

Original Article

Uncovering the relationship between light intensity and *Tripneustes gratilla* (collector sea urchin): implications for aquaculture

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Abstract

The sea urchin species *Tripneustes gratilla* has high aquaculture potential. It is known for using debris to cover itself, likely to avoid light, which suggests that high light levels may impact its production. This study aimed to validate these concerns through four experiments. The first experiment assessed if *T. gratilla* preferred opaque or transparent covering materials, finding no evidence that shading ability of the material influenced their choice ($F_{(2, 33)} = 0.27, p = 0.765$). The second experiment investigated the impact of shade on the number of covering materials collected, showing that higher solar radiation correlated with increased material collection per urchin ($H_{(3)} = 7.844, p = 0.049$). The third experiment evaluated righting response time as a fitness trial, finding that high light intensity reduced fitness to urchins acclimated to low light ($H_{(2)} = 5.615, p = 0.020$). The fourth long-term experiment demonstrated that *T. gratilla* can acclimatize to higher light intensities, with no significant differences in fitness, mortality, disease susceptibility, growth, and gonadosomatic index ($p > 0.128$). While this suggests that shading may not be a strict requirement for *T. gratilla* aquaculture, it is recommended for consideration. These findings underscore the importance of acclimatization when altering light conditions for *T. gratilla*.

Keywords: aquaculture, sea urchin, *Tripneustes gratilla*, echinoderm aquaculture

Introduction

The gonads (uni) of sea urchins such as *Hemicentrotus pulcherrimus*, *Heliocidaris crassispinam*, *Paracentrotus lividus*, *Strongylocentrotus droebachiensis* and *Tripneustes gratilla* are in high demand and greatly valued as a culinary delicacy (Tsukiji Market, 2020). *Tripneustes gratilla* is a circumglobal, tropical and sub-tropical sea urchin species that holds significant commercial and ecological importance (Toha *et al.*, 2017). This species is considered a promising candidate for full-lifecycle aquaculture due to their high value, fast growth rates, palatability of both sexes and large marketable gonads (Cyrus *et al.*, 2013; Shpigel *et al.*, 2018). However,

commercial production of *T. gratilla* uni is not yet at scale, partly due to knowledge gaps regarding optimal conditions to promote high survival and growth and therefore increasing yield. A factor potentially influencing the production of *T. gratilla* may be the intensity of solar radiation upon the urchins (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al.*, 2021).

The urchin's behavioural tendency to shade itself by actively utilizing materials from the substratum to cover its aboral surface suggests that there may be a negative correlation between light intensity and *T. gratilla* production. This process involves the use of

its podia (tube feet) and spines to move material actively onto its aboral surface (Ziegenhorn, 2016). This covering behaviour has been observed, to a lesser extent, in other urchin species where it has been demonstrated to correlate with temperature, solar radiation, feeding, availability of cover material, predation, surge and suspended particles (Adams, 2001; Sigg *et al.*, 2007; Dix, 1970; Dennis and Gerald, 1972; Sharp and Gray, 1962; James, 2000; Agatsuma, 2001, Kehas *et al.*, 2005).

There is some evidence for *T. gratilla* that the covering behaviour serves to assist urchins against the effects of currents and waves, preventing them from becoming detached from the substrate (Park and Cruz, 1994), but the most substantiated reason is that the urchin will cover itself to avoid light (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al.*, 2021). This is physiologically feasible due to the urchins' ability to sense light using photo-receptors located on the podia (Millott *et al.*, 1975). Ziegenhorn (2016) demonstrated that *T. gratilla* prefers opaque over translucent materials to cover itself, which implies this is either a photo-defensive behaviour (protecting itself from solar radiation) or a cryptic behaviour (avoiding predation). Li *et al.* (2021) investigated the influence of different spectrums of light on urchin fitness, which the current study defines as the overall health, adaptability, and performance of *T. gratilla*. The metric used for fitness in Li *et al.* (2021) is righting response which is the time sea urchins take to return to their normal posture after being inverted (Brothers and McClintock, 2015). Li *et al.* (2021) found that *T. gratilla* exposed to short-wavelength irradiation (blue light) had significantly reduced fitness compared to higher wavelength irradiation (red and full spectrum light). There is further evidence suggesting that without sufficient shading *Tripneustes ventricosus* will use their feed (e.g., macroalgae) to cover their bodies, presenting a trade-off between feeding and ingesting, negatively impacting growth and gonad quality (Kehas *et al.*, 2005). These studies (Park and Cruz, 1994; Kehas *et al.* 2005; Ziegenhorn, 2016; Li *et al.*, 2021) provide evidence that *T. gratilla* are influenced by light conditions. However, it is unclear if these relationships between light, covering behaviour, and fitness are relevant to *T. gratilla* aquaculture or what specific actions aquaculture operators should take to maximize *T. gratilla* production, such as shading culture facilities.

