

# Finding the sweet spot: The use of dissolved glucose in the commercial rearing of post-settlement Greenshell™ mussels

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## ABSTRACT

The widespread and extensive reliance on wild-sourced spat, which are inconsistent in supply and variable in nutritional condition, is a major factor limiting the growth of bivalve aquaculture globally. Producing spat in hatcheries can circumvent these issues, although the costs associated with feeding juvenile bivalves with high-quality cultured microalgae have constrained the expansion of hatchery and nursery production. One promising alternative to reduce reliance on cultured microalgae is to provide dissolved sugars alongside microalgae. This study aimed to determine whether dissolved glucose could be used in the commercial rearing of Greenshell™ mussels in New Zealand hatcheries, specifically during the first two weeks following larval settlement, when microalgal feeding costs are highest. Replacing 10 % of live microalgae with dissolved glucose (100 µg mL<sup>-1</sup>) for 2 h daily resulted in no differences in the growth, microalgae consumption, spat densities (a proxy for survival), or elemental composition of spat when compared with those spat in the 100 % microalgae ration over the 14 day period. Furthermore, supplementing a 100 % microalgae ration with dissolved glucose showed a trend towards greater spat growth without affecting performance. These findings demonstrate that dissolved glucose is an effective feed in bivalve hatcheries to replace or enrich microalgae as the only food. However, further research is needed to determine the most effective combination of dissolved nutrients to meet the precise nutritional requirements of Greenshell™ mussel spat. This will allow commercial operators to choose valuable alternatives to enhance mussel spat growth and hatchery profitability without compromising performance.

## 1. Background

Global marine mussel aquaculture provides an important source of protein for humans, with total global production in 2022 reaching 1.93 million tonnes. However, the growth of the farmed mussels has lagged behind that of other aquaculture sectors, with total global production down 15 % in 2022 compared to the annual average between 2010 and 2020 (FAO, 2025). This decline has been particularly pronounced in key producing nations, such as New Zealand, Spain, Netherlands and France, due in a large part to inefficiencies in the early stages of mussel seed or spat production and utilisation (Avdelas et al., 2021; Kamermans and Capelle, 2019; Skelton et al., 2022; South et al., 2022; Wijsman et al., 2019). For many mussel aquaculture industries, a primary dependence on the wild harvest of spat to supply the industry has resulted in an inconsistent supply which often is unable to meet industry demands (Avdelas et al., 2021; South et al., 2022), due to the seasonal and unpredictable nature of wild spat fall (Alfaro et al., 2010; Peteiro et al.,

2007; Soria et al., 2015). Additionally, wild-sourced spat are often highly variable in nutritional condition and are seeded out at small and vulnerable sizes (i.e., < 1 mm) which contributes to high losses shortly after they are initially seeded onto grow out substrate (Carton et al., 2007; Skelton et al., 2024; Supono et al., 2020).

One approach to mitigating the losses of early-stage juvenile mussels and improving the efficiency of mussel aquaculture is the hatchery production of spat. Hatcheries can offer a consistent and reliable supply of mussel spat, reducing reliance on unpredictable natural spat fall events and allowing farmers to optimise stocking densities and farm management strategies year-round. Further, hatchery reared spat are in superior condition compared to wild-sourced, because they are typically fed high-quality cultured microalgae through larval development and post-settlement (Ragg et al., 2010; Skelton et al., 2024). Producing spat with better nutritional condition from a hatchery can also reduce spat losses on farms because spat with greater energy reserves can withstand adverse environmental and feeding conditions, resulting in a greater

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chance of remaining attached to aquaculture substrate (Babarro et al., 2008; Supono et al., 2021). Despite these advantages, widespread adoption of the use of hatchery production of mussel spat remains limited due to the high costs associated with hatchery production. A significant portion of these costs (typically 30 to 40 %) is attributed to the production of live microalgae required as feed for juvenile mussels (Borowitzka, 1997; Brown and Blackburn, 2013; Coutteau and Sorgeloos, 1993). As a result, developing cost-effective alternative feeds to supplement or replace live microalgae is a key priority for improving the economic viability of hatchery production and supporting the sustainable growth of global mussel aquaculture.

New Zealand's Greenshell™ mussel industry is the country's largest aquaculture sector by volume, with 92,967 t harvested in 2023, generating NZD \$423 million and accounting for 55 % of the country's total aquaculture production (Aquaculture New Zealand, 2023). Approximately 80 % of mussel spat used in New Zealand's industry comes from wild sources, primarily washing ashore at Te Oneroa-a-Tōhē (Ninety Mile Beach) attached to drift seaweed (Aquaculture New Zealand, 2022; Jeffs et al., 1999). The remaining spat is sourced from spat-catching ropes, with only a small proportion currently supplied from a single commercial hatchery (Aquaculture New Zealand, 2020). This reliance on wild spat fall events, which are both seasonal and unpredictable (Alfaro et al., 2010), has been one of the primary factors limiting the expansion of mussel aquaculture in New Zealand, despite the availability of large areas of coastal water space for mussel farming (Ministry for Primary Industries, 2024).

Increasing hatchery production of spat could address this issue by providing a year-round supply of spat, which also produce more uniform spat in terms of condition, age, size and genetics, all factors believed to influence retention in the early stages of grow out (Buchanan and Babcock, 1997; Carton et al., 2007; South et al., 2020; Supono et al., 2021). However, hatchery production in New Zealand is also limited by the high cost and labour-intensive nature of live microalgae feed needed to rear hatchery raised juveniles to a sufficient size to be seeded onto coastal mussel farms, i.e., > 1.5 mm in shell length (Aquaculture New Zealand, 2020; South et al., 2022). Efforts to produce more cost-effective feeds for juvenile Greenshell™ mussels have focused on replacing live phytoplankton with both formulated and natural alternative feeds (i.e., Gui et al., 2016a; Gui et al., 2016b; Skelton et al., 2021). These alternatives have shown some promise in experimental evaluation, although they have lacked the nutritional value of live microalgae and typically lead to decreased growth and survival of the mussel spat.

