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Growth dynamics and the proximate biochemical composition and fatty acid profile of the heterotrophically grown diatom *Cyclotella cryptica*

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Abstract To investigate the nutritional value of the diatom *Cyclotella cryptica* as an alternative feed for aquaculture, its heterotrophic growth characteristics were studied. First, the proximate biochemical composition and fatty acid profiles were studied under a controlled heterotrophic growth condition. The approximate total ash, carbohydrate, lipid, and protein content were 245 mg g^{-1} (dry weight), 360 mg g^{-1}, 165 mg g^{-1} and 260 mg g^{-1}, respectively. Polyunsaturated fatty acids accounted for 24.5, 31.3, 45.1 and 17.3% of the total lipids in the phospholipid, sterol, free fatty acid and triglyceride classes. Secondly, the effect of aeration and agitation rates on the specific growth rate of *C. cryptica* under heterotrophic conditions was studied. The maximum specific growth rate was not significantly affected ($P>0.05$) by the rate of agitation within the range of 100 to 160 rpm, but it was significantly affected ($P>0.05$) by the rate of aeration. Optimal growth occurred when the aeration rate was within the range of 0.44 to 1.07 *v*/*v*/min. Viability measurements throughout the growth period showed that the *C. cryptica* cells remained viable in spite of the varied cultivation conditions. Hydrodynamic forces are an important parameter within biological systems, and optimisation is crucial for the successful scale-up of microalgal cultivation systems. Whilst the investigation was preliminary in nature, the information gained in this study will be useful for the continual development of an alternative and cost-effective feed for bivalve spat rations.

Keywords Ash · Carbohydrate · Lipid · Mixing · Protein · PUFAs · Bacillariophyta

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Introduction

There is a growing body of evidence that indicates the importance of polyunsaturated fatty acids (PUFAs) in human diets. PUFAs have specific physiological functions, and clinical studies have shown that an increase in consumption of the essential fatty acids, eicosapentaenoic acid (EPA, 20:5 *n*−3) and docosahexaenoic acid (DHA, 22:6 *n*−3), can result in improved general health and well-being. At present, these essential fatty acids are largely obtained through the consumption of seafood. However, many fish stocks are currently exploited above the maximum sustainable level, and many species could be vulnerable to extinction if current practices are maintained. In 2007, the Food and Agriculture Organisation of the United Nations reported that approximately 75% of the native fish stocks were fully exploited, overexploited, depleted or recovering from depletion (FAO Fisheries and Aquaculture Department 2007).

It has been widely suggested that the increased demand for seafood may be met by the expanding aquaculture industry. However, despite significant investments to date, the consistent supply of highly nutritious live microalgae remains a major 'bottleneck' and can affect the productivity and profitability of many commercial hatchery and nursery operations. Furthermore, the cultivation of microalgae is likely to remain a vital process within most aquaculture

facilities as microalgae are the biological starting point for energy flow through aquatic ecosystems (De Pauw et al. 1984), and live microalgae are an essential feed source for molluscs, crustaceans and fish species, for at least part of their life cycle.

The nutritional value of the microalgal biomass depends on several physiological and biochemical attributes including microalgae size and shape, digestibility, non-toxicity and biochemical composition. Furthermore, the nutritional value of the microalgal biomass should match the nutritional requirements of the target organism (Webb and Chu 1983; Brown et al. 1989). The biochemical composition of microalgae is influenced by environmental conditions (light, temperature, salinity, pH, aeration), nutritional factors (sources and availability) and culture age (Brown et al. 1989; Dunstan et al. 1992; Renaud and Parry 1994).

Traditional microalgae cultivation techniques, which rely on photosynthetic microalgae (grown in outdoor ponds or indoors under artificial lights), are generally expensive due to the relatively low biomass concentrations achieved, large operating costs, culture inefficiencies and unexplained crashes. It has been estimated that microalgal production costs can be as high as US$ 600 kg^{-1} (Borowitzka 1997). Several reports including Gladue and Maxey (1994), Duerr et al. (1998), Heasman et al. (2002), Brown (2002) and Harel et al. (2002) have outlined that heterotrophically grown microalgal biomass is potentially a cost-effective alternative. The advantages to the use of heterotrophically grown microalgal biomass have previously been reviewed (Gladue and Maxey 1994; Chen 1996; Wen and Chen 2003). However, oxygen is frequently a limiting factor during the heterotrophic cultivation of microalgae (Beherns 2005), although this limitation is often preventable by adequate mixing.

