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# Evaluating the flexibility of a reflex action mortality predictor to determine bycatch mortality rates: A case study of Tanner crab (*Chionoecetes bairdi*) bycaught in Alaska bottom trawls

Noëlle Yochum<sup>a,\*</sup>, Craig S. Rose<sup>b,1</sup>, Carwyn F. Hammond<sup>b</sup>

<sup>a</sup> Oregon State University, 104 Nash Hall, Corvallis, OR 97331, USA

<sup>b</sup> Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115, USA

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## ABSTRACT

To quantify total fishing mortality it is necessary to incorporate mortality rates attributed to bycatch, including animals that are discarded and that interact with the gear without being caught. The Reflex Action Mortality Predictor (RAMP) approach has been increasingly used to determine bycatch mortality rates in fisheries. This methodology creates a RAMP that relates reflex impairment to probability of mortality. As the RAMP approach becomes more prevalent it becomes important to evaluate the efficacy of its application. We evaluated the flexibility of this methodology by creating a RAMP for Tanner crab (*Chionoecetes bairdi*) discarded from the groundfish bottom trawl fishery in the Gulf of Alaska and comparing it to a previously established RAMP for unobserved Tanner crab bycatch (encountered gear and remained on the seafloor) from the bottom trawl fishery in the Bering Sea. The two RAMPs and the overall mortality rates calculated using these predictors were comparable. However, we detected significant differences between RAMPs. While probabilities of mortality were similar between the two studies for crab with all or no reflexes missing, discarded crab with intermediate reflex impairment had lower mortality probabilities than those from the unobserved-bycatch study. Our results indicate that a RAMP may produce more accurate mortality estimates when applied to animals experiencing similar stressors as those evaluated to create the RAMP, through similar methodology.

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## 1. Introduction

### 1.1. Reflex Action Mortality Predictor

It is valuable to have a method for evaluating the way fisheries impact their associated ecosystems and for ameliorating any negative impacts to promote sustainability in fisheries. One such impact is bycatch of non-target animals. The process of encountering fishing gear and being captured, exposed to air and sunlight, handled, injured, and left on deck before being returned to the water can be fatal for bycaught animals. Death that results from this process is referred to as “discard fishing mortality”. Mortality can also

result from an animal encountering fishing gear without being captured or from entering the gear and escaping (termed “unobserved bycatch mortality”). Both types of mortality can happen immediately or after a delay. Injuries sustained during these processes may also contribute indirectly to mortality through changes in behavior or impediment to feeding, mating, or defense (He, 2010). As bycatch (unused or unmanaged catch) comprises 40.4% of global marine catches (Davies et al., 2009), it is important to have effective tools to estimate immediate and delayed mortality rates of these non-targeted animals to understand the true impact of a fishery.

There are a variety of tools that have been utilized to estimate rates of bycatch mortality. These tools include direct observation: mark-recapture (Kruse et al., 1994; Trumble et al., 2000; Watson and Pengilly, 1994), acoustic telemetry (Pepperell and Davis, 1999), and captive holding studies (Bergmann and Moore, 2001; Kennelly et al., 1990; Parker et al., 2003). Researchers have also quantified impairment attributed to fishing stressors based on physiological parameters, including metabolic, biochemical, and immune responses (Aparicio-Simón et al., 2010; Bergmann et al., 2001; Broadhurst et al., 2009; Leland et al., 2013; Mercier et al., 2006;

\* Corresponding author. Tel.: +1 916 719 5920; fax: +1 541 737 3590.

E-mail addresses: [noelle.yochum@oregonstate.edu](mailto:noelle.yochum@oregonstate.edu) (N. Yochum), [fishnextresearch@gmail.com](mailto:fishnextresearch@gmail.com) (C.S. Rose), [carwyn.hammond@noaa.gov](mailto:carwyn.hammond@noaa.gov) (C.F. Hammond).

<sup>1</sup> Present address: Fish Next Research, 4707 238 Place SW, Mountlake Terrace, WA 98043, USA.

Parker et al., 2003; Uhlmann et al., 2009). While these methods have proven effective at measuring stress, they have associated disadvantages. For example, sampling methods are invasive to the animal, costly, labor intensive, difficult to perform at sea, or are time consuming, and it is generally difficult to attribute physiological changes directly to stress caused by fishing or to relate these changes to actual mortality (Cooke et al., 2013; Davis and Schreck, 2005; Stoner, 2012a). The drawbacks of these tools can lead to reduced sample sizes or inconclusive results.

The Reflex Action Mortality Predictor (RAMP) is an alternative approach for assessing bycatch mortality (Davis, 2007; Davis and Ottmar, 2006; Stoner, 2012a). RAMP is a methodology that relates reflex impairment to a probability of mortality. This is accomplished by, first, establishing a set of reflexes that are present in a minimally stressed individual and that give a consistent response to stimulation. Animals that are bycaught (either directly during fishing operations or through laboratory simulation) can then be scored by evaluating whether each of these reflexes is present or absent, and summing the number of missing reflexes. If five reflexes are used in the assessment, an individual that is in the healthiest condition would receive a reflex impairment score (“Score”) of zero (i.e., no reflexes are absent) and an individual lacking a response for all five reflexes would receive a Score of five. To relate the Scores to a probability of mortality, Scored individuals are held for a period of time to determine delayed mortality. The relationship between each Score and probability of mortality is then explained with RAMP, a predictor of mortality. Multiplying the probabilities of mortality associated with a given Score by the number of animals with that Score, summing over all Scores, and dividing by the total number assessed generates overall discard mortality rates for a given fishery.

