Abstract—Millions of crabs are sorted and discarded in freezing conditions each year in Alaskan fisheries for Tanner crab (Chionoecetes bairdi) and snow crab (C. opilio). However, cold exposures vary widely over the fishing season and among different vessels, and mortalities are difficult to estimate. A shipboard experiment was conducted to determine whether simple behavioral observations can be used to evaluate crab condition after low-temperature exposures. Crabs were systematically subjected to cold in seven different exposure treatments. They were then tested for righting behavior and six different reflex actions and held to monitor mortality. Crabs lost limbs, showed reflex impairment, and died in direct proportion to increases in cold exposure. Righting behavior was a poor predictor of mortality, whereas reflex impairment (scored as the sum of reflex actions that were lost) was an excellent predictor. This composite index could be measured quickly and easily in hand, and logistic regression revealed that the relationship between reflex impairment and mortality correctly predicted 80.0% of the mortality and survival for C. bairdi, and 79.4% for C. opilio. These relationships provide substantial improvements over earlier approaches to mortality estimation and were independent of crab size and exposure temperature.

Prediction of discard mortality for Alaskan crabs after exposure to freezing temperatures, based on a reflex impairment index

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Fishes and invertebrates are discarded from fishing operations in ever increasing quantities (Alverson et al., 1994; Cook, 2003; Broadhurst et al., 2006), and the various components of bycatch-related mortality, including detected, undetected, immediate, and delayed forms of mortality have been discussed in recent reviews (Hall et al., 2000; Davis, 2002). Crabs, shrimps, and lobsters are discarded in high proportions in relation to landings in both the directed fisheries for crustaceans and in the prosecution of finfish fishing (Cook, 2003), and many of those discards die from stressors including physical injuries to the carapace, lost and broken limbs, and physiological stress associated with temperature changes and air exposure.

Davis (2002) reviewed general principles of bycatch-related stressors and concluded that some aspects of handling and discard mortality can be simulated in the laboratory. This simulation has been undertaken extensively for fishes in recent years (see Davis, 2002), and controlled laboratory exposures to stressors relevant to fishing, such as air exposure and dropping of crabs (during handling on ships), have been conducted for lobsters (Brown and Caputi, 1983; DiNardo et al., 2002; Harris and Ulmestrand, 2004) and crabs (Zhou and Shirley, 1995; Grant 2003). Field studies designed to test different handling methods for discards typically employ either a tag and recovery approach (Brown and Caputi, 1983; Watson and Pengilly, 1994) or some means of holding the test animals in field enclosures (Kennelly et al., 1990; Grant, 2003; Broadhurst et al., 2009) or tanks (DiNardo et al., 2002; Stoner et al., 2008). Although direct experimental observations on mortality are useful, tag studies often yield relatively low returns, and experiments requiring holding can ordinarily accommodate only a relatively low number of treatment types and limited replication.

As an alternative to observing mortality directly it is sometimes possible to apply a measure of animal condition that is closely associated with delayed mortality. For example, Shirley and Stickle (1982) suggested that righting behavior (i.e., an animal’s ability to turn from a ventrum-up position to normal orientation) is a complex reflex requiring muscle coordination and neurological control that can be a sensitive measure of well-being. Righting behavior has been observed in several studies with Alaskan crabs (Stevens, 1990; Carls and O’Clair, 1995; Zhou and Shirley, 1995; Warrenchuk and Shirley, 2002), and others have scored vitality of crabs on the basis of spontaneous movements of the appendages in order to predict delayed mortality (Stevens, 1990; Purves et al., 2003). More recently, Stoner et al. (2008) developed an extension of the vitality metric, exploring a suite of six reflex actions (Table 1) that reflect the condition of Chionoecetes bairdi (Tanner crab) and C. opilio (snow crab) injured in bottom trawl operations. These reflexes are stereotypic and can be evaluated rapidly in the tester’s hand (out of water) during shipboard operations,
Table 1
Reflexes identified as useful for assessing stress in Chionoecetes spp. “Test” is the manipulation required to elicit a stereotypic positive response. No response was recorded when no motion was detected in response to repeated testing (modified from Stoner et al., 2008)

