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Evaluation of fast green uptake as a simple fitness test for spat of *Perna canaliculus* (Gmelin, 1791)

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Abstract

The method described here gauges marine mussel (*Perna canaliculus*) spat health by observing their declining ability to isolate themselves from hyposmotic water after exposure to apparently deleterious agents such as exposure to air, elevated temperature, and ethanol. This inward movement of water is disclosed by Fast Green stain. Although individual spat were found to stain either markedly or not at all, test groups showed variation in proportions staining with different treatments thus giving an indication of group ($n=50$) fitness. Control and lethally stressed spat groups showed low and high staining proportions, respectively, that corresponded with group differences ($P<0.001$) in physical activity and valve closure in freshwater. Furthermore, staining and activity levels in a range of spat samples from controls to lethal exposures show a highly significant correlation ($r=-0.967$, $P<0.001$). Thus staining is a good surrogate for activity as an indicator of group health. The advantage of staining over activity assessment is its ease and brevity. A range of conditions caused by exposure to air, ethanol and nutrient loaded (hypoxic) water were detectable ($P<0.05$): normal health, sublethal and lethal conditions were statistically distinguishable. Such a test may have application in the mussel industry as currently there is no quick means of testing spat viability. This is needed because spat at this stage are commonly distributed from nursery or wild settlement site to grow-out locations; different handling and transport regimes may impact on spat viability. Successful grading and ensuing pricing based on viability will encourage best practice in maximizing spat quality thereby extending a currently limited spat supply.

Keywords: Lethal; Mussel; *Perna canaliculus*; Spat; Stain; Stress test; Sublethal

1. Introduction

Bivalve spat are an important intermediate product in aquaculture. At this stage they are commonly distributed from nursery or settlement sites to grow-out locations (Laing and Spencer, 1997; Guo et al., 1999; Jeffs et al., 1999). The unavoidable handling, with attendant stress, at this time may result in discrepancy between counted spat and those that are actually viable. This will impact on profitability, thus a method to assess spat viability is clearly desirable.

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Spat of the Greenshell™ mussel (*Perna canaliculus*) were chosen for experimentation as this mussel is a major aquaculture species in New Zealand (Jeffs et al., 1999) and hatchery grown spat are regularly available for experimental work. A further reason for the use of *P. canaliculus* is to investigate causes of spat losses: only about 2% of spat applied to grow-out ropes remain and grow to harvestable size. Critical events for these losses appear to be associated with collection and post collection handling regimes. Spat wash ashore attached to seaweed; once on the beach, overheating and desiccation may take their toll if collection is not prompt. In addition, transport takes up to 6 h from nearby sites in Golden Bay to the Marlborough Sounds; from Kaitaia in the north it may take 24 h to 36 h.

It is clear that a test for spat viability (a "stress" test) would be useful to help identify events and conditions in the transport process that have the greatest effect on spat mortality. For such an application, a simple, easily executed and inexpensive test that yields a meaningful comparative index of spat condition is needed.

There is a plethora of technology-intensive procedures represented as molluscan stress tests (see Gosling, 1992; Webb, 1999, 2001 for reviews). Most are complicated and time consuming; few appear to be appropriate for rapidly testing mussel spat. Brunner (2003) evaluated a test based on adhesion of *P. canaliculus* spat by foot, mucus or byssus. This test proved insensitive and suffered from excessive variation in control values. For larger mussels, Harding et al. (2002) assessed the retention of neutral red stain in *Mytilus* hemocyte lysosomes as a stress indicator. Application of this test for spat would founder on devising a method to extract hemolymph from such small individuals and the time required to process a suitable sample of spat. Maguire et al. (1999) determined that behavioural performances in righting and recession were accurate indicators of quality in juvenile scallops (*Pecten maximus*). Recession performance, in particular, could be used distinguish different quality levels in long-term stress such as density and short-term stress such as desiccation stress. These behavioural tests, though they are simple and easy to perform, are not applicable to mussel spat, which exhibit a different and far less dynamic activity repertoire. Selin (1999) studied the effects of environment and physiology on burrowing in the clam *Ruditapes philippinarum*. The clams became fatigued from repeated efforts to burrow. Up to about cycle 24 the clams took about 500–700 s to complete the burrowing. From cycle 25 to 30 the time increased to about 2500 s, as did variability. This again is not applicable to spat but these last two works exploited measurement of a fitness enhancing activity. To apply this concept it is necessary to find some measurable critical performance parameter in spat.

The stain test described here assesses shell sealing efficiency. It exploits the propensity of marine bivalves to isolate themselves from hyposmotic water. Such isolation is a prerequisite for survival as death through osmotic shock will ensue rapidly if isolation is incomplete. Thus the closure function might be expected to approach the highest efficiency achievable in very healthy spat and any degradation in this function might thus be attributable to compromised health. Deficiency in the isolation function is marked by the ingress of hyposmotic water; this can be tracked by the addition of a suitable vital stain to the test water.

The use of a stain was prompted by the work of Murray and Bowser (2000) who used staining methods for distinguishing living and dead foraminiferans. Vital stains are widely used in fields as diverse as ecology (Fleming and Coughlan, 1978), ophthalmology (McEnerney and Peyman, 1978) and parasitology (Holliman, 1961).

2. Materials and methods

The experiments were performed at the Glenhaven Aquaculture Centre near Nelson, on the north coast of South Island, New Zealand (41°11′30″ S, 173°21′20″ E) who also supplied the hatchery produced spat.

2.1. Spat activity

This performance parameter indicates life in individuals and levels of health in groups of spat. It can be inferred that non-performing spat are unhealthy spat and, by implication, samples with a high proportion of non-performers are unhealthy samples.