A promising alternative feeding method for juvenile Greenshell™ mussels is enriching seawater with dissolved carbohydrates as the mussels are capable of taking up and using dissolved carbohydrates to enhance growth and improve nutritional condition when used as a supplementary feed (Jordan et al., 2024; Jordan et al., 2025). Carbohydrates, like glucose, are an effective, cheap source of energy for juvenile mussels that are often metabolised to meet immediate energy needs, allowing protein and lipids supplied from live microalgal diets to be conserved for growth (Anderson et al., 2004). This has been reinforced in experiments where carbohydrates have been preferentially depleted over lipids and proteins during periods of starvation (Sim-Smith and Jeffs, 2011; Supono et al., 2022).

Despite the success of dissolved carbohydrates as a supplementary feed in experimental studies, it is unknown if a simple monosaccharide, like dissolved glucose, can also be used to partially replace live microalgae to maximise cost-savings in nursery rearing of spat in a hatchery. One of the most costly periods of mussel rearing is the first two weeks after larval settlement when post-settlement spat are typically fed on high-quality cultured live microalgae before they can be weaned onto less costly feeding options, such as pond-reared mixed microalgae or preserved microalgae products (Helm et al., 2004; Supono et al., 2023).

Experiments have shown that enriching seawater with glucose at a concentration of 100  $\mu\text{g mL}^{-1}$  can significantly enhance Greenshell™ mussel spat growth (Jordan et al., 2024). This was the lowest effective

concentration of glucose previously tested, making it the most suitable option due to its lower cost and reduced risk of bacterial proliferation during exposure. Furthermore, short exposure windows of 2 h are sufficient for spat to uptake dissolved sugars, as longer exposure times have been found to have limited influence on improving the growth of spat (Jordan et al., 2025). If dissolved glucose proves effective as a supplement or partial replacement for live microalgae during this period of early spat development, it will provide the potential for cost-effective dissolved feeds to reduce the reliance on live microalgae as a sole feed input in commercial-scale hatchery operations.

This study aims to determine the optimal substitution or supplementation level of dissolved glucose for live microalgae, with the goal of reducing hatchery costs while maintaining spat nutritional condition and growth.

## 2. Materials and methods

### 2.1. Spat source

Broodstock Greenshell™ mussels were conditioned in a semi-recirculating system where wild microalgae were delivered via an artificially fertilised saltwater pond at the Te Huata Ltd. hatchery, allowing mussels to feed ad libitum. The ponds were initially fertilised upon filling with urea (5.45  $\text{g m}^{-3}$ ), superphosphate (1.52  $\text{g m}^{-3}$ ), and sodium metasilicate (9.55  $\text{g m}^{-3}$ ). On subsequent days, only sodium metasilicate was added, with dosing rates adjusted according to pond fluorometer readings to maintain optimal microalgal productivity.

Spawning of broodstock was induced by thermal shock, and the initial gamete release allowed individuals to be sexed and then isolated. Egg density was stocked at 1000  $\text{mL}^{-1}$ , and sperm was added at a ratio of 500  $\text{egg}^{-1}$ . Following fertilisation, the embryos were incubated for approximately 48 h until embryos had transformed into early D veligers.

After the 48 h incubation, the veligers were transferred into a high density larval rearing system (King et al. (2005)). The larvae were fed on a diet consisting of a mixture of axenically cultured microalgae. The mixed microalgae diet consisted of *Chaetoceros calcitrans*, *Chaetoceros muelleri*, and *Tisochrysis lutea*. The ratio of these microalgae species was adjusted throughout larval development. Initially, *C. calcitrans* was the only species provided, followed by the introduction of *T. lutea* and then *C. muelleri*, resulting in larvae receiving a mixed diet of all three species by the end of their development.

After 21 days, larvae displayed signs of settling and were transferred into rectangular tanks (1000 L capacity) that were equipped with frames wound with stretched coir rope, providing substrate for larval settlement. Larvae were introduced at a target density of 6000 individuals per metre of rope to optimise settlement and post-larval development. After 5 days of continued feeding with a mixture of axenically cultured microalgae and daily seawater flushes, there were no longer larvae washing out from the tank and onto the catch screen, indicating that all larvae had successfully settled onto the coir rope. The mixed microalgal diet consisted of *T. lutea* and *C. muelleri* at a ratio of 1:1, and fluorescence readings were taken each day to determine the equivalent cell density of the microalgae to reach the desired fluorescence reading for feeding to spat. Fluorescence readings were taken using a handheld fluorometer that consisted of a Cyclops-7 chlorophyll-a fluorometer (Turner Designs, Sunnyvale, CA, USA) with a 30 mL black polyethylene sample chamber coupled to a generic digital voltmeter, which provided readings in mV.

### 2.2. Experimental set up

Once larvae had successfully settled, 18 randomly selected 5 m sections of the coir rope (~ 10,100 individual spat) were each transferred into 18 separate 10 L cylindrical tanks ( $\phi$  150 mm), resulting in each tank containing 5 m of coir rope. The section of coir rope was suspended from the top of the tank and dangled down the centre of the cylindrical tank, which was aerated from the base.

Six feeding treatments were used to investigate the impact of supplementing and replacing microalgae with dissolved glucose. Three replicate tanks were randomly assigned to each treatment. The concentrations of microalgae were set to provide restricted feeding rations, where a 100 % ration corresponded to a fluorescence measurement of 40, a 90 % ration to a fluorescence measurement of 36, and a 60 % ration to a fluorescence measurement of 24. Fluorescence readings were used as a proxy for microalgae concentration.