The main goal of this study was to determine if and how solar radiation affects *T. gratilla* in Seychelles and to provide subsequent management advice to *T. gratilla*

production facilities. It is important to note that while *T. gratilla* covers itself to avoid light in other regions (Park and Cruz, 1994; Ziegenhorn, 2016; Li *et al.*, 2021), this has not been proven for the *T. gratilla* population from Mahe, Seychelles, used in this study. Due to significant geographic differences in *T. gratilla* behaviour, such as spawning time and feed preferences (Toha *et al.*, 2017, personal observation, 2023), it was necessary to confirm if the *T. gratilla* in Seychelles covers itself to provide shading and not for another reason.

Methods

Experimental design and system

A total of four experiments were conducted in this study, the first three were short-term experiments focussing on behavioural responses to light, whereas the fourth experiment assessed the long-term influence of light on *T. gratilla* production. All experiments were conducted in fiberglass raceways with dimensions 6.2 m x 2 m x 0.8 m filled with seawater to a working depth of 60 cm fiberglass raceways. The exchange rate during all experiments was approximately 0.25 full tank exchanges per hour (1860 L.hr⁻¹). Fresh seawater was passed through a sand filter prior to entering the tanks. There was constant aeration distributed evenly across the raceway. For all experiments the urchins were held in plastic crates that measured 50 x 32 x 26.5 cm, with a different number of urchins in each crates depending on the experiment.

Once a week the tank was cleaned by draining the raceway completely and scrubbing it. Prior to draining the raceway all baskets with sea urchins were transferred to a clean raceway already filled with filtered seawater and aerated. The temperature and dissolved oxygen levels were measured daily and on average (\pm standard deviation) were 28.97 (\pm 2.89) °C and 5.2 (\pm 0.772) mg.L⁻¹ respectively. Light intensity was measured in LUX with a Lutron LX-1108 and UV was measured with a Lutron UV-340A. The measurements for LUX and UV were taken daily by positioning the meter beneath each crate shading materials (Table 1), during the different experiments.

Experimental animals

T. gratilla were collected from the wild typically at water depths ranging from 1 to 2 meters. They were then transported to the sea urchin research facility situated at the Seychelles Maritime Academy in Providence, Mahe, Seychelles. During transport, the urchins were distributed into separate containers to avoid overcrowding and covered with *Sargassum spp.*

Table 1. The shading treatments, and the materials used to achieve the shading levels and the observed mean LUX and UV values beneath the materials.

Treatment (% shaded)	Shading material	Mean LUX (lm/m ²)	Min LUX (lm/m ²)	Max LUX (lm/m ²)	Average UV
0	None	116300	126,500	112900	4939
32	Mesh	36675	78500	102000	2738
41	Green corrugated roofing	47241	28200	62000	120
77	Shade cloth	89708	28300	41600	515

to shield them from direct sunlight exposure. Upon arrival, they were transferred to plastic crates with 41 % shading and placed in a raceway where they were allowed to acclimate for a period of 14 days.