Mixing affects the hydrodynamic conditions and is a critical parameter within biological systems and is important to: (1) prevent cell sedimentation; (2) prevent the formation of environmental, nutritional and gaseous gradients, thereby reducing boundary layers and improving mass and energy transfer rates; and (3) move cells through optical gradients, thereby varying the light regime (quantity and quality of light and frequency of the light/dark cycles) experienced by the microalgae cells (phototrophic and mixotrophic growth modes only).

Mixing can be accomplished via mechanical agitation, aeration or a combination of both. Mechanical agitation, through the use of impellers and baffles, often provides sufficient mixing (Beherns 2005); however, aeration is frequently required to satisfy the oxygen demand associated with the high cell densities achieved during heterotrophic cultivation. Unfortunately, excessive mixing may generate high shear conditions, which can lead to impaired cell growth, cell damage and eventually cell death. The tolerance to shear stresses and turbulence is species-dependent and rates of shear are influenced by the rate and method of mixing, geometry of vessel, vessel surface, nutrient composition and cell concentration.

The aim of the present work is to investigate the heterotrophic growth characteristics and nutritional value of the diatom *Cyclotella cryptica*. This species is capable of heterotrophic growth (Hellebust 1971; White 1974; Gladue and Maxey 1994), has previously been used within the aquaculture industry (Loosanoff and Davis 1963; Laing and Millican 1992) and has been recommended as a species worthy of further investigation. The work presented in this paper details the proximate biochemical composition and fatty acid profile of *C. cryptica* under a controlled heterotrophic growth condition. Although this species is known to grow heterotrophically and has previously been used in aquaculture, its proximate biochemical composition and the fatty acid distribution under heterotrophic conditions have never been reported. The present paper also examines the individual effects of agitation and aeration rates on the specific growth rate of the diatom *C. cryptica* when cultivated heterotrophically.

Materials and methods

The diatom *Cyclotella cryptica* (UTEX 1269) was cultured heterotrophically in a modification of SK medium reported by Gladue and Maxey (1994). The modified medium used during the investigation into the proximate biochemical and fatty acid profile consisted of (per litre) 27.2 g synthetic sea salt (Taikong Corp., Taipei, Taiwan), 2.17 g $MgSO_4 \cdot 7H_2O$, 1.6 g tryptone, 917 mg $NaNO_3$, 892 g Tris buffer, 800 mg yeast extract, 50.5 mg KH_2PO_4, 34 mg H_3BO_3, 20 mg $FeSO_4 \cdot 7H_2O$, 15 mg $NaH_2PO_4 \cdot 2H_2O$, 6 mg thiamine·HCl, 5 mg Na_2EDTA, 4.3 mg $MnCl_2 \cdot 4H_2O$, 0.3 mg vitamin B_{12}, 0.3 mg biotin, 0.3 mg $ZnCl_2$, 0.26 mg $NiSO_4 \cdot 6H_2O$, 0.13 mg $CoCl_2 \cdot 6H_2O$, 0.03 mg $Na_2MoO_4 \cdot 2H_2O$, 0.017 mg Na_2SeO_3 and 0.01 mg $CuSO_4 \cdot 5H_2O$ and was supplemented with 10 g L^{-1} glucose and 480 mg L^{-1} $Na_2SiO_3 \cdot 5H_2O$. The pH of the medium was adjusted to 7.5 prior to autoclaving at 121°C for 20 min. The medium used during the investigation of the effects of agitation and aeration was the same as above, with the following exceptions: 6.8 g synthetic sea salt and the omission of the Tris buffer, tryptone and yeast extract.

Cultivation systems

Three different cultivation systems were investigated: 250 mL Erlenmeyer flasks containing 100 mL sterilised medium were incubated on orbital shakers at five mixing rates (100, 115, 130, 145 and 160 rpm); 500 mL Schott

bottles containing 400 mL sterilised medium were incubated at three aeration rates (0.28, 0.44 and 1.07 *v*/*v*/min); and 19 L carboys containing 16 L sterilised medium were incubated with an aeration rate of 0.25 *v*/*v*/min. All cultivation systems were inoculated with 6–10% exponentially growing culture and were incubated at 25°C in darkness. Air (if supplied) was filtered through 0.22-μm filters.

