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Evaluating vitality and predicting mortality in spot prawn, *Pandalus platyceros*, using reflex behaviors

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ABSTRACT

Evaluating vitality and predicting mortality in commercially exploited crustaceans is increasingly important for reducing discard mortality and for improving handling and shipping for live markets. A suite of 10 reflex actions were identified in spot prawns (*Pandalus platyceros*) that vary in sensitivity to injury and type of stressor. After establishing a baseline for stereotypic reflexes, prawns were subjected to physiological stress (emersion up to 60 min) and physical trauma (dropping). The prawns were tolerant of air exposure up to 40 min, but susceptible to injuries from dropping and the results within treatments were variable. However, mortality of individuals over a 30-day recovery period was closely related to a simple reflex impairment score calculated as the sum of reflexes lost (range = 0–10) and the effects of different injuries were additive. Logistic regression indicated that reflex impairment was an excellent predictor of delayed mortality (87.5% correct predictions) across prawn size (35–48 mm carapace length) and treatment types. A sigmoid curve describing the relationship between impairment and mortality was termed a Reflex Action Mortality Predictor. This RAMP approach should be a valuable tool in practical experiments related to both discard mortality and handling live crustaceans without the need for tagging or long-term holding.

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1. Introduction

The importance of reducing unintentional mortality of fishery resource species associated with both bycatch and live transport is intensified by an ever increasing demand for seafood and the associated fishing pressure (Alverson et al., 1994; Hall et al., 2000; Broadhurst et al., 2006). Decapod crustaceans make up a large part of the global demand, and many crabs, lobsters, and prawns are sold live. Evaluating the general condition of large crustaceans and predicting their likely survival intersects with fishery science when undersized or illegal bycatch are discarded at sea. The issue is especially relevant for prawns and shrimp; in a review exploring the magnitude of bycatch mortality, Cook (2003) reported that the ratio of discarded to landed shrimp in worldwide fisheries was five to one, and that discards from shrimp trawl fisheries averaged nearly twelve to one. Fishery models usually incorporate parameters for discard-related mortality, and there is increased motivation to develop fishing methods and gear to reduce injury to the non-target individuals (Broadhurst, 2000). In practice, mortality of discarded animals is usually evaluated with field tagging studies, by holding groups of animals in tanks or underwater enclosures, or with rough estimates of likely mortality based upon observations of animal condition before they are discarded (Broadhurst et al., 2006).

Recently, methods were developed to evaluate condition and to predict survival in Alaskan Tanner crabs (*Chionoecetes* spp.) in the field (Stoner et al., 2008; Stoner, 2009). The general approach involves observing a suite of reflex behaviors for presence or absence and developing a curve that describes the relationship between mortality and a composite score for reflex impairment. This reflex action mortality predictor (RAMP) can then be used in subsequent observations to determine the probability of delayed mortality without the need for tagging or long-term holding. Consumers have an intuitive understanding for evaluating live lobsters (e.g., *Homarus* and *Nephrops* spp.), and professional seafood handlers routinely grade rock lobsters (*Panulirus* spp.) for condition and likely survival in transport (Paterson et al., 2005). Consequently, the RAMP approach is a quantitative extension of the more general evaluation practiced by consumers and seafood handlers, and has been applied successfully to fishing gear experiments with the Alaskan crabs (e.g., Hammond, 2009; Rose et al., unpublished data).

Pandalus platyceros (spot prawn) is the largest species (to 61 mm carapace length, CL) in the family Pandalidae which sustains a small but valuable fishery ranging from California through Alaska (Mormorunni, 2001). The current value of whole fresh spot prawns is ~US\$22–26 kg^{-1}, and more in the live market (S. Groth, Oregon Department of Fisheries and Wildlife, pers. comm.). Spot prawns are protandric hermaphrodites, maturing first as males, and then

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entering a transition phase to become functionally female sometime between the third and fourth years, near 40 mm CL (Butler, 1964). While young spot prawns can be found in inshore waters in Alaska (Butler, 1970; Marliave and Roth, 1995), most fishing through the species' range occurs in deep water (90–240 m) where they tend to be associated with complex habitats with gravel and cobble (Schlining, 1999; Mormorunni, 2001). They consume small invertebrate prey including polychaetes, mollusks, and crustaceans. Spot prawns may live 7–11 years in Prince William Sound (Kimker et al., 1996). A long period to female maturity, relatively low fecundity (1000–5000 eggs per female once per year) (Butler, 1964), specific habitat requirements, and intensive fishery for the valuable product makes the spot prawn vulnerable to overexploitation. Therefore, it is important to develop tools that can be used to evaluate likely discard mortality and improve handling, holding, and shipping practices for this and other large prawns.