Experiment 1: Covering material opacity preference trial

To examine the correlation between light and covering of *T. gratilla* from Seychelles, an experiment was conducted to determine the preference for selecting opaque or transparent covering materials by *T. gratilla*. On the day of this experiment, 12 *T. gratilla* were removed to a separate raceway and were placed individually in uncovered crates. These urchins had an average (\pm SD) wet weight, test diameter and test height of 353.9 ± 69.87 g, 87.1 ± 7.51 mm and 61.8 ± 7.0 mm respectively. The height and test diameter were determined using vernier callipers. An average LUX of $117,450 \pm 937.54$ lm/m² was recorded during the study. Each crate was randomly scattered with three different types of covering materials of various opacities, with 15 pieces of each type. The covering materials used were corrugated plastic roofing sheets cut into 2.5 x 2.5 cm pieces of either blue (opaque), green (partially transparent) or clear (fully transparent). After 3 hours, the number and type of covering materials collected by each *T. gratilla* in each crate were recorded.

Experiment 2: Influence of solar radiation on quantity of covering materials collected by *T. gratilla*

This experiment was conducted to determine if solar radiation intensity would influence the quantity of covering material collected by *T. gratilla*. Four treatment levels of solar radiation intensity were achieved by placing different types of shading materials (mesh, green corrugated roofing, shade cloth), or lack therefore, on top of the crates and above the water surface. The light intensity beneath the shading material within each crate was measured daily and the average value calculated (Table 1).

Twelve *T. gratilla* were measured for wet weight, test diameter and test height, with mean \pm SD values of 368.8 ± 60.1 g, 89.4 ± 7.8 mm, and 65.3 ± 7.0

mm respectively before each was placed individually into a crate. Following this, 45 pieces of 2.5 x 2.5 cm opaque (not transparent) plastic sheet covering materials were added inside the crates amongst the urchins. Besides these green plastic sheets, no other covering materials were provided. Note that this covering material differs from the shading material (Table 1) which is above the water surface and out of the urchins' reach. After 30 minutes, the number of covering materials collected by each *T. gratilla* in each crate was recorded. Thirty minutes was required for this trial (as opposed to the three hours of the previous trial), as the urchins did not need time to select different materials.

Experiment 3: Short-term effects of solar radiation on *T. gratilla* fitness

In this experiments *T. gratilla* were placed in crates that had three different light regimes: full light (control), 37 % light reduction and 100 % light reduction. The amount of light was controlled using different covering materials (none, corrugated roofing and black plastic sheeting respectively). A total of 60 *T. gratilla* were included in the study, conducted over five experimental runs, with four *T. gratilla* randomly allocated to each crate per run. The experiment was only undertaken on days with no cloud cover (between 10:00 hrs and 14:00 hrs). *T. gratilla* in this experiment had an average \pm SD of wet weight of 399.42 ± 71.35 g, diameter of 77.51 ± 23.33 mm and, height of 55.43 ± 8.79 mm.

At the start of the experiment, LUX and UV radiation intensity were recorded, using the same methods previously described, at the water surface in all the crates. After three hours they were removed to be tested for righting response time. To do so, *T. gratilla* was gently placed in individual plastic containers of 50 cm x 40 cm x 30 cm with their aboral side down. The urchins were positioned in the centre of the container so they could not access the sidewalls to assist with righting themselves. The containers were filled with seawater. Aeration and water flow were not used to avoid potential impacts on the righting behaviour. The time taken

Table 2. The shading treatments, and the materials used to achieve the shading levels and the observed LUX and UV values beneath the materials.

Treatment (% shaded)	Shading material	Mean LUX (lm/m ²)	Min LUX (lm/m ²)	Max LUX (lm/m ²)	Average UV
0	None	188298	7982	351700	6824
37	Corrugated roofing	69128	1608	272400	238
100	Black plastic sheeting	0	0	0	0

for the sea urchins to right themselves, with their aboral side facing upwards was measured and recorded.