<table>
<thead>
<tr>
<th>Reflex</th>
<th>Test</th>
<th>Positive response</th>
<th>Lost response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg flare</td>
<td>Lift crab by the carapace, dorsum up</td>
<td>Legs spread wide and to near horizontal orientation in strong crabs</td>
<td>Legs droop below horizontal, with no attempt to raise them</td>
</tr>
<tr>
<td>Leg retraction</td>
<td>While holding crab as above, draw the forward-most walking legs in the anterior direction</td>
<td>Legs retract in the posterior direction, or present resistance to the motion in weakened crabs</td>
<td>No resistance to the manipulation occurs</td>
</tr>
<tr>
<td>Chela closure</td>
<td>Observe for motion or hold the chelae in the fingers</td>
<td>Chelae open and close with or without manipulation. In weakened crabs the chelae may close slowly, or show low resistance to manual opening</td>
<td>No motion is detected in the chelae under manipulation</td>
</tr>
<tr>
<td>Eye retraction</td>
<td>Touch the eye stalk with a blunt probe, or lift the eye stalk from its retracted position</td>
<td>Eye stalk retracts in the lateral direction below the carapace hood, or shows resistance to lifting</td>
<td>No motion or resistance to manipulation occurs in the eye stalk</td>
</tr>
<tr>
<td>Mouth closure</td>
<td>If closed, attempt to open (extend) the 3rd maxillipeds with a sharp dissecting probe. If open, draw the maxillipeds downward</td>
<td>3rd maxillipeds retract to cover the smaller mouth parts. The maxillipeds droop open or move in an agitated manner in weakened crabs</td>
<td>No motion in the maxillipeds occurs</td>
</tr>
<tr>
<td>Kick</td>
<td>With the crab in ventrum-up position, use a sharp dissecting probe to lift the abdominal flap away from the body</td>
<td>One or more legs or chelipeds move quickly in the ventral direction, particularly in males. Motion in the hind most legs is retained in weakened crabs</td>
<td>No motion in the legs or chelipeds occurs</td>
</tr>
</tbody>
</table>

with a high degree of reliability. More importantly, a composite index of reflex impairment (i.e., the simple sum of reflex actions lost) provided a graded metric for the animal’s condition [0 to 6], and was a good predictor for mortality in field trials. Relationships between reflex impairment and mortality were first explored for fishes (Davis, 2002, 2007) and the results were termed a reflex action mortality predictor (RAMP) by Davis and Ottmar (2006). These recent experiments have shown that RAMP models, once established for a species, provide reliable predictors independent of crab size, molting stage, gender, and injury. The RAMP approach has the added benefit of eliminating the need for holding the animals tested.

The subject species of this study were C. bairdi and C. opilio. Landings of C. bairdi reached maximum values (>100 million pounds) in Alaska during the late 1970s but have been relatively low since a fisheries collapse in the mid-1980s (Herrmann and Greenberg, 2007). Chionoecetes opilio is distributed more widely, from the Sea of Japan to Alaska in the North Pacific and through the Arctic Ocean to Atlantic Canada. During the early 1990s landings for C. opilio exceeded 300 million pounds in Alaska and over 100 million pounds in the North Atlantic (Herrmann and Greenberg, 2007). Peak value in Alaska occurred in 1994 at $200 million, then declined sharply, with Canadian fisheries in the Atlantic now representing >80% of the catch.

Fishing for Chionoecetes spp. in Alaska is limited to baited pots and only males of minimum size may be retained. Consequently, all crabs captured in trawl fishing are returned to the sea, and large numbers of sub-legal-size males and all female crabs caught in pots must be returned to the water. Many males exceeding minimum size are also returned. MacIntosh et al. (1996) discussed the magnitude of discards in the Bering Sea fishery, noting that discards could represent a large source of population mortality if these incidentally captured crabs die. Because directed fisheries for C. bairdi and C. opilio occur primarily during winter months in Alaska, the crabs can experience periods of freezing temperature and wind chill before being discarded. For calculations of catch limits for US-managed crab fisheries in the Bering Sea, both the retained commercial catch and the numbers of discarded crabs which die due to handling mortality are considered to be part
of the total catch (NPFMC, 2008). Currently, a rate of 50% mortality is applied to the crab discarded from pot fisheries in Alaska (Turnock and Rugulo, 2008), but it is widely recognized that more research is needed to refine this value and to improve handling methods to reduce discard mortality.

The goal of this study was to determine if the RAMP approach can be applied to thermal stress experienced by C. bairdi and C. opilio in Alaska fisheries prosecuted during winter months. In a shipboard experiment, males of both species at submarket size were subjected to different subfreezing temperatures and exposure times to test for possible relationships between simple reflex actions, righting behavior, autotomy, and subsequent immediate mortality and delayed mortality.

Materials and methods

Field collections and experimental animals

Crabs for this study were collected with the 50-m fishing vessel Pacific Explorer in waters east of St Paul Island (Pribilof Islands), in the Bering Sea near 57°03'N, 168°20'W, at 80 m depth. During the August 2008 field work, bottom water temperature was 1.1°C to 1.5°C. Crabs were collected in various locations around a commercial trawl with a recapture net previously described for studies of crab bycatch injury (Rose, 1999; Stoner et al., 2008). The main trawl was a two-seam Alfredo bottom trawl (with headrope and footrope lengths of 36 m and 54.6 m, respectively) similar to that used by many vessels in the Bering Sea. This trawl was towed with an open codend. The recapture net was a small 2-seam trawl with a longer headrope than footrope (14.3 m and 12.0 m, respectively). The long headrope maximized escape of fish, and a small-diameter (5 cm) footrope was used to enhance crab capture. For the broader role of the research cruise, evaluating crab injury caused by various gear components, crabs were collected at various locations around the primary net (e.g., behind the main net sweeps, wings, or footrope). As a control for damage in the recapture nets, crabs were also fished directly in the recapture nets, wings, or footrope). As a control for damage in the recapture nets, crabs were also fished directly

