AN ABSTRACT OF THE DISSERTATION OF

Noëlle Yochum for the degree of Doctor of Philosophy in Fisheries Science presented on May 20, 2016.

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All animals that interact with fishing gear are not necessarily captured, and all animals that are captured are not necessarily retained. Fishing practices and gear configuration, management regulations, and markets dictate which animals ultimately are retained or discarded. The impact of a fishery and the efficacy of management regulations can depend on the mortality rate of the animals that interact with the gear or are discarded. The Reflex Action Mortality Predictor (RAMP) is a simple, non-invasive, and inexpensive approach that has been used to evaluate this component of fishing mortality. The RAMP approach relates the degree of reflex impairment in an animal to the probability the animal will die. Since its introduction in 2006, the RAMP approach has been utilized in the U.S. and abroad to evaluate mortality for a variety of species, fishing gear types, and stressors. Although there have been numerous applications of the RAMP approach in mortality estimation studies, there has been limited research to directly evaluate RAMP estimates and some skepticism remains in the fisheries science and management communities about the reliability and accuracy of the approach. The goal of this dissertation was to conduct research to assess RAMP and to synthesize findings from previously completed RAMP studies.

The three research studies described in this dissertation consider: (1) the accuracy of applying an established relationship between reflex impairment and mortality

probability to predict overall mortality attributed to novel stressors; (2) the development and utilization of a RAMP relationship to evaluate discard mortality in a fishery with management regulations that mandate discarding of certain categories of animals; and (3) whether the RAMP approach produces accurate estimates of mortality if survival is determined through laboratory captive holding.

The first study estimated a relationship between reflex impairment and mortality probability for Tanner crab (*Chionoecetes bairdi*) discarded from the groundfish bottom trawl fishery in the Gulf of Alaska. This relationship was then compared to one previously established for Tanner crab in the Bering Sea bottom trawl fishery that encountered the fishing gear, but remained on the seafloor ('unobserved bycatch'). While mortality probabilities were similar between the two studies for crab with no or full reflex impairment, discarded crab with intermediate levels of reflex impairment had lower mortality probabilities than those from the unobserved bycatch study. Results from this study indicate the importance of describing all stressors to which animals are exposed and detailing the study methodology when initially creating a RAMP relationship. Failure to do so may result in inaccurate mortality estimates when the RAMP is applied to animals exposed to stressors not included in the original calibration.

The second study developed a RAMP relationship using laboratory captive holding for Dungeness crab (*Cancer magister*) discarded in the Oregon commercial and recreational Dungeness fisheries and estimated that the discard mortality rate is lower than previously determined. This supports the goal of the '3-S' management strategy currently employed for these fisheries to protect sub-legal males (Size), females (Sex), and soft-shell (Season) crab by discarding them from the catch. For the commercial ocean Dungeness fishery, the estimated overall discard mortality rates (five days after release) varied by sex and shell-hardness, and reflex impairment was a significant predictor of mortality for both the commercial and recreational fisheries. In addition, results indicated that, when evaluating the role of discard mortality in '3-

S' management with respect to fishery impact and sustainability, it is important to look not only at mortality rates, but also at the mortality- and bycatch- per retained ratios, and temporal trends relative to changes in effort, animal condition, and catch composition. This study also highlighted the (*i*) importance of evaluating the influence of biological, environmental, and fishing variables on mortality, (*ii*) complications that arise when establishing a RAMP relationship for a low impact fishery, and (*iii*) limitations of determining mortality through laboratory captive holding.

The third study used mark-recapture methods to evaluate the reliability of results generated using the RAMP relationship established in the second study, which was based on the survival of crab held in captivity in the laboratory. Given the unnatural conditions for determining survival in captivity and the short-term duration of the experiment, mortality probability estimates may be biased. Similarities in patterns of relative survival rates between the studies lend support to the ability of the RAMP relationship to estimate discard mortality rates using captive holding. The laboratory-based RAMP approach was superior in its ability to provide direct estimates of mortality rates, whereas the mark-recapture study was limited to providing relative survival rates between reflex impairment levels that were imprecise due to low numbers of recaptured crab. This study highlighted the complications associated with tagging discarded animals and conducting a RAMP study with a fishery that has highly variable seasonal fishing effort.

