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Exposure of Greenshell Mussel Spat (*Perna canaliculus*) to Stressors for Producing Single-Spat From Collector Ropes

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ABSTRACT

Nursery culture can improve the efficiency of mussel aquaculture by reducing the high losses of spat and overcoming inconsistencies in wild-spat supplies. The success of nursery systems depends on the availability of unattached single or singulated spat, which requires the separation of spat from their associated settlement substrates (i.e., singulation). This study investigated whether sub-lethal environmental stressors could facilitate the singulation of Greenshell mussels (*Perna canaliculus*) spat from their attachment to collector ropes. Segments of spat-collector ropes were exposed to stressors consisting of either high (45 ppt) or low (25 ppt) salinity for 2 h, periods of emersion (air exposure) for 2 or 4 h, or combinations of reduced aeration and limited food availability for 12 h. Spat singulated from the collector rope were returned to clean seawater and reared for 24 h, after which their survival rates were measured. The results indicated that up to 80% of spat could be singulated from the ropes after a 12 h exposure to seawater with no aeration and no food (i.e., NA-NF). However, although survival rates following singulation ranged from 60% to 90% across treatments, overall recovery of viable, unattached spat was lower, reaching a maximum of 65% relative to the initial number of settled spat. Singulation efficacy was influenced by treatment and spat size, with smaller spat (< 3.5 mm) more easily singulated than larger ones (> 5 mm). Variations in the spat responses to the stressors indicate a need for further research to optimise singulation techniques and better understand the mechanisms driving the attachment and detachment responses in the mussel spat. Regardless, the study demonstrates that up to 65% of viable spat can be effectively removed from collector ropes using environmental stressors, making them readily available for rearing in nursery systems and reducing spat losses from production.

1 | Introduction

Nursery culture offers a promising solution to inefficiencies in mussel aquaculture, mainly for mitigating high losses of spat (i.e., juvenile mussels between 0.3 and 5 mm in shell length [SL]) that frequently occur during the early stages of production (Capelle et al. 2016; Molinet et al. 2021; South et al. 2020). These losses are costly and often strain the already limited supply of wild-caught spat (Avdelas et al. 2021; Filgueira et al. 2007; South et al. 2022). Constrained wild spat supplies combined with high spat losses have significantly contributed to stagnation in production in significant mussel aquaculture-producing countries, including New Zealand, Spain, France, Italy, Korea, Netherlands and Thailand (OCED 2024). However, nursery rearing of spat to larger sizes in containment, whether land or sea-based, can reduce spat losses whilst also improving their nutritional condition, making them more resilient once seeded onto coastal mussel farms (Phillips 2002; Skelton et al. 2021; Supono et al. 2022). This

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approach ensures more efficient use of spat while providing a stable, reliable and accessible source of healthy spat to mitigate inconsistent quality and availability in wild supplies (Heasman et al. 2002; Holliday et al. 1991; Skelton and Jeffs 2022).

Innovations such as floating upwelling systems (FLUPSYs) and land-based recirculating aquaculture systems (RAS) have been adapted for mussels, building on the success of nursery systems used in other forms of bivalve aquaculture (Kamermans et al. 2016; Skelton et al. 2021). However, a critical factor in the success of any nursery culture operation is the availability of unattached single-spat or singulated spat for stocking the nursery systems. Single-spat makes nursery culture more manageable by removing bulky settlement substrate that spat often settles onto (e.g., plastic ropes or macroalgae) while providing spat in a format that facilitates optimal growth and survival (Sanjayasari and Jeffs 2019). Without the inclusion of settlement materials, the space required and time it takes to rear spat can be reduced, thereby reducing the prohibitive operational costs often associated with bivalve nurseries. Furthermore, once reared, single-spat allows for more efficient farming practices by eliminating the need to transport the bulky wet material that spat are attached to. Single-spat also enables more even distribution of the mussel spat onto growing ropes when seeding onto coastal mussel farms, leading to better utilisation of available space and resources (Skelton and Jeffs 2022).

Unfortunately, there are currently no widely adopted methods for sourcing single-spat directly from the wild. Most spat naturally settle in clumps or on various substrates, which often accumulate a broad size range due to multiple settlement events and the continued growth of earlier settlers (Alfaro and Jeffs 2003; Filgueira et al. 2007). While some larger individuals may reach a size suitable for direct seeding, many remain too small or are tightly clustered, making them difficult to separate without causing damage and compromising their viability (Heasman et al. 1994; Himiona et al. 2024). This presents a significant challenge for aquaculture operations that wish to adopt nursery culture on a large scale, as the lack of available single-spat can negate many of the potential benefits of this approach. To overcome this obstacle, practical methods for separating spat from their settlement substrate must be developed, a process that is called singulation. This process involves carefully separating individual spat from the substrates they attach to without causing harm. Several approaches for spat singulation have been explored, including methods that seek to degrade the strength of byssus threads, such as, mechanical separation, and chemical pretreatment and methods that seek to capitalize on the natural migratory behaviour of spat by encouraging them to crawl off substrates by immersing them in static seawater (i.e., 'spat walking') (Bunter et al. 2006; Heasman et al. 1994; Himiona et al. 2024; Taylor et al. 1997). Each method comes with challenges, such as ensuring the spat remains healthy and viable after singulation, scaling the process to be cost-effective for commercial operations, and adapting techniques to different settlement substrates.

