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RESEARCH

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Effect of dietary seaweed *Caulerpa racemosa* on growth, biochemical, non-specific immunity, and disease resistance to *Pseudomonas aeruginosa* in *Cirrhinus mrigala*

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Abstract

Background The green seaweed *Caulerpa racemosa* possesses highly potential elements in animal forages and human use since ancient times. The current study was designed to investigate the antioxidants, phytochemical properties of *C. racemosa*, as well as their effects on growth parameters, biochemical components, non-specific immunological parameters, and disease resistance to *P. aeruginosa* in *Cirrhinus mrigala*. The experimental group, divided into five groups as T1–T5. T2–T5, was given *C. racemosa* extract at concentrations of 0.5%, 2.5%, 4.5%, & 6.5% supplemented with basal diets. T1's group food is devoid of extract and acts as a control, and the trial lasted forty-five days.

Results *C. racemosa* exhibited dose-dependent antioxidant activity. The highest levels of DPPH (61.21%), ABTS (70.55%), and H_2O_2 (66.55%) scavenging activities were obtained at 100 µg/ml 100 µg/ml. GC–MS analysis revealed phytoconstituents in the crude extract, such as palmitolinoleic acid, butanoic acid, arachidonate, linoleic acid, vaccenic acid, octadecenoic acid, trans-phytol, and eicosanoids. Among these different concentrations, 4.5% C. racemosa supplemented diet produced a significantly higher growth metrics of *C. mrigala's* (WG, SGR, FCR), biochemical constituents, when compared to other concentrations. In a post-challenge trial, 4.5% *C. racemosa* extract meals increased *C. mrigala* SOD, CAT, non-specific immune response (lysozyme, NBT, phagocytic activity), and haematological (RBC, WBC, HCT & Hb) parameters when compared to other experiments.

Conclusions The findings revealed that 4.5% of *C. racemosa* may be supplied in the diet of *C. mrigala* to regulate better survival growth performance and haematological parameters.

Keywords *C. racemosa*, *C. mrigala*, *P. aeruginosa*, Non-specific immunity

Background

Seaweeds inhabit watery environments and are mostly used by communities living near the shore. Seaweed is often used in its raw form for medicinal purposes, human consumption, animal feed, and fertilizer. Seaweed biomass is a valuable animal feed supplement. Seaweeds have a high mineral content because they absorb inorganic chemicals from their surroundings. They include polyunsaturated fatty acids but are heavy in carbs. Seaweed is an excellent source of natural antioxidants and antimicrobials. Seaweed farming accounts for around 20% of all marine aquaculture output. Seaweed farming is a major aspect of worldwide aquaculture, producing 27.3 million tons in 2014 (Mustafa & Nakagawa, 1995; Ragunath et al., 2020; Shapawi & Zamry, 2015).

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Compared to terrestrial plants, seaweed composites have infinite non-carbon compounds and organic constituents, which can benefit the human nutraceutical and pharmaceutical sectors (Lavanya & Veerappan, 2011). Seaweeds are a source of several biologically active quarantine compounds; those composites have been possibly exploited as active elements for human and animal health purposes. The sulphated polysaccharides fucoidan, β-glucans, polyphenols, and laminarin have received the most attention in bioactive composites, which contain antioxidants, antimutagenic agents, antidiabetic agents, anticoagulants, antibacterial agents, diterpenes, and antitumor properties (Freitas et al., 2021; Kolanjinathan & Stella, 2009; Kolanjinathan et al., 2009; Ragunath & Ramasubramanian, 2022).

The use of nutritional immunostimulants has been seen as a realistic addition or alternative to traditional methods, and it has gained widespread acceptance among fish breeders. Immune enhancers, when used as nutritional enhancements, may increase fish resistance during times of extreme pressure or increase strength during periods of dissolving water management (Bagni et al., 2001). In vivo experiments with bacteriological challenges identified immunostimulants as antiparasitic and neutralizer creation advertisements (Bricknell & Dalmo, 2005). The use of dietary seaweed has been shown to have a significant influence on fish growth, feed consumption, stress reactivity, physiological condition, and resistance to refined fish.

Seaweeds have many extra-nutritional constituents, and those compounds have stimulated feed consumption, growth, immune activity, inhibitor factors, fatty acid metabolism, under-stress conditions, and disease resistance in fish and other aquatic animals (Mohan et al., 2019; Wan et al., 2019). Previous studies showed that inclusion of various marine algae supplements, like such as *S. polycystum* (Shapawi & Zamry, 2015), *S. ilicifolium* (Zeynali et al., 2020), *K. alvarezii* (Zuldin et al., 2016), *K. alvarezii*, *E. denticulatum*, *U. ohnoi* (Jones et al., 2016), and *G. pulvinata* (Morshedi et al., 2017) can improve the growth performance, immune parameters, and disease resistance of various aquatic species.

Current research has shown that seaweeds have positive effects on both humans and aquatic species. Consequently, experts have focused on using kelp (algae) as an immunostimulant to enhance health and prevent disease outbreaks in animals, thus reducing the need for antibiotics and chemotherapeutics (Mohan et al., 2019).

The increasing spread of aquatic infections has posed a significant economic threat to the aquaculture sector. It is thought that aquatic infections have hampered the aquaculture industry's expansion, resulting in annual financial losses. In recent years, antibiotics and chemotherapy have become popular treatments for bacterial infections. Furthermore, the continued use of antibiotics and chemotherapy has led in the emergence of drug-resistant microorganisms. These bacteria may damage the host's immune system and contaminate the aquatic environment. This necessitates the development of innovative ways for managing bacterial infections. Pseudomonas, which causes ulcerative syndrome and hemorrhagic septicemia, is one of the deadliest pathogens found in freshwater fish. *Pseudomonas aeruginosa* is a gram-negative aerobic bacterium that produces catalase and oxidase. This bacterium also possesses several virulence-related determinants, including secreted and cell-mediated virulence. The patterns of antibiotic resistance in Pseudomonas species are diverse. In particular, *P. aeruginosa* can develop congenital and acquired resistance. Today, extensive efforts are being made to stumble on *P. aeruginosa*, which is no longer the most effective for financial and public fitness reasons (Eissa et al., 2010). Pseudomonas infection, commonly resulting from *P. aeruginosa*, is a public fitness hassle that causes many healthcare-associated ailments in patients. Currently, Pseudomonas species are recognised as a foodborne infection that infects people via the intake of spoiled ingredients and ready-to-consumer products and the manipulation of infected seafood (Algammal et al., 2020), even though only a few research studies, like *C. racemosa*, fed with *Penaeus monodon* and *Chanos chanos* (Puspitasari et al., 2019).

