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Cultivating Microalgae With Pickled Cabbage Wastewater: A Sustainable Alternative to Replacing Fishmeal in Sea Urchin (*Tripneustes gratilla*) Diets

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ABSTRACT

Recent studies have suggested that plant-based proteins, such as algal powder, can partially replace fishmeal used in formulated aqua feeds while maintaining the growth performance of aquaculture species. In Taiwan, the traditional pickled cabbage industry produces high-salinity wastewater rich in nutrients and microelements essential for plant growth. Reusing this nutrient-rich brine as a medium for microalgae cultivation offers a cost-effective and sustainable solution. This study aimed to evaluate the feasibility of using Taiwanese pickled cabbage wastewater (TPCW) to cultivate the halotolerant microalgae (*Dunaliella salina*), and of partially replacing fishmeal in *Tripneustes gratilla* (TG) feed with the resulting algal powder. The experiment consisted of two phases: (1) cultivation of *D. salina* using seawater partially substituted with varying proportions of TPCW and (2) assessment of the effects of replacing fishmeal with different levels of algal powder on the growth, survival, and gonad index of *T. gratilla* (0%: TG0, 30%: TG30, 35%: TG35, 40%: TG40, 45%: TG45, and 50%: TG50). Results showed that TPCW significantly enhanced the growth of *D. salina*, with the TPCW100 group (100% wastewater) displaying the highest growth rate, yielding 10.07 g of algal powder with 59.6% protein. Besides, a notable CO₂ decline in late culture stages indicated strong carbon uptake. For *T. gratilla*, the TG0 group exhibited the lowest weight gain and gonad index among all treatments, while TG40 showed the highest, significantly exceeding TG0 and TG30 ($p < 0.05$). Moreover, higher replacement levels of fishmeal with algal powder also increased gonad redness and yellowness, suggesting greater accumulation of pigments such as astaxanthin and carotenoids. Overall, this study confirms that agricultural waste can be effectively recycled for algal cultivation, contributing to carbon reduction, and that algal protein can successfully replace fishmeal in sea urchin feed, enhancing both growth and gonad coloration.

1 | Introduction

Tripneustes gratilla (TG), commonly known as the collector sea urchin, is an ecologically important species distributed throughout the Indo-Pacific. It plays a key role in seagrass beds and coral reefs by contributing to biomass cycling and maintaining habitat

health [1, 2]. As a major herbivore, *T. gratilla* grazes on macroalgae, preventing overgrowth that could smother seagrass habitats, thereby promoting biodiversity and supporting overall ecosystem function [2, 3]. The aquaculture of *T. gratilla* is receiving increasing attention due to rising global seafood demand and

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the decline of wild populations from overfishing and habitat loss [4, 5]. Its high market value and ecological importance highlight the need for sustainable aquaculture practices.

With the rapid expansion of global aquaculture, including sea urchin farming, the demand for formulated aquafeeds has increased significantly. A recent review study reported that ~87% of global fishmeal and 74% of global fish oil production are directed to aquaculture [6]. However, the overall global production of fishmeal and fish oil has shown a declining trend over the past two decades [7]. In response to these challenges, developing novel feed ingredients to replace fishmeal has emerged as a major priority for sustainable aquaculture. Various animal (e.g., meat and bone meal, poultry meal, and blood meal) and plant protein sources (e.g., legumes, oilseeds, algae, and cereal proteins) are abundant, cost-effective, and stable in supply and have thus been widely used in aquafeeds as alternatives to fishmeal [7]. However, these alternatives have several limitations. Animal by-products often have unbalanced amino acid profiles and high saturated fatty acid levels, requiring careful formulation. Plant-based proteins may contain antinutritional factors, low palatability, and high fiber content. Prolonged use can impair nutrient absorption and utilization in aquatic animals, ultimately reducing the flavor quality of the final products [8].

Recent studies on the culture of sea urchins have focused on optimizing dietary formulations to enhance growth, reproductive performance, and nutritional quality [4, 5, 9–12]. A key focus has been the inclusion of macroalgae, microalgae, and algal powders in formulated feeds to replace fishmeal and fish oil. These plant-based protein sources not only provide essential nutrients but have also shown potential to promote better growth performance and gonadal development in sea urchins. For example, some research has demonstrated that the incorporation of specific algae and algal powders, such as *Ulva* (green macroalgae), in the diets of *T. gratilla* can significantly improve the palatability and digestive efficiency of these diets and enhance the growth rates and gonadal development of *T. gratilla* [9, 10]. Moreover, a previous study indicated that some microalgae, including *Isochrysis* sp., *Chaetoceros muelieri*, and *Skeletonema pseudocostatum* are suitable feeds for rearing *T. gratilla* larvae [13]. However, limited research has examined the effects of incorporating microalgae or microalgal powders into formulated feeds as a replacement for fishmeal on the growth and gonadal development of *T. gratilla*.

Microalgae, a major component of marine phytoplankton, contribute to over 40% of global carbon fixation through photosynthesis each year [14, 15], with carbon sequestration rates estimated to be four times higher than those of tropical rainforests [16]. Microalgae have also been applied to reduce carbon emissions from fossil fuel power plants [17–19]. In addition to their environmental benefits, microalgae such as *Arthrospira maxima*, *Arthrospira platensis*, *Chlorella pyrenoidosa*, and *Spirogyra* spp. contain protein levels comparable to fishmeal [20–22]. Moreover, the protein and fatty acid contents of microalgae vary with culture conditions; for instance, salinity significantly affects the fatty acid profile of halotolerant *Dunaliella salina* [23]. Furthermore, microalgae offer key advantages in aquaculture due to their minimal land requirements and high adaptability.

To date, over 40 microalgal species have been mass-cultivated for aquaculture applications, including larval rearing of finfish,

crustaceans, and mollusks; live feed enrichment; and as ingredients or additives in formulated aquafeeds [24–30]. Among these, *D. salina* has attracted considerable attention due to its high nutritional value and bioactive compounds. Studies have shown that salinity and light stresses enhance the accumulation of β -carotene in *D. salina* [31–33]. β -Carotene has been reported to exhibit immunomodulatory [34], antioxidant [35, 36], antimutagenic [37], and antitumor [38] properties.

Taiwanese pickled cabbage is a distinctive local industry in Dapi Township, Yunlin County, Taiwan, with ~1500 hectares of farmland annually dedicated to cultivating mustard greens for processing. The production of pickled cabbage generates a large amount of high-salinity acidic wastewater, called Taiwanese pickled cabbage wastewater (TPCW), which can lead to soil acidification/salinization if discarded indiscriminately [39]. Moreover, substantial financial resources are required for the treatment and disposal of TPCW. Proper treatment and utilization of TPCW could significantly reduce disposal costs and potentially enable its commercialization, creating additional economic value. Compositional analyses of TPCW have shown that it contains organic acids and inorganic salts (Table 1), which may be beneficial for the growth of algae. As a result, this study aimed to develop a cultivation technique for the *D. salina* using TPCW as a culture medium. We evaluate the growth performance and nutritional composition of the resulting

TABLE 1 | The salinity, biochemical oxygen demand, pH, nutrient salt (ion) composition, and organic acids of Taiwanese pickled cabbage wastewater (TPCW).

