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Anaesthetic induced relaxation of the winged pearl oyster, *Pteria penguin*, varies with oyster size and anaesthetic concentration

Pranesh Kishore [a,1], Max Wingfield [a], Thane A. Militz [a,2], Tracy Aisea [b], Paul C. Southgate [a,*,3]

[a] School of Science, Technology & Engineering and Australian Centre for Pacific Islands Research, University of the Sunshine Coast, 90 Sippy Downs Drive, Sippy Downs, Queensland 4556, Australia
[b] Aquaculture Section, Ministry of Fisheries, Tonga

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ABSTRACT

Stress and mortality of pearl oysters during nucleus implanting for round pearl and mabé pearl production can be reduced using appropriate anaesthetics that allow improved access to nucleus implanting sites. This study evaluated the efficacy of three different concentrations of benzocaine (0.25, 0.50 and 1.20 g L^{-1}) and 1-propylene phenoxetol (2.50, 3.00 and 3.50 mL L^{-1}) when presented to 'small' (dorso-ventral height [DVH], 78.7 ± 1.6 mm), 'medium' (DVH, 118.2 ± 2.0 mm) and 'large' (DVH, 149.3 ± 1.1 mm) cohorts of the winged pearl oyster, *Pteria penguin*. Results showed the following general trends across treatments with both anaesthetics: (1) greater proportions of large oysters became relaxed compared to small oysters; (2) large oysters required shorter exposure times to become relaxed than small oysters; (3) for each size class of oyster, an increase in anaesthetic concentration resulted in an increased proportion of relaxed oysters; and (4) 'mantle collapse' (where the mantle collapses away from the shell) was only recorded in large oysters in treatments with higher concentrations of anaesthetics. The most effective concentration of benzocaine to use with small, medium and large *Pt. penguin* was the highest level tested in this study (1.20 g L^{-1}). Similarly, the highest concentration of 1-propylene phenoxetol tested (3.5 mL L^{-1}) was also the most effective with all three size classes of *Pt. penguin*. These treatments caused mantle collapse in large oysters, for which use of lower, less effective anaesthetic concentrations may be considered preferable, to avoid potentially negative impacts of mantle collapse on subsequent mabé pearl production. As well as efficacy, choice of anaesthetic should consider ease of preparation and preparation time. Benzocaine requires dissolving in methyl alcohol and heating to 88–92C, while 1-propylene phenoxetol is readily soluble in seawater.

1. Introduction

The winged oyster, *Pteria penguin* (Röding 1798), is now cultured widely within the Asia-Pacific region to produce high quality mabé pearls that are also known as 'half-pearls'. Mabé pearl culture has become an important livelihood activity in some Pacific island countries, where community-level production is possible because of a relatively simple culture procedure, that is compatible with local lifestyles (Kishore et al., 2015; Gordon et al., 2019; Johnston et al., 2019, 2020) and supports value adding activities (Southgate et al., 2019). Production of mabé pearls involves adhesion of hemispherical nuclei to the inner surfaces of pearl oyster shells (Haws et al., 2006; Kishore et al., 2015; Kripa et al., 2008), a process called nucleation or seeding, followed by an oyster culture period of 10–12 months. During this time, successive layers of mother-of-pearl, or nacre, are deposited onto the nuclei to form mabé pearls with an appropriate nacre thickness. Resulting pearls are then cut from the shells for processing and value-adding (Gordon et al., 2019; Southgate et al., 2019). The process of nucleus implantation is simple compared to round pearl production (Haws et al., 2006; Kishore et al., 2015) and can be undertaken by local people following appropriate training (Southgate et al., 2019). However, forceful opening of the oyster shell is required to facilitate nucleus implantation, and this is

* Corresponding author.
E-mail address: psouthgate@usc.edu.au (P.C. Southgate).
[1] https://orcid.org/0000-0002-7421-8892
[2] https://orcid.org/0000-0002-6476-8559
[3] https://orcid.org/0000-0002-3781-2606

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concerning because the large, non-nacreous shell margin of *Pt. penguin* is relatively thin and fragile, and prone to breakage. Shell damage during nucleation causes stress to the oyster, may increase susceptibility to predation and biofouling, and potentially affects pearl yield and quality.

Stress and mortality of pearl oysters during the nucleus implanting procedure, for both round pearl and mabé pearl production, has prompted studies into potential uses of anaesthetics (relaxants) to reduce stress during this process (e.g., Acosta-Salmón and Rangel-Dávalos, 1997; Norton et al., 1996; O'Connor and Lawler, 2002; Monteforte et al., 2004; Kishore, 2011). Effective anaesthetics cause the adductor muscle of pearl oysters to relax, prevent muscle contractions, and allow improved access to nucleus implanting sites (Norton et al., 1996; O'Connor and Lawler, 2002). Several anaesthetics have been assessed for pearl oysters, with results varying between species and according to the particular anaesthetics used and their concentrations (e.g., Ehteshami, 1993; Norton et al., 1996; O'Connor and Lawler, 2002; Acosta-Salmon and Southgate, 2006; Mamangkey et al., 2009; Kripa et al., 2008; Kishore, 2011). Relaxation of *Pinctada margaritifera* (Norton et al., 2000), *P. maxima* (Mills et al., 1997; Mamangkey et al., 2009), *P. imbricata* and *P. albina* (O'Connor and Lawler, 2002) and *Pt. penguin* (Kishore et al., 2015) has been reported when exposed to 1-propylene phenoxetol at concentrations of 2–3 mL L^{-1}. Similarly, benzocaine at concentrations of 500–1200 mg L^{-1} has been shown to be effective at inducing relaxation in *P. maxima* (Mamangkey et al., 2009), *P. margaritifera* and *P. fucata* (Acosta-Salmón et al., 2005), *Pt. sterna* (Acosta-Salmón and Rangel-Dávalos, 1997) and *Pt. penguin* (Kishore, 2011; Wan et al., 2012).