The concentration of dissolved glucose ( $100 \mu\text{g mL}^{-1}$ ) was selected based on prior laboratory trials demonstrating its effectiveness as a supplementary feed without inducing mucus production or bacterial proliferation during a 2-h exposure (Jordan et al., 2024).

The six feeding treatments were as follows: **M100**: 100 % microalgae ration (fluorescence 40) with no glucose; **M100 + G**: 100 % microalgae ration (fluorescence 40) plus dissolved glucose ( $100 \mu\text{g mL}^{-1}$ ); **M90**: 90 % microalgae ration (fluorescence 36) with no glucose; **M90 + G**: 90 % microalgae ration (fluorescence 36) plus dissolved glucose; **M60**: 60 % microalgae ration (fluorescence 24) with no glucose; **M60 + G**: 60 % microalgae ration (fluorescence 24) plus dissolved glucose.

Each day the seawater in each tank was changed by flushing each tank with fresh seawater and draining the tanks through a  $250 \mu\text{m}$  mesh to retain any loose spat which were then returned to the tank upon refilling the tank with seawater. Once all spat were returned into their tank they were fed their corresponding microalgae ration and left to feed for the following 22 h. At this time dissolved glucose was added to those tanks with glucose feeding treatments, exposing the spat to dissolved glucose for a period of 2 h before the seawater in all the tanks were changed. The experiment was conducted over 14 days, and every 2 days, each tank was exchanged for a sterilised tank to reduce any risk from bacterial contamination in tanks.

### 2.3. Spat performance

#### 2.3.1. Growth

Fifty randomly selected spat from each replicate tank were measured for shell length using an inverted microscope at the outset of the experiment (i.e., day 0) and at the end of the experiment (i.e., day 14) by taking the longest distance across the shell from the umbo. Measurements were recorded individually, and the mean shell length was calculated per tank. Treatment means were then calculated as the average of the three replicate tank means.

#### 2.3.2. Coir density

At the beginning of the experiment one 10 cm section of coir rope from the 5 m section was sampled from each replicate tank and the spat were washed off the rope onto a  $250 \mu\text{m}$  mesh and counted to estimate the starting density of spat. At the end of the experiment (day 14), spat counts were taken in the same manner from five 10 cm sections of coir rope **sampled separately** from each replicate tank. The counts from these five sections were averaged to estimate the final density of spat per tank.

#### 2.3.3. Microalgae consumption

Microalgae were fed out each day after the seawater exchange in the experimental tanks. For each tank, a **single fluorescence reading** was taken immediately after microalgae addition and again 24 h later. The difference between the fluorescence reading for each tank taken immediately after the microalgae were administered and 24 h later enabled the daily estimation of the total percentage of the ration of microalgae that were consumed by the spat in each experimental tank.

### 2.4. Elemental analysis

At the end of the experiment spat from each replicate tank were washed off the coir rope and separated from the rope debris, washed in deionised water, frozen at  $-80 \text{ }^\circ\text{C}$  and then lyophilised for 24 h.

Elemental analysis was then used to assess potential differences in the nutritional condition of early post-settlement spat by determining total carbon, nitrogen and hydrogen concentrations. The lyophilised mussel spat were ground into a fine powder using a small Eppendorf pestle and 10 mg was loaded into tin capsules per replicate tank and analysed using an elemental analyser (Vario El Cube Element Analysensysteme, Langensfeld, Germany). The combustion and reduction temperatures were  $950 \text{ }^\circ\text{C}$  and  $600 \text{ }^\circ\text{C}$ , respectively. The analytical error was  $<1 \%$ .

### 2.5. Statistical analysis

Shell length data of mussel spat collected on Day 0 and Day 14 were used to estimate the average daily growth rates across treatments. A linear model was fitted with shell length as the response variable and day (treated as a numeric covariate), glucose supplementation (present or absent), microalgae ration (60 %, 90 %, or 100 %), and all interactions among these factors as fixed effects. The model was specified as: shell length  $\sim$  day  $\times$  glucose  $\times$  ration.

Analysis of variance (ANOVA) was used to assess the main effects and interactions, including whether growth rates differed among treatments. Estimated daily growth rates ( $\mu\text{m day}^{-1}$ ) for each treatment combination were extracted using the `emmeans()` function in the `emmeans` R package. Pairwise comparisons of the estimated slopes were performed using Tukey-adjusted  $p$ -values to identify significant differences in daily growth rates among the treatments.

Separate two-way analysis of variance (ANOVA) was used to compare the consumption of microalgae, and elemental composition of the spat (i.e., C, N, H) among treatments. One-way ANOVAs were used to compare the shell length and spat densities on coir rope at the beginning of the experiment. All data were checked for normality and homogeneity of variances, and if they did not conform with parametric assumptions, they were log-transformed and re-assessed to ensure they conformed. The percentage data (i.e., microalgae consumption & elemental analysis) were transformed using an arcsine transformation prior to analysis by ANOVA. Where significant main effects were identified with the ANOVA, pairwise comparisons of estimated marginal means (`emmeans`) were conducted using Tukey's tests with adjustment for multiple comparisons.

## 3. Results

### 3.1. Daily growth

Daily growth rates differed significantly among treatments, with significant interactions between day and microalgae ration ( $F_{(2,1788)} = 33.38$ ,  $P < 0.001$ ), and day and glucose ( $F_{(1,1788)} = 10.40$ ,  $P = 0.0013$ ), indicating that both microalgae ration and glucose supplementation influenced spat growth (Fig. 1). Growth increased overall with increasing microalgae ration, with spat fed 100 % ration without glucose growing on average  $8.1 \mu\text{m day}^{-1}$  greater than those fed 60 % ration without glucose ( $P < 0.001$ ). At the 90 % ration level, glucose supplementation significantly improved growth compared to the no glucose 90 % microalgae ration ( $4.5 \mu\text{m day}^{-1}$  greater  $P = 0.011$ ), resulting in growth rates similar to both 100 % ration treatments. In contrast, within the 60 % and 100 % ration levels, glucose did not significantly affect spat growth although in both instances there was a trend towards increased daily growth in the glucose treatment.