Name of nutrient salt (ion)	Concentration	Unit
Salinity	266	Permillage (%)
Biochemical oxygen demand	0.12	ppm (mg/L)
pH	4.6	—
Na ⁺	79,046.31	ppm (mg/L)
K ⁺	2213.64	ppm (mg/L)
Mg ²⁺	259.61	ppm (mg/L)
NH ₄ ⁺	0	ppm (mg/L)
Li ⁺	45.42	ppm (mg/L)
Ca ²⁺	40.83	ppm (mg/L)
Cl [−]	123,754.85	ppm (mg/L)
SO ₄ ^{2−}	701.40	ppm (mg/L)
NO ₃ [−]	183.40	ppm (mg/L)
PO ₄ ^{3−}	153.88	ppm (mg/L)
NO ₂ [−]	84.00	ppm (mg/L)
Br [−]	20.48	ppm (mg/L)
Lactic acid	30.70	Percentage (%)
Malic acid	15.58	Percentage (%)
Acetic acid	13.68	Percentage (%)
Citrate acid	6.35	Percentage (%)
Succinic acid	1.23	Percentage (%)
Tartaric acid	0.14	Percentage (%)

algal biomass and further assess the feasibility of using the resulting *D. salina* powder (rich in algal protein) as a partial replacement for fishmeal in the formulated diets of *T. gratilla*.

2 | Materials and Methods

2.1 | Experiment on the Cultivation of *D. salina* Using TPCW

The original TPCW was obtained from the Taiwanese pickled cabbage production park in Dapi Township, Yunlin County. The salinity, biochemical oxygen demand, pH, nutrient salt (ion) composition, and organic acids of TPCW are shown in Table 1. Before conducting the analysis of cations and anions, the TPCW was prediluted 1000-fold to ensure that analyte concentrations remained within the instrument's working range and to prevent potential inaccuracies in measurement. The concentrations of sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), ammonium (NH_4^+), lithium (Li^+), calcium (Ca^{2+}), chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), phosphate (PO_4^{3-}), nitrite (NO_2^-), and bromide (Br^-) in the diluted TPCW were measured using ion chromatography (Eco IC, Metrohm, Herisau, Switzerland) [40, 41]. Ion concentrations were calculated using calibration curves established for each standard solution, and the resulting values were multiplied by 1000 to determine the original ion concentrations in the original TPCW. The contents of lactic acid, acetic acid, and malic acid were determined using a high-performance liquid chromatography analyzer (Hitachi 7200 HPLC). The TPCW was filtered through a sieve with a pore size of 45 μm to remove solid matter, diluted to 3.4% with deionized water, adjusted to pH 7 using calcium carbonate, and sterilized in an autoclave before use.

In this experiment, TPCW was used to replace seawater at substitution levels of 0% (TPCW 0), 10% (TPCW 10), 20% (TPCW 20), 40% (TPCW 40), 80% (TPCW 80), and 100% (TPCW 100), resulting in six treatment groups. An additional group using pure seawater without algae was included as a blank control. Each treatment had six replicates, totaling 42 algae culture barrels in the experiment. The water nutrient composition and F2 medium level for each TPCW/seawater substitution level were shown in Appendix A.

D. salina was obtained from the Tungkang Aquaculture Research Center, Ministry of Agriculture, Taiwan. Prior to cultivation, 2.5 mL of f/2 medium was added per liter of culture water. *D. salina* was grown in a 120 L closed cylindrical photoreactor (diameter: 0.5 m; height: 1.7 m) containing 100 L of culture water. The initial optical density at 680 nm (OD_{680}) was 0.10 ± 0.01 , corresponding to a cell dry weight of ~ 0.007 g/L. Cultivation was conducted at $25 \pm 1^\circ\text{C}$ under a 12 h light:12 h dark photoperiod, with a light intensity of $92 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a fixed CO_2 input (5715–5801 ppm) from the bottom. The culture period was 14 days, with samples collected every 2 days to monitor the growth of microalgal cells using a UV-2450 spectrophotometer (OD_{680}). CO_2 concentrations were recorded using a GC2028 CO_2 detector.

2.2 | Crude Protein Content and Growth Analysis of Microalgal Cell

The crude protein content of microalgal cells was determined using the AOAC [42] analytical method and was calculated as following:

$$\text{Crude protein (\%)} = 14.007 \times 5.95 \times ((b - a)/S) \times 100,$$

a: volume of blank titration (mL); b: volume of sample titration (mL); S: sample weight (g); 14.007: molecular weight of nitrogen; 5.95: protein conversion factor of *D. salina*.

The method for determining microalgal cell growth involved a cellulose acetate membrane (Whatman 0.45 μm), which was pre-weighed ($W1$). A 10 mL water sample (V) containing *D. salina* was filtered through the membrane, which was then dried at 105°C until a constant weight ($W2$) was obtained. The dry weight concentration of *D. salina* (DW, g/L) was calculated using the following formula:

$$\text{DW (g/L)} = \frac{(W2 - W1)}{V}.$$

2.3 | Evaluating the Effects of *D. salina* Powder as a Partial Fishmeal Substitute on the Growth and Gonadal Development of *T. gratilla*

2.3.1 | Experimental Organism

Healthy sea urchins (*T. gratilla*) without apparent injuries were selected for this experiment. Sea urchins used in this experiment with an initial average weight of 31.10–31.13 g and a shell diameter of 28.4–28.6 mm were originally cultured in outdoor ponds and transferred to indoor aquaria before the experiment.

2.3.2 | Experimental Design

Experimental design was shown in Appendix B. The required protein and lipid levels in the experimental feed were determined based on the research of Agnette et al. [43]. Accordingly, the protein and lipid contents were set at 32% and 10%, respectively. A preliminary experiment was conducted before the main experiment in this study. This preliminary experiment included one control group (0% replacement) and five treatment groups in which *D. salina* powder replaced 10%, 15%, 20%, 25%, and 30% of fishmeal. During the acclimatization period, sea urchins were fed the diet (0% replacement). At the start of experiments, sea urchins were starved for 2 days and an average initial body weight of 30.2 g, shell diameter of 28.3 mm of sea urchins were randomly distributed into 18 fiberglass aquaria (56 cm $\times$ 56 cm $\times$ 30 cm) with 10 sea urchins in each aquarium. Triplicate groups of sea urchins were fed an experimental diet by satiation at 09:00 h by hand for 8 weeks. Feeding was stopped 2 days before weighing, and sea urchins were individually weighed at the start and end of the feeding trial. The experiment was conducted in a recirculating aquaculture system. During the feeding period, water temperature and salinity ranged from 28°C to 32°C and 3.0‰ to 3.3‰, respectively. After the feeding trial, the result indicated that the control group showed a weight gain rate of 62.3%, which was significantly lower than those of all treatment groups. The treatment groups exhibited weight gain rates of 104.7%–104.9% and feed efficiencies of 1.2–1.3. No significant differences in growth efficiency were observed among the treatment groups.

Based on the preliminary results, diets of six experimental groups for the main experiment were formulated: one control group (TG0, 0% replacement) and five treatment groups in which 30% (TG30), 35% (TG35), 40% (TG40), 45% (TG45), and 50% (TG50) of

fishmeal were replaced with *D. salina* powder. All feed ingredients were premixed and combined with 30% deionized water, then pelletized using a feed extruder into 0.5 cm granules. Pellets were dried at 30°C to a final moisture content of ~7% and stored at -20°C until use. The ingredient composition and major amino acid composition of each diet were presented in Tables 2 and 3, respectively.