This study is the first to evaluate feasibility of using a suite of reflex actions to predict delayed mortality in a large prawn. Individual spot prawns were subjected to controlled levels of physiological and/or mechanical stress, reflexes were tested, and their subsequent survival was monitored with the goal of developing a RAMP that can be applied for rapid assessment of discard and handling mortality.

2. Methods and materials

2.1. Prawn collections and holding

Spot prawns were captured in baited pots deployed from a commercial fishing vessel (F/V *Shenanigan*) in 200 m depth offshore from Bandon, Oregon on 8 March 2011. The prawns were maintained alive in the vessel's live tank which was supplied with a continuous flow of refrigerated (2 °C) seawater. The prawns were delivered to the dock on the next morning, and 120 individuals were immediately transported in insulated containers (5 °C) to the Hatfield Marine Science Center (HMSC) in Newport, Oregon. Upon arrival at HMSC the prawns were divided evenly among five circular pools (2.3 m diam.; 1.0 m depth) supplied with a constant flow of seawater (9 L min^{-1}) at 5 °C, and the room was kept dark (9×10^{-7} μmol photons m^{-2} s^{-1}). The prawns were fed a combination of clam (*Mactra solidissima*), herring (*Clupea pallasii*), and krill (*Euphausia pacifica*) three times per week, and they were allowed to acclimate to the laboratory environment for at least 9 weeks before experimentation. No mortality occurred between transport and the first experiments, and molting was observed occasionally indicating excellent health. Prawns 35–48 mm CL were chosen for the handling experiments.

2.2. Reflex actions

Reflexes that might be useful in evaluating spot prawn condition were explored before initiating experiments on handling stress. This was guided by earlier reflex observations on crabs (Stoner et al., 2008) and other practical experience handling large shrimp. The goal was to identify simple reflex actions that are consistent stereotypic responses that can be tested rapidly in hand either at sea or in commercial packing facilities. A long list of tests was performed for 10 individuals on two consecutive days, scoring each response as strong, weak, or no response. Responses to manipulations of the uropods, scaphocerites, secondary antennae, and other structures were not consistent, while 10 reflex actions were found to be reliable (Table 1). Preliminary experiments revealed that some reflexes were lost with prolonged air exposure and physical trauma associated with dropping on a hard surface, as expected.

2.3. Experimental treatments and protocol

Prawns were subjected to two kinds of stress—physiological stress associated with air exposure (emersion) for specific periods, and physical trauma caused by dropping onto a concrete floor from standard heights. These stressors were tested both individually and in combination to test for possible additive effects of physical and physiological injury. Air exposures were made for periods of 10, 20, 40 and 60 min at room temperature (13–14.5 °C) (treatments identified as A10, A20, etc.), and drops were made from 2 m and 3 m heights (D2, D3). Combinations of 2 m drop followed by 20 min emersion (D2 + A20) and 3 m drop followed by 40 min emersion (D3 + A40) were included as mixed treatments. As intended this group of eight treatments provided a broad range of stress and associated losses in reflex actions. Ten prawns were subjected to each of the experimental treatments. In the A10 treatment, six prawns lost no reflexes, four lost one reflex, and none died over the subsequent 60 days (see below). With 100% survival in this treatment, no other form of experimental control was deemed necessary.

For a handling experiment, three prawns were dip netted from a holding tank with the objective of gathering individuals with firm exoskeletons (i.e., not recently molted) and sufficient range in size (CL) such that they could be identified without tagging. Each set of prawns was moved in a closed black container (with 5 °C seawater) to a dimly lit seawater laboratory (6×10^{-2} μmol photons m^{-2} s^{-1}) where the handling experiments were conducted. Air exposure was carried out by placing a prawn in a plastic tray (50 cm × 37 cm × 13 cm), timing each emersion period with a stop-watch. Drop treatments were conducted by holding a prawn in a horizontal and ventrum-up orientation and dropping from the prescribed height. Mixed treatments were performed by dropping followed immediately by timed air exposure. Immediately following a handling treatment the prawn was measured for carapace length and assessed for all 10 reflexes, recording each as present or absent. Weak reflexes were sometimes observed, but were scored as present or absent following the protocol of Stoner (2009). It was then placed in a large recovery tank supplied with a constant flow of seawater (9 L min^{-1}) at the standard holding temperature (4.5–5.5 °C). Each recovery tank (2.3 m diam.; 1.0 m depth) was divided into two halves with a plastic barrier, each side holding a three-prawn group. Prawns in a weakened state generally landed on their sides, and the time taken to turn dorsum up was observed and recorded for each individual. Light level in the recovery tanks was dim (4×10^{-4} μmol photons m^{-2} s^{-1}). Observations for mortality were made throughout the first 8 h and daily thereafter to determine approximate time of death. Among all of the treatments, just two mortalities occurred after 4 days, one at 9 days and one at 19 days, and observations on individuals ended at 30 days. During the post-handling recovery period each group of prawns was fed as described above and observations on survival and molting were recorded daily for each individual. All surviving prawns were re-tested for reflex actions 7–9 days following the handling treatment. After the initial 30-day recovery period, the prawns were moved to one of three large community tanks where no mortality occurred during the subsequent 30 days (total post-treatment period = 60 days).

