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Research Article

Potential for Concentrated Microalgae as Replacement Diets for Juvenile Green-Lipped Mussels, *Perna canaliculus*

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The nursery culture of bivalves typically relies on the feeding of costly live microalgae, while the use of natural sources of phytoplankton for feed is uncertain due to their variable quality and abundance. Replacement diets have been applied in bivalve nursery culture to replace live microalgae with varying success. This study investigated the potential use of two concentrated microalgal diets at a range of levels of substitution with live microalgae. Shellfish Diet 1800® (called SD) and LPB™ Frozen Shellfish Diet® (called LPB) were fed to juvenile green-lipped mussels (*Perna canaliculus*) at five levels of substitution for live microalgae (i.e., 0, 25, 50, 75, and 100%) for 27 days. The mortality of mussels fed with 100% LPB replacement was significantly higher than the mortality of mussels fed at the lower levels of replacement, i.e., 0 and 25%. The overall final size of spat tended to decrease with the increasing level replacement of live microalgae. Proximate analysis (i.e., crude ash-free dry weight, crude protein, crude lipid, and carbohydrate) showed that only the proportion of carbohydrate content of spat was influenced by feeding treatments, with the mean total carbohydrate content of mussels tending to decrease with increasing levels of replacement of live microalgae. The results indicate that both concentrated microalgal feeds (SD and LPB) are effective at replacing live microalgae by up to 50% without compromising the survival and nutritional profile (AFDW, protein, lipid, and carbohydrate content) of juvenile green-lipped mussels and are therefore a useful resource for improving the efficiency of production.

1. Introduction

The suspended culture of mussels is normally reliant on seeding the farms with juvenile mussels, known as spat, which are most commonly harvested from the wild using a variety of techniques [1–3]. In New Zealand, an intermittent supply of juveniles or spat of the green-lipped mussel, *Perna canaliculus*, that are attached onto drifting seaweed that is washed ashore at Ninety Mile Beach has supplied around 70% of spat resource for all mussel farms in the country for at least 40 years [4–6]. In 2019, the supply of spat from the wild has contributed to the production of 98 t of market-size mussels (4.7% of global mussel production in the same year), with the total export reaching~NZ$300 million [7, 8].

A large number of mussel spat, consisting of 200 to 2 million of spat per kg of spat material (e.g., seaweed fragments and debris) are routinely harvested from Ninety Mile Beach and transported to mussel farms across the country of New Zealand [9]. The physiological and nutritional condition of these spat during transportation has been reported to be compromised and may affect their subsequent performance once seeded onto coastal mussel farms [10]. Similarly, the transportation of spat of the blue mussel, *Mytilus edulis*, for more than 24 hours can compromise their physiological and nutritional condition [11]. The poor nutritional condition of juvenile mussels has been reported to negatively affect the number and quality of byssus threads that they produced, with starved mussels producing fewer byssus threads that are also weaker [12]. Therefore, a period of

feeding for wild spat has been suggested to improve their nutritional condition prior to seeding onto mussel farms to increase their subsequent retention and survival under aquaculture conditions [13].

Including a nursery culture step for wild mussel spat would significantly increase the cost of production, especially through the provision of large quantities of cultured live microalgal food, which typically constitutes 30–50% of the total cost of hatchery production of shellfish [14, 15]. Moreover, shellfish hatcheries most often need to produce a range of species of microalgae for feeding larvae and juveniles, in order to provide a nutritionally complete diet for ensuring adequate growth and survival [16, 17]. The most common microalgae species used in shellfish hatcheries belong to the genera *Tisochrysis*, *Diacronema*, *Chaetoceros*, *Tetraselmis*, *Nanochloropsis*, *Thalassiosira*, *Skeletonema*, and *Chlorella* [18]. Of these, *Tisochrysis lutea*, *Diacronema lutheri*, and *Chaetoceros calcitrans* are the most commonly used in multispecies microalgae diets in bivalve hatcheries and nurseries [19].

