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Optimizing Prepared Feed Ration for Somatic Growth and Gonad Production of the Sea Urchin *Tripneustes gratilla* (Linnaeus, 1758)

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Abstract

Somatic growth and gonad production and quality of the sea urchin *T. gratilla* fed with prepared diets with *Sargassum sp*. as feed base at different feeding rations was studied *in vitro* using glass aquaria from April to August 2006. The trial consisted of five treatments with three equal replications arranged in Completely Randomized Design as follows: I-Fresh *Sargassum sp.* (control), II-Dried pellets at 2% body weight (BW) day^{-1}, III-Dried pellets at 3% BW day^{-1}, IV-Dried pellets at 4% BW day^{-1}, and V-Dried pellets at 5% BW day^{-1}. Results show that Treatments I, IV and V significantly ($p<0.05$) gave better results than Treatments II and III. The highest monthly mean growth increment was observed in Treatment V which was significantly higher ($p = 0.05$) than all the treatments except for Treatment IV. These results show that dried pellets feeding ration was optimized at 4 to 5 % BW day^{-1} for somatic growth and gonad production and quality. Significantly higher gonadosomatic index ($p=0.05$) was also observed in Treatments I, IV and V than Treatments III and II, respectively. Treatments II and III did not differ significantly.

Introduction

The increasing demand for sea urchin roe in both the local and world markets necessitates the development of extensive fishery enhancement techniques such as land-based and field aquaculture, selective breeding, gonad enhancement through prepared diet feeding and others.

Pearce et al. (2002a) noted that a number of studies have examined the effect of manipulating source and/or concentration of various dietary components of prepared feeds on gonad quantity and/or quality but surprisingly little is known about how prepared feed ration may affect gonad development. For example, McBride et al. (1999) reported significantly greater gonad indices in adult *Strongylocentrotus franciscanus* given a prepared feed ration of 1.02% BW day^{-1} than those fed only 0.34% BW day^{-1}. Little is known about the effect of prepared feed ration on gonad yield or quality of sea urchins with no information being available for *T. gratilla*.

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This study was undertaken to determine the optimum feeding ration using prepared diets for the somatic growth and gonad production and quality of the animal in land-based facilities. Specifically, it aims to: (1) determine the somatic growth (wet weight and test diameter) performance of the animal fed with prepared diets at different feeding rations; (2) determine the gonad production (gonadosomatic index) and gonad quality (color) of the animal fed with prepared diets at different feeding rations; and (3) monitor the ecological parameters affecting the somatic growth performance and gonad maturity and quality of the sea urchin. Likewise, determining the minimum feed ration required for effectively enhancing somatic growth and gonad yield and quality will minimize feed wastage which is critical in achieving profitability especially when the culture technology of the animal will have been developed at a commercial scale.

Materials and Methods

Location of the Study

The experiment was set-up and conducted within the premises of the MMSU College of Aquatic Sciences and Applied Technology campus, Pias Sur, Currimao 2903, Ilocos Norte, Philippines.

Experimental System

Three glass aquaria measuring 30 cm x 20 cm x 20 cm were used in the experiment. Each of the glass aquaria was sub-divided into 6 equal compartments with Amazon plastic net in order to contain the 5 feeding ration treatments. Seawater was pumped from the coast in front of the college building using a diesel water pump into four concrete tanks measuring 4.0 m x 2.5 m x 2.0 m (L x W x D). Unfiltered seawater from the concrete tanks was pumped using a 0.5 HP electric water pump into two circular fiberglass tanks measuring 1.0 m diameter x 1 m depth provided with gravel and sand serving as filtration tanks that supply the nine experimental basins on a flow-through system. The seawater was aerated with NSB electric air pumps.