There are many advantages of RAMP for estimating bycatch mortality rates. Rates estimated using RAMP can be applied regardless of environmental or biological factors; the approach is relatively inexpensive; assessments can be done rapidly and with little training (applications for observers and citizen scientists, and involvement of fishermen); and results are generated quickly and reflect physiological damage that cannot be seen (Stoner, 2012a). Advantages of the RAMP approach have made it an increasingly utilized methodology. It has successfully been used on a variety of species, including fishes (Barkley and Cadrin, 2012; Brownscombe et al., 2013; Davis, 2007; Davis and Ottmar, 2006; Humborstad et al., 2009; Raby et al., 2011) and invertebrates (Chilton et al., 2011; Hammond et al., 2013; Rose et al., 2013; Stoner et al., 2008; Stoner, 2012b). In addition to estimating bycatch mortality rates, RAMP can be used as a tool for the development, modification, and evaluation of fishing gear and techniques to mitigate incidental effects of fishing (conservation engineering) through quantifying bycatch mortality rates (Hammond et al., 2013; Rose et al., 2013).

## 1.2. Case study: discarded Tanner crab

Like many fisheries, the management of the Alaska bottom trawl fishery is influenced by bycatch levels and mortality rates generated by the bycatch. Suitable habitat for fish targeted by the bottom trawl industry is similarly favored by Tanner and snow crab (*Chionoecetes bairdi* and *Chionoecetes opilio*), which results in the incidental capture of both species. To mitigate this impact on the crab populations in the Bering Sea and Gulf of Alaska there are regulations on the bottom trawl fishery: no bycaught crab may be retained; fishing gear configurations to reduce crab mortality are required; prohibited fishing grounds were established; and there are crab bycatch limits that, when met, result in the closure of the trawl fishery (NOAA, 2014; NPFMC, 2013a,b). To better inform management decisions regarding restrictions on bottom trawling and to

promote sustainable crab populations and fisheries, it is important to know rates of mortality for bycaught crab, both those discarded and those contributing to unobserved fishing mortality.

Studies have been conducted to estimate both discard and unobserved bycatch mortality rates of Tanner crab in the Alaska bottom trawl fishery. A research study was completed using RAMP to compare mortality rates for unobserved Tanner and snow crab bycatch in the Bering Sea with different trawl gear configurations. Rates were determined by applying RAMP methodology to crab that had encountered a trawl, and subsequently were captured in an auxiliary net behind the trawl immediately after passing under trawl groundgear and then brought on deck for assessment (Hammond et al., 2013; Rose et al., 2013). Assessments from crabs captured with the auxiliary nets, but with no groundgear exposure, were used to account for effects of capture and handling. Assessments were made as soon as possible upon retrieval (less than 15 min) to minimize air exposure, and some crab were held in water prior to assessment.

For discarded Tanner crab, Blackburn and Schmidt (1988) estimated mortality to be 17% in the bottom trawl fishery in the Gulf of Alaska by recording the viability of a subset of crab intended for discard. Viability was based on injuries, missing appendages or mouthparts, and strength of movement. In this study only a portion of the catch was sampled, the results were heavily influenced by one tow, and the methods did not factor in the relationship between viability and mortality. Stevens (1990) estimated mortality rates to be 78% for discarded Tanner crab in the Bering Sea bottom trawl fishery through a holding experiment. This value, however, is outdated given that the study was completed more than two decades ago, using fishing gear that was configured differently than what is currently used, and using fishing practices that have changed (current tow durations and captivity times are shorter). An updated, more systematic estimate of mortality for discarded Tanner crab is needed.

The need for an updated mortality rate estimate of discarded Tanner crab in the Alaska bottom trawl fishery and the existence of a RAMP for unobserved bycatch provided an opportunity to evaluate the flexibility of the RAMP approach. As RAMP use becomes more prevalent, an assessment of the strengths and limitations of this methodology is increasingly important. We contributed to this assessment by evaluating whether a RAMP, once established for a species in a given fishery, can be broadly used for a fishery and for different types of fishing mortality (discard and unobserved bycatch). We did this by creating a RAMP for Tanner crab that are discarded in the bottom trawl fishery in the Gulf of Alaska and then comparing this to the previously established RAMP for unobserved Tanner crab bycatch in the bottom trawl fishery in the Bering Sea (Hammond et al., 2013). From here on, our study will be referred to as the “Discard-mortality” study and the study completed by Hammond et al. (2013) as the “Unobserved-mortality” study.

The comparison between the Discard- and Unobserved-mortality studies will evaluate differences in RAMPs from the same fishery, but for two different types of bycatch (discard and unobserved). There were additional differences between the studies. These include that crab for the Unobserved-mortality study were assessed less than 15 min after being landed on deck. The crab were in air only briefly and were in water for the remainder of that time. The time in water was intended to reduce air exposure, but may have inadvertently served as a recovery period. In contrast, crab from the Discard-mortality study were exposed to air for 90 min on average (range from 9 to 230 min) without time in water.

While overall mortality rates for the two studies are likely to differ given that the two types of bycatch experience different stressors and given that there are typically more mortalities for discards than for animals that escape fishing gear (Broadhurst et al.,

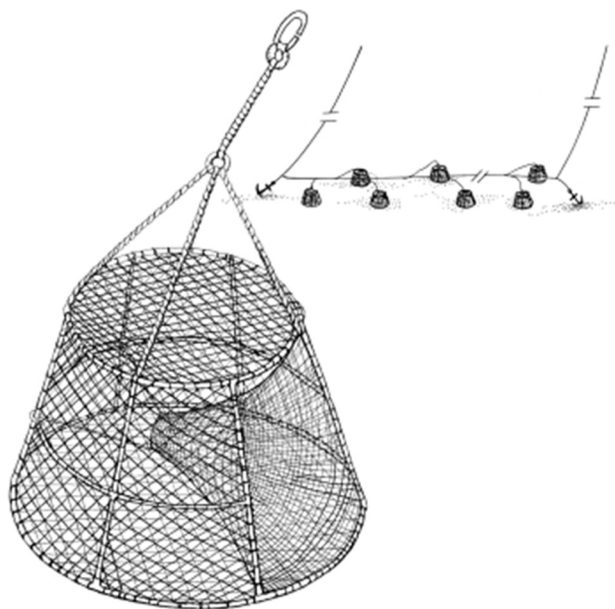
2006), we hypothesized that the relationship between Score for Tanner crab bycaught in the Alaska bottom trawl fishery and probability of mortality would be the same regardless of the type of bycatch (discard vs. unobserved). The goals of this study were to test this hypothesis to determine the extent to which RAMP can be applied once created, to assess components of the RAMP approach to clarify the methodology, and to evaluate the requirements and limitations of RAMP. The more that is known about RAMP the more useful it will be as a tool for promoting sustainability in fisheries by reducing uncertainty associated with bycatch mortality.