### 3.2. Spat density on coir rope

At the beginning of the experiment (i.e., day 0), the mean density of spat on the coir rope was not different among the six treatments ( $F_{(5,12)} = 0.835$ ,  $p = 0.55$ ). The overall mean density of spat on the coir was  $2018 \text{ m}^{-1}$  ( $\pm 200.0 \text{ SE}$ ).

There were no differences in the mean density of spat on coir rope at the end of the experiment due to the interaction between the microalgae

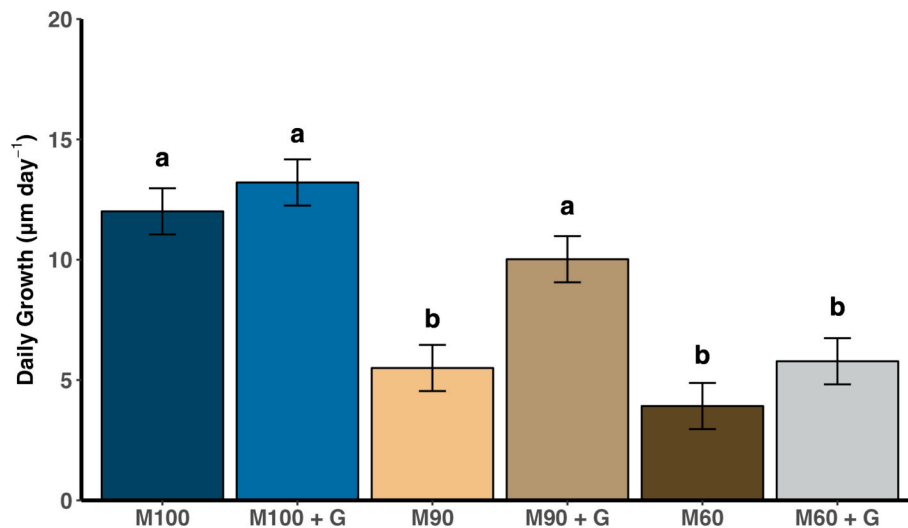


Fig. 1. The daily growth of spat in the six treatments over the 14 d experiment. Daily growth values with different letters are different ( $P < 0.05$ ).

ration and glucose treatment ( $F_{(2,84)} = 2.10, P = 0.13$ ) (Fig. 2). However, there were differences in final spat densities due to the main effect of feed ration ( $F_{(2,84)} = 3.67, P = 0.03$ ), with the two 100 % feed ration treatments (i.e., M100 & M100 + G) having  $328 (\pm 122 \text{ SE})$  more spat  $\text{m}^{-1}$  of coir rope than the 60 % feed ration treatment groups (i.e., M60 & M60 + G) ( $P = 0.02$ ). There was an overall trend for the mean density of spat to decline with decreasing feeding ration and their associated water transparency.

### 3.3. Microalgae consumption

There was no difference in the mean percentage of the provided ration of microalgae that was consumed daily by spat due to the interaction between the feed ration and glucose treatment ( $F_{(2,138)} = 0.91, P = 0.41$ ) (Fig. 3). However, there was a difference in the daily consumption of microalgae by spat due to the main effect of feed ration ( $F_{(2,138)} = 5.15, P = 0.007$ ), with mussel spat in the 100 % feed treatments consuming more microalgae than those spat in the 60 % ration treatments ( $P = 0.006$ ).

### 3.4. Elemental analysis (CHN)

#### 3.4.1. Carbon

There was no difference in the mean carbon content of whole spat due to the interaction between the feed ration and glucose treatment ( $F_{(2,12)} = 1.98, P = 0.18$ ). Additionally, there was no difference in carbon content due to the main effects of feed ration ( $F_{(2,12)} = 3.41, P = 0.06$ ) or glucose treatment ( $F_{(1,12)} = 0.70, P = 0.42$ ) (Fig. 3). The overall mean carbon content of whole spat among the six treatments was  $14.4\% (\pm 0.11 \text{ SE})$  (Fig. 4).

#### 3.4.2. Nitrogen

There was no difference in the mean nitrogen content of whole spat due to the interaction between the feed ration and glucose treatment ( $F_{(2,12)} = 0.15, P = 0.86$ ). Additionally, there was no difference in mean nitrogen content of whole spat due to the main effects of feed ration ( $F_{(2,12)} = 1.79, P = 0.21$ ) or glucose treatment ( $F_{(1,12)} = 0.59, P = 0.46$ ) (Fig. 3). The overall mean nitrogen content of whole spat among the six treatments was  $0.7\% (\pm 0.02 \text{ SE})$  (Fig. 5).