Experiment 4: The long-term influence of light on *T. gratilla* production

A long-term experiment was conducted to assess the influence of light on *T. gratilla* production over 67 days. *T. gratilla* (n=90) was collected from the wild and acclimated for 14 days in crates covered with corrugated roofing. To begin the experiment, nine crates were placed in the raceway with 10 *T. gratilla* in each with an average \pm SD wet weight, test diameter and test height of 336 ± 82.3 g, 71.4 ± 6.6 mm, 43.8 ± 15.2 mm, respectively. Three of the crates were covered with corrugated roofing material, three with black plastic sheeting and three crates had no shading. The solar radiation intensities under each level of shading are shown in Table 2. LUX and UV were measured using the same instrument and method mentioned above. Mortalities and incidence of disease (bald spot) during the experiment were recorded. The appearance of bald spot disease (Becker et al., 2007) was considered a sign of stress in *T. gratilla*. At the end of the experiment, three urchins were randomly chosen from each basket to test their righting response time. The same method as described above was used. Also, urchins from each replicate were measured for height, diameter and mass. Subsequently, the specific growth rate (SGR) was determined for each replicate (Equation 1).

$$\text{SGR} = 100 \times \ln(L_T/L_0)/t \quad (1)$$

Where:

L_0 = initial length/mass

L_T = final length/mass

t = days of culture

The urchins were then dissected to determine the gonadosomatic index (GSI, Equation 2).

$$\text{GSI} = 100 \times (\text{GW}/\text{WW}) \quad (2)$$

Where:

GW = Gonad weight

WW = Wet weight

Statistical analysis

The statistical programming environment R (R Development Core Team, 2017) was employed in all the analytical procedures. Excel was utilized for data organization and presentation. It was ensured that the assumptions regarding independence and non-selectivity were satisfied, as elucidated within the context of the experimental designs. Significance was attributed to p-values below the threshold of 0.05.

All the data was tested for homogeneity of variance and normality of distribution using Levene's test and the Shapiro-Wilk test respectively. The data stemming from the experiments on cover preference and long-term specific SGR, GSI and fitness was found to be homogenous ($p > 0.29$) and parametric ($p < 0.056$) and therefore one-way ANOVAs were applied. When treatments were found to have a significant influence on the mean, pairwise Tukey tests were applied for multiple comparisons. The data from the experiments quantifying the covering response to light, short-term fitness, long-term survival and disease was found to be homogenous ($p > 0.091$) but not parametric ($p < 0.015$), subsequently, a Kruskal-Wallis test was applied. When the tests were found to be significant, Dunn post-hoc analyses were conducted.

Results

Experiment 1: Covering material opacity preference trial

At the end of the three-hour trial, all urchins had collected covering materials of various transparencies to cover themselves with a mean \pm standard deviation (SD) of 1.72 ± 1.92 pieces per urchin (Fig. 1). While the average number of opaque pieces (2.33 ± 1.92) collected by the sea urchins was greater than that of the fully (1.83 ± 1.89) and partially transparent (1 ± 1.94) pieces (Fig. 1), there is no conclusive evidence that *T. gratilla* preferred covering material due to their ability to provide shade. There were instances where the individual urchins collected only transparent pieces, but no opaque pieces and vice versa. There was no significant influence of material transparency on the number of pieces collected by *T. gratilla* ($F_{(2,33)} = 0.27$; $p = 0.765$).

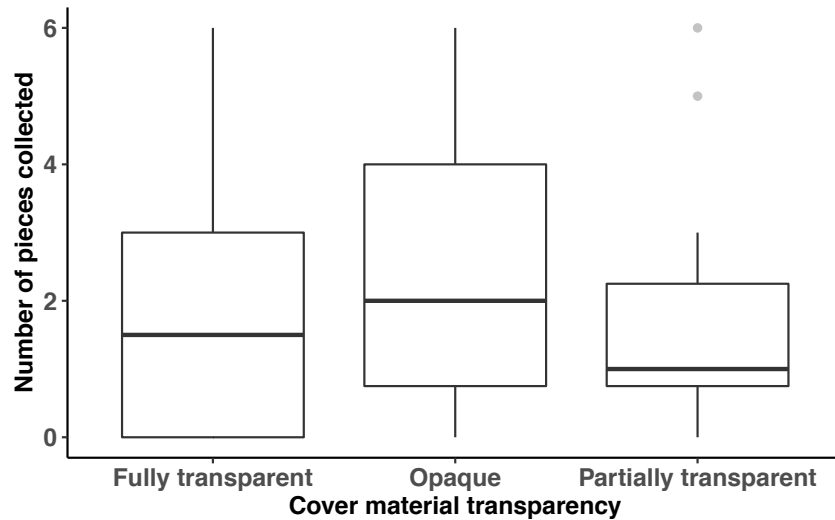


Figure 1. The number of covering material pieces of various transparencies collected by *T. gratilla* after three hours. The covering material pieces (n=45) provided to each urchin (n=12) were small sheets of hard plastic, either fully transparent (clear), partially transparent (green) or opaque (blue), with equal quantities of each type.