## 2. Materials and methods

### 2.1. Collection and assessment of discarded Tanner crab

Data to create a RAMP for Tanner crab discarded in the commercial shallow-water flatfish bottom trawl fishery were collected in May 2011 by scientists during a three-day trip aboard a Gulf of Alaska trawler. Regular commercial fishing operations took place targeting shallow-water flatfish, including rock sole (*Lepidopsetta bilineata*), along with Pacific cod (*Gadus macrocephalus*) and pollock (*Gadus chalcogramma*). For every completed tow, water depth and temperature at depth where the trawl was towed, tow duration, and total catch size were recorded. The crab were subjected to the full range of stressors experienced when bycaught because fishing operations (e.g., fishing gear, tow duration, etc.) were representative of commercial practices. At the point where the Tanner crab would be discarded (i.e., after crab were released from the trawl and sorted out of the catch), each crab was measured (carapace width, mm), and sex, loss of chela, and the condition of the shell were noted. Shell condition was scored from 0 to 5 indicating newer to older shell; 0: individuals that were molting or had recently molted; 1: those that had a soft shell (0–2 weeks after molting); 2: crab that were fully hard; and scores 3–5: crab with old (3) to “graveyard” (5) shell indicating degree of discoloration and encrustation (Jadamec et al., 1999). Shell condition was noted because of findings that crab close to molting may be more susceptible to mortality from trawling (Wassenberg and Hill, 1989), and that softshell crab have higher mortality rates than hardshell crab (Kruse et al., 1994).

In addition, the crab were given a Score based on how many of the six RAMP reflexes established for Tanner crab by Stoner et al. (2008) were absent: leg flare, leg retraction, chela closure, eye retraction, mouth closure, and kick. The Score is equal to the count of the number of negative responses to the reflex assessments, regardless of which of the six reflexes, or combination of reflexes, were absent. It was determined by Stoner et al. (2008) that RAMP for Tanner crab was not significantly improved if the reflexes were evaluated as present, lost, or weak, as opposed to present or absent. They also found that determining what constituted “weak” could be ambiguous. Therefore, we Scored the crab based only on absent reflexes, and only considered the reflex absent when there was a complete lack of reaction to the stimulus. Dead crab were given a Score of six. However, we differentiated between dead crab (immediate mortality) and those that died after the initial assessment (delayed mortality). Assessments, which took less than a minute per crab, did not substantially increase total air exposure. All assessments were done just prior to returning the crab to water. Therefore, assessments were not completed and crab were left on-deck until fishing operations resumed if the catch was left unsorted on-deck for a period of time. This allowed us to assess the crab after experiencing stressors representative of fishing practices. We noted the amount of time each crab spent out of water



**Fig. 1.** Longline sablefish survey pots, modified by the addition of weight to the base and closure of the openings, used as at-sea holding cages for bycaught Tanner crab (*Chionoecetes bairdi*) to determine delayed mortality for the Discard-mortality study. Image courtesy of Alaska Fisheries Science Center (AFSC), NOAA-NMFS.

to include with additional stressors to evaluate their influence on survival.

### 2.2. Determination of delayed mortality for discarded Tanner crab

To establish the relationship between the seven reflex impairment scores (0–6) and probability of mortality, crab were held to determine delayed mortality. All live crab with Scores greater than zero (with the exception of those with apparent parasites or disease) and a large sample ( $n > 75$ ) of Score-zero crab were held. Held crab were tagged at the base of the third walking leg with a cable tie that had an attached RFID chip (Hallprint, [www.hallprint.com](http://www.hallprint.com)), and were kept in plumbed (constant inflow and outflow of sea water), on-board, 100 cm × 68 cm × 58 cm (inside dimensions) holding tanks. The crab were held in these tanks, without food, until the completion of the three-day fishing trip. Daily observations were made to determine if any crab had died. Dead crab were removed from the tanks and number of days until death was recorded. At the end of the fishing trip, before returning to port, all of the surviving held crab were divided into two groups: (1) crab that would be transported to the National Marine Fisheries Service (NMFS) laboratory in Kodiak, AK; and (2) crab that would be put in cages (Fig. 1) and lowered to the sea floor for holding to determine delayed mortality.

### 2.3. Comparison of holding types and duration

We evaluated the RAMP methodology with respect to the influence on delayed mortality of different mechanisms for captive holding. This was done by comparing mortality rates and trade-offs among the different holding types. There is potential for a holding type to contribute to mortality through the spread of disease or parasites, microbial infection, antagonistic interactions between the animals, or stress from captivity. For the Discard-mortality study the first one to three days of holding (depending on when the crab were collected) were in on-board, plumbed holding tanks. Water in the on-board tanks for this study was slightly warmer than the crab would have experienced in their natural environment

(approximately one degree Celsius difference). Subsequently, holding was either in a laboratory tank or in at-sea cages. Holding for the Unobserved-mortality study was entirely in on-board tanks with a difference of several degrees between holding and surface water temperatures. Despite this, previous studies concluded that on-board tanks were a suitable mechanism for holding and did not contribute to crab mortality (Stoner et al., 2008; Stoner, 2009).

We utilized both at-sea holding cages and a laboratory tank subsequent to on-board holding to evaluate if holding type contributes to mortality. The crab were divided so that 28 of those that survived on-board holding, of mixed Score, size, and sex, were taken to the NMFS laboratory in Kodiak, AK, where they were held for 12 days. This number of crab was selected based on laboratory tank capacity. Crab held in the laboratory were kept in a 2479 L, 1.8 m × 0.9 m circular seawater holding tank with constant flowing, unfiltered seawater, maintained at between 6 and 7°C. The crab were fed squid every four days; and the females were kept separate from the males in a floating basket due to size differences. Observations were made daily to see if any of the crab had died and dead crab were removed. The remaining 92 crab that survived after on-board holding were divided into eight modified, longline sablefish pots (Fig. 1; females were kept separate from the males), which were lowered to the sea floor in Kalsin Bay, AK, in an area with suitable crab habitat and segregated from fishing gear. After 11 days the at-sea cages were retrieved and all of the live crab were Scored and then released back into the water.

