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Propylene Phenoxetol as a Relaxant for the Pearl Oysters *Pinctada imbricata* and *Pinctada albina*

W.A. O' CONNOR* and N.F. LAWLER

NSW Fisheries
Port Stephens Research Centre
Taylors Beach, NSW, 2316
Australia

Abstract

The responses of pearl oysters *Pinctada imbricata* and *Pinctada albina* to the relaxant, propylene phenoxetol (PP) were similar to those reported for other members of the genus. Wedges that keep the valves of oysters open were unnecessary as most values opened readily in the presence of PP (2 mL L^{-1} seawater). Relaxation generally occurred within 15 min and, upon removal from the relaxant bath, oysters recovered within 10 min without any sign of ill-effects. In general, both relaxation and subsequent recovery times decreased with increasing water temperature. The size of oysters had little effect on the time taken to open the valves in the presence of PP, the time to relax nor the time to recover after exposure. Prolonged exposure to PP (90 min) significantly increased the recovery time, but no mortality or apparent ill effects were observed in the week following exposure.

Introduction

In both pearl culture and pearl oyster research, the invasive nature of some procedures has led to the evaluation of potential relaxants to reduce stress to oysters (Tranter 1957, Hildemann et al. 1974, Dev 1994, Norton et al. 1996, 2000). In particular, relaxants have been suggested as a means of reducing oyster mortality and enhancing pearl quality by preventing muscle damage during nuclei insertion operations; reducing muscularly induced haemolymph loss and increasing the ease and accuracy of surgery and biopsy by preventing muscular contractions (Norton et al. 1996). Among the relaxants tested, propylene phenoxetol (PP) is particularly useful to relax the oysters *P. margaritifera* (Hildemann et al. 1974, Norton et al. 1996), *P. albina* (Norton et al. 1996) and *P. maxima* (Norton et al. 1996, Mills et al. 1997). In these species, 1.5 to 2.5 $ml \cdot l^{-1}$ PP produces relatively rapid relaxation (generally <15 min, Mills et al. 1997) with a short recovery period, although its effectiveness was reduced at lower temperatures (Norton et al. 1996).

*Corresponding author

P. imbricata is native to the New South Wales coast (Australia) and is the subject of research that would benefit from the availability of a suitable relaxant, notably during experimentation where handling could reduce growth rates or induce spawning. PP was suggested based on its success with other pearl oyster species, but the possibility of species-specific differences in responses to relaxants (Runham et al. 1965, Kaplan 1969, Heasman et al. 1995) required the evaluation of PP prior to routine use on *P. imbricata. P. albina* is also native to NSW and the efficacy of PP has previously been evaluated with this species. However, PP had only previously been used on larger oysters (>90 mm or >120 g) at higher water temperatures (>21ºC). The *P. imbricata* and *P. albina* of interest were routinely between 40 and 90 mm shell height (20 to 120 g total weight) and commonly required relaxation at temperatures of <20ºC.

Materials and Methods

All pearl oysters used in this study were collected from Port Stephens, New South Wales (32º44'S, 152º08'E). To prepare relaxant baths, PP (1-phenoxy-propan-2-ol ($C_9H_{10}O_2$), Nipa Laboratories, UK) was added to a small quantity (100 to 200 mL) of seawater in a bottle and shaken vigorously to aid dispersion before being added to 4 l of seawater (35 $g \cdot kg^{-1}$ salinity). The term "relaxant" has been used in preference to anaesthetic" to acknowledge the difficulties in differentiating between muscular paralysis and anesthetization in pearl oysters (after Norton et al. 1996).

In all the experiments, the concentration of PP was 2 $ml \cdot l^{-1}$ of seawater and unless otherwise specified the temperature of baths was 18ºC. Pearl oysters were placed in the baths vertically on their hinge and leaned against the wall of the bath. To recover following relaxation, oysters were placed into a 200 l aerated tank of seawater that was held at the same temperature as the relaxant bath.

