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Emersion Tolerance of Pearl Oyster, *Pinctada imbricata* Röding, Spat and Juveniles

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Abstract

Regular air exposure of spat and juvenile pearl oysters, *Pinctada imbricata*, during culture prompted an evaluation of their tolerance to emersion. Oysters were emersed under conditions chosen to simulate the harshest experienced in Port Stephens, New South Wales, Australia. Temperatures tested were in the range 12 to 36°C and fans were used to simulate the desiccative effects of winds. Spat (4.8 mm) and juvenile (12.3 mm) survivals were greatest in the range 16 to 24°C. At 20°C survival was size dependent, varying between 4 h for 4.8 mm spat to 30 h for 37 mm juveniles. Any additional stress that may be imposed by breaking the byssal attachment of the oysters prior to emersion had no significant effect on survival. In attempts to increase oyster tolerance to emersion, protection against desiccation was of particular importance and significantly increased tolerance. Protection of spat (4.8 mm) from airflow by placing them in plastic bags increased survival times three-fold. Tolerance was further increased if oysters were wrapped in damp paper toweling inside the bags, but the replacement of air with O_2 in bags did not significantly increase survival. With the practical application of these results, oysters (12 to 35 mm) are now routinely emersed and transported for up to 30 h without significant loss.

Introduction

The pearl oyster, *Pinctada imbricata* Röding, is among the most widespread of the Pteriid species and, in Australia, occurs from Shark Bay in the west, around the northern coastline and down the east coast as far south as northern Victoria (Hynd 1955). While *P. imbricata* has been used for pearl culture in Asia for decades, the species has only recently been the subject of commercial interest in Australian waters.

Although predominantly subtidal in nature, *P. imbricata* is occasionally subject to periods of emersion. In wild populations, oysters are emersed relatively briefly and infrequently by spring low tides (Hynd 1955), but in culture the frequency and duration of emersion can increase greatly because oysters are regularly taken from the water for procedures such as cleaning, grading and nuclei insertion. As optimum conditions for successive stages of pearl production commonly occur in different parts of particular estuaries or in different estuaries, extended emersion associated with transportation cannot be avoided.

Given the need for farmed *P. imbricata* to endure protracted exposure to air and the paucity of information regarding its impacts on any *Pinctada spp.*, this study was done to provide an indication of the tolerances of both *P. imbricata* spat and juveniles to emersion. In Port Stephens the harshest conditions are probably in summer when temperatures can rise to >35°C and hot dry winds from the west can persist for weeks. Under these circumstances, emersed oysters, particularly spat and small juveniles encounter high temperatures and an increased risk of desiccation; both factors which have been identified in reducing the viability of emersed bivalves (Davenport and Wong 1992; McMahon and Payne 1992; Maeda-Martinez et al. 2000).

To reflect conditions in New South Wales, oysters were emersed at temperatures in the range 12 to 36°C for periods of up to 60 h and emersion experiments were done under conditions that varied from those thought to be conducive to oyster survival, to those considered to be harsh. To account for desiccation, oysters were emersed in incubators in which fans were used to circulate the air.

Materials and Methods

All oysters used in this study were produced in the hatchery at Port Stephens Fisheries Centre and, in the case of juveniles, grown in nursery facilities located on Port Stephens. Prior to each experiment, oysters were brought to the hatchery and divided into groups of ten. Each group was placed on an individual mesh screen (90 mm diameter) and the screens were stacked so that spat were confined to the screen

on which they had been placed. Screen stacks containing the groups of oysters were then placed in a 200 L seawater bath at 24°C. An airlift pump was used to ensure water flow through the stacks (Fig. 1) and the oysters were allowed to acclimatise to their surroundings for a minimum of 24 h before experimental emersion. Oysters held in the 200 L bath were fed mixtures of Tahitian *Isochrysis aff. galbana*, P*avlova lutheri* and *Chaetoceros muelleri* ad libitim before and after experimental emersion.

