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Research Article

It is all in the looks: a rapid field-based visual assessment tool for evaluating the spawning likelihood of the Asian green mussel, *Perna viridis* (Linnaeus, 1758)

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Abstract

Numerous interceptions of *Perna viridis* on vessels entering Western Australian waters prompted the development of a rapid field-based assessment technique for determining reproductive status and hence spawning likelihood of *P. viridis*. The visual assessment tool and spawning likelihood matrix were developed using correlations between laboratory-based assessments of *P. viridis* size, colour and egg size in combination with field-based validations from mussels collected on vessels in Western Australian waters. The spawning likelihood matrix provides an immediate indicator of whether the mussel is low, medium or high likelihood of spawning. Mussels were recorded initiating gonad tissue development from approximately 6.5 mm in length, with the mean size of mature animals 59.6 mm. There was a positive correlation between mussel size and stage of reproductive development. Gonad colour, however, appeared to be a more accurate indicator of gonad maturity than mussel size. Female mussels showed a decrease in gonad colour intensity following spawning. Mussels that scored 1 for colour (potential score 1–3) generally had a low proportion of mature eggs (< 70 % mature eggs). Over 60% of the mussels with a colour score of 2 contained 70–100% mature eggs, indicating the capacity for further spawning. Mussels were assigned an overall spawning likelihood score (through the spawning likelihood matrix) based on the proportion of the visceral mass occupied by gonad tissues (% gonad cover, value from 1–3) and overall colour of gonads (value from 1–3). The spawning likelihood score was significantly related to the percentage of mature eggs present, and hence the spawning potential of the mussel. The matrix provides an immediate indicator of the risk of spawning posed by the sample. As such, it is expected that application of the matrix *in situ* would enable the potential likelihood posed by *P. viridis* translocated on vessels to be determined quickly and efficiently.

Key words: spawning risk, biosecurity, rapid technique, reproductive condition, gonad colour

Introduction

Ocean-going vessels are a leading cause of species introductions world-wide (Bax et al. 2003; Godwin 2003; Hewitt and Campbell 2008). Vessels may translocate marine species either as planktonic larvae in ballast water, or as biofouling organisms attached to hulls, other submerged structures and internal seawater systems (review in Hewitt et al. 2009). Some of these translocated organisms survive the voyage and establish to form reproductive populations in regions where they did not previously occur. Increasing the frequency with which a species is introduced to an area (propagule pressure) enhances the likelihood of the species becoming established (Kolar and Lodge 2001; Lockwood et al. 2009). The ensuing success or otherwise of these introduced species depends upon their interaction with both locally available resources and the resident indigenous species (Shea and Chesson 2002). Introduced species

that are able to survive and proliferate in their new habitat have the potential to seriously impact upon local biodiversity and ecosystem function, as well as have an economic impact on fisheries, aquaculture and tourism (e.g. Pimentel et al. 2000; Williams and Grosholz 2008).

The biosecurity response to an introduced species—which may include exclusion, control or potential eradication—will depend upon the introduced marine species (IMS), the pathway, the surrounding environment, as well as economic and political factors (Kaiser and Burnett 2010; Simberloff et al. 2013). The most effective and cost-efficient way to manage introduction of IMS is to prevent their initial introduction through vector regulation (Floerl et al. 2005; Hulme et al. 2008; Briski et al. 2012). In the case of vessels, while there has been progress at an international level towards regulating ballast water discharge (IMO 2017), there has been little towards developing a system for global biofouling compliance, resulting in individual countries developing their own regulatory systems. Australia, along with other locations such as New Zealand and California, have or are developing their own biofouling regulation (Fisheries Western Australia 2017; MPI 2017).