Oysters were described as "relaxed" when they gaped and gentle probing of the mantle failed to induce the oyster to either withdraw the mantle or close the shell and when the oyster could also be removed from the relaxant bath without closing its shell (after Heasman et al. 1995, Norton et al. 1996). Oysters were considered to have recovered when any handling or disturbance induced the shell to close. All oysters were maintained within the hatchery for at least 24 h after experimentation to monitor any resultant mortality.

Experiment 1: Valve wedges and relaxation

Wedges are commonly used in the pearl industry to prevent oysters from closing their shells prior to nuclei insertion. These can also be experimentally useful to ensure that an oyster is immediately exposed to the relaxant (Norton et al. 1996). However, wedges can also be difficult to use in smaller oysters and on occasions are considered to be impractical and potentially stressful to the oyster. We avoided using wedges and inducing any asso- ciated stress to oysters during routine assessments of the reproductive condition. The time the

12 oysters took to relax and the subsequent time for them to recover with their valves wedge opened were compared with those of the 12 oysters placed directly in the relaxant baths. In the latter treatment, the time to relax was defined as the time that elapsed when the oysters first opened their valves up to the time that they subsequently relaxed.

Experiment 2: Prolonged exposure and oyster survival

Ten oysters were placed in individual baths of PP solution and retained in the bath for 90 min after relaxation. Each oyster was then removed and placed in a recirculating holding system to recover. Individual response times (opened, relaxed and recovered) were recorded and oyster survival was monitored for one week following exposure to PP.

Experiment 3: The effect of temperature on relaxation and recovery

Oysters were held in the hatchery in 200 l tanks of seawater for 24 h at one of four temperatures, 14, 18, 22 or 26°C. A mixture of *Pavlova lutheri* and *Chaetoceros calcitrans* was added to each tank to allow the oysters to filter. Each oyster was then placed in an individual PP bath and the lengths of time taken to open the valves, to relax and to recover following removal from the bath were recorded. The response times (opened, relaxed and recovered) of the 10 oysters were recorded for each temperature.

Experiment 1 used only *P. albina*, while Experiments 2 and 3 were repeated using both *P. imbricata* and *P. albina*.

Statistical analysis

The effects of wedging oyster valves on response times (Exp. 1) and of prolonged exposure on response times (Exp. 2) were evaluated using ANOVA (Sokal and Rholf 1981) after homogeneity had been confirmed with Cochran's Test (Winer et al. 1991). For Experiments 1, 2 and 3, the relationships between shell height and response times were investigated using Pearson product-moment correlations (Sokal and Rholf 1981). Due to the number of correlation coefficients calculated (33), α was set *a priori* at 0.01 to reduce the possibility of Type I error (Sokal and Rholf 1981). The effects of temperature on response times (Exp. 3) were analyzed using linear regression analysis (Sokal and Rohlf 1981).

Results

Experiment 1: Valve wedges and relaxation

For *P. albina* without wedged valves, the time taken to open the valves ranged between 0.5 to 33 min, but generally occurred between 3 to 6 min after immersion. Having opened their valves, the time taken for oysters to relax (5.5

± 0.6 min) did not differ significantly (F =2.199, df 1/22, P>0.05) from the time taken for relaxation in oysters with valves wedged open (8.8±2.1 min). The time taken to recover was, however, significantly faster for oysters with valves wedged open (3.31±0.4 min and 5.45±0.8 min, respectively; F =5.423, df 1/22, P<0.05). No significant correlations were found between the shell height of oysters and either the time taken to open in the bath, to relax in the presence of PP nor to recover following exposure to the relaxant (Table 1).

Experiment 2: Prolonged exposure and oyster survival

For both *P. imbricata* and *P. albina*, the time taken to open in the presence of PP and the time to relaxation did not differ significantly from those observed at the same temperature (18°C) in Experiment 3. However, recovery following prolonged exposure to PP was significantly protracted (*P. imbricata*, F =10.372, df 1/18, P<0.05; *P. albina*, F =4.755, df 1/18, P<0.05). There were no significant correlations between shell height and response times for either species (Table 1) and no mortality occurred in the week following prolonged exposure. Indeed, throughout these experiments, all oysters were retained for a minimum of 24 hr after exposure to PP, during which no mortality occurred.

