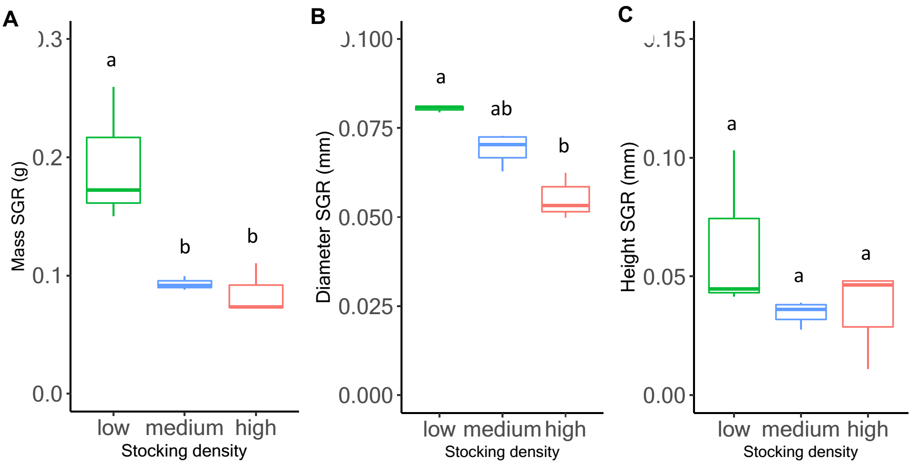

Fig. 5 The influence of low (4 kg.m^{-2}), medium (6 kg.m^{-2}) and high (8 kg.m^{-2}) stocking densities on the specific growth rate (SGR) of mass (**A**), diameter (**B**) and height (**C**) of *Tripneustes gratilla* fed *Ulva lacinulata* over a period of three months. Different lowercase letters above box plots represent significant differences between the mean SGR of urchins held at different stocking densities

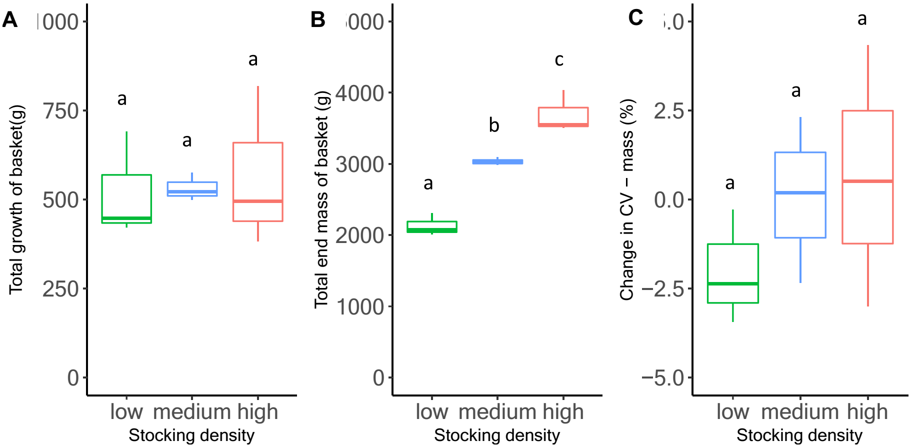

Fig. 6 The influence of stocking density on the difference of the total mass of *Tripneustes gratilla* in a basket (total basket mass) at the beginning and end of the grow-out experiment (**A**), the total basket mass at the end of the experiment (**B**) and the change in the coefficient of variation (CV) between urchins within each treatment from the beginning to the end of the experiment (**C**). Different lowercase letters above box plots represent significant differences between means of the stocking densities

required to apply any known statistical test correctly. The GSI became more variable with increasing stocking densities (Fig. 7A). Similarly, gonad redness and yellowness did not meet the required assumptions for an ANOVA, but there is indication these values may be reduced at the high stocking density (Fig. 7C and D). Gonad lightness (L*) data did meet the assumptions of the ANOVA, but there was no significant change in lightness with an increase in density ($F_{2,4} = 0.286$; $p = 0.765$).