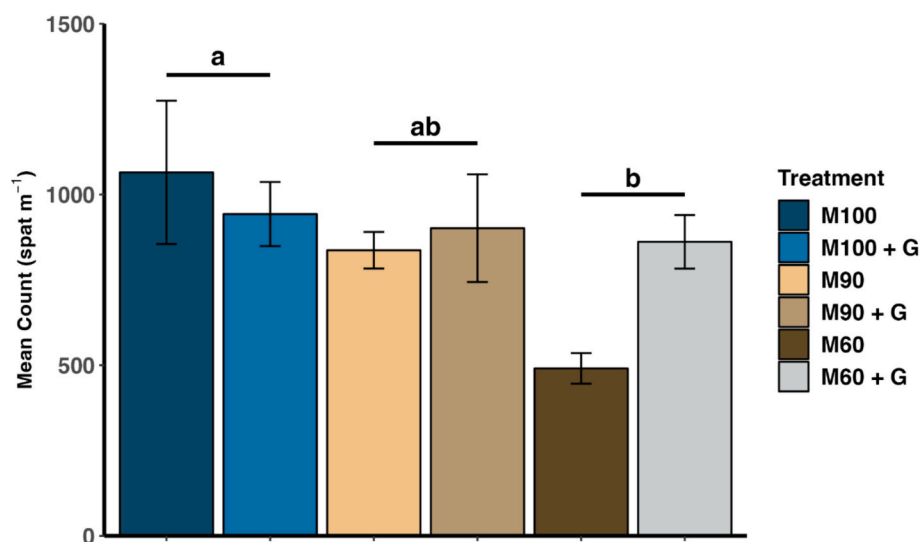
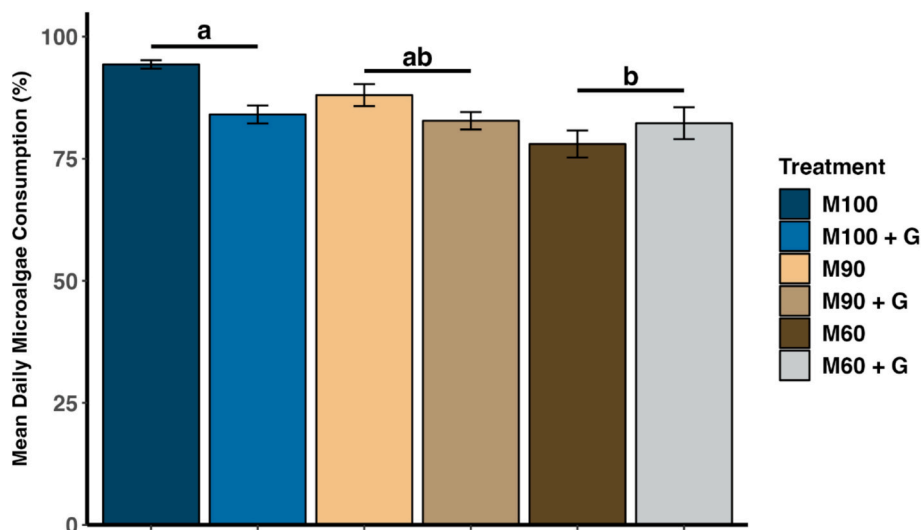
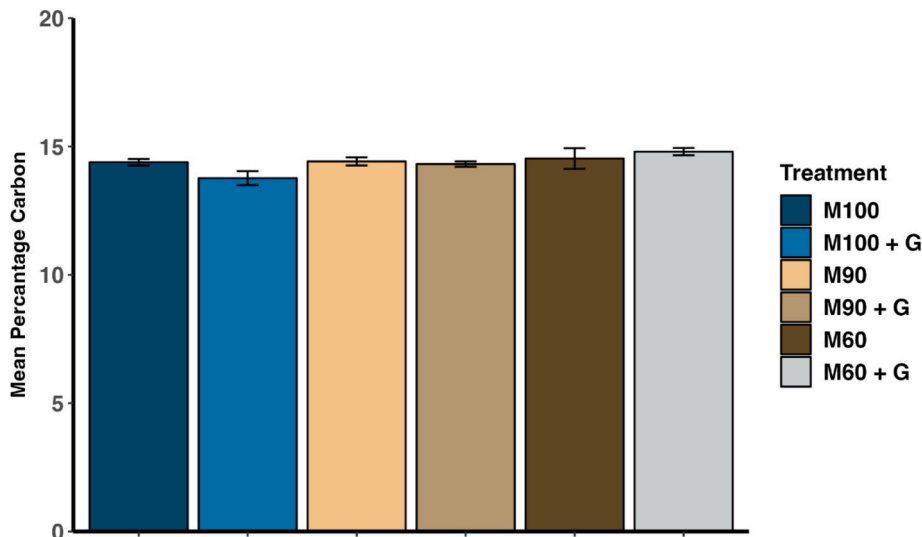


Fig. 2. The mean density ( $\pm \text{SE}$ ) of spat ( $\text{m}^{-1}$ ) on the coir rope for the six treatments after 14 days at the end of the experiment. Means with different letters are different ( $P < 0.05$ ).



**Fig. 3.** The mean percentage ( $\pm$ SE) of microalgae consumed daily by mussel spat for the six treatments over the 14 day experiment. Means with different letters are different ( $P < 0.05$ ).



**Fig. 4.** The mean percentage ( $\pm$ SE) of carbon in mussel spat for the six feeding treatments at the end of the 14 day experiment. There were no differences among the means ( $P > 0.05$ ).

### 3.4.3. Hydrogen

There were differences in the mean percentage of hydrogen in whole spat among the six feeding treatments due to the interaction between the feed ration and glucose treatment ( $F_{(2,12)} = 6.16$ ,  $P = 0.01$ ). Spat in the 60 % Ration + Glucose exhibiting higher hydrogen content than those spat in the 100 % Ration + Glucose treatment ( $P = 0.045$ ) (Fig. 6).

### 3.4.4. Carbon & nitrogen ratio

There was no difference in the in the mean C:N ratio of whole spat due to the interaction between the feed ration and glucose treatment ( $F_{(2,12)} = 0.34$ ,  $P = 0.72$ ), and there was no difference in mean nitrogen content of whole spat due to the main effects of feed ration ( $F_{(2,12)} = 1.39$ ,  $P = 0.29$ ) or glucose treatment ( $F_{(1,12)} = 0.01$ ,  $P = 0.95$ ) (Fig. 3). The mean C:N ratio for mussel spat was  $21.6 (\pm 0.52 \text{ SE})$  (Fig. 7).