In New Zealand, the Greenshell mussel (*Perna canaliculus*) industry has developed several techniques for singulating spat from spat-laden macroalgae that was collected from Ninety Mile Beach, the primary source of wild-spat used by this industry, for nursery culture (Hickman 1976; Jeffs et al. 1999, 2018). Currently, the most successful of these methods involves using dilute chlorine solutions. This highly effective approach results in up to 94% singulation without adverse impacts on spat survival (Skelton and Jeffs 2023). However, the availability of macroalgae that washes ashore on Ninety Mile Beach and the number of spat attached is variable and unpredictable, posing a challenge for a consistent spat supply to nurseries (Skelton et al. 2022). To address this issue and diversify spat sources, research has begun exploring methods for singulating spat from alternative spat sources, such as from spat-collector farms, which use fibrous ropes to attract and collect spat in coastal waters, or from hatcheries, where spat are settled onto coir.

Techniques similar to those used for macroalgae, such as dilute chlorine solutions and protease enzymes, have previously been tested to dissolve the byssus threads of mussel spat attached to spat-collector ropes (Himiona et al. 2024). While these methods are effective for singulation, when used with high chemical concentrations or prolonged exposure durations they can result in high spat mortality (i.e., up to 97%). As a result, there is often a trade-off between achieving high singulation at the cost of increased mortality, and achieving lower singulation with correspondingly lower rates of mortality. This highlights the need for alternative methods that minimise spat mortality while maintaining the effectiveness of singulation. A potential alternative approach is to exploit the natural detachment response of mussel spat to sub-lethal environmental stressors. When exposed to sub-lethal stressors, spat may modify their production of byssus threads to either produce fewer, or even stop producing byssus threads, or they may actively sever their threads, enabling them to be attached from their settlement substrate (Buchanan and Babcock 1997; Duchini et al. 2015). For instance, while byssus formation is a continuous process in mussels, exposure of *M. coruscus* adults to hyposalinity (10 and 5 ppt) led to the complete suppression of byssus thread secretion, suggesting that exposure to significant physiological stress may be a viable option for achieving high rates of spat singulation (W.-Y. Wang et al. 2024). However, exposure of spat to sub-lethal stressors may also encourage spat to actively detach from their settlement substrate and migrate elsewhere. This migration may occur over short distances as spat crawl and explore potential settlement substrata using their muscular foot, or over longer distances via passive drifting on fine mucous threads, a process called secondary migration (Bayne 1964; Buchanan and Babcock; 1997; Sigurdsson et al. 1976). Conversely, stressful conditions may severely impact the physiological state of spat to such an extent that secondary migration is no longer possible. However, their attachment to substrates may still be compromised. During this time, spat may not be securely attached, potentially making them easier to singulate from collector ropes.

Factors such as lack of aeration (reduced water flow and low dissolved oxygen), limited food supply, prior aerial exposure and salinity changes have been attributed to detachment and reduced attachment performance in mussel spat. For instance, mussels seeded in long-line culture naturally exhibit crawling behaviour, moving outward along the ropes in search of space and better access to food (Carl et al. 2012; MacNeill 2005; Karayücel et al. 2002). This movement is aided by water movement, which provides favourable conditions for attachment and repositioning to optimise nutrient flow. However, when water motion is absent, oxygen levels drop, and food circulation becomes limited, leading to decreased byssal thread production, which may cause spat to migrate in search of more favourable conditions (Alfaro 2005, 2006; Hayden and Woods 2011; Sanjayasari and Jeffs 2019).

Salinity changes are another effective method for singulating bivalve spat from their settlement substrates, commonly used to remove spat from hatchery tank walls (Christophersen and Strand 2003; Heasman et al. 1994; Taylor et al. 1997). In mussels, exposure to excessively high or low salinities weakens existing byssus threads and halts the production of new ones, significantly reducing their adhesion strength (Allen et al. 1976; Rice et al. 2016; Rajagopal et al. 1996; W.-Y. Wang et al. 2024). In farming environments, salinity gradients influence spat distribution, with spat often migrating vertically to more favourable positions. This movement becomes more pronounced with increasing salinity differences between water layers, suggesting that sudden salinity shifts encourage spat movement (Molinet et al. 2021; Sameoto and Metaxas 2008). Thus, salinity changes may be helpful for singulating spat from collector ropes by weakening their attachment and encouraging movement, allowing them to reposition themselves.

Exposure to air, or emersion, can also influence the detachment of mussel spat from substrates. When spat are exposed to air, they often experience changes in environmental conditions such as dehydration, temperature extremes and reduced oxygen availability (South et al. 2021; Delorme et al. 2021). The effect of emersion has been tested in several bivalve species, including scallops (Christophersen 2000; Heasman et al. 1994), oysters (Taylor et al. 1997) and mussels (Carton et al. 2007; South et al. 2021), showing that it is an effective method for inducing detachment from their settlement substrates.

These prior findings suggest that by temporarily creating sublethal, stressful environments, spat can be induced to release or loosen their attachment to the substrate in preparation to initiate migration, or their attachment may become partially compromised, allowing for gentler and more efficient removal. Through the experimental manipulation of environmental factors, such as altering salinity, emersion (i.e., air exposure), reducing water motion and food availability, this study aimed to investigate potential techniques for effectively singulating spat from collector ropes while maintaining high survival. These approaches could provide more sustainable and less invasive mussel spat singulation methods, minimising labour and spat mortality. The insights gained from this study could drive innovations that enhance the efficiency of mussel aquaculture, particularly in regions that depend on spat-collector systems to supplement nursery spat supply.

2 | Methods

2.1 | Source of Mussel Spat

To collect spat, spat-collector ropes were deployed in a commercial Greenshell mussel spat-collecting site in Aotea Harbour on the west coast of the North Island, New Zealand. The spat collector-ropes used in this experiment were left in situ for up to 8 weeks prior to their recovery to obtain spat of various sizes, ranging from recent settlers (< 1 mm SL) to larger spat (> 3 mm SL). Approximately 10 m of closed-looped spat-collector rope (product code R50QE#LS#L Quality Equipment Ltd.) was collected and packed into an insulated bin from the spat-collection site in Aotea Harbour on 28 July 2023 before being immediately transported for 3 hours to the seawater laboratory at the University of Auckland. Upon arriving at the laboratory, spat-collector ropes were cut into 0.2 m length segments and returned to the insulated bin for less than 1 hour until their use for the experiment. The segments of spat ropes were lightly shaken to dislodge spat that detached before the experiment (i.e., dropped off during transport or moribund) and discarded.