2.4. Analysis

The number of lost reflexes was summed for each prawn to provide an impairment score which could range from 0 to 10. The composite provides a robust index of overall condition for the animal and has the advantage of reducing the weight of any one reflex (Davis, 2007; Stoner et al., 2008).

Logistic regression was used to model mortality, using potential predictors and mediators including the composite reflex

Table 1
Reflexes identified as useful for assessing stress in spot prawns. "Test" is the manipulation required to elicit a stereotypic positive response. No response was recorded when no action was detected in response to repeated stimulus. The reflexes are listed in the order that they were tested. The first nine reflexes are tested while grasping the prawn by the carapace and holding it dorsum up in horizontal orientation.

Reflex	Test	Positive response	Lost response
Abdomen turgor	Lift the prawn by the carapace, dorsum up	Abdomen is extended to horizontal position, or a tail flip occurs	Abdomen hangs limply and without motion
Abdomen extension	While holding the prawn as above, manually depress the abdomen	Abdomen shows resistance and extends outward or a tail flip occurs	Abdomen shows no resistance to being flexed and does not re-extend
Leg motion	Manually stimulate the pereiopods	Pereiopods move spontaneously when the prawn is lifted or upon stimulation	Pereiopods are motionless upon stimulation
Leg retraction	Draw the 1st or 2nd pereiopods in the anterior direction	Pereiopods retract in the posterior direction or present resistance to the motion	No resistance to manipulation occurs
Maxilliped motion	Manually stimulate the 3rd maxillipeds	Maxillipeds move spontaneously when the prawn is lifted or upon stimulation	Maxillipeds are motionless upon stimulation
Maxilliped retraction	Draw the 3rd maxillipeds in the anterior direction	Maxillipeds retract in the posterior direction or present resistance to the motion	No resistance to manipulation occurs
Antenna motion	Manually stimulate the antenna	Antennae move spontaneously or upon stimulation	Antennae are motionless upon stimulation
Eye turgor	Touch the eye stalk with a blunt probe, or lift it from retracted position	Eye stalk retracts, moves, or shows normal turgor when stimulated	Eye stalk shows no motion or resistance to manipulation and hangs limply
Pleopods retraction	Extend the pleopods downward with a blunt probe	Pleopods are retracted close to the abdomen	Pleopods hang limply from the abdomen and do not retract upon stimulation
Mouth closure	Hold the prawn in ventrum-up position. If closed, open the 2nd maxillipeds with a blunt probe	Maxillipeds move spontaneously or move actively upon stimulation	Maxillipeds are motionless upon stimulation

impairment index, carapace length and treatment type. For modeling purposes treatments were combined under three categorical variables including emersion (i.e., the four air exposure treatments), dropping (two treatments), and mixed treatments where the prawns were subjected to both dropping and emersion. Models were fit by the method of maximum likelihood for binary data (i.e., dead or alive) using the regression module of Systat 13 (Peduzzi et al., 1980). A backward stepwise approach was used to determine the most parsimonious model for mortality, with an alpha value of 0.15 to remove a variable from the full model. This model for mortality was described by:

$$\log_e\left(\frac{p}{1-p}\right) = \alpha + \beta' x$$

where $p = \Pr(y=1)$, $y=1$ if dead and 0 if alive, α = intercept, x = the model matrix of explanatory variables, and β' = model coefficients.