A synthesis of the research described in this dissertation and published work by other researchers highlights the limitations of the RAMP approach so that future researchers can avoid pitfalls in its application, and leads to suggestions on how to standardize some of the methodological steps. This analysis aims to increase the reliability of future RAMP studies and their production of high quality estimates of discard mortality rates that promote sustainable fisheries.

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Evaluating the Efficacy of Predicting Bycatch Mortality Using Reflex Impairment through an Assessment of Crab Discards

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Noëlle Yochum, Author

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CONTRIBUTION OF AUTHORS

Dr. Craig Rose was involved in project design, overview, and assessment, and editing for Chapters 2-4, and was involved with data collection for Chapter 2. Carwyn Hammond provided data, assisted with data collection, and was involved in project assessment for Chapter 2. Dr. Allan Stoner was involved in project design, overview and assessment, and editing for Chapters 3 and 4. Dr. David Sampson was involved in project overview, assessment, and data analysis, and editing for Chapters 3 and 4. Alan Pazar and Robert Eder provided insight into project design, assisted with data collection, and participated in project assessment for Chapter 3.

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Dedicated to my husband, Ben, and to those,

like him, who support moms in science

CHAPTER 1

THE REFLEX ACTION MORTALITY PREDICTOR AS AN APPROACH FOR EVALUATING BYCATCH MORTALITY

1.1 Research Overview

While fishing gear selectivity varies by fishery and gear type, all fisheries are susceptible to bycatch; the fate of which is largely determined by management regulations. Bycatch comprises animals that encounter the gear and die as a result (‘unobserved bycatch mortality’) and those that are captured, but are not retained. The latter are discarded because either they lack commercial value or are not economical to retain, are protected, are not the target size or sex, or have been landed after a catch quota has been met (Cook 2003). ‘Discard fishing mortality’ and/ or injury can result from the handling and discard process for these non-target animals. Both discard- and unobserved- mortality can happen immediately or after a delay. Encounters with fishing gear and discard practices can also contribute to mortality indirectly through impairment to feeding, mating, or defense behaviour (Juanes and Smith 1995, Davis and Ottmar 2006, He 2010, Wilson et al. 2014).

Bycatch mortality rates support fisheries management by providing a metric for measuring successful adherence to laws requiring bycatch mitigation (16 U.S.C. § 1801-1981 2007), and they factor into stock assessments and the understanding of how a fishery impacts the ecosystem and endangered or threatened animals. Having an understanding of bycatch mortality also allows for an evaluation of the efficacy of the fishing gear and practices being employed. In addition, animals taken as bycatch in one fishery can be targeted in another, thereby linking the profitability and sustainability of the two fisheries. Along these lines, knowing mortality rates of discarded animals can inform managers about strategies for managing non-target catch with respect to sustainability and maximizing economic gains (e.g., bycatch quotas and catch restrictions). To do this, trade-offs between bycatch mortality rates and harvest (i.e., 100% probability of mortality) must be evaluated (Benaka et al.

2014, 2016). For example, the European Union (EU) created regulations that prohibit discarding. This Landing Obligation (LO), which began in 2015 and will be fully phased in by 2019, necessitates full catch retention for a list of vertebrate and invertebrate species caught by a variety of fishing gears. While the LO aim is to radically cut discards, it also imposes 100% mortality for any specified animal captured, even those likely to survive post-discard. To address this, the 'high survival' exemption was enacted, allowing for discarding of animals with low discard mortality rates (STECF 2015). To facilitate the evaluation of potential 'high survival' species for the EU, and to address management requirements with respect to bycatch in general, it is important to have an effective, reliable, and respected method to quantify bycatch mortality rates.