Holding duration for the Discard-mortality study was determined by logistics (e.g., how long we were able to use the laboratory facilities, etc.) and based on the determination by Wassenberg and Hill (1993) that four days is an adequate holding time when assessing survival of discarded animals from trawl bycatch. They asserted that, while the animals may die over a longer period, delayed mortality beyond this time frame cannot be determined in holding tanks. Studies determining animal survival have spanned a range of holding time: hours (Wassenberg and Hill, 1989), days (Moreira et al., 2011; Uhlmann et al., 2009), and months (Leland et al., 2013; Zhou and Shirley, 1995). Given that long-term captivity can induce stress and/or infections (He, 2010) and can lead to antagonistic interactions among the animals, longer term holding can make it difficult to interpret results. Moreover, laboratory holding may overestimate or underestimate survival given that the animal does not have to endure all variables that may affect its survival (e.g., predation, finding food, etc.). We therefore held crab for two weeks to determine mortality.

## 2.4. Data analysis

### 2.4.1. Reflex Action Mortality Predictors

Logistic regression was used to determine if there was a relationship for discarded Tanner crab between reflex impairment (Score) and mortality (proportion of dead crab). We included fishing and biological variables in the model to determine if these influenced mortality. We performed model selection in R (R Development Core Team, 2011) to determine the most parsimonious logistic model for the data using a backward stepwise model selection technique (dropterm), and drop-in deviance tests.

For model selection we began with a rich model that included explanatory variables: (1) reflex impairment score (a continuous variable equal to the count of absent reflexes, 0–6); (2) sex (male or female); (3) shell condition (0–5); (4) air exposure (amount of time a crab was out of water before assessed, minutes); (5) tow duration (amount of time the trawl gear was towed, minutes); and (6) carapace width (mm, either continuous or binned). In a previous study where bycaught Tanner and snow crab in the Bering Sea bottom trawl fishery were given reflex impairment scores and held,

it was noticed that crab smaller than 90 mm had a higher probability of survival than those above this size for all Scores (Yochum, unpublished data). A similar phenomenon was observed by Rose et al. (2013) where snow crab above 95 mm had higher mortality rates than smaller snow crab regardless of Score. We therefore ran model selection with carapace width as a continuous variable, and separately with binned-widths (“small”: <90 mm; and “large”: ≥90 mm). We did not include water depth or temperature at which the crab were caught, total catch size, or missing chelae as variables due to limited sample size. Explanatory variables were included in the logistic model. In addition, we used drop-in deviance tests to determine if interactions between the explanatory variables and Score significantly improved model fit.

We repeated the logistic regression model selection analysis on the Unobserved-mortality study data and on those data combined with the Discard-mortality study data. For the Unobserved-mortality and combined datasets, air exposure was not included in the full model. For the combined data, an additional variable that differentiated the studies (“Study”) was included in the full model to test for a difference, after accounting for other variables, in the probability of mortality between Discard- and Unobserved-mortality studies for Tanner crab. We also used a drop-in deviance test to determine if an interaction between Score and Study improved model fit, indicating that, after accounting for other variables, the relationship between probability of mortality and Score depends on the study.

The final RAMP that was selected by Hammond et al. (2013) for the Unobserved-mortality study was the actual proportion of held crab that died in captivity for each Score (i.e., not fitted with a model; “discrete RAMP”) rather than a continuous logistic model determining probability of mortality by Score (“logistic RAMP”). To directly compare with that selected model we also created a discrete RAMP for the Discard-mortality study crab. We tested for differences between the probabilities of mortality for each Score for the Discard- and Unobserved-mortality studies with a Fisher’s exact test. A Mantel–Haenszel test was used to determine if there were significant differences in the proportion of those that died between studies.

### 2.4.2. Estimation of mortality rates

To evaluate the potential for different RAMPs to affect the estimation of bycatch mortality rates, we used the discrete and logistic RAMPs from both the Discard- and Unobserved-mortality studies to estimate a mortality rate for our research trip. This was done by multiplying the probabilities of mortality associated with a particular RAMP for each Score by the number at that Score, summing over all Scores, and dividing by total crab assessed.

## 3. Results

### 3.1. Assessment of discarded Tanner crab

Tanner crab were assessed and collected aboard the F/V *Sea Mac* during six fishing tows south of Kodiak Island, AK, at an average depth of 52 fathoms. During our research trip a total of 261 Tanner crab were captured in the trawl gear, landed, and assessed; 12 died before assessment (immediate mortality), and 153 were held to determine delayed mortality. Of the bycaught crab, males were predominant (92%), and had, on average, lower Scores than female crab. The average Score for female crab was three, and one for males. Similarly, only 32% of female crab were Score-zero, compared to 71% for males. Bycaught crab ranged in size from 74 to 171 mm, with females being smaller on average (89 mm) than the average male crab (142 mm). The majority of crab had old shells (Shell Condition 3, 78%) and were Score-zero (67% of crab caught).

**Table 1**

Number of Tanner crab (*Chionoecetes bairdi*) from the Discard-mortality study research trip missing each reflex when only one reflex was absent. This is considered to be the first reflex lost (left column). The percent of total losses (right column) is the percentage of all lost reflexes attributed to each reflex. For this analysis Score-six crab (those with all reflexes absent) were not included.

Reflex	1st reflex lost n = 7	% of losses n = 161
Leg retraction	4	34
Leg flare	1	31
Chelae closure	2	17
Eye retraction	0	1
Mouth closure	0	3
Kick	0	14

Of the six RAMP reflexes, leg retraction and leg flare were those most often lost, while eye retraction and mouth closure were most seldom lost. When only one reflex was lost it was most frequently leg retraction (Table 1).