The maximum likelihood estimates of mortality (p) were calculated as:

$$\rho = \frac{e^{(\alpha+\beta' x)}}{1+e^{(\alpha+\beta' x)}}$$

Initially, the data were split randomly into equal halves, one representing a learning set and the other a test set. The most parsimonious logistic model was developed using the learning set, and validated with the test set. After cross-validation, a final model was fit to the entire data set. Finally, the logistic model was used to develop a curve showing the probability of mortality based upon fixed values for the key observations of prawn condition.

3. Results

3.1. Relationships between stressors, mortality and impairments

Mortality in spot prawns occurred in all of the experimental treatments except A10 and A40, and only one in 10 prawns died after A20 treatment (Fig. 1). The combination treatment D2 + A20 resulted in 20% mortality; the same as D2. D3 resulted in 60% mortality, and no mortality occurred with A40. However, the combination of dropping and air exposure in the D3 + A40 treatment showed the additive effects of the two different stressors and resulted in 100% mortality.

The most commonly lost reflex actions in prawns subjected to emersion and dropping treatments were retraction of the 3rd maxilliped and tail extension (Table 2). However, some differences also occurred. Air-exposed prawns lost antenna motion and leg-retraction reflex at a relatively high frequency while stress from dropping resulted in reduced abdominal turgor and eye reflex. When just one reflex was missing (impairment score = 1) (n = 8), the reflex lost was retraction of the 3rd maxilliped in all cases except one where tail extension was lost. The least sensitive indicator of stress appears to be spontaneous leg motion which was lost in very few individuals (n = 13); most of these prawns (n = 9) subsequently died.

Average reflex impairments associated with the experimental treatments generally increased with the severity of handling treatment, from just 0.4 after A10 treatment to 8.7 (maximum possible = 10) after D3 + A40 (Fig. 2). The scores from singular treatments of D3 (4.8) and A40 (3.3), summed closely (8.1) to the

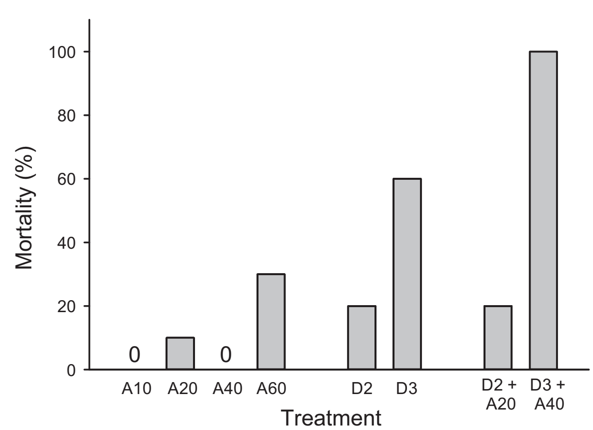

Fig. 1. Mortality of spot prawns subjected to handling treatments including air exposure and dropping tested both individually and in combination. Air exposures were 10, 20, 40 and 60 min long (A10, A20, etc.). Drops were made from 2 and 3 m height (D2, D3). Mortalities are reported as the percent of total numbers tested (n = 10 for each treatment).

Table 2
Summary of reflexes lost by spot prawns in association with handling treatments. Results are shown for prawns subjected to singular treatments including emersion (A10, A20, A40, A60; $n=40$) and dropping (D2, D3; $n=20$) (see text). The values reported are percentages of total reflexes counted as lost by each subgroup.

Treatment type	Emersion		Dropping	
Reflex	No. losses	% of losses	No. losses	% of losses
Abdomen turgor	6	4.2	11	15.7
Abdomen extension	20	13.8	15	21.4
Leg motion	4	2.8	0	0
Leg retraction	19	13.1	2	2.9
Maxilliped motion	17	11.7	7	10.0
Maxilliped retraction	29	20.0	15	21.4
Antenna motion	20	13.8	5	7.1
Eye turgor	15	10.3	10	14.4
Pleopods retraction	7	4.8	1	1.4
Mouth closure	8	5.5	4	5.7
Totals	145	100	70	100

score recorded for the combination treatment. The same was not observed for the less stressful combination of D2 and A20 min emersion, scoring 2.2 and 3.0 when tested individually, and 3.6 when tested in combination. Impairments tended to be slightly more variable for treatments including dropping than those with air exposure alone.

Most of the mortality observed (75%) occurred within the first 24 h following experimental handling, increasing slowly to 96% by 9 days (Fig. 3). Only one individual died subsequent to that time, at 19 days. There was no clear treatment-related pattern in time to mortality. Shortest times were associated with 3-m drops (D3 and D3 + A40) where four individuals died within 1 h of handling. However, four other individuals in the same treatments survived from 3 to 19 days.