Determination of biomass, growth rate and viability

The biomass concentration was determined spectrophotometrically from the culture absorbance measured at 675 nm and was compared to a correlation curve that was previously developed (Pahl, unpublished data). At the termination of the experiment, the biomass was harvested by centrifugation (15,700 rcf [relative centrifugal force] for 10 min), rinsed twice with purified water, dried in a freeze drier and stored at −18°C prior to analysis. The specific growth rate was determined by plotting the natural logarithm of culture dry weight against time. Readings within the exponential phase were then used to obtain the maximum specific growth rate by linear regression. Cell viability was determined by the method reported by Crippen and Perrier (1974).

Determination of nutrient status

Throughout the cultivation, samples of the culture suspension were centrifuged (15,700 rcf for 10 min) and the supernatant stored at 4°C until analysed. The residual glucose concentration in the media was determined based on the dinitrosalicylic acid assay (Miller 1959). A 1-mL sample and 5-mL dinitrosalicylic acid reagent was heated to 95°C for 10 min in tightly capped tubes. Tubes were allowed to cool to room temperature and absorbance at 570 nm was measured and compared to known glucose standards. The residual sodium metasilicate concentration in the media was determined based on the method reported by Hansen and Koroleff (1999).

Determination of proximate biochemical composition and fatty acid profile

Ash was determined gravimetrically using a method based on ASTM E-1755 (1995). Freeze-dried biomass was heated to 575ºC and held until constant weight in a muffle furnace. Carbohydrate content was determined using the phenol–sulphuric acid method with the absorbance measured at 485 nm following extraction with 0.5 M sulphuric acid at 90°C for 60 min (Dubois et al. 1956). Total nitrogen was determined with a Leco TruSpec CHN analyser (Leco Australia, Castle Hill, NSW, Australia), and crude protein calculated by total nitrogen multiplied by a conversion factor of 6.25 (Dorsey et al. 1978). Total lipids were extracted with 6 mL of chloroform/methanol (2:1, *v*/*v*) and 1.5 mL saline. Lipid extracts were evaporated to dryness in a vacuum concentrator at 60°C prior to being weighed.

The lipids were separated by thin layer chromatography (TLC) into phospholipid, triglyceride, cholesterol ester, free fatty acid and sterol classes on silica gel plates. The solvent system for TLC was petroleum spirit/diethyl ether/glacial acetic acid (180:30:2, *v*/*v*/*v*). Lipid classes were visualised with fluorescein 5-isothiocyanate against TLC standard 18-5 (Nu-Chek Prep, Elysian, USA). All solvents contained the antioxidant butylated hydroxy anisole at 0.005% (*w*/*v*). Lipid fractions were transesterified by methanolysis (1% H_2SO_4 in methanol) for 3 h at 70°C (Blank et al. 2002). After cooling, the resulting fatty acid methyl esters (FAMEs) were extracted with *n*-heptane and dried over anhydrous sodium sulphate. The FAMEs were analysed with a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionisation detector and a BPX-70 (50 m × 0.32 mm ID × 0.25 μm film thickness) capillary column (SGE Pty Ltd, Australia). Helium was used as the carrier gas with a column flow rate of 34 cm/s. The injector and detector temperatures were set at 250°C and 300°C, respectively. Samples (5 μL) were injected with a split ratio of 20:1. The initial oven temperature was 140°C and programmed to rise to 220°C at 5°C min^{-1}, held for 1 min, followed by a further rise to 260°C at 20°C min^{-1}. FAMEs were identified by comparison of retention times to authentic lipid standards (Nu-Chek Prep, USA).

Statistical analysis

Significant differences between experimental conditions were detected by means of one-way analysis of variance (ANOVA), followed by pairwise comparisons using

Table 1 Proximate biochemical composition (percentage of dry weight) of *Cyclotella cryptica* biomass (n=2, unless otherwise specified)

Mode of growth	Heterotrophic (present study)	Photoautotrophic (Darley 1977)
Protein	26.0±0.2	31
Carbohydrate	35.8±1.2	26
Lipid	16.5[a]	12
Ash	24.6±0.2	n.r.