Experimental systems and rationale for temperature exposures

Crabs were exposed to freezing temperatures in a standard chest freezer (internal dimensions 76 × 46 × 71 cm deep) secured below deck on the FV Pacific Explorer. Temperature was monitored with a digital instrument and a cabled resistance temperature detector (RTD) platinum probe (0.1°C resolution, Fisher Scientific, Pittsburgh, PA) fastened at the bottom of the freezer compartment near the subject crabs. This digital thermometer was calibrated against a standard mercury thermometer. A 5-cm-thick layer of ice in the bottom of the freezer helped to stabilize the air temperature on the bottom of the compartment where crabs were placed ~3 mm off the bottom surface in an open mesh plastic rack. Preliminary experimentation with a small fan inside the freezer compartment showed that temperature stability at the bottom of the freezer was greatest without air circulation, and this eliminated the complicating factor of wind chill. Although crabs in the fishing conditions would rarely be exposed to freezing temperatures without at least some wind, the primary objective of this study was to determine how well reflex actions would reflect thermal stress in crabs and predict mortality, not to provide absolute values of mortality under specific outdoor conditions.

The strategy for thermal exposures was guided by the results of earlier cold-exposure experiments conducted by Carls and O’Clair (1990, 1995). They found that responses to cold air in C. bairdi and Paralithodes camtschaticus were best described when the units of exposure were considered as the product of temperature and duration of exposure (h), over a range of temperatures from –20°C to 5°C. For example, short exposures at low temperature caused the same effects as long exposures at a higher temperature when the units of exposure (degree hours, h) were equal. The same units of measurement were used in this study.

The objective of this investigation was to determine whether stress (and mortality) caused by cold exposure can be predicted from a reflex impairment index; therefore, it was important to generate variable levels
of cold-related stress that would result in a full range of impairments (0 to 6; see below). Mortality in *C. bairdi* observed by Carls and O’Clair (1990) increased in a clear sigmoid pattern from 0% to 100% with increasingly severe exposures from approximately –2 to –7°h. Therefore, exposures of –2, –3, –4, –5, –6, –8 and –10°h were chosen for this study. These exposures were achieved with two nominal temperatures, –20°C for primary experiments, and additional tests at –10°C to test for the generality of the degree-hour approach (Table 2). Temperature excursions of 1–3°C sometimes occurred during a run, particularly when the largest crabs were placed in the small freezer, but the degree-hour exposure desired could be easily achieved by systematically increasing or decreasing exposure time for an individual run.

**Experimental protocol**

Crabs for this study were held for at least 48 h before initiating experiments on cold stress. Each crab was inspected for injury and retested for reflexes just before experimentation to insure that only crabs in perfect condition were used. In fact, few crabs in diminished condition were found among those initially selected for holding (<1%), indicating that the holding environment was suitable.

Experiments were typically conducted with pairs of crabs. This procedure ensured that exposures to freezing temperatures and subsequent handling for each crab could follow a strictly timed protocol, and that uniform postexposure testing was possible. First, the test crabs were marked with uniquely numbered vinyl spaghetti tags tied securely but loosely around the basi-ischium of the third or fourth leg. The crabs were then quickly placed on the freezer rack (at a preset temperature), ventrum up to prevent excessive movement. Temperature of the freezer was closely monitored during the exposure, and exposure times were adjusted to maintain the prescribed degree-hour treatment. The seven different exposures were interspersed over the course of runs made at each experimental temperature (–20° and –10°C) until 10 or 12 replicates of each exposure were completed (Table 2).

After exposure to the cold each crab was evaluated with a series of three basic tests of behavioral capabilities. 1) Immediately following removal from the freezer the crab was tested for the presence or absence of the six reflex actions (Table 1). 2) Then, the crab was laid ventrum up in a flowing seawater bath (dimensions= 80×50×30 cm deep; 8–9°C) and observed for 120 seconds. Attempts by the crab to turn dorsum up were recorded, and if it was successful the time to right was recorded. This procedure was identical to that used by Carls and O’Clair (1990). Although crabs placed in this position in the freezer normally lay quietly, the natural tendency of crabs in water is to quickly turn dorsum up. 3) After precisely 120 sec in the water bath, the crab was removed from the water and reflex actions were retested. After these tests, any autotomies were recorded (identifying the specific limbs missing), and the crab was returned to a large recovery tank for monitoring of mortality. Mortality was assessed and dead crabs were removed each day until the end of the experiment when reflexes were re-assessed for all of the living crabs. Autotomies were recorded for dead crabs and for all live crabs at the end of the experiment. Tests for the two species spanned six days, and each individual was