3.2. Righting behavior and mortality

Ninety percent of the prawns eventually moved to an upright orientation after experimental handling, but success and time to righting varied with treatment (Table 3). All of the prawns subjected to air exposure righted eventually in times ranging from immediate to 40 min. Lack of righting occurred least frequently in prawns that died; however, seven of the individuals in the D3 + A40 min treatment died after orienting properly. Initially, fast righting appeared to be an indication of good condition, and there was only one mortality among the 37 individuals that righted in <1 min. The converse was not true. Twenty of 43 individuals that did not right within 1 min survived. Therefore, successful righting was a good predictor of survival, but not righting within 1 min was not a good predictor of mortality. The time taken to assume an upright orientation showed a somewhat better relationship with mortality (Fig. 4), but mortalities associated with righting times >1 min ranged from 20 to 67%.

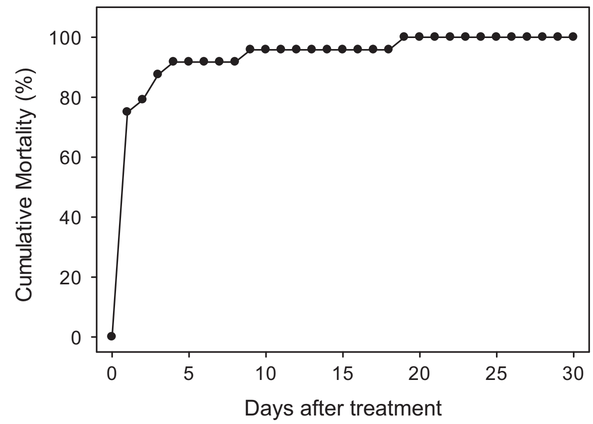

Fig. 3. Cumulative mortality of spot prawns subjected to handling treatments including air exposure and dropping (see Fig. 1). Eighty prawns were tested and 24 died (30%) during a 30-day holding period. None died after 19 days.

Table 3
Righting behavior exhibited by spot prawn after experimental handling. Treatments included air exposure and dropping tested both individually and in combination. Air exposures were 10, 20, 40 and 60 min long (A10, A20, etc.). Drops were made from heights of 2 and 3 m (D2, D3). Most prawns landed on their sides on the recovery tank bottom, and the time taken to achieve the typical dorsum-up orientation was recorded. Mortality was tracked for 30 days. Ten prawns were tested for each treatment type.

Treatment	Prawns righting (% of total)	Time to right (min) (range)	Mortality (% of total)
A10	100	0–3.0	0
A20	100	0–32	10
A40	100	0.2–10	0
A60	100	1.2–40	30
D2	90	0–10	10
D3	60	0.3–3.4	60
D2 + A20	100	0–80	20
D3 + A40	70	1.2–300	100

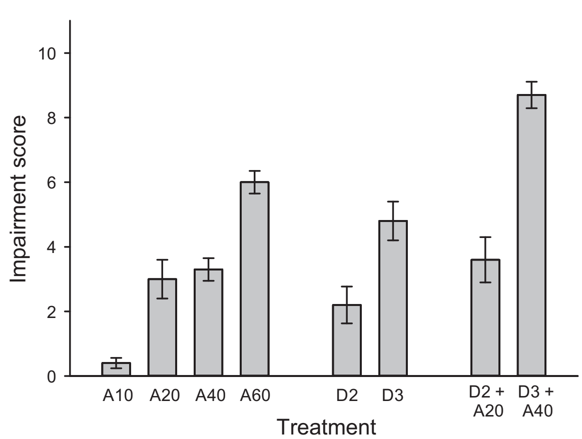

Fig. 2. Reflex impairments observed in spot prawns subjected to handling treatments including air exposure and dropping tested both individually and in combination (see Fig. 1). Impairments are reported as the mean and standard error for each treatment ($n=10$ for each treatment).

