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Any Sugar with That? Assessment of Dissolved Sucrose as Supplementary Feed in Nursery Rearing of Juvenile Bivalves

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Abstract: The costly production of live microalgal feed prevents the inclusion of an extended nursery phase in bivalve aquaculture. One method of feeding juvenile bivalves that has received minimal attention is the use of dissolved nutrients to reduce the reliance on live microalgae as a sole feed input. This study aimed to determine whether dissolved sucrose could work as a supplement to live microalgae. Two different concentrations of dissolved sucrose (i.e., 100 μg mL^{-1} and 1 mg mL^{-1}) were each provided daily for 2 h and 4 h to juvenile Greenshell™ mussels as a supplement to a diet of live microalgae. The growth and survival of the mussels were measured over three weeks. All combinations of sucrose concentrations and exposures improved the growth of spat compared with the control without sucrose. However, the best-performing spat were provided with a concentration of 1 mg mL^{-1} of dissolved sucrose for an exposure time of 4 h, which induced 57% greater spat growth daily compared with the control diet. The mussel spat supplemented with dissolved sucrose also accumulated greater carbohydrate content compared with those in the control treatment, indicating they were in greater nutritional condition. This demonstration that dissolved sucrose can significantly improve the growth and nutritional composition of mussel spat over periods as short as 2 h shows promise for the commercial application of sucrose as low-cost supplementary feed in bivalve nurseries.

Keywords: nursery culture; mussel; spat; juveniles; sucrose; nutrient uptake

Key Contribution: The use of dissolved sucrose as a supplementary feed with live microalgae can improve the growth of and carbohydrate content in juvenile Greenshell mussels over 21 days. Dissolved glucose may present a cost-effective alternative feed for bivalve nursery culture.

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1. Introduction

Over the past two decades, the global production of marine bivalves by volume has grown annually by 3.5%, with mussel aquaculture production tripling over the same period [1,2]. Mussels now constitute an important source of protein globally, with annual production being estimated to be 1.8 million t, valued at USD 2.7 billion [3]. Moreover, the increase in the farming of marine mussels offers more than global food security, as there is a growing recognition of the broader ecological benefits of bivalve aquaculture [4]. These benefits include regulatory functions such as carbon sequestration and nutrient remediation, as well as the indirect ecosystem benefits that stem from shellfish beds and reefs [5,6].

Despite the growth of mussel aquaculture globally and its ecological benefits, many mussel-producing nations in Europe and Oceania are currently experiencing stagnating production [7,8]. For example, European mussel aquaculture production peaked at 600,000 t in the late 1990s and has since decreased by 20% [2]. The reasons for stagnation or decline in production across many mussel-farming countries is varied, with industries reporting significant impacts from the spread of diseases, algal blooms, fish predation and reduced profitability [7]. However, one consistent issue across many mussel aquaculture industries is the loss of seed mussels, or 'spat', during the early stages of production [3]. This issue of high losses of juveniles is not unique to mussel aquaculture, being widely shared by many other bivalve aquaculture sectors, including scallops, clams and oysters [9].

Greenshell™ mussels (*Perna canaliculus*) are New Zealand's primary aquaculture species, generating an annual revenue of NZD 347 M in 2021 [10]. Most of the spat (~80%) used by the industry are wild spat that wash ashore on Te Oneroa-a-Tōhē (Ninety Mile Beach) attached to drift seaweed [10]. The remaining balance of the spat supply for the industry is sourced from spat-catching ropes and from a small number of hatcheries. The spat are transported to farms located around the country, where they are seeded onto coastal longlines at small sizes (i.e., 1–2 mm in shell length (SL)) and typically grow for 15 to 18 months until reaching a harvestable size of 90–110 mm in SL [11].

New Zealand's overall use of mussel spat for seeding its mussel aquaculture is extremely inefficient [8]. Similar to other mussel-producing nations throughout Oceania and Europe, the primary challenge for New Zealand farmers is poor spat retention during early grow-out stages, with reported losses as high as 99% on some farms [12]. Two key factors that impact the retention of spat on coastal farms are the size and nutritional condition of individuals when they are initially seeded onto longlines [13,14]. At small sizes (i.e., <6 mm in SL), Greenshell™ mussel spat are highly mobile and can detach from settlement substrate, using mucus threads to passively drift to new settlement sites, a process called secondary settlement [13,15]. Consequently, a significant proportion of mussel spat migrate off farms shortly after seeding out due to secondary settlement [8,16].

The nutritional condition of spat when they are initially seeded out onto longlines is another critical factor affecting spat retention [14,17]. Wild-caught spat from Te Oneroa-a-Tōhē often spend days to weeks in the turbulent surf zone without access to food before being harvested and transported to farms [18]. This period has been shown to result in significant variability in the nutritional condition among wild-sourced spat [19]. Spat in poor nutritional condition are less likely to tolerate the stress of transport when they are transported to farms [20] and poor feeding or adverse environmental conditions during the initial grow-out period [13,14]. Additionally, depleted energy reserves can negatively impact the ability of juvenile mussels to produce byssus threads, hindering their ability to remain attached to settlement substrate [21].