### 3.2. Comparison of holding types and duration

Delayed mortality for our study occurred for 33 crab during holding in the on-board tanks, followed by 4 crab in the laboratory tank and 3 crab in the at-sea cages (68% of held crab survived until the end of the study). In the laboratory setting, deaths occurred for up to 12 days (total holding, including in on-board tanks). However, 86% of all mortalities occurred within the first day of holding, and 92% within the first two days. Score-zero crab died at a slower rate than crab with higher Scores (Fig. 2). Of the six Score-zero crab that died in holding, three were held in on-board tanks and died after one day of holding, and three died in the laboratory tank after 2,

**Table 2**

Results from binary logistic modeling for the Discard-mortality study data and that combined with the Unobserved-mortality study data ("Combined"), including estimates, standard errors (SE), and *P*-values for the intercept and coefficients of the explanatory variables. The most parsimonious model was determined by backward model selection and drop-in deviance tests. The logistic reflex action mortality predictor (RAMP) was created from data that included a reflex impairment score (Score) equivalent to the total number of reflexes absent out of six reflexes. Binned width separates carapace width into two bins: "small" (<90 mm) and "large" (≥90 mm).

	Estimate	SE	<i>P</i> -value
<b>Discard-mortality study parameters</b>			
Most parsimonious model			
Intercept	-2.60	0.36	4.55E-13
Score	0.82	0.12	1.61E-12
Alternative model			
Intercept	-2.60	0.36	9.60E-13
Score	0.87	0.13	3.72E-12
Binned-width:Small	-1.81	0.95	0.06
<b>Combined study parameters</b>			
Most parsimonious model			
Intercept	-2.59	0.36	6.72E-13
Score	0.83	0.12	9.59E-13
Binned-width:Small	-0.65	0.26	0.012828
Study: Unobserved-mortality	0.46	0.41	0.263821
Score * Study	0.50	0.15	0.000532

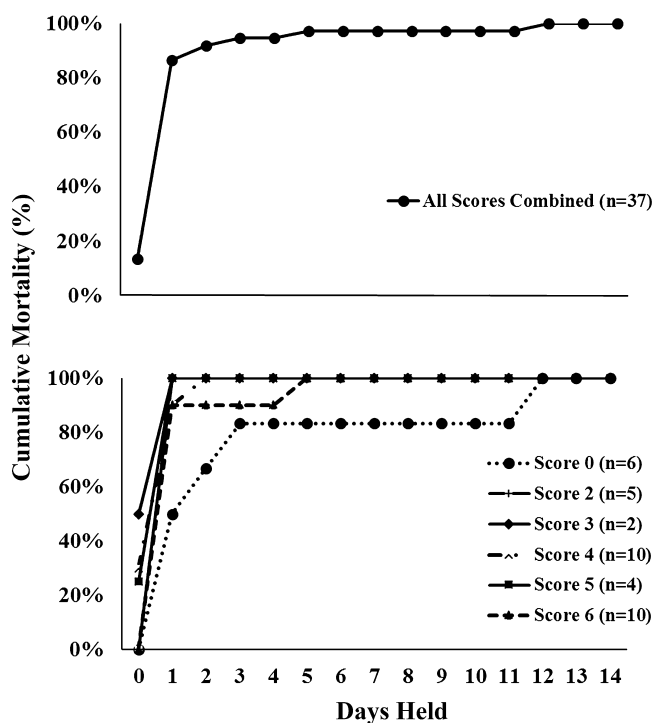
and 12 days of total holding. In the at sea cages, three crab died (Scores 1, 2, and 6), and none were Score-zero. Days until death for crab held in the at-sea cages could not be determined. Moreover, the three crab that were found dead in the at-sea cages had been consumed by amphipods. Without video footage it was not possible to verify whether these crab died from fishing stressors before predation commenced. However, we made the assumption that the mortality occurred before predation given that only crab with Scores greater than zero were eaten.

### 3.3. Reflex Action Mortality Predictors

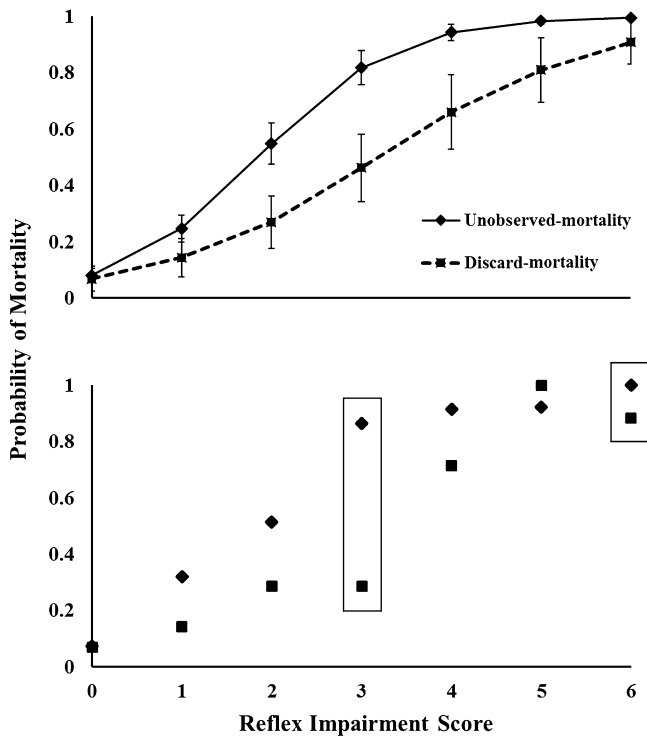
Logistic regression model selection and drop-in deviance tests determined that the most parsimonious model for Tanner crab from the Discard-mortality study included only Score as a predictor (Table 2). This indicates a relationship between Score and probability of mortality where each one unit increase in Score is associated with an increase in the odds of mortality (the ratio of the probability of mortality to the probability of survival) by 2.26 (95% Confidence Interval 1.80–2.83, logistic regression; Table 2). There was not convincing evidence that, after accounting for Score, binned or continuous width significantly improved model fit (*p*-values 0.05 and 0.24, respectively, drop-in deviance tests).