To evaluate these rates, a variety of methods have been employed, including direct observation and physiological assessments. Methods of the former include mark-recapture (Kruse et al. 1994, Watson and Pengilly 1994, Trumble et al. 2000), acoustic telemetry (Pepperell and Davis 1999, Yergey et al. 2012), and captive holding (Kennelly et al. 1990, Bergmann and Moore 2001, Parker et al. 2003). Impairment attributed to fishing stressors has also been assessed through measuring physiological changes (e.g., metabolic, immune, and stress responses; Crear and Forteath 2001, Parker et al. 2003, Mercier et al. 2006, Ridgway et al. 2006, Lorenzon et al. 2008). Obtaining samples, however, to detect these changes can be invasive to the animal, costly and labor intensive, and/or difficult to perform at sea. More importantly, it is difficult to attribute physiological changes directly to fishing stressors, or to use these changes to quantify mortality rates (Davis and Schreck 2005, Stoner 2012b, Cooke et al. 2013). Similarly, it can be difficult to collect direct observation data that are representative of a fishery or are able to predict delayed mortality, and direct observation methods are subject to bias based on the animals selected for holding or tagging.

An alternative approach for quantifying bycatch mortality that remedies some of the concerns with the aforementioned approaches is the Reflex Action Mortality Predictor (RAMP; Davis and Ottmar 2006, Davis 2007, Stoner 2012b). RAMP relates impairment in reflex actions to the probability of mortality. This is accomplished by, first, establishing a set of reflexes (i.e., involuntary responses to a stimulus) that are present in a minimally stressed individual and that give a consistent response to stimulation. Animals enduring the stressor(s) of interest (e.g., discard; either directly during fishing operations or through laboratory simulation) can then be evaluated by determining whether each of these reflexes is present or absent. To relate the levels of impairment to the probability of mortality, mortality rates are determined through captive holding in situ (e.g., net pens or cages) or in a controlled, laboratory setting, or though mark-recapture or telemetry. The relationship between impairment level and probability of mortality is then explained with a RAMP relationship, a predictor of mortality (Davis and Ottmar 2006).

Since the inception of RAMP, its advantages have become evident and the methodology has increasingly been applied, which has both expanded its potential for application and highlighted variation within the approach. Applications, with respect to fishing stressors, have included evaluating the effects of environmental variables, including: (1) cold temperature exposure (Stoner 2009, Szekeres et al. 2014, Urban 2015); (2) air exposure (Davis 2007, Humborstad et al. 2009, Stoner 2012a, Barkley and Cadrin 2012, Donaldson et al. 2012, McArley and Herbert 2014, Brownscombe et al. 2015, Bower et al. 2016); (3) water temperature (Danylchuk et al. 2014, McArley and Herbert 2014, Brownscombe et al. 2015); (4) wind speed (Urban 2015); and (5) capture depth (Hochhalter 2012). Applications also have been made to assess biological variables, including: (6) barotrauma (Campbell et al. 2010a) and (7) the ability of a fish to attain refuge (Brownscombe et al. 2014). Moreover, RAMP has been utilized to evaluate fishing gear configuration and use, including: (8) duration of gear interaction or use (Davis 2007, Humborstad et al. 2009, Barkley and Cadrin 2012, Donaldson et al. 2012, Danylchuk et al. 2014, McArley and Herbert 2014, Uhlmann et al. 2015, Bower et al. 2016); (9) encounter with bottom trawl gear (Stoner et al. 2008, Rose et al. 2013); (10) injury from fishing gear or handling (Davis and Ottmar 2006, Humborstad et al. 2009, Stoner 2012a, Nguyen et al. 2014); (11) handling duration (Raby et al. 2012); and (12) gear modification or alternatives (Hammond et al. 2013, Humborstad et al. 2016). Lastly, RAMP has been applied to studies evaluating post-release survival, including (13) facilitated recovery (e.g., reuperation in water before release; Hochhalter 2012, LeDain et al. 2013, Nguyen et al. 2014) and (14) discard practices (Davis 2007, Diamond and Campbell 2009, Campbell et al. 2010b, Raby et al. 2012, Braccini et al. 2012, Barkley and Cadrin 2012, Depestele et al. 2014, Gallagher et al. 2014, Nguyen et al. 2014, Danylchuk et al. 2014, Urban 2015, Uhlmann et al. 2015). Reflex impairment also was correlated with the effect of triclosan (an antibacterial compound) on Atlantic croaker (*Micropogonias undulatus*) (Hendrick-Hopper et al. 2015). These studies were conducted on a variety of fish (round and flat), invertebrate, and chondrichthian species, captured using a variety of fishing gears, including: bottom and beam trawl; hook-and-line; pots; and beach and Danish seine, fyke, gill, and tangle nets.