The most promising way to circumvent secondary settlement and compromised nutritional condition at seeding is to grow spat to larger sizes in contained nursery systems prior to seeding them out onto farms. Introducing nursery culture for both hatchery and wild-caught Greenshell™ mussel spat is an immediate measure that industry could take to reduce spat losses. However, the greatest obstacle preventing the industry from using the nursery rearing of mussels is the high cost of large-scale production of live microalgal feed. It has been estimated the microalgal production can account for up to 50% of a hatchery's operating cost [22,23]. In response to this challenge, a significant amount of research has been directed at finding more cost-effective alternative feed types to live microalgae. Many feed types have been experimentally tested; however, these alternative products typically lead to either decreased growth or increased mortality with the increase in the substitution of live microalgal feed [24–27]. Consequently, the development of alternative feed that can

act as a substitute or supplement to live microalgae is an important next step for the New Zealand Greenshell™ mussel industry to make nursery culture economically viable.

It is well established that bivalves have the capability to uptake dissolved organic material from seawater, as a number of studies have demonstrated the phenomenon in bivalve larvae, spat and adults [28–32]. However, the significance of dissolved nutrients in meeting the overall nutritional requirements of bivalves throughout their development remains largely unknown. Recent work has shown that Greenshell™ mussel spat can uptake dissolved glucose from seawater, using this additional source of nutrients to fuel growth and improve the nutritional condition [33]. To support the commercial implementation of sugars as supplementary feed, it is important to investigate whether other types of more cost-effective carbohydrates can also positively impact growth and the nutritional condition. Furthermore, understanding what impact the time of exposure to dissolved sugars has on the performance of Greenshell™ mussel spat is crucial for successful commercial implementation.

A potentially cost-effective alternative to glucose with potential as a supplementary feed is sucrose. Sucrose is a naturally occurring sugar produced by many photosynthetic organisms, although it is not commonly found in microalgae, making it a somewhat novel component in shellfish diets [34,35]. Consequently, the potential impact of dissolved sucrose on the performance of juvenile shellfish is unknown. Structurally, sucrose differs from glucose, as it is a disaccharide composed of a glucose and a fructose molecule linked by a glycosidic bond. Due to this more complex molecular structure compared with glucose, it has been suggested that sucrose as a disaccharide may protect the glucose component from metabolic breakdown until it reaches specific sites for growth or storage [36]. Moreover, sucrose as a product is readily available, of comparatively lower cost than glucose and highly soluble in seawater and has the potential to serve as a low-cost source of additional carbohydrates, with the potential to increase growth and the levels of stored glycogen in juvenile bivalves. If sucrose can be effectively absorbed and assimilated by Greenshell™ mussel spat, it would present a cost-effective feed option for bivalve nurseries on a commercial scale. A downside of the use of dissolved sucrose in bivalve nursery culture systems is its potential to trigger bacterial growth, which could impact the survival of spat. Short periods of exposure to dissolved sugar (2 h) followed by seawater changes appear to have prevented this issue previously [33]; however, a longer period of exposure to sugar has the potential to improve outcomes for spat and merits testing.

The objective of this study was to evaluate whether dissolved sucrose can enhance the growth and nutritional condition of Greenshell™ spat when supplemented for different periods with a diet of live microalgae.

2. Materials and Methods

2.1. Spat Source

A commercial shellfish hatchery (SPATnz, Nelson, New Zealand) provided approximately 30 g (*w*/*w*) (~37,500 individuals) of hatchery-reared spat, 1.8–2 mm in SL. The spat were air-freighted to the laboratory in Auckland in 2 h in a cool, damp and insulated polystyrene box.

2.2. Experimental Design

Each experimental tank was made from a 3 L polyethylene bottle with its base removed which was inverted on a wooden rack and contained 2 L of filtered and sterilized seawater (UV, 5 µm, 19 °C), which was aerated by an air stone inserted into the neck of the bottle. A small piece of plastic mesh was added to each tank to provide an attachment substrate for spat. To ensure that feeding quantities were calculated accurately, the number of spat

per gram was estimated by counting 10 randomly selected sub-samples of 100 mg of spat. Then, an aliquot of 2 g wet weight of Greenshell™ spat was added to each tank (~1500 individuals).

Five sucrose concentrations were used, with each concentration being randomly assigned to three replicate tanks; these were control—no sucrose; Sucrose 1 (2 h exposure)—100 μg mL^{-1}; Sucrose 2 (4 h exposure)—100 μg mL^{-1}; Sucrose 3 (2 h exposure)—1 mg mL^{-1}; and Sucrose 4 (4 h exposure)—1 mg mL^{-1}. These experimental concentrations of sucrose were based on the results of previous research providing dissolved glucose to Greenshell™ mussel spat (Jordan et al., 2024 [33]). To deliver the sucrose to the tanks, a measured aliquot of concentrated sucrose solution was added to the seawater at the start of the experimental exposure period. Live axenically cultured microalgae were also fed in all five treatments and consisted of *D. lutheri*, *C. muelleri* and *T. suecica*, at a rate of 3×10^5 cells $mussel^{-1}$ day^{-1} in a ratio of 2:1:1, determined by cell count daily (Muse® Cell Analyser).

Seawater changes were undertaken daily after spat were exposed to the dissolved nutrient (i.e., 2 or 4 h) to prevent bacterial contamination in tanks. Seawater changes were conducted by draining seawater from bottles through a 250 μm mesh. Once the tanks were cleaned and replenished with fresh seawater, spat were rinsed with clean seawater and returned to the tanks, where they were fed their microalgal ration and left to consume this for the following 24 h.

The experiment was conducted over 21 d, and at the outset and subsequently every 7 days, 50 randomly selected spat from each tank were placed on a plastic tray with a reference grid and photographed with a digital camera, and the shell length of the spat was subsequently determined by using image analyses (ImageJ V1.46, NIH).