2.2 | Singulation Treatments

To assess the effectiveness of various treatments for singulating spat from spat-collector ropes, three replicate 0.2 m segments of rope were subjected to one of nine treatments, and each was applied for varying durations. Following treatment, the rope segments were washed in a standardised manner to recover detached spat. The nine treatments were grouped into four broad categories:

1. Salinity treatment: Segments of rope were immersed in either low (25 ppt, S25) or high (45 ppt, S45) salinity seawater at 17°C for 2 h. For the salinity treatments, high salinity (45 ppt, S45) was achieved by adding 99.5% NaCl (Sigma-Aldrich) to UV-treated and filtered seawater (5 µm), and low salinity (25 ppt, S25) was achieved by adding deionised water. Salinity was confirmed using a YSI Professional Plus water quality instrument before immersion of the spat. The pH was not measured following salinity adjustment, and any effects of salinity manipulation on pH were not accounted for in this experiment.
2. Emersion treatment: Segments of ropes were removed from insulated bins, placed in empty, unsealed tanks and exposed to 17°C room temperature for either 2 h (E2) or 4 h (E4).
3. Immersion treatment: Segments of ropes were immersed in seawater (17°C, 35 ppt, UV-treated and filtered 5 µm) without aeration and food (NA-NF), with aeration and food (A-F) or without aeration but with food (NA-F) for 12 h. For A-F and NA-F, a mixture of axenically cultured microalgae mix was provided at a density of 150,000 cells per spat of *Diacronema lutheri*, 75,000 cells of *Chaetoceros muelleri* and 75,000 cells of *Tetraselmis suecica*. The microalgae cell densities were measured using a flow cytometer, and the entire ration of food was added at the start of the 12-h immersion.
4. Control Treatment: Segments of rope were immersed in aerated seawater (17°C, 35 ppt, UV-treated and filtered 5 µm) for 2 h (C2) or 12 h (C12). The control treatments (C2 and C12) involved immersion in aerated seawater without food and are therefore equivalent to an A-NF condition for the respective exposure durations.

2.3 | Experimental Setup

Each replicate rope segment was placed in an individual inverted 1.5 L plastic tank (Kiwi Blue BPA-free plastic) with the base removed (Gui et al. 2016). A small air stone was sealed at the base of each tank, providing continuous aeration throughout the experiment in all treatments except for NA-F and NA-NF. All tanks were housed in a temperature-controlled room to maintain a constant room and water temperature of 17°C.

At the end of the experiment, the contents from each tank were emptied into a 200 µm mesh sieve and washed with flowing seawater (UV treated and 5 µm filtered) for 60 s. During the washing process, the fibres of spat-collector ropes were teased apart by hand to dislodge any singulated spat retained within the ropes. The washed rope and the spat that remained attached were immediately frozen for later analyses. At the same time, all spat that had singulated and were caught by the sieve were added back into their respective tank and refilled with clean, UV treated and filtered (5 µm) seawater. Aeration was provided for all tanks for an additional 24 h of rearing.

2.4 | Spat Singulation, Survival, Recovery and Size

After 24 h of rearing, the singulated spat from each rope segment were removed from their tank and transferred into a 14 cm diameter petri dish filled with enough seawater to fully submerge the spat. Spat that were clumped together were first carefully separated using fine-tipped tweezers or gently squirted with additional seawater if they were too small, then spread evenly across the dish using a metal spatula to achieve a uniform distribution across the petri dish for counting. Spat were counted in five randomly selected 4 cm^2 sub-samples, averaged and then multiplied by the area of the petri dish to determine the total number of spat singulated from the sample rope.

Spat viability, defined as the presence of active physiological responses indicating the individual is alive and in good condition, was assessed visually in each of the same five subsamples as above. Spat were considered viable if they closed their shells in response to gentle poking or exhibited movement of the foot, valve or tissue visible through the shell. In contrast, spat that showed no response, had no visible tissue movement, or consisted of empty shells were recorded as non-viable. The survival rate (%) was then calculated by the proportion of viable individuals relative to non-viable individuals.

The frozen ropes were rigorously washed and stripped of all spat, then counted in the same manner as above to determine the number of spat that had remained attached to the rope. Counts of spat that had remained attached to the ropes were added to the counts of spat that had been singulated from the treatment to determine the total number of spat on the rope at the experiment's outset. The number of spat that were singulated from the rope was then compared to the total number of spat to determine the percentage (%) of spat that were successfully singulated from the experiment and was calculated by:

$$\text{Singulation rate } (\%) = \frac{\text{No. of spat singaulted}}{\text{Total no. of spat}} \times 100$$

Lastly, spat recovery efficiency, which denotes the percentage yield of viable spat expected to be recovered from the collector rope, was calculated by:

$$\text{Recovery efficiency } (\%) = \frac{\text{Singualtion rate } \times \text{ Survival rate}}{100}$$

All spat counted from each replicate (both for spat that were singulated and remained attached) were photographed under a dissecting microscope. Their SL were then measured using image analysis software (Image J Software).