Each group of this experiment was replicated three times, resulting in a total of 18 aquaria, which were integrated into an indoor recirculating aquaculture system. Each aquarium (45 cm × 30 cm × 30 cm, 40 L volume) contained 10 individuals. The culture water was filtered through a filter bag, then treated with an ozone

generator and an immersed biological filter tank to maintain optimal water quality. Salinity was maintained at 3.4‰, and water temperature ranged from 24 to 27°C. A photoperiod of 12 h light and 12 h dark was applied. Experimental organisms were fed to apparent satiation one time (09:00 h) per day by hand, and the bottom of each aquarium was siphoned at 16:30 to remove uneaten feed and waste. The amount of feed consumed by the sea urchin in each tank was recorded weekly. The experimental period lasted 180 days. All animal experiments in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of National Chiayi University (NCYU) (Approval Number NCYU-IACUC-11101). All procedures were performed in accordance with the NCYU guide for the care and use of laboratory animals. Efforts were made to minimize the number of animals used and their suffering.

TABLE 2 | Ingredient composition of experimental diets for sea urchins (*T. gratilla*).

Ingredient (%)	Treatments					
	TG0	TG30	TG35	TG40	TG45	TG50
Algae powder ^a	0	16.6	19.0	21.5	24.5	27.2
Fishmeal ^b	47.2	33.0	30.7	28.5	25.9	23.6
α-starch	15	15	15	15	15	15
Gelatin	5	5	5	5	5	5
Agar	5	5	5	5	5	5
Fish oil	5.9	4.8	3.7	2.5	2.0	1.5
Yeast ^c	1	1	1	1	1	1
Vitamin mix ^d	1	1	1	1	1	1
Mineral mix ^e	0.2	0.2	0.2	0.2	0.2	0.2
MgCl ₂	2.75	2.75	2.75	2.75	2.75	2.75
CaH ₂ PO ₂	2	2	2	2	2	2
Cellulose	15.0	15.4	15.8	16.0	16.3	16.5
Analyzed composition (as fed)						
Moisture	7.56	7.54	7.45	7.44	7.44	7.56
Crude protein ^f	32.10	32.20	32.10	32.00	32.10	32.10
Crude fat ^f	10.14	10.13	10.13	10.15	10.13	10.14
Ash ^f	9.51	9.46	9.50	9.45	9.56	9.54
Crude fiber ^f	16.15	16.04	16.57	17.12	17.56	17.51
β-carotene ^g	0.29	1.14	1.34	1.52	1.73	1.95

^aAlgae powder: *D. salina*.

^bLipid-extracted fishmeal (CP 68%, Liandong Industry Co., Ltd., Taiwan).

^cYeast (Sun Right foods Co., Ltd., Taiwan).

^dMineral mix ([44] modified; calcium phosphate 0.0735%, potassium phosphate 0.0081%, potassium sulfate 0.0068%, sodium chloride 0.0031%, calcium carbonate 0.0021%, sodium phosphate 0.0022%, magnesium oxide 0.0025%, trace element mix 0.0018%; trace element mix: ferric citrate 0.0031%, zinc carbonate 0.0005%, manganese carbonate 0.0024%, copper carbonate 0.0002%, potassium iodide 0.0005%, citric acid 0.0039%).

^eVitamin mix ([45] modified; vitamin D3 0.001%, vitamin A 0.06%, α-tocopheryl acetate 0.45%, vitamin K3 0.4%, thiamine-HCl 0.5%, riboflavin 0.5%, calcium pantothenate 1%, niacin 2%, biotin 0.4%, pyridoxine-HCl 0.06%, folic acid 0.15%, B12 0.001%, Inositol 20%, ascorbic-monophosphate-Mg 2.5%, choline chloride 40%, α-cellulose 31.978%) 1%.

^fExpressed as percent dry weight.

^gmg/100 g.

2.3.3 | Growth Analysis

At the end of the feeding trial, all individuals were collected from each aquarium for measurement of body weight, shell diameter, and gonad weight. Gonads were also excised for color analysis. The growth parameters of sea urchins were calculated as follows:

$$\text{Weight gain (WG, \%)} = 100 \times (\text{Wt} - \text{W}_0) / \text{W}_0.$$

TABLE 3 | Major amino acid composition (%) of diets.

Amino acid	Treatments					
	TG0	TG30	TG35	TG40	TG45	TG50
Arginine	2.3	3.4	3.6	3.8	3.9	4.1
Histidine	1.2	1.4	1.5	1.5	1.6	1.6
Isoleucine	2.4	2.8	2.8	2.9	2.9	3.0
Leucine	3.9	5.7	6.0	6.3	6.6	6.9
Lysine	3.6	4.6	4.7	4.9	5.0	5.2
Methionine	1.4	1.0	0.9	0.8	0.8	0.7
Phenylalanine	2.1	3.4	3.6	3.8	4.0	4.3
Threonine	1.9	3.0	3.2	3.3	3.5	3.7
Valine	2.7	3.4	3.5	3.6	3.7	3.8
Tryptophan	0.4	0.3	0.3	0.3	0.2	0.2
EAA	22.0	28.9	30.0	31.2	32.3	33.5
Tyrosine	1.5	2.3	2.4	2.6	2.7	2.8
Alanine	2.1	4.3	4.6	5.0	5.4	5.7
Aspartate	4.0	6.1	6.4	6.8	7.1	7.4
Cystine	0.1	0.2	0.3	0.3	0.3	0.3
Glutamate	8.6	10.3	10.6	10.8	11.1	11.4
Glycine	2.1	3.3	3.5	3.7	3.9	4.1
Proline	3.3	2.9	2.9	2.8	2.8	2.7
Serine	2.2	3.2	3.3	3.5	3.7	3.8
TAA	23.9	32.6	34.1	35.5	37.0	38.4

Note: n = 1.

Abbreviations: EAA, essential amino acid; TAA, total amino acid.

Specific growth rate (SGR, % day⁻¹) = 100 × [(ln Wt – ln W₀) / t],

where W₀ is the initial average body weight (g), Wt is the final average body weight (g), and t (days) is the feeding period.

Feed conversion ratio (FCR) = Feed intake (g) / weight gain (g),

Diameter gain (DG, %) = [(Final diameter – initial diameter) / initial diameter] × 100,

Gonad somatic index (GSI, %) = Gonad wet weight / body weight × 100.

2.3.4 | General Component Analysis

The general component analysis of diets and sea urchin gonads was performed according to the AOAC [42] analytical method. Crude protein content was determined after acid digestion using the Kjeldahl System 1002 (Tecator, Sweden). Crude lipid was measured using the chloroform–methanol extraction method (2:1, v/v) according to Folch et al. [46]. Crude fiber content was analyzed using the Fibertec System M1020 (FOSS Tecator, Sweden) following sequential acid and alkali digestion. Moisture content was determined by drying at 105°C for 24 h, and ash content was measured using a muffle furnace at 540°C for 6 h.

2.3.5 | Determination of Gonad Color

Gonad colors were measured using a method described by Baião et al. [47]. The gonads of the various treatment groups were first photographed. Then, the lightness (*L**), redness (*a**), and yellowness (*b**) were analyzed using a chromameter (Minolta CR-10 handheld chromameter) to evaluate the gonad color of sea urchins. *L** represents brightness, indicating the permeability of light to the gonad. Higher permeability of light corresponds to a higher *L** value. A positive *a** value indicates a red hue, while a negative value indicates green. Higher *a** values correspond to increased redness, whereas lower values indicate increased greenness. A positive *b** value indicates a yellow hue, while a negative value indicates blue. Higher *b** values correspond to increased yellowness, whereas lower values indicate increased blueness. The color parameters are as follows:

Overall color difference value $\Delta E = \sqrt{(a^* - a^{*0})^2 + (b^* - b^{*0})^2 + (L^* - L^{*0})^2}$,

Red color difference value $\Delta E_a = \sqrt{(a^* - a^{*0})^2}$,

Yellow color difference value $\Delta E_b = \sqrt{(b^* - b^{*0})^2}$,

where *a**⁰, *b**⁰, and *L**⁰ are the *a**, *b**, and *L** values of control group.