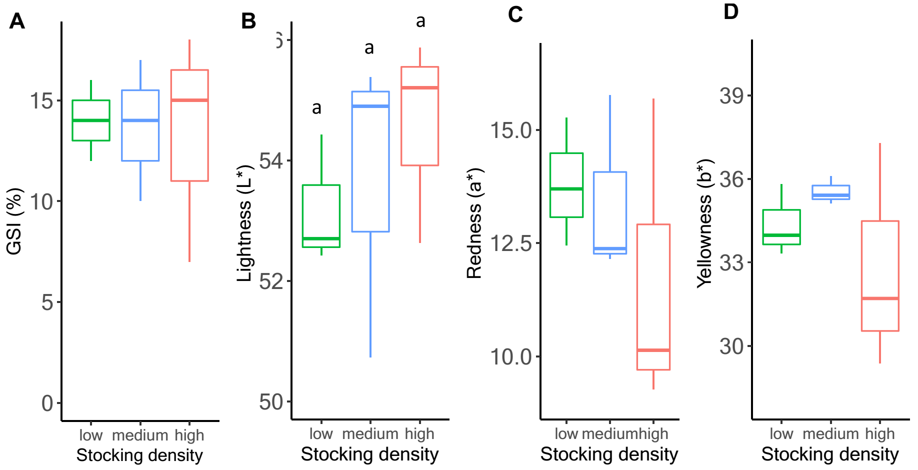

Fig. 7 The effect of various stocking densities on gonadal somatic index (**A**), gonad colour lightness (**B**), gonad redness (**C**) and gonad yellowness (**D**) when fed *Ulva* over a three-month period. Matching letters denote a lack of significant differences in gonad lightness among treatments. Graphs lacking letters did not meet ANOVA assumptions and were excluded from statistical analysis

Effect of stocking density on the gonad enhancement phase

At the beginning of the experiment, the average mass of an individual urchin was 169.69 g. By the end of the two-month period of being fed pellets every second weekday and *Ulva* over the weekend, the average individual urchin mass across all treatments was 185.41 g. Stocking density had a significant effect on the specific growth rate (SGR) of mass ($F_{3, 9}=5.048$; $p=0.044$). The SGR in mass of individually housed urchins was significantly higher than that of urchins maintained at a high stocking density (Fig. 8A, $p=0.035$), whereas no significant difference in mass SGR was recorded between individually held urchins and those at the low or medium stocking densities (Fig. 8A, $p=0.110$ and $p=0.066$ respectively). Furthermore, there was no significant difference between the treatments (low, medium and high stocking density) when multiple urchins were maintained in a single basket ($p>0.647$). There was no clear statistical evidence that stocking density affected growth of urchin diameter (Fig. 8B, F3, 9 = 3.412; $p=0.093$) or the difference in coefficient of variance from the beginning to the end of the experiment (Fig. 8C, F3, 9 = 1.121; $p=0.412$).

While the number of urchins in each basket between treatments differed greatly, the net increase in weight of each basket from the beginning to the end of the experiment was not significantly affected ($F_{3, 9}=1.765$; $p=0.254$) (Fig. 9A). There was high variation in GSI across all treatments. At the beginning of the experiment, the average GSI of the urchins was 9.15%, whereas by the end of the experimental period the average GSI across all treatments was 12.16% (Fig. 9B). However, GSI of *T. gratilla* was not influenced by stocking density ($F_{3, 9}=0.594$; $p=0.642$). The histological analysis revealed that most of the gonads examined from urchins were in a premature stage (stage 3), as described in Cyrus et al. (2015), regardless of stocking density (Fig. 9C; $F_{3, 9}=1.875$; $p=0.235$). Conversely, 6.7% of animals were in the recovery, partially spawned, or spent stages. There was no indication that stocking density influenced the colour (lightness (L*), redness (a*) or yellowness(b*)) of the gonads ($F_{3, 9}>0.781$; $p>0.546$).