Experiment 2: Influence of solar radiation on covering materials collected.

All urchins collected some covering material (7.71 ± 5.38 pieces per urchin) regardless of shading, except for one individual in an unshaded crate which did not collect any pieces. Overall, the amount of shading over the crate containing the urchins had a significant influence on the number of pieces collected ($H_{(3)} = 7.844$, $p = 0.049$). The average number of materials collected by urchins in the unshaded crates (11 ± 6.85 pieces) was greater than that of the treatments with various levels of shade (6.42 ± 4.15 pieces; Fig. 2).

However, the urchins in the unshaded crates collected significantly more covering pieces than those in the maximally (77 % shade) shaded crates ($p = 0.039$), but not significantly more than in the other treatments ($p > 0.098$).

Experiment 3: Short-term effects of solar radiation on *T. gratilla* fitness

After three hours of being exposed to three different light intensities, the righting response of *T. gratilla* was recorded. The urchin's ability to right themselves took the longest in the crates without shade

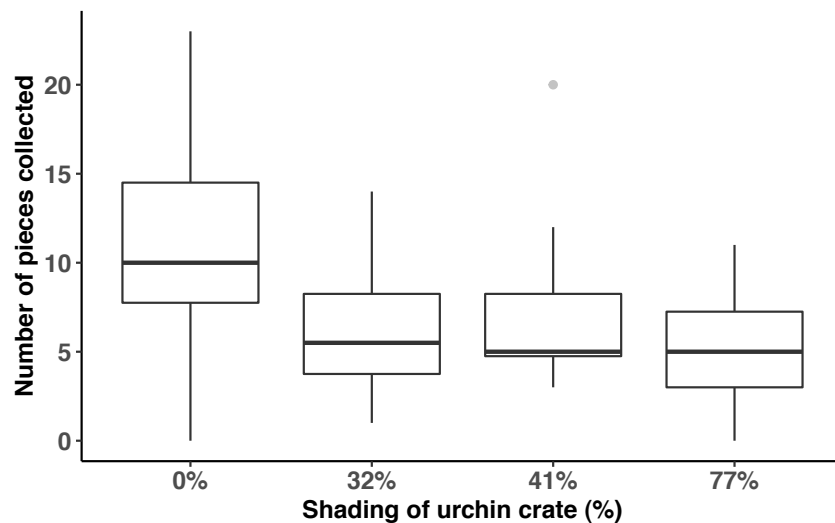


Figure 2. The number of covering materials (plastic sheets) collected by *T. gratilla* in crates with varying levels of shading. The 0 % shaded treatment had no shading, the 32 % shaded treatment had the crates covered by a fine mesh, the 41 % used an opaque plastic sheet, and the 77 % shade treatment was achieved by covering with a shade cloth.

