Use of 1-propylene phenoxetol and benzocaine to anaesthetise *Pteria penguin* (Röding, 1798) for mabe production

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Abstract

Mortality among *Pteria penguin* can be caused by severe stress during the half-pearl (mabe) implantation procedure. Anaesthetics can play a major role in reducing stress and resulting mortality. Three different concentrations of 1-propylene phenoxetol (2.8 mL L^{-1}, 3.0 mL L^{-1}, 3.2 mL L^{-1}) and two different concentrations of benzocaine (500 mg L^{-1}, 1200 mg L^{-1}) were assessed for their effectiveness as anaesthetics for *P. penguin*. Different concentrations and anaesthetics tested had an influence ($P<0.05$) on the period required for relaxation of the oysters. However, the recovery time and oyster mortalities at different concentrations were not significantly different ($P>0.05$). 1-propylene phenoxetol at a concentration of 3.0 mL L^{-1} was the best of the anaesthetics tested and brought about anaesthesia after an average of 15 minutes with an average recovery time of 12.5 minutes. This treatment also recorded the lowest mortality of the various treatments tested.

Introduction

Pteria penguin is commonly used for the production of half pearls (mabe), which are highly regarded (Tanaka and Yamamoto 1997). The process of mabe production involves adhesion of nuclei to the inner surface of the oyster's shell (Haws et al. 2006; Kripa et al. 2008) and nuclei may vary in shape (e.g. hemispherical, heart, tear drop, oval). The nucleus implantation process is simple compared with the round pearl production process (Haws et al. 2006); however, there are concerns regarding the difficulty of forcefully opening *P. penguin* shells prior to implanting, because the relatively large, non-nacreous shell margin in this species makes it difficult to use shell openers and is easily broken causing stress to the oyster. Stress during nucleus implantation has been reported for other pearl oyster species and it often results in mortality (Norton et al. 1996; O'Connor and Lawler 2002).

Stress and mortality of pearl oysters during the nucleus implanting procedure has prompted studies into the potential for using anaesthetics (relaxants) to reduce stress (Norton et al. 2000; O'Connor and Lawler 2002). Relaxants cause the adductor muscles of pearl oysters to relax, reducing muscularly stimulated haemolymph wastage, preventing muscle contractions, and allowing better access to the implanting site (Norton et al. 1996; O'Connor and Lawler 2002). Several relaxants have been assessed for pearl oysters, to which each species responded differently according to relaxant type and the concentrations used (Mamangkey et al. 2009). Kripa et al. (2008) used menthol crystals and clove oil on *Pinctada margaritifera*, *Pinctada maxima* (Jameson 1901) and *Pteria penguin*. 1-propylene phenoxetol was used on *Pinctada margaritifera* (Norton et al. 2000), *Pinctada maxima* (Mamangkey et al. 2009), and on *Pinctada imbricata* and *Pinctada albina* by O'Connor and Lawler (2002). MS 222 was used on *Pinctada radiata* by Ehteshami (1995) while benzocaine has been used on *Pinctada maxima* (Mamangkey et al. 2009) and on *Pteria sterna* (Acosta-Salmón and Rangel-Davalos 1997).

Exposing oysters for too long to a relaxant solution can cause mantle retraction, mantle and body collapse and excessive mucus production (Norton et al. 1996; Mamangkey et al. 2009). However, while appropriate relaxants applied at the correct concentration facilitate pearl production in oysters, knowledge of appropriate relaxants and effective concentrations of those relaxants needs to be known in order to obtain favourable results. This paper determined the most appropriate of two commonly used relaxants for *Pteria penguin* at varying concentrations. Relaxants tested were 1-propylene phenoxetol at 2.5 mL L^{-1}, 3.0 mL L^{-1} and 3.2 mL L^{-1}, and benzocaine at 500 mg L^{-1} and 1200 mg L^{-1}.

Materials and method

The experiment was carried out at J. Hunter Pearls in Savusavu, Fiji (16°49'S, 179°19'E). *P. penguin* were collected from spat collectors and maintained on a long line until they were three years old and had a mean dorso-ventral measurement (DVM) of 250 ± 6.5 mm. They were scrubbed to remove unwanted fouling organisms before the

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experiment. Six randomly selected oysters were exposed to one of the five treatments tested in 20-L aquaria. Another six oysters were held in ambient seawater as controls.