<table>
<thead>
<tr>
<th>Nominal temperature (°C)</th>
<th>Exposure treatment (°h)</th>
<th>Actual temperature (°C)</th>
<th>Exposure time (min)</th>
<th>C. bairdi</th>
<th>C. opilio</th>
</tr>
</thead>
<tbody>
<tr>
<td>–20</td>
<td>–2</td>
<td>–17.6 ±3.1</td>
<td>7.3 ±1.8</td>
<td>–17.2 ±3.7</td>
<td>7.3 ±1.8</td>
</tr>
<tr>
<td>–3</td>
<td>–2</td>
<td>–17.6 ±2.2</td>
<td>10.5 ±1.5</td>
<td>–17.0 ±2.9</td>
<td>10.5 ±1.9</td>
</tr>
<tr>
<td>–4</td>
<td>–2</td>
<td>–17.0 ±2.8</td>
<td>14.5 ±2.6</td>
<td>–16.6 ±2.9</td>
<td>14.8 ±2.4</td>
</tr>
<tr>
<td>–5</td>
<td>–2</td>
<td>–19.0 ±1.6</td>
<td>15.8 ±1.2</td>
<td>–17.8 ±3.1</td>
<td>17.2 ±3.4</td>
</tr>
<tr>
<td>–6</td>
<td>–2</td>
<td>–18.2 ±1.8</td>
<td>20.0 ±2.2</td>
<td>–16.6 ±2.3</td>
<td>22.2 ±3.3</td>
</tr>
<tr>
<td>–8</td>
<td>–2</td>
<td>–19.6 ±0.5</td>
<td>24.2 ±0.5</td>
<td>–17.2 ±2.4</td>
<td>28.6 ±4.3</td>
</tr>
<tr>
<td>–10</td>
<td>–2</td>
<td>–16.3 ±3.5</td>
<td>40.8 ±9.3</td>
<td>–17.0 ±2.5</td>
<td>36.6 ±4.4</td>
</tr>
<tr>
<td>–2</td>
<td>–10</td>
<td>–10.0 ±0.7</td>
<td>12.0 ±0.7</td>
<td>–10.2 ±0.5</td>
<td>12.0 ±1.6</td>
</tr>
<tr>
<td>–6</td>
<td>–11.0 ±0.7</td>
<td>33.0 ±2.0</td>
<td>–10.6 ±0.5</td>
<td>33.6 ±1.2</td>
<td></td>
</tr>
<tr>
<td>–8</td>
<td>–10.4 ±0.5</td>
<td>46.4 ±2.1</td>
<td>–10.4 ±0.5</td>
<td>46.4 ±2.0</td>
<td></td>
</tr>
</tbody>
</table>
monitored for nine days. This monitoring period was determined by the duration of the vessel charter, and justified by Carl and O’Clair’s (1995) observation that “almost all mortality occurred 1–2 days after exposure to freezing temperatures.”

To ensure that holding conditions did not affect the condition of crabs during posttreatment monitoring, additional crabs collected in the trawls were set aside for routine handling and monitoring in the same manner as that for the test subjects. Males (n=20 C. bairdi and n=34 C. opilio) with perfect reflex scores and in size ranges equivalent to the experimental crabs were removed from the holding tanks daily and monitored for mortality. None of these crabs died and all had perfect reflex scores at the end of the experimental period, indicating that the holding conditions were adequate despite water temperature higher than where the crabs were collected.

**Reflex impairment indices and statistical procedures**

Scores for reflex actions were combined into a composite impairment index. This index, simply the sum of reflex actions lost, ranges from 0 to 6. Composites provide robust indices of overall condition for the animal and have the advantage of reducing the weight of any one reflex (Davis, 2007). Analyses described below were conducted with two different impairment indices, one calculated for reflexes assessed immediately after removal from the freezer (index A) and the other after two minutes in a water bath (index B) (see above).

As with the statistical approach of Stoner et al. (2008), a logistic regression was used to model mortality with potential predictors and mediators, namely reflex impairment, experimental temperature, and crab size. Models were fitted by the method of maximum likelihood for binary data (i.e., dead or alive) by using the regression module of Systat 12 (SYSTAT Software, Inc., San Jose, CA) (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 the following equation:

\[
\log\left(\frac{p}{1-p}\right) = \alpha + \beta^t x, \tag{1}
\]

where \( p \) = proportion of y=1; 
\( y = 1 \) if dead and 0 if alive; 
\( \alpha \) = intercept; 
\( \beta \) = model coefficients; and 
\( x \) = the model matrix of explanatory variables.