Failure to stop the initial introduction of a IMS requires action further along the invasion timeline in order to prevent the species becoming established. Biosecurity measures must include early detection of IMS, and fast and efficient biosecurity response procedures (Coutts and Forrest 2007; Kaiser and Burnett 2010; Simberloff et al. 2013). Given the potential environmental and economic costs associated with introduction of a marine species (e.g. Bax et al. 2003; Galil 2007; Williams and Grosholz 2008), agencies responsible for biosecurity must ensure that decisions made regarding an IMS are the most appropriate for the situation. Fast and efficient action following a biosecurity incident is key (Kaiser and Burnett 2010; Simberloff et al. 2013). As such, agencies must rely on gathering the most comprehensive information as quickly as possible. Consequently, having a rapid means of verifying the identification of a species, the size of the cohort (or cohorts), the IMS' reproductive maturity and hence potential spawning risk, will make a substantial impact on the response speed to a biosecurity incident.

Perna viridis Linnaeus, 1758, the Asian green mussel, is well-recognised outside its native range as a fecund and highly invasive species (Rajagopal et al. 2006). *P. viridis* is a sessile filter-feeding bivalve native to the tropical western Pacific region, with high ecological significance and aquaculture value in South East Asia. The characteristics of fast growth rate, high fecundity, wide tolerances to temperature, salinity, and pollutants all contribute to the success of *P. viridis* as an aquaculture species. These same characteristics, however, also contribute to *P. viridis* being a highly successful invasive species outside its native region. The likelihood of spawning is dependent upon a range of environmental conditions, cooler, low-nutrient waters are less conducive to reproductive development, compared with warm nutrient rich-waters.

After managing numerous interceptions of *P. viridis* on vessels entering Western Australian (WA) waters, the authors identified that strategies for identifying reproductive characteristics were needed to aid in quantifying the potential biosecurity risk posed by this species. These included identifying size to reproductive potential ratios and use of reproductive stage colour markers. Histological methods for determining reproductive condition have been discussed by several authors (*Perna canaliculus* Gmelin, 1791: Buchanan 2001; review in Rajagopal et al. 2006). While histological methods provide quantitative information of the reproductive status of an organism, they are slow and time consuming. There is a need for a rapid field-based assessment method to determine reproductive status of *P. viridis* during vessel inspections and in an early biosecurity response and management contexts. Given the high biosecurity risk posed by *P. viridis*, any method that enables a faster response to potential incursions will be valuable to management authorities in helping to prevent establishment of this species.

This aim of this paper is to provide a rapid assessment technique for determining reproductive status of *Perna viridis* in the field. Here we correlate laboratory-based assessments of *P. viridis* size, colour and egg size to develop a visual assessment of mussel reproductive condition, comparable to the method developed by Buchanan (2001) for *Perna canaliculus* in aquaculture facilities. We then relate measurements of mussel size and colour made in the laboratory to field observations from mussels collected on vessels in Western Australian waters. Using the data, a rapid visual assessment tool and set of simple criteria was developed for use under field conditions that can be used to determine the spawning likelihood of *P. viridis*, particularly in areas where it has the potential to become an invasive species.

Methods

Developing a mussel assessment system that could utilise frozen or fresh samples was necessary because in Western Australia samples arrive for assessment in varied condition (fresh or frozen) due to the often-vast distances samples must travel across WA. As such, live fresh samples were dissected, or animals

Table 1. Assignment of reproductive stage of *Perna viridis* based on observations of mantle and gonad conditions.

Mantle and gonad condition	Assessment of Reproductive Stage
Mantle is transparent, no sign of gonadal tissue	Stage 0: Not developed
Mantle is mostly transparent but white/cream/light yellow 'webbing' pattern of developing gonadal tissue clearly visible – cannot always clearly determine sex	Stage 1: Early stage of gonad development
Mantle is now semi-transparent with gonadal tissue; gonadal tissue becoming thicker. Females can now be clearly identified by their light orange gonadal tissue; males white/light cream gonadal tissue	Stage 2: Developing gonads becoming evident
Mantle is opaque with gonadal tissues, gonadal tissue is thick, may appear granular looking. Fully mature females readily identified by dark orange to brick red gonadal tissue. Males cream to dark cream colour.	Stage 3: Reproductively mature gonads present