Pearl oyster age or size may also influence the efficacy of anaesthetics and a size dependent response has been suggested for *P. margaritifera* (Kripa et al., 2008). This has important implications for the mabé pearl sector because although the preferred minimum dorso-ventral shell height (DVH) of *Pt. penguin* used for mabé pearl production is around 150 mm (Gordon et al., 2019), much smaller oysters (e.g., from ~70 mm DVH; Kripa et al., 2008) can be used for pearl production. The latter approach is often attractive to subsistence level mabé pearl farmers where income generation over the shortest possible culture period may be favoured in place of potentially greater returns, but greater risk, associated with the use of larger oysters and a longer culture period. The possibility of a size dependent response to anaesthetics by *Pt. penguin* has not previously been investigated but, given recent expansion of the community-based mabé pearl sector in the South Pacific (Johnston et al., 2019), there is need to determine if the same anaesthetics and concentrations used successfully with larger *Pt. penguin* (e.g., Kishore, 2011) are appropriate for smaller oysters.

Knowledge of species- and age-specific efficacy of anaesthetics, their appropriate doses and exposure times are key to their effective use. For example, extended exposure of pearl oysters to an anaesthetic, once relaxed, or exposure to too high a concentration of an anaesthetic can also cause 'mantle collapse', where the mantle retracts and collapses away from the inner surfaces of the shells, and 'body collapse', where soft tissues become flaccid and lose their muscular strength, accompanied by excessive mucus production (Acosta-Salmón et al., 2005; Mamangkey et al., 2009; Norton et al., 1996); these reactions increase the time required for oyster recovery (Norton et al., 1996). Exposure to some chemicals assessed for their anaesthetic potential with pearl oysters (e.g., menthol) has also resulted in significant oyster mortality (Mamangkey et al., 2009). Desirable characteristics of anaesthetics used for pearl oysters therefore include: (1) induced relaxation within a short exposure period; (2) relaxation of a high proportion (preferably 100%) of the population; (3) rapid oyster recovery following exposure; and (4) no adverse physiological effects or mortality.

Given the potential benefits of using anaesthetics during mabé pearl nucleation, but considering the potential risks of incorrect use, and size dependent response to anaesthetics shown by some species, there is a need for further research to develop appropriate and size-specific anaesthetic procedures for *Pt. penguin*. To address this research need, we evaluated the efficacy of two anaesthetics (benzocaine and 1-propylene phenoxetol) for different age cohorts of this species. Both have proven to be effective relaxants of pearl oysters with a brief exposure period and allow rapid recovery without mortality (e.g., Norton et al., 1996; Acosta-Salmon and Southgate, 2006).

2. Materials and methods

2.1. Pearl oysters

Hatchery-produced *Pteria penguin* were obtained from the government-operated hatchery at Sopu, on the island of Tongatapu in the Kingdom of Tonga. Oysters were spawned from broodstock collected at Vava'u Island and were cultured using standard hatchery procedures (Southgate et al., 2016). All oysters were progressively grown out in oyster baskets and then on chaplets suspended from a longline at a depth of 5 m (Southgate, 2021; Gordon et al., 2020) in nearshore waters adjacent to the hatchery facility.

A total of 315 cultured *Pt. penguin* were selected for the study and allocated to one of three age cohorts, corresponding to 'small' (dorso-ventral height [DVH], 78.7 ± 1.6 mm), 'medium' (DVH, 118.2 ± 2.0 mm) and 'large' (DVH, 149.3 ± 1.1 mm) groupings (Table 1). Small oysters represent the suggested minimum size of *Pteria* spp. appropriate for mabé pearl production (Ruiz-Rubio et al., 2006; Kripa et al., 2008), while medium and large groupings correspond to the sizes of cultured *Pt. penguin* more commonly used for mabé pearl production (Gordon et al., 2019). Oysters were scrubbed to remove unwanted fouling organisms and rinsed with seawater before the experiment.