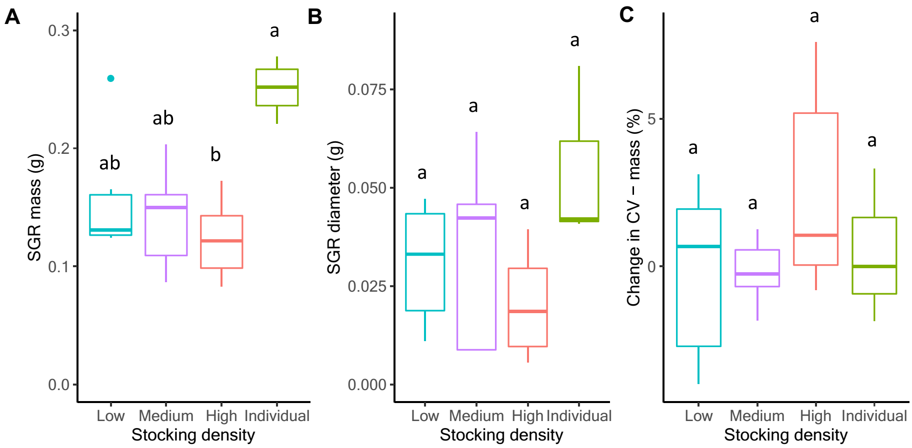

Fig. 8 The effect of stocking density on weight gain (**A**), diameter gain (**B**) or change in the coefficient of variance (CV) of the mass (**C**) of *Tripneustes gratilla*. The contrasting letters in A indicate a significant difference between treatments. There were no significant differences in the data for B and C

The effect of stocking density on urchin behaviour (consumption, faecal production and spine loss)

The average daily consumption rate of *Ulva* by an individual urchin was 4.52% (wet weight) of their own body mass across all stocking densities (Fig. 10A). Stocking density had no effect on the consumption of *Ulva* ($F_{2, 9}=0.833$; $p=0.466$). The percentage of faecal production relative to body weight (Fig. 10B) also had no relationship with stocking density ($F_{2, 9}=0.639$; $p=0.550$). There was a significant correlation between faecal production and consumption ($p=0.017$), but the relationship is not very strong (Pearson's correlation coefficient = 0.550). Daily individual spine loss, shown here as a percentage

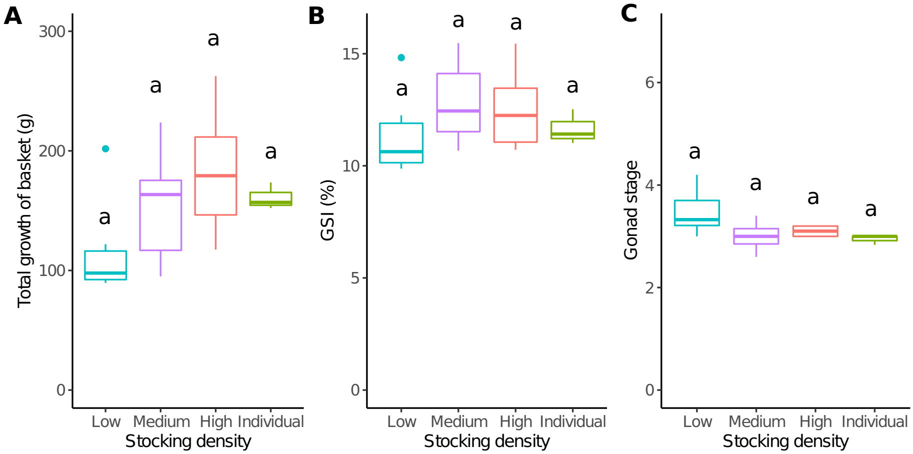

Fig. 9 The influence of various densities of *Tripneustes gratilla* on their total basket mass increase (**A**), GSI (**B**), and average gonad stage per basket (**C**) over a period of two months while being fed pellets with 20% *Ulva* inclusion. Different letters above bars represent significant differences

of urchin body mass (Fig. 10C), significantly increased at higher stocking densities ($F_{2,\,9}=9.551$; $p=0.005$). In a basket stocked at a high density, the individuals on average lost spines totalling 0.03% of their body weight daily, with a maximum of 0.04%. This was significantly higher than spine loss recorded in the low-stocking density baskets ($p=0.005$), which averaged at 0.013%. The medium-density baskets were not significantly different to the low- or high-density treatments ($p<0.124$).