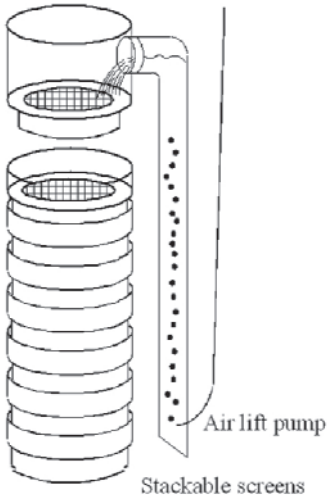


Fig. 1: Screen and stack arrangement used to hold spat and juveniles

Experiment 1 *The effect of duration of emersion and temperature on oyster survival*

Spat (4.8 ± 1.0 mm, dorso-ventral shell height ± SD) were emersed at one of seven temperatures {12, 16, 20, 24, 28, 32 or 36°C (± 0.5°C)} in temperature-controlled, incubator cabinets with fan forced airflows to promote even temperature. In each case, six screens were placed in an incubator. At intervals of 2 h over a 12 h period, one screen was removed from the incubator and returned to the 200 L seawater bath. A seventh screen of spat remained in the seawater bath as a control. The procedure was repeated three times with new spat on each occasion so that three replicate results were recorded for each time and temperature combination. Due to the limited number of incubators available, only two emersion series could be done at one time, thus treatments (temperature) were randomised in order and with respect to incubator. Two days after emersion, each replicate was removed from the water bath and each screen was inspected with the aid of a binocular microscope to determine the survival of spat.

The previously described experimental procedure was repeated using juveniles (12.3 ± 2.1 mm, mean ± SD) with the exception that additional replicate screens were used on each occasion so that emersion times could be extended to 20 h.

Experiment 2 *Extending emersion tolerance*

Seventy-two groups of *P. imbricata* (n = 5) were divided among two temperature controlled incubators set at 20°C - the temperature found to be most conducive to oyster survival in Experiment 1. The 36 groups in each incubator were then divided equally among four treatments. Nine groups were held in air filled plastic bags so that the oysters were protected from the fan forced airflows. Nine were

placed in plastic bags filled with medical grade O_2 to increase partial pressures for O_2 diffusion across the gill surface. Nine groups were wrapped in damp paper toweling to keep the air moist and placed in air filled bags and the remaining nine groups remained on mesh screens (as in Experiment 1). After 12 h, one group of oysters from each treatment was removed from each incubator and returned to a seawater bath to recover. Every 6 h thereafter, up until 60 h, an additional group of oysters from each treatment was removed and returned to seawater. An additional two groups of oysters remained on screens in the seawater baths as controls.

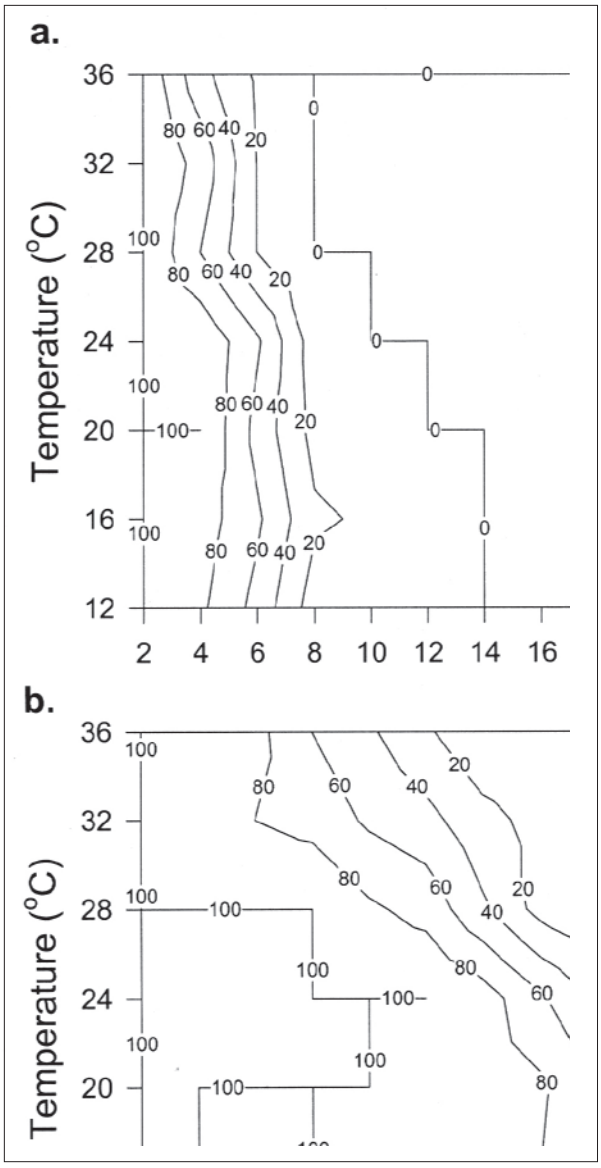
The experimental procedure was repeated on three occasions using either spat (4.8 ± 1.0 mm; x ± SD) or juveniles of one of two size classes (15.5 ± 2.6; 36.7 ± 1.8 mm; x ± SD). For juveniles the number of groups initially placed in each incubator was reduced to 28 because the minimum time for emersion was increased to 24 h.