2.2. Anaesthetic solutions

Anaesthetic solutions were prepared in 20 L basins containing 1 μm filtered, non-aerated seawater maintained at 26 °C. Six anaesthetic solutions were prepared in triplicate: benzocaine (CAS Number: 94-09-7) at three concentrations of 0.25 g L^{-1}, 0.5 g L^{-1}, and 1.2 g L^{-1}, and 1-propylene phenoxetol (CAS Number: 770-35-4) at three concentrations of 2.5 mL L^{-1}, 3.0 mL L^{-1}, and 3.5 mL L^{-1}. These concentrations were chosen on the basis of their successful use to induce relaxation in *Pinctada* spp. (e.g., Mills et al., 1997; Acosta-Salmon and Southgate, 2006; Mamangkey et al., 2009) and *Pt. penguin* (Kishore, 2011; Wan et al., 2012).

Preparation of anaesthetic solutions followed the methods of Acosta-Salmón et al. (2005). Preparation of the 0.25 and 0.5 g L^{-1} benzocaine solutions require benzocaine to be first dissolved in methanol to a saturation of 0.25 g mL^{-1}. This mixture was then poured into a small container (0.5 L) of heated (88–92 °C) seawater to completely dissolve the benzocaine crystals and the resulting solution was then added to the basins containing seawater. For the 1.2 g L^{-1} concentration, a benzocaine solution in ethanol (100 g L^{-1}) was prepared and then directly added to the basins containing seawater. To prepare the 1-propylene phenoxetol solutions, the required volume of 1-propylene phenoxetol was agitated with seawater in a small container (0.5 L) to disperse the chemical into small droplets before adding to basins containing seawater. Further dispersal of the 1-propylene phenoxetol was aided by swirling the anaesthetic solution in each basin.

Table 1
Characteristics of the *Pteria penguin* size classes examined in the study. Mean (± SE) measurements of dorso-ventral height (DVH; mm) and wet weight (g) are shown.

Size class	DVH	Wet weight	Age (months)	Number
Small	78.7 ± 1.6	44.5 ± 2.5	14	105
Medium	118.2 ± 2.0	108.2 ± 3.4	28	105
Large	149.3 ± 1.1	349.1 ± 5.6	40	105

Table 2
The response of *Pteria penguin* of different sizes following exposure to benzocaine and 1-propylene phenoxetol at the specified concentrations. Asterisks (*) denote a statistical difference ($p < 0.05$) from the corresponding control.

Treatment	Size	Relaxed (%)	$\bar{X}$ induction time (min)	Mantle collapse (%)	Body collapse (%)
Control:					
	Small	0	NA	0	0
	Medium	0	NA	0	0
	Large	0	NA	0	0
Benzocaine:					
0.25 g L^{-1}	Small	0	NA	0	0
	Medium	20	86.0	0	0
	Large	73*	46.8	0	0
0.50 g L^{-1}	Small	73*	64.9	0	0
	Medium	73*	70.9	0	0
	Large	87*	31.0	0	0
1.20 g L^{-1}	Small	80*	26.0	0	0
	Medium	87*	34.2	0	0
	Large	100*	21.5	13	0
1-propylene phenoxetol:					
2.50 mL L^{-1}	Small	40*	32.8	0	0
	Medium	53*	39.6	0	0
	Large	67*	24.7	0	0
3.00 mL L^{-1}	Small	73*	28.5	0	0
	Medium	80*	32.8	0	0
	Large	73*	20.3	13	0
3.50 mL L^{-1}	Small	93*	27.4	0	0
	Medium	93*	29.6	0	0
	Large	100*	18.0	13	7

2.3. Experimental design

Fifteen oysters from each size class were randomly selected and immersed in one of the six anaesthetic solutions or in 1 μm filtered seawater (control). Prior research with *Pt. penguin* showed that their shell valves may remain tightly closed for long periods, which hinders direct contact of anaesthetic solution with oyster tissues and lengthens the time required for relaxation to be achieved (Kishore, 2011). This was overcome by placing oysters upright (on their hinges) in the sun, out of water, for a few minutes prior to exposure to anaesthetic solutions. This caused the shell valves to open slightly allowing a wooden wedge to be inserted anteriorly between the shell valves to allow penetration of anaesthetic solution into the shell cavity (Kishore, 2011). Once wedges were in place, oysters were positioned vertically upon immersion within a basin of anaesthetic solution, resting on their hinges and leaning against the sides of the basins (O'Connor and Lawler, 2002).

To assess the effectiveness of anaesthetic treatments, the mantles of exposed oysters were gently probed with forceps each minute after immersion in the anaesthetic solutions. Oysters were considered fully relaxed if there was no reaction (i.e., no tissue contraction or tensing of adductor muscle) upon probing (Norton et al., 1996). The time taken for each oyster to fully relax was recorded, as were any instances of mantle or body collapse (*sensu* Acosta-Salmón et al., 2005). Oysters with mantle or body collapse were removed from the solution and placed into recovery basins. Oysters failing to fully relax within 90 min of immersion were censored. Once fully relaxed, oysters were removed from the anaesthetic solutions and placed in aquaria containing aerated seawater for recovery. Oysters in recovery were probed gently with forceps at five-minute intervals, and individuals were considered to have recovered if they reacted (i.e., tissue contraction or tensing of adductor muscle) upon probing. The time taken for each oyster to recover was recorded. Oysters failing to recover within 30 min were censored. All oysters were subsequently returned to ocean-based farm conditions where they were monitored for a further seven days.