2.3. Growth and Survival

At the end of the experiment, subsamples of 100 randomly selected spat from each experimental tank were inspected under a dissecting microscope to determine the number of alive and dead spat, to provide an estimate of mortality (i.e., the proportion of the total count that were found to be dead). Empty shells from deceased spat were removed from the samples, and the remaining live spat were photographed for digital size measurement. Spat were then washed in deionized water, drained onto laboratory tissue, placed into 50 mL Eppendorf tubes and frozen at −80 °C for subsequent biochemical analyses.

2.4. Biochemical Analysis

2.4.1. Ash-Free Dry Weight

Once spat were removed from the −80 °C freezer, they were freeze-dried for 24 h and then weighed to establish their dry weight. Three replicate samples of 100 mg of freeze-dried spat from each replicate tank were then used to obtain their ash-free dry weight (AFDW). The samples were burnt in a muffle furnace (Nabertherm LT15/11 B410; Germany) at 450 °C for 4 h. The residual ash was then weighed to calculate the proportion of AFDW in relation to total dry weight.

2.4.2. Calorific Content

Three 100 mg subsamples of freeze-dried spat from each tank were used for measuring the calorific content of the spat by using a semi-microcalorimeter (Parr 6725; Parr Instrument Company, Moline, IL, USA). The calorific value per gram of dry mass of spat from each replicate of each treatment was then converted into calorific content per gram of organic tissue by using the proportion of AFDW to dry tissue mass of the corresponding spat sample.

2.4.3. Protein, Lipid and Carbohydrate Contents

To determine protein content in spat, three subsamples of 50 mg of lyophilized spat from each tank were incubated in 0.1 M NaOH for 16 h at 50 °C and then centrifuged at 10,000 rpm at 4 °C for 10 min. The protein in each subsample was then quantified by the BCA (bicinchoninic acid) method [37] by using a micro BCA protein assay kit (ThermoFisher Scientific, Waltham, MA, USA) and read against the bovine serum albumin (BSA) standard at 562 nm.

The lipid content in the spat was determined for three subsamples, each of 300 mg of freeze-dried spat for each tank with a modification of the methanol–chloroform solvent extraction method by Bligh and Dyer [38]. The total lipid extracted from each sample was quantified following the removal of the residual solvent by using a stream of nitrogen gas following the method by Wang et al. (2013) [39].

A subsample of 100 mg of spat from each replicate tank was used to determine total carbohydrate content in spat. The freeze-dried spat were ground in liquid nitrogen and subsequently homogenized in 1 mL of distilled water by using a homogenizer (Polytron PT 1200; US). The solutions were then centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant was transferred into a 1.5 mL Eppendorf tube and stored at −20 °C prior to analysis. The total carbohydrate content in the solutions was determined by using the phenol sulfuric acid reagent method [40] and was read against the D-glucose standard curve by using a spectrophotometer at 490 nm (Multiskan™ Sky; Thermo Scientific Inc., Waltham, MA, USA) [41].

2.5. Statistical Analyses

A linear mixed-effects model was fitted to the shell length measurements that were taken for spat sampled from each experiment initially on day 0 and then subsequently every 7 days. The shell length of the spat was predicted by the fixed effect of time, as well as the interaction between sucrose concentration and exposure, and replicate tank was included as a random effect. The slope of the regression from the mixed-effects model was used to determine daily growth for each treatment.

Two-way analysis of variance (ANOVAs) was used to compare how spat mortality and each biochemical component (i.e., AFDW, lipid, protein, carbohydrate and calorific content) as measured at the end of the experiment was influenced by the concentration and exposure to sucrose among treatments. Where significant interactions or main effects were identified with ANOVA, post hoc pairwise comparisons were used to identify differences among individual means. Percentage data (mortality and AFDW) were calculated as arcsine transformed prior to analysis.

All data were checked for normality and homogeneity of variances by using measures of skewness and kurtosis. Additionally, graphical methods such as histograms and Q-Q plots were employed to visually assess the distribution of the data. If the data defied these parametric assumptions, they were log-transformed and rechecked for conformity to the assumptions.

3. Results

3.1. Spat Growth

Overall, there were differences in the mean daily growth of spat among the five treatments ($F_{(4, 5988)} = 17.2$, $p < 0.001$) (Figure 1). All four sucrose treatments led to greater daily growth compared to the control ($p < 0.001$), but there were no differences among any of the sucrose treatments. Daily spat growth on average was 9.3 μm day^{-1} higher for all sucrose treatments versus the control, which showed a value of 18.9 μm day^{-1} (±3.5 CI).