Experiment 3 *Byssal attachment and emersion tolerance*

In contrast to Experiment 1, spat used to investigate effects of protection from desiccation in Experiment 2 required detachment from the screens on which they had been acclimated. This raised concerns that the additional stress imposed by breaking the byssus to remove the oysters from the screens could affect emersion tolerance. This possibility was investigated by emersing both byssally attached and detached juvenile oysters (25.1 ± 2.7 mm; x ± SD) for periods of either 24, 30, 36, 42, 48 or 54 h at a temperature of 20°C. Three replicate screens of juveniles for both treatments (attached or detached) were emersed for each time period. Another three screens of attached oysters and three screens of detached oysters remained in the seawater bath as controls.

Results

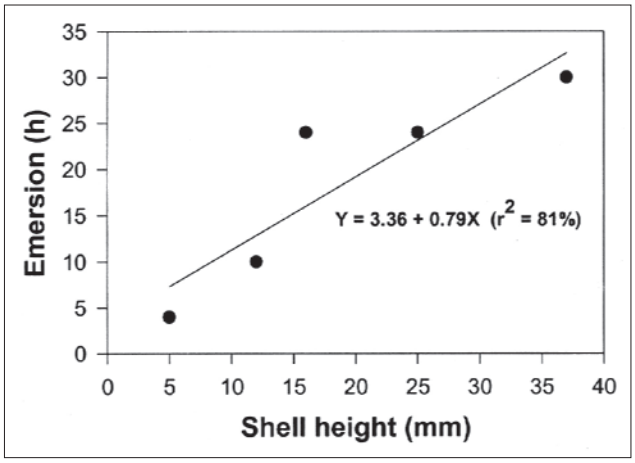
While no mortality occurred among oyster spat or juveniles held in non-emersed control screens in any of the trials conducted, emersion tolerance of *P. imbricata* was affected by temperature, oyster size and the conditions under which the oysters were held. In Experiment 1, the survival of both spat and small juveniles was greatest in the range 16 to 24°C and poorest at the highest temperature tested, 36°C (Fig 2). Spat (4.8 mm DVH) were capable of surviving up to 4 h in the incubator at 20°C without mortality, while 12 mm juveniles could survive 10 h at the same temperature. This trend for increasing tolerance to emersion continued in Experiments 2 and 3, where 16 mm, 25 mm and 37 mm juveniles survived 24 h, 24 h and 30 h, respectively, at 20°C without mortality (Fig. 3).



<< Fig. 2: The effect of temperature and duration of emersion on survival of *Pinctada imbricata* a) spat (4.8 ± 1.0 mm) and b) juveniles (12.3 ± 2.1 mm). Isopleths indicate the number of live oysters expressed as a percentage of the initial number emersed.

The effects of treatments to increase emersion tolerance, data from Experiment 2, were compared using three-way-Anova (Sokal and Rohlf 1981). To permit a balanced design, survival data for spat prior to 24 h were not included in the analysis. Emersion treatment differences were evaluated using the Student-Newman-Kuels procedure (Winer et al. 1991). Oyster tolerance of emersion increased significantly as the size of the oysters increased and as the time of emersion decreased ($F = 345.02$; $df\ 2/84$; $P < 0.001$ and $F = 146.28$; $df\ 6/84$; $P < 0.001$, respectively; Fig. 4). The treatment used during emersion also significantly affected survival ($F = 75.72$; df 3/84; $P < 0.001$). Oysters protected from airflow with plastic bags showed significant improvements in emersion tolerance (SNK, $P < 0.05$), with those wrapped in moist toweling inside the bags showing significantly greater tolerance than any other treatment (SNK, $P < 0.05$). The use of oxygen to fill the bags did not significantly improve survival (SNK, $P > 0.05$).