2.4. Assessment

In this study, 'Size' was a fixed factor with three levels, corresponding to the oyster size classes used in this study (Table 1). 'Treatment' was a fixed factor with six levels, comprising the three benzocaine (0.25, 0.5, and 1.2 g L^{-1}) and the three propylene phenoxetol (2.5, 3.0, 3.5 mL L^{-1}) anaesthetic solutions. For each unique combination of Size and Treatment levels the percentage of oysters (n = 15) induced to fully relax was compared to the corresponding control using a χ^2 test (R Core Team, 2021). An ANOVA, constructed as a linear model with a Gaussian distribution, was employed to determine the effects of Size and Treatment on the time taken to induce full relaxation (R Core Team, 2021). Censored oysters were excluded from this analysis under the premise that only fully relaxed oysters would have nuclei implanted (Kishore et al., 2015). Model fit was validated through examination of diagnostic plots. Following detection of a significant interaction term ($R^2 = 0.77$, $F_{9,173} = 8.9$, $p < 0.001$), pairwise comparisons between levels of Treatment and Size were made using the estimated marginal means (Russel, 2020), controlling for the familywise error rate using the Bonferroni correction. Differences in the recovery time of oysters among levels of Size and Treatment were compared using a nonparametric log-rank test of significance (Therneau, 2020). Following detection of significant differences among groups ($\chi^2 = 37.5$, $p = 0.002$), pairwise comparisons were made using log-rank tests, controlling for the false discovery rate using the Benjamini-Hochberg procedure.

3. Results

All treatments, with the exception of the 0.25 g L^{-1} benzocaine treatment, were effective in inducing a significant proportion ($\geq 40\%$; $\chi^2 \geq 5.2$, $p \leq 0.02$) of oysters to fully relax (Table 2). For both benzocaine and 1-propylene phenoxetol treatments, higher concentrations improved effectiveness. For example, the 2.5 mL L^{-1} 1-propylene phenoxetol treatment induced 40 – 67% of oysters to relax, with this percentage increasing to 73 – 80% of oysters at a concentration of 3.0 mL L^{-1} and higher still, to 93 – 100% of oysters, at a concentration of 3.5 mL L^{-1}. A similar trend was apparent for the three benzocaine treatments with 0 – 73% of oysters induced to relax at a benzocaine concentration of 0.25 g L^{-1}, 73 – 87% induced at a concentration of 0.5 g L^{-1}, and 80 – 100% induced at the highest benzocaine treatment of 1.2 g L^{-1}. The small oyster size class had the lowest proportion of oysters responding to a given treatment, while large oysters were most often (in five of the six treatments) the size class with the highest proportion of oysters

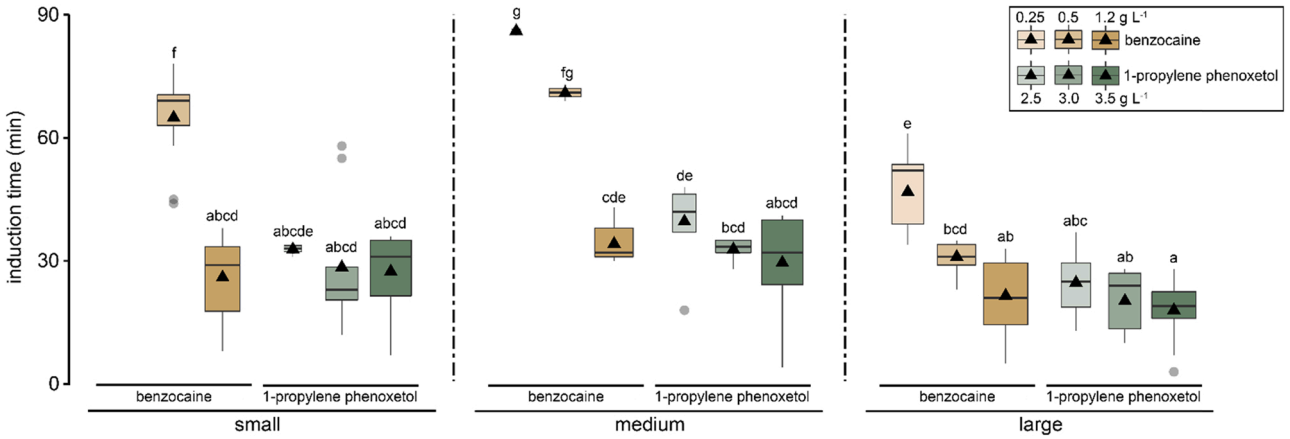

Fig. 1. Mean (▲) induction time for *Pteria penguin* ($n = 15$) to fully relax following immersion in an anaesthetic solution, faceted by relative oyster size. Boxplots illustrate range and distribution of response, where box width is scaled to reflect the percentage of oysters induced to fully relax. Labels with shared letters denote statistically similar (Bonferroni-corrected p-value: $p \geq 0.05$) induction times.

responding to a given treatment. Thus, the effectiveness of both benzocaine and propylene phenoxetol appears to be positively associated with both oyster size and the concentration of the anaesthetic solution (Table 2).