Biomass cultivated in 250 mL Erlenmeyer flasks containing 100 mL media and agitated on an orbital mixer at 100 rpm

n.r. not recorded

[a] n=1

Table 2 Fatty acid composition (percentage of individual lipid class) of *Cyclotella cryptica* biomass grown under heterotrophic conditions (n=1)

	Phospholipid	Sterol	Free fatty acid	Triglyceride
9:0	n.d.	n.d.	0.1	tr.
10:0	n.d.	n.d.	tr.	tr.
12:0	tr.	n.d.	0.1	tr.
13:0	0.1	0.3	n.d.	tr.
14:0	1.8	1.4	1.2	2.0
15:0	1.7	2.4	0.7	0.9
16:0	38.2	13.0	16.5	17.9
17:0	0.9	0.9	0.3	0.8
18:0	0.8	1.5	1.0	0.6
20:0	tr.	n.d.	n.d.	tr.
22:0	tr.	n.d.	0.1	0.1
24:0	0.1	n.d.	0.2	0.1
Sum saturated	43.6	19.4	20.3	22.3
Trans 16:1	tr.	n.d.	n.d.	n.d.
Trans 18:1 n−9	tr.	n.d.	n.d.	tr.
Trans 18:2	0.1	n.d.	0.1	tr.
Sum trans	0.1	0.0	0.1	0.0
12:1	tr.	n.d.	n.d.	tr.
13:1	tr.	0.1	n.d.	n.d.
14:1	n.d.	0.1	n.d.	tr.
16:1 n−9	0.2	0.3	0.3	0.2
16:1 n−7	26.8	45.5	31.0	57.6
17:1	0.1	0.2	n.d.	0.3
18:1 n−9	1.6	1.6	1.7	1.1
18:1 n−7	1.1	1.0	1.1	0.8
19:1	tr.	n.d.	n.d.	tr.
20:1 n−11	n.d.	n.d.	n.d.	tr.
22:1 n−11	0.6	0.5	0.5	0.3
22:1 n−9	n.d.	n.d.	n.d.	tr.
24:1 n−9	n.d.	n.d.	n.d.	tr.
Sum MUFA	30.5	49.3	34.5	60.3
18:2 n−9	1.1	1.0	0.4	0.6
20:2 n−9	tr.	n.d.	n.d.	tr.
20:3 n−9	tr.	n.d.	n.d.	tr.
Sum n−9	1.1	1.0	0.4	0.7
18:2 n−6	0.8	0.6	0.7	0.2
18:3 n−6	0.7	0.5	0.2	0.3
20:3 n−6	tr.	n.d.	n.d.	tr.
20:4 n−6	0.1	0.1	0.2	0.1
22:2 n−6	n.d.	n.d.	n.d.	tr.
22:5 n−6	1.7	3.8	2.5	0.4
Sum n−6	3.3	5.1	3.7	1.0
16:2 n−3	1.8	3.3	0.2	0.4
18:3 n−3	0.1	0.2	n.d.	tr.
18:4 n−3	1.9	1.4	0.5	0.9
20:5 n−3	15.8	17.8	38.0	13.9
22:5 n−3	tr.	n.d.	n.d.	tr.
22:6 n−3	1.8	2.7	2.2	0.5
Sum n−3	21.4	25.3	41.0	15.7
Sum PUFA	25.7	31.3	45.1	17.3

Biomass cultivated in a 250-mL Erlenmeyer flask containing 100 mL media and agitated on an orbital mixer at 100 rpm

n.d. not detected, *tr.* trace, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

Tukey's test, where appropriate. Equality of variance was checked using the modified Levene procedure.

Results

Proximate biochemical composition and fatty acid profile

Cyclotella cryptica cells were harvested during the early stationary phase of growth. The onset of the stationary phase of growth was caused by a depletion of sodium metasilicate (data not shown). The proximate composition of the harvested biomass is shown in Table 1. The phospholipids, sterols, free fatty acids and triglycerides were extracted from *C. cryptica* and separated by TLC. Only a minor quantity of the cholesterol ester class was detected and consequently could not be analysed. The fatty acid composition of each lipid class is shown in Table 2. The major fatty acids in each lipid class are palmitic acid (16:0), palmitoleic acid (16:1 n−7) and EPA (20:5 n−3).