Experimental Design

The experiment consisted of five feeding treatments, namely: Treatment I - control consisting of fresh unformulated *Sargassum sp* diet; Treatment II – dried *Sargassum sp* pellets at 2% wet BW day^{-1}; Treatment III - dried *Sargassum sp* pellets at 3% wet BW day^{-1}; Treatment IV - dried *Sargassum sp* pellets at 4% wet BW day^{-1}; and Treatment V - dried *Sargassum sp* pellets at 5% wet BW day^{-1} with three equal replications (Table 1). Asia and Tabije (2003) found out that *Sargassum* is a better natural feed affecting the growth and gonad development of *T. gratilla* as compared with the seagrass *Enhalus acoroides*. Treatment I was contained in one aquarium

while Treatments II to V were arranged in a completely randomized design (CRD) in the other two aquaria (Fig. 1).

Table 1. Feeding ration treatments and stocking density used in the study.

Treatments	Stocking density	Replicates
I - Control (Fresh Sargassum)	3 individuals/compartment	3
II - Dried Sargassum sp pellets at 2% BW day^{-1}	3 individuals/compartment	3
III - Dried Sargassum sp pellets at 3% BW day^{-1}	3 individuals/compartment	3
IV - Dried Sargassum sp pellets at 4% BW day^{-1}	3 individuals/compartment	3
V - Dried Sargassum sp pellets at 5% BW day^{-1}	3 individuals/compartment	3

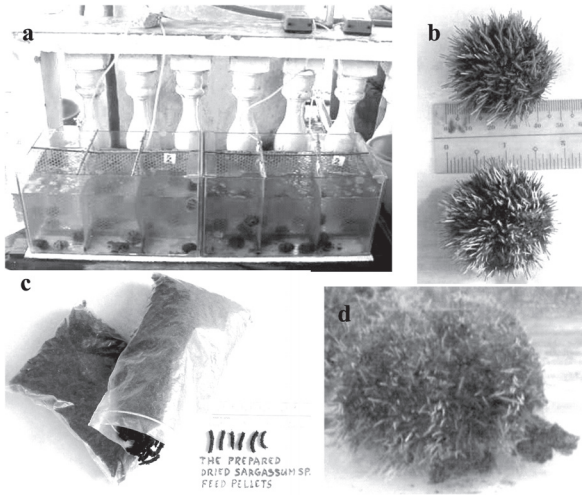

Figure 1. **(a)** The Experimental set-up, (b) post-juvenile *T. gratilla*, (c) prepared diet, (d) feeding organism

Experimental Animals

Forty-five post-juvenile *T. gratilla* with equatorial test diameter ranging from 34.00 to 40.00 mm and weighing from 24.40 to 39.10 g (wet weight) were used in the experiment (Fig. 1). These were taken from the wild stock in Dadalaquiten, Sinait, Ilocos Sur, a nearby municipality of about 15 kilometers from the experimental site.

Feed Preparation

The dried pellets were composed of finely ground dried *Sargassum sp.* (sun-dried for 1-2 days before grinding) with 6% binder consisting of corn starch and gelatin.

The steps in feed preparation following that of Alava (1996) were observed. The finely ground seaweed was sieved into uniform particle size. The binder was suspended into half of the required water (the required water for a kilo of prepared feed is 1,200 ml) while the remaining half was boiled and then the suspended binder was poured slowly to the boiled water stirring constantly with low flame until jelly-like consistency was obtained. The gelatinized binder was added to the ground *Sargassum* and mixed. The feed mixture was pelletized at a size of 5mm diameter using a manually-operated pelletizer, steamed with sea water for 5 minutes to enhance the binding of ingredients with an improvised steamer set-up, fan-cooled and sun dried for one day. The pelletized feeds were placed and sealed in plastic containers and refrigerated (Fig. 1).

Experimental Procedure and Sampling

Three sea urchins were placed in each compartment. They were fed with the fresh *Sargassum* diet daily on an *ad libitum* basis and with the prepared diets daily at the specified ration per treatment. The daily dried pellet rations were divided into equal proportions and given to the animals in the morning and in the afternoon. Pearce et al. (2002b) concluded in their research that gonad yield of the green sea urchin *Strongylocentrotus droebachiensis* is maximized at 0.5 to 1.0% BW day^{-1} feeding of prepared diets. Since *T. gratilla* is a tropical species, which presumably has a higher metabolic rate than *S. droebachiensis* which is a temperate species, feeding rations of 2.0 to 5.0 % BW day^{-1} were evaluated. Daily cleaning and maintenance of the culture system were also done.