2.3.6 | Carotenoid Analysis

Carotenoid contents were measured using a method described by Okada et al. [48]. Gonad samples were rinsed with 0.45 μm-filtered sterilized seawater, frozen at –80°C for 2–3 days, and then freeze-dried. A 0.1 g sample was placed in a test tube, mixed with 2 mL of

acetone, and homogenized for 1 min. The mixture was then left to stand for 30 min, followed by the addition of 2.4 mL of n-hexane and 5% sodium chloride. Samples were centrifuged at 5000 rpm for ~10 min, and the upper pigment-containing phase was collected and stored at –20°C for spectrophotometric analysis.

An equal volume of the pigment-containing supernatant was transferred into each colorimetric tube. n-Hexane was used as the blank, and absorbance was measured at 450 nm to determine β-carotene content. The concentration was calculated using the following formula:

$$\frac{\text{mg carotenoid}}{100 \text{ g tissue}} = \frac{A \times V \times 1000}{E \times W},$$

where *A* = absorbance at 450 nm, *V* = volume of extract (mL), *E* = extinction coefficient of β-carotene in n-hexane (2500), and *W* = weight of tissue sample (g).

2.3.7 | Analyses of Amino Acid

The amino acid compositions of the experimental diets were performed in accordance with the modified procedures described by Chu and Huang [45]. Each sample added 1 mL of 6-N HCl solution containing 1% phenol after freeze-dried and degreased. The following procedure involved removing oxygen, followed by hydrolysis with 6 M hydrochloric acid, heating at 105°C for 24 h, filtration through a 0.22 μm filter, reaction with ophthalaldehyde, and analysis using HPLC (Hitachi L-8900). The analysis equipment column was Gemini 5u NX-C18. Mobile phase A used a mixture of phosphoric acid, tetrahydrofuran, and methanol, and mobile phase B used a mixture of methanol and water. The flow rate was set at 1 mL/min, and the excitation and emission wavelengths were set at 340 nm and 455 nm, respectively.

2.4 | Statistical Analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) with the statistical software SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) to assess significant differences among treatments. If significant differences were found (*p* < 0.05), Duncan's new multiple-range test was used for post hoc comparisons among different groups. To determine the optimal substitution level of *D. salina* in the formulated feed, a quadratic regression analysis was performed using the relationship between the *D. salina* substitution percentage and the weight gain rate of *T. gratilla* (SAS software, version 9.4). The regression model was fitted using least squares estimation, and the substitution level corresponding to the maximum predicted weight gain rate was identified as the optimal value.

3 | Results

3.1 | Cultivation of *D. salina* Using TPCW

The results showed that TPCW had a positive effect on the growth of *D. salina* (Figure 1). In the TPCW100 group, where seawater was fully replaced by TPCW, the algal concentration (OD₆₈₀) reached a peak of 0.215 after 14 days, which was significantly higher than in the other treatment groups (Figure 1). This corresponded to a dry weight of 10.07 g/L. In contrast, the

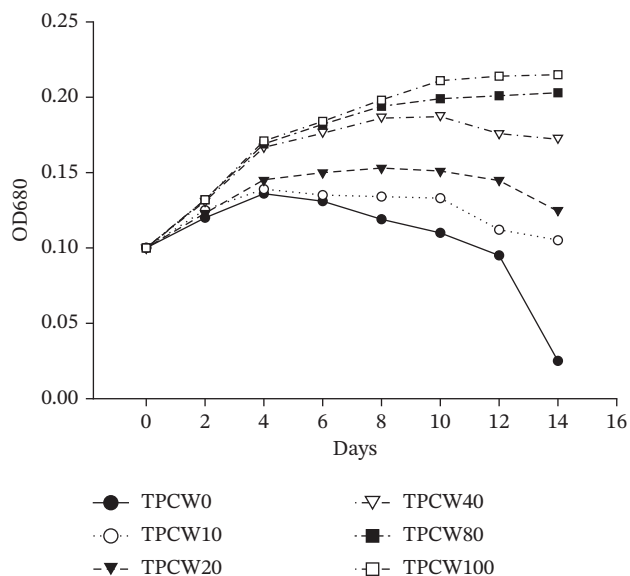


FIGURE 1 | Growth of *D. salina* cultivated in different ratios of TPCW substitution for seawater for 14 days ($n=3$).

TPCW0 group (with full seawater) exhibited the lowest algal concentration, with an OD₆₈₀ of 0.025 at the end of the 14-day culture period.

Changes in CO₂ concentration within the cylindrical photoreactor are shown in Figure 2. Initial CO₂ concentrations across all groups ranged from 5715 to 5801 ppm. As the culture progressed, CO₂ concentrations declined in all groups except the control. In the control group, CO₂ levels decreased slightly from 5801 ppm on to Day 1 5756 ppm on Day 14, with no significant change observed. In contrast, the TPCW80 and TPCW100 groups showed a significant decrease, reaching 1799 ppm and 1604 ppm, respectively (Figure 2), indicating substantially higher CO₂ absorption efficiency compared to the other treatments. In the TPCW0 group, CO₂ concentration decreased from 5732 to 3831 ppm during the first 4 days, followed by a slight increase to 4124 ppm between Day 4 and Day 14 (Figure 2).

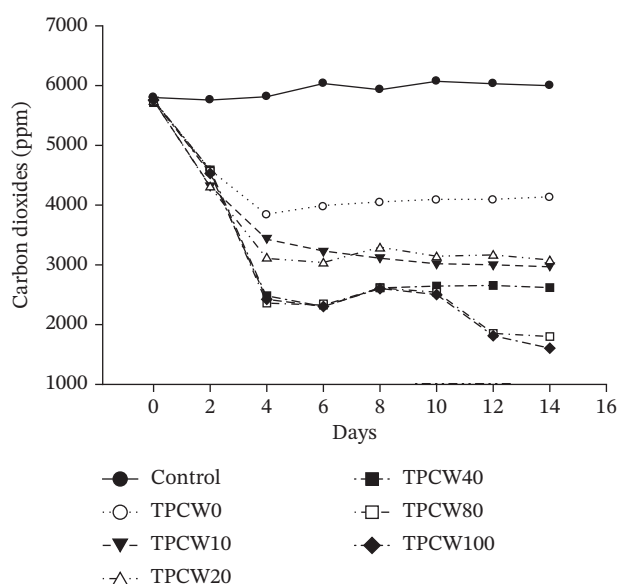


FIGURE 2 | Changes of carbon dioxide levels during the 14-day cultivation period of *D. salina* ($n=3$).

At the end of cultivation, the crude protein content of *D. salina* was shown in Figure 3. Protein content increased with rising TPCW concentrations. The TPCW100 group exhibited the highest protein content at 59.6%, which was significantly greater than that of the other groups (Figure 3).

3.2 | Effects of *D. salina* Powder as a Partial Fishmeal Substitute on the Growth and Gonadal Development of *T. gratilla*

Following the general component analysis, the composition of each diet was presented in Table 3. The moisture content of the feed ranged from 7.44% to 7.56%, and the ash content from 9.46% to 9.56%. Crude protein content was between 32.1% and 32.2%, crude fat between 10.13% and 10.14%, and crude fiber ranged from 16.04% to 17.56%. The amino acid composition of the feed was shown in Table 3. Regarding essential amino acids (EAAs), the contents of methionine and tryptophan decreased with increasing levels of *D. salina* powder substitution. In contrast, the levels of other EAAs increased. Both total EAAs and total AAs (TAAs) increased as the proportion of *D. salina* powder substitution rose.