Water quality monitoring

These analyses were conducted to test the assumption that varying production between treatments was primarily the result of behavioural interactions between urchins and not changes in water quality. Generalised linear models showed that the number of urchins in a basket did not significantly influence the pH or oxygen levels ($Z_{(26)}=1.285$; $p>0.200$). The dissolved oxygen remained almost constant throughout the experiment, at a mean of 78% and a minimum value of 75%. The pH of the inflowing water and water in the tank ranged from 7.87 to 7.61. The ammonium levels remained below detectable levels (<0.005 mg.l^{-1}) throughout the experiment.

Discussion

Effects of basket height and feed type on spine loss

This study found that deep baskets did not result in higher spine loss in *Tripneustes gratilla* due to collisions between individuals, contrary to Siikavuopio's (2009) theory. However, feeding kelp led to significantly higher spine loss compared to animals fed pellets or fresh *Ulva lacinulata*, regardless of basket height. The decomposition of kelp (*Ecklonia maxima*) in warm water causes mucus formation and affects water quality (Fleischman et al. 2019; personal observation, de Vos), but also leads to the breakage of spines when removing the

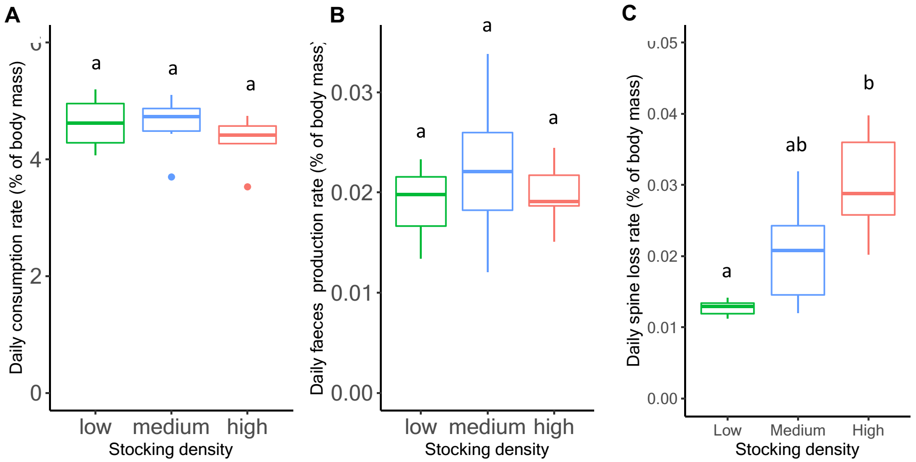

Fig. 10 The influence of stocking density on various daily consumption (**A**), faecal production (**B**) and spine loss (**C**) while fed *Ulva*. Different letters indicate significant differences between treatments

kelp. This breakage occurs due to the firm, almost leather-like, texture of kelp and when it is removed it runs along the test of the urchin and breaks its spines. Although the study couldn't quantify the impact of spine loss on growth reduction, previous research suggests spine loss negatively affects urchin reproductive and somatic growth (Ebert 1967, 1968; Edwards and Ebert 1991; Haag et al. 2016). The observed spine loss in the kelp treatment could potentially reduce growth rates, emphasizing the importance of monitoring spine loss and avoiding degrading or firm feeds like kelp for echinoculture.

Effects of basket removal and height on spine loss

Spine loss was significantly higher in baskets removed from rearing tanks for longer periods, suggesting that prolonged removal results in more damage. As short removal periods (approximately 5 s) or no removals had no significant impact on spine loss, we suggest that maintenance practices, such as avoiding long removal periods, are crucial for reducing damage and maximizing urchin production. Preventing urchins from falling to the bottom of the basket when they are removed from tanks (which can be achieved by gently shaking the basket prior to its removal), may be more practical than adjusting basket heights.