Prior to stocking in the aquaria, the initial test diameter and weight of each experimental animal were measured and recorded. To monitor the somatic growth of sea urchin, the experimental animals were measured in terms of their test diameter (mm) both equatorial and polar using a Vernier caliper and wet weight with triple beam balance of 1.0 g sensitivity. Measurements were taken once a week for a period of four months.

The test animals were shucked upon the termination of the study to determine gonad growth in terms of the gonadosomatic index (GSI) and quality (color). The GSI of the animals was determined using the following formula (Lozano 1995):

$$GSI = \frac{\text{Wet weight of gonad}}{\text{Wet weight of sea urchin}} \times 100$$

Gonad quality in terms of colour was determined using the Munsell Color Guide. The guide has three simple variables that combine to describe colors as hue, value and chroma. This study used the hue Yellow-Red (10YR) which is preceded by numbers

from 0 to 10 where the hue becomes more yellow and less red as the numbers increase. The value notation used was 8 (near white) and the chroma notation used ranged from 1 to 8 where the colour becomes more yellow with increasing number. The values based on the chroma notation were the ones recorded in this study for gonad color comparison. The combination of a bright yellow colour and fine granularity of gonad is most preferred by the market (Blount and Worthington 2002).

Monitoring of Water Parameters

The water parameters that may affect the somatic growth and gonadal growth and quality of the experimental animals were measured. Dissolved oxygen and temperature were measured by a YSI Dissolve Oxygen Meter (Model 55); salinity by an Atago refractometer; and pH by a pH paper.

Analysis of Data

Means and ranges were used to describe growth parameters of the test animals and the water parameters. Analysis of covariance (ANCOVA procedure of the SAS System) was used to determine differences of somatic growth parameters among treatments with wet weight and test diameter as covariates. Analysis of variance (ANOVA procedure of the SAS System) was also used to determine the differences of the GSI and gonad color among treatments. A post test using the Duncan's Multiple Range Test (DMRT) was used to further test significant findings.

Further, a correlation analysis using the Proc Corr procedure of the SAS System was used to determine the strength of relationship of the somatic growth parameters, gonad growth and quality parameters, and water parameters.

Results and Discussion

Somatic Growth

Growth increments in wet weight of the experimental animals in the four months culture period (MCP) at different feeding ration treatments are shown in Table 2. Figure 2 also shows samples of matured animals per treatment. The values are adjusted means with initial weight as covariate. After one MCP, Treatments I, III, IV and V with 10.36 g, 9.66 g, 13.29 g and 13.05 g, respectively significantly ($p = 0.05$) had higher weight increments than Treatment II with weight increment of 6.19 g. Treatment IV which generally had the highest weight increment in all treatments was also significantly ($p = 0.05$) higher than Treatment III. After two MCP, although all the treatments did not significantly differ, Treatment V generally had the highest observed weight growth increment followed by Treatments IV, I and III in decreasing order. Treatment II had the least growth increment.

Table 2. Mean growth increment in wet weight* (g) of the sea urchin *T. gratilla* at different feeding ration treatments for four months culture period.

	Months After Stocking						
	Initial	1	2	3	4		
I – Fresh Diet (Control)	32.71	10.36^{ab}	12.03^{a}	9.99^{ab}	11.66^{a}	44.04	11.01^{bc}
II – Dried Pellets at 2% BW day^{-1}	31.99	6.19^{c}	9.91^{a}	8.33^{b}	8.89^{a}	33.32	8.33^{c}
III – Dried Pellets at 3% BW day^{-1}	30.80	9.66^{abc}	10.54^{a}	13.16^{ab}	6.91^{a}	40.27	10.07^{bc}
IV – Dried Pellets at 4% BW day^{-1}	31.74	13.29^{ab}	12.16^{a}	15.93^{ab}	8.73^{a}	50.11	12.53^{ab}
V – Dried Pellets at 5% BW day^{-1}	30.14	13.05^{ab}	14.27^{a}	19.22^{a}	13.30^{a}	59.84	14.96^{a}

Means with different superscripts are significantly different at 5% level.
*Adjusted mean with initial weight as covariate.