Despite the fact that only a few research studies have been conducted on *C. racemosa*, in this respect, the current study focused on assessing the phytochemical and antioxidant content of *C. racemosa* and its immunostimulatory action on *C. mrigala* in response to infection with *P. aeruginosa.*

Materials and methods

Algal sample and experimental animal collection

The green macroalgae *C. racemosa* collected from Mandapam, Rameshwaram (079° 20`E longitude: 09° 25`N latitude) regions, South-east coast of India. These species were identified with help of Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu. The collected *C. racemosa* thoroughly washed with seawater followed by freshwater to eliminate debris. Samples were dried under room temperature. Dried *C. racemosa* was pulverized to powder.

Preparation of *C. Racemosa* extract

Thirty grams of the pulverized sample was extracted with 250 ml of methanol in a thimble for 24 h. Methanol was evaporated from the extract using rotary evaporators.

They were preserved in refrigerator-airtight bottles for further research (Laher et al., 2013).

Phytochemical analysis

To analyse phytochemicals, alkaloids, flavonoids, saponin, terpene, phenolic compounds, carbohydrates, amino acids, glycosides, and tannins used standard methods (Sadasivam & Manickam, 1992).

Analysis of the antioxidant activity of *C. racemosa*

The DPPH, ABTS, and H_2O_2 scavenging tests (Freitas et al., 2021) were used to measure the in vitro antioxidant activity of C. *racemosa* extract.

Gas chromatography and mass spectrometry analysis (GC–MS)

The phytoconstituents bioactive components of *C. racemosa* extract were studied using gas chromatography (GC) and mass spectrometry. The extracted material was analysed using a Perkin Elmer Clarus SQ8C-gas chromatographic system and a capillary column.

Diet preparation and proximate analysis

Five experimental diets were formulated to meet fish requirements using known substances: Rice bran, Fishmeal, Soybean, Groundnut oil cake, Wheat flour, Tapioca flour, Cod liver oil, and Vitamins & mineral mixture. Egg albumin was used as binding agent. Different concentrations of *C. racemosa* extract (0.5%, 2.5%, 4.5% and 6.5%) incorporated with basal diets. The prepared diets feed was analysed for crude protein (AOAC 984.13), crude fibre (AOAC 962.09), ether extract (AOAC 2003.06), ash (IS 14827-2000), moisture (AOAC 930.15), and total energy (Table 1).

Maintenance of experimental animal and feeding trial

The *C. mrigala*, 300 numbers average weight (g) of 0.32 ± 0.15 and length (cm) of 4.0 ± 0.42, was collected from the Bhavani Sagar farm in Erode, Tamil Nadu. The experimental animals were acclimated to laboratory conditions for ten days, and water quality parameters were analysed (temperature 27.5 ± 1.73^{0}C, pH 7.5 ± 1.32, BOD 13 ± 1.53 mg/l, COD 65 ± 5.30 mg/l, chloride 0.1 ± 0.25 mg/l, DO_2 6.5 ± 0 0.13 mg/L). During the acclimatisation period, fish were fed with rice bran for fifteen days before the feeding trial. Every day, 80 per cent of the water was drained and replaced with fresh water.

The experiment was divided into five groups. Each group contained twenty fish (0.32 ± 0.15 and length (cm) 4.0 ± 0.42), with triplicates. *C. racemosa* extracts (0.5%, 2.5%, 4.5%, and 6.5%) were mixed into the basal diets of experiments T2 to T5, while the control group (T1) received no *C. racemosa* extract. The feeding trials were carried out for forty-five days.

Growth parameters

The specific rate of growth (SRG), final weight (FW), weight gain (WG), final length (FL), survival rate, and feed conversion ratio (FCR) are all calculated according to (Prabhu et al., 2018).

The specific rate of growth (SRG) = Final mean deviation of Weight (g) − Initial mean deviation of Weight (g)/experimental period × a hundred.

Table 1 Feed ingredients and proximate composition of experimental diets

Ingredients	T_1 (C)	T_2	T_3	T_4	T_5
Fish meal	30	30	30	30	30
Vitamin and mineral	4	4	4	4	4
Soybean	21.5	21.5	21.5	21.5	21.5
Tapioca starch	15	14.5	12.5	10.5	8.5
Groundnut oil cake	10	10	10	10	10
Binder	1	1	1	1	1
Cod liver oil	6.5	6.5	6.5	6.5	6.5
Wheat meal	12	12	12	12	12
Seaweed powder	0	0.5	2.5	4.5	6.5
Proximate compositions					
Moisture %	8.13 ± 0.23	8.02 ± 0.50	8.34 ± 0.21	8.46 ± 0.12	8.95 ± 0.28
Crude protein	34.93 ± 0.17	35.32 ± 0.14	35.92 ± 0.14	38.32 ± 0.14	39.72 ± 0.45
Crude fibre	5.24 ± 0.06	5.49 ± 0.03	5.69 ± 0.17	5.12 ± 0.23	6.11 ± 0.58
Ether extract	9.16 ± 0.06	9.73 ± 0.07	11.76 ± 0.10	11.92 ± 0.08	11.30 ± 0.05
Total ash	7.07 ± 0.13	7.33 ± 0.05	7.98 ± 0.08	7.43 ± 0.08	7.75 ± 0.12

Weight gain (WG) % = Final mass (g) − Initial Mass (g)
Survival rate = No of animals treated − No of animals remaining × 100
Feed conversion ratio (FCR) = Feed given (g)/Weight gathered (g)

Muscle biochemical constituents analysis and digestive enzyme activity

Following the experimental period, the levels of total proteins (Lowry et al., 1951), total carbohydrates (Roe, 1955), total lipids (Folch et al., 1957), protease activity (Esakkiraj et al., 2008), amylase activity (Bernfeld, 1955), and lipase activity (Adel et al., 2015) were analysed.