The maximum likelihood estimates of mortality (\( \rho \)) were calculated as

\[
\rho = e^{(\alpha + \beta^t x)} / (1 + e^{(\alpha + \beta^t x)}). \tag{2}
\]

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

**Results**

**Autotomy**

Autotomy during and immediately after freezing conditions increased in direct proportion to cold exposure and was generally higher in C. opilio than C. bairdi (Fig. 1). *Chionoecetes opilio* lost two legs on average in exposures ranging from –4 to –10°C, and none at –2°C. Average losses in C. bairdi were less than one across the
exposure range. When two different temperatures (–10° and –20°C) were used to create fixed exposure levels (°h) differences in initial autotomy were not different for either species (ANOVA, \(P > 0.60\)). In \textit{C. opilio}, the chelipeds were infrequently lost (4.8%) and limb losses increased in the anterior to posterior direction (Fig. 2). Losses were more uniformly distributed in \textit{C. bairdi}. Although direct comparisons with the initial limb losses were not possible because of high mortalities in some treatments, autotomies increased substantially over the 9-day holding period, especially in crabs exposed to freezing temperatures for longer periods (Fig. 1).

### Righting behavior

Crabs of both species attempted to right themselves when placed in the water bath after exposure to freezing temperatures (Table 3), but the total number of successful crabs was relatively low (10–11%). Most of the crabs able to right themselves survived over the 9-day follow-up period (90.9% of \textit{C. opilio}, 80% of \textit{C. bairdi}). However, neither attempts to right nor time to right were good predictors of subsequent survival because 52.6% of surviving \textit{C. opilio} and 60.9% of surviving \textit{C. bairdi} made no attempt to right. Also, crabs demonstrating fast righting times (e.g., 12 sec) sometimes died. Overall, successful righting was a relatively good predictor of survival, but neither the lack of righting nor attempts to right were good predictors of mortality.

#### Cold exposure, mortality, and reflex impairment

Crab mortality increased substantially with increasing exposure to freezing stress (Fig. 3), as expected. With exposures ranging from –2 to –4°h, mortalities...
were relatively similar in *C. bairdi* and *C. opilio*, then rose more quickly in *C. opilio*, reaching 100% at an exposure of –6°h. The results for crabs exposed at –10° and –20°C were nearly identical. Large proportions of the total mortality values for both *C. bairdi* and *C. opilio* occurred within 24 hours and then continued in a near linear pattern until the end of the 9-day holding period (Fig. 4). First-day mortality was especially high in *C. opilio*, accounting for more than 60% of the total mortalities observed. Eighty percent of mortality was observed by day 5 and day 6 for *C. opilio* and *C. bairdi*, respectively.

Exposure to freezing temperatures resulted in substantial impairments of reflex actions, both immediately after removal from the freezer (index A) and after the 2-minute warming period in a water bath (index B). However, preliminary analyses showed that index B had two critical limitations in terms of association with subsequent crab mortality. First, 32–35% of crabs with apparently perfect reflex scores (index B=0) died over the following 9 days. Second, mortality in *C. opilio* reached 100% at all impairment scores greater than 1.

These flaws make index B a weak predictor of mortality, and this impairment index was considered no further. The results from index A were far superior. Mortality increased smoothly from an impairment of 0 to 6 in both species, and relatively little mortality occurred with an index value of 0. All of the following results are reported with respect to index A.

Reflex impairment score increased in a near linear pattern with increasing exposure to freezing temperatures (Fig. 5). For both *C. bairdi* and *C. opilio*, mean impairments were just above 0 with an exposure of –2°h and increased steadily to 5.5 and 6.0 (loss of all reflexes tested) at –10°h. The variation was remarkably small for *C. opilio*, although crabs in the two longest exposures conducted at –10°C (–6 and –8°h) fell well below the curve for trials conducted at –20°C. The effect of the test temperature on impairment was significant for *C. opilio* (ANOVA, *P*<0.001), but not for *C. bairdi* (*P*=0.435).

Patterns occurred with reflex impairments. The most sensitive reflex, lost first in 40% or more cases, was the ability to close chelae (Table 4). Kick and eye retraction were also lost first in substantial numbers of crabs. Reflex action of the mouth was least likely to be the first reflex lost, and among all of the losses observed this response was lost in just 0.4% (*C. bairdi*) and 1.0% (*C. opilio*) of individuals with impairments scoring 1 to 5. All other reflexes were lost in substantial numbers for both species (Table 4).

Reflex impairment (index A) was a good predictor of mortality for *C. bairdi* and *C. opilio* and was independent from test temperature and crab size (logistic regression). The most parsimonious model for *C. bairdi*, containing only a constant and reflex impairment, correctly predicted 80.0% of the mortality and survival (Table 5), whereas the full model (with all variables)
Table 4
Percentages of reflex actions lost in Chionoecetes bairdi (Tanner crab) and C. opilio (snow crab). When only one reflex was absent in a crab it was considered the 1st reflex lost. In all cases where only one reflex remained that reflex was mouth closure. The right column represents the percentage of specific reflexes that were lost among all of the crabs where between one and five reflexes were lost.