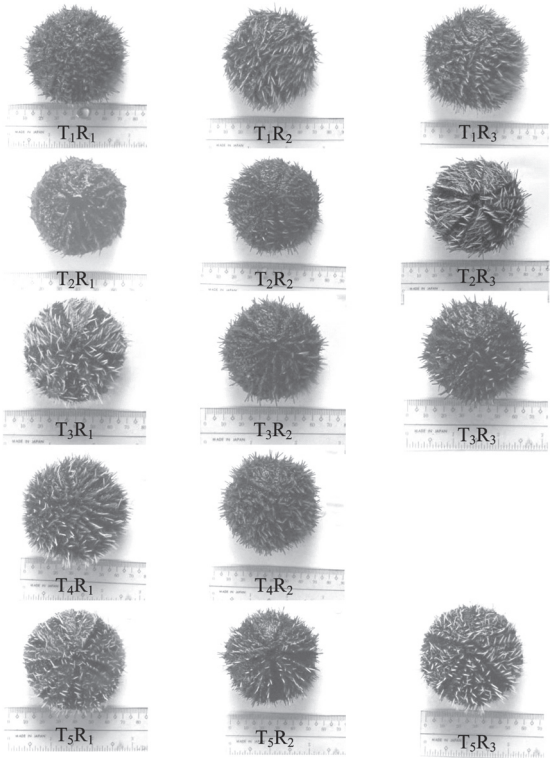

Figure 2. Samples of matured *T. gratilla* from the different treatments

After three MCP, only Treatments V and II were observed to have significant differences with Treatment V having a higher growth increment. Treatment V also generally had the highest growth increments in all treatments. No significant differences were observed among all the treatments after the last MCP, however Treatment V generally had the highest growth increment.

Overall, the highest mean growth increment was observed in Treatment V with 14.96 g which was significantly higher ($p = 0.05$) than all the treatments except for Treatment IV. Treatment II significantly had the lowest mean growth increment. This result shows that feeding ration per day with the dried pellets for the test animals was optimized at 4 to 5 % BW day^{-1}.

The results of the study likewise show that the test animals fed with prepared diets at 4 to 5 % BW day^{-1} had considerably higher growth in wet weight than those fed with fresh *Sargassum*. Similarly in their experiments, Pearce et al. (2004a) and Daggett et al. (2005) observed that the green sea urchin *Strongylocentrotus droebachiensis* fed prepared diets were significantly larger and heavier than those fed with fresh kelp.

Growth increments in equatorial test diameter of the sea urchins during the four MCP are shown in Table 3. The values are adjusted means with initial equatorial test diameter as covariate. It was observed during the first MCP that Treatment II significantly ($P = 0.05$) had the lowest growth increment in equatorial test diameter of 4.70 mm. The other treatments had no significant differences in equatorial test diameter growth

Table 3. Mean growth increment in equatorial test diameter* (mm) of the sea urchin *T. gratilla* at different feeding ration treatments for four months culture period.

	Months After Stocking						
	Initial	1	2	3	4		
I – Fresh Diet (Control)	38.34	7.80^{a}	6.78^{a}	1.84^{a}	2.72^{a}	19.14	4.79^{a}
II – Dried Pellets at 2% BW day^{-1}	37.11	4.70^{b}	4.30^{a}	1.85^{a}	3.37^{a}	14.22	3.56^{a}
III – Dried Pellets at 3% BW day^{-1}	37.67	7.79^{a}	3.42^{a}	3.12^{a}	3.16^{a}	17.49	4.37^{a}
IV – Dried Pellets at 4% BW day^{-1}	37.78	9.04^{a}	5.25^{a}	3.20^{a}	4.74^{a}	22.23	5.56^{a}
V – Dried Pellets at 5% BW day^{-1}	37.00	7.53^{a}	4.23^{a}	4.56^{a}	4.54^{a}	20.86	5.22^{a}

Means with different superscripts are significantly different at 5% level.