As a consequence of the significant costs involved in feeding live microalgae for the nursery culture of mussel spat, there has been growing interest in their replacement with artificial diets, which have generally been found to produce unsatisfactory results. For example, some artificial diets are difficult to resuspend without disintegration or rapidly drop out of suspension and do not support good growth and survival of shellfish spat [15]. For example, feeding the formulated diet (i.e., MySpat®, INVE Aquaculture, Inc.) was found to negatively affect the feeding activity of green-lipped mussel spat (7 mm in shell length) and often caused mortality among smaller spat [20].

Partial replacement of live microalgae using different types of formulated diets has been reported for facilitating the aquaculture of a number of species of bivalves. For example, yeast-based diets [21], lipid emulsion [22], microcapsule [23], bacteria [24], and microalgal pastes [25, 26] have been used to partially substitute live microalgae for rearing juvenile shellfish. For example, a replacement of 20% of the dried microalgae *Tetraselmis suecica,* 80% of yeast and up to 81% of mixed microcapsules and yeast has been found to be a satisfactory diet for juvenile oysters (*Crassostrea gigas* and *Ostrea edulis*) and the clams (*Mercenaria mercenaria* and *Ruditapes philippinarum*) [15]. In a spat of the mediterranean mussel, *Mytilus galloprovincialis*, feeding of 75,000 cells·ml^{-1} of live microalgae and 2.5% MySpat® on a mussel live weight basis resulted in similar weight gain to the spat fed with 150,000 cells·ml^{-1} of pure live microalgae over a three-week experimental period [27]. In green-lipped mussel spat, a replacement of live microalgae with up to 50% fabricated liposomes or up to 75% MySpat® diet did not compromise the survival of spat over 32 days [28]. However, these previous studies mostly determined the performance of replacement feeds by measuring the survival and growth of the shellfish, rather than examining differences in their final nutritional status. Mussel spat are known to be highly resistant to short-term starvation, exhibiting low mortality (i.e., less than 1.5%) and with their growth only compromised after 9 days without food [29], despite a marked decrease in endogenous energy reserves evident after 8 days of starvation [30]. The nutritional status of spat (i.e., protein, carbohydrate, lipid, and fatty acids) is critical to their subsequent performance in aquaculture and, in turn, is dependent on the abundance and nutritional qualities of their food supply [31]. Therefore, this study aims to investigate the potential for using two commercially available preserved concentrated microalgal shellfish diets for maintaining the growth and survival of green-lipped mussel spat, while also improving their nutritional condition prior to seeding out onto coastal farms.

2. Materials and Methods

2.1. Spat Collection. About 50 g wet weight of hatchery spat (~1.2 mm shell length) sourced from SPAT NZ Ltd. was used for the experiment. They were placed on fine plastic mesh and covered with a cloth wetted with seawater, packed in a polystyrene box, and airfreighted to Leigh Marine laboratory for ~5 h. On arrival, spat were randomly subdivided into 27 aliquots of 1.6 g wet weight (~2000 spat), off which ~75 mg (~100 spat) from each aliquot were randomly subsampled for shell length measurement using image analysis. Those 27 aliquots were then placed into 27 conical bottles of 1.5 L volume containing 1 μm filtered and UV sterilised seawater. Aeration of 18 ml·s^{-1} was supplied from the centre of the base of the conical bottles [32, 33]. The spat were then fed using experimental combinations of live microalgae and concentrated microalgal diets during feeding treatments.

2.2. Marine Microalgal Culture and Commercial Feeds. Axenic cultures of three species of microalgae: *Tisochrysis lutea*, *Diacronema lutheri*, and *Tetraselmis suecica* obtained from CSIRO culture collection were used for the experiment. Starter cultures of *T. lutea* and *T. suecica* were grown in Guillard media, while *D. lutheri* was grown in Walne media under standardised environmental conditions described by Kaplan et al. [34] and Kaspar et al. [35].

Two commercial shellfish diets recommended for feeding juvenile bivalves were sourced from Reed Mariculture; (1) Shellfish Diet 1800® consisting of concentrated and preserved cultured microalgal cells comprising a mix of five microalgae species, i.e., *Isochrysis* sp., *Diacronema* sp., *Tetraselmis* sp., *Thalassiosira weissflogii,* and *Thalassiosira pseudonana* and (2) LPB™ Frozen Shellfish Diet® consisting of a mix of *Tetraselmis* sp., *T. weissflogii, T.*, and *Schizochytrium* sp.