## 4. Discussion

The highly unpredictable supply of wild mussel spat for seeding farms is a common problem throughout many mussel industries

globally. Increasing the supply of hatchery produced mussel spat would overcome this issue. However, the expansion of hatchery production of spat is currently constrained by the high costs associated with culturing high-quality live microalgae feed for feeding the early stages of the mussel spat. High feeding costs for mussel spat occurs in the first few weeks after settlement, when the newly-settled spat are weaned off high-quality cultured microalgae onto lower quality microalgae, such as that supplied from pond microalgae culture systems. One possible way of reducing the feeding costs of spat after settlement is by either increasing growth rates so that spat spend less time in land-based systems before reaching appropriate sizes or by substituting more costly cultured microalgae with lower cost alternatives. This study evaluated the use of dissolved glucose as a potential additional food source to replace or enrich live microalgae as the only food and increase spat growth while reducing the costs of land-based nursery culture for Greenshell™ mussel spat.

The addition of dissolved glucose at  $100 \mu\text{g mL}^{-1}$  to a full ration of microalgae (M100 + G) did not increase the growth of mussel spat compared to the Control without the addition of glucose (M100),

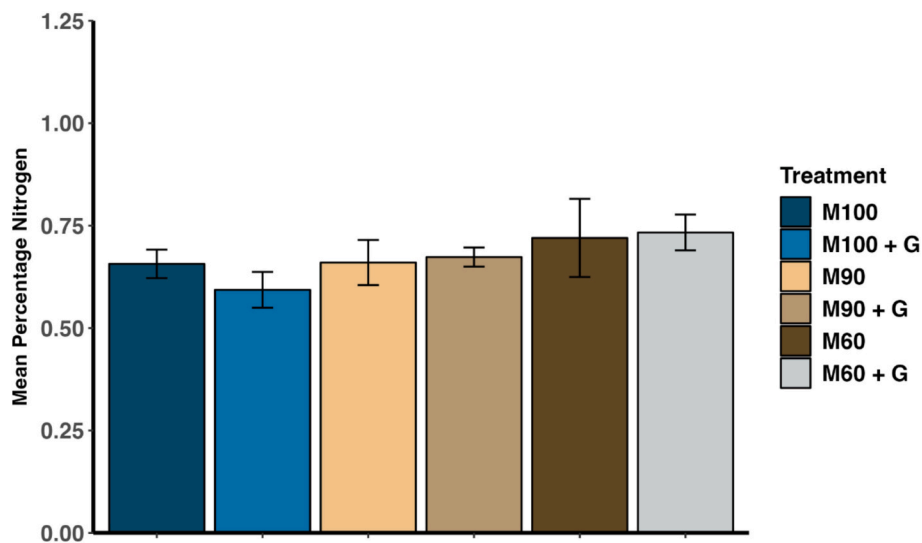


Fig. 5. The mean percentage ( $\pm$ SE) of nitrogen in mussel spat for the six feeding treatments at the end of the 14 day experiment. There were no differences among the means ( $P > 0.05$ ).

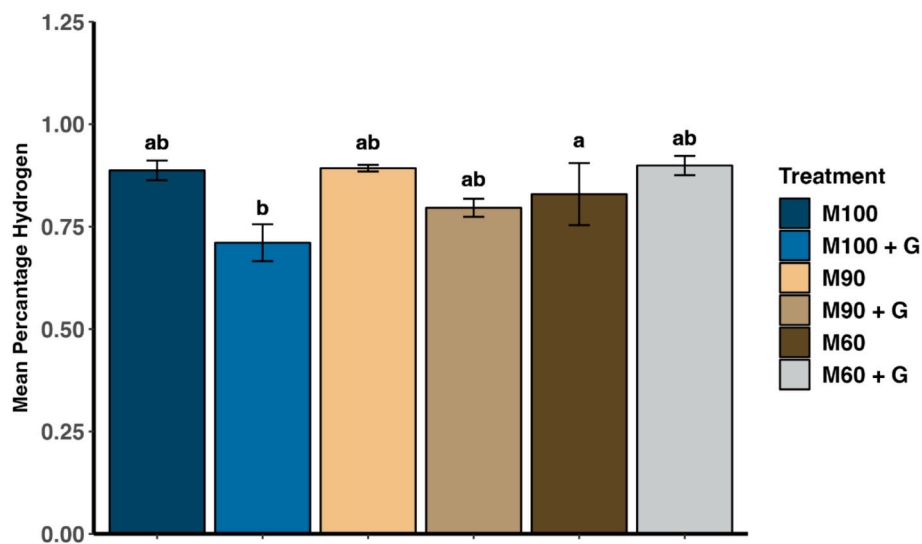


Fig. 6. The mean percentage ( $\pm$ SE) of hydrogen in mussel spat for the six feeding treatments at the end of the 14 day experiment. Means with different letters are different ( $P < 0.05$ ).

although there was a trend towards higher growth from those spat that received supplemental glucose. This suggests that at larger commercial scales using glucose as a supplemental feed may be a viable way to reduce costs by improving spat growth. A 10 % reduction in the ration of live microalgae to spat (M90) resulted in a 56 % decline in growth of the spat (i.e., on average a  $7.1 \mu\text{m d}^{-1}$  reduction in growth in shell length) compared to those provided with the full ration, indicating a nutritional limitation. This reduction in the growth of spat was partly recovered with the addition of glucose to spat experiencing a 10 % reduction in their ration of microalgae, indicating that carbohydrate supply was recovering some of the nutritional limitation. A 60 % Ration of macroalgae (M60 & M60 + G) markedly reduced the growth of spat by 63.5 % compared to the 100 % Ration treatments. Supplementing glucose to spat with a 40 % reduction in the ration of macroalgae made no difference to their growth rate, indicating that carbohydrate supply alone was not the nutrient limiting spat growth.

Carbohydrates appear to be the primary energy source for Green-shell™ mussel spat, as they are preferentially depleted over lipids and proteins during starvation (Sim-Smith and Jeffs, 2011; Supono et al.,

2022) and can make significant differences to growth when used as a supplementary source of nutrition (Jordan et al., 2024; Jordan et al., 2025). The results of this study indicate that dissolved glucose can only be used as a partial replacement for microalgae before the growth of the mussel spat is affected. This finding suggests that while carbohydrate supplementation may be effective in supporting immediate energy demands and maintaining basic physiological functions, it is likely insufficient on its own to sustain long-term growth and tissue development, which also depends on protein and lipid availability (Bayne, 1976). Future studies could examine whether combining dissolved carbohydrates with other macronutrient supplements supports greater substitution away from microalgae.