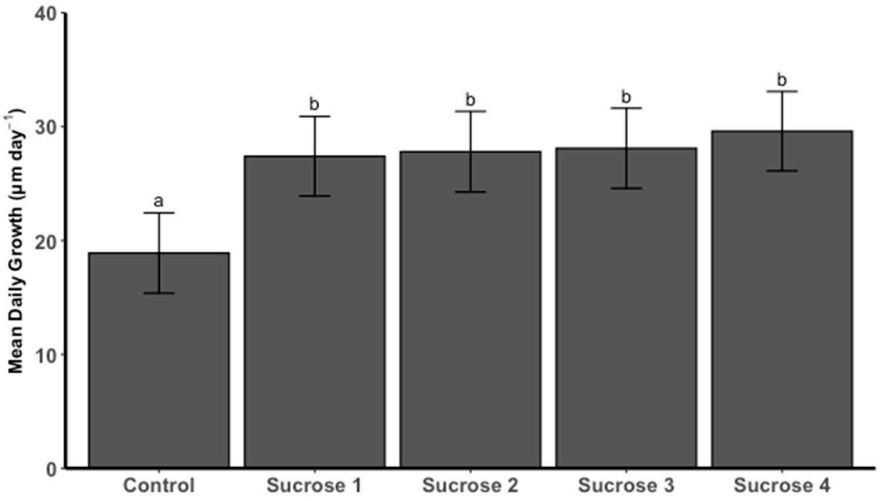

Figure 1. Mean daily growth (±95% CI) of Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose for two different periods (Sucrose 1 = 100 µg mL^{-1}, 2 h exposure; Sucrose 2 = 100 µg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Mean spat growth was derived from a linear mixed-effects model taken from measurements taken at 0, 7, 14 and 21 days over the 21-day experiment. Means with different letters are different ($p < 0.05$).

3.2. Spat Mortality

The mean mortality of spat among the five treatments ranged from 4% (±1.73 SE) in the control to 16.67% (±9.56 SE) in Sucrose 3 (Figure 2). There was no significant difference in mortality due to the interaction between the concentration of the dissolved nutrient and the exposure time ($p = 0.72$). Additionally, there was no significant main effect of nutrient concentration ($p = 0.16$) or exposure time ($p = 0.77$) on the mean percentage mortality of mussel spat among sucrose treatments.

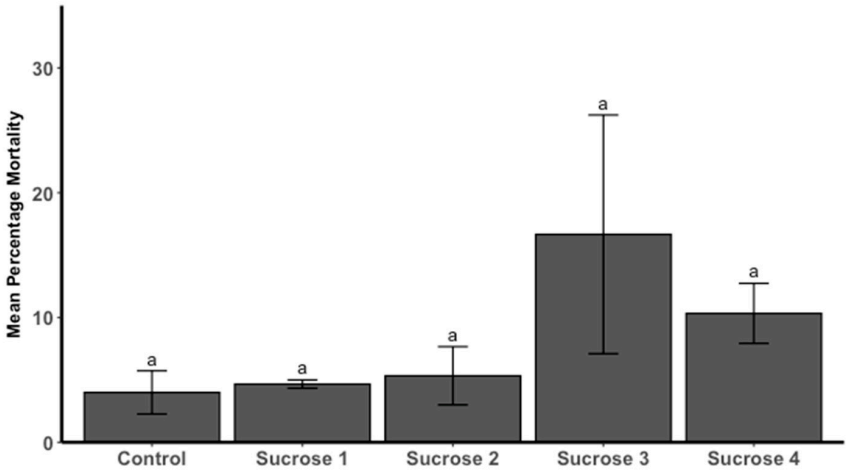

Figure 2. The mean percent mortality (±SE) of Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose each for two different exposure periods (Sucrose 1 = 100 µg mL^{-1}, 2 h exposure; Sucrose 2 = 100 µg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Means with different letters are different ($p < 0.05$).

3.3. Biochemical Analysis

3.3.1. Ash-Free Dry Weight

The mean percentage of AFDW of spat ranged from 19.8% (±0.5 SE) in Sucrose 3 to 26.0% (±2.0 SE) in Sucrose 4 (Figure 3). There was no difference in the mean percentage of AFDW due to the interaction between the concentration of dissolved sucrose and the

exposure time ($F_{(1, 10)} = 3.8$, $p = 0.07$). However, there was a significant difference due to the time of exposure to sucrose ($F_{(1, 10)} = 6.7$, $p = 0.03$), although the post hoc comparison failed to isolate any significant differences among the pairs of means.

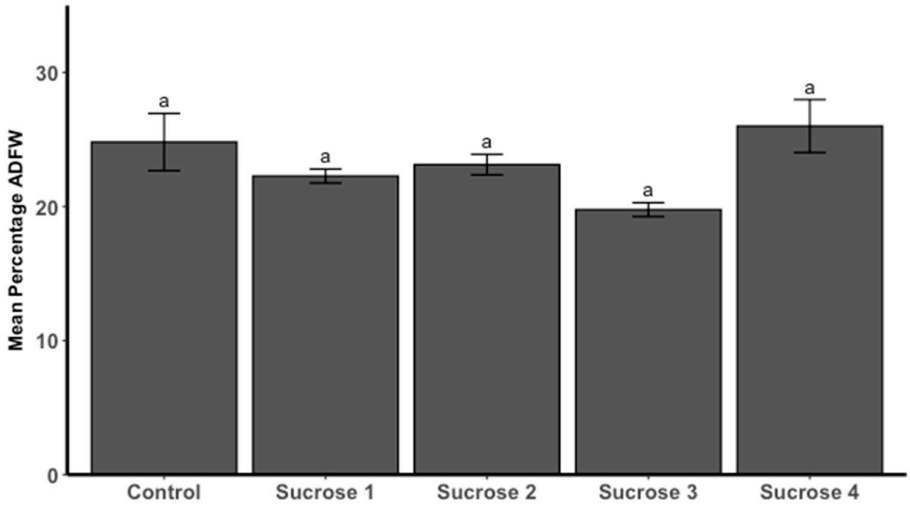

Figure 3. The mean percentage of AFDW (±SE) of Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose each for two different exposure periods (Sucrose 1 = 100 μg mL^{-1}, 2 h exposure; Sucrose 2 = 100 μg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Means with different letters are different ($p < 0.05$).