Given the diverse applications of the RAMP approach and the number of researchers who have utilized it, there has been variation in its implementation. One example is in how reflex impairment has been used to quantify reflex impairment. In some previous RAMP studies, a reflex impairment score (‘Score’) has been quantified by scoring present reflexes (i.e., unimpaired) as “0” and absent reflexes as “1”, and summing over all reflexes tested (i.e., a Score of zero would be given to the animals with no impairments; Stoner et al. 2008, Stoner 2009, 2012a, Hammond et al. 2013, Rose et al. 2013, Brownscombe et al. 2014, Urban 2015). Other researchers have calculated a Score by summing the “0”s and “1”s over all reflexes, and dividing by the total number of reflexes (i.e., the proportion of reflexes that were impaired; Raby et al. 2012, Barkley and Cadrin 2012, Donaldson et al. 2012, Depestele et al. 2014, Nguyen et al. 2014, Danylchuk et al. 2014, McArley and Herbert 2014, Bower et al. 2016). Additional methods include subtracting the proportion of impaired reflexes from one

(Davis and Ottmar 2006, Humborstad et al. 2009, LeDain et al. 2013), and including a multiplier for replicates within a treatment group (Davis 2007). Lastly, some studies have calculated a Score that includes reflex impairment along with an additional impairment index (e.g., buoyancy status, barotrauma, injury; Campbell et al. 2009, 2010, Diamond and Campbell 2009, Hochhalter 2012, Humborstad et al. 2016).

Similar to calculating the reflex impairment score, there has been no established practice for how to score reflexes that are neither clearly present nor absent, nor for dealing with immediate mortalities (i.e., died before assessment). The majority of previous RAMP studies have not specified how the researchers have scored an animal if the impairment was ambiguous. In some studies, 'weak' reflexes were combined with those 'present' (Stoner 2012a, Hammond et al. 2013) or they were scored separately (Stoner et al. 2008), and, in other studies, ambiguous or weak reflexes were combined with 'absent' reflexes to be "conservative" (Humborstad et al. 2009, Raby et al. 2012, Depestele et al. 2014, Danylchuk et al. 2014, McArley and Herbert 2014). In addition, researchers have not consistently described whether immediate mortalities were given a reflex impairment score (Barkley and Cadrin 2012, Hammond et al. 2013) or were treated separately from animals being assessed for delayed mortality (Davis and Ottmar 2006, Davis 2007).

Additional variation in RAMP studies has come from how mortality of animals with varying levels of impairment was determined, including observation method and duration. Methods have included captive holding in on-board holding tanks (Stoner et al. 2008, Hammond et al. 2013, Rose et al. 2013, Depestele et al. 2014, Humborstad et al. 2016) and laboratory tanks (Davis and Ottmar 2006, Davis 2007, Humborstad et al. 2009, Stoner 2009, 2012a, Braccini et al. 2012, Barkley and Cadrin 2012, McArley and Herbert 2014, Hendrick-Hopper et al. 2015, Uhlmann et al. 2015). Telemetry has been utilized, including radio (Raby et al. 2012, Nguyen et al. 2014), acoustic (Donaldson et al. 2012), and satellite (Gallagher et al. 2014). Mortality rates have been determined in-situ in net pens or cages (Diamond and Campbell 2009, Brownscombe et al. 2015, Bower et al. 2016), and through visual monitoring (Campbell et al. 2010b, Hochhalter 2012, Danylchuk et al. 2014, Brownscombe et al. 2014). Depending on the observation method, the duration has lasted from minutes to months.