2.3. Feeding Treatment. Nine different feed treatments, each with three replicate tanks, were used to experimentally compare the performance of the mussel spat, i.e., control treatment (a mixture of three species of live microalgae *T. lutea, D. lutheri,* and *T. suecica,* (0% replacement) and feed treatments (25, 50, 75, and 100% replacement of dry biomass of mixed live microalgae for each of both SD and LPB). The three microalgal species used for the experiment have different nutritional compositions and are commonly used for feeding juvenile shellfish in hatcheries and nurseries

[36–38]. Spat in the control treatment were fed using the three microalgal species at a combined concentration of 200,000 cells spat^{-1}·day^{-1} (required to reach the DW feeding target for this experiment), which was reported to promote optimum feeding for green-lipped mussel spat of 0.5–1.2 mm [39]. The proportion of a mixture of live algae for feeding spat in the control group was calculated based on the ratio of their algal cell volumes (i.e., 1 : 1: 0.1, for *T. lutea, D. lutheri*, and *T. suecica* respectively). Therefore, feeding of 200,000 cells spat^{-1}·day^{-1} consisted of 95,238 cells of *T. lutea* and *D. lutheri* and 9,524 cells of *T. suecica.* The feeding concentration was increased every week following the methods of Sanjayasari [40]. The proportion of replacement of SD and LPB was calculated based on the dry mass of microalgae cells following Helm et al. [41] and Utting and Spencer [37]. The feeding experiment was conducted over 27 days. Seawater changes were undertaken on a daily basis by draining the conical tanks through a 250 μm mesh size to retain the spat. At the end of feeding treatment, the spat that had died in each tank were sorted and counted under a dissecting microscope. The dead spat were then photographed for digital size measurement. Live spat from each tank were counted and 100 spat were randomly subsampled for size measurement using digital image analyses ImageJ software. The spat were then washed using deionised water and dried using a paper towel. They were then stored in −80°C for later biochemical analysis.

2.4. Biochemical Analyses. Prior to biochemical analyses, 10 ml of each SD and LPB diets were centrifuged at 4000 rpm at 4°C for 10 minutes to separate the seawater content and concentrate the microalgae. The concentrated SD and LPB (without seawater content) and spat from each treatment were freeze-dried for 16 h. Triplicate samples of 100 mg dry weight (hereafter called DW) of SD, LPB, and spat were used for each analysis of ash-free dry weight (AFDW), total carbohydrate, and crude lipid content. For AFDW, 50 mg of spat from each treatment was burned in a muffle furnace at 450°C for 4 h, reweighed, and the results were used to calculate the proportion of AFDW of spat. For total carbohydrate analysis, the freeze-dried SD, LPB, and spat from each treatment were prepared following Wang et al. [42]. The total carbohydrate content of spat, SD, and LPB were determined using the phenol sulphuric acid reagent method of Dubois et al. [43] by reading the absorbance against a D-glucose standard at 490 nm [44]. Crude lipid content was extracted using a modified methanol-chloroform solvent extraction of Wang et al. [45]. A 50 mg freeze-dried lipid-free residue from the lipid assay was used for protein analysis. Crude protein content was measured using the bicinchoninic acid (BCA) method with a micro-BCA protein assay kit (ThermoFisher Scientific, USA) following Wang et al. [42]. The absorbance of samples was read against a bovine serum albumin (BSA) standard as a reference at 562 nm.

2.5. Statistical Analyses. One-way ANOVAs were used to compare the mean values of all parameters observed (i.e., size of dead spat, increase in shell length of spat, proportion of AFDW, total carbohydrate, crude protein, and crude lipid) among spat from different feeding treatments after confirming the normality (Kolmogorov–Smirnov test) and homogeneity (Levene's test) of variances. In addition, the mortality of spat in relation to the increasing replacement of live microalgae with the commercial shellfish diets for the two feeding treatments were compared using linear regression. All percentage data were arcsine transformed prior to use in ANOVA. A Kruskal–Wallis test was used to compare the experimental results among groups for those data where the assumptions of normality and homogeneity of variances could not be met. Pairwise Tukey's posthoc tests were used for parametric analyses and Dunn's test for nonparametric analyses at a significance level of $\alpha = 0.05$ were used to identify differences among pairs of means where the analyses indicated an overall significant difference among treatments.