2.5 | Statistical Analyses

Three one-way analyses of variance (ANOVA) were conducted to determine the effects of the nine singulation treatments (i.e., S25, S45, E2, E4, NA-NF, NA-F, A-F, C2 and C12) on the mean spat singulation (%), mean spat survival (%) and mean recovery efficiency of spat (%). A two-way ANOVA was conducted to test the combined effect of singulation treatment and singulation state (i.e., singulated vs. attached) on the mean size of the spat. The data were transformed before analysis to satisfy parametric assumptions (i.e., arcsine transformation for percentage data). The distribution of residuals and the residuals versus means were plotted and examined visually to confirm the parametric assumptions for all ANOVAs. Post hoc pairwise comparisons were conducted using Tukey HSD tests, in which significant main effects were detected (i.e., $p < 0.05$). All statistical analyses were conducted in R (version 4.4.1) using the base stats package.

3 | Results

3.1 | Spat Singulation

The mean proportion of spat successfully singulated from spat-collector ropes differed among treatments ($F_{(8,18)} = 22.88$, $p < 0.001$). All treatments had higher mean singulation rates compared to C2 (28.5% ± 3.7 SE), C12 (36.2% ± 3.1 SE) and A-F (45.2% ± 3.1 SE) ($p < 0.05$). Notably, NA-NF achieved a mean singulation rate of 80.1% (± 0.7 SE), which was higher than NA-F (63.8% ± 4.4 SE, $p = 0.02$), E2 (63.0% ± 3.8 SE, $p = 0.01$) and E4 (58.3% ± 1.6 SE, $p = 0.02$). However, no other pairwise comparisons were different (Figure 1a).

3.2 | Spat Survival

The mean proportion of spat surviving 24 h post-singulation was different among treatments ($F_{(8,18)} = 7.54$, $p < 0.001$). Specifically, C2 (96.2% ± 1.9 SE) had higher survival than all other treatments ($p < 0.05$), except S45 (90.1% ± 1.4 SE, $p = 0.44$), A-F (85.1% ± 1.3 SE, $p = 0.07$) and E4 (84.3% ± 2.7 SE) where there was no difference. The E2 (59.8% ± 10.8 SE) treatment had lower survival compared to E4 ($p = 0.02$), S25 (83.0% ± 2.3 SE, $p = 0.04$), S45 ($p < 0.001$) and A-F ($p = 0.01$), but was not different compared to NA-NF (80.1% ± 2.2 SE, $p = 0.99$). In addition, no other comparisons were made (Figure 1b).

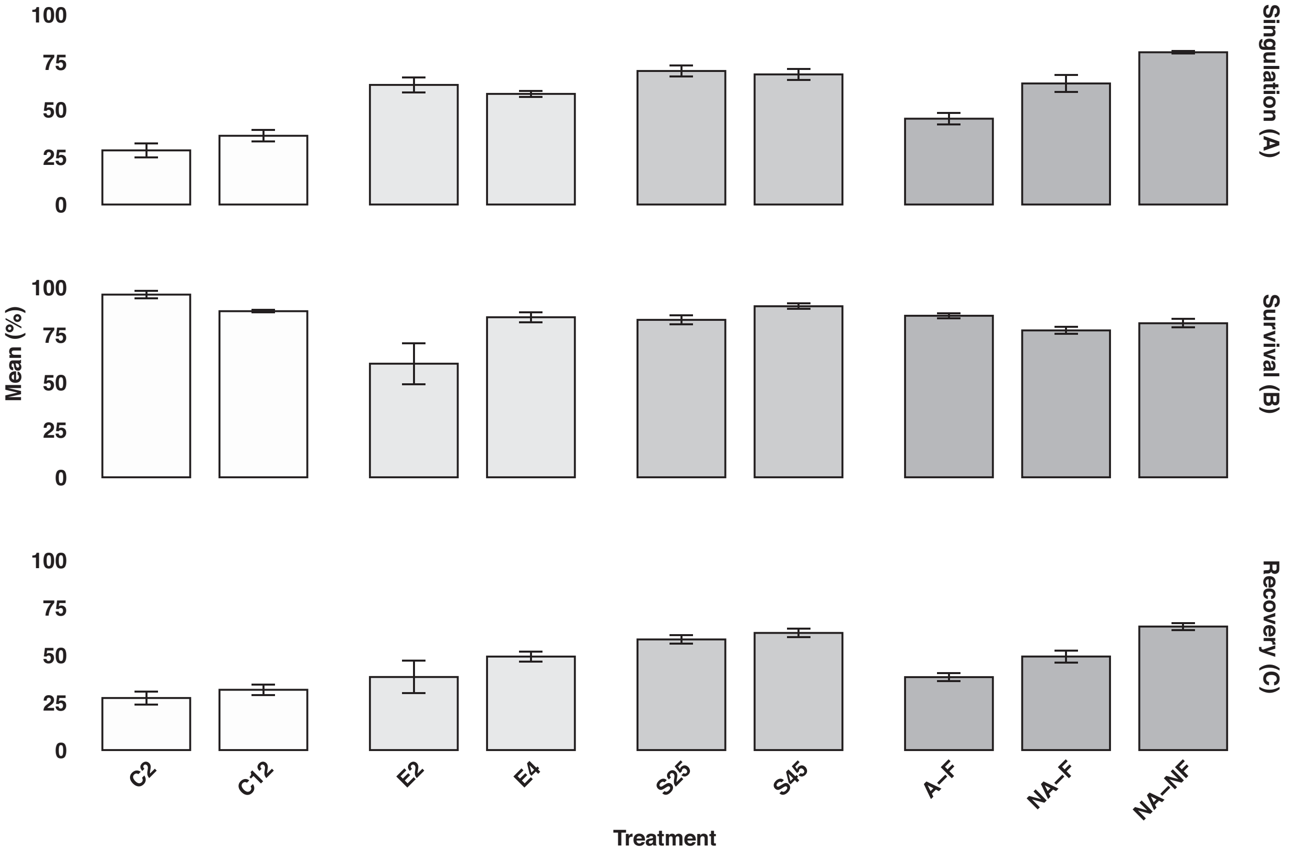

FIGURE 1 | Mean percentage (± SE) of spat singulation (A), survival (B) and recovery (C) in response to exposure to various singulation treatments. The *x*-axis labels represent distinct singulation treatments, listed from left to right: 2 and 12 h seawater control (C2, C12), 2 and 4 h emersion (E2, E4), low and high salinity (S25, S45), aeration with food (A–F), no aeration with food (NA-F), and no aeration with no food (NA-NF). Lowercase letters above the bars indicate significant differences between treatment groups, with different letters representing statistically distinct groups.