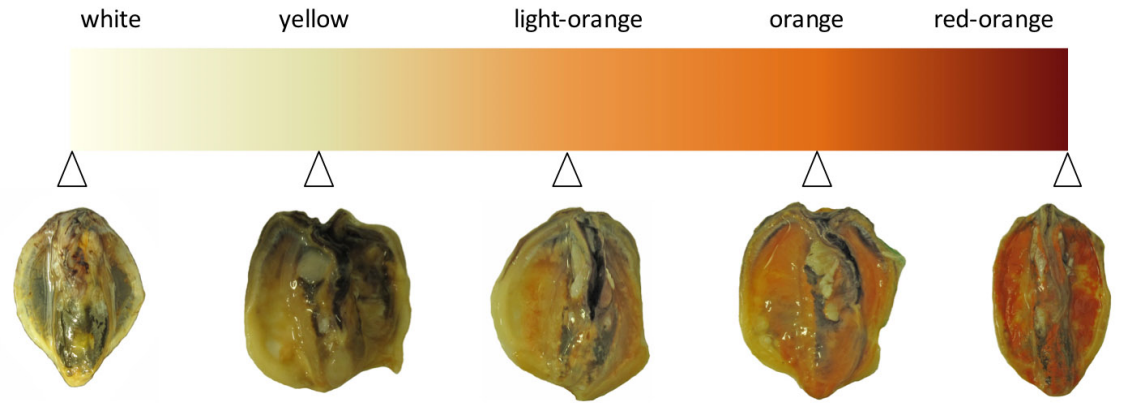

Figure 1. Visual guide for determining colour of *P. viridis* female gonads. RGB colour scores have been included for correct colour interpretation of each gonad colour stage: white (RGB: 211, 204, 123), yellow (RGB: 166, 151, 58), light orange (RGB: 136, 101, 19), orange (RGB: 138, 73, 5), red-orange (RGB: 94, 27, 8). Photographs by Serina Lee.

were frozen on the day of collection and assessed at a later date, allowing mussels to be assessed under varied states of preservation.

Assessment of mature males required checking for sperm motility. Sperm, however, lose motility after freezing or preservation. Consequently, while the visual staging guide for males can provide an indicator of reproductive development, the authors could not reliably produce a visual-histological correlation. As such, only females were used for our assessment.

Relationship between Perna viridis *size and stage of reproductive development*

A range of *P. viridis* were collected regularly from fish farms in East and West Johor Straits of Singapore for examination of reproductive condition (150 from each location). From each batch of mussels, 50 individuals of each of the 3 class sizes (< 10 mm, 10–20 mm and > 20 mm) were selected for dissection. This allowed for any differences in reproductive development with size to be determined. After the length of each mussel was recorded, the mussel was then dissected and scored for stage of reproductive development using the entire internal body of the mussel (gonad tissue development) under the stereoscope following the descriptors in Table 1. Visceral mass was visually scored and photographed. Visual assessment of the condition of female gonads was based on two criteria: 1) proportion of the visceral mass (area of mussel containing the internal organs) occupied by gonad tissues (% gonad cover – assigned a value from 1–3) (based on Table 2), and 2) overall colour of gonads using the colours described in Figure 1 (assigned a value from 1–3) (based on Table 2). This was done rapidly by eye upon opening the mussel, with estimates of percent cover of the

Table 2. Scoring criteria for visual assessment of the condition of *Perna viridis* female gonads. "% Gonad Cover" indicates the proportion of the visceral mass occupied by gonads.

% Gonad Cover	Cover Score	Gonad Colour	Colour Score
< 25%	1	White, Yellow, Light Orange	1
25–50%	2	Orange	2
> 50%	3	Reddish Orange	3

gonad tissue based on general principles of proportional area estimates (for example, see methods in McDonald et al. 2006). In total, 300 mussels were assessed. Correlation analysis were conducted to determine if there was a relationship between *P. viridis* size and stage of reproductive development.