3.3.2. Carbohydrate Content

The mean carbohydrate content in spat among the sucrose treatments varied from 32.7 mg g^{-1} (±3.4 SE) of AFDW in the control to 84.5 mg g^{-1} (±5.6 SE) in Sucrose 4 (Figure 4). There was a significant interaction between the sucrose concentration and exposure time ($F_{(1, 10)} = 24.5$, $p < 0.001$). The spat in the Sucrose 4 treatment contained higher carbohydrate content than the spat from all other treatment groups ($p \leq 0.001$). Additionally, the spat from Sucrose 3 had mean carbohydrate content greater than the spat in the control group ($p \leq 0.05$).

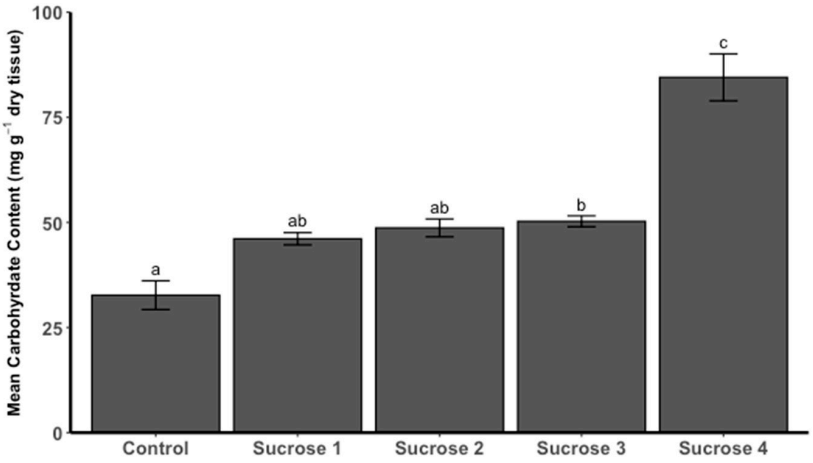

Figure 4. Mean carbohydrate content (mg g^{-1}) (±SE) in Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose each for two different exposure periods (Sucrose 1 = 100 μg mL^{-1}, 2 h exposure; Sucrose 2 = 100 μg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Means with different letters are different ($p < 0.05$).

3.3.3. Protein Content

The mean protein content in spat among the sucrose treatments ranged from 381.0 mg g^{-1} (±18.59 SE) to 536.9 mg g^{-1} (±20.51 SE) of AFDW (Figure 5). There was no difference in the mean protein content due to the interaction between sucrose concentration and exposure time ($F_{(1, 10)}$ = 1.7, p = 0.06). Additionally, there was no significant main effect of nutrient concentration ($F_{(1, 10)}$ = 0.6, p = 0.4) or exposure time ($F_{(1, 10)}$ = 4.5, p = 0.06) on mean protein content in mussel spat among the sucrose treatments.

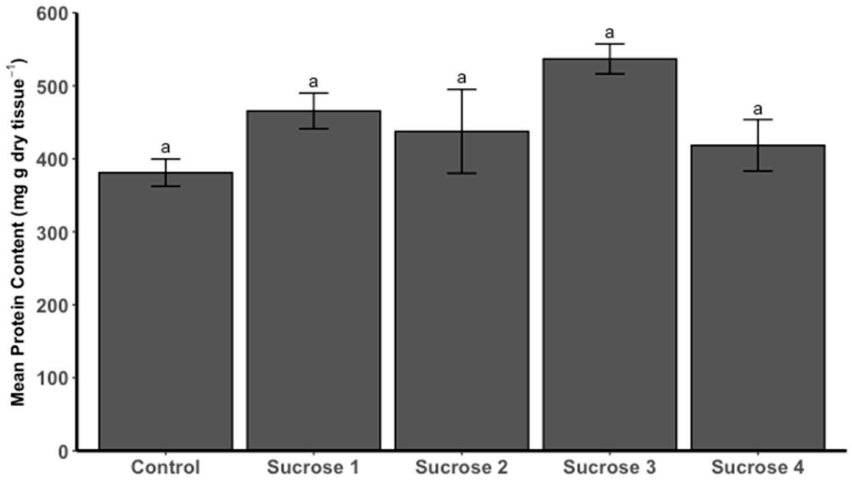

Figure 5. Mean protein content (mg g^{-1}) (±SE) in Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose each for two different exposure periods (Sucrose 1 = 100 µg mL^{-1}, 2 h exposure; Sucrose 2 = 100 µg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Means with different letters are different (p < 0.05).

3.3.4. Lipid Content

The mean lipid content in spat among the sucrose treatments ranged from 60.2 mg g^{-1} (±3.4 SE) to 91.8 mg g^{-1} (±7.9 SE) (Figure 6). There were no significant differences due to the interaction between sucrose concentration and exposure time ($F_{(1, 10)}$ = 0.6, p = 0.47). However, there was an overall difference in mean lipid content in spat due to the duration of exposure to dissolved sucrose ($F_{(1, 10)}$ = 12.2, p = 0.005), with those spat exposed to sucrose for two hours containing higher mean lipid content than those exposed for four hours ($p \leq 0.05$).