Effects of basket height on consumption

Significantly lower consumption in deep baskets for both pellets and fresh *Ulva* (Fig. 4A-B) provides a clear justification to culture *T. gratilla* in shallow baskets (ca. 15 cm deep) for optimal production. This finding supports Devin's (2002) theory that urchin production is greater in shallow systems because the animals are largely sedentary and more likely to consume when feed is more accessible. While the urchins were observed to feed on all surfaces (the sides and bottom of the basket) both pellets and *Ulva* gradually sink and accumulate on the bottom of the basket. As the urchins prefer to attach to the upper portions of the basket, the majority of the feed is less accessible (further away) in deeper baskets. As such, while the available feeding surface area is the same between the deep and shallow baskets, in deeper baskets urchins are less likely to travel the greater distance to the bottom of the basket to collect settled feed due to the aforementioned sedentary behavioural trait of the urchins. For various urchin species, it has been observed that higher consumption results in faster growth rates (Thompson 1983; McCarron et al. 2009; Cárcamo 2015). It can therefore be assumed lower consumption rates will result in lower production for *T. gratilla*. In the case of feeding *Ulva*, it may be possible to ameliorate this effect by keeping *Ulva* suspended by providing aeration directly beneath the urchin baskets.

Effects of stocking density on the grow-out phase

There were no instances of mortality over the three-month experiment in any of the treatments. While higher stocking density reduced the individual growth of *Tripneustes,* it also allowed for a net increase in production (total mass per basket). Stocking densities of 22.77% coverage (medium) or greater significantly reduced the weight gain of individual urchins (Fig. 5A), while densities greater than 29.87% (high) significantly reduced the diameter SGR (Fig. 5B). The total mass of urchins by the end of the experiment, however, was significantly larger in the greater stocking densities (Fig. 6B). There was no clear evidence that stocking density influenced the quantity (GSI) or quality of gonads (Figs. 7A-D).

These various metrics indicate the optimal stocking density will differ depending on the objective of the culture activity. If the objective is to have each individual urchin gain as much mass as possible, then a low density of approximately 15% cover is ideal. Conversely, if the objective is to gain as much total urchin mass as possible, a density of approximately 30% cover would likely be optimal. The objective will generally depend on the resource limitations of the aquaculture facility. For example, if juvenile supply and/or feed is limited or expensive, then low densities should be used to maximise the potential of each urchin. If there are no limitations of feed, but only running cost and spatial limitations (number of baskets etc.), then higher densities should be used. Each aquaculture facility will need to consider their specific limitations to determine their own optimal density. For simplicity, this study assumes the sole objective of the grow-out phase is to increase the size of the test, thus providing a maximum test volume for the sequential gonad enhancement stage, as discussed in the introduction. The increase in height was not influenced by density (Fig. 5C) but the diameter gain was significantly reduced when urchins were stocked above the medium stocking density (Fig. 5B). This medium density was initially stocked to 19% cover and ended as 22.77% cover. Therefore, this study suggests that if there are no resource limitations on the facility, a stocking density of approximately 20% cover should be used during the grow-out phase of a *T. gratilla* production cycle.

Effect of stocking density on the gonad enhancement phase

There were two cases of mortality (0.98% mortality over two months), one in a basket with a medium stocking density and the other with a high density. These events occurred shortly after the experiment began and were therefore probably the result of handling stress. This suggests stocking density up to the high-density levels put forward by this study, that is, approximately 21% cover, and possibly higher, will not contribute to mortality. The individually housed urchins had significantly greater mass growth than those in the high-density treatment (Fig. 8A), but there was no significant difference between the high-, medium- and low-density treatments. As growth of the individually housed urchins will not be influenced by behavioural interactions, this implies that negative behavioural interactions induced by greater stocking density will only begin to limit urchin growth when density exceeds approximately 15.24% cover, while being fed pellets. It should be noted that while significantly different, the extent of this difference between stocking densities is very small (Fig. 8A-C). Furthermore, the mass SGR was the only metric (based on individual urchins) that showed a significant difference between stocking densities. The objective of the gonad enhancement period is to produce a high quantity and quality of urchin gonads, and there was no evidence of individual metrics related to gonads (GSI, colour or gonad stage) being influenced by stocking density (Figs. 9B-C). This implies that urchins could be stocked to the high density (21.06% cover) or possibly greater during the gonad enhancement phase.