2.6. Ethics Statement. This work did not require ethics approval.

3. Results

3.1. Proximate Analysis of SD and LPB. The nutritional compositions of SD and LPB were both dominated by crude protein content of 30.3% and 20.0% DW, respectively, followed by crude lipid and carbohydrate. The proportion of crude lipid were 6.7% DW for SD and 7.6% DW for LPB. The carbohydrate content of SD and LPB were both only 0.6% DW (Table 1).

3.2. Spat Mortality. The mean percentage of dead spat in each of the experimental feeding combinations following 27 days of culture ranged from 12 ± 1% to 43 ± 2%. There was a linear relationship between the proportion of dead spat and the increasing replacement of live microalgae with commercial shellfish diet regardless of the type of commercial diet used, i.e., SD ($F_{(4,10)} = 5.19$, $P < 0.05$, $R^2 = 0.55$) and LPB ($F_{(4,10)} = 11.20$, $P < 0.01$, $R^2 = 0.74$) (Figure 1). However, pairwise comparisons among means for feeding treatments showed that there was no statistical difference in the percentage of dead spat for 0%, 25%, and 50% replacement treatments for either the SD or LPB diets, while the replacement of live microalgae using 100% and 75% LPB and 100% SD resulted in higher mortality of spat, i.e., 41 ± 5%, 36 ± 2% and 43 ± 2%, respectively, compared to the 0 and 25% replacement treatments. The size of dead spat was different among feeding treatments ($\chi^2_{(8)} = 18.32$, $P < 0.05$). Overall, the size of dead spat tended to increase with the increasing level of replacement but a significant difference was only observed between spat fed with 0% replacement (1.3 ± 0.0 mm) and spat fed with 100% SD (2.0 ± 0.0 mm).

3.3. Spat Growth. The growth rate of spat overall treatments following 27 d of experimental feeding was significantly different ($F_{(8,26)} = 16.30$, $P < 0.01$), ranging from 0.02 ± 0.00

Table 1: Proximate composition for the mixture of live microalgae used in the control treatment [48] and SD and LPB diets as determined in this study.

Composition	Mg·g dry weight^{-1} (% DW)		
	Mixture of live algae (*T. iso*, *D. lutea*, and *T. suecica*)	SD	LPB
Crude protein	430 (43.0)	302.6 ± 35.4 (30.3)	206.4 ± 13.1 (20.6)
Crude lipid	121 (12.1)	66.6 ± 1.5 (6.7)	75.8 ± 4.3 (7.6)
Carbohydrate	151 (15.1)	5.6 ± 0.1 (0.6)	5.6 ± 0.3 (0.6)
Crude ash	—	465.7 ± 6.0 (46.6)	295.3 ± 5.4 (29.5)

to 0.07 ± 0.00 mm·day^{-1} (Figure 2). The growth rate of spat fed with a mixture of live microalgae (control group) was the highest (0.07 ± 0.00 mm·day^{-1}), and it decreased when the live algae were replaced by 25% of SD (0.04 ± 0.00 mm·day^{-1}). The growth rate of spat fed with LPB showed a decreasing trend with the increasing proportion of replacement of live microalgae but was only statistically significant after the live microalgae feeding was replaced by 75% LPB (0.03 ± 0.00 mm·day^{-1}). Spat fed with 100% of SD and LPB showed the lowest growth rate at 0.02 ± 0.00 mm·day^{-1} for both SD and LPB diets.

3.4. AFDW. The mean percentage of AFDW of spat from all treatments ranged from 26.4 ± 0.6% to 36.1 ± 1.9% and was significantly different among feeding treatments ($\chi^2_{(8)} = 17.70$, $P < 0.05$) (Figure 3). There was an overall trend for AFDW to decrease with increasing replacement of live microalgae with either of the two commercial shellfish diets. However, the only statistical difference in AFDW was between spat from 0% replacement (26 ± 0.6%) compared to spat from 100% replacement with SD (36 ± 1.9%). The small number of differences detected among the treatments will be due in part to the low statistical power of the nonparametric comparisons.