The results for final weight, weight gain rate, feed conversion ratio, feed intake, specific growth rate, and protein utilization rate were presented in Table 4. The final weight and weight gain rates of the TG0 treatment group were 51.6 g and 65.8%, respectively, indicating the poorest growth. In comparison, the TG40 group exhibited a final weight of 104.3 g and a weight gain rate of 235.3%, both significantly higher than those of the other treatment groups ($p < 0.05$), except for the TG45 group. The feed intake of the TG0 treatment group was 34.7 g, the lowest among the groups. Feed intake increased with the substitution amount of *D. salina* powder. The TG50 group had the highest feed intake, which was 74.3 g, significantly higher than the TG0, TG30, and TG35 groups ($p < 0.05$). However, there was no significant difference in feed intake among the TG40, TG45, and TG50 groups ($p > 0.05$). In addition, there were no significant differences in feed conversion ratio and protein utilization rate between the TG40 and TG45

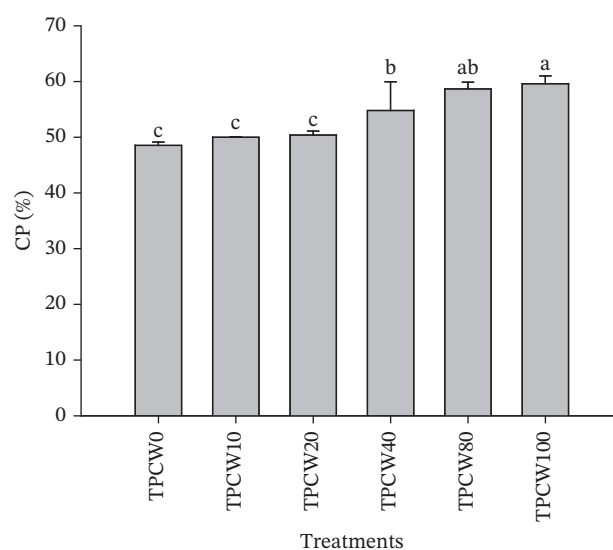


FIGURE 3 | Crude protein (CP) percentage content of *D. salina* after 14-day cultivation in different ratios of TPCW substitution for seawater. Data are expressed as means $\pm$ S.E. ($n=3$).

TABLE 4 | Final weight (FW, g), weight gain (WG, %), feed intake (FI, g), feed conversion ratio (FCR), specific growth rate (SGR, % day⁻¹), and protein efficiency ratio (PER, %) of sea urchins (*T. gratilla*) fed experimental diets.

Diet	FW	WG	FI	FCR	SGR	PER
TG0	51.6 ± 3.1 ^d	65.8 ± 9.4 ^d	34.7 ± 1.5 ^c	1.7 ± 0.2 ^a	0.9 ± 0.1 ^d	1.8 ± 0.3 ^d
TG30	63.9 ± 3.1 ^c	105.5 ± 10.4 ^c	46.0 ± 3.6 ^b	1.4 ± 0.1 ^b	1.3 ± 0.1 ^c	2.2 ± 0.1 ^c
TG35	77.53 ± 2.1 ^b	149.0 ± 6.6 ^a	52.0 ± 1.0 ^b	1.1 ± 0.1 ^c	1.6 ± 0.1 ^b	2.8 ± 0.1 ^b
TG40	104.3 ± 6.5 ^a	235.3 ± 22.1 ^a	72.3 ± 6.7 ^a	1.0 ± 0.1 ^c	2.2 ± 0.1 ^a	3.2 ± 0.1 ^a
TG45	100.0 ± 1.0 ^a	221.5 ± 2.3 ^a	70.0 ± 2.0 ^a	1.0 ± 0.1 ^c	2.1 ± 0.1 ^a	3.1 ± 0.1 ^a
TG50	74.3 ± 4.3 ^b	138.6 ± 14.1 ^b	74.3 ± 5.9 ^a	1.7 ± 0.2 ^a	1.6 ± 0.1 ^b	1.8 ± 0.2 ^d

Note: Mean in the same column with the different superscript letters are significantly different ($p < 0.05$). Data are expressed as means ± S.E. ($n = 3$).

groups ($p > 0.05$); however, both values were significantly higher than those observed in the TG0, TG30, and TG50 groups ($p < 0.05$). The specific growth rates of the TG40 and TG45 groups of *T. gratilla* were 2.2% day⁻¹ and 2.1% day⁻¹, respectively. Although there was no significant difference between these two groups, both rates were significantly higher than those of the other treatment groups ($p < 0.05$). Among the remaining groups, TG35 and TG50 exhibited the next highest specific growth rate of 1.6% day⁻¹.

The results for initial shell diameter, final shell diameter, and shell diameter growth rate were presented in Table 5. The TG0 group showed significantly lower values than all other groups ($p < 0.05$), with a final shell diameter of 36.5 mm and a diameter gain of 27.4%. In the TG40 group, the final shell diameter and diameter gain were 40.4 mm and 41.2%, respectively, significantly higher than those of the TG0, TG30, and TG35 groups ($p < 0.05$), but not significantly different from the TG45 and TG50 groups ($p > 0.05$). No mortality was observed during the experiment.

The results of gonad color analysis after the experiment were presented in Table 6. There were no significant differences in L^* values among the groups, which ranged from 45.6 to 47.2. However, both a^* and b^* values increased with the substitution level of *D. salina*. The a^* value of the TG50 group was 1.7, significantly higher than those of the other treatment groups ($p < 0.05$), except for TG45 ($p > 0.05$), followed by TG40. In contrast, the TG0 group had the lowest a^* value at -0.6, which was significantly lower than those of all other groups ($p < 0.05$). Similarly, the b^* value of the TG50 group was 25.5, significantly higher than those of the other treatment groups ($p < 0.05$), followed by TG45. The TG0 group

exhibited the lowest b^* value at 18.0, which was also significantly lower than those of the other groups ($p < 0.05$).

The gonad composition of *T. gratilla* from each group was summarized in Table 7. Gonad moisture content decreased with increasing levels of *D. salina* substitution. The TG0 group had the highest moisture content at 70.63%, which was significantly higher than that of the other groups ($p < 0.05$). In contrast, crude protein and lipid contents increased with higher substitution levels. The TG50 group showed the highest crude protein and lipid contents at 36.97% and 19.56%, respectively, both significantly

TABLE 6 | Gonad integument $L^*a^*b^*$ color values of sea urchins (*T. gratilla*) fed experimental diets.

Diet	L^* (lightness)	a^* (redness)	b^* (yellowness)
TG0	45.6 ± 0.8	-0.6 ± 0.1 ^d	18.0 ± 0.4 ^e
TG30	46.1 ± 1.9	1.1 ± 0.1 ^c	19.6 ± 0.9 ^d
TG35	46.6 ± 1.7	1.1 ± 0.1 ^c	20.1 ± 0.1 ^d
TG40	46.8 ± 1.9	1.4 ± 0.2 ^b	21.7 ± 1.8 ^c
TG45	47.2 ± 0.3	1.6 ± 0.1 ^a	23.8 ± 0.3 ^b
TG50	47.2 ± 2.3	1.7 ± 0.1 ^a	25.5 ± 0.3 ^a

Note: Mean in the same column with the different letters are significantly different ($p < 0.05$). Data are expressed as means ± S.E. ($n = 3$). Letters a–e indicate groups with significant differences.

TABLE 7 | Proximate analysis (%) and β -carotene (mg/100 g) of gonads of sea urchins (*T. gratilla*) fed experimental diets.