<table>
<thead>
<tr>
<th>Reflex</th>
<th>1st reflex lost</th>
<th>% of total losses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C. bairdi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chela closure</td>
<td>40.0</td>
<td>25.6</td>
</tr>
<tr>
<td>Kick</td>
<td>35.0</td>
<td>24.7</td>
</tr>
<tr>
<td>Eye retraction</td>
<td>20.0</td>
<td>18.9</td>
</tr>
<tr>
<td>Leg flare</td>
<td>5.0</td>
<td>14.1</td>
</tr>
<tr>
<td>Leg retraction</td>
<td>0</td>
<td>16.3</td>
</tr>
<tr>
<td>Mouth closure</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>C. opilio</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chela closure</td>
<td>42.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Kick</td>
<td>14.3</td>
<td>20.9</td>
</tr>
<tr>
<td>Eye retraction</td>
<td>21.4</td>
<td>16.4</td>
</tr>
<tr>
<td>Leg flare</td>
<td>14.3</td>
<td>16.9</td>
</tr>
<tr>
<td>Leg retraction</td>
<td>7.1</td>
<td>16.9</td>
</tr>
<tr>
<td>Mouth closure</td>
<td>0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 5
Results of logistic modeling for mortality in Chionoecetes bairdi (Tanner crab). 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Z</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model constant</td>
<td>-7.466</td>
<td>-1.789</td>
<td>0.074</td>
</tr>
<tr>
<td>temperature</td>
<td>0.008</td>
<td>0.013</td>
<td>0.990</td>
</tr>
<tr>
<td>crab size</td>
<td>0.058</td>
<td>1.321</td>
<td>0.187</td>
</tr>
<tr>
<td>reflex impairment</td>
<td>0.877</td>
<td>5.900</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Most parsimonious model constant</td>
<td>-1.953</td>
<td>-4.427</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>reflex impairment</td>
<td>0.833</td>
<td>5.568</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Prediction matrix for the most parsimonious model

<table>
<thead>
<tr>
<th></th>
<th>Dead predicted</th>
<th>Live predicted</th>
<th>Actual total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Die</td>
<td>42</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>Live</td>
<td>9</td>
<td>38</td>
<td>47</td>
</tr>
<tr>
<td>Total no. predicted</td>
<td>51</td>
<td>49</td>
<td>100</td>
</tr>
<tr>
<td>Correct (%)</td>
<td>79.2</td>
<td>80.9</td>
<td></td>
</tr>
<tr>
<td>False (%)</td>
<td>26.2</td>
<td>33.9</td>
<td></td>
</tr>
<tr>
<td>Total correct (%)</td>
<td></td>
<td></td>
<td>80.0</td>
</tr>
</tbody>
</table>

Figure 5
Reflex impairment observed in Chionoecetes bairdi (Tanner crab) and C. opilio (snow crab) immediately after exposure to seven treatments at different freezing conditions. Exposure treatments were made at two temperatures and are plotted in units of degree-hours (°h, the product of temperature [in degrees Celsius] and time [in hours]). Predicted 81.0% correctly. The model for a random learning set (one half of the data) incorporated the same statistically significant variables (constant and reflex impairment) and correctly predicted 75.5% of the results for an independent test set. A similar analysis for C. opilio resulted in a full model that predicted 80.4% of mortality and survival correctly, with only reflex impairment having a significant effect (Table 6). Restricted to a constant and reflex impairment, the model predicted 79.4% of the results correctly. Cross-validation for C. opilio with a random learning set resulting in 80.4% correct predictions for the test set. Consequently, models for both species are robust for the experiments. Curves of the calculated probabilities of mortality based upon the logistic models with specific reflex impairments revealed very similar patterns for the two crab species (Fig. 6). Both plots rise in smooth sigmoid form from 13% to 17% mortality in crabs with impairments equal to zero to near total mortality with impairments equal to six.
When reflexes were reassessed at the end of the 9-day holding period average impairments had decreased (i.e., condition improved) since the first assessment. This was true for both crab species and for both –20°C and –10°C temperature treatments. However, most of the crabs with high initial reflex impairments died. More importantly, most individuals demonstrated either no change in reflex actions (85.1% of *C. bairdi*; 94.9% of *C. opilio*) or improvement during the holding period, and only a few individuals showed a one point increase in impairment. None of the control crabs died in holding and all had perfect reflex scores at the end of the experiment.