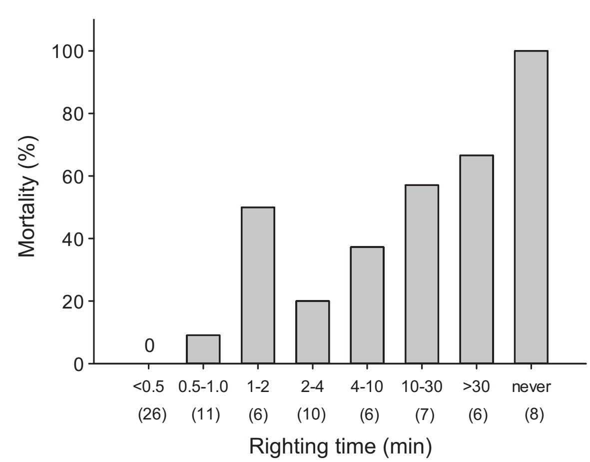

Fig. 4. Relationship between righting time and mortality in spot prawns subjected to handling treatments (see Fig. 1). The numbers of individuals representing each block of righting times are shown in parentheses. All of the prawns that did not right within 1 h died within the following 17 h.

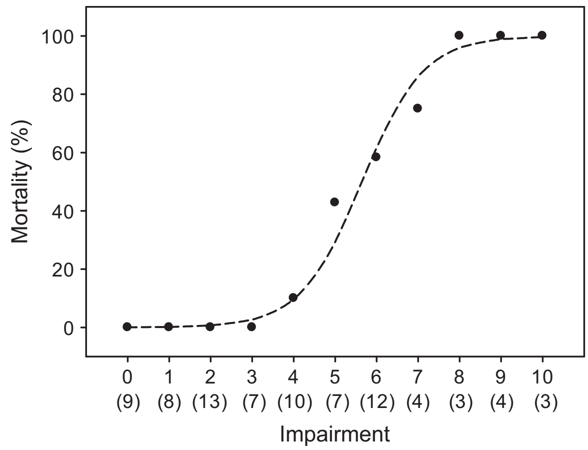

Fig. 5. Mortality of spot prawns shown as a function of reflex impairment. The numbers of individuals representing each score are shown in parentheses below the *x*-axis. Closed circles represent the observed percentages of mortality for each impairment score. The dashed line is the curve fit from logistic regression, and represents the probability of observing mortality in a prawn with the associated impairment.

3.3. Reflex impairment and mortality

Mortality was closely associated with reflex impairment score (Fig. 5). No mortality occurred in prawns with reflex scores ranging from 0 to 3 immediately following handling, but increased rapidly among prawns scoring between 4 and 8 reflexes lost. No prawn with reflex impairment higher than 7 survived in recovery tanks during the subsequent 30-day recovery period. There was no clear relationship between time to mortality and impairment score. Short-term survivals (<1 day) occurred at every impairment level from 4 to 10, and long delays in mortality (>3 days) occurred at all levels from 5 to 10, except 9.

Logistic regression using impairment score, carapace length, and treatment type revealed that impairment had the greatest value in predicting prawn mortality and that prawn size had no significant effect (Table 4). Treatment type (i.e., emersion vs. dropping vs. combination treatments) had marginal significance in predicting mortality. This occurred because emersion-related treatments resulted in somewhat lower mortality than did dropping treatments when impairment scores were similar. However, there were only four mortalities associated with all of the emersion treatments, and the most parsimonious logistic model included just the impairment variable and a constant. This model yielded 87.5% correct predictions of overall mortality and survival, and fit for the logistic model is shown in Fig. 5.

Table 4
Results of logistic modeling for mortality in spot prawns. A backward stepwise approach was used to determine the most parsimonious model for mortality, with an alpha value of 0.15 to remove a variable from the full model.

Parameter	Estimate	Z	p value
Full model			
Constant	21.657	2.446	0.014
Treatment 1	1.456	1.071	0.284
Treatment 2	−4.129	−1.665	0.096
Carapace length	−0.169	−1.101	0.271
Impairment	−2.609	−2.715	0.007
Most parsimonious model			
Constant	7.634	3.932	<0.001
Impairment	−1.351	−3.878	<0.001

Prediction matrix for the most parsimonious model

	Dead predicted	Live predicted	Actual total
Dead	20	4	24
Live	6	50	56
Total predicted	26	54	80
Correct (%)	83.3	89.3	
False (%)	23.1	7.4	
Total correct (%)			87.5

Table 5
Molting observed in spot prawns observed individually for 30 days following handling treatments (see Table 2). Results are summarized by treatment and by reflex impairment score.