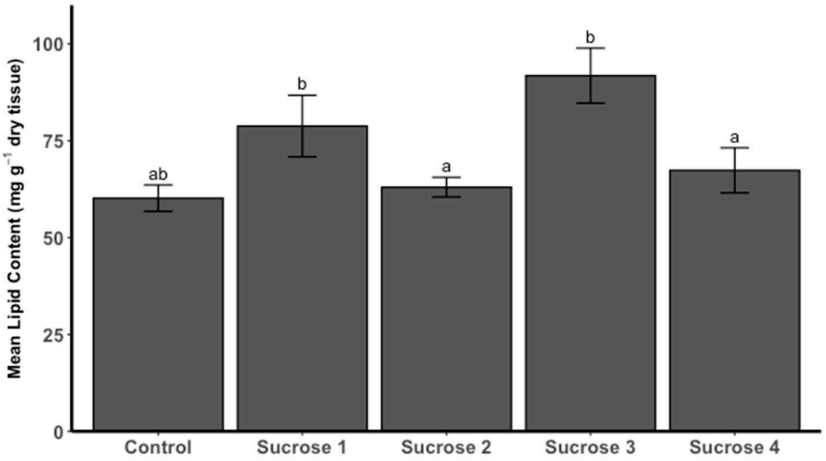

Figure 6. Mean lipid content (mg g^{-1}) (±SE) in Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose each for two different exposure periods (Sucrose 1 = 100 µg mL^{-1}, 2 h exposure; Sucrose 2 = 100 µg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Means with different letters are different (p < 0.05).

3.3.5. Calorific Content

The mean calorific content of spat ranged from 1209 to 1598 calories g^{-1} of AFDW, and there was no difference due to the interaction between the sucrose concentration and exposure time ($F_{(1, 10)}$ = 0.8, p = 0.38) (Figure 7). Additionally, there was no significant main effect of nutrient concentration ($F_{(1, 10)}$ = 0.3, p = 0.62) or exposure time ($F_{(1, 10)}$ = 2.1, p = 0.18) on the mean calorific content of mussel spat among the sucrose treatments.

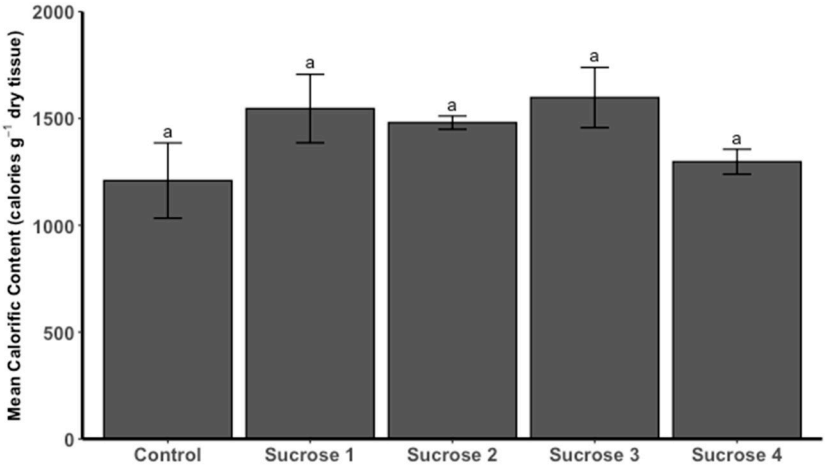

Figure 7. Mean calorific content of AFDW (cal g^{-1}) (±SE) of Greenshell™ mussel spat fed live microalgae alone (control) or two concentrations of dissolved sucrose each for two different exposure periods (Sucrose 1 = 100 µg mL^{-1}, 2 h exposure; Sucrose 2 = 100 µg mL^{-1}, 4 h exposure; Sucrose 3 = 1 mg mL^{-1}, 2 h exposure; Sucrose 4 = 1 mg mL^{-1}, 4 h exposure). Means with different letters are different (p < 0.05).

4. Discussion

The high cost associated with culturing live microalgae is a major constraint on the use of a nursery culture phase for bivalves. To date, efforts to develop cost-effective alternative feed types have focused on microencapsulated or dried and preserved microalgal products, and these have had mixed success in juvenile shellfish [24,25,42,43]. One potential low-cost method for providing additional nutrients to juvenile shellfish, such as mussel spat, in nursery culture is to enrich seawater with dissolved organic material. Previous research has demonstrated the ability of bivalves to uptake dissolved sugars [32,44], amino acids [28,45] and fatty acids from seawater [31,46]. However, the potential use of dissolved nutrients as supplementary feed for juvenile bivalves had not been explored prior to a recent investigation on the potential to use dissolved glucose as a supplementary feed in Greenshell mussel spat [33]. This previous study found that 2 h of exposure to glucose each day could boost the growth of Greenshell™ mussel spat and improve their nutritional condition. The current study aimed to assess whether dissolved sucrose could provide similar growth benefits to Greenshell™ mussel spat and whether extending periods of exposure to the dissolved nutrient might further increase the nutritional benefits without compromising survival or contaminating aquaculture systems due to potentially triggering bacterial blooms.