Detachment from settlement substrate results in spat being lost from culture systems, hence attachment in this study is interpreted as a proxy for spat survival. Spat in the 60 % ration treatments (i.e., M60 & M60 + G) had a lower attachment to coir rope at the end of the experiment compared to those spat fed the 100 % ration (i.e., M100 & M100 + G). Food availability and energy reserves have been shown to impact the byssogenesis rate and attachment strength in juvenile mussels, with

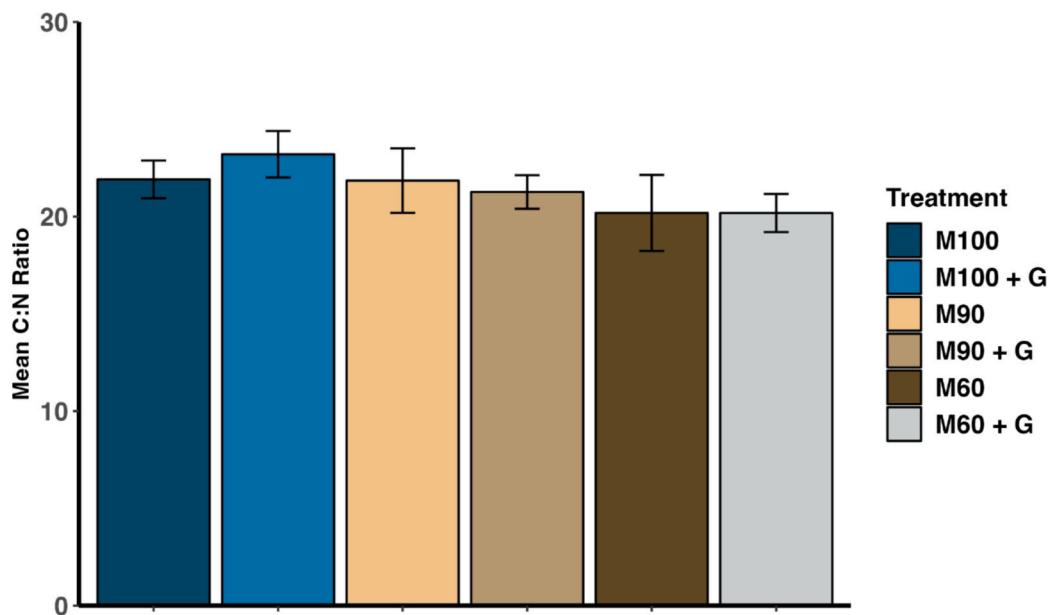


Fig. 7. The mean carbon to nitrogen (C:N) ratio ( $\pm$ SE) in mussel spat for the six feeding treatments at the end of the 14 day feeding experiment. There were no differences among the means ( $P > 0.05$ ).

fasted juvenile *Mytilus galloprovincialis* displaying reduced byssus production and attachment strength (Babarro et al., 2008). Additionally, Greenshell™ mussel spat have previously been shown to have lower rates of attachment to substrata after periods of starvation which was suggested to be due to the food limitation triggering migration behaviour (Supono et al., 2021). However, the influence of tank dynamics, such as flow, aeration, and water transparency cannot be ruled out as possible experimental artefacts causing the spat to detach from coir rope, with spat detachment also shown to decrease with increasing water velocity (Alfaro, 2005; Hayden and Woods, 2011). The lower densities of Greenshell™ mussel spat resulting from a 60 % ration of microalgae is consistent with explanations that byssus production or migration behaviour of the spat is affected by either feeding conditions or environmental conditions. Spat retention during the initial weeks after seeding onto Greenshell™ mussel farms is extremely low (i.e., < 1 %) and is reported to be even lower for spat sourced from the wild for seeding, which often spend weeks in the turbulent surf zone before they are harvested off Ninety Mile Beach and are known to be in poor nutritional condition (Alfaro et al., 2010; Skelton and Jeffs, 2021). These results highlight the potential benefits of supplying hatchery-reared spat in good nutritional condition for seeding out to coastal mussel farms to ensure high initial rates of attachment of the spat to the aquaculture substrates.

The proportion of the ration of microalgae consumed by mussel spat was not affected by the addition of dissolved glucose despite prior research evidence for dissolved carbohydrates increasing bivalve filtration rates, leading to higher capture efficiency and greater total microalgae consumption (Collier et al., 1953). Spat in the two treatments where the microalgal ration was reduced to 60 % consumed a lower percentage of their available feed compared to the 100 % Ration treatment. This was likely due to the lower initial microalgae concentration in their feeding ration, as spat with lower experimental rations of microalgae reduced overall microalgal cell densities to similar low levels after 24 h as those in treatments with higher feeding rations.

Elemental (CNH) analysis is used to assess body tissue composition in marine invertebrates in relation to available food sources, providing insight into nutrient assimilation and allocation (Anderson et al., 2004; Arranz et al., 2021; Urabe and Watanabe, 1992). In this study, a shift in the carbon content of spat from the glucose treatments would indicate increased carbon incorporation from glucose, reflecting changes in

metabolic pathways or nutritional status (DeNiro and Epstein, 1978). There were no differences in the carbon or nitrogen content of spat among the feeding treatments, suggesting that substituting and enhancing a live microalgae diet with dissolved glucose at  $100 \mu\text{g mL}^{-1}$  for  $2 \text{ h day}^{-1}$  did not affect overall carbon or nitrogen assimilation on a proportional tissue dry mass basis. However, the hydrogen content of spat was only different between two treatments, with spat in M60 exhibiting higher hydrogen content than those in M100 + G. Hydrogen is a major component of both lipids and carbohydrates, so elevated hydrogen content may indicate a greater proportion of stored lipids or carbohydrates relative to proteins (Gnaiger and Bitterlich, 1984). However, the spat in M60 were significantly smaller in size than those in M100 + G at the end of the treatment, so that the absolute hydrogen content may not have been different due to size differences in the spat.