The effect of stocking density on urchin behaviour (consumption, faecal production and spine loss)

There were no differences in the coefficient of variance of mass between stocking densities during both experiments (Figs. 6C and 8C). As such, there is no evidence that variation between individual urchin mass within experimental units was significantly affected by stocking density. There was also no evidence that differences in production between stocking densities were the result of changes in consumption (Fig. 10A-B). The clear relationship between spine loss and stocking density implies that reduced performance at higher stocking densities was likely due to negative physical interactions between urchins (Fig. 10C). There is substantial evidence that spine loss in urchins negatively impacts gonadal and somatic growth (as previously described). A similar observation was made in a stocking density analysis of *Strongylocentrotus droebachiensis* (Siikavuopio et al. 2007).

Additional observations and improvements

The observed low SGRs in this study relative to previous studies (Table 1) can be attributed to the urchins being beyond their optimal growth phase (Dafni 1992; Shpigel et al. 2018) and the low pH recorded in the system (Mos et al. 2015; Shpigel and Erez 2020). The influence of behavioural interactions on stocking density is assumed consistent across different ages of urchins (personal observations, de Vos), thus the findings in this study are still relevant for stocking density recommendations, especially when using a percentage cover metric. Water quality was recorded, finding no significant differences between treatments or blocks (tanks) implying the contrast in urchin production between stocking densities was the result of behavioural interactions and not a confounding factor. The gonad size observed in the gonad enhancement trial was lower compared to a previous study (Cyrus et al. 2015), which used feed with an identical composition. This contrast between studies may be due to feed stability issues with this particular feed formulation, as experienced in another study (Shpigel et al. 2018). Since all urchins in this study received the same pellets, the stability of the feed was not a confounding factor in this study. The observed metrics may not be absolute but still correlate, implying that the optimal stocking densities and management recommendations identified in this study are applicable in more ideal aquaculture conditions.

Conclusion

This study successfully achieved its objectives by determining the influence of basket depth and stocking density on the production of *T. gratilla*. We found that while basket depth does not influence spine loss or mortality it does affect consumption, which is reduced in deeper baskets. Subsequently, we suggest shallower baskets ca. 15cm in height should be used to optimise the production of *T. gratilla* and possibly other urchin species. Our experiments on basket height revealed additional insights that are valuable recommendations to aquaculture operators of *T. gratilla*. The choice of feed significantly impacts spine loss, suggesting large and firm feeds such as kelp may not be optimal for production of *T. gratilla*. Furthermore, we emphasized the importance of handling baskets by demonstrating that removing them from the water for extended periods can damage the urchins and should be avoided.

When addressing the stocking density limitations due to behavioural interactions of *T. gratilla* we determined that an optimal stocking density of approximately 20% cover is ideal for both the grow-out (*Ulva*-fed) and gonad enhancement (pellet-fed) growth phases. While individual growth slightly decreased at this stocking density, it had no significant impact on mortality or gonad quality and led to greater net production of urchins. The results of this study offer practical guidance that can be of significant value to producers of *T. gratilla*, ultimately playing a role in the advancement of more sustainable, ethical and economically viable practices for urchin aquaculture.

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Author contributions Bas C. De Vos was responsible for project conceptualisation, experiment execution, data analysis, and manuscript composition. All authors participated in manuscript editing. Brett M. Macey, Mark D. Cyrus and John J. Bolton made substantial contributions in securing project funding, the projects conceptualisation and provided valuable input on technical aspects.

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Data availability The data sets generated or analysed during this study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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