Visual assessment of Perna viridis *reproduction: relationship between gonad colour and egg maturity*

Walter (1982) reported that female gonads showed appreciable colour changes with maturity (light orange to reddish orange). Size of eggs also changes with maturity. As a result, it should be possible to identify a mature female ready to spawn by assessing gonad colour and egg size. To determine if there was a relationship between gonad colour and maturity of the gonad tissue, individual mussels collected from East and West Johor Straits of Singapore were firstly visually assigned a colour score from 1 to 3 depending upon gonad colour, using the schema in Table 2. A 5 × 5 mm piece of gonad tissue was then excised from the coloured area of the gonad from each mussel. The isolated gonad tissue was macerated and suspended in 3 ml of 30 ppt, 0.2 µm filtered seawater to release the eggs. A calibrated inverted microscope was used to assess the first 100 eggs encountered within a Sedgwick rafter. Only one count was taken from each piece of tissue as preliminary studies showed very low variation (≤ 5%) between multiple counts.

Only eggs that were intact, covered with a layer of membrane, pigmented (light orange or yellowish) and at least 10 µm were scored so as to avoid counting cell debris or artefacts. Freshly spawned eggs swell in seawater to take on a spherical shape, with mature eggs measuring 45–50 µm wide (Tan 1975; Rajagopal et al. 2006). When assessing egg maturation, eggs ≥ 40 µm were scored as mature, while eggs between 10 µm to < 40 µm were scored as immature. Eggs were scored as mature at 40 µm rather than 45 µm, as used by Tan (1975) and Rajagopal et al. (2006), to function as a conservative cut off and to compensate for any potential shrinkage during freezing.

To determine if there were differences in gonad colouration pre- and immediately post-spawning, the above procedures were conducted for spawned females and randomly collected females. Spawning was induced for the spawned females using thermal shock, whereby the aquaria water in which the mussels were maintained in the laboratory was cooled to 22–25 °C (following the protocol in Rajagopal et al. 2006). Correlation analyses were conducted to determine if there was a relationship between the colour of gonads and proportion of mature eggs.

Table 3. *Perna viridis* reproductive stage, based on description of gonad development and colour from Table 1, and associated size range (mm)

Reproductive stage	Size range (mm) Min–max
Stage 0: No observable differentiation in tissue	3–30
Stage 1: white-yellowish tinge to tissue	6.5–46
Stage 2: light orange (female); light cream (male)	15–89
Stage 3: red orange (female); dark cream (male)	34–96

Visual assessment of Perna viridis *reproduction: spawning likelihood score*

Results from statistical analyses were used to test the validity of the scoring criteria for *P. viridis*. Using results from above, the gonad cover score was multiplied by the gonad colour score to arrive at a spawning likelihood score. From this, a *P. viridis* spawning likelihood assessment matrix was constructed.

Comparison of Perna viridis *collected in Singapore with* Perna viridis *collected from vessels entering Western Australian waters*

To verify if the results obtained from *P. viridis* collected in Singapore were comparable with those collected from vessels that had entered into WA waters, samples were collected and assessed using the criteria in Table 1. Samples removed from the vessel were examined by the lead author *in-situ* to determine any reproductive development and relate these back to mussel size. In total 562 mussels were examined from various vessels. The length of each mussel was measured. As above, each mussel was then assigned a score from 1–3 based on the proportion of visceral mass covered by gonads (% gonad cover score) and a score from 1–3 based on the colour of the gonads (gonad colour score) (see Table 2). Spawning likelihood scores were then calculated by multiplying the gonad colour score by the gonad cover score to determine if the mussels were low, medium or high likelihood of spawning.

Results

Relationship between Perna viridis *size and stage of reproductive development*

Mussels were recorded initiating gonad tissue development from approximately 6.5 mm in length (Stage 1) under optimal conditions for mussels in their native range in Johor Straits of Singapore (Table 3). At this stage, however, sex of the mussels could not be determined. It was not until mussels reached approximately 15 mm that sexual differentiation was possible, though these animals were far

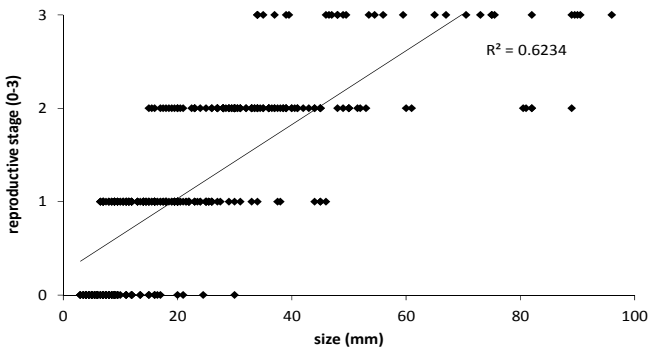

Figure 2. Relationship between mussel size and stage of reproductive development (based on stages of gonad condition described in Table 1) (Stage 0 n = 109; Stage 1 n = 152; Stage 2 n = 108; Stage 3 n = 31).