There were no differences in the C:N ratio of post-settlement mussel spat among the six treatments. While the C:N ratio of bivalve tissue typically ranges from 4.1 to 6.2 (Bayne, 2009), the mean values in this study were considerably higher, ranging from 20 in M60 + G to 23 in M100 + G. These elevated values likely reflect, at least in part, the inclusion of shell material in the analysis, which would have contributed inorganic carbon from calcium carbonate ( $\text{CaCO}_3$ ) and inflated overall carbon content relative to nitrogen. Carbon is a key component of carbohydrates and lipids, while nitrogen is primarily associated with protein (Arranz et al., 2021; Mariotti et al., 2008). The high C:N ratios observed may still suggest a relatively low protein content in the spat compared to the lipid and carbohydrate reserves. However, due to the influence of shell derived carbon, these results are not directly comparable to values reported for soft tissue alone. This methodological consideration may also explain the lower C:N ratios previously reported for newly settled Greenshell mussel spat reared on the same microalgal diet (Jordan, *in press*), where the influence of shell material would have been less than the current study due to spat being 14 days younger. Those spat had C:N ratios of 11.7 (microalgae only) and 12.4 (microalgae + glucose), suggesting that protein may play a larger role in larval nutrition. The shift towards carbon rich energy reserves in these older spat may reflect the transition in nutritional demands post-metamorphosis, consistent with previous findings (Sim-Smith and Jeffs, 2011; Supono et al., 2021; Supono et al., 2022).

Dissolved glucose presents a viable partial substitute (i.e., 10 %) to live microalgae provided to spat for  $2 \text{ h day}^{-1}$  at  $100 \mu\text{g mL}^{-1}$ , as it does

not impact the growth or settlement densities (a proxy for retention on aquaculture substrates) of the mussels. Finding alternatives to live microalgae that can be used on a commercial scale has presented a significant challenge for expanding hatchery culture for New Zealand's Greenshell™ mussel industry and in many other mussel production industries around the world (Kamermans and Capelle, 2019; Knauer and Southgate, 1999; South et al., 2022; Willer and Aldridge, 2019). This study has demonstrated the ability of glucose to allow significant cost savings in microalgae production by substituting it with an off-the-shelf product that is readily available and significantly more cost-effective. There is also the need to explore optimal substitution further, as although glucose substitution was ineffective when replacing 40 % of live feed, it is likely that further optimisation can occur beyond 10 %.

This study has demonstrated the ability of glucose as a cost-effective alternative feed option, that is a readily available off the shelf option. In similar hatchery operations internationally, microalgae feed costs have been reported to account for approximately 20–40 % of total operating expenses (Coutteau and Sorgeloos, 1993; Helm et al., 2004; Oostlander et al., 2020). This range suggests that reducing microalgae requirements by subsisting with dissolved glucose alone could yield meaningful cost savings of between 2 and 4 %. Although larger scale experiments would need to be conducted in experimental tanks with greater volume, and with seawater changes after exposure to nutrients to minimise impacts on water quality and microbial conditions in larger culture systems. With this, there would also be need to be further work to quantify the long term effects of dissolved glucose on microbial dynamics and water quality.

Given the ability of bivalves to uptake a range of different dissolved organic material, such as amino acids (Gorham, 1988; Manahan et al., 1983), vitamins (Langdon, 1983), and other forms of carbohydrates (Nell et al., 1983; Swift et al., 1975), there is potential to optimise the formulation of a dissolved feed so it aligns with the nutritional requirements juvenile mussels and that substitution of microalgae can occur beyond 10 % so cost savings on microalgae can be further optimised. Different sugars follow distinct metabolic pathways in Greenshell™ mussel spat, with glucose promoting greater growth and sucrose enhancing glycogen storage (Jordan et al., 2024; Jordan et al., 2025). Therefore, dissolved feeds may have the potential to be tailored to specific nursery phases. Initially, maximising growth may be the priority, but before seeding on farms, commercial operators should ensure that spat have adequate glycogen reserves, as this improves retention during early grow-out on mussel farms (Supono et al., 2021).

## 5. Conclusions

Many mussel aquaculture industries worldwide rely primarily on wild-sourced spat, resulting in a highly inconsistent supply of juvenile mussels that are also variable in size, age, condition, and genetics. Expanding the hatchery culture of juveniles is a key step to ensuring the long term sustainable growth of mussel aquaculture globally. However, the reliance on live microalgae as the sole commercial feed for juvenile mussels has hatchery expansion cost-prohibitive. This experiment demonstrated the potential of glucose as both a supplement and partial replacement to live microalgae. Spat supplemented with dissolved glucose (i.e., M100 + G) for 2 h daily over the first 14 days of spat rearing showed a trend towards higher daily growth, without affecting the microalgae consumption, spat densities, or elemental composition of the mussels. Additionally, replacing 10 % of live microalgae with dissolved glucose resulted in no differences in spat performance compared to the 100 % Ration control (i.e., M100). This study highlights the potential of dissolved glucose as a feed in bivalve hatcheries, although further research is needed to identify optimal combinations of dissolved nutrients so that commercial operators can choose to either supplement and enhance spat growth or partially substitute away from microalgae feeds without compromising spat performance.

## CRediT authorship contribution statement

**Andy Jordan:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Data curation, Conceptualization.  
**Kim Thompson:** Resources, Project administration, Methodology.  
**Andrew Jeffs:** Writing – review & editing, Validation, Resources, Project administration, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

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