Enzymatic antioxidants assay

The levels of superoxide dismutase (SOD) and catalase (CAT) were quantified using a standardized methodology outlined by Marklund and Marklund (1974) and Aebi (1984). The muscle tissues of the chosen groups were blended at a concentration of 10% weight-to-volume in a chilled 50 mM Tris buffer with a pH of 7.4. The combination underwent centrifuged with a force of 10,000 times the acceleration due to gravity for 20 min at a temperature of 4 degrees Celsius. The liquid recovered from the layer above the silt was used to assess the enzyme activity.

Catalase (CAT)

In the phosphate buffer, the activity of the catalase (CAT) enzyme was determined by utilising H2O2 as the substrate. After adding progressively 0.5 ml of tissue homogenate, 1.0 ml of phosphate buffer with a pH of 7.1 and a concentration of 0.01 M, 0.5 ml of hydrogen peroxide with a concentration of 0.2 M, and 0.4 ml of distilled water, the reaction was kicked off. After sixty seconds, the reaction was terminated by adding two millilitres of a reagent containing dichromate and acetic acid. After 10 min in the boiling water bath, the test tubes were removed and allowed to cool. At a wavelength of 620 nm, the absorbance of the chromophore was measured. The level of catalase activity was measured in micromoles of hydrogen peroxide consumed per minute per milligram of protein.

Superoxide dismutase activity (SOD)

The 0.5 mL samples were mixed with 0.25 mL of ethanol that was very cold and 0.15 mL of chloroform. Next, the mixture was centrifuged at a speed of 13,000 revolutions per minute for a duration of 15 min. The 0.5 mL of liquid remaining after sedimentation was combined with 2 mL of Tris buffer. The total amount was then increased to 4.5 mL by adding distilled water and 0.5 mL of freshly made pyrogallol solution. The blank solution was produced by combining 2.5 mL of distilled water with 2 mL of Tris HCl. In addition, standards were made by mixing 2 mL of Tris HCl with distilled water, 2 mL of deionized water, and 0.5 mL of pyrogallol. Subsequently, these criteria were appropriately categorized. The presence of a brown tint shows the process of oxidation. The samples were seen at a wavelength of 470 nm for a period of 3 min, with measurements recorded every 60 s. The level of superoxide dismutase (SOD) activity was measured and reported as international units per milligram of protein.

Antibacterial activity and challenging study

The PSG Institute of Medical Sciences and Research, Peelamedu, Coimbatore, Tamil Nadu, provided the P. aeruginosa bacterial strain.

In our earlier studies, Ragunath et al., (2020) described the antibacterial assay of C. racemosa in the methanol extract.

We sub-cultured the bacteria in Pseudomonas broth for 24 h at 28 degrees Celsius. *The experimental groups received intraperitoneal administration of P. aeruginosa (1.8 × 107 CFU/mL) mixed with PBS saline. Both pre- and post-challenging studies analysed the WBC, lysozyme activity, NBT, phagocytic activity, and survival rate.*

Haematological and immunological assays

Following the infection, three fish from each experimental group were selected at random and anesthetized in order to collect blood samples for hematological tests (Palanisamy et al., 2017). The syringe washed with an anticoagulant was used to extract body fluid from the caudal vein. The following parameters were measured: red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hb) g/dL, packed cell volume (PCV) (hematocrit) percent, mean corpuscular volume (MCV) f/L, mean corpuscular haemoglobin (MCH) g/dL, mean corpuscular haemoglobin concentration (MCHC), and lysozyme activity (Anderson & Siwicki, 1995). Mbokane and Moyo (2018) used the NBT test to evaluate the oxidative burst generated by leucocytes. The phagocytic activity was conducted using the technique developed by Pratheepa et al. (2011).

Statistical analysis

The data were reported as the arithmetic mean ± standard error (SE), with a sample size of n = 3. The data underwent statistical analysis using one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests using SPSS software (version 21). A P-value less than 0.05 was deemed significant, while a P-value less than 0.01 was regarded extremely significant.

Results

Preliminary qualitative phytochemical analysis

The *C. racemosa* demonstrates presence of alkaloids, flavonoids, saponins, terpenoids, phenolic compounds, carbohydrates, and amino acids (Table 2). *C. racemosa* does not contain glycosides or tannins.

Antioxidant activities

The *C. racemosa* extract exhibited significant DPPH scavenging activity, with the highest scavenging effect (61.21%) seen at a concentration of 100 µg/ml (Fig. 1). Figure 2 illustrates the radical scavenging capabilities of *C. racemosa* extracts. With a concentration of 100 g/ml, it exhibited a very effective scavenging effect of 70.55%.

Table 2 Qualitative phytochemical analysis

S. no.	Phytochemicals	Inference of *C. racemosa*
1	Alkaloids	+ +
2	Flavonoids	+ + +
3	Saponins	+ +
4	Phenolic compounds and tannins	+ + +
5	Glycosides	–
6	Terpenoids	+ + +
7	Phlobatannins	+ +
8	Amino acid and protein	+ + +
9	Carbohydrate	+ + +
10	Tannins	–

+ + + Highly presence of the compound, + + moderately presence, – absence of the compound

Figure 3 shows that the H_2O_2 was suppressed with a 66.55% inhibitory effect when applied at a dose of 100 µg/ml. The findings demonstrated that extracts of *C. racemosa* had potent antioxidant activities.

Gas chromatography-mass spectroscopy analysis

Fifteen bioactive compounds were discovered from the partly purified fractions of the *C. racemosa* based on the GC–MS data. Seaweeds possess a greater concentration of fatty acids in comparison with terrestrial plants. The present work used GC–MS analysis to identify several metabolites in *C. racemosa*, including eicosenoic acid, methyl palmitate, hexadecanoic acid, linoleic acid, methyl ester, methyl oleate, phytanic acid, linolenic acid, oleic acid, palmitic acid, and other metabolites (Table 3).

Source of antibacterial activity

Our earlier study reported that C. racemosa showed the highest antibacterial activity (21.0 ± 0.2) against Gram-negative bacteria *P. aeruginosa* at 50 mg/ml concentration.