The time taken for oysters to fully relax differed depending on treatment and oyster size ($R^2 = 0.77$, $F_{9,173} = 8.9$, $p < 0.001$; Fig. 1). Both the mean and minimum induction time tended to decrease with increasing concentrations of benzocaine and 1-propylene phenoxetol (Fig. 1). The influence of oyster size was only apparent at low concentrations of benzocaine (0.25 g L^{-1}) and 1-propylene phenoxetol (2.5 mL L^{-1}), with smaller oysters experiencing significantly longer induction times. At the highest concentrations tested, neither benzocaine nor 1-propylene phenoxetol treatments showed this trend, with both anaesthetics resulting in comparable induction times for small and large oysters.

All oysters in all treatments survived immersion in the anaesthetic solutions. Relaxation coincided with mantle collapse for 13% (n = 2) of large oysters immersed in certain benzocaine (1.2 g L^{-1}) and 1-propylene phenoxetol (3.0 and 3.5 mL L^{-1}) solutions, but this was not observed with smaller (small or medium) oysters exposed to the same concentrations (Table 2). Body collapse accompanied relaxation for only a single (7%) large oyster immersed in the 3.5 mL L^{-1} 1-propylene phenoxetol solution (Table 2). Despite instances of mantle and body collapse, these oysters were still alive, as were all oysters, seven days

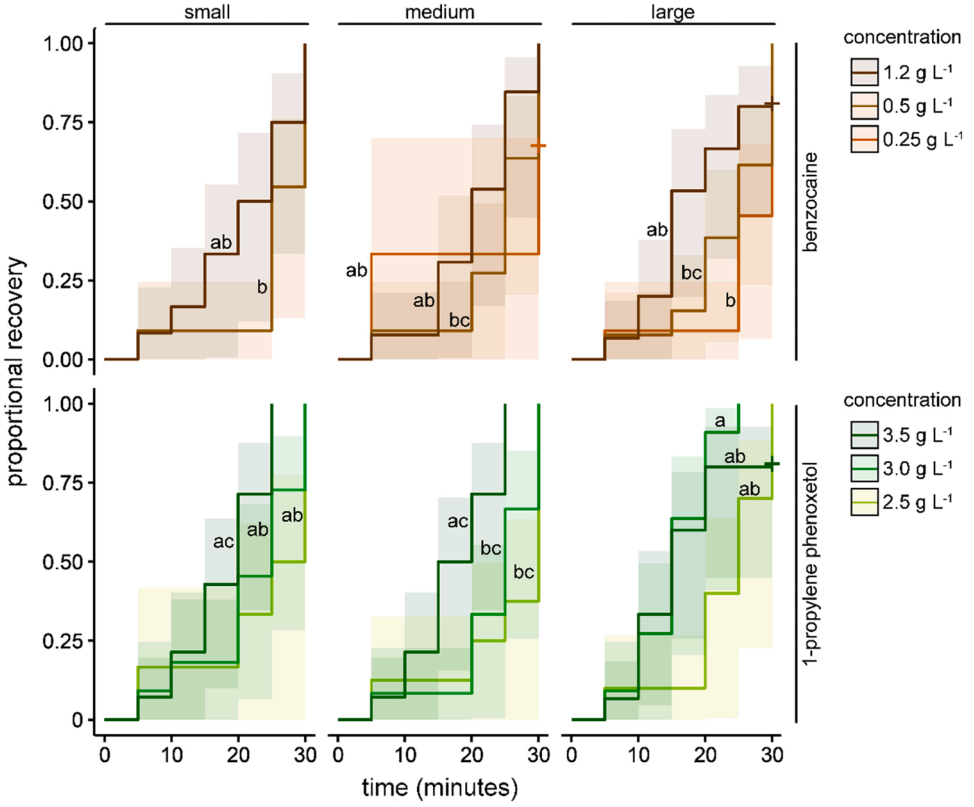

Fig. 2. Kaplan-Meier recovery curves (± 95% confidence intervals) for *Pteria penguin* following relaxation, faceted by relative oyster size and anaesthetic. Oysters failing to recover within 30 min were censored (+). Left-adjacent labels with shared letters indicate statistically similar (Benjamini-Hochberg adjusted p-value: $p \geq 0.05$) recovery curves.

after their return to oceanic farm conditions.

Nearly all oysters induced to full relaxation had recovered within 30 min (Fig. 2). Exceptions included 20% (n = 3) of large oysters exposed to the highest concentrations of benzocaine (1.2 g L^{-1}) and 1-propylene phenoxetol (3.5 mL L^{-1}), and a single medium sized oyster exposed to 0.25 g L^{-1} of benzocaine. Oyster recovery from exposure to anaesthetic solutions was size-specific ($\chi^2 = 37.5, p = 0.002$; Fig. 2). The lowest median recovery time was 15 min, shared by large oysters relaxed with certain concentrations of benzocaine (1.2 g L^{-1}) and propylene phenoxetol (3.0 and 3.5 mL L^{-1}). In contrast, the highest median recovery time was 30 min for both medium and large oysters relaxed with 0.25 g L^{-1} benzocaine, and for medium oysters relaxed with 2.5 mL L^{-1} 1-propylene phenoxetol. Oyster size alone ($\chi^2 = 0.1$, $p = 0.93$) was unable to explain the observed differences in recovery curves, but treatment was independently significant ($\chi^2 = 23.7$, $p < 0.001$), with lower concentrations of both benzocaine and 1-propylene phenoxetol associated with delayed recovery.