A comparison of the logistic RAMP for the Discard- and Unobserved-mortality studies (Fig. 3) with Score as the only predictor indicated that the probabilities of mortality were lower for all Scores for the Discard-mortality study. This is further highlighted by the fact that Scores two through six did not have overlapping 95% Confidence Intervals, and that the Score at which a crab has a 50% or greater probability of mortality (Score<sub>50</sub>) is Score-three for the Discard-mortality study, and Score-two (1.9) for the Unobserved-mortality study. Moreover, logistic model selection on the combined datasets determined that the most parsimonious model included Score, binned-width, Study, and an interaction between Study and Score as predictors (Table 2). The significant interaction indicates that Study has a measurable influence on the relationship between Score and probability of mortality, after accounting for binned-width. Results from the logistic regression analyses indicate significant differences between RAMPs for the Discard- and Unobserved-mortality studies.

A comparison of the discrete RAMPs (the actual proportion of held crab that died in captivity for each Score) between the



**Fig. 2.** Mortality curves for the Discard-mortality study indicating the cumulative percent of bycaught Tanner crab (*Chionoecetes bairdi*) that died per number of days of holding in on-board holding tanks (0–3 days, 33 crab) or in a laboratory tank (11 days following on-board holding, 4 crab) for all reflex impairment scores ("Score") combined (above), and individually (below). These curves do not include immediate mortality (12 crab) or mortality in at-sea cages (3 crab out of 92 held, Scores-one, two, and six) as the day on which the crab died is unknown. "Days Held" was calculated as the difference between the date on which the crab died and was captured, regardless of time of day. Total mortalities by Score are indicated in parentheses.



**Fig. 3.** Logistic reflex action mortality predictors (RAMPs, top) with 95% confidence intervals for the Discard- and Unobserved-mortality studies for Tanner crab (*Chionoecetes bairdi*) in the Alaska bottom trawl fishery relating probability of mortality to reflex impairment score (Score). The discrete RAMPs for both studies are shown (bottom) with boxes indicating significant differences in probability of mortality between Scores based on results from a one-way Fisher’s exact test ( $p < 0.05$ ). Number of crab held to determine delayed mortality by Score for all holding types combined was 86, 7, 21, 7, 14, 4, and 14 (Scores 0–6, respectively).

Discard- and Unobserved-mortality studies (Fig. 3) indicate probabilities of mortality are significantly higher for the Unobserved-mortality study for Scores three and six (one-sided  $p$ -values 0.004 and 0.0002, respectively, Fisher’s exact test). Moreover, there was convincing evidence of a higher proportion of mortalities for the Unobserved-mortality study than would have been expected if the odds of mortality were equal for both studies, after controlling for Score (one-sided  $p$ -value 0.0002, Mantel–Haenszel test). Comparable to the logistic RAMP, a comparison of discrete RAMPs revealed similarity between studies at high and low Scores, but divergence at intermediate Scores.

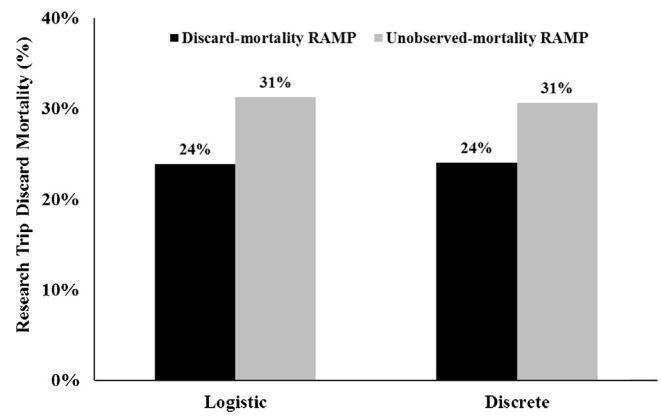
3.4. Estimation of mortality rates

To evaluate how different RAMPs affect the estimation of bycatch mortality rates, we calculated rates for our research trip in the Gulf of Alaska using both the logistic and discrete RAMPs from both the Discard- and Unobserved-mortality studies. For both studies the logistic and discrete RAMPs estimated the same mortality rates. For the research trip, the discard mortality rate was estimated to be 24% from the Discard-mortality study RAMPS, and 31% from the Unobserved-mortality study RAMP (Fig. 4).

4. Discussion

4.1. Comparison of holding types and duration

There were differential mortality rates by holding type. Higher mortality rates occurred in the on-board tanks (where the crab were held for the first few days) and in the laboratory tank. Moreover, Score-zero crab died in the holding tanks, but not in the at-sea



**Fig. 4.** Mortality rate estimates for the research bottom trawl fishing trip in the Gulf of Alaska from this study, using the logistic RAMP and discrete RAMP (the actual percent of crab that died per reflex impairment score, “Score”, without modeling) from both the Discard- and Unobserved-mortality studies.

cages. These results indicate that holding tanks contribute additional stressors, either due to transport, additional handling, or stress from being held in an unnatural setting or at temperatures greater than what was experienced in their natural environment.

Our holding duration of two weeks was sufficient to determine mortality for all Scores. Given that it can take longer for Score-zero animals to die than those with higher Scores (Fig. 2) our holding period allowed us to sufficiently capture Score-zero mortalities. However, the death of a Score-zero crab at day 12 may indicate that holding for more than a week confuses mortality attributed to fishing stressors with that from captivity.

4.2. Evaluation of RAMP flexibility

Despite remarkable similarities between the Discard- and Unobserved-mortality RAMPs, we feel that differences in probabilities of mortality for the intermediate Scores and in mortality rate estimates for the Discard-mortality research trip indicate that the RAMPs from these studies should not be used interchangeably. To evaluate the divergence between the RAMPs we analyzed the differences between the studies. The primary difference was in experimental methods, namely the treatment of the crab before assessment. Crab from the Discard-mortality study were exposed to air for 90 min on average (range from 9 to 230 min) without any “recovery” in water. In contrast, crab from the Unobserved-mortality study had only brief air exposure and were held in water while awaiting assessment (generally less than 15 min), which may have allowed some recovery.