3.3 | Spat Recovery

The mean proportion of recovery of viable spat was different among treatments ($F_{(8,18)} = 11.85$, $p < 0.001$), with all treatments showing higher mean recovery compared to C2 (27.4% ± 3.4 SE, $\alpha = 0.05$), except between A-F where there was no difference ($p = 0.55$). However, only three treatments (i.e., S25 (58.3% ± 2.3 SE), S45 (61.7% ± 2.2 SE) and NA-NF (65.1% ± 1.86 SE)) had higher recovery than C12 (31.7% ± 2.2 SE). In addition, S25, S45 and NA-NF exhibited higher mean recovery of singulated spat compared to A-F and E2 ($p < 0.05$), with no other comparisons revealing differences ($p > 0.05$) (Figure 1c).

3.4 | Size

The mean size of spat differed significantly for the interaction of singulation status and treatments (singulation status × treatment interaction, $F_{(8,\ 1803)} = 3.67$, $p < .001$). Post hoc comparisons indicated that the mean size of spat remaining attached to the collector ropes was larger than those that detached in all treatments (i.e., at the $\alpha = 0.05$ level), but for S45 and A-F, where there was no difference ($p = 0.99$ and 0.98, respectively). Furthermore, while there was no difference in the mean size of spat successfully singulated from the collector ropes among treatments, the mean size of spat that remained attached was different among the treatments ($p < 0.05$) (Figure 2).

4 | Discussion

The results of this study indicate that the general approach of manipulating the environmental conditions in which spat are held is a practical method for singulating up to 80% of spat, and after accounting for mortalities, recovering up to 65% nursery-ready spat from spat collector ropes. These rates of singulation are comparable to the ~60% recovery achieved from macroalgae using chlorination and represent an improvement over the ~35% recovery achieved previously from chlorination of spat collector ropes (Skelton and Jeffs 2023; Himiona et al. 2024). However, the effectiveness of this general approach is dependent on several factors, including the specific environmental parameters adjusted, the duration of exposure to these conditions and the initial size of the spat present on the ropes.

4.1 | Immersion Treatment

The results of this study indicate that spat exposed to non-aerated immersion treatments (i.e., NA-NF and NA-F) were more