3.5. Biochemical Composition of Spat. The mean crude protein content of spat as a proportion of tissue dry mass was not significantly different among feeding treatments ($F_{(8,18)} = 1.25$, $P = 0.32$) (Figure 4). The average of crude protein content across nine feeding treatments was 591.3 ± 28.5 mg·g^{-1} dry tissue.

The mean crude lipid content of spat as a proportion of tissue dry mass was not different among different feeding treatments ($\chi^2_{(8)} = 14.6$, $P = 0.07$) (Figure 5). The mean crude lipid content of spat from 0% replacement was 141.6 ± 41.5 mg·g^{-1} dry tissue. The mean crude lipid content of spat fed with 25, 50, 75, and 100% SD replacement was 77.3 ± 21.9, 101.3 ± 5.9, 66.4 ± 2.3, and 61.1 ± 1.1 mg·g^{-1} dry tissue, respectively. Meanwhile, the mean crude lipid content of spat fed with replacement of LPB was 73.7 ± 21.7, 91.0 ± 14.2, 58.1 ± 0.2, and 59.6 ± 4.4 mg·g^{-1} dry tissue, respectively.

The mean total carbohydrate content as a proportion of tissue dry mass of spat from all feeding treatments ranged from 26.5 ± 2.3 to 63.8 ± 12.7 mg·g^{-1} of dry tissue (Figure 6). The mean of the total carbohydrate content of spat was

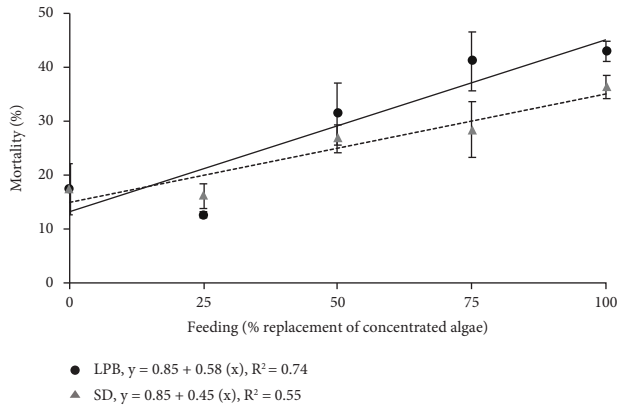

Figure 1: Mean (±SE, $n = 3$) percentage of dead spat for feeding five levels of replacement (i.e., 0, 25, 50, 75, and 100%) of live microalgae with either of two commercial shellfish diets (i.e., LPB and SD) for 27 days. There was a significant relationship between the proportion of dead spat and the increasing replacement of live algae with either SD or LPB ($P < 0.05$).

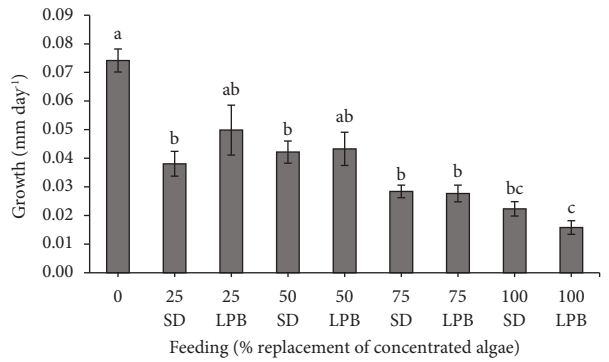

Figure 2: Mean (±SE, $n = 3$) shell length of spat at day 0 and day 27 for feeding five levels of replacement (i.e., 0, 25, 50, 75, and 100%) of live microalgae with either of two commercial shellfish diets (i.e., LPB and SD) for 27 days. Means with different superscript letters are significantly different between feeding treatments and time of measurement ($P < 0.05$).

different among feeding treatments ($F_{(8,18)} = 4.82$, $P < 0.01$). Overall, the mean carbohydrate content of spat tended to decrease with increasing replacement of live microalgae regardless of the type of concentrated preserved microalgae used as a replacement. There were differences between 0% versus 100% SD, 25% SD, and LPB vs. 100% SD and LPB). Spat fed with 100% SD replacement had a lower mean total