*Adjusted mean with initial equatorial test diameter as covariate.

increment. The overall means also show no significant differences among treatments, although comparatively, Treatments IV and V had generally higher growth increments.

Highest wet equatorial test diameter growth increment in all treatments was observed during the first MCP and had a decreasing trend towards the end of the study.

Table 4 shows the data on growth increments in polar test diameter of the test animals during the four MCP. The values are adjusted means with initial polar test diameter as covariate. Results show that there were no significant differences among the treatments from the first to the third MCP. However, during the last MCP, Treatment II significantly ($p = 0.05$) had the lowest growth increment in polar test diameter. The overall means also show no significant differences among treatments, although comparatively, Treatments V generally had the highest growth increments followed by Treatments III and IV. It was also observed that the highest growth increment in polar test diameter was during the first month culture period which follows the trend on the growth in equatorial test diameter.

Table 4. Mean growth increment in polar test diameter* (mm) of the sea urchin *T. gratilla* at different feeding ration treatments for four months culture period.

	Months After Stocking						
	Initial	1	2	3	4		
I – Fresh Diet (Control)	22.00	5.68^{a}	1.34^{a}	1.66^{a}	2.25^{a}	10.93	2.73^{a}
II – Dried Pellets at 2% BW day^{-1}	21.44	5.24^{a}	2.20^{a}	1.33^{a}	0.64^{b}	9.41	2.35^{a}
III – Dried Pellets at 3% BW day^{-1}	22.22	5.06^{a}	2.44^{a}	1.35^{a}	2.48^{a}	11.33	2.83^{a}
IV – Dried Pellets at 4% BW day^{-1}	22.67	6.09^{a}	1.44^{a}	0.36^{a}	3.44^{a}	11.33	2.83^{a}
V – Dried Pellets at 5% BW day^{-1}	22.33	5.86^{a}	2.27^{a}	1.14^{a}	3.20^{a}	12.47	3.12^{a}

Means with different superscripts are significantly different at 5% level.

*Adjusted mean with initial polar test diameter as covariate.

Gonad Growth and Quality

Gonad growth and development measured in terms of gonadosomatic index and gonad quality determined in terms of color were assessed upon the termination of the study (Table 5). Figure 3. also shows samples of gonads taken per treatment. Results

Table 5. Mean gonadosomatic index (GSI) and gonad color of the sea urchin *T. gratilla* at different feeding treatments after four months culture period.

	Gonad Growth and Quality Parameters	
	GSI (%)	Color
I – Fresh Diet (Control)	4.80[a]	7.67[a]
II – Dried Pellets at 2% BW/day	2.24[b]	4.00[b]
III – Dried Pellets at 3% BW/day	3.29[ab]	4.50[b]
IV – Dried Pellets at 4% BW/day	4.59[a]	7.50[a]
V – Dried Pellets at 5% BW/day	4.49[a]	7.00[a]

Means with different superscripts are significantly different at 5% level by DMRT.