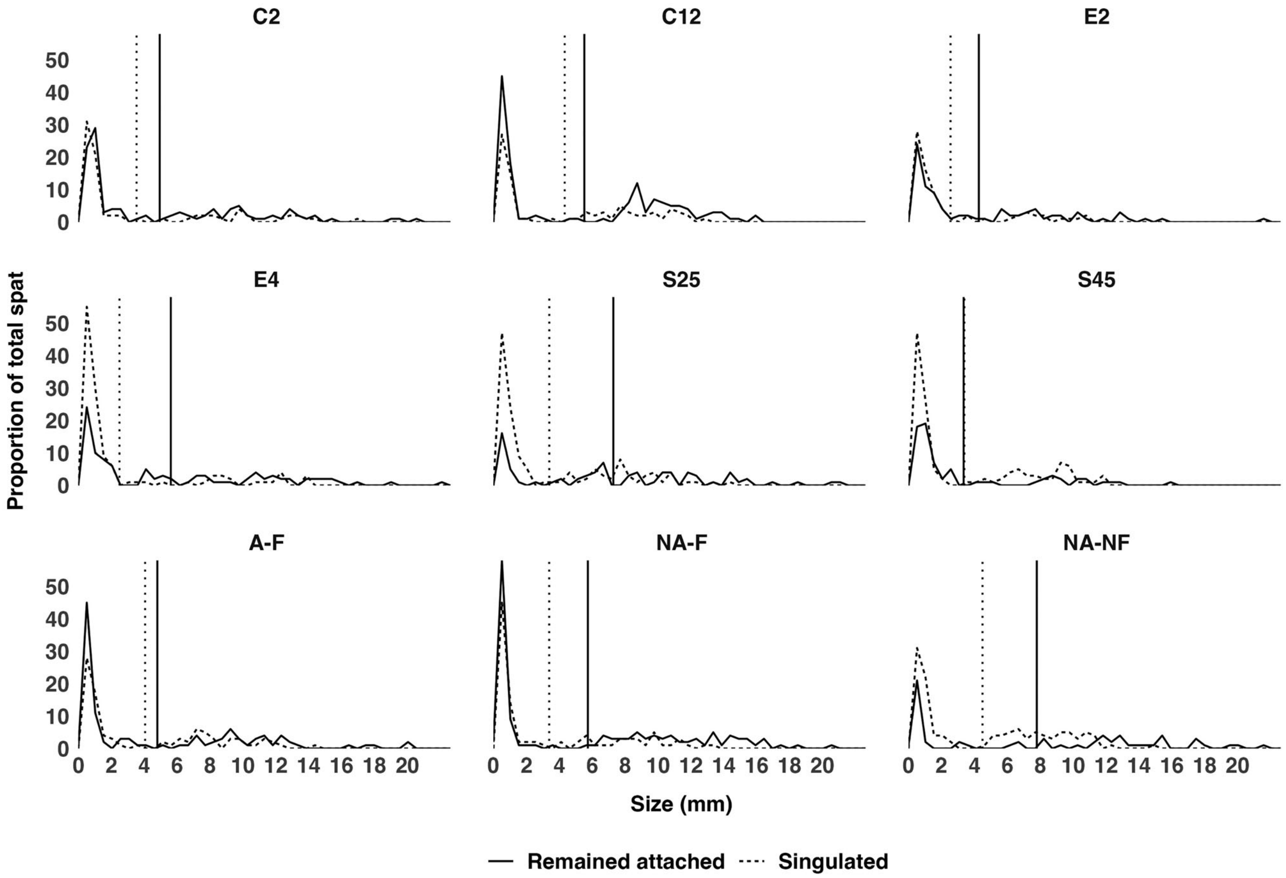

FIGURE 2 | Spat size frequency histogram of spat exposed to singulation treatments. Vertical lines represent the mean size of spat for each respective state (i.e., singulated vs. remained attached).

effectively singulated from the collector ropes than those in aerated treatments. Aeration is critical in maintaining spat attachment to substrates by providing mechanical stimulation from the surrounding water turbulence, sustaining dissolved oxygen levels and facilitating nutrient circulation (Caceres-Martinez et al. 1994; Mero et al. 2019). Conversely, when aeration is absent, these processes can be disrupted, leading to the weaker attachment of spat to collector ropes and higher rates of singulation. For example, research shows that water movement enhances mussel attachment by stimulating increased byssal thread production, likely as a defence mechanism against dislodgement by predators or hydrodynamic forces (Alfaro 2006; Eyster and Pechenik 1988; Sanjayasari and Jeffs 2019). Conversely, without water movement, spat are more likely to be singulated from collector ropes, potentially as a preparatory response to migrate and seek better settlement locations (Caceres-Martinez et al. 1994). This is consistent with observations of spat crawling in search of more suitable substrates in low-flow or static environments (Bayne 1976).

In addition, it is probable that dissolved oxygen levels declined more rapidly in non-aerated treatments, although this was not directly measured in this current study. Lower oxygen levels have been linked to reduced byssal thread production and weaker adhesion in several mussels species, potentially making spat more susceptible to singulation (Clarke and McMahon 1996; George et al. 2018; Y. Wang et al. 2010, 2012). For example, experiments with Greenshell mussel spat showed lower retention (i.e., higher detachment) on plastic mesh in low-oxygen conditions than treatments with higher oxygen levels over 25 days (Sanjayasari and Jeffs 2019).

Food availability also affects spat attachment, although its influence appears to depend on the presence or absence of aeration. For example, in the non-aerated, non-fed (NA-NF) treatment, the mean singulation rate was 80%, which decreased to 64% when food was introduced (NA-F). In contrast, the C12 treatment (equivalent to A-NF) showed a lower singulation rate of 48%, similar to the aerated, fed (A-F) treatment at 45%. This pattern indicates that the effect of food is conditional, as it reduces singulation only when aeration is absent. When water circulation is low, limited food access may drive spat to migrate outward in search of resources, increasing the chance of detachment. In contrast, when aeration is present, food availability has little effect, possibly because improved flow ensures more consistent access to food within the rope fibres (MacNeill 2005; Kobak and Kakareko 2009). For example, Sullivan and Couturier (2004) observed increased crawling speed in *Mytilus edulis* and *M. trossulus* spat in the absence of food. These findings suggest that food deprivation may promote singulation, but only under low-flow conditions

Despite variations in aeration and food availability across the different singulation treatments, spat survival remained consistently high, ranging from 77% in the NA-F treatment to 85% in the A-F treatment. This outcome contrasts with previous studies that highlight the importance of aeration in maintaining dissolved oxygen levels, facilitating nutrient transport and ultimately supporting spat health and survival. For instance, Sanjayasari and Jeffs (2019) reported a 22.8% reduction in survival for Greenshell mussel spat reared without aeration over 25 days, while Alfaro (2005) observed approximately 23% lower survival in juvenile *P. canaliculus* exposed to combined low-flow and low-oxygen conditions over 24 h. The relatively high survival rates observed in the present study may be attributed to the shorter exposure duration of 12 h, which is likely insufficient to induce substantial mortality. However, mussels have demonstrated resilience to extended hypoxia (low oxygen) and anoxia (total oxygen depletion). They can adjust their metabolic rates, switching to anaerobic metabolism after some time to survive in low-oxygen environments for extended periods (W. Wang and Widdows 1991, 1993). Mussels, including Greenshell spat, can also close their shells and periodically gape to reduce exposure to stressful conditions (Delorme et al. 2021; Reyden et al. 2023; Webb and Heasman 2006). This inherent resilience likely explains the high survival rates of spat despite variations in aeration and food availability.

4.2 | Salinity Treatment

This study found that spat exposed to high and low salinity levels exhibited similar mean singulation rates (68% and 70%, respectively). This suggests that salinity extremes—elevated or reduced—disrupt spat attachment comparably, promoting easier singulation from collector ropes. The likely explanation is that salinity fluctuations interfere with byssal thread production, weakening attachment strength. Altered salinity has previously been demonstrated to suppress or even halt byssal thread production in various mussel species, and the strength of existing byssus threads and their plaque adhesive can be significantly reduced, indicating that both byssus production and mechanical integrity are sensitive to salinity stress (Allen et al. 1976; Rice et al. 2016; Van Winkle 1970; Wang. Y, et al. 2012; W.-Y. Wang et al. 2024; Young 1985). Such stress enable mussels to actively release their entire byssus complex, though the exact mechanism remains unclear for spat (Sivasundarampillai et al. 2023; W.-Y. Wang et al. 2024). As a result of exposure to unfavourable salinities, mussel spat may prepare for movement by reducing byssus formation and weakening their attachment. For example, in *M. edulis* and *M. trossulus* spat, reducing salinity from 30 ppt to 15 ppt was associated with increased crawling behaviour and fewer byssal threads produced daily (Sullivan and Couturier 2004). This behavioural response to salinity stress may explain the high rates of singulation observed at both high and low salinity levels in the current study. It appears that spat may actively self-detach and migrate under salinity stress, contributing to the high singulation rates observed.