Each aquarium was filled with 20 L of seawater. The different concentrations of 1-propylene phenoxetol (2.5 mL L^{-1}, 3.0 mL L^{-1}, 3.2 mL L^{-1}) were each vigorously shaken with seawater in a measuring cylinder before being added to aquaria. The solutions in each aquarium were swirled for an even dispersion of relaxants and solutions were brought to the desired concentrations. To prepare a benzocaine solution of 500 mg L^{-1}, 9.75 g of benzocaine was first dissolved in methyl alcohol to a saturation of 250 mg mL^{-1}. The solution was then poured into a small container (0.5 L) of hot (88–92˚C) seawater to completely dissolve the benzocaine crystals (Acosta-Salmón and Davis 1997; Acosta-Salmón et al. 2005; Mamangkey et al. 2009). The solution was then poured into a container with 19.5 L of seawater to obtain the desired concentration. The 1,200 mg L^{-1} solution of benzocaine was prepared by dissolving benzocaine in ethanol first (100 mg L^{-1}) and then mixing the solution with seawater (Acosta-Salmón et al. 2005; Mamangkey et al. 2009).

P. penguin shells were observed to be tightly closed during the first trial with 2.5 mL L^{-1} of 1-propylene phenoxetol. The relaxants could not enter the shells to contact oyster tissues. This increased the time required for the oysters to relax. To counter this *P. penguin* were placed on their hinges out in the sun for a little while prior to exposure to anaesthetic solutions. This caused the valves to open slightly, and a wooden wedge was inserted in between to prevent the oysters from closing their valves. This method allowed direct contact of the anaesthetic solution with oyster tissues, which resulted in less time taken by oysters to relax. *P. penguin* were placed vertically resting on their hinges and leaned against the sides of aquaria (O'Connor and Lawler 2002).

Seawater temperature was maintained at 25.5˚C while pH was maintained between 7.9 and 8.3. *P. penguin* exposed to relaxants were observed for 25 minutes (min). The mantles of the oysters were probed with forceps to determine whether the oysters had relaxed. The oysters were considered to be fully relaxed if there was no reaction by the mantle upon probing (Mamangkey et al. 2009). The time taken for *P. penguin* to fully relax in each of the relaxant solutions was recorded. Relaxed *P. penguin* from each aquarium were then placed in different containers with freshly aerated seawater for recovery. *P. penguin* were considered to have recovered when their mantles reacted or when they closed their shells upon being probed. The time taken for *P. penguin* to recover from each relaxant treatment was recorded.

Some *P. penguin* also experienced mantle and body collapse during the relaxation period. Mantle collapse is when mantle tissues are not rigid enough to adhere to the inner surfaces of the shells, while body collapse is characterised by all soft body parts losing their muscular strength (Acosta-Salmón et al. 2005; Mamangkey et al. 2009). Such oysters were removed from the relaxant solution and placed into containers with clean seawater. Recovered *P. penguin* were returned to farm conditions where they were further observed for seven days to note if there was any mortality.

Kruskall-Wallis analysis was done to determine the difference in time taken for the oysters to relax in each treatment. Pearson's Chi-square test was used to determine the difference between the recovery rates and mortality of the oysters for each of the different concentrated solution (Mamangkey et al. 2009). Both of the analyses were carried out using SPSS Version 13.

Results

All *P. penguin* became fully relaxed in 2.5, 3.0 and 3.2 mL L^{-1} solutions of 1-propylene phenoxetol within 40 min (Fig. 1). Additionally, oysters exposed to 1,200 mg L^{-1} of benzocaine were also fully relaxed after the same amount of time (Fig. 2). In contrast, only 12 *P. penguin* were fully relaxed when exposed to 500 mg L^{-1} of benzocaine within the 40 min time allocation. 1-propylene phenoxetol, at a concentration of 3.0 mL L^{-1} required an average of 15 min to bring about relaxation in all *P. penguin*. *P. penguin* in this treatment required a mean recovery time of 12.5 min (Fig. 3) and showed the lowest mortality (1 oyster after 7 days) (Fig. 4). *P. penguin* exposed to a 2.5 mL L^{-1} concentration of 1-propylene phenoxetol required the longest mean time period to be relaxed (26.4 min) and a shortest time to recover (10.7 min) with mortality of 3 oysters after 7 days. 1-propylene phenoxetol, at a concentration of 3.2 mL L^{-1} recorded the shortest mean time period (10 min) for the oysters to relax; however, this treatment also required the longest recovery time of 14.1 min and recorded the highest mortality (7 oysters) after 7 days. In contrast, oysters exposed to 500 mg L^{-1} and 1,200 mg L^{-1} concentrations of benzocaine required mean times of 25.0 min and 16.1 min to relax, respectively (Fig. 3), with average recovery times of 14.2 min and 13.9 min, correspondingly (Fig. 4).