**Discussion**

**Cold exposure and mortality**

Although numerous studies have explored the effects of air exposure on economically significant crustaceans (e.g., Brown and Caputi, 1983; Vermeer, 1987; DiNardo et al., 2002; Lorenzon et al., 2007), and fishes (Olla et al., 1998; Davis et al., 2001; Gingerich et al., 2007) few experiments have been conducted to evaluate the effects of freezing temperatures relevant to high-latitude fisheries. In the present study, exposure to cold air caused high levels of mortality in *C. bairdi* and *C. opilio*, and the relationships to degree-hours of exposure were similar to those observed by Carls and O’Clair (1995) for *C. bairdi*. They reported a median lethal exposure for *C. bairdi* equal to –3.3°C•h for juveniles (46–74 mm CW) and –4.3°C•h for adult females (85–124 mm CW). The latter value corresponds closely with a median of approximately –4.8°C•h for male *C. bairdi* and –4.3°C•h for male *C. opilio*. Although the degree-hour exposure metric used in this and earlier studies provides a good integration of temperature and time, it is also clear that variation in mortality occurs with animal size (Carls and O’Clair, 1995), wind chill effect (Warrenchuk and Shirley, 2002), test temperature (this study), and other environmental variables (van Tamelen, 2005). Furthermore, large differences in mortality rates for *C. opilio* between this study and that conducted by Warrenchuk and Shirley (2002) illustrate the importance of specific environmental conditions. For example, in their study only five minutes exposure to windchill of –16°C (–1.33°C•h) resulted in 100% mortality. In comparison, the closest...
test equivalent in this new study (–2°C) resulted in no mortality. Van Tamelen (2005) pointed out that simple wind chill estimates are not particularly useful in the context of heat exchange for crabs and he developed a thermal model for *C. opilio* that accommodates crab size and incorporates the numerous elements of chilling, such as convection, radiation, evaporation, and conduction.

Air exposure itself probably did not cause death. Earlier observations indicate that both *C. bairdi* and *C. opilio* are highly tolerant of handling and air exposures above freezing (MacIntosh et al., 1996; Grant, 2003). For example, McLeese (1965) observed no mortality in *C. opilio* after 4-day exposures to air in two above-freezing temperatures (3°C and 8°C). Stevens (1990) reported a median lethal holding time of 8.3 hours in air for *C. bairdi*, and preliminary experiments at the Kodiak Laboratory (Stoner and Munk, unpubl. data) showed that *C. bairdi* could recover and survive for months after air exposures (15–18°C) up to seven hours. Although it is possible that mortality was caused by impaired oxygen delivery because of freeze-damaged gills, Carls and O’Clair (1995) argued convincingly against that mechanism, and Warrenchuk and Shirley (2002) proposed that mortality associated with freezing temperatures was caused by neurological damage. Their conclusion arose from observations of jerky, uncoordinated, and uncontrolled motions that would indicate nerve damage. Crabs in this study also commonly presented the same symptoms.

**Autotomy**

Shedding damaged limbs (autotomy) is an adaptation triggered by stress detectors in the limbs of crustaceans to prevent blood loss (Wales et al., 1971). The long, narrow form of the walking legs results in relatively rapid cooling (van Tamelen, 2005), and autotomy is commonly observed in *Chionoecetes* spp. exposed to freezing temperatures. In the present study, autotomy increased with increasing cold exposure, especially in *C. opilio*—a reaction analogous to that of earlier experiments with Alaskan crabs (Carls and O’Clair, 1995; Warrenchuk and Shirley, 2002). The proportions of types of limbs lost varied substantially between the two subject species for unknown reasons; however, the distribution of losses by *C. opilio* was similar to observations reported by Warrenchuk and Shirley (2002) who noted the relatively rare loss of chelipeds.

Delays in limb autotomy appear to be common among crabs and may have important consequences. As with the present study, others have observed that limb loss continues in holding tanks after exposure to cold temperatures (Carls and O’Clair, 1995; Warrenchuk and Shirley, 2002). The problem is exacerbated when limb losses increase during subsequent molting cycles (Carls and O’Clair, 1995). Given the apparent delays in autotomy, it is likely that limb losses observed before discard in typical fishing operations are substantially lower than the actual number. Autotomy may be especially critical for crabs that have reached a terminal molt or that molt infrequently. For example, *C. opilio* >90 mm CW appear not to regenerate limbs, and smaller crabs do so slowly, over at least two molt cycles (Miller and Watson, 1976). Therefore, Warrenchuk and Shirley (2002) considered autotomy to be a permanent injury for large *C. opilio*, and multiple limb losses create an obvious impediment for crabs returning to the bottom with regard to feeding, predator avoidance, reproductive behavior, and other ecological functions (see below).

**Behavioral and reflex predictors of mortality**

Righting ability depends upon a complex integration of neurological and mechanical systems, and several biologists have suggested that this may be a good condition index for Alaskan crabs (Stevens, 1990; Carls and O’Clair, 1995; Zhou and Shirley, 1995). As earlier, crabs demonstrating positive righting results in this study survived in high proportion. However, righting was a poor predictor of mortality because more than half of surviving crabs (both species) made no attempt to right. Also, Warrenchuk and Shirley (2002) found that the ability of crabs to right often recovered after several days. Although Stevens (1990) suggested that the time required to right might provide a useful predictor of survival, no such association was found in this study. Observation of righting behavior has two further limitations for assessing crab condition. First, because exposure to freezing temperatures and other forms of injury frequently result in autotomy, righting ability can be impaired by the lack of certain limbs. Second, the use of tanks of water to observe righting behavior on a moving vessel can be difficult or impractical.