Multivariate analysis of the effects of the duration of emersion on



<< Fig. 3: Emersion tolerance of *Pinctada imbricata* spat and juveniles as a function of size. Points represent the maximum duration of emersion without mortality at 20°C.

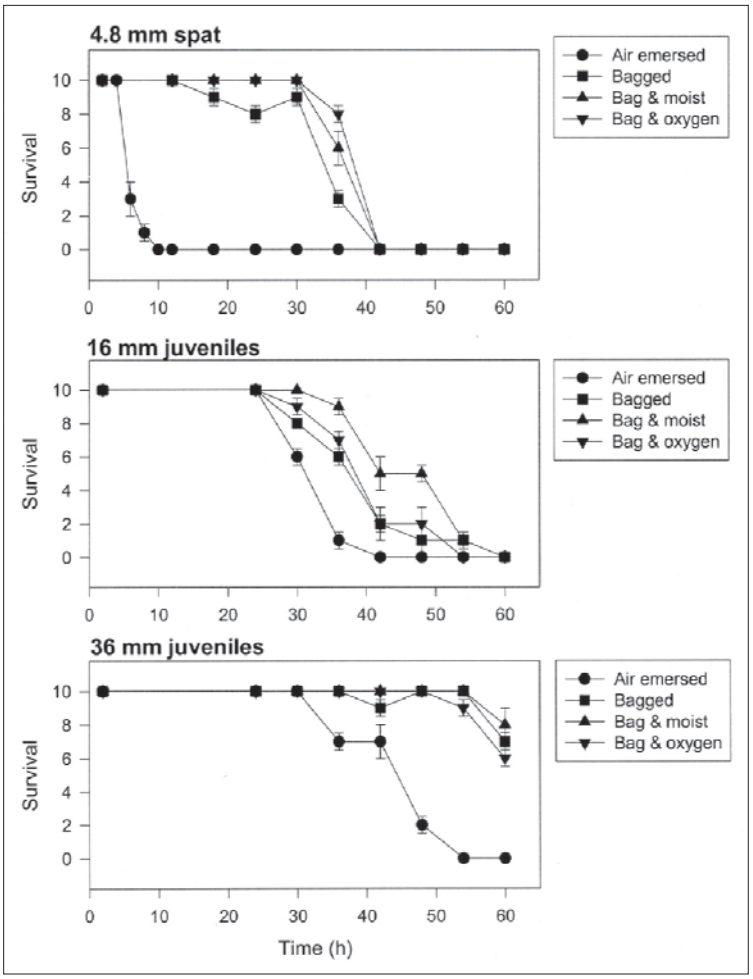


Fig. 4: A comparison of survival of three size classes of *Pinctada imbricata* emersed at 20°C using one of four treatments: 1) emersed and exposed to airflow; 2) emersed in a protective plastic bag; 3) emersed wrapped in moist paper in a plastic bag, 3) emersed an oxygen filled plastic bag. Survival data for 4.8 mm spat emersed and exposed to airflow has been drawn from Experiment 1. Values are the mean number of oysters surviving per replicate ± SE.

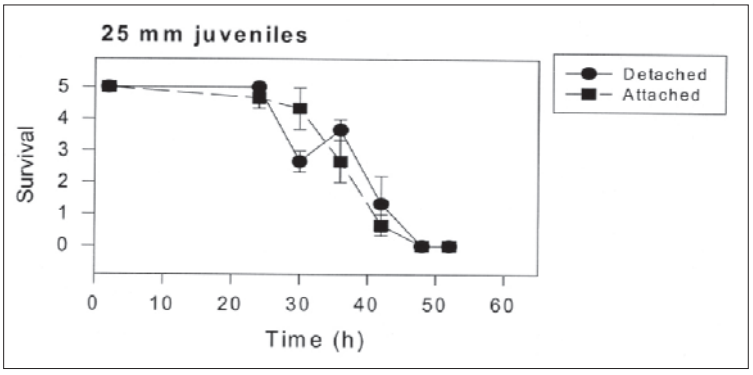


Fig. 5: Emersion tolerance of byssally attached and detached *Pinctada imbricata* juveniles (25 mm shell height) at 20°C. Values are the mean number of oysters surviving per replicate ± SE.

attached and detached 25 mm juveniles found survival decreased significantly with time (F = 37.45; df 5/29; $P < 0.001$; Fig. 5), however severing the byssal attachment prior to emersion did not significantly affect survival (F = 0.04; *df* 1/29; $P > 0.05$).