The Kruskal–Wallis ($\varkappa$) analysis, showed that the different concentrations of 1-propylene phenoxetol ($\varkappa = 36.55$, $p = 0.00$) and benzocaine ($\varkappa = 19.40$, $p = 0.00$) had an influence on the time required for *P. penguin* to be fully relaxed. However, the Chi-square analysis showed that the different concentrations of these relaxants did not have significance difference, 1-propylene phenoxetol ($\chi^2 = 0.17$, $p = 1.0$) and benzocaine ($\chi^2 = 0.05$, $p = 1.0$), on the recovery times

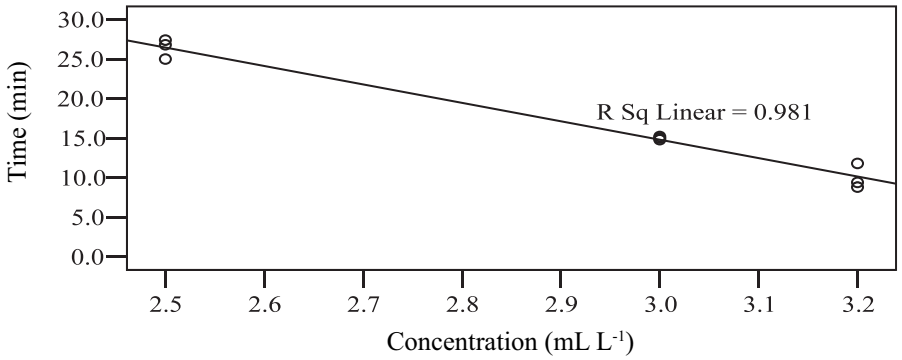

Figure 1. Mean time taken for *P. penguin* to relax at different concentrations in 1-propylene phenoxetol.

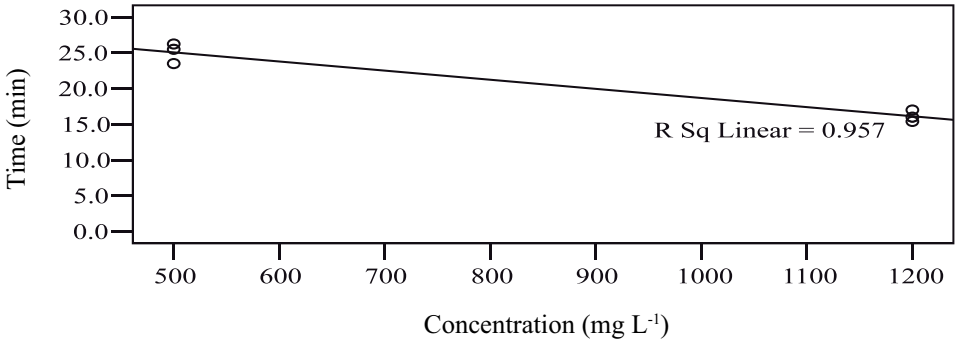

Figure 2. Mean time taken for *P. penguin* to relax at different concentrations of benzocaine.