Agitation and aeration

Cyclotella cryptica was cultivated under a variety of heterotrophic growth conditions and was scaled up to a 16-L working volume. The maximum specific growth rates and dry weights at harvest are shown in Table 3. The maximum specific growth rate was not significantly affected (P>0.05) by the rate of agitation within the range of 100 to 160 rpm. Agitation rates above 160 rpm could not be undertaken with the available equipment. Minimal growth occurred when the flasks were manually shaken twice a day (0 rpm). The rate of aeration significantly affected (P>0.05) the maximum specific growth rate. The maximum specific growth rate was higher when aerations rates were within the range of 0.44 to 1.07 v/v/min, as compared to 0.28 v/v/min. There was no significant

Table 3 Maximum specific growth rate of *Cyclotella cryptica* and dry weight at harvest under heterotrophic growth conditions (n=3, unless otherwise specified)

Cultivation condition	Maximum specific growth rate (h^{-1})	Dry weight at harvest (g L^{-1})	Duration of growth (days)
250 mL Erlenmeyer flasks			
0 rpm, no aeration	0.007±0.003	0.17±0.01	3
100 rpm, no aeration	0.052±0.000	1.33±0.04	3
115 rpm, no aeration	0.050±0.001	1.28±0.01	3
130 rpm, no aeration	0.051±0.001	1.36±0.08	3
145 rpm, no aeration	0.051±0.002	1.29±0.18	3
160 rpm, no aeration	0.053±0.002	1.39±0.02	3
500 mL Schott bottles			
0 rpm, 0.28 *v*/*v*/min	0.026±0.000	0.81±0.06	4
0 rpm, 0.44 *v*/*v*/min[a]	0.030±0.001	1.09±0.126	4
0 rpm, 1.07 *v*/*v*/min[a]	0.031±0.000	1.01±0.11	4
19 L carboys			
0 rpm, 0.25 *v*/*v*/min[a]	0.025±0.002	2.11±0.18	12

[a] n=2

difference (P>0.05) in the maximum specific growth rate when the aeration rate was 0.44 *v*/*v*/min or 1.07 *v*/*v*/min. The *C. cryptica* cells remained uniformly suspended within the culture media under all culture conditions except when mixing was absent (i.e. manually shaken flasks). Viability measurements throughout the growth period showed that the *C. cryptica* cells remained viable in spite of the varied culture conditions (data not shown). At the lowest aeration rate (0.25–0.28 *v*/*v*/min), the maximum specific growth rate was not significantly affected (P>0.05) by the culture volume and consequently the heterotrophic cultivation of *C. cryptica* was successfully scaled-up from 400 mL to 16 L without an observed decrease in the maximum specific growth rate.

Discussion

The continuous cultivation of microalgal biomass is a method which is frequently used in aquaculture facilities. This mode of cultivation is beneficial as the microalgal biomass can be continuously harvested whilst maintaining the biomass in the exponential growth phase and minimising rapid changes in biochemical composition and nutritional valve. For these reasons, *C. cryptica* cells used in this study were harvested after they entered the early stationary growth phase. This ensured that sufficient biomass was produced and that the fatty acid profile would be similar to that of the exponential growth phase. The onset of the stationary phase was caused by silicon deficiency. Silicon is often a limiting nutrient for many diatom species as they require silicon to form their silicified cell walls. Silicon deficiency has been shown to induce lipid accumulation in a number of diatoms, including *C. cryptica* (Roessler 1988a), and this may have altered the proximate biochemical composition.

Despite the heterotrophic growth, the proximate biochemical composition outlined in Table 1 is comparable to the values reported under photoautotrophic conditions. This contradicts the generalised belief that lipid content is markedly reduced under heterotrophic conditions (Borowitzka 1997) and enforces that cell metabolism responses are often species specific and should be studied individually. Furthermore, the major fatty acids synthesised under heterotrophic growth are similar to that of photoautotrophic reports (Kates and Volcani 1966; Roessler 1988b) with the exception that hexadecatrienoic acid (16:3 n−3) was not identified. *C. cryptica* was capable of synthesising EPA and DHA under heterotrophic growth conditions. While EPA and DHA are essential fatty acids for bivalves, the reported fatty acid composition is qualitative in nature. Before the nutritional value of *C. cryptica* can be estimated, a detailed quantitative analysis should be undertaken.