While there has been variation in the methods used to employ RAMP, one consistency links all of the studies: benefits of the approach compared to physiological assessment or traditional direct observation studies were exemplified. Advantages of RAMP are that the approach allows for the determination of variables that significantly affect survival outcomes, and it can be a good predictor of mortality across a range of biological, environmental, and fishing variables. In addition, the RAMP approach allows for the quantification of mortality rates attributed to a stressor(s), and, once a RAMP relationship is established, it can be used to broaden the scope of the mortality rate estimate and can be applied in future applications for similar studies without requiring additional observation. Through previous research, RAMP has proven to be a non-invasive, quick, and simple approach for evaluating the relationship between mortality and fishing stressors (Stoner 2012b). Given these attributes, there is the potential for employing RAMP in observer and survey programs or involving citizen scientists. In addition, there are potential applications for helping fishermen decide whether to retain, release, or facilitate recovery of animals that have been bycaught, and to decide how effective their fishing performance is relative to mitigating discard mortality. Moreover, the breadth of successful applications of RAMP suggests that this method could be applied for additional purposes, including determining optimal shipping and handling procedures, animal husbandry and aquaculture practices, and evaluating currently unassessed fishing related stressors.

While benefits of RAMP were highlighted in previous studies, limitations to the approach were also specified. This includes the potential for getting unrepresentative results if test animals did not endure the full suite of stressors that they would experience under typical fishing or discard conditions (Davis and Ottmar 2006, Braccini et al. 2012, Barkley and Cadrin 2012). In addition, estimated mortality rates are likely conservative in short-term monitoring studies given that indirect or delayed effects (e.g., impairment that affects an animal's ability to feed, grow, avoid predation, etc.) are not considered (Stoner 2009, 2012b, Uhlmann et al. 2009, Benoît et al. 2010, Urban 2015). Similarly, if a RAMP study only evaluates animals representing a limited range of the biological, environmental, or fishing variables, it cannot be known for certain if the resultant RAMP relationship can be extrapolated to the fishery or population (e.g., applied to animals outside the range of sizes that was studied; Davis and Ottmar 2006, Davis 2007, Stoner 2012a).

An additional limitation of RAMP is the potential for results to be influenced by bias in reflex assessment. This includes the potential for observer bias in scoring impairment, given that the way a fish is handled may affect a reflex response, and the determination of 'presence' and 'absence' of a reflex can be subjective (Benoît et al. 2010, Depestele et al. 2014, Uhlmann et al. 2015). Bias may also result from the methodology with respect to when an animal is assessed post stressor(s). Reflexes and the relationship between reflex impairment and mortality can change if facilitated recovery in water is allowed before assessment (Stoner 2009, Nguyen et al. 2014), or if animals are not tested shortly after exposure to the stressor(s) of interest (Davis 2007, Depestele et al. 2014). Also, without control data, mortality from the scientific process (e.g., captivity or tagging) may be confounded with mortality attributed to the stressor(s) of interest (Davis and Ottmar 2006).