4. Discussion

Use of anaesthetics with pearl oysters has proven beneficial in a number of procedures associated with cultured pearl production, including surgical removal of saibo tissue from donor oysters for round pearl production (Acosta-Salmón et al., 2005; Acosta-Salmón and Southgate, 2005, 2006), relaxing host oysters prior to the invasive nucleation procedure for round pearl production (Mamangkey et al., 2009; Mills et al., 1997; Norton et al., 1996, 2000), and to ease placement of nuclei on the inner shell surface for mabé pearl production (Kishore et al., 2015). All have found commercial application. The outcomes of these and similar studies have also provided a basis for anaesthetic use in experimental and commercial production of pearls from other molluscan taxa, such as scallops (Torres-Martínez et al., 2012) and gastropods including abalone and conch (Acosta-Salmon and Davis, 2007). Despite considerable research attention on this topic, however, more effective use of anaesthetics for mabé pearl production using *Pt. penguin*, required more focused assessment of effective concentrations of proven anaesthetics, and knowledge of size dependent responses to them.

The results of this study show the following general trends across treatments for both anaesthetics tested in this study: (1) greater proportions of 'large' oyster became relaxed compared to 'small' oysters; (2) 'large' oysters required shorter exposure times to become relaxed than 'small' oysters; (3) for each size class of oyster, an increase in anaesthetic concentration resulted in an increased proportion of relaxed oysters; and (4) mantle collapse and/or body collapse only occurred in 'large' oysters in treatments with higher concentrations of anaesthetics. Of the anaesthetic treatments tested in this study, results show that 1-propylene phenoxetol at a concentration of 3.5 mL L^{-1} was very effective for *Pt. penguin.* This treatment required the least time to relax all oyster sizes tested, did not result in oyster mortality and supported favourable oyster recovery times that were well within the 30 min recommended by prior studies (Kishore, 2011; Mamangkey et al., 2009; Norton et al., 1996). It is interesting to note that Kishore (2011) assessed 2.5, 3.0 and 3.2 mL L^{-1} concentrations of 1-propylene phenoxetol for their anaesthetic efficacy for larger *Pt. penguin* (250 mm, DVH) than those used in this study. Unlike the current study, oyster mortality was reported in all treatments, with the highest rate of mortality associated with the highest concentration of 1-propylene phenoxetol (Kishore, 2011). The reasons for this are unclear but results may have been influenced by factors such as underlying oyster health and physiological condition, as well as season and its influence on oyster condition (e.g., Saucedo and Southgate, 2008). As outlined above, our results indicate greater susceptibility of larger oysters to the anaesthetics tested, and this point is supported when considering Kishore's findings and potentially relates to the greater tissue area exposed to the anaesthetic solution in larger oysters.

Only one prior study has reported a size dependent response of pearl oysters to anaesthetics, where smaller *Pinctada margaritifera* relaxed faster, and required a lower concentration of anaesthetic (menthol) to achieve this, than larger individuals (Kripa et al., 2008). These findings are contrary to those reported here for *Pteria penguin*, where an opposing size related response was shown for both benzocaine and 1-propylene phenoxetol, with improved levels of relaxation (as a proportion of oysters) and shorter times to relaxation, generally shown by larger oysters. The reasons for this are unclear and potentially reflect physiological differences between species and differences in experimental conditions between studies. It is notable that the anaesthetic used by Kripa et al. (2008) was menthol, which has been shown to cause significant mortality of *Pinctada maxima* (22%) following exposure (Mamangkey et al., 2009). Despite this, Kripa et al. (2008) reported full relaxation of *Pteria penguin* within 75 min when exposed to a menthol solution of 145 mg L^{-1}. It should also be noted that the efficacy of anaesthetics with pearl oysters (including benzocaine) is influenced by environmental factors, such as water temperature which, as it increases, reduces time to relaxation and time for recovery (Wan et al., 2012).

Mantle collapse and body collapse are thought to result from reduced haemolymph pressure (Norton et al., 1996) induced by exposure to relatively high concentrations of anaesthetics (Kishore, 2011) or prolonged anaesthetic exposure (Acosta-Salmón et al., 2005; Granados-Amores et al., 2017). As outlined above, our results indicate that the occurrence of mantle collapse and body collapse may also be size related. Despite anaesthetic concentrations and exposure times being the same for all size categories of *Pt. penguin* used in this study, mantle/body collapse occurred only in large oysters suggesting that older oysters are more responsive to anaesthetic exposure than smaller/younger oysters. While Acosta-Salmon and Southgate (2006) recommended that oysters with collapsed mantle should be avoided as potential donors for round pearl production, their use for mabé pearl production, which relies on nacre secretion from epithelial cells within the mantle, onto adjacent nuclei (Gordon et al., 2019; Taylor and Strack, 2008), requires consideration. In this study, oysters with anaesthetic-induced mantle collapse fully recovered on return to oceanic conditions, however, any resulting long-term physiological impairment to mantle tissue function is unknown, and further research is required to assess potential impacts of mantle collapse on subsequent nacre secretion rates and quality.