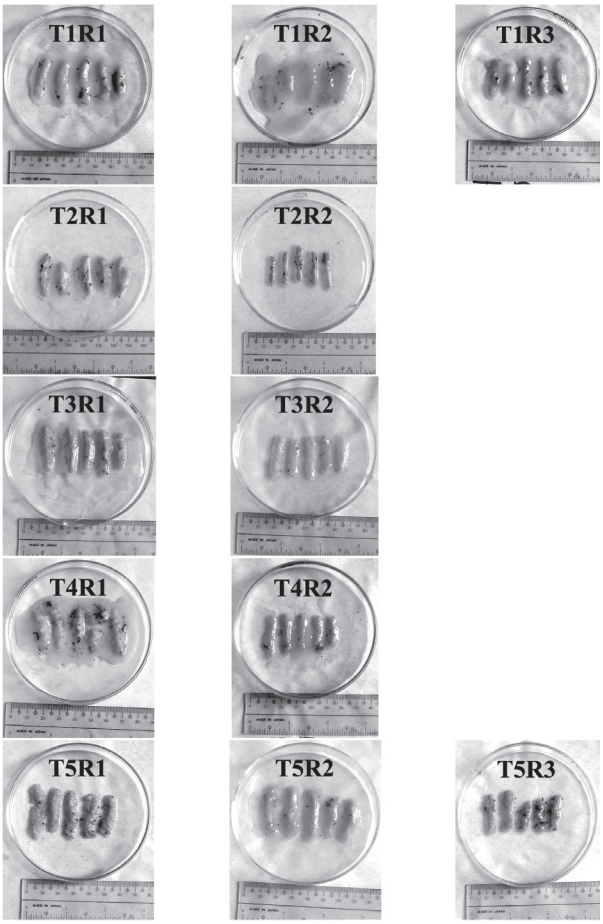

Figure 3. Samples of gonads of *T. gratilla* taken from the different treatments

revealed that the test animals did not have fully developed gonads. The probable reason is that the animals were observed to have frequently spawned prior from the second MCP until the end of the study. Garvida and Asia (2004) also noted that *T. gratilla* reached maturity and spawned at about 45 days culture fed with different species of *Sargassum* reared in cages placed in land-based concrete tanks.

The data showed that Treatments I, IV and V had a significantly ($p = 0.05$) higher GSI of 4.80%, 4.59% and 4.49%, respectively than Treatments III and II with 3.29% and 2.24%, respectively. Treatments II and III did not differ significantly. Asia et al. (2006) noted a mean gonad index ranging from 3.44% to 5.77% for animals which are also grown *in vitro* conditions and fed with prepared diets. Bangi (2001), in her study on the cage culture of the same species under sea-based conditions, observed a mean gonad index ranging from 2.70 % to 5.16 % for the low *Sargassum* diet and from 5.38 % to 8.14 % at the end of the six month culture period. Garvida and Asia (2004) also observed an average GSI of the animal reared for 75 days in land-based cages placed in concrete tanks ranging from 5.60 to 7.47 %.

The above results also indicate that the test animals fed the 4 to 5 % BW day^{-1} prepared diets had comparable average GSI with those fed fresh *Sargassum sp.* In the experiments of Pearce et al. (2002a; 2002b; 2002c; 2003) on *S. droebachiensis*, the animals fed with prepared diets had significantly higher percent gonad yields than those fed with fresh kelp. Olave et al. (2001) also observed that *Loxechinus albus* fed artificial diet had higher gonad production than those given macroalgae.

Gonad quality was measured by determining the gonad color of the test animals. For gonad color, higher values indicate brighter yellow colors which are considered of better quality for the gonads. In the Munsell Colour Chart, the value of 8 has the brightest yellow and becomes pale yellow or yellow brown as the value decreases to 1. Results show that Treatments I, IV and V with values of 7.67, 7.50 and 7.00, respectively, significantly had brighter yellow color ($p = 0.05$) than Treatments II and III. Treatments II and III with values of 4.00 and 4.50, respectively, did not differ significantly. In the studies of Pearce et al. (2002b; 2002c; 2003) on *S. droebachiensis*, animals fed prepared diets and fresh kelp had comparable gonad colors while their experiment in 2004b, gonad color was significantly better in urchins fed prepared diets than those given with kelp.

Water Parameters

The water parameters believed to have influences on the growth of the test animals such as dissolved oxygen, water temperature, salinity and pH were monitored during the course of the study (Table 6). The dissolved oxygen ranged from 2.42 to 5.98 mg L^{-1}

throughout the culture period with an average of 4.16 mg L^{-1} while the temperature ranged from 27.40 to 29.30 °C with an average of 28.89 °C. Salinity and pH did not appreciably change throughout the duration of the study at 35.00 ppt and 7.5, respectively. The observed measurements of said parameters are within the favorable range for the growth and survival of the test animals.

Table 6. Water parameters monitored in the culture of the sea urchin *T. gratilla* for four months culture period.