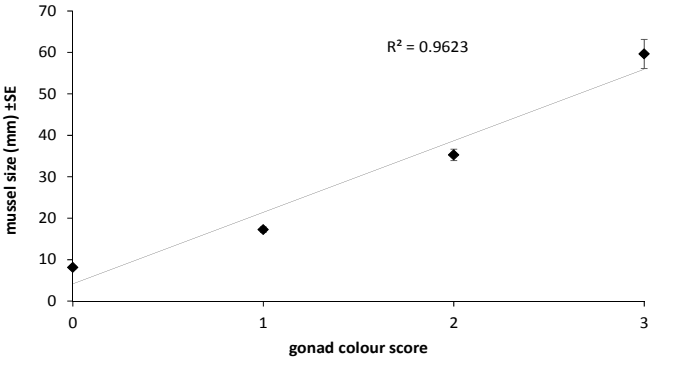

Figure 3. Relationship between mean *Perna viridis* size (mm) (± SE) and evidence of reproductive development based on gonad colour score (using reproductive stage schema from Table 2) (Score 0 n = 109; Score 1 n = 152; Score 2 n = 108; Score 3 n = 31).

from mature. The smallest sized mussel showing evidence of complete reproductive maturity (based upon gonad cover and colour, and containing mature eggs) was 34 mm. Mean size of mature animals was almost 60 mm long (59.6 mm).

The smallest clearly identifiable female specimen was 30 mm, with thin patches of very light orange gonad tissue observable (Stage 2). At 33 mm, sperm development was recorded in animals (observable only under microscope). Visually these animals were classed as Stage 2 with cream/white gonads developing (Table 3).

There was a positive correlation between mussel size and stage of reproductive development ($R^2 = 0.62$) (Figure 2), with a high degree of variability within each of the reproductive stages associated with size (Table 3, Figure 2). Overall there was a strong positive correlation between mussel size and the gonad colour score despite the high degree of variability in mussel size within each reproductive stage ($R^2 = 0.96$) (Figure 3).

Visual assessment of Perna viridis *reproduction: relationship between gonad colour and egg maturity*

The was a significant relationship between the mean proportion of mature eggs and the gonad colour score ($R^2 = 0.96$) (Figure 4). The proportion of mature eggs increased as the colour of the gonads changed from white, to yellow, then to orange and red (colour scores 1–3) (Figure 1, Tables 1 and 2).

Overall, female mussels showed a decrease in gonad colour intensity following spawning. However, over 60% of spawned females with a colour score of 2 contained 70–100% mature eggs (Table 4), indicating the potential for further spawning. Mussels that scored 1 for colour had a lower proportion of mature eggs (17/22 contained fewer than 30% mature eggs) (Table 4).

To determine if results for spawned females differed from randomly selected females, a random pool of females was collected, and the colouration and gonad condition scoring system was applied to them. Of these random females, the mean proportion of mature eggs for colouration scores of 1 and 2 was generally similar to that obtained for spawned females (Table 4). For colour score 1, over three-quarter of mussels contained < 30% mature eggs, and for colour score 2, over half individuals contained 70%–100% mature eggs (Table 4). More than three-quarters of mussels with a colour score of 3 contained a high proportion (70–100%) of mature eggs. The only differentiation between spawned and random females