The marked increase in the daily growth of spat in all four sucrose treatments in the current study relative to the control indicates that supplementing a diet of mixed microalgae with dissolved sucrose can significantly contribute to the nutritional requirements of Greenshell™ mussel spat. On average, exposure to sucrose increased the growth of spat to 29.6 µm day^{-1}, which was a 57% increase compared with the spat fed microalgae alone (18.8 µm day^{-1}). Previous studies on the role of dietary sources of carbohydrates in other marine invertebrates have indicated that achieving optimal carbohydrate ratios can lead to

improved growth and feed utilization [47,48]. For instance, it was documented that a diet consisting of 38% carbohydrates in abalone (*H. discus hannai*) led to increased blood glucose levels and the activation glycolysis pathways, which helped in the efficient breakdown and use of glucose, leading to superior growth and survival [49]. Those studies that have assessed the role of carbohydrates in Greenshell™ mussel spat have also indicated that they are key nutritional requirements and are rapidly depleted preferentially over protein and lipid during periods of nutritional stress [17,50]. Furthermore, when glucose was used as supplementary feed for Greenshell™ mussel spat at concentrations of 100 μg mL^{-1} and 1 mg mL^{-1} for 2 h day^{-1}, the growth of mussel spat increased by 2.7 times compared with that induced by a microalgae-only control diet [33].

The total carbohydrate content in spat varied among the treatments. The spat in Sucrose 3 showed increased carbohydrate content compared with the control group, while those in Sucrose 4 exhibited the highest carbohydrate content among all treatments. This indicates that both the concentration of and the time of exposure to dissolved sucrose affects the ability of Greenshell™ mussel spat to uptake the dissolved nutrient and ultimately the total carbohydrate content in spat. The accumulation of carbohydrates in the mussel spat in these two treatments, which did not show differences in growth compared with the other sucrose treatments, indicates that the sucrose supply exceeded their immediate energetic demands for somatic growth. The carbohydrate content in the spat in Sucrose 4 was higher than that in hatchery-reared spat of the same size documented by both Supono, Yu [17] and Skelton, Múgica [19] and was also higher than that in spat that were given glucose as supplementary feed at the same concentration [33]. The hydrolysis of the glycosidic bond of sucrose releases glucose and fructose molecules. It appears that the greater use of fructose derived from sucrose versus glucose for the synthesis of glycogen for energy storage results in higher carbohydrate content in spat than when spat are fed glucose alone [33,51]. Alternatively, this may relate to the disaccharide structure of sucrose protecting the glucose component from metabolic breakdown until it reaches specific sites for storage [36]. Given that one of the primary goals of nursery culture should be to increase the amount of stored glycogen in spat prior to seeding them on farms, higher concentrations and longer times of exposure to sucrose may enable spat to accumulate greater carbohydrate reserves.

The dry tissue mass of spat among all the sucrose treatments consisted predominantly of protein, relative to carbohydrates and lipids. There was no difference in protein content in spat as a portion of dry tissue mass among the five sucrose treatments. However, given that the spat in the sucrose treatments showed greater growth than those in the control, the variation in the shell length of the spat (i.e., mineral content) relative to dry tissue mass (organic content) would result in proportionally greater total organic tissue and its associated proximate components. This is because proportional values do not reflect absolute differences in proximate components that may exist due to the differences in mussel size at the end of the experiment. Previous studies that have documented total protein content in Greenshell™ mussel spat have reported high variability [17,19,33]. For example, where glucose has been used as supplementary feed, the maximum protein content in mussel spat, as a proportion of AFDW, was 341 mg g^{-1} [33]. In this study, the spat across all treatments displayed protein content ranging from 381 to 536 mg g^{-1}, indicating that the spat used in this experiment were in superior condition compared with the spat reported in other studies. However, given that the control treatment also showed high protein content, the elevated protein content across all five sucrose treatments were not due to other experimental factors, such as microalgal quality and seawater temperature.

The lipid content in mussel spat in the groups with 2 h exposure to sucrose (Sucrose 1 and Sucrose 3) was greater than that in those in the 4 h exposure groups (Sucrose 2 and Sucrose 4). A similar lipid-sparing response was documented when glucose was used as

supplementary feed for 2 h in Greenshell mussel spat of the same size [33]. However, this increase in lipid content did not lead to higher AFDW or total energy content (calories g^{-1}) in the spat exposed to sucrose for 2 h. The spat in the Sucrose 4 treatment accumulated higher total carbohydrate content, although a lipid-sparing effect was not observed, as had been documented when glucose was used as a supplementary feed [33]. Lipids are typically the most important part of the diet for bivalve larvae, as they are crucial for their growth and development because they are essential to the construction of cell membranes during early development [52–54]. However, there is evidence that the nutritional composition of mussel spat shifts after metamorphosis and carbohydrates become the primary source of energy [14,17,47,50,55,56]. Furthermore, although the Greenshell™ mussel spat in the Sucrose 3 and Sucrose 4 treatments had proportionately 1.5 and 2.6 times higher total carbohydrate content compared with the control, there was no difference in the total energy content (calorific content) of spat among all five sucrose treatments. Given that carbohydrates provide approximately 4 cal g^{-1} [57], even the additional 51 mg g^{-1} of dry tissue in Sucrose 4 compared with the control would have resulted in a difference of 0.2 cal g^{-1}, which was undetectable by the microcalorimetry methods.