	Survivors	Percent molting
Treatment		
A10	10	30.0
A20	9	44.4
A40	10	30.0
A60	7	42.9
D2	8	50.0
D3	4	0
D2 + A20	8	75.0
D3 + A40	0	–
Impairment		
0	9	22.2
1	8	62.5
2	13	38.5
3	7	57.1
4	9	33.3
5	4	25.0
6	5	40.0
7	1	100.0
8, 9, 10	0	–
Overall	56	41.1

3.4. Recovery from stress

After 7–9 days of recovery in the large holding tanks, every surviving prawn had a reflex impairment score of 0 (no impairment). Recovery was also indicated by successful molting in a large number of prawns observed in the weeks following handling. Forty-one percent of the survivors molted within 30 days, including the one individual that had an impairment score of 7 after handling, and two of the five survivors with impairments of 6 (Table 5). Molting occurred between 2 and 27 days following handling treatments. None of the survivors from treatment D3 ($n = 4$) molted but molts occurred in all of the other treatments with survivors. Despite recoveries in reflexes, two individuals died subsequent to the follow-up testing, both from dropping treatments, suggesting that some delayed effects of trauma were no longer revealed by diminished reflex actions.

4. Discussion

Spot prawns were tolerant to emersion, with all but one individual surviving exposure up to 40 min. This was somewhat unexpected for a deepwater crustacean given both the rapid rise in temperature (to 14 °C in air) and long emersion times. Crustaceans rapidly accumulate lactate and ammonium in their hemolymph with air exposure, and the effects tend to be exacerbated by increases in temperature (Vermeer, 1987; Woll et al., 2010). Numerous physiological studies have been aimed at understanding the physiological consequences of emersion because of relevance to live shipment for rock lobsters (Spanoghe and Bourne, 1997; Paterson et al., 2005), clawed lobsters (Ridgway et al., 2006; Lorenzon et al., 2007), and edible crab (Barrento et al., 2009). Acidosis is a common effect of emersion that can have lasting effects on various tissues. However, the long-term survival of spot prawns and continued molting observed in this experiment indicate that the effects of emersion for <1 h were temporary.

The prawns were more susceptible to injury from dropping than from emersion. External injuries such as broken pereiopods or rostrum occurred occasionally, but cracks in the carapace were never apparent, even with a 3-m drop, and deaths appeared to result from bleeding or internal injury. The physical injuries associated with dropping also resulted in more variable impairment scores than emersion, probably as a result of uncontrolled differences in the types and severities of injuries received. Similar experiments were conducted with snow crabs (*C. opilio*), combining dropping and emersion treatments (Grant, 2003). In both snow crabs and spot prawns, dropping caused greater injury and mortality than emersion, and the effects appeared to be additive, at least in the more severe conditions.

Righting behavior should be a good indicator of condition in benthic marine animals because regaining a proper orientation requires the integration of sensory, nervous, and locomotor systems. Several studies have used righting as an index of vitality in crabs (Stevens, 1990; Carls and O'Clair, 1995; Warrenchuk and Shirley, 2002); however, Stoner (2009) found that righting was not a reliable predictor of mortality in cold-stressed *Chionoecetes* spp. Similarly, in this study, the relationship between righting and survival was not clear. For example, some prawns slow to right themselves survived long-term, while others that oriented quickly died.

Simple reflexive behaviors are often useful in evaluating animal condition. "Tail flip" has been used to characterize condition in rock lobsters (*Panulirus argus* and *P. cygnus*) (Vermeer, 1987; DiNardo et al., 2002), and professional graders in Australia routinely evaluate the condition of *P. cygnus* on the basis of behavioral characteristics to predict survival for the live market trade (Paterson et al., 2005). Spanoghe and Bourne (1997) used a combination of tail flip response, and movements of the appendages and eye stalks to classify the condition of *P. cygnus* as "dead", "moribund", "weak", "healthy", or "very healthy", and Harris and Ulmestrand (2004) used a similar system of observations to evaluate the condition of Norwegian lobsters (*Nephrops norvegicus*). Others have incorporated behavior-based vitality indices to evaluate handling stress in lobsters (Brown and Caputi, 1983; Castro et al., 2003) and crabs (Stevens, 1990; Tallack, 2007). In all of these cases, different handling treatments resulted in different condition indices, but the authors did not rigorously test for relationships between condition and subsequent mortality.