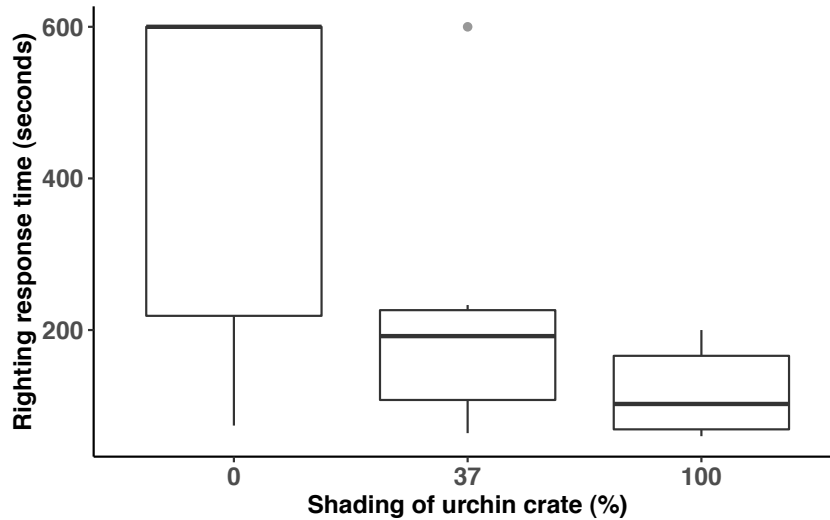


Figure 3. The righting response time (seconds) of *T. gratilla* after three hours exposed to three different light intensities. A total of 20 sea urchins exposed to each of the different light intensity either black plastic sheeting (0 % light intensity), corrugated roofing (37 % light intensity) and none (100 % light intensity).

(282 ± 221 seconds), followed by those in 37 % shade under corrugated roofing material (241.4 ± 197.12 seconds; Fig. 3). The urchins in 100 % shade under black plastic sheeting took the least time (143 ± 113.97 seconds) to right themselves. The results showed that light intensity had a significant influence on righting response in *T. gratilla* ($H_{(2)}=7.182$, $p = 0.027$). A significant difference was observed between treatments 0 % and 100 % shading ($p = 0.032$).

Experiment 4: The long-term influence of light on *T. gratilla* production

At the end of the long-term trial, during which *T. gratilla* was exposed to three different light intensities for 67 days, the urchins' righting response was assessed to quantify their degree of fitness. The righting response of urchins exposed to a lower light intensity (100 % shade) was faster (128 ± 92.79 seconds) than those under 37 % shade (285 ± 205.17 seconds) and 0 %

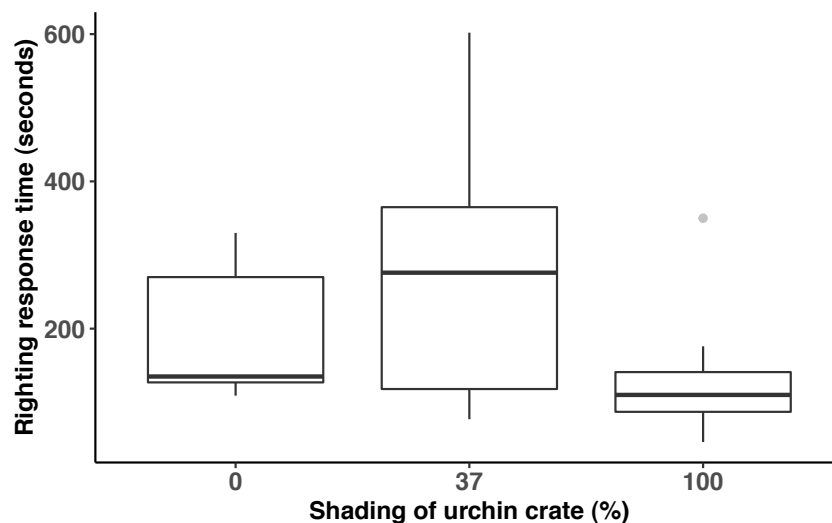


Figure 4. The fitness of *T. gratilla* (n=27) after long-term exposure to three different light intensities; 0 %, 37 % and 100 % shade. Fitness was assessed by the time taken for to right themselves from being inverted.