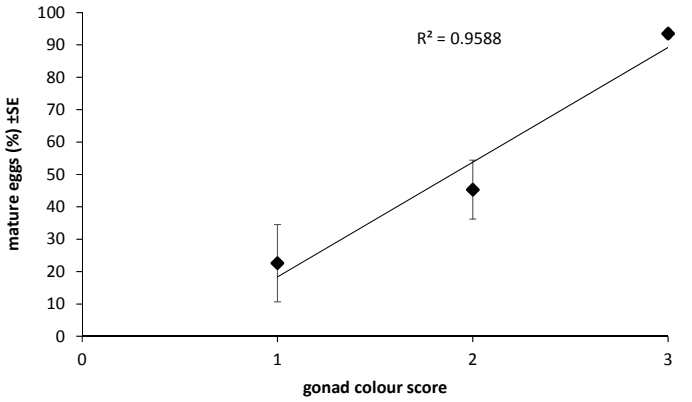

Figure 4. The relationship between mean proportion of *Perna viridis* mature eggs (%) (± SE) in the gonads and the gonad colour score (using the reproductive stage schema from Table 2) (Score 1 n = 9; Score 2 n = 19; Score 3 n = 11).

Table 4. Comparison of number of mature eggs present in random (randomly collected) and spawned mussels (numbers in parentheses indicate the percentage of mussels in that egg category from the total mussels for that particular gonad colour).

Mussel type	Gonad colour	Number of mussels	Too few or no eggs	< 30% mature eggs	30–69% mature eggs	70–100% mature eggs
Spawned females	3	3	0 (0%)	0 (0%)	1 (33%)	2 (66%)
	2	50	2 (4%)	8 (16%)	8 (16%)	32 (64%)
	1	22	8 (36%)	9 (41%)	5 (23%)	0 (0%)
Total females		75				
Random females	3	72	0 (0%)	0 (0%)	13 (18%)	59 (82%)
	2	50	2 (4%)	14 (28%)	8 (16%)	26 (52%)
	1	54	11 (20%)	30 (56%)	2 (4%)	11 (20%)
Total females		176				

was for those random mussels that were assigned score 1: 20% of score 1 random mussels contained 70–100% of mature eggs compared to 0% for spawned females (Table 4).

Visual assessment of Perna viridis *reproduction: the spawning likelihood score*

The spawning likelihood score (i.e. the gonad colour score multiplied by the percentage gonad cover score) was significantly related to the percentage of mature eggs present (R^2 = 0.96) (Figure 5). There was no significant relationship between mussel size and spawning likelihood score (R^2 = 0.13), or between mussel size and the number of mature eggs present in the visceral mass (R^2 = 0.07).

When mussels were assigned stage categories based on gonad colour, a large number of mussels were assigned scores of 2 and 3 due to the presence of an orange colouration on gonad tissue. This resulted in the inclusion of many immature mussels. When the gonad tissue was examined, approximately 10% of the samples had very few or no eggs. When only percentage gonad cover was used as a scoring criterion, almost all those individuals were scored 3. If only colour is used during visual assessment, most of the mussels scored 1. A combination of gonad colour and percentage gonad cover is necessary to provide a more accurate descriptor of reproductive maturity, and hence likelihood of spawning.

Following validation of the relationships between mussel size, gonad colour and egg maturity, the spawning likelihood matrix was constructed by using percentage gonad cover and gonad colour score as indicators of reproductive risk (Figure 6). Under the matrix, scores between 1–3 indicate low likelihood of spawning, 3–4 moderate likelihood of spawning, and 6–9 high likelihood of spawning.

Comparison of Perna viridis *collected in Singapore with* Perna viridis *collected from vessels entering Western Australian waters: application of the spawning likelihood matrix*

Mussels from fish farms in Singaporean waters showed evidence of reproductive maturity (Stage 2) in animals as small as 30 mm. The 562 specimens removed from vessels in WA waters ranged in size from 7.39 mm to 54.53 mm in length (mean size