Experiment 3: The effect of temperature on relaxation and recovery

The responses of both *P. imbricata* and *P. albina* to immersion in PP baths were similar with the exception of the time taken for oysters to open when initially placed in the bath. *P. imbricata* generally opened valves within 10 min irrespective of the temperature of the bath (Fig. 1). *P. albina*, however, showed a significant reduction in the time taken to open their valves as water temperatures increased (Fig. 2).

Both *P. imbricata* and *P. albina* commonly relaxed within 15 min of valve opening and both species showed significant reductions in the time taken to relax as water temperatures increased (Figs. 1 and 2). Recovery for both species was rapid with a

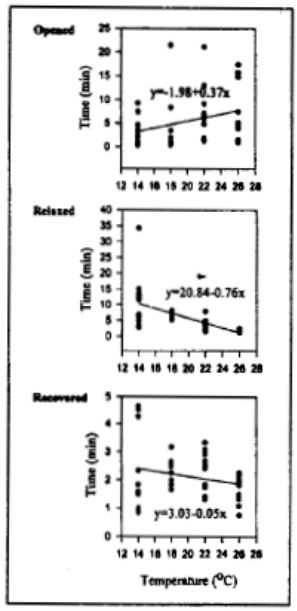

Fig. 1 The time taken for valve opening, relaxation and subsequent recovery of *P. imbricata* exposed to a 2 mL·L^{-1} propylene phenoxytol solution.

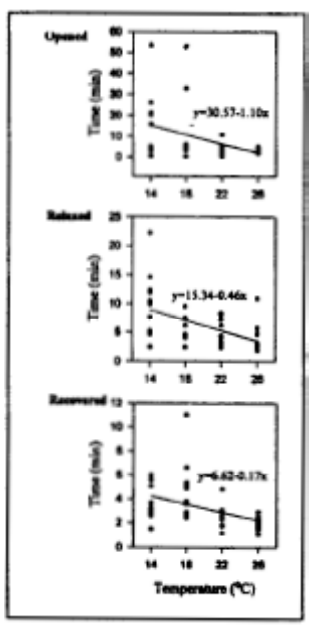

Fig. 2. The time taken for valve opening, relaxation and subsequent recovery of *P. albina* exposed to a 2 mL·L^{-1} propylene phenoxetol solution.

tendency for reduced recovery times with increasing water temperature, although this trend was significant only in the case of *P. albina* (Figs. 1 and 2).

Response times were again independent of shell height with the exception of time to relaxation of *P. imbricata* at 18ºC, where a significant positive correlation was found (Table 1).

Discussion

While the response of other molluscs to PP varies, there would appear to be a degree of uniformity in response among members of the genus *Pinctada*. In all species evaluated thus far, relaxation and recovery from the influence of PP are relatively rapid and without obvious negative consequences (Table 2). For all species tested, response times are unaffected by oyster size, but can vary according to water temperature (Norton et al. 1996, Mills et al. 1997, this study). In all cases, reductions in temperature can extend the time taken to relax and in most cases protract the time taken to recover.

Table 1. Correlation coefficients for oyster shell height and response times during and after exposure to propylene phenoxetol baths (2 $mL \cdot L^{-1}$) at one of four temperatures.

	Experiment 1	Experiment 2		Experiment 3							
Species	*P. albina*	*P. albina*	*P. imbricata*	*P. albina*				*P. imbricata*			
Size range	39-115 mm	49-83 mm	54-88 mm	46-115 mm				49-95 mm			
Temperature	18ºC	18ºC	18ºC	14ºC	18ºC	22ºC	26ºC	14ºC	18ºC	22ºC	26ºC
Time to open	-0.33	-0.34	-0.36	0.00	-0.34	-0.36	-0.49	-0.41	-0.35	-0.07	0.45
Time to relax	0.00	0.31	0.52	-0.28	0.64	-0.14	0.64	0.15	0.85*	0.02	0.66
Time to recover	-0.38	-0.14	-0.43	0.05	0.34	0.35	-0.24	0.36	0.04	-0.22	0.25

Values are Pearson product-moment correlation coefficients.
*Significant at P<0.01.