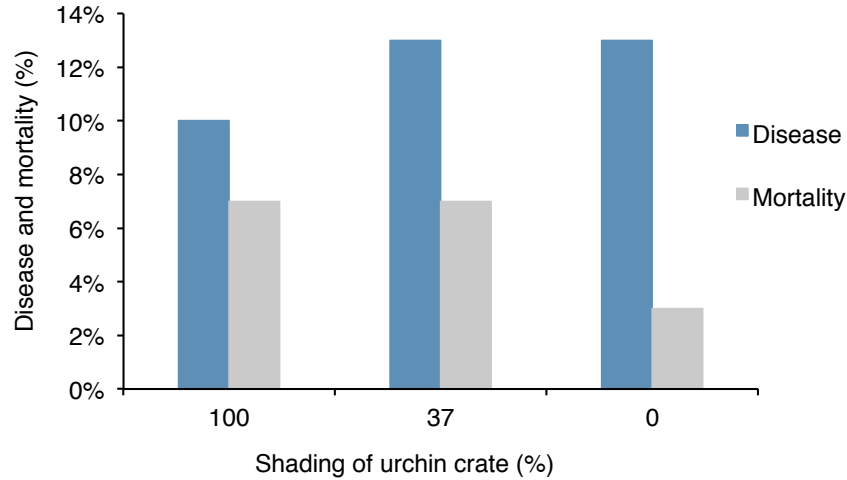


Figure 5. The disease and mortality rate (%) of *T. gratilla* after 67 days of exposure to three different light intensities; 0 %, 37 %, and 100 % shading.

shade (184 ± 83.04 ; Fig. 4). However, there is no statistical evidence that light intensities influenced the righting abilities of *T. gratilla* ($H_{(2)} = 4.108$, $p = 0.128$).

Across all treatments, the average specific growth rates (SGR) were low over the long-term trial. The mean \pm SD SGR of the test diameter, height and wet mass was 0.04 ± 0.06 mm, 0.04 ± 0.07 mm, 0.07 ± 0.15 g respectively. There were no significant differences between treatments for the SGR of height ($F_{(2,6)} = 0.782$; $p = 0.499$), diameter ($F_{(2,6)} = 0.279$; $p = 0.766$) or mass ($F_{(2,6)} = 0.829$; $p = 0.481$).

During the 67-day trial, the lowest light intensity treatment (100 % shade) had the lowest mortality rate (3.5 %), while the two treatments at higher light intensities had an equally greater mortality (7 %; Fig. 5). However, there was no evidence of a significant influence of light intensity on mortality ($F_{(2,12)} = 0.09$, $p = 0.913$). Similarly, even though fewer urchins with symptoms of the disease were recorded in fully shaded crates (Fig. 5), there were no significant differences between the number of diseased individuals and light intensity ($F_{(2,12)} = 0.09$, $p = 0.913$).

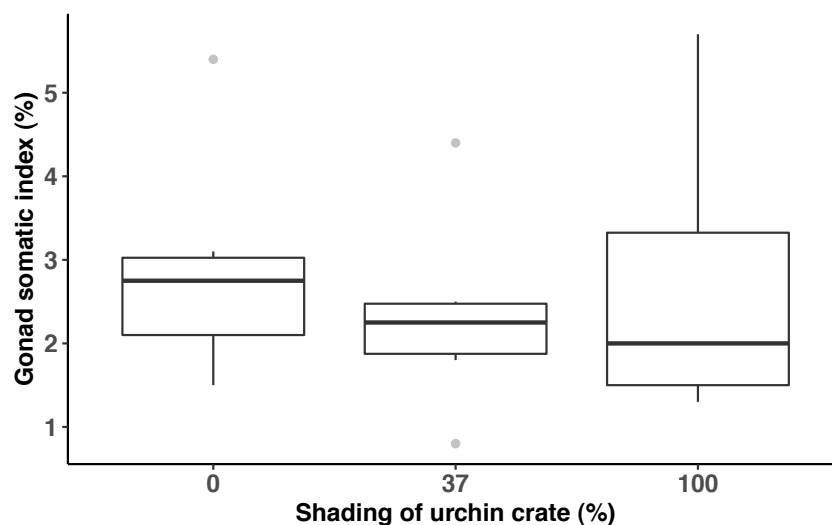


Figure 6. Gonad somatic index (%) of *T. gratilla* after being exposed in three different light intensities for 67 days. The covering materials were black plastic (0 % light intensity), corrugated roofing (37 % light intensity) and none (100 % light intensity).

The average gonad somatic index (GSI) was not significantly different under the three different light intensities ($H_{(2)} = 0.877$ $p = 0.644$). The mean \pm SD GSI values for 100 %, 37 % and 0 % shaded treatments were low (Fig. 6), 2.6 ± 1.73 %, 2.3 ± 1.20 % and 2.9 ± 1.36 % respectively.