The RAMP approach is also limited by the quality of the reflexes that are selected, and the analyses that are completed to determine the relationship between reflex impairment and probability of mortality. RAMP reflexes are species specific and, therefore, must be selected based on characteristics specific to the animal (Davis and Ottmar 2006, Davis 2007, LeDain et al. 2013, Rose et al. 2013, Depestele et al. 2014, Gallagher et al. 2014).Reflexes could be biased if they are linked with the stressor being studied (e.g., tail grab reflex to test swimming exhaustion; Szekeres et al. 2014), and reflexes may be differentially sensitive to stressors or the degree of the stressor (Stoner 2009, Bower et al. 2016). Successful application of the RAMP approach relies on the selection of reflexes that give consistent response to stimulation, or bias may be introduced (Depestele et al. 2014), and using only one reflex is less effective than if multiple are utilized (Gallagher et al. 2014). It is equally important to be aware of the potential for bias depending on how the data are analyzed. For example, model selection can be affected by whether the reflex impairment score is evaluated as a categorical or continuous variable (Hammond et al. 2013). Along these lines, reflex impairment may not be the sole predictor of mortality; therefore, it is important to evaluate how environmental, biological, and fishing variables influence the accuracy of mortality predictions (Campbell et al. 2010a, Urban 2015). Moreover, the relationship between reflex impairment and mortality can be difficult to determine if only a few test animals die (McArley and Herbert 2014) or if the majority of mortality is immediate rather than delayed (Davis 2007).

Through the completion of three research studies assessing crab discard mortality and the synthesis of research completed by other scientists, I evaluated the RAMP approach to provide a clearer understanding of how and where to apply this methodology. This research involved bycaught Tanner crab (*Chionoecetes bairdi*) in the Alaska bottom trawl fishery, and discarded crab in the commercial and recreational Dungeness (*Cancer magister*) fisheries in Oregon. The existing need for discard mortality rates for these fisheries provided a rationale for selecting them for this RAMP assessment. Also, management implications for this research were sufficiently important to generate research funding and involvement from the fishing community. In addition, this work was feasible given available resources with respect to both laboratory facilities and access to fishing vessels. Despite the focus on these fisheries, my results extend beyond discarded crab and are relevant to the broader body of bycatch mortality research. Lessons learned from the three studies described in this dissertation provide an evaluation of RAMP that may highlight applications of the technique to other species, stressors, or gear types.

1.2 RAMP Flexibility: Tanner Crab Bycatch in Alaska Bottom Trawls

In *Chapter 2*, results are presented for a study evaluating how broadly an established RAMP relationship can be applied once created. I determined the relationship between reflex impairment and probability of mortality for Tanner crab discarded from the Alaska bottom trawl. This RAMP was then compared to one created for unobserved bycaught Tanner crab in the same fishery. This research allowed me to answer the question of whether a RAMP relationship, once created for a given species, fishery, and stressor, can be applied to additional stressors that were not represented when creating the relationship.

1.3 RAMP Application for Management: Dungeness Crab Bycatch in Oregon Pot Fisheries

In *Chapter 3,* I demonstrate a new application for RAMP: assessing the role of discard mortality in a fishery managed primarily by regulations that require discarding of a subset of the catch. Specifically, I estimated bycatch and discard mortality rates for the commercial and recreational Oregon Dungeness crab fisheries. These fisheries are managed by the ‘3-S’s: Size, Sex, and Season. The efficacy of this management approach relies heavily upon low bycatch and/or discard mortality rates for the discarded sub-legal (‘Size’), female (‘Sex’), and soft-shell (‘Season’) crab. This study demonstrates the application of RAMP for assessing discard in this new context.

1.4 RAMP Field Validation Study: Comparing Mark-Recapture and Captive Holding to Determine Mortality

Chapter 4 presents results for a field validation study that evaluates discard mortality of Dungeness crab in the directed recreational and commercial Oregon fisheries. Described is a study using mark-recapture to evaluate mortality rates. This is then compared to the results from the study described in Chapter 3, which were determined through the use of laboratory captive holding. This comparison informs the question of whether or not mortality rates estimated using captive holding are biased due to unnatural conditions and because delayed mortality is only evaluated in the short term.

1.5 Conclusions

Chapter 5 provides a summary of the benefits and limitations of RAMP as determined through the findings from Chapters 2-4 and from research completed by other scientists. Suggestions are provided on ways to effectively utilize the RAMP approach. Through this synopsis and a detailed methodological description, I provide a guide for applying the RAMP approach in future research. Also provided are suggested future studies to further evaluate and understand the methodology.

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CHAPTER 2

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

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2.1 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 differences between the two 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.