Diet	Moisture	Crude protein	Crude lipid	β -carotene
TG0	70.63 ± 0.06 ^a	36.20 ± 0.10 ^d	18.19 ± 0.17 ^c	0.27 ± 0.01 ^f
TG30	70.09 ± 0.09 ^b	36.27 ± 0.21 ^{cd}	18.39 ± 0.16 ^c	1.11 ± 0.06 ^e
TG35	70.01 ± 0.11 ^{bc}	36.47 ± 0.06 ^c	18.63 ± 0.06 ^{bc}	1.33 ± 0.04 ^d
TG40	69.96 ± 0.06 ^{bc}	36.73 ± 0.12 ^b	19.05 ± 0.82 ^{ab}	1.52 ± 0.07 ^c
TG45	69.97 ± 0.07 ^{bc}	36.83 ± 0.06 ^{ab}	19.15 ± 0.14 ^{ab}	1.74 ± 0.03 ^b
TG50	69.92 ± 0.07 ^c	36.97 ± 0.12 ^a	19.56 ± 0.02 ^a	1.94 ± 0.05 ^a

Note: Mean in the same column with the different letters are significantly different ($p < 0.05$). Data are expressed as means ± S.E. ($n = 3$). Letters a–f indicate groups with significant differences. Groups sharing the same letter are not significantly different.

TABLE 5 | Initial diameter, final diameter, and diameter gain of sea urchins (*T. gratilla*) fed experimental diets.

Diet	Initial diameter (mm)	Final diameter (mm)	Diameter gain (%)
TG0	28.5 ± 0.1	36.5 ± 0.8 ^c	27.4 ± 3.0 ^c
TG30	28.4 ± 0.0	37.2 ± 1.3 ^{bc}	30.1 ± 4.4 ^{bc}
TG35	28.6 ± 0.1	38.5 ± 1.2 ^b	34.6 ± 4.1 ^b
TG40	28.6 ± 0.0	40.4 ± 0.7 ^a	41.2 ± 2.5 ^a
TG45	28.5 ± 0.1	40.3 ± 0.3 ^a	40.7 ± 0.9 ^a
TG50	28.5 ± 0.2	38.8 ± 0.7 ^{ab}	35.7 ± 2.6 ^{ab}

Note: Mean in the same column with the different superscript letters are significantly different ($p < 0.05$). Data are expressed as means ± S.E. ($n = 3$).

higher than those of the other groups ($p < 0.05$). Similarly, β -carotene content in the gonads increased progressively with substitution, reaching 0.27, 1.11, 1.33, 1.52, 1.74, and 1.94 mg/100 g across the treatment groups. The gonad somatic index results were shown in Figure 4. The TG40 group exhibited the highest gonad somatic index at 5.3%, significantly higher than those of the TG0 (2.9%) and TG30 (4.2%) groups ($p < 0.05$). Figure 5 displayed the optimal substitution level of *D. salina*, determined through quadratic regression analysis of weight gain rate versus substitution percentage. The optimal substitution level of *D. salina* in *T. gratilla* feed was estimated at 41.5%.

4 | Discussion

4.1 | Cultivation of *D. salina* in TPCW

To ensure the biosafety of the feed ingredients, analyses of pesticide residues and heavy metals were performed for the TPCW. No

pesticide residues were detected, and only trace amounts of lead, copper, barium, arsenic, and hexavalent chromium were found. All measured concentrations were far below the permissible limits established by the Taiwan Food and Drug Administration (TFDA) and the FAO/WHO food safety standards. Furthermore, because TPCW is a by-product derived from food-grade materials and its high-salt and high-acidity properties inherently inhibit the growth of harmful microorganisms, microbial contaminants such as *Escherichia coli* were not examined in this study. Additionally, although the nutritional composition of TPCW used for *D. salina* cultivation (including organic acids and inorganic salts) may exhibit slight variations among production batches, all processing and fermentation procedures within the Taiwanese pickled cabbage production in Dapi Township follow standardized operating protocols. Therefore, we consider the overall nutritional composition of TPCW from different batches to be largely comparable, and the use of a single batch in this study should not compromise the relevance of our findings. Accordingly, the experimental results still provide valuable and representative information.

The results of this study demonstrated that the growth of *D. salina* increased with higher substitution levels of TPCW. When seawater was fully replaced by adjusted TPCW, 10.07 g of high-quality algal biomass (L^{-1}) with a protein content of 59.6% was obtained after 14 days of cultivation. The enhanced growth of *D. salina* in this study may be primarily attributed to the presence of organic acids in TPCW, which are byproducts of lactic acid bacteria during the pickling process [49]. Li et al. [50] found that organic acids such as acetic, propionic, and malic acids serve as effective carbon sources for *Chlorella* growth, enhancing biomass production. Similarly, Cho et al. [51] and Hou et al. [52] reported that valeric acid produced from anaerobic digestion of organic wastewater promotes algal growth. In our TPCW100 group, the culture medium contained 4.06% lactic acid, 1.86% acetic acid, and 2.05% malic acid. After 14 days, the elevated cell density of *D. salina* confirmed that these organic acids significantly enhanced the growth of *D. salina*.

Cultivating algae in TPCW can help mitigate environmental concerns related to its direct discharge. In this study, the CO_2 concentration in the culture container decreased during *D. salina* cultivation. After 14 days, the TPCW100 group reduced CO_2 levels by 4152 ppm and produced 10.07 g of algal biomass with a crude protein content of 59.6%. Parmar et al. [53] reported that algae can reduce atmospheric CO_2 through photosynthesis, incorporating it into biomass. Yen et al. [54] further demonstrated that 1.83 kg of CO_2 can yield 1 kg of microalgae. Similarly, Xu et al. [55] found that *Cylindrospermopsis raciborskii* significantly lowered CO_2 levels while enhancing biomass accumulation. CO_2 not only serves as a substrate for photosynthesis but also influences the nutritional demands and chemical composition of algal cells, affecting the synthesis of carbohydrates, proteins, and fatty acids [56]. However, in this study, the efficiency of CO_2 reduction declined after 72 h, and CO_2 levels began to rise, likely due to nutrient depletion, reduced algal growth, and subsequent cell death. The decomposition of algal biomass by microbes may have contributed to secondary CO_2 release. As carbon fixation is directly linked to algal growth, various environmental factors—such as temperature [57, 58], light intensity and photoperiod [59], and pH [60]—play critical roles in determining carbon fixation efficiency. In the later stages of this experiment, variations in light, nutrient availability, and pH likely

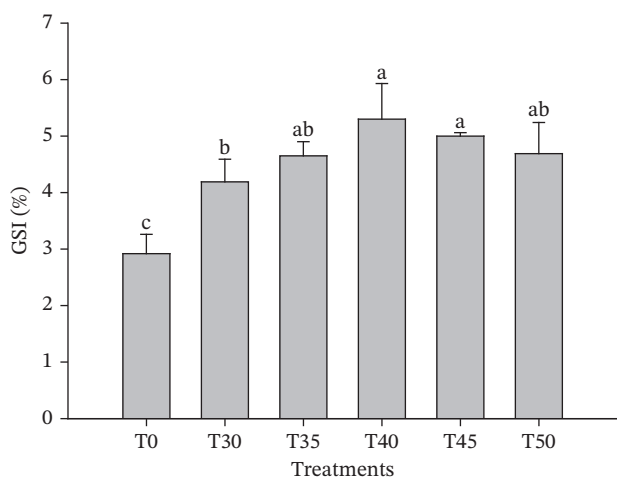


FIGURE 4 | Gonad somatic index (GSI) of sea urchins (*T. gratilla*) after 180-day feed trial of experimental diets. Groups labeled with different letters are significantly different from each other ($p < 0.05$), while groups sharing the same letter do not differ significantly. Data are expressed as means $\pm$ S.E. ($n = 3$).