These differences in air exposure and recovery in water probably affected the relationship between observed reflex impairments and delayed mortality and hence accounted for the discrepancy between RAMPs. Prolonged air exposure and experiencing cold temperatures was linked with increased delayed and instant mortality, number of autotomies for crab, as well as reduced vigor, juvenile growth, and feeding rates (Carls and O’Clair, 1995; Giomi et al., 2008; Grant, 2003; Stoner, 2009; Warrenchuk and Shirley, 2002). Stoner (2009) found that reflex impairment score and exposure to freezing temperatures were nearly linearly related for Tanner crab. Moreover, he found that the different RAMP reflexes had variable sensitivity to freezing temperatures, namely that the chela closure reflex was the most sensitive reflex, and mouth closure was least. Similarly, Van Tاملen (2005) found that the legs and eyes of snow crab cooled faster than the body, perhaps making them more susceptible to cold air exposure. We hypothesize that the prolonged air exposure for the Discard-mortality study likely impaired the crabs’ reflexes and resulted in higher Scores.

**Table 3**  
A comparison of three types of holding for determining delayed mortality of bycaught animals when using the reflex action mortality predictor (RAMP) approach.

Holding type	Advantages	Disadvantages
On-board tanks	<ul style="list-style-type: none"> <li>• Can be used during fishing operations</li> <li>• Is relatively inexpensive</li> <li>• Allows for easy monitoring</li> </ul>	<ul style="list-style-type: none"> <li>• Cannot easily regulate water temperature or quality</li> <li>• Subjects animals to vessel's motion</li> <li>• Requires deck space and plumbing</li> <li>• Requires monitoring and maintenance</li> </ul>
Laboratory tanks	<ul style="list-style-type: none"> <li>• Can regulate water temperature and quality</li> <li>• Can control the environment</li> <li>• Allows for easy monitoring</li> </ul>	<ul style="list-style-type: none"> <li>• Requires transport and additional handling of the animals</li> <li>• Cannot be used during fishing operations</li> <li>• Requires a wet-laboratory near a fishing port</li> <li>• Requires monitoring and maintenance</li> </ul>
At-sea cages	<ul style="list-style-type: none"> <li>• Can be used during fishing operations</li> <li>• Provides more natural holding conditions</li> <li>• Does not require monitoring, feeding, or maintenance</li> </ul>	<ul style="list-style-type: none"> <li>• Requires additional handling of the animals</li> <li>• Prohibits knowing when or how an animal died (unless cameras are used)</li> <li>• Can become lost</li> <li>• Must be retrieved</li> <li>• Makes animals vulnerable to predation</li> </ul>

Mortality for the Unobserved-mortality study could have been influenced by including a “recovery” period in water before assessment. Stoner (2009) found that recovery in water before assessment can change the reflex impairment score and that RAMP was more successful in predicting mortality when assessments were made subsequent to exposure than after a period of soaking in water to recover. Methodological differences between the Discard- and Unobserved-mortality studies in stressors imposed on and treatment of the crab before assessment may have resulted in dissimilar relationships between Score and mortality. Further study is needed to directly evaluate the influence of air exposure and recovery in water on reflex impairment scores.

We evaluated additional methodological discrepancies between the Discard- and Unobserved-mortality studies to determine if there were additional variables that may have contributed to the between-study variability in RAMP, including that data from the Unobserved-mortality study (1) were collected by different scientists who made judgments regarding presence or absence of reflexes (although there was overlap in scientists making assessments between the two studies); (2) included information on the presence of visible injuries on the crab; (3) were based on holding only in on-board holding tanks (as opposed to a laboratory tank or at-sea cages following on-board holding for the Discard-mortality study); and (4) were from tows that lasted for only 10–20 min (Hammond et al., 2013). Tows for the Discard-mortality study lasted for over 3 h on average.

With respect to (1) different scientists making the assessments, subjectivity error should be considered when results are based on assessments (Benoit et al., 2010). Regardless, we do not feel that the difference in RAMPs was attributed to a difference in scientists making the assessment given that the reflexes are determined to be fully absent or present, which reduces subjectivity in assessment. With respect to (2) injury affecting survival, it has been shown that lost limbs, removal of dactyli, damaged chelipeds, and wounds, especially with continuing loss of hemolymph, can lead to mortality (Kennelly et al., 1990; Uhlmann et al., 2009). It has also been shown that injured Tanner crab have higher mortality rates than uninjured crab (MacIntosh et al., 1996) and large crab (those over 90 mm carapace width) do not regenerate limbs (Miller and Watson, 1976), indicating that autotomy can be a permanent injury and could impede movement, mating, and/or predator avoidance. We do not feel, however, that our exclusion of injuries from the model affected the difference between RAMPs given that in analyzing a subset of the Unobserved-mortality study data Stoner et al. (2008) determined that injury had only a small influence on probability of mortality and Hammond et al. (2013) found similar results and did not include injury in their final RAMP for Unobserved-mortality. We therefore ruled out injury as being a contributing factor to differences between RAMPs.

We similarly ruled out holding type (3), because the majority of mortality, for both studies, occurred while the holding mechanism was the same (on-board holding tanks). Only 13% of mortalities from the Discard-mortality study occurred when crab were held in the laboratory tank or at-sea cages. Moreover, while survival of animals can be affected by trawl tow duration (Ridgway et al., 2006; Stevens, 1990), we did not feel that this was a contributing factor to the differences between the two studies given that it did not improve the logistic model for determining mortality. We determined that none of these additional methodological differences contributed to the discrepancy in RAMPs for the Discard- and Unobserved-mortality studies. Rather, we suspect that differences in RAMPs between studies were likely attributed to differences in the stressors experienced by the crab before assessment (i.e., air exposure and recovery in water).