Growth parameters

The current study discovered that different levels of *C. racemosa* extract in the diet affected *C. mrigala* growth and feeding intake in a dose-dependent manner. The growth indices WG (weight gain), LG (length gain), and SRG (specific growth rate) increased significantly in a dose-dependent manner with the addition of *C. racemosa* to the fish meal. The highest growth was seen in fish given diets supplemented with 4.5% *C. racemosa*. The fish-fed control and 0.5 diets had equal FCR values, but

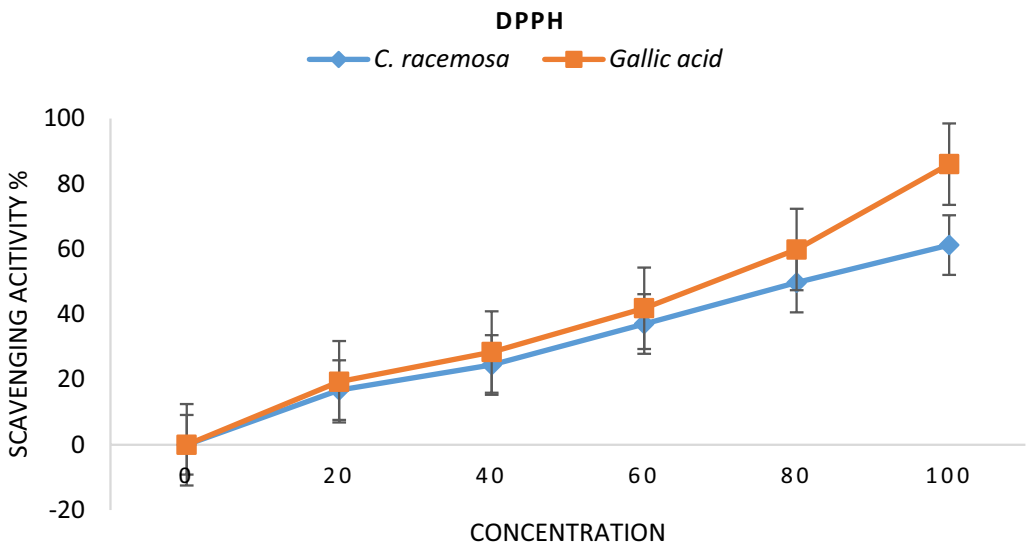

Fig. 1 Comparison of the DPPH activity of *C. racemosa* (20–100 µg/mL) with conventional gallic acid (20–100 g/mL) has been made. The results are provided as the mean standard deviation (n = 3)

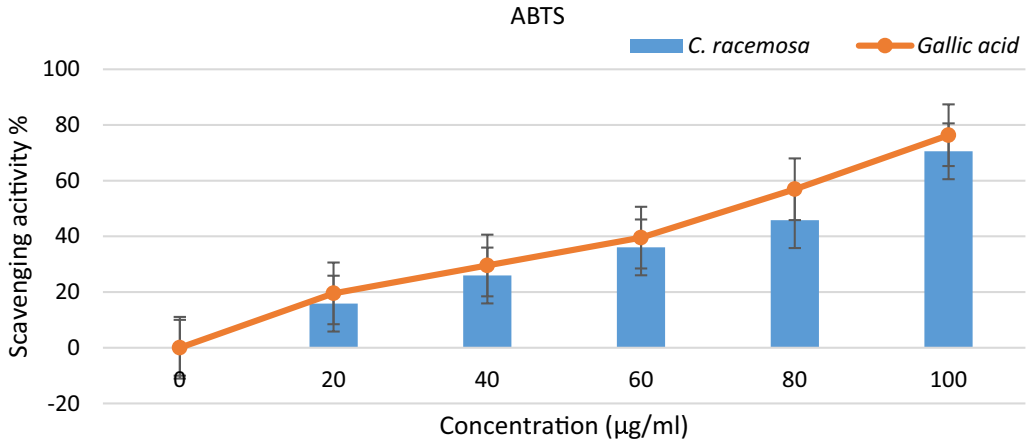

Fig. 2 ABTS activity of *C. racemosa* (20–100 µg/ml), with standard gallic acid (20–100 µg/ml). Data expressed as mean ±SD, n = 3

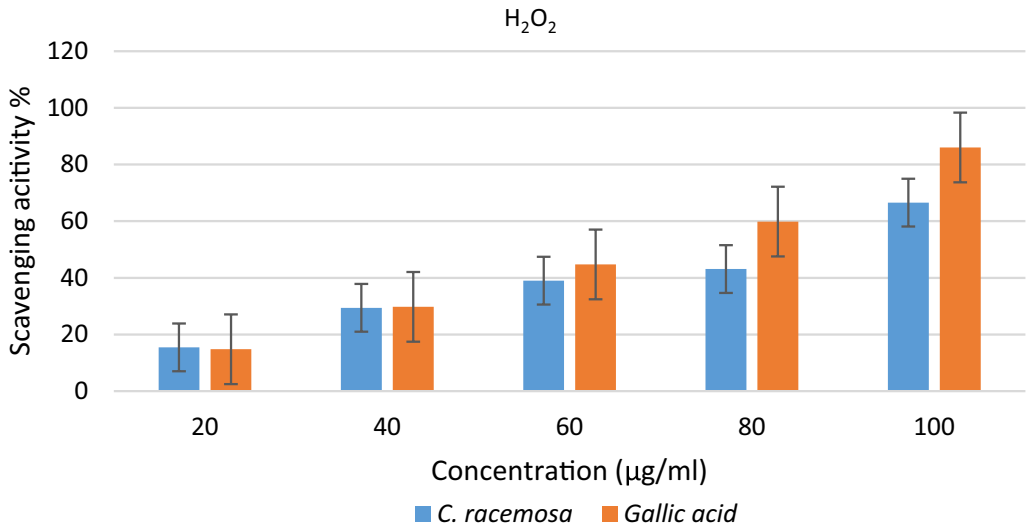

Fig. 3 H2O2 radical scavenging activity of *C. racemosa* (20–100 µg/ml), with standard gallic acid (20–100 µg/ml) . Data expressed as mean ± SD, n = 3

the meals supplemented with 2.5%, 4.5%, and 6.5% *C. racemosa* had lower FCR values (Table 4). The results of this study show that fish given diets supplemented with 4.5% *C. racemosa* (T4) had better growth performance indicators, such as final weight, weight gain, and SGR, than compared to control and other experimental groups.