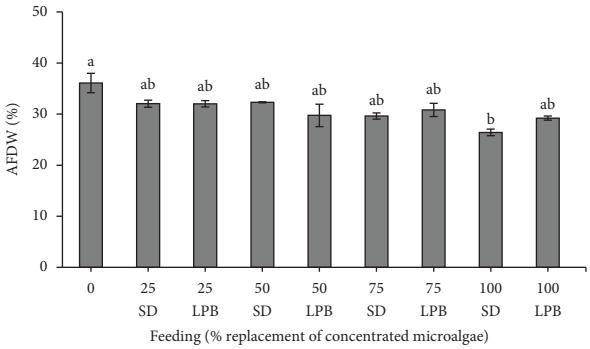

Figure 3: Mean (±SE, $n = 3$) percentage of AFDW of spat in nine experimental feeding treatments after 27 days. Means with different superscript letters are significantly different between feeding treatments ($P < 0.05$).

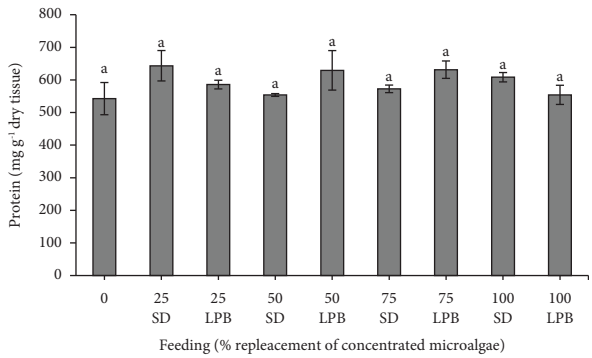

Figure 4: Mean (±SE, $n = 3$) crude protein content of spat as a proportion of tissue dry mass in nine experimental feeding treatments after 27 days. Means with same superscript letters are not significantly different between feeding treatments ($P > 0.05$).

carbohydrate content (26.5 ± 2.2 mg·g^{-1} dry tissue) than spat fed with 0% (59.8 ± 3.5 mg·g^{-1} dry tissue), 25% SD (63.8 ± 12.7 mg·g^{-1} dry tissue) and 25% LPB replacement (61.2 ± 5.8 mg·g^{-1} dry tissue). The mean of total carbohydrates of spat fed with 50%, 75%, and 100% replacement for both SD and LPB were not different to each other.

4. Discussion

The inclusion of an extended nursery stage in the aquaculture production of juvenile bivalves is problematic because of the high cost of providing sufficient live microalgal food [15]. However, transferring early juvenile bivalves to grow-out in coastal farms, in an effort to reduce or eliminate the cost of nursery rearing, can result in high losses of the bivalves. For example, consistently high losses of green-lipped mussel spat have been reported in New Zealand within a few weeks after seeding of the spat onto coastal farms [3, 46, 47]. Replacing the costly production of live microalgae in spat nurseries by either full or partial substitution with more cost-effective replacement diets is therefore a pressing need [28, 48]. The results of the current study indicate that two commercially available diets, LPB and SD, can substitute live microalgae for feeding spat of the green-lipped mussel by up to 50% without significantly

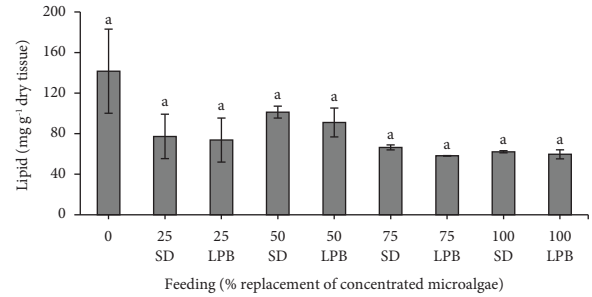

Figure 5: Mean (±SE, $n = 3$) crude lipid content of spat as a proportion of tissue dry mass in nine experimental feeding treatments after 27 days. Means with same superscript letters are not significantly different between feeding treatments ($P > 0.05$).