In considering both the proportion of oysters achieving relaxation and the time taken to achieve this, our results indicate that the most effective concentration of benzocaine to use with small, medium and large *Pt. penguin* is the highest level tested of 1.20 g L^{-1}. Similarly, the highest concentration of 1-propylene phenoxetol tested (3.5 g L^{-1}) was also the most effective with all three size classes of *Pt. penguin*. In selecting these treatments, however, the user should be aware that both anaesthetics, when used at these concentrations, brought about mantle collapse in large size oysters. As such there may be prolonged recovery time (and attention) required for such oysters and the possibility of longer-term effects on nacre secretion, as discussed above, should be considered. On this basis, it may be more appropriate to consider using a lower, less effective anaesthetic concentrations for larger (ca. 149 mm, DVH) oysters to avoid mantle collapse. This may be a pertinent approach given that Kishore (2011) reported considerably higher mortality of very large *Pt. penguin* (250 mm, DVH) when exposed to 3.2 mL L^{-1} 1-propylene phenoxetol, when compared to lower concentrations (2.5 and 3.0 mL L^{-1}).

As well as efficacy, availability and price, the choice of anaesthetic for relaxing pearl oysters should also consider ease of preparation and preparation time. Preparation of 1-propylene phenoxetol solutions is simpler than that for benzocaine. It is readily soluble in seawater while benzocaine requires dissolving in methyl alcohol before being heated to 88–92C to completely dissolve the crystals. Mamangkey et al. (2009) also highlighted the need to consider potential health hazards and toxicity to human users when assessing the potential of various anaesthetics. While Mills et al. (1997) reported that 1-propylene phenoxetol

in solution is non-toxic, non-irritating and does not induce skin hypersensitivity, material safety data sheets advise potential for eye irritation and recommend the use of appropriate protective equipment.

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CRediT authorship contribution statement

Pranesh Kishore: Conceptualization, Data curation, Methodology, Investigation, Writing – original draft. **Max Wingfield**: Conceptualization, Methodology, Investigation. **Thane A. Militz**: Formal analysis, Visualization, Writing – original draft. **Tracy Aisea**: Conceptualization, Investigation. **Paul C. Southgate**: Conceptualization, Methodology, Writing – original draft, Supervision, Funding acquisition.

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.

Data availability

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

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References

Acosta-Salmon, H., Davis, M., 2007. Inducing relaxation in the queen conch *Strombus gigas* (L.) for cultured pearl production. Aquaculture 262, 73–77.

Acosta-Salmon, H., Southgate, P.C., 2006. Would healing after excision of mantle tissue from the Akoya pearl oyster, *Pinctada fucata*. Comp. Biochem. Physiol. A 143 (2), 264–268.

Acosta-Salmón, H., Rangel-Dávalos, C., 1997. Benzocaine (ethyl-p-aminobenzoate) as anesthetic for surgical implantation of nucleus in the pearl oyster *Pteria sterna* (Gould, 1851). World Aquaculture Society. World Aquaculture'97, Book of Abstracts. Seattle, WA, U.S.A., Feb. 19–23, 1997.

Acosta-Salmón, H., Southgate, P.C., 2005. Mantle regeneration in the pearl oysters *Pinctada fucata* and *Pinctada margaritifera*. Aquaculture 246, 447–453.

Acosta-Salmón, H., Martínez-Fernández, E., Southgate, P.C., 2005. Use of relaxants to obtain saibo tissue from the blacklip pearl oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*). Aquaculture 246, 167–172.

Ehteshami, F., 1993. Anaesthetizing *Pinctada radiata* with MS 222. Iran. Sci. Fish. J. 1 (3), 1–6.

Gordon, S.E., Malimali, S., Wingfield, M., Kurtböke, D.I., Southgate, P.C., 2019. Effects of nucleus position, profile and arrangement on the quality of mabé pearls produced by the winged pearl oyster, *Pteria penguin*. Aquaculture 498, 109–115.

Gordon, S.E., Wingfield, M., Malimali, S., Halafihi, T., Southgate, P.C., 2020. Assessing protective mesh cylinders for chaplet-based culture of the winged pearl oyster, *Pteria penguin*. J. Shellfish Res. 39 (1), 59–66.

Granados-Amores, A., Campa-Córdova, Á.I., Rodríguez-Jaramillo, C., Mazón-Suástegui, J.M., Tovar-Ramírez, D., Saucedo, P.E., 2017. Physiological response to relaxation as pre-operative therapy to pearl formation in the winged pearl oyster *Pteria sterna*. Aquaculture 473, 145–152.