Discussion

The observations from this study confirmed that *T. gratilla* covering behaviour is stimulated by greater light intensity (Experiment 2, Fig. 2), aligning with prior studies (Park and Cruz, 1994; Ziegenhorn, 2016; Li et al., 2021). Yet, evidence could not be provided that *T. gratilla* prefers opaque materials for covering (Experiment 1, Fig. 1), unlike Ziegenhorn (2016). Therefore, it cannot be concluded that *T. gratilla* in the Seychelles selected material for their ability to provide shade. Thus, the purpose of this covering behaviour remains unclear. While it may protect from UV radiation or have a cryptic function, they may also collect objects as a physical barrier against predators or to stabilize the urchins during surges. The contrasting finding of cover material opacity preference between this study and that of Ziegenhorn (2016) may stem from regional behavioural differences among *T. gratilla* populations (Toha et al., 2017; personal observation, 2023). This underscores the imperative to validate proposed aquaculture practices, findings, or technologies within the region of application, prior to upscaling for commercial implementation in general. More specifically, given the spatial variations in *T. gratilla* behaviour, it is essential to conduct similar investigations in different regions to obtain a comprehensive understanding of their responses to light.

The results of the short-term fitness trial (Experiment 3) complemented those of Li et al. (2021), where greater light exposure significantly reduces the fitness of urchins (Fig. 3). However, it should be noted that urchins in this study were acclimatised to lower light levels prior to this experiment and Li et al. (2021) did not specify what lighting the urchins were acclimatised to before experimentation. This is relevant as this fitness and light relationship was not observed when urchins were acclimatised to specific lighting conditions over a longer-term experiment (Fig. 4). This indicates that *T. gratilla* fitness will only be affected adversely by light when shocked by it, but they can acclimatise to higher light intensities. This emphasizes the importance of gradual acclimatization when altering light conditions for *T. gratilla*, where care should be taken by aquaculture operators to not rapidly change environmental conditions. Additionally,

this finding does not provide conclusive evidence that *T. gratilla* production facilities require shading. This is further supported by the lack of evidence that light intensity impacted urchin mortality, disease susceptibility (Fig. 5), growth or gonad somatic indices (Fig. 6), suggesting that *T. gratilla* can adapt to higher light levels over an extended period, a novel finding that carries important implications for aquaculture practices.

While there were no significant differences in disease and mortality between the different light treatments, which suggests that *T. gratilla* can adjust to higher light intensity, it is important to note some limitations regarding urchin growth and GSI results. The GSI results might have been affected by spawning events that occurred before the dissections. Even though no spawning was observed during the trial, the very low GSI values (average of 2.84 %) suggest that spawning might have happened. Previous trials at this facility under similar conditions and feed showed much higher GSI values, around 10 % (personal observation, 2023). The lack of significant difference in growth rates is likely due to the urchins starting at a size where they no longer experience high growth rates (Dafni, 1992; Shpigel et al., 2018). Additionally, the measurement methods used may not have been precise enough (de Vos et al., 2023), making it unlikely to detect a significant difference. Future research should use smaller urchins that can continue to grow during the experiments and ideally control for spawning events.

Although these experiments did not establish a substantial influence of light intensity on *T. gratilla* production, the authors are not necessarily advising that shading should not be implemented in land-based urchin production facilities. There are likely alternative advantages of shading such as preventing salinity fluctuations due to rainwater, slowing the growth of epiphytes, and reducing temperature fluctuations in tanks.

In conclusion, this research contributes to a deeper comprehension of the complex relationship between light exposure, covering behaviour, and the production of *T. gratilla*. While the motives behind *T. gratilla*'s covering behaviour remain somewhat enigmatic and are likely dynamic, the findings from this study underline the importance of gradual acclimatization to changing light conditions and regional variation in *T. gratilla* behaviour. This study does not provide definitive evidence that light directly hampers urchin production thus implying that shading of urchin production facilities may not be an absolute requirement

for production. This research contributes another piece of the puzzle to advancing *T. gratilla* aquaculture by offering valuable insights into optimizing production practices while ensuring the welfare of this economically important species.

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