There is a possibility that the harvested cells contained residual glucose and the reported carbohydrate level could be overestimated and should be viewed with care. The high ash content shown in Table 1 is typical of many diatom species due to their silicon-based frustules. Further optimisation of the culture and environmental conditions may reduce the ash content, as faster dividing cells have thinner frustules (Lewin and Guillard 1963). *C. cryptica* has been reported to have a specific growth rate of 0.138 h^{-1} (Werner 1977), but the ash content was not reported.

Aeration and agitation are important parameters to ensure optimal growth and productivity of microalgal

cells. The rates of agitation and aeration were investigated separately as combining agitation and aeration can have an increased detrimental effect on the growth of microalgae than with either mechanism alone (Yang and Wang 1992; Joshi et al. 1996). The effect of aeration on the maximum specific growth rate of *C. cryptica* was investigated. While the optimal aeration rate remains unknown, the higher aeration rates (0.44 *v*/*v*/min and 1.07 *v*/*v*/min) increased the maximum specific growth rate. The optimal aeration rate under heterotrophic cultivation would be dependent on the biological oxygen demand (biomass concentration and metabolic rate) of *C. cryptica*. While the range of aeration rates was narrow, a maximum aeration rate of 1 *v*/*v*/min was set as it is typical in the cultivation of other microorganisms. Future investigations should also monitor the concentration of dissolved oxygen in the growth media.

The maximum specific growth rate was higher in the agitated vessels than in the aerated vessels. However, the agitation mechanism used (placing the cultivation vessel on an orbital mixer) is not suitable for large-scale cultivation systems. Mechanical agitators are typical in large cultivation systems; however, the sterility requirements of the culture media necessitate that the mechanical seals surrounding the agitator shaft are often complex and expensive, and therefore, mechanical agitators are not suited to low-cost operations. Continuous recirculation of the culture media can also be used to provide the agitation; however, this adds to the complexity of the cultivation process, and the response of the microalgal cells to the shear generated within a recirculation system is unknown. Consequently, aeration is likely to be the preferred method of mixing, and many existing microalgal cultivation systems utilise bubble-column reactors.

In this investigation, aeration was achieved by pumping filtered air through an in-house sparger. Spargers were used as, for a given air flow rate, they emit a larger number of smaller bubbles than a capillary tube, which emits a smaller number of larger bubbles. The smaller bubbles provide a higher surface area per unit volume, and this should improve the mass and energy transfer rates. However, cell damage primarily occurs at the culture media–air interface when the bubble film ruptures (Joshi et al. 1996; Mazzuca Sobezuk et al. 2006). Consequently, the microalgal growth rate and cell viability will depend on the bubble size, bubble frequency and the shear tolerance of the individual microalgal cells.

Rocha et al. (2003) investigated the effect of aeration by sparging and capillary on the growth of *Nannochloropsis gaditana*. The authors reported that *N. gaditana* responded better when air was supplied via capillary compared to the sparger. However, no actual information was presented on bubble size or shear rates.

This paper has investigated the effects of agitation and aeration on the specific growth rate and cell viability of heterotrophically cultivated *C. cryptica*. Ultimately, aeration and/or agitation rates should be sufficient to provide adequate mixing, whilst not inhibiting growth or causing cell damage or death by lysis. No critical or detrimental growth conditions were uncovered. In order to improve the productivity of large-scale cultivation systems, future investigations should quantify the critical shear stresses for different microalgae species. In addition, agitation and aeration can affect the biochemical composition of microalgae cells, and future research should investigate the effect of aeration rates on the biochemical composition of *C. cryptica*. Whilst the investigation was preliminary in nature, this paper has reported the growth rate, proximate biochemical composition and fatty acid profile of *C. cryptica* under heterotrophic growth conditions. It was outside the scope of this study to determine if the nutritional value of *C. cryptica* could be enhanced by heterotrophic cultivation. In order to be nutritionally sufficient, microalgal biomass must supply a balanced mixture of nutrients that matches the nutritional requirements of the target organism. More studies are needed for optimisation of the culture medium and environmental conditions associated with the heterotrophic growth and resulting nutritional value of *C. cryptica*. Further research may show that consistent and high-quality biomass can be economically produced and used as an alternative feed for bivalve spat.

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