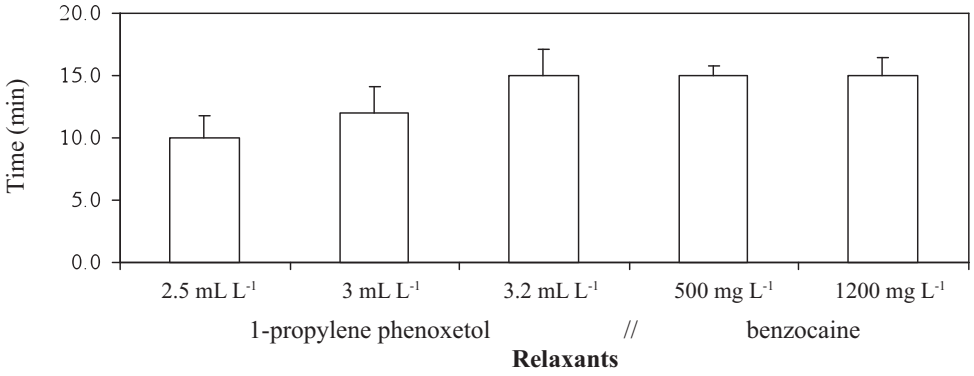

Figure 3. Recovery times of *P. penguin* after being relaxed at different concentrations in both of the relaxants.

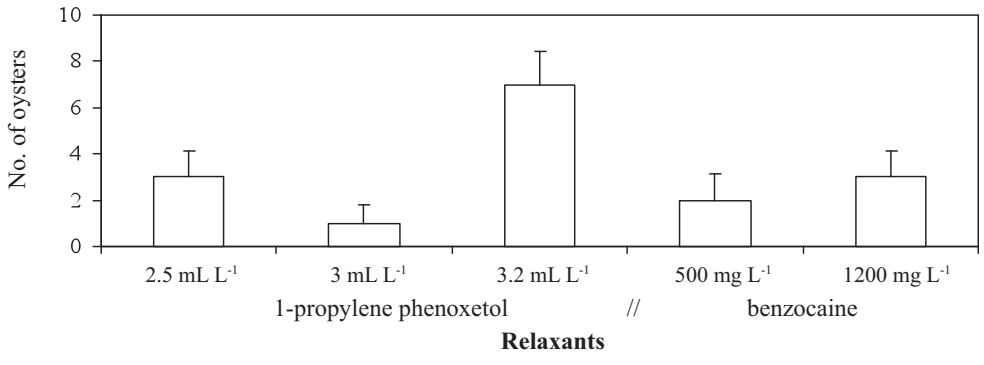

Figure 4. The number of oysters that died at different concentrations in both of the relaxants after 7 days.

required by the oysters. Moreover, the mortality rate of the oysters also did not differ significantly, 1-propylene phenoxetol (χ^2 = 0.17, p = 0.92) and benzocaine (χ^2 = 0.00, p = 1.00) after 7 days.

Discussion

Numerous methods are used to relax marine invertebrates (Sendall 2003). The effectiveness of the different relaxant on molluscs is species and concentration specific (Aquilina and Roberts 2000; Acosta-Salmón and Davis 2007; Mamangkey et al. 2009). The results presented here show that *P. penguin* responded differently to dissimilar relaxants with varying concentrations. As might be expected, as the concentrations of relaxants increased, the time required by *P. penguin* to relax decreased. A relaxant was considered to be most efficient if it induced relaxation in less than 15 min and had a recovery time of less than 30 min with minimum mortality among the oysters (Norton et al. 1996; Mamangkey et al. 2009). The 3 mL L^{-1} treatment of 1-propylene phenoxetol proved to be the most appropriate concentration to be used with *P. penguin*. It relaxed the required number of *P. penguin* and had a reasonable and economical recovery time. There was also the least number of mortalities with this treatment.

1-propylene phenoxetol is a commonly used relaxant for molluscs. *P. penguin* required a higher concentration (3.0 mL L^{-1}), of 1-propylene phenoxetol compared to *Pinctada margaritifera* (Norton et al. 1996) and *Pinctada maxima* (Mamangkey et al. 2009), to bring about relaxation; 2.5 mL L^{-1} provided favourable results for *Pinctada* species. According to O'Connor and Lawler (2002), 2.2 mL L^{-1} of 1-propylene phenoxetol relaxed *Pinctada imbricata* and *Pinctada albina* within 15 min and the oysters had recovered within 10 min. However, 2.5 mL L^{-1} of 1-propylene phenoxetol used to relax abalone, *Haliotis iris*, resulted in muscle contraction followed by mortality (Aquilina and Roberts 2000).