The nutrient absorption and digestion of sugars in bivalves begins on the surface of the gills and mantle, with digestion being subsequently completed in the hepatic caeca [58,59]. Bivalves can absorb dissolved material passively when concentrations are high or actively when concentrations are low [32,60]. Despite this versatility, relatively little research has been conducted on how uptake and metabolism vary among different organic molecules. Furthermore, the uptake and metabolism of sucrose and its constituent monosaccharides (i.e., glucose and fructose) have not been previously documented in marine mollusks. However, it is likely that these sugars undergo metabolic processing similar to that of other invertebrates [51,61]. Unique transporter proteins facilitate the capture and transport of carbohydrate molecules across the lipid bilayer of cell membranes due to their hydrophilic nature [61,62]. An examination of the metabolism of glucose and fructose labelled with an isotope in the mussel *Mytilus californianus* found that both were rapidly metabolized to CO_2 with fructose about three times faster than glucose [51]. Therefore, although there is evidence to suggest that fructose metabolism is faster than glucose metabolism in some species of mussels, it appears that in Greenshell mussels, both carbohydrates are metabolized to meet immediate energy needs. However, a greater proportion were converted into glycogen rather than CO_2 when they were provided in conjunction as sucrose, as opposed to when only glucose was provided at the same concentration [33]. It is also possible that the extra metabolic step of hydrolysis to break down the glycosidic linkage between the fructose and glucose molecules in sucrose requires energy that may be otherwise used for growth [63].

While supplementary sucrose did not increase growth to the same magnitude as previously reported with glucose when using spat of the same size [33], the daily growth of the spat in the control group in this experiment was significantly higher than that reported in the previous glucose study (i.e., 9.5 µm day^{-1} vs. 18.8 µm day^{-1}). One possible reason for this difference could be the 2 °C warmer seawater used in this experiment (19 vs. 17 °C). Studies that have assessed the impact of temperature on the growth of juvenile bivalves have found that water temperatures close to 20 °C provide optimum growth, potentially due to higher microalgal ingestion rates [64,65]. Similarly, temperatures of 20 °C have been reported as optimal for later-stage larval rearing in Greenshell mussels™ [66]. Furthermore, the quality of the microalgae may have been responsible for the differences in growth between the control groups in the two studies, as microalgae are known to constantly vary in their nutritional condition due to culture conditions. However, the mean AFDW values of the spat in the control group between the two studies were different, with the

spat in the control group of this study having a higher mean AFDW of 24%, compared with 19.9% in the glucose study [33]. It is unknown if the nutritional condition of the spat used in these two experiments was different from the outset of the experiment, causing a discrepancy in growth despite the same combination of microalgal species and daily cell quantity (3×10^5 cells mussel^{-1} day^{-1}).

Considering that supplementary sucrose was able to improve the performance of mussel spat, this may suggest that the carbohydrate content in the microalgal species used in this study is insufficient to meet the requirements of Greenshell™ mussel spat of this size, which are thought to be relatively high [50,67]. The microalgae used for this experiment were species that are commonly used in bivalve hatcheries but contain relatively low carbohydrate content, with *D. lutheri* containing 9%, *T. suecica* 12%, and *C. muelleri* 11% of their total dry mass [35,68]. This is typical of microalgal species that are used in commercial bivalve hatcheries, as carbohydrates are not generally considered a key dietary component in larval rearing [69,70]. Using dissolved sucrose as supplementary feed gave the mussel spat in the sucrose treatments an additional source of carbohydrates that they metabolized to fuel their growth. This suggests that the carbohydrate requirement of Greenshell™ mussel spat is likely to exceed 10% of their nutritional intake, as estimated from the proximate composition of the microalgae in the control treatment.

Investigation into the biochemical composition of mussel spat in this experiment has demonstrated that the delivery of dissolved sucrose as a supplementary nutrient to live microalgae can improve the carbohydrate content of hatchery-reared GSM spat over three weeks. In New Zealand, 65 to 80% of Greenshell™ mussel spat that are supplied to mussel farms are sourced from the wild by harvesting at Te Oneroa-a-Tōhē (Ninety Mile Beach) [71], with high variability in their nutritional status. For example, spat collected from Ninety Mile Beach between 2014 and 2021 showed huge variability in their amount of stored glycogen, with total carbohydrate contents ranging from 0.24 to 95 mg g^{-1} of their AFDW [19]. Those wild-caught spat with low carbohydrate content often have depleted glycogen stores due to the extended periods they face without food and the anaerobic stress experienced during harvesting and transport [17,50,72]. Spat that are in poor condition with reduced glycogen stores are likely to be impaired in their ability to produce byssus threads and remain attached to settlement substrates [14,21], increasing the likelihood that they are lost from the production cycle after initial seeding. Enhancing the carbohydrate content in these wild spat in nurseries would help to ensure that when spat are seeded onto coastal farms, they are better able to cope with periods of nutritional stress and can successfully attach to grow rope. These findings suggest that exposing spat to dissolved sucrose would be a cost-efficient approach to improving the amount of stored energy in Greenshell™ mussel spat prior to their seeding on coastal farms while also boosting growth.

5. Conclusions

Our study has demonstrated that supplying dissolved sucrose as a supplementary feed to live microalgae at concentrations between 100 µg mL^{-1} and 1 mg mL^{-1} provides Greenshell™ spat with additional nutrition, which supports superior growth. Furthermore, a dissolved sucrose concentration of 1 mg mL^{-1} with an exposure time of 2 or 4 h increased the nutritional condition of spat through higher total carbohydrate content. These findings can be applied for reducing feeding costs in nursery culture by increasing growth and also for improving the nutritional condition of spat prior to transfer to coastal farms. The results of this study present opportunities to further optimize the delivery of sucrose during the nursery phase of Greenshell™ spat by exploring intermediate concentrations and exposure windows to those used in this study. Given the similarities in bivalve metabolic physiology,

it is likely that dissolved sucrose has the potential to be used effectively in the nursery culture of other bivalve species.

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