Haws, M., Ellis, S., Ellis, E.P., 2006. Producing Half-Pearls (Mabé). Western Indian Ocean Marine Science Association. University of Hawaii, Hilo and the Coastal Resources Centre University of Rhode Island.

Johnston, W.L., Hine, D., Southgate, P.C., 2019. Overview of the development and modern landscape of marine pearl culture in the south Pacific. J. Shellfish Res. 38 (3), 499–518.

Johnston, W.L., Kishore, P., Vuibeqa, G.B., Hine, D., Southgate, P.C., 2020. Economic assessment of community-based pearl oyster spat collection and mabé pearl production in the western Pacific. Aquaculture 514, 734505.

Kishore, P., 2011. Use of 1-propylene phenoxetol and benzocaine to anaesthetise *Pteria penguin* (Röding, 1798) for mabe production. SPC Pearl Oyster Inf. Bull. 19, 29–33.

Kishore, P., Southgate, P.C., Seeto, J., Hunter, J., 2015. Factors influencing the quality of half-pearls (mabé) produced by the winged pearl oyster, *Pteria penguin* (Röding, 1758). Aquac. Res. 46, 769–776.

Kripa, V., Abraham, K.J., Libini, C.L., Velayudhan, T.S., Radhakrishnan, P., Mohamed, K. S., Modayil, M.J., 2008. Production of designer mabe pearls in the black-lipped pearl oyster, *Pinctada margaritifera*, and the winged pearl oyster, *Pteria penguin*, from Andaman and Nicobar Islands, India. J. World Aquac. Soc. 39, 131–137.

Mamangkey, N.G.F., Acosta-Salmon, H., Southgate, P.C., 2009. Use of anaesthetics with the silver-lip pearl oyster *Pinctada maxima* (Jameson). Aquaculture 288 (3–4), 280–284.

Mills, D., Tlili, A., Norton, J., 1997. Large-scale anesthesia of the silver-lip pearl oyster, *Pinctada maxima* Jameson. J. Shellfish Res. 16 (2), 573–574.

Monteforte, M., Bervera, H., Saucedo, P., 2004. Response profile of the Calafia pearl oyster, *Pincatda mazatlanica* (Hanley, 1856), to various sedative therapies related to surgery for round pearl induction. J. Shellfish Res. 23 (1), 121–128.

Norton, J.H., Dashorst, M., Lansky, T.M., Mayer, R.J., 1996. An evaluation of some relaxants for use with pearl oysters. Aquaculture 144, 39–52.

Norton, J.H., Lucas, J.S., Turner, I., Mayer, R.J., Newnham, R., 2000. Approaches to improve cultured pearl formation in *Pinctada margaritifera* through use of relaxation, antiseptic application and incision closure during bead insertion. Aquaculture 184, 1–17.

O'Connor, W.A., Lawler, N.F., 2002. Propylene phenoxetol as a relaxant for the pearl oysters *Pinctada imbricata* and *Pinctada albina*. Asian Fish. Sci. 15, 53–59.

R Core Team, 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ruiz-Rubio, H., Acosta-Salmón, H., Olivera, A., Southgate, P.C., Rangel-Dávalos, C., 2006. The influence of culture method and culture period on quality of half-pearls ('mabé') from the winged pearl oyster *Pteria sterna*, Gould, 1851. Aquaculture 254, 269–274.

Russel, L., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.2-1. https://CRAN.R-project.org/package=emmeans.

Saucedo, P., Southgate, P.C., 2008. Reproduction, development and growth. In: Southgate, P.C., Lucas, J.S. (Eds.), The Pearl Oyster. Elsevier, Oxford, pp. 131–186.

Southgate, P.C., 2021. Pearl culture and pearl production. In: Shumway, S. (Ed.), Molluscan Shellfish Aquaculture: A Practical Guide. 5m Books, Sheffield, pp. 205–232.

Southgate, P.C., Beer, A.C., Ngaluafe, P., 2016. Hatchery culture of the winged pearl oyster, *Pteria penguin*, without living micro-algae. Aquaculture 451, 121–124.

Southgate, P.C., Kishore, P., Sharma, S., Simos, T., 2019. Empowering women through pearl industry-based livelihoods in Fiji. SPC Women Fish. Inf. Bull. 29, 24–29.

Taylor, J., Strack, E., 2008. Pearl production. In: Southgate, P.C., Lucas, J.S. (Eds.), The Pearl Oyster. Elsevier, Oxford, pp. 273–302.

Therneau, T., 2020. A package for Survival Analysis in R. R package version 3.2-7. ⟨https://CRAN.R-project.org/package=survival⟩.

Torres-Martínez, J.A., Saucedo, P.E., Rangel-Dávalos, C., Acosta-Salmón, H., 2012. Advances in pre-operative techniques for pearl production in the lions-paw scallop *Nodipecten subnodosus*: relaxation and mantle excision. Aquaculture 356, 279–283.

Wan, Z.P., Wang, M.F., Li, S.B., Yu, X.Y., 2012. Anaesthesia effect of two anaesthetic chemicals, 2-phenoxyethanol and benzocaine, on the winged pearl oyster (*Pteria penguin* (Roding)). J. Trop. Org. 1, 16–21.