	Months After Stocking				
	Initial (April)	1 (May)	2 (June)	3 (July)	4 (August)
Dissolved Oxygen (mg L^{-1})					
Range	4.57 – 5.98	3.34 – 4.69	3.41 – 4.75	2.63 – 4.71	2.42 – 4.30
Mean	5.15	3.98	4.14	3.95	3.58
Water Temperature (^{0}C)					
Range	27.40-27.80	28.80-29.30	28.70-29.20	28.80-29.40	28.80-29.30
Mean	27.67	29.73	28.97	29.07	29.03
Salinity (ppt)					
Range	-	-	-	-	-
Mean	35.0	35.0	35.0	35.0	35.0
pH					
Range	-	-	-	-	-
Mean	7.5	7.5	7.5	7.5	7.5

Correlation Analyses

In order to determine the degree of relationships that exist among the somatic and gonad growth and quality and the various water parameters, a correlation analysis was done as shown in Table 7. Results showed a significantly ($p = 0.01$) high positive correlation between wet weight and equatorial and polar test diameters. Similarly, Asia et al. (2006) noted the same observations. Hagen (1998) also observed that gonad size increases with increasing test diameter in his study on *S. droebachiensis* from different habitats.

Equatorial test diameter had a significant ($p = 0.01$) positive correlation with polar test diameter. Dissolved oxygen had a significant ($p = 0.04$) negative correlation with temperature.

Table 7. Correlation analysis of the different somatic and gonad growth and quality with the various water parameters.

	Pearson Correlation Coefficients, N = 9 Prob > |r| under H0: Rho=0						
	Wwt	Etd	Ptd	GSI	Color	DO	Temp
Wwt	1.00000	0.91824	0.70544	0.41096	0.56580	-0.14498	0.17882
		<.0001	0.0104	0.1845	0.0552	0.6530	0.5782
Etd	0.91824	1.00000	0.69320	0.48050	0.49651	-0.25570	0.04505
	<.0001		0.0124	0.1138	0.1006	0.4225	0.8894
Ptd	0.70544	0.69320	1.00000	0.46579	0.38662	-0.17140	-0.01628
	0.0104	0.0124		0.1270	0.2144	0.5943	0.9600
GSI	0.41096	0.48050	0.46579	1.00000	0.87692	0.30202	0.25024
	0.1845	0.1138	0.1270		0.0002	0.3400	0.4328
Color	0.56580	0.49651	0.38662	0.87692	1.00000	0.34009	0.49575
	0.0552	0.1006	0.2144	0.0002		0.2794	0.1012
DO	-0.14498	-0.25570	-0.17140	0.30202	0.34009	1.00000	-0.03587
	0.6530	0.4225	0.5943	0.3400	0.2794		0.9119
Temp	0.17882	0.04505	-0.01628	0.25024	0.49575	-0.03587	1.00000
	0.5782	0.8894	0.9600	0.4328	0.1012	0.9119	

Legend: Wwt = Wet weight, g; GSI = Gonadosomatic Index, %; Etd = Equatorial test diameter, mm; Color = Gonad color; Ptd = Polar test diameter, mm; DO = Dissolved oxygen, mg/L; Temp = Water temperature, ^{o}C

Conclusion

Results of the study suggest that the best ration for prepared feed with *Sargassum sp.* as feed base would be at 4.00-5.00 % BW day^{-1} to optimize somatic growth and gonad production and quality. At this feed ration, both the somatic growth and gonad production and quality performances of the animal are comparable with the fresh *Sargassum sp*. Further, the study had shown the viability of growing *T. gratilla* in a land-based facility using both the fresh natural food and prepared diets. Such information is very important in future research studies regarding the use of prepared diets for *T. gratilla* and in the development of the grow-out culture of the animal both in land-based and on-site conditions.

Recommendations

The following recommendations are suggested: (1) The same study should be performed for different feed formulations other than *Sargassum sp*. as feed base in order to determine optimum feed requirement and proper nutrition of the animal; (2) The use of gonad color enhancers such as carotenoids or locally available pigment sources such as tomato, squash and the like in the diet of the animal necessitates further studies on optimizing feed requirements for better results. Further refinements in the feed formulation are necessary to optimize both gonad color, granularity, firmness and taste; and (3) Further research will be required at a commercial scale to determine if the recommended level as a result of this study is still optimal when urchin numbers and densities are increased.

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