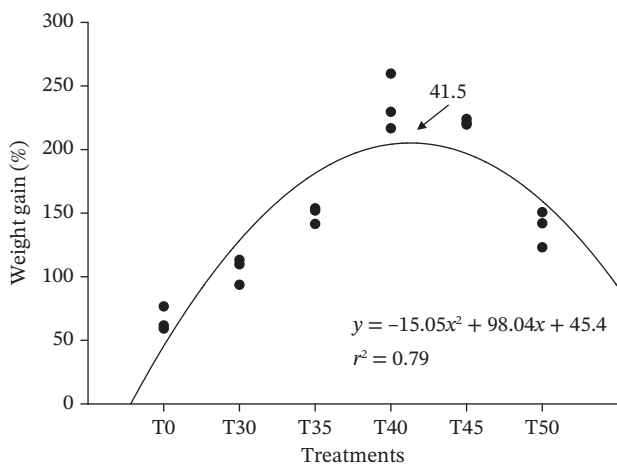


FIGURE 5 | Quadratic regression analysis between the substitution ratio of *D. salina* powder to fishmeal and the weight gain of sea urchins (*T. gratilla*) after a 180-day cultivation period. ($n = 3$).

influenced algal performance. Further studies are necessary to identify the optimal cultivation conditions for *D. salina*.

Additionally, previous studies have reported that various microalgae, including *Chlorella*, *Spirulina*, *Oscillatoria*, *Scenedesmus*, and *Anabaena*, can remove nitrogen and phosphorus from municipal, agricultural, and industrial wastewater while producing valuable biomass [61]. For example, *Scenedesmus* sp. treated municipal wastewater and effectively removed phosphate and ammonium nitrogen, yielding 22.2 g/m²/day of algal biomass [62]. Similarly, *Monoraphidium* sp. removed PO₄³⁻ and NO₃⁻ from municipal effluent while increasing intracellular carotenoids and oil content by 2.0-fold and 3.72-fold, respectively [63]. *Chlorella* was also reported to treat high-ammonium industrial wastewater, producing biomass, protein, and lipid yields of 58.4 g/L, 5.56 g/L, and 5.66 g/L/day, respectively [64]. Therefore, the cultivation of *D. salina* as a sustainable biofilter for effluent treatment represents a promising avenue for further investigation in future studies.

4.2 | Experiment on Partial Replacement of Fishmeal With *D. salina* Powder in *T. gratilla* Culture

Microalgae are valuable sources of protein, lipids, vitamins, carotenoids, and energy in aquafeeds. During the late logarithmic growth phase, they typically contain 30%–40% protein, 10%–20% lipids, and 5%–15% carbohydrates [65]. Due to their complete amino acid profiles, comparable to conventional protein sources, microalgal proteins are suitable substitutes for fishmeal [20]. For example, *Arthrospira* spp. contain all EAAs and up to 68% protein [66], and most microalgal species have similarly high EAA levels [65]. *T. gratilla* requires around 32% dietary protein [43], and its primary food sources in the wild are macroalgae such as *Sargassum* and *Ulva*, which are nutritionally limited, requiring high consumption to meet nutritional demands. Therefore, juvenile sea urchins fed *Sargassum* show low growth rates, as observed in *S. droebachiensis*, *P. lividus*, and *T. gratilla* [67–69]. Microalgae such as *Dunaliella*, *Chaetoceros*, *Isochrysis galbana*, and *P. tricornutum* may be the suitable food sources for larvae and juveniles of sea urchins [70].

This study assessed the effects of partially replacing fishmeal with *D. salina* powder on *T. gratilla* growth and found that the TG40 group significantly improved both growth and gonad index. Similar findings have been reported in other species. For instance, *Spirulina* successfully replaced 10%–20% of fishmeal in *Oncorhynchus mykiss* and *Carassius auratus gibelio* [71, 72], and *Nannochloropsis* replaced 10% in *S. ocellatus* diets [73]. *Scenedesmus* and *P. tricornutum* have also shown promise as fishmeal substitutes without compromising nutrient utilization [74, 75]. Appropriate microalgae inclusion enhances growth in many species. For example, 10 g/kg *Spirulina* improved growth and survival in *O. niloticus* [76], and 10% inclusion benefited *H. huso* and *P. leopardus* [77, 78]. *Chlorella* replacing 6%–8% of fishmeal improved *M. rosenbergii* growth [79].

In comparison with natural algae, compound feeds are probably more effective in promoting rapid somatic growth, making them more suitable for artificial breeding, where the primary goal is to shorten the time to market by maximizing somatic cell development [68, 80–82]. Numerous studies have shown that both the

quantity and quality of food directly influence the development and quality of sea urchin gonads [80, 82, 83]. In particular, high-quality proteins in formulated feeds have been found to significantly enhance gonad growth [83–90]. Cirino et al. [91] demonstrated that sea urchin feed formulated with *Spirulina* yielded better growth performance compared to wild individuals in the natural environment. The time required for gonads to reach maturity was significantly reduced, indicating that microalgae-based feeds provide more balanced nutrition than natural diets and lead to improved feeding outcomes.

To meet the nutritional needs of aquatic organisms, feed formulations often emphasize nutritional balance while overlooking palatability, energy content, and bioavailability. This can lead to uneaten or poorly digested feed, resulting in waste and reduced growth performance [92]. Seymour et al. [93] reported that brown algae have an attractive effect on *T. gratilla*, and the addition of brown algae powder to artificial feed promotes growth. Similarly, Dworjanyn et al. [67] found that including 5% *Ecklonia radiata* in feed increased the growth rate of *T. gratilla* by 12% compared to the control. To evaluate the potential of microalgae as feed attractants, this study incorporated varying levels of *D. salina* powder into sea urchin diets. Results showed that feed intake increased with higher *D. salina* content, supporting the notion that microalgae can act as effective feed attractants for sea urchins.

In addition to the attractant properties of microalgae, the digestibility by sea urchins is also important. Horn and Messer [94] noted that in herbivorous fish, gastric acid breaks down algal cell walls, enabling nutrient absorption. However, echinoderms have different digestive systems, and the *D. salina* used in this study, being a halophilic unicellular alga without cell walls [95, 96], likely enhanced nutrient availability. This study demonstrated that *D. salina* powder protein can partially replace fishmeal protein in *T. gratilla* diets, particularly in early developmental stages, and sea urchin growth was significantly improved. Basch [97] identified *Dunaliella tertiolecta* as an optimal food source for starfish larvae, promoting their growth and development. Similarly, Brundu et al. [98] reported that a mix of microalgae, including *Isochrysis*, *D. tertiolecta*, and *Chaetoceros gracilis*, supported sea urchin larval development. Bai [99] also found *D. salina* most effective for early *Stichopus japonicus* larvae, likely due to the lack of cell walls, improving digestibility.