In addition, the average time taken for 1,200 mg L^{-1} of benzocaine to relax *P. penguin* was slightly longer then the time taken to relax *Pinctada maxima* (10.5 min) (Mamangkey et al. 2009), *Pinctada fucata* (10.27 min) and *Pinctada margaritifera* (9 min) (Acosta–Salmón et al. 2005). Despite the slightly extended time required to relax *P. penguin*, the results were rather positive. Few cases of body and/or mantle collapse were noted among *P. penguin* exposed to 1,200 mg L^{-1} of benzocaine, as reported by Acosta–Salmón et al. (2005) for *Pinctada fucata* and *Pinctada margaritifera*. Body and mantle collapse was mainly noted in the 3.2 mL L^{-1} concentration of 1-propylene phenoxetol that resulted from the strong concentration of the solution and prolonged exposure of *P. penguin* to the relaxant. Excessive mucus production by *P. penguin* was also noted in this treatment. *P. penguin* with body and mantle collapse had wider valve openings than normal and took more time to recover, or in most cases, died.

The type of relaxant used should also consider labour cost and preparation period. Preparation of 1-propylene phenoxetol solutions was simpler than that for benzocaine. 1-propylene phenoxetol was readily soluble in seawater while benzocaine required dissolving in methyl alcohol before being heated to 88–92˚C to completely dissolve the crystals. Mamangkey et al. (2009) stated that the potential health hazards and toxicity to human users should also be considered when assessing the potential of various relaxants; however, 1-propylene phenoxetol and benzocaine pose very little risk to human health.

This research was performed as a part of a larger research project, which aimed to improve the quality mabe pearls. Mabe pearls are currently considered to be a future alternative livelihood for coastal communities in the region. The procedure and recommended relaxant reported in this paper will be of assistance to people at rural community level concerned with mabe production from *P. penguin*.

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References

Acosta-Salmón H. and Davis M. 2007. Inducing relaxation in queen conch *Strombus giga* (L.) for cultured pearl production. Aquaculture 262(1):160–170.

Acosta-Salmón H., Martinez-Fernandez E. and Southgate C.P. 2005. Use of relaxants to obtain saibo tissue from the blacklip pearl oyster (*Pinctada margaritifera*) and the Akoya pearl oyster (*Pinctada fucata*). *Aquaculture* 246(1–4):160–170.

Acosta-Salmón H. and Rangel-Davalos C. 1997. Benzocaine (ethyl p-Aminobenzoate) as anesthetic for surgical implantation of nucleus in the pearl oyster *Pteria sterna* (Gould, 1851). SPC Pearl Oyster Information Bulletin 10:68.

Aquilina B. and Roberts R. 2000. A method for inducing muscle relaxation in the abalone, *Haliotis iris*. Aquaculture 190(3–4):403–408.

Ehteshami F. 1995. Anaesthetizing *Pinctada radiata* with MS 222. Iranian Fisheries Bulletin, Jahad Sazandegi Ministry, Iranian, Fisheries Research and Training Organisation, No. 3. SPC Pearl Oyster Information Bulletin 8:51.

Haws M., Ellis C.S. and Ellis P.E. 2006. Producing half-pearls (mabe). Western Indian Ocean Marine Science Association, University of Dar es Salaam, University of Hawaii, Hilo and the Coastal Resources Center, University of Rhode Island: 5–14.

Kripa V., Abraham J.K., Libini L.C., Velayudhan S.T., Radhakrishan P., Mohamed S.K. and Modayil J.M. 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. Journal of World Aquaculture Society 39(1):132–134.

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

Norton H.J., Lucas S.J., Turner I., Mayer J.R. and 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(1–2):1–17.

Norton H.J., Dashorst M., Lansky M.T. and Mayer J.R. 1996. An evaluation of some relaxants for use with pearl oysters. Aquaculture 144(1–3):39–52.

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

Sendall K. 2003. Phenoxyethanol as a relaxant before fixation in the sea cucumber *Cucumaria miniata* (Echinodermata). Collection Forum 18(1–2):98–103.

Tanaka H. and Yamamoto T. 1997. Potential of commercial development of mabe pearl farming in Vava'u islands, Kingdom of Tonga. Food and Agriculture Organization of the United Nations. Suva. Project reports (not in a Series) - No. 5. Accessed 28 July 2008 from < http://www.fao.org/docrep/005/AC.htm>