Various other indicators have been used to assess the condition of crustaceans toward the goal of predicting survival or mortality. Stevens (1990) evaluated the vitality of Alaskan crabs, scoring them as alive and active, moribund, or dead. Purves et al. (2003) used a similar three-tier index of vitality (i.e., lively, limp, or dead) in three lithodid crabs (*Paralomis* spp.) to evaluate how different fishing modes used in the South Atlantic toothfish (*Dissostichus eleginoides*) fishery affect the bycatch mortality of crabs. Criteria for the index incorporated four reflexes, namely actions by the mouth parts, chelae, and legs, and leg flare. Therefore, the vitality index used by Purves et al. (2003) could have been expanded to a reflex impairment score with five increments (0–4) instead of just two for live crabs. The reflex impairment score reported in this study was based upon the same principle of considering each reflex independently and providing a seven-increment resolution (0–6).

Davis (2007) and Stoner et al. (2008) discussed the merits of using multiple reflexes and a higher resolution for a reflex impairment index to predict discard mortality. One advantage is that the composite score reflects animal condition and the likelihood of survival over a wide range of stressor types and environmental exposures (i.e., physiological stress as well as physical injuries). This result is true because different forms of stress can have different impacts on the reflexes tested,
but considered together, they reflect overall condition. For example, chela closure was the most sensitive indicator of freezing stress observed in this study, but chela function was affected in only 14% of crabs (C. bairdi and C. opilio) injured in trawl capture (Stoner et al., 2008). Impairment of reflex action in eye stalks was also elevated in crabs exposed to freezing temperatures but was rare in trawl-captured crabs. Conversely, leg retraction and leg flaring actions were impaired infrequently under freezing temperatures but were commonly impaired in trawl-captured crabs. Despite these differences, mortality was closely associated with reflex impairment in both the freezing-stress and trawl-stress experiments, and the logistic regressions for C. bairdi and C. opilio in this study were nearly identical. This result indicates that the reflex impairment score is a robust tool for predicting mortality in Chionoecetes species.

Limitations of reflex action mortality predictors

The RAMP approach does have certain limitations because, as with other measures of animal condition, some of the indirect and delayed effects of the discarding process and the behavioral impairments were not considered. 1) Predation on discarded catch can occur in the water column. Marine mammals, large fish, and birds often follow fishing vessels, scavenging injured and uninjured discard. 2) Impairments in defensive behaviors can result in predation once a crab has reached its benthic habitat. Some of these discards would otherwise survive their injuries. 3) Impairments in sensory apparatus, feeding appendages, or locomotory functions (e.g., because of limb damage or autotomy), can result in starvation. Carls and O'Clair (1995) showed that sublethal effects of freezing on C. bairdi can include reduced feeding rate and growth. In addition, mortality observed in laboratory experiments may not reflect the lost function of sensory appendages that aid in locating food or avoiding predators. Eye stalks are particularly susceptible to freezing (van Tamelen, 2005), but it is unknown how vision may be impaired by exposure of crabs to freezing temperatures. The eyes of deep-dwelling decapods can also be damaged by exposure to sunlight (Gaten 1988). Future experiments should be designed to test for losses in vision, chemoreception, and other sensory functions after exposures of crabs to air and freezing temperature. 4) Freezing temperatures can also cause long-term injury resulting in unsuccessful molting (O’Brien et al., 1986). Carls and O’Clair (1995) reported increased limb loss at molting after exposure to freezing, and deaths during molting are a common occurrence in Alaskan crabs (Stoner, pers. observ.). 5) Freezing and other forms of injury may also raise the animal’s susceptibility to disease.

RAMP curves as currently developed do not account for these forms of discard mortality, and do not provide an absolute value for discard mortality. However, it is likely that the last four sources of mortality discussed above will be directly proportional to the reflex impairments observed. Longer term holding or tagging experiments could provide greater insight into the relationships between reflex impairment and long-term survival of discarded animals.

Conclusions

Fisheries for Chionoecetes spp. in Alaska are centered on the winter season, and the threat of cold-related mortality in the face of wind chill conditions is real. One could employ van Tamelen’s (2005) thermal model to obtain crab mortality estimates in the field; however, this would require continuous monitoring of a wide array of environmental conditions as well as crab measurements. In addition, mortality of crabs in the field results from various combinations of physiological stressors and physical injuries (not only thermal stress) for which appropriate mortality rates are not known. A simpler and more direct measure of crab condition and viability is provided by the RAMP approach. Impaired reflexes reveal stress and the composite reflex impairment index allows a calculated probability of mortality that is independent of crab size, physical injuries, and exposure conditions (Stoner et al., 2008; this study). Representative crabs are observed in hand, the reflex actions are summed, and probability of mortality for an individual or a treatment population can be extracted quickly from the RAMP curve with reasonable precision. Once a robust RAMP curve is established, presence or absence of reflex actions are the only observations required to calculate probability of mortality. The approach should be equally applicable to the wide variety of crabs, lobsters, and shrimps that are routinely discarded as unwanted or illegal bycatch, or in instances where an immediate assessment of animal condition or discard mortality is required. Greatest use for the RAMP approach will occur in experiments with fishing gear or handling methods aimed at reducing bycatch or discard mortality.

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