Gonads are the primary edible part of sea urchins, and their color and moisture content are critical factors influencing market value [100]. Numerous studies have demonstrated that feeds significantly affect the wet weight, somatic index, moisture, and color of sea urchin gonads, implying that feed composition directly influences gonad quality [101–104]. In this study, increasing the replacement of fishmeal with microalgal powders significantly altered the moisture, crude protein, and lipid content of collector sea urchin gonads. Robinson et al. [105], Shpigel et al. [106], and Symonds et al. [107] reported that dietary β -carotene enhances gonad coloration. Shpigel et al. [108] further noted that *Echinone* levels in *Paracentrotus lividus* gonads are positively correlated with coloration, and Robinson et al. [105] confirmed that echinone is derived from β -carotene in the feed. These findings align with the present study, where microalgae protein substitution above 45% (β -carotene: 1.73 mg/100 g) resulted in significantly improved gonad coloration compared to other groups.

Different feeds significantly influence the fatty acid composition of sea urchin gonad [109, 110]. Feeding various algae or artificial diets can thus affect gonad quality. Carboni et al. [111] compared artificial feed with brown algae and found that sea urchin larvae utilize specific fatty acids during growth. The DHA/EPA and EPA/ARA ratios in feed are key factors affecting larval development. Liu et al. [112] also reported that high levels of LC-PUFA in diets enhance seedling growth, with $20:3n-3$ and $20:4n-3$ being particularly important. Carboni et al. [113] showed that sea urchins can convert 18-carbon polyunsaturated fatty acids into LC-PUFA. Furthermore, studies indicate that $18:2n-6$ and $18:3n-3$ in feed are positively correlated with highly unsaturated fatty acid content in gonads [114]. These findings support this experiment's results, which confirm that dietary unsaturated fatty acids significantly impact gonad quality.

In summary, this study demonstrated that TPCW can serve as an effective medium for cultivating *D. salina*, providing high-quality biomass rich in protein, while partially substituting fishmeal with *D. salina* powder in *T. gratilla* diets significantly enhanced growth performance, gonad development, feed intake, and gonad nutritional quality, including fatty acid composition and coloration. These findings highlight the dual benefits of integrating microalgae cultivation with sustainable aquaculture practices, simultaneously addressing effluent management and feed resource optimization. Future research should explore the scale-up potential of this approach, conduct cost-benefit analyses, and evaluate long-term feeding trials to assess growth performance, gonad quality, and overall economic feasibility under commercial farming conditions.

Author Contributions

Conceptualization, funding acquisition: Sheng-Wei Ho and Jen-Hong Chu. Methodology: Hsiang-Yi Hsu, Mei-Ying Huang, Yu-San Han, and Jen-Hong Chu. Investigation: Sheng-Wei Ho, Hsiang-Yi Hsu, Sheng-Yu Ho, and Jen-Hong Chu. Data curation, writing – review and editing: All authors. Formal analysis, visualization: Sheng-Wei Ho, Hsiang-Yi Hsu, Yu-San Han, and Jen-Hong Chu. Resources: Mei-Ying Huang and Sheng-Yu Ho. Software: Sheng-Wei Ho and Hsiang-Yi Hsu. Writing – original draft: Sheng-Wei Ho, Hsiang-Yi Hsu, and Jen-Hong Chu. Supervision: Jen-Hong Chu.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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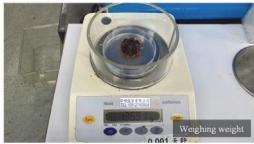
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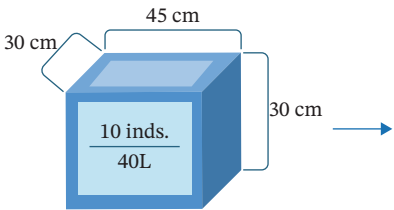
TABLE A1 | The nutrient salt (ion), organic acids, and Guillard's f/2 medium composition for each TPCW/seawater substitution level.

Name of nutrient	Unit	Treatments					
		0	10	20	40	80	100
Na ⁺	ppm (mg/L)	10,500.00	17,354.63	24,209.26	37,918.52	65,337.05	79,046.31
K ⁺	ppm (mg/L)	380.00	563.36	746.73	1113.46	1846.91	2213.64
Mg ²⁺	ppm (mg/L)	1280.00	1177.96	1075.92	871.84	463.69	259.61
NH ⁴⁺	ppm (mg/L)	0.0001	0.00009	0.00008	0.00006	0.00002	0
Li ⁺	ppm (mg/L)	0.17	4.70	9.22	18.27	36.37	45.42
Ca ²⁺	ppm (mg/L)	400.00	364.08	328.17	256.33	112.66	40.83
Cl ⁻	ppm (mg/L)	19,000.00	29,475.49	39,950.97	60,901.94	102,803.88	123,754.85
SO ₄ ²⁻	ppm (mg/L)	2700.00	2500.14	2300.28	1900.56	1101.12	701.40
NO ₃ ⁻	ppm (mg/L)	0.0001	18.34	36.68	73.36	146.72	183.40
PO ₄ ³⁻	ppm (mg/L)	0.001	15.39	30.78	61.55	123.10	153.88
NO ₂ ⁻	ppm (mg/L)	0.0001	8.40	16.80	33.60	67.20	84.00
Br ⁻	ppm (mg/L)	65.00	60.55	56.10	47.19	29.38	20.48
Lactic acid	percentage (%)	0	3.07	6.14	12.28	24.56	30.70
Malic acid	percentage (%)	0	1.56	3.12	6.23	12.46	15.58
Acetic acid	percentage (%)	0	1.37	2.74	5.47	10.94	13.68
Citrate acid	percentage (%)	0	0.64	1.27	2.54	5.08	6.35
Succinic acid	percentage (%)	0	0.12	0.25	0.49	0.98	1.23
Tartaric acid	percentage (%)	0	0.01	0.03	0.06	0.11	0.14
NaNO ₃	g/L	7.5	7.5	7.5	7.5	7.5	7.5
NaH ₂ PO ₄ ·H ₂ O	g/L	0.5	0.5	0.5	0.5	0.5	0.5
CuSO ₄ ·5H ₂ O	g/L	0.98	0.98	0.98	0.98	0.98	0.98
ZnSO ₄ ·7H ₂ O	g/L	2.2	2.2	2.2	2.2	2.2	2.2
CoCl ₂ ·6H ₂ O	g/L	1	1	1	1	1	1
MnCl ₂ ·4H ₂ O	g/L	18	18	18	18	18	18
Na ₂ MoO ₄ ·2H ₂ O	g/L	0.63	0.63	0.63	0.63	0.63	0.63
Cyanocobalamin	mg/L	0.1	0.1	0.1	0.1	0.1	0.1
Biotin	mg/L	0.1	0.1	0.1	0.1	0.1	0.1
Thiamine	mg/L	20	20	20	20	20	20

Experimental Design

1. preliminary experiment

Feed preparation	Experimental organism
TG0 (0% fish meal protein replacement) and five treatment groups in which <i>D. salina</i> powder replaced 10% (TG10), 15% (TG15), 20% (TG20), 25% (TG25), and 30% (TG30).	<div></div> <p>The experiment will begin by measuring the sea urchins shell diameter and weight.</p>

Experimental aquaria	Feeding
 <p>Each treatment with 3 replicates.</p>	<div></div> <p>Triplicate groups of sea urchins were fed experimental diet by satiation at 09:00 h by hand for eight weeks.</p>

After feeding trial, the result indicated that the TG0 showed a weight gain was significantly lower than those of all treatment groups. No significant differences in growth efficiency were observed among the treatment groups.

2. Main experiment

Based on the preliminary results, diets of six experimental groups for the main experiment were formulated: one control group (TG0, 0% fish meal replacement) and five treatment groups in which 30% (TG30), 35% (TG35), 40% (TG40), 45% (TG45), and 50% (TG50) of fish meal were replaced with *D. salina* powder. Experimental organism, Experimental aquaria and Feeding are the same as in preliminary experiment.