Water temperature also influences the effectiveness of salinity in singulating spat from substrates. For example, in *Pecten maximus* scallop spat, a three-degree increase in water temperature at the same salinity (30 ppt) resulted in a 50% reduction in attachment (Christophersen and Strand 2003). However, in this current study, the temperature was constant at 17°C, which falls within the optimal range for rearing Greenshell spat (Buchanan 1999; Jeffs et al. 1999). This suggests that while temperature fluctuations can impact spat attachment, they were not implicated in this experiment. However, it is possible that combining salinity variations with temperature changes may further enhance spat singulation. In adult Greenshell mussels, for instance, elevated temperatures have been associated with reduced byssal thread production and weaker attachment (Copedo et al. 2023), suggesting that thermal stress may lower the energetic investment in adhesion and make detachment easier under certain salinity conditions.

This study found no significant difference in survival between spat exposed to high salinity (S45; 90%) and the seawater control (C2; 96.2%, P = 0.44). However, survival was lower in the low salinity treatment (S25; 83%) compared to the control ($P < 0.05$). This suggests that while salinity extremes cause detachment from ropes, survival rates vary, with low salinity having a more significant negative impact than high salinity or control conditions. Nonetheless, the high survival rates observed under both salinity treatments indicate that detachment is likely a behavioural response rather than a result of direct physiological harm. These findings are consistent with observations in other species, such as *Pecten fumatus* scallops and *Pinctada maxima* pearl oysters, where exposure to 45 ppt seawater for 2 hours led to over 90% singulation without increased mortality (Taylor et al. 1997; Heasman et al. 1994). Mussels, like other bivalves, demonstrate mechanisms to cope with acute salinity change such as by their ability to close their valves and reduce heart rate to protect against short-term osmotic stress (Kholodkevich et al. 2009; Kilgour et al. 1994; Picoy-Gonzales and Laureta 2022). Similar resilience has been documented in oysters and scallops, where salinity-induced singulation occurred without associated mortality (Christophersen and Strand 2003; Heasman et al. 1994; Taylor et al. 1997). The results suggest that Greenshell mussel spat can tolerate short-term exposure to extreme salinity and undergo salinity-induced detachment from substrates without immediate mortality. This indicates that salinity changes may offer a safe method for singulating spat from collector ropes, which warrants further development toward commercial application.

4.3 | Emersion Treatment

Emersion led to high spat singulation from collector ropes, though increasing emersion durations did not increase the singulation rate. The 2-h and 4-h emersion treatments collectively resulted in 60% singulation, suggesting that air exposure triggers a rapid response in the spat, after which the singulation rate plateaus. This contrasts with expectations from previous studies, where increasing emersion duration progressively increased singulation rates in *P. fumatus* and *P. maximus* spat (Heasman et al. 1994; Taylor et al. 1997). However, even brief emersion periods (i.e., 15 min) have been shown to cause over 40% singulation of *P. canaliculus* spat from coir ropes, indicating that emersion, regardless of duration, may serve as a trigger for secondary migration in this species (South et al. 2021).

In this study, when emersion was prolonged from 2 to 4 h, there was a 24.4% increase in the mean survival. These findings contradict previous research that indicates that more prolonged periods of emersion usually lead to higher spat mortality over similar periods (i.e., a factor of hours) (Waller et al. 1995; Delorme et al. 2021). This is likely due to prolonged air exposure, which causes the depletion of energy reserves in the spat, resulting from starvation and desiccation when they are out of water (Andrade et al. 2018). In addition, spat are likely to shift to anaerobic metabolism, causing the accumulation of toxic by-products that can induce cellular damage, even after a few hours (W. Wang and Widdows 1993; Delorme et al. 2021). However, while survival under the 4-h emersion treatment was consistent across replicates, survival following 2-hour emersion was highly variable across replicates. Therefore, although increased spat survival with longer emersion was observed, the underlying cause remains unclear.

Nonetheless, there are several potential mechanisms which may explain the unexpected increase in spat survival during extended emersion. Firstly, the collector ropes likely retained residual moisture, creating a humid microenvironment that buffered spat from desiccation. For instance, Greenshell spat (~1 mm in SL) exposed to high relative humidity (> 90%) during emersion exhibited significantly lower mortality, reduced oxidative damage and improved recovery once immersed in seawater compared to those held under low humidity (< 30%) conditions (Delorme et al. 2021). However, collector ropes are not structurally homogeneous. Moisture retention varies across their surface, with inner fibres typically remaining cooler and more saturated than the exposed outer layers. This creates a microclimatic gradient, where spat located deeper within the rope may experience less desiccation. Therefore, it could be likely that more spat in the 4-h immersion remained nestled deep in the collector ropes compared to the 2-h immersion, resulting in the differences in survival.