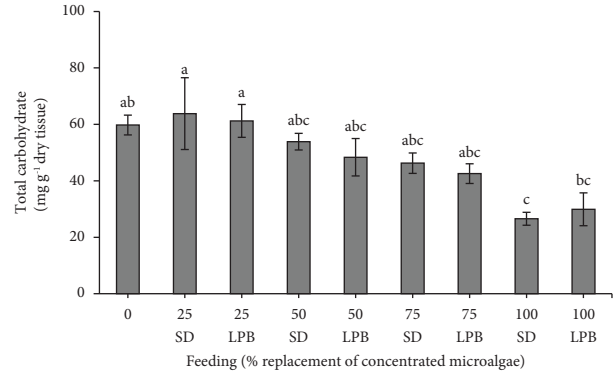

Figure 6: Mean (±SE, $n = 3$) total carbohydrate content of spat as a proportion of tissue dry mass in nine experimental feeding treatments after 27 days. Means with different superscript letters are significantly different between feeding treatments ($P < 0.05$).

compromising the survival and nutritional condition of the spat.

The mortality of spat fed with SD and LPB in this study did not significantly differ from the mortality of spat in the control (0% replacement) until live microalgae were replaced with 75% LPB and 100% SD. This is consistent with previous studies that have reported increased mortality of spat following partial replacement of live microalgae with alternative diets. For example, there was no difference in the mortality of green-lipped mussel spat after being fed with formulated diets, MySpat®, at 0, 25, 50, 67, 75, 90, and 100% replacement for 21 days (ranging from 27 to 52% mortality) [48] or fed with 25% and 50% of fabricated liposomes for 32 days (ranging from 18 to 20% mortality) [28]. In mediterranean mussel spat, no mortality was observed in a spat when they were fed with 16% of live microalgae and up to 4.8% MySpat® on a mussel live weight basis [27]. The size of dead spat recovered at the end of the experiment for 0%, 25% SD (1.3 ± 0.1 mm), and 25% LPB (1.4 ± 0.1 mm) replacement were not different from the size of spat in these treatments at day 0 (1.2 ± 0.0, 1.3 ± 0.0, and 1.3 ± 0.0 mm, respectively). In contrast, the size of dead spat fed with 50% or higher percentage of replacement were larger (>1.5 mm) than their size at day 0. This suggests that the dead spat from 0% and 25% replacement might have died before the start of the experiment. While there was no difference in spat mortality

between 0% and up to 50% replacement of SD and LPB, the growth of spat decreased when they were fed with 25% replacement of SD and LPB.

The growth of spat in the present study tended to decrease with an increasing percentage of live microalgal feed replacement using either SD or LPB. The nutritive value of SD and LPB per DW basis (Table 1) are lower than the nutritive value of live microalgae as reported in Gui et al. [48]. Therefore, it is possible that the lower nutritional density or the nutritional composition of SD and LPB have resulted in lower growth of spat. However, it has been reported that the growth of spat is not always influenced by the nutritional composition of feeds [49]. There are several factors affecting the growth of bivalves when fed alternative diets, especially the extent of particle retention during filter feeding, which is related to the size of particles, chemical cues, and nutritional composition of diets [20, 23, 27]. The size of concentrated microalgae in SD and LPB diets in this study range from 4– 20 μm (Reed Mariculture Inc., California, USA); however, food particles larger than 15 μm have been shown to be not well tolerated by green-lipped mussel spat of this size range, interrupting feeding and possibly resulting in mortality [20]. Consequently, this disparity in meeting the specific particle size requirements of the spat may have resulted in lower growth of the spat with the increasing percentage of replacement of SD and LPB.

Overall, there was largely no difference in the nutritional profiles of spat following different ranges of replacement of SD and LPB (i.e., the percentage composition of AFDW, protein, and lipid) except for the total carbohydrate content, which tended to decrease with increasing replacement of live microalgae. This decreasing trend is likely to be a result of the relative carbohydrate content present in the SD and LPB diets (~0.6% DW), which is 25 times lower than the carbohydrate content of 100% live microalgal feed used in this study (15.1% DW) [48]. This may be due to losses of some proportion of carbohydrate content associated with a concentration of live microalgae. The measured carbohydrate content of the microalgal concentrates presented in SD and LPB in the present study were over 10 times lower than for live microalgae of the same species used to make these concentrated microalgal products [38, 50, 51]. A decrease of carbohydrate content was also reported in concentrates of the microalgae *Chaetoceros calcitrans, Skeletonema costatum*, and *T. lutea* which were on average 50% lower than live microalgae of the same species and may be a result of soluble carbohydrates not being retained in the concentrated microalgal cells [25].