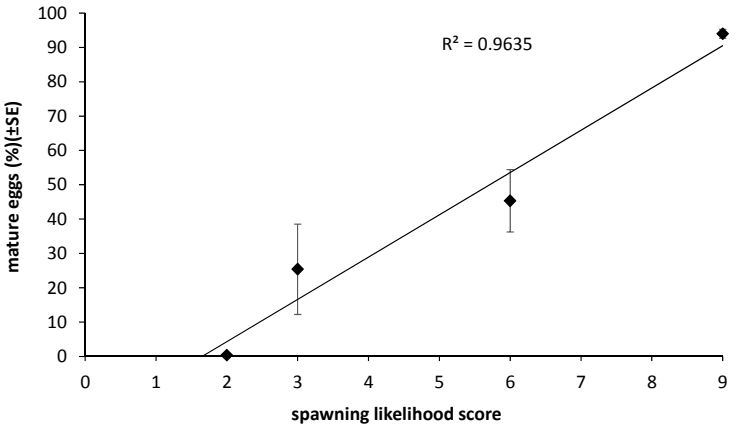

Figure 5. Relationship between the proportion of mature *P. viridis* eggs in the gonads (%) and the spawning likelihood score (based on scoring criteria in Table 2).

20.12 mm). Of the 562 mussels, only 19 showed evidence of reproductive development, with the smallest a female 28.3 mm in length (Stage 2). Five mussels were Stage 1 (sex indeterminate) and 12 were Stage 2 (7 males and 5 females). Only two animals were collected that were Stage 3 (one female at 30.05 mm and one male at 54.53 mm). The reproductive maturity exhibited in these small animals was the exception. The spawning likelihood matrix was successfully applied to all 562 specimens collected with consistent outcomes as those assessed in Singapore populations.

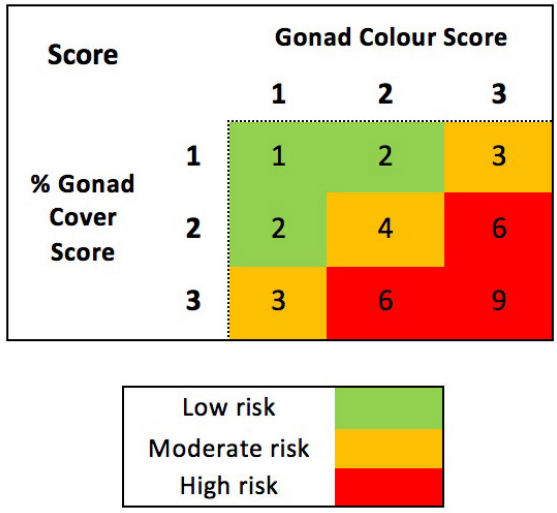

Figure 6. Spawning likelihood matrix for *Perna viridis* based on the outcome of field observations of "gonad colour score" and "% gonad cover score". Low risk equates to limited spawning likelihood, a high risk indicates imminent spawning likelihood.

Discussion

To date there have been crucial and potentially unnecessary delays in determining the risk of *Perna viridis* to environments when it is unintentionally transported outside its native range. These delays are often due to the necessity for sending samples to experts far afield for verification of mussel spawning likelihood. This verification often requires time consuming histological examination of mussels to determine reproductive status. Such delays are impractical when dealing with commercial vessels. Likewise, acting in an overly precautionary manner by assuming all animals pose a spawning risk could have important ramifications for vessel operators and the subsequent flow of activities. Consequently, development of this rapid spawning likelihood assessment technique to determine the potential risk posed by translocated *P. viridis* is valuable from both environmental and economic perspectives. The technique outlined in this paper was developed using gonad colour, mussel size and laboratory examination of spawned and randomly collected *P. viridis* specimens as indicators of mussel reproductive maturity and hence their spawning likelihood. It will provide rapid response units, such as biosecurity compliance teams, with a user-friendly approach to aid in *in-situ* vessel management i.e. turn away, clean, quarantine.