2.2 Introduction

2.2.1 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, Watson and Pengilly 1994, Trumble et al. 2000), acoustic telemetry (Pepperell and Davis 1999), and captive holding studies (Kennelly et al. 1990, Bergmann and Moore 2001, Parker et al. 2003). Researchers have also quantified impairment attributed to fishing stressors based on physiological parameters, including metabolic, biochemical, and immune responses (Bergmann et al. 2001, Parker et al. 2003, Mercier et al. 2006, Broadhurst et al. 2009, Uhlmann et al. 2009, Aparicio-Simón et al. 2010, Leland et al. 2013). 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 (Davis and Schreck 2005, Stoner 2012b, Cooke et al. 2013). 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 and Ottmar 2006, Davis 2007, Stoner 2012b). 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 2012b). Advantages of the RAMP approach have made it an increasingly utilized methodology. It has successfully been used on a variety of species, including fishes (Davis and Ottmar 2006, Davis 2007, Humborstad et al.

2009, Raby et al. 2012, Barkley and Cadrin 2012, Brownscombe et al. 2013) and invertebrates (Stoner et al. 2008, Chilton et al. 2011, Stoner 2012a, Hammond et al. 2013, Rose et al. 2013). 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.

2.2.2 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 *C. 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 (NPFMC 2013a, 2013b, National Oceanic and Atmospheric Administration 2014). 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 minutes) 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 studies evaluating 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 minutes 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 minutes on average (range from 9 to 230 minutes) 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.

2.3 Material and Methods

2.3.1 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 soft-shell crab have higher mortality rates than hard-shell 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. 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 to include with additional stressors to evaluate their influence on survival.

2.3.2 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), and were kept in plumbed (constant inflow and outflow of sea water), on-board, 100 x 68 x 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 (Figure 2.1) and lowered to the sea floor for holding to determine delayed mortality.

2.3.3 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 2,479 L, 1.8 m x 0.9 m circular seawater holding tank with constant flowing, unfiltered seawater, maintained at between 6 and 7 degrees Celsius. 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 (Figure 2.1; 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 (Uhlmann et al. 2009, Moreira et al. 2011), and months (Zhou and Shirley 1995, Leland et al. 2013). 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.

2.3.4 Data Analysis

2.3.4.1 *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.

2.3.4.2 *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.

2.4 Results

2.4.1 <u>Assessment of Discarded Tanner Crab</u>

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). 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 2.1).

2.4.2 <u>Comparison of Holding Types and Duration</u>

Delayed mortality for our study occurred for 33 crab during holding in the on-board tanks, followed by four crab in the laboratory tank and three 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 (Figure 2.2). 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, 3, and 12 days of total holding. In the at sea cages, three crab died (Scores 1, 2, and 6), 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.

2.4.3 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 2.2). 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 to 2.83, binary logistic regression; Table 2.2). 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 (Figure 2.3) 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 ($Score_{50}$) 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 2.2). 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 Discard- and Unobserved-mortality studies (Figure 2.3) 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.

2.4.4 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 RAMP, and 31% from the Unobserved-mortality study RAMP (Figure 2.4).

2.5 Discussion

2.5.1 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 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 (Figure 2.2) 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.

2.5.2 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 minutes on average (range from 9 to 230 minutes) 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 minutes), 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, Warrenchuck and Shirley 2002, Grant 2003, Giomi et al. 2008, Stoner 2009). 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 Tamelen (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.

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 tow that lasted for only 10-20 minutes (Hammond et al. 2013). Tow for the Discard-mortality study lasted for over three hours on average.

With respect to (1) different scientists making the assessments, subjectivity error should be considered when results are based on assessments (Benoît 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 (Stevens 1990, Ridgway et al. 2006), 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 was likely attributed to differences in the stressors experienced by the crab before assessment (i.e., air exposure and recovery in water).

2.6 Conclusions and Recommendations

2.6.1 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 2.3). 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.

2.6.2 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 *Chionoecetes* 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.

2.6.3 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 (Figure 2.5), 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 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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2.9 Figures