The nutritional composition, particularly the carbohydrate content of SD and LPB in the present study, appears to be insufficient for green-lipped mussel spat to maintain growth and nutritional condition achievable on live microalgae feed at the same DW basis. Carbohydrate content has been previously reported to be the main energy reserves utilised by the spat of the same species when food supplies were limited [30, 52]. A marked decrease in the growth of spat was evident in this current study when 25% of live microalgae was replaced with SD and LPB. In contrast, spat of the same species fed with formulated diets, MySpat®, did not show a decrease in the growth of spat until 90% of live microalgae was replaced [48]. Spat fed with 100% live microalgae in the present study achieved the best growth among all feeding treatments, with spat being 1.5 times larger than spat fed with 25–75% replacement and nearly double the size of spat fed with 100% replacement. The mix of live microalgae used in this study has relatively high nutritive values by DW (43% protein, 12.1% lipid, and 15.1% carbohydrate) [38, 48]. In contrast, the protein, lipid, and carbohydrate content extracted from SD and LPB in this study were 20.6–30.3% DW, 6.7–7.6% DW, and 0.6% DW, respectively. This suggests that for SD and LPB to be effective replacement diets their feeding rates may need to be increased to achieve a similar level of dietary nutrient delivery on a dry weight basis compared to feeding pure live microalgae.

The nutritional requirements of juvenile bivalves have been reported to be highly varied. For example, a juvenile European flat oyster, *Ostrea edulis*, showed good growth rates when fed with live microalgae containing 15.5% protein and 59.4% DW of carbohydrates [53]. Juveniles of the Pacific blue mussel, *Mytilus trossulus*, achieved optimal growth when the diets contained higher than 40% DW of protein [54]. In contrast, high carbohydrate content (74% DW) together with 18% DW protein and 10% DW lipid content in the diets were sufficient to achieve optimum growth in Sydney rock oyster juvenile, *Saccostrea commercialis* [55]. In juvenile grooved carpet shell clam, *Ruditapes decussatus,* a minimum of 22% DW protein and 18% DW lipid content, by replacing live microalgae with 50% cornmeal were required to achieve similar growth to feeding only live microalgae [56]. These previous studies suggest that the nutritional requirements of juvenile bivalves are likely to be species-specific and could be related to the differences in the storage and utilisation of energy reserves among different bivalve species [57, 58]. Therefore, the nutritional composition of SD and LPB in the present study needs to meet the nutritional requirement of green-lipped mussel spat to achieve optimum growth. Nutritional composition of SD and LPB, particularly their carbohydrate content needs to be at least at a similar level of the carbohydrate content in 100% live microalgae (~15% DW). In contrast, the protein content of SD and LPB (30.3 and 20.6% DW) and lipid content of SD and LPB (6.7 and 7.6% DW) in this study did not appear to significantly influence the overall proportions of protein and lipid content accumulated by the growing spat when fed up to 100% of both diets over 27 days but did result in significantly reduced growth of spat overall, i.e., the total accumulation of nutrients as tissue biomass in the spat. Therefore, both concentrated algae used in this study may need to be delivered at higher feeding rates to meet the nutritional requirement (carbohydrate, protein, and lipid content) of spat to achieve a similar level of growth of the spat fed with a mixture of live microalgae.

5. Conclusion

The current study confirms the potential for commercial diets made from concentrated and preserved microalgae (i.e., SD and LPB) to partially replace live microalgae for

nursery feeding of green-lipped mussel spat. Under the condition of this study, the replacement of live microalgae using up to 50% SD and LPB did not compromise the survival and proportional biochemical composition (i.e., AFDW, lipid, protein, and carbohydrate) of spat, showing similar levels to spat fed with 100% live microalgae. However, the marked decrease in growth of spat fed with higher replacements of live microalgae with SD and LPB, and the lower proportional carbohydrate content of spat fed with 100% SD suggests that spat may require the delivery of higher dietary nutrients levels, especially carbohydrates. Therefore, future studies need to investigate the performance and biochemical composition of green-lipped mussel spat fed at higher feeding rates of SD and LPB.

Data Availability

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

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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