Body composition

C. mrigala fed with *C. racemosa*-enriched meals showed a significant increase in body composition, specifically the levels of protein, carbohydrate, and lipid, as well as the activity of digestive enzymes such as protease, amylase, and lipase. The study scrutinised the variations in the digestive enzyme activity levels in *C. mrigala* fish-fed diets enriched with varying concentrations of *C. racemosa*. The fish that were fed diets enriched with 4.5% *C. racemosa* (T4) showed the highest levels of enzyme activity for protease, amylase, and lipase compared to the control group and other experimental groups (Table 4). Experiment T4 had the highest concentration of muscle proteins and carbohydrates in comparison with the control group. Experiment T5 demonstrated that fish-fed diets containing 6.5% C. *racemosa* extract exhibited greater fat consumption compared to the other groups.

Serum antioxidant activity

Figure 4a, b illustrates the activity of antioxidants (SOD and CAT) in *C. mrigala*. In both pre- and post-challenge

Table 3 GC–MS analysis of *C. racemosa* and their biological activity, molecular are shown

S. no.	Peak%	Compound	Molecular formula	Biological activity
1	44.798	Chloro-xylenol	$C_8H_9C_{10}$	Antibacterial (Capkin et al., 2017)
2	0.476	6-Hydroxy-4,4,7a trimethyl-5,6,7,7a,tetrahydro-benzofuran-2(4H)-1	$C_{11}H_{16}O_{3,}$	Antipyretic (Yang et al., 2011)
3	2.097	Hexadecanoic acid	$C_{17}H_{34}O_2$	Antifungal and antibacterial (Shaaban et al., 2021)
4	8.634	n-Hexadecanoic acid	$C_{17}H_{34}O_2$	Antioxidant (Shaaban et al., 2021)
5	0.887	Palmitic anhydride	$C_{32}H_{62}O_3$	Anticancer (Harada et al., 2002)
6	0.308	Rhodovibrin	$C_{41}H_{60}O_2$	Anticancer (Sakshi et al., 2018)
7	1.580	Phytol	$C_{20}H_{40}O$	Anticancer, anti-inflammatory (Shariare et al., 2021)
8	1.826	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Antimicrobial, anticancer, hepatoprotective, antiarthritic, antiasthma, diuretic (Sergio et al., 2007)
9	6.069	Oleic acid	$C_{18}H_{34}O_2$	Antioxidant(Wei et al., 2016)
10	0.802	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	Anticancer (Harada et al., 2002)
11	0.399	17-Octadecynoic acid, TMS derivative	$C_{21}H_{40}O_2Si$	Antioxidant, hepatoprotective, antihistaminic, and antieczemic activities (Sergio et al., 2007)
12	0.399	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	Antihistaminic, anticoronary, insectifuge, and antieczema (Huang et al., 2010)
13	0.623	Glucobrassicin	$C_{16}H_{20}N_2O_9S_2$	Antioxidant, antimicrobial (Nguyen et al., 2020)
14	0.410	Oleic acid, eicosylester	$C_{38}H_{74}O_2$	Anti-inflammatory, cancer preventive (Wei et al., 2016)
15	0.496	5(a)Pregane-3,20adiol,	$C_{28}H_{43}NO_6$	Antioxidant, antimicrobial (National Center for Biotechnology Information (2022)

investigations, the tissue of *C. mrigala* exhibited a considerable increase in the activity of SOD and CAT enzymes across all experimental groups. The fish that received meals with 4.5% *C. racemosa* supplementation showed a notable enhancement in SOD and CAT activity, in contrast to the control group.

Haematological and immunological parameters post-infection with *P. aeruginosa*

C. mrigala fed with *C. racemosa* diets exhibited significant improvement in haematological and immunological indices ($P < 0.05$) in both pre- and post-challenge phases, with dose influencing the improvement. Fish given diets supplemented with 4.5% (T4) of *C. racemosa* had the greatest levels of haematological parameters (as reported in Table 5) and immunological activity (NBT, lysozyme, and phagocytic activity) compared to both the control and other experimental groups. Similarly, both experimental groups revealed that feeding a meal supplemented with *C. racemosa* resulted in an increase in both haematological and immunological indices (Fig. 5a–c), as reported in Table 5.

The fish given diets supplemented with 4.5% (T4) of *C. racemosa* exhibited the best survival rate. The fish meal with the least quantity of *C. racemosa* had lower haematological values, whereas the control group had the lowest survival rate.

Discussion

Currently, microbial infectious diseases are a powerful barrier to fish farming. This circumstance necessitates using a significant number of antibiotics and antiseptics to reduce fish fatalities and avoid financial losses. Recent research has reignited interest in seaweed as a secure substitute for preventive and therapeutic medications in farmed fish, with the aim of diminishing economic losses resulting from diseases (Nagappan et al., 2021; Thepot et al., 2021).

The phytochemical investigation indicated the presence of alkaloids, flavonoids, saponins, terpenoids, phenolic compounds, carbohydrates, and amino acids. *C. racemosa* lacks glycosides and tannins. Saponin, terpenoid, phenolic compounds, and carbohydrates were not present in the methanol extract of *C. racemosa* in the previous study done by Louiza Belkacemi et al., (2020). According to Sandhya Madan Mohan et al., (2015), phytochemical compounds provide a variety of biological and therapeutic benefits, as well as antioxidant capabilities. Jamuna et al., (2014) discovered a new class of secondary metabolites that had both antibacterial and therapeutic characteristics. Green seaweeds have a higher fatty acid content than other plants (Cardoso et al., 2017). Our findings are congruent with those of Luigi Ornano et al., (2014) and Widjanarko (2016), who found comparable outcomes to our findings.