Table 2. Response of pearl oysters of the genus Pinctada to the relaxant propylene phenoxetol.

Pinctada species	Size	Water temperature ºC	Concentration ($mL \cdot L^{-1}$)	Time to relaxation (min)	Time to recovery (min)	Mortality	Author
P. margaritifera	—	—	2.5	10-20	10-15	0	Hildemann et al. (1974)
	90-170 mm	21-31	2.0-3.0	1-48	4-57	0	Norton et al. (1996)
	100-150 mm	—	2.0	—	—	18-22%*	Norton et al. (2000)
P. albina	70-100 mm	21-31	2.0-3.0	1-40	6-39	0	Norton et al. (1996)
P. maxima	120-2000 g	24-32	1.5-2.5	6-15	—	"negligible"	Mills et al. (1997)
	"large"	—	2.5	<15	—	—	Norton et al. (1996)
P. imbricata	49-95 mm (20-100 g)	14-26	2.0	2-15	1 - 5	0	This study
P. albina	39 -115 mm (20-120 g)	14 - 26	2.0	2-15	1 - 8	0	This study

— Data unavailable
* Mortality when anaesthetised in conjunction with nuclei insertion.

Stress in pearl oysters has provided both the impetus for relaxant studies and has been identified as a factor influencing the efficacy of PP induced relaxation (Mills et al. 1997). For this reason, we have tried to avoid the use of wedges as they are considered to be both stressful and could cause damage to the shell and mantle. Although no significant differences were found between the time taken to relax in *P. albina* with wedged valves and those introduced directly to the bath, relaxation times in the latter treatment were, on the average, faster (8.8 min versus 5.5 min, respectively). This is thought to reflect the PP solution entering the shell cavity, mostly through the byssal notch and initiating relaxation, prior to the shell first opening. Given that both oyster species will open in the presence of PP, we have found that the time, difficulty and potential stress and damage to experimental oysters associated with wedging is unwarranted.

In previous studies, response times for other pearl oysters have been independent of shell height and this was the case for *P. albina*. However, a significant correlation was found between time to relaxation at 18ºC and the shell height of *P. imbricata*. Rather than being indicative of any underlying trend, this result is thought to have arisen as product of the number of comparisons made (33) and to have occurred by chance alone (Type I error). Relaxation times for *P. imbricata* at other temperatures were not significantly correlated with shell height, nor were relaxation times for oysters held at 18ºC in Exp. 2. Notably, the oysters used in Exp. 2 were also representative of a larger size range and were therefore considered more likely to exhibit size-related effects if such an effect did exist.

Overall this study has reflected the findings of previous researchers, particularly those of Norton et al. (1996). Approximately 2.0 $mL \cdot L^{-1}$ PP is an effective relaxant for use with pearl oysters which induces rapid relaxation with short recovery periods and without subsequent mortality. However, while this study and that of Mills et al. (1997) found PP to be useful in the laboratory, particularly with reproductive studies, care should be taken with certain applications. One of the proposed uses of relaxants has been to reduce stress during nuclei insertion for pearl production (Norton et al., 1996; Mills et al., 1997). Recent observations with *P. margaritifera* have suggested that the use of PP in this regard is not without adverse effects on oyster survival, and pearl weights (Norton et al. 2000). While we have not reached the stage in our research where we can comment on the ultimate effects of the use of PP in operations, we do note that seeding technicians have commented that PP makes pearl insertion more difficult. In particular, technicians noted that the mantle of the oyster occasionally collapsed or retracted obstructing the body of the oyster and, second, that speculums would occasionally fall out of the relaxed oysters. These physical problems are not insurmountable, but warrant acknowledgment.

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