The merits of using multiple reflexes to provide accurate mortality predictors were discussed first for fish (Davis, 2007, 2010), and the same principles apply to crustaceans (Stoner et al., 2008). The strength of combining reflex observations into a composite score lays in the fact that the composite integrates the effects of different stressors on the host of physical and physiological systems that determine whether an animal lives or dies. The relationship between the composite reflex impairment score (inverse of vitality) and mortality observed directly in controlled experiments provides the Reflex Action Mortality Predictor (RAMP). In this study, a group of 10 stereotypic reflex actions were identified for spot prawns. Both emersion and dropping treatments resulted in losses of the most sensitive 3rd maxilliped reflex and abdominal extension; however, loss of leg retraction and antenna motion was associated with the physiological stress of emersion, and abdominal turgor was lost with the physical trauma of dropping. Even larger variation in reflex losses was observed in an earlier study of *Chionoecetes* spp. (Stoner, 2009). In the end, no one or two reflexes provided a definitive predictor of mortailty for *Chionoecetes* spp. or for spot prawn; rather, good predictions depended upon the combination of reflex observations. This study showed that a RAMP approach is feasible for spot prawn and 10 reflexes yielded excellent resolution of prawn condition and mortality.

The real benefits of a RAMP lay in its application to fishing and bycatch experiments and as a tool in perfecting handling associated with live-market trade. More specifically, once a RAMP curve is established field experiments designed to explore differential mortality can be conducted without long-term holding in tanks or field enclosures, and tag returns following field releases are less critical. For example, after the relationships between reflex impairment and mortality were established for *Chionoecetes opilio* and *C. bairdi* (Stoner et al., 2008), Hammond (2009) explored how modifications to footrope and sweeps on bottom trawls could assist in reducing injuries to crabs in the Bering Sea flatfish fishery. Crabs collected in field experiments were assessed for probability of mortality using RAMP curves, quickly yielding the results for different gear configurations. Rose et al. (unpublished data) expanded the study to include red king crab (*Paralithodes camtschaticus*). A third investigation, currently under analysis (Alaska Fisheries Science Center, Kodiak Laboratory, unpublished data), used the RAMP curve for *C. opilio* to explore likely mortality of crabs in the winter pot fishery and how differences in handling practices and season might impact discard mortality. In all of these studies several crabs per minute could be processed for reflex impairment, thousands of crabs were assessed, and their mortality probabilities were determined from the RAMP curves, requiring no subsequent tagging or holding. A similar approach should be possible for prawns now that feasibility has been demonstrated for *P. platyceros*. For example, experiments were conducted recently to evaluate stress and mortality in school prawns (*Metapenaeus macleayi*) discarded from seine and trawl capture (Broadhurst and Uhlmann, 2007). Prawns captured in the two types of gear were held in underwater cages to observe physiological indicators of stress and to observe mortality directly. Cages were also used to evaluate different forms of handling in trawl-captured *M. macleayi* (Broadhurst et al., 2009). Once RAMP curves are developed for a wide range of stressors, mortality can be assessed quickly and without the need for holding systems. The RAMP approach also has potential for experimental evaluation of holding or transport procedures used in the live market trade and aquaculture.

Stoner (2009) discussed limitations of the RAMP approach for crustaceans as it relates to fishery discards, including delayed mortality that is caused by predation and disease. However, these losses should be proportional to reflex impairment and the relative index is likely to be robust. The RAMP approach does not provide absolute measures of bycatch or discard mortality in the field, but is a powerful tool for field experiments comparing variables such as different types of gear and handling methods. A RAMP approach allows users to explore a wide range of fishing or handling conditions with a high degree of replication unlike either holding or tagging experiments.

This study provides the first proof of principle for the use of RAMP with a prawn. RAMP approach worked well for spot prawn in the size range from 35 to 48 mm CL, but a broader size range should be considered along with other intrinsic variables such as shell condition and gender. Extrinsic variables such as temperature variation, dense packing, and low oxygen/high ammonium stress that might occur with shipping spot prawns also need to be explored to obtain a truly universal RAMP curve. Preliminary experiments indicated that soft-shelled spot prawns are very vulnerable to both emersion and physical trauma, as might be expected. These prawns had high reflex impairment scores when exposed to stressors that would present minor stress for hard-shelled individuals, but the soft-shelled individuals died as predicted by the RAMP curve derived for hard-shelled prawns. This suggests that the curve reported here is relatively robust to a wide range of prawn stages and exposure conditions.

Studies of animal stress are conducted with different goals. Traditional physiological studies are important in understanding the biochemical reasons for stress and mortality (i.e., why and how an animal might die). However, biochemical indicators of stress often do not translate directly to mortality or "fitness outcomes" as discussed by Davis (2010). In contrast, RAMP methods are designed to provide a rapid assessment of which and how many animals will die, while the mechanisms are left mostly unexplained. However, this simple reflex-based predictor of likely mortality worked well for spot prawn and holds great promise in experiments and applications where reducing bycatch-related mortality or where perfecting handling associated with live-market trade or aquaculture is an important goal.

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