Table 4 Growth parameters of experimental fish *Cirrhinus mrigala* fed with *C. racemosa* extract

Diets	SGR	FCR	WG	LG	Protein	Carbohydrates	Lipid	Amylase	Lipase	Protease
T_1 (C)	0.91 ± 0.03^{e}	2.76 ± 0.19^{a}	1.07 ± 0.21^{c}	5.60 ± 0.30^{c}	11.89 ± 0.43^{e}	16.40 ± 0.28c	4.51 ± 0.40^{ab}	3.26 ± 0.15^{d}	11.5 ± 0.18^{d}	10.9 ± 0.11^{d}
T_2	2.12 ± 0.03^{d}	2.55 ± 0.16^{ab}	1.64 ± 0.21^{b}	5.96 ± 0.40^{bc}	18.92 ± 0.10^{d}	19.57 ± 0.13b	4.93 ± 0.22^{ab}	4.78 ± 0.17^{c}	18.32 ± 0.11^{c}	12.38 ± 0.26^{c}
T_3	2.95 ± 0.06^{c}	2.37 ± 0.12^{bc}	1.99 ± 0.25^{b}	6.43 ± 0.25a	21.60 ± 0.34^{b}	21.57 ± 0.37[a]	7.58 ± 0.39^{a}	6.92 ± 0.33^{b}	21.05 ± 0.36^{b}	15.71 ± 0.14^{b}
T_4	**4.77 ± 0.08^{a}**	**2.09 ± 0.14^{c}**	**2.81 ± 0.23^{a}**	**6.63 ± 0.30^{a}**	**24.12 ± 0.13^{a}**	**21.57 ± 0.37[a]**	**7.80 ± 0.34^{a}**	**9.44 ± 0.21^{a}**	**28.65 ± 0.21^{a}**	**19.87 ± 0.22^{a}**
T_5	4.11 ± 0.17^{b}	2.18 ± 0.25^{c}	2.51 ± 0.19^{b}	6.60 ± 0.36^{a}	19.89 ± 0.43^{c}	21.36 ± 1.65[a]	8.0 ± 0.61^{a}	7.77 ± 0.10^{a}	20.1 ± 0.44^{ab}	16.87 ± 0.11^{a}

Data expressed as Mean ± SD (n = 3) within the same row sharing the same superscript are statistically significant level ($P < 0.05$)

Different superscript letter indicates significant differences ($p < 0.05$)

T_1-Control group fishes fed with devoid of *C. racemosa* extract

T_2 = the experimental groups fed with 0.5% *C. racemosa* incorporated fed

T_3 = the experimental groups fed with 2.5% *C. racemosa* incorporated fed

T_4 = the experimental groups fed with 4.5% *C. racemosa* incorporated fed

T_5 = the experimental groups fed with 6.5% *C. racemosa* incorporated fed

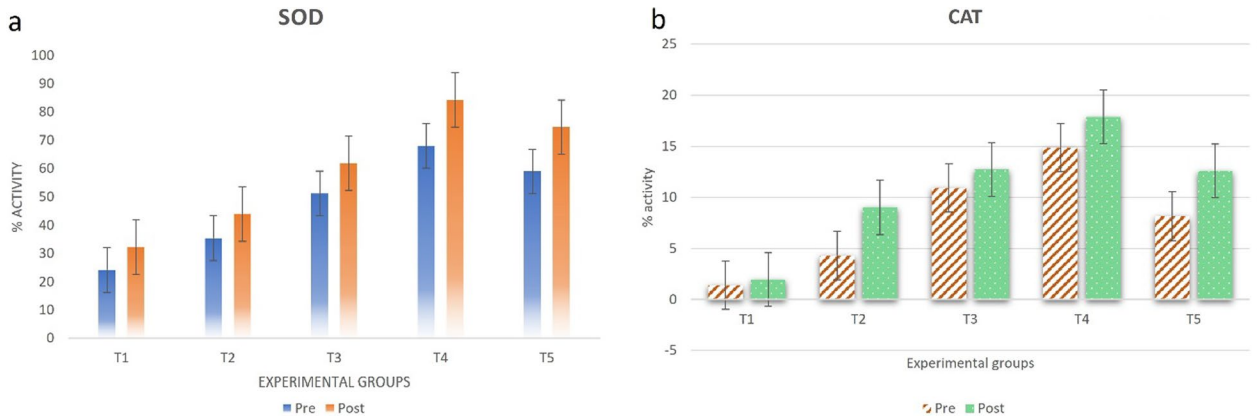

Fig. 4 Serum antioxidant activity (SOD (**a**) and CAT (**b**)) of *C. mrigala*. Data expressed as mean ± SD, n = 3

Table 5 The effect of *C. racemosa* on hematological parameters of *Cirrhinus mrigala*. The mean ± SD (n = 3) values within the same row sharing the same superscript are statistically significant level ($p < 0.05$)

Diets	RBC	WBC	HB	PCV	MCV	MCH	MCHC
T_1(C)	1.05 ± 0.09^{c}	13.66 ± 0.30^{c}	7.82 ± 0.41^{c}	24.29 ± 0.62^{e}	230.50 ± 1.90^{bc}	75.02 ± 4.98^{a}	32.21 ± 1.58^{a}
T_2	1.17 ± 0.17^{abc}	14.58 ± 0.11^{bc}	8.65 ± 0.28^{b}	30.56 ± 0.56^{d}	264.42 ± 0.32^{a}	74.75 ± 0.46^{ab}	28.29 ± 0.40^{bc}
T_3	1.51 ± 0.19^{ab}	15.13 ± 0.23^{ab}	8.72 ± 0.28^{b}	32.14 ± 0.43^{c}	214.97 ± 0.26^{c}	58.40 ± 0.82^{c}	27.13 ± 1.08^{cd}
T_4	1.84 ± 0.32^{a}	15.87 ± 0.94^{a}	9.57 ± 0.28^{a}	37.07 ± 0.30^{a}	205.18 ± 0.45^{d}	53.21 ± 1.72^{d}	25.83 ± 0.92^{d}
T_5	1.62 ± 0.18^{a}	15.61 ± 0.23^{ab}	9.68 ± 0.24^{a}	34.14 ± 0.21^{b}	231.89 ± 0.10^{bc}	60.21 ± 3.72^{b}	28.36 ± 0.61^{b}

Different superscript letter indicates significant differences ($p < 0.05$)