## 5. Conclusions and recommendations

### 5.1. Comparison of holding types and duration

We recommend that the holding method employed for a RAMP study be determined based on the goal of minimizing stress experienced by the study animals within the logistic constraints of the study (Table 3). For example, if there are no wet-laboratory facilities near the fishing port then it is preferable to use on-board holding or at-sea cages to minimize time that the animal spends out of water and in transport. Similarly, if the fishing trips associated with a fishery of interest are long in duration, it is not feasible to bring animals to a laboratory and would therefore require on-board holding or at-sea cages depending on available deck space and plumbing, and ability to return to a location to retrieve the cages. Moreover, we recommend evaluating the mortality of Score-zero and, when possible, control animals as a potential indicator of stressful holding conditions. For this study, 5 days was sufficient time to determine delayed mortality for Tanner crab with all Scores, and was short enough that it did not confuse mortality attributed to fishing stressors with that attributed to holding for longer periods.

### 5.2. RAMP selection for discarded Tanner crab

The RAMP selected to model the relationship between reflex impairment score and probability of mortality for Tanner crab discarded from the bottom trawl fishery in the Gulf of Alaska is the logistic model with the only predictor being Score, which was calculated by summing the number of reflexes absent out of six possible reflexes (0–6). Despite the similarity in mortality estimates, the logistic RAMP, rather than the discrete RAMP, was selected given the small sample size of crab with intermediate Scores that were held in the Discard-mortality study. With few crab held, the death



of a single crab has more influence on the shape of the RAMP than if the sample size was large. This, however, is mitigated better with a logistic relationship than discrete. Because of the large sample size, the logistic and discrete RAMPs are more similar for the Unobserved-mortality study and either can be used. The discrete RAMP was selected, however, for the Unobserved-mortality study because sample sizes at each Score were considered sufficient to support Score-specific estimates, reducing the requirement for a general model (Hammond et al., 2013).

Binary logistic regression was a good predictor of delayed mortality independent of fishing or biological predictor variables (e.g., sex and carapace width). These variables did not significantly improve model fit and were therefore not included in the RAMP model. Regardless, it is worth noting the possibility that small crab (<90 mm) may survive at a higher rate than larger crab with the same Score and, therefore, that mortality for these crab may be overestimated with our selected RAMP. The reason for reduced mortality of the small crab is unknown, but Miller and Watson (1976) found that snow crab >90 mm did not regenerate limbs in captivity. This may indicate that large *Chionoectes* crab have a reduced ability to recover. Nonetheless, we feel that our RAMP can be applied regardless of size.

The RAMP selected for discarded Tanner crab can be utilized to estimate discard mortality rates for the Alaska bottom trawl fishery, given that assessed crab experience stressors similar to the crab assessed to generate this RAMP. A mortality rate for the fishery should not be based on this one trip, but should be calculated from data on reflex assessments gathered during a number of trips that incorporate the variability of the fishery.

### 5.3. Evaluation of RAMP flexibility

Results from this study indicate that bias can be introduced in mortality rate estimates when using a RAMP created for one study to estimate mortality rates for a different study where the experimental methods differ, especially with respect to air exposure and recovery in water before assessment. However, when RAMP is used only to approximate mortality rates or to make comparisons between gear types or uses, a previously established RAMP could be used with caution, especially if animals with intermediate Scores are not predominant. For more accurate bycatch mortality rate estimates, our results indicate the importance of using a RAMP that was created by assessing animals that experienced similar stressors to those which the RAMP will be applied. Namely, the procedure for assessing the animals should be similar. We feel that the amount of time the animal spends out of water before assessment be standardized within a time range, along with whether or not the animal is allowed to recover in water before assessment, unless these variables are the treatments being studied.

These conclusions are further supported by Stoner's research on mortality rates of Tanner crab attributed to freezing stress (2009). In this study, Stoner created a RAMP to predict mortality for Tanner and snow crab exposed to different treatments of temperature stress (cold) and air exposure. The RAMP generated from this research was dissimilar to both the Discard- and Unobserved-mortality studies (Fig. 5), again highlighting the importance of applying RAMP under conditions that correspond with those applied when the RAMP was created.

Our results indicate that consistency in methodology and relevance with respect to mimicking actual fishing stresses for the RAMP approach increases the flexibility of RAMP. It is therefore important, when creating a RAMP, to create repeatable methods that are well documented when publishing. RAMP reflexes should be assessed in a specified order to prevent bias from reflexes that are physiologically linked. If there is a reflex that influences the determination of other reflexes it should be assessed last or not

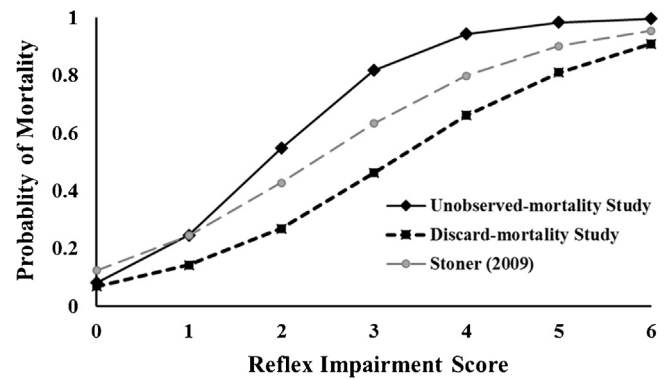


Fig. 5. Logistic reflex action mortality predictors (RAMPs) for the Discard- and Unobserved-mortality studies and that completed by Stoner (2009) for Tanner crab (*Chionoectes bairdi*) in the Alaska bottom trawl fishery relating probability of mortality to reflex impairment score.

at all. Reflexes that are difficult to determine presence or absence should not be used, and it should be clear in the methods what constitutes an “absent” reflex and how immediate mortalities are treated (are they given a Score or classified separately?). In addition, when a RAMP is being created, data should be recorded on all possible stressors, including injury, and evaluated for their contribution to mortality. Moreover, effort should be made (within the logistical constraints of field and laboratory research) to minimize additional stressors that are unrelated to the fishing stressors of interest.

Despite the incomplete flexibility that we discovered when comparing RAMPs for the Discard- and Unobserved-mortality studies, we feel RAMP is a powerful and effective methodology for estimating and evaluating bycatch mortality. With improved understanding of this methodology, RAMP will be increasingly useful as a tool for quantifying discard mortality and consequently promoting fisheries sustainability.

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