Discussion

Despite occurring almost exclusively in subtidal areas, *P. imbricata*, are tolerant of emersion, at least to the extent that extended handling and transport without seawater is possible. By the time *P. imbricata* reach 16 mm in size, they can be emersed for 24 h, even under conditions designed to simulate among the harshest likely to be encountered in Port Stephens. But, emersion in incubators in which fans were used to circulate the air has meant that the tolerance of the various sized oysters used was frequently less than we had expected from our experiences in the field. Regardless, this study was thought to provide useful baseline data for safe emersion under most circumstances.

Despite the severity of the conditions, responses of *P. imbricata* were similar to those of other bivalves in several respects. Initially, *P. imbricata* commonly responded to emersion by gaping as has been observed with bivalves such as oysters and scallops (Brand and Roberts 1973; Littlewood and Young 1994, Maeda-Martinez et al. 2000) and has been reported in other Pteriid oysters (Hancock 1973). Second, *P. imbricata* emersion tolerance was influenced by temperature, with the mortality rates increasing at elevated temperatures (Davenport and Wong 1992; McMahon and Payne 1992). Third, like emersed clams, *Tapes philippinarum* (Richardson 1988), *P. imbricata* that survive prolonged emersion show a distinct layer in the shell that has been reported by farmers to be typical of the response to stressful events. Finally, as reported for the hard clam *Mercenaria mercenaria* and the scallop, *Argopecten irradians* (Rhodes and Manzi 1988), the size of *P. imbricata* significantly affected their tolerance to emersion. Tolerance increased from 4 h for 4.8 mm spat to 30 h for 37 mm juveniles (Fig. 3) without signs of a significant plateau in survival. This suggests that larger oysters (> 37 mm) may be capable of withstanding even greater periods of emersion.

As noted earlier, the risk of desiccation in these trials was likely to be greater than generally experienced in Port Stephens. It is therefore not surprising to find that treatments that reduced the risk of desiccation greatly increased emersion tolerance. Simply protecting oysters from airflow was particularly effective in this respect, trebling the tolerance of spat. Smaller, but significant improvements in tolerance were achieved by wrapping spat in moist substrate within the bag, which was thought to assist in the maintenance of humidity and thus prevent desiccation.

The use of an O_2 rich environment is common during the transport of aquatic organisms and has been found to be useful with some subtidal bivalves (Maeda-Martinez et al. 2000) by increasing partial pressures for diffusion across the gill surfaces. However, this was not observed in this study because the inclusion of O_2 failed to infer any advantage. The reasons for this are unclear, although it may serve to further reinforce the importance of preventing desiccation. Any advantage that O_2 might provide may be ameliorated by the fact that when it was supplied in bottled form it is largely moistureless (<20 ppm, John Manfred, Air Liquide, pers. comm.) and may have exacerbated desiccation. Alternatively, the amount and diffusion rate oxygen in air simply may not have been a limiting factor under the experiment conditions applied. Further trials could be done to test if there is some synergistic advantage in the use of a moist, oxygen enriched environment.

In practice we have combined techniques to enhance survival of juvenile *P. imbricata* during transport. Oysters have been placed upon several layers of wet absorbent paper in a plastic bag that is subsequently filled with oxygen and sealed with elastic bands. O2 has been used, as the use of wet paper is considered sufficient to offset any initial reduction in humidity. Oyster numbers are such that they occupy no

more 30% of the total volume of the bag and are in a layer two to three oysters deep. The bag is placed in a polystyrene box for transport and if temperatures are likely to increase beyond 20°C, a frozen block is taped inside the lid of the polystyrene container. Under these conditions, juvenile oysters (12 to 35 mm) have been emersed during transport for up to 30 h without significant mortality.

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