T_1-Control group fishes fed with devoid of *C. racemosa* extract

T_2 = the experimental groups fed with 0.5% *C. racemosa* incorporated fed

T_3 = the experimental groups fed with 2.5% *C. racemosa* incorporated fed

T_4 = the experimental groups fed with 4.5% *C. racemosa* incorporated fed

T_5 = the experimental groups fed with 6.5% *C. racemosa* incorporated fed

Fatty acids play a crucial role in animal nutrition by providing the necessary fatty acids and transporting fat-soluble vitamins. It contains biological properties such as antibacterial, anticancer, and antioxidant activity. Seaweed contains several fatty acids. Seaweeds have been shown to promote growth, stimulate non-specific host immunity, and inhibit bacterial activity (Karthikeyan et al., 2015; Kolanjinathan & Stella, 2009b; Kolanjinathan et al., 2009). The current study showed *C. racemosa* contains eicosenoic acid, methyl palmitate, hexadecanoic acid, linoleic acid, methyl ester, methyl oleate, phytanic acid, linolenic acid, oleic acid, and palmitic acid fatty acids. The use of edible seaweed as a feed additive has been shown to have a significant influence on the growth, feed intake, pressure condition, and meaty quality of fish and other aquatic animals (Ochang et al., 2007; Ruperez, 2002; Wang et al., 2016).

Natural antioxidants derived from seaweed and other natural sources are more environmentally friendly and less hazardous than manufactured antioxidants. Many studies have shown that seaweeds have strong antioxidant capabilities (Palanisamy et al., 2017). The extract of *C. racemosa* showed an increase in antioxidant activity with increasing concentration. The methanol extract of *C. racemosa* had the best effectiveness at 100 µg/ml against DPPH, ABTS, and H_2O_2 radicals. Our findings align with prior research by Louiza Belkacemi et al., (2020), indicating that increased total phenolic content generally leads to greater antioxidant activity. Polyphenols are a type of flavonoid that exhibits antioxidant, chelating, and cytotoxic activities. Our results agree with those of earlier research (Aftab Uddin et al., 2020), which found that methanolic extracts of *C. racemosa* and *U. intestinalis* were better at fighting free radicals because they contained more flavonoids. Kind of extract, season, location, and species all influence the free radical scavenging activity of extracts (Freitas et al., 2021). Previous research (Ragunath et al., 2020) demonstrated that *C. racemosa* has antibacterial activity against a wide range of pathogens. Yap et al., (2019)

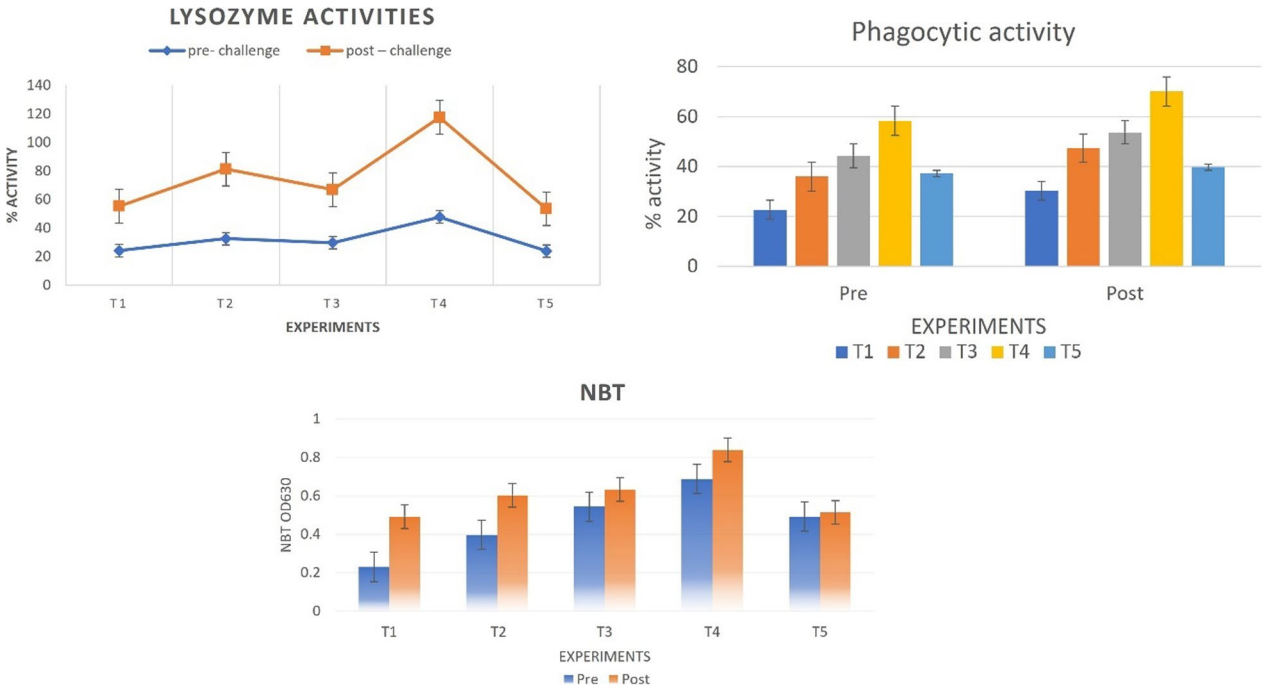

Fig. 5 The effect of *C. racemosa* on lysozyme activity, phagocytic, and NBT activity of *C. mrigala* for pre-challenge and post-challenge in each sampling period. Data expressed as mean ± SD, n = 3. Different superscript values are statistically significant P ≤ 0.05

and Zainuddin et al., (2019) revealed similar findings that corroborate our results.

The effects of the seaweed *C. racemosa* on carp growth performance have yet to be extensively studied. *C. racemosa*-fed groups were shown to have significantly improved growth metrics and non-specific immune responses in *C. mrigala*. In the present investigation, varied dietary amounts of *C. racemosa* extract affected *C. mrigala* growth and feed consumption in a dose-dependent manner. The growth parameters WG and SRG increased considerably when compared to the control and other experimental groups. The experiment with *C. racemosa*: 4.5% enriched meals (T4) revealed the greatest proportion of WG, SRG, and FCR. The FCR showed that diets containing algal extract were efficient for the growth of *C. mrigala*. Although early research showed that high lipid levels reduce FCR, the FCR index demonstrated that diets including algae were effective. Flavonoids, saponins, tannins, linolenic acid, and phytic acid are secondary metabolites derived from natural sources. These substances have the potential to stimulate the growth of fish and other aquatic animals (Azaza et al., 2007; Francis et al., 2001; Jomeh et al., 2021; Nasmia et al., 2022). Our findings are consistent with Citarasu (2010), who reported a natural chemical capable of improving growth indices in fish and prawns. Our results are congruent with the findings of Fang et al. (2023), who discovered that *Cyprinus carpio* growth performance may be enhanced by supplementing diets with seaweed fucoidan at doses ranging from 1.6 to 1.75 mg/kg^{-1}.