Gonad colour and reproductive maturity

From this study, it can be concluded that gonad colour and stage of gonad development combined are the most useful indicators for assessing *P. viridis* likelihood of spawning. Visual colour grading of *P. viridis* gonads correlated with the quantitative measures for gonad condition i.e. the proportion of mature eggs in gonads. The orange colour in the female gonad indicates the presence of mature eggs,

and the intensity of the colour provides an indication of the maturity and quantity of eggs present. Buchanan (2001) reported that gonad visual grading system developed for *Perna canaliculus* clearly identified broad changes in reproductive development. Walter (1982) also reported that female gonads showed appreciable colour changes with maturity (light orange to reddish orange), with gonad colour intensifying as the eggs mature, and decreasing as mussels commence spawning. Likewise, the amount of mature eggs present in a single mussel increases and decreases as it goes through its reproductive cycle. For mature females, spawning can occur progressively over time and as observed from the assessment of spawned females, the majority of animals with reduced colour intensity still contained large proportion of mature eggs. Based on our observations, even if mussels have recently spawned, they often still contain a large proportion of mature eggs. Light-coloured individuals contained predominantly immature eggs which may be assumed to mature in a short period of a time (days to weeks) depending on environmental conditions (temperature, salinity and available food for adult). The size of eggs also increases with maturity. As such, it should be possible to identify a mature female ready to spawn by assessing gonad colour and egg size.

Relationship between Perna viridis *size and stage of reproductive development*

Identifying *P. viridis* cohorts is important when conducting vessel inspection for IMS as it provides valuable information with regards to how many settlement events may have occurred on the vessel. This is important in determining the degree of risk a vessel may pose, as smaller settlement events (cohorts) are likely to pose less of a propagule pressure risk than larger settlement events. Gathering data on mussel cohorts on a vessel entails collecting and measuring size classes of the mussels present and can occur at the same time as collection of mussels to determine likelihood of spawning.

The spawning likelihood posed by *P. viridis* is difficult to determine using only mussel size as an indicator. This is due to the high variability associated with mussel size and reproductive stages, and the lack of relationship between mussel size and the proportion of mature eggs present in gonads. In Singapore, mussels more than 15 mm in size may be expected to contain some gonad tissue though they may not be reproductively mature. *P. viridis* becomes sexually mature at 15–30 mm shell length, which equates to approximately 2–3 months of age (Siddall 1980). In Tampa Bay, Florida, Power et al. (2004) reported sexual maturity in as little as 1–2 months. Hence size alone is not truly indicative of age or reproductive potential, as the conditions in which the mussel is growing can significantly influence both these factors. This is exemplified by the smaller size of reproductively mature mussels collected in Singapore compared to those collected from vessels arriving in WA waters. Mussels collected from vessels that have been in sub-optimal thermal or nutrient conditions have been observed with prominent bands of thickened shell caused by slower growth (R. Willan, J. McDonald pers. obs.). Although this study revealed a positive correlation between mussel size and stage of reproductive development, there was high variability within each of the reproductive stages associated with size.

Application of the Perna viridis *spawning likelihood matrix to mussel detections on vessels*

Specific application of the spawning likelihood matrix is a simple procedure involving four discrete steps. 1) mussels need to be collected from the vessels and clearly identified as both *P. viridis* and female. This can be determined based on the colour of the gonads. If the colour of the gonads is creamy or very pale, then the mussels are either immature females, and hence not at risk of spawning or are male. If the mussels are clearly identified as female, 2) the colour of gonads needs to be checked against the colour guide (Figure 1, Table 2) to determine the gonad colour score, 3) the percentage of gonad cover needs to be recorded and scored (Table 2). Both scores are applied to the matrix (Figure 6) to determine the likelihood of spawning posed by the mussel. This procedure needs to be repeated for a range of mussels collected from various locations on the vessel.

Once a spawning likelihood score has been calculated, investigators need to understand the implications of the score. Under Singaporean conditions, high risk indicates imminent likelihood of spawning i.e. within one day. Under Western Australian conditions, high risk of spawning may indicate spawning in as little as 3 to 4 days, depending upon location and environmental conditions (including water temperature, nutrient levels and salinity). When using the matrix, the following key questions need to be addressed to determine the spawning likelihood of translocated *P. viridis* and subsequent rapid management: 1) what is the colour and coverage of the gonads?, 2) what size are the mussels?, 3) where have the mussels come from?, 4) what length of time will the vessel will be in port?, and importantly, 5) what are the environmental conditions of the vessel's current location?

We are confident that this rapid assessment tool can be used with ease under field conditions. This will aid first responders in making rapid and better-informed decisions regarding the spawning likelihood of *Perna viridis*.

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