4.4 | Spat Size and Treatment Efficacy

Smaller-sized spat were more likely to be singulated from the collector ropes than larger spat, regardless of treatment. The mean size of spat successfully singulated was approximately 3.5 mm, whereas the mean size of those that remained attached was 5.4 mm. In addition, of the spat successfully singulated, 65% had a SL of less than 3.5 mm, compared to 45% of the same-sized spat that remained attached. This aligns with previous findings, which indicate that larger spat are more challenging to remove from their attachment to natural and artificial substrates than smaller ones (Himiona et al. 2024; Skelton and Jeffs 2023). The difference in singulation between spat of different sizes may be due to several factors: larger spat are more resilient to stress due to ontogenetic changes, are more firmly attached because byssal thread number and strength increase with mussel size, and are less likely to undergo secondary settlement (Babarro and Carrington 2013; Hunt and Scheibling 2001; Buchanan and Babcock 1997). The increased strength and number of byssal threads in larger spat provide a stronger attachment, making them less prone to singulation.

Exposing the spat to the A-F treatment or 2-h air exposure resulted in recovery rates of viable spat similar to those of the control (i.e., 38% in both treatments). In contrast, 4-h air exposure, S25, S45 and NA-NF resulted in higher recovery rates of viable spat (i.e., 49%, 58%, 61% and 65%, respectively). However, among the treatments explored, salinity adjustments emerge as the most effective and practical method for commercial spat singulation. The rapid and high recovery rates and minimal impact on spat survival make salinity treatments particularly suitable for the Greenshell mussel industry. Achieving significant singulation in a shorter period enhances the efficiency of spat handling and processing, leading to potential cost savings and increased productivity. While aeration and food availability adjustments and air exposure treatments are effective, their slower impact on singulation or their potential to induce deleterious stress makes them less optimal than salinity treatments. Further research to optimise salinity levels and exposure durations could further enhance the efficiency and practicality of this method, solidifying its role as an efficacious choice for commercial spat singulation.

4.5 | Nursery Implications and Spat Losses

Nursery culture presents promising solutions for reducing spat losses, particularly in the Greenshell mussel industry (Skelton et al. 2021). However, practical methods for singulating spat from spat-collector ropes must be developed for this potential to be fully realised. The results of this study demonstrate that subjecting spat to environmental stresses is a viable method, achieving up to 80% singulation from collector ropes without causing increased mortality relative to seawater controls. Once singulated, these spat can be transferred to nursery culture systems, where they are reared to a larger size before seeding. This approach has already shown success, with nursery-reared single-spat larger than 6 mm reducing losses to as low as 60%, compared to losses exceeding 99% when spat are directly seeded onto coastal farms (Skelton et al. 2021).

While it is possible to grow spat to larger sizes on collector ropes before harvest, a common practice in some mussel-producing regions, this approach is less compatible with New Zealand's operational model. Here, spat collectors typically harvest spat at smaller sizes to enable rapid turnaround and redeployment of collector ropes, thereby maximising spat catches and the number of collection events per season. The findings from this study underscore the potential to improve spat production efficiency by singulating spat early and selling them as a single-seed product. This strategy would allow ropes to be quickly reused, supporting multiple settlement cycles within a season and increasing total amount of spat collected per deployment effort. However, further research is required to address challenges in scaling up these methods for commercial operations to maximise this potential. Specifically, there is a need to investigate how these singulation techniques can be applied to large quantities of spat (i.e., singulating spat from hundreds of kilometres of collector ropes at a time). In addition, exploring whether these methods apply to alternative spat sources will be crucial for diversifying the industry's supply and ensuring long-term sustainability.

Spat are exposed to various stressors throughout the farming process, beginning before harvest and continuing during transport to farms, where they endure prolonged periods of emersion and exposure to adverse environmental conditions, such as elevated temperatures and desiccation (Delorme et al. 2021, 2024; South et al. 2020). Once at the farm, they face dynamic and often harsh environmental conditions, including severe weather events, salinity fluctuations, food availability variability and hydrodynamic forces. The results of this study indicate that most spat remaining attached to collector ropes were viable shortly after collection; however, under the conditions of a variable farm environment, many spat will detach, leading to potentially considerable losses. However, they become more resilient to these stressors by rearing spat to a larger size and less prone to secondary migration. Secondary migration of spat, a common phenomenon in Greenshell mussels, is particularly prevalent in smaller spat and appears to decrease as they grow. Once the spat reaches a size of 6 mm or more, secondary settlement is thought to cease entirely (Buchanan and Babcock 1997; Skelton et al. 2021; Smith 2018). These findings reinforce the need for singulating and on-growing spat to larger sizes before seeding. Doing so can significantly reduce losses and improve production efficiency. Producers can minimise losses by prioritising spat growth to more robust sizes, enhancing mussel farming operations' overall viability and sustainability.

5 | Conclusion

This study demonstrates the potential of environmental stressors to facilitate the singulation of Greenshell mussel (*Perna canaliculus*) spat from collector ropes, providing a viable source of unattached spat for nursery systems. This approach achieved singulation rates of up to 80% and recovery rates of up to 65%. These results highlight the feasibility of using sub-lethal stressors to enhance spat availability for further rearing, offering a pathway to reduce early-stage losses and improve production efficiency.

The simplicity and scalability of this broad approach to spat singulation makes it an attractive option; however, with further investigation this approach could be improved further. Firstly, the long-term impact (> 24 h) of exposure to environmental stressors on spat survival should be evaluated. Secondly, identifying the specific mechanisms responsible for singulation in each treatment could help to develop more effective strategies for spat singulation. Each of these factors would therefore need to be addressed before any methods can be applied at scale. Nevertheless, this study offers practical steps toward incorporating singulation strategies into mussel farming practices, with the potential to improve spat supply consistency and support more effective use of nursery systems.

Author Contributionsm

Kayleb Himiona: Conceptualisation; investigation; methodology; data curation; formal analysis; visualisation; writing – original draft. **Bradley M. Skelton**: Writing – review and editing. **Andrew G. Jeffs**: Funding acquisition; writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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