In this study, we discovered that the fish feed included *C. racemosa* at a concentration of 6.5%, which may be over the recommended range, resulting in decreased growth indices. Our results were similar to those of Zhou et al., (2007), who indicated that fish and shrimp have an ideal amount of dietary lipids for development; when they exceed this level, their growth decreases. Our results supported those of Al-Asgah et al. (2016), who found that 10% *G. arcuata* diets inhibited the growth of *C. gariepinus*.

Body composition is like protein, carbohydrate, fat, and ash, indicating the animal's physiological status and health (Younis et al., 2018). The current study found that dose-dependent protein, carbohydrate, and fat increased in meals containing *C. racemosa* extract. The lipid content increased during the experiment compared to the control group, and a higher lipid content was observed in the T5 groups. This study backs up Mohan et al., (2019), findings that *Ganoderma lucidum* consumption improves aquatic animal development and body composition. Our findings complement Gora et al., (2018) study that feeding *Sargassum wightii* to *Labeo rohita* improves growth performance and carcass composition.

The present investigation discovered that consuming *C. racemosa* extract significantly enhanced the activity of digestive enzymes (amylase, protease, and lipase). When compared to the control and other experimental groups, 4.5% of the *C. racemosa*-incorporated diets had a significant influence on the observed digestive activity. The digestive enzyme activity of fish and other aquatic animals is essential for their nutritional physiology and development (Wang et al., 2016). The present investigation demonstrated significant increases in digestive enzyme levels in the groups treated with *C. racemosa* extract. These results clearly show that *C. racemosa* extract has the ability to boost food intake and *C. mrigala* growth. Our results support the findings of Mohan et al., (2019), who observed that ingesting *Ganoderma lucidum* enhances development and digestive enzymes in aquatic animals.

In response to oxidative stress, the development of superoxide dismutase (SOD) is an important antioxidant defence enzyme in fish. Similarly, CAT and peroxidase play important roles in the physiological process and contribute to the defence mechanism (Sivagurunathan et al., 2012). In a post-challenge study, meals supplemented with *C. racemosa* showed increased levels of SOD and CAT compared to the control group. The findings are consistent with earlier research by Prabhu et al., (2018) and Zeynali et al., (2020). *Syzygium cumini* increased SOD activity in *Litopenaeus vannamei* when exposed to *Vibrio parahaemolyticus*. Similarly, Vinosha et al., (2020) found that when Tilapia were given galactan and subsequently infected with *A. hydrophila*, the activity of antioxidant enzymes increased.

Hematological indicators are critical tools for evaluating the health of fish and mammals. *C. racemosa*-treated tests resulted in enhanced hematological parameters. These studies demonstrated that *C. mrigala* has an enhanced non-specific immune system. The non-specific immune system relies heavily on lysozyme, NBT, and phagocytic activity. Our findings corroborate Natify et al., (2015), who found that the combination of fish meal and dietary *Ulva lactuca* modifies the hematological parameters of *O. niloticus*. Our findings are consistent with Sivagurunathan et al., (2012) finding that *C. carpio* hematological parameters improved after receiving *Nelumbo nucifera*.

The increase in hematological and immunological markers shows that *C. racemosa*-supplemented diets boost the fish's immune system. Leukocyte, lysozyme, NBT, and phagocytic activity were greater in the T4 group compared to the control group and other experiments, which might be associated with an increase in non-specific immunity. Vazirzadeh et al., (2020) research is consistent with our findings. Incorporating algae into the diet of *Oncorhynchus mykiss* resulted in a notable increase in immunological parameters. In line with the findings of Yang et al., (2014), our study observed elevated levels of antioxidant enzymes in fish that were given seaweed meal supplementation. Similar findings are reported to support our results: incorporating *Ulva lactuca* and *Ulva intestinalis* into the diet resulted in an elevation of antioxidant, immunological, and biochemical activities (Abdel-Warith et al., 2015; et al., 2022).

In the present investigation, feed supplementation with *C. racemosa* extract promoted considerable growth and also improved immunological parameters against the pathogenic bacteria *P. aeruginosa*. As a consequence, the study's results imply that fed with 4.5% *C. racemosa* extract into diet might boost immune system and resistance to infection. More study is required to identify and isolate the active components in *C. racemosa*. This work might lead to new uses for seaweed in aquaculture.

Conclusion

In conclusion, dietary supplementation with *C. racemosa* can induce positive effects on growth performance, feed utilization, innate immunity, digestive enzyme activity, and disease resistance to *P. aeruginosa*. In addition, it can be used as an alternative to antibiotics and decrease chemical usage in *C. mrigala* aquaculture. However, more research on the dosage of *C. racemosa*, extraction techniques, and immune genes is required. This research may lead to identifying new novel compounds from the findings.

Abbreviations

DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS	%, 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
H_2O_2	Hydrogen peroxide
%	Percentage
µg/ml	Microgram per millilitre
GC–MS	Gas chromatography–mass spectrometry
WG	Weight gain,
SGR	Specific growth rate
SOD	Superoxide dismutase,
CAT	Catalase
RBC-	Red blood cells
WBC	White blood cells
HCT	Hematocrit
Hb	Haemoglobin
NBT	Nitroblue tetrazolium test
g	Gram
hrs	-Hours
BOD	Biological oxygen demand
COD	Chemical oxygen demand
Do_2	Dissolved oxygen
cm	Centimetre
FCR	Feed conversion ratio
FL	Final length

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Author contributions
V. Ramasubramanian and C. Ragunath conceptualised and designed the study, and Cholaraj Ragunath conducted the research, experimented, gathered data, and prepared the text, figures, and table. The manuscript was reviewed and approved for submission by all authors.

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Availability of data and materials
The authors confirm that the manuscript contains all relevant data.

Declarations

Competing interests
The authors declare no conflict of interest.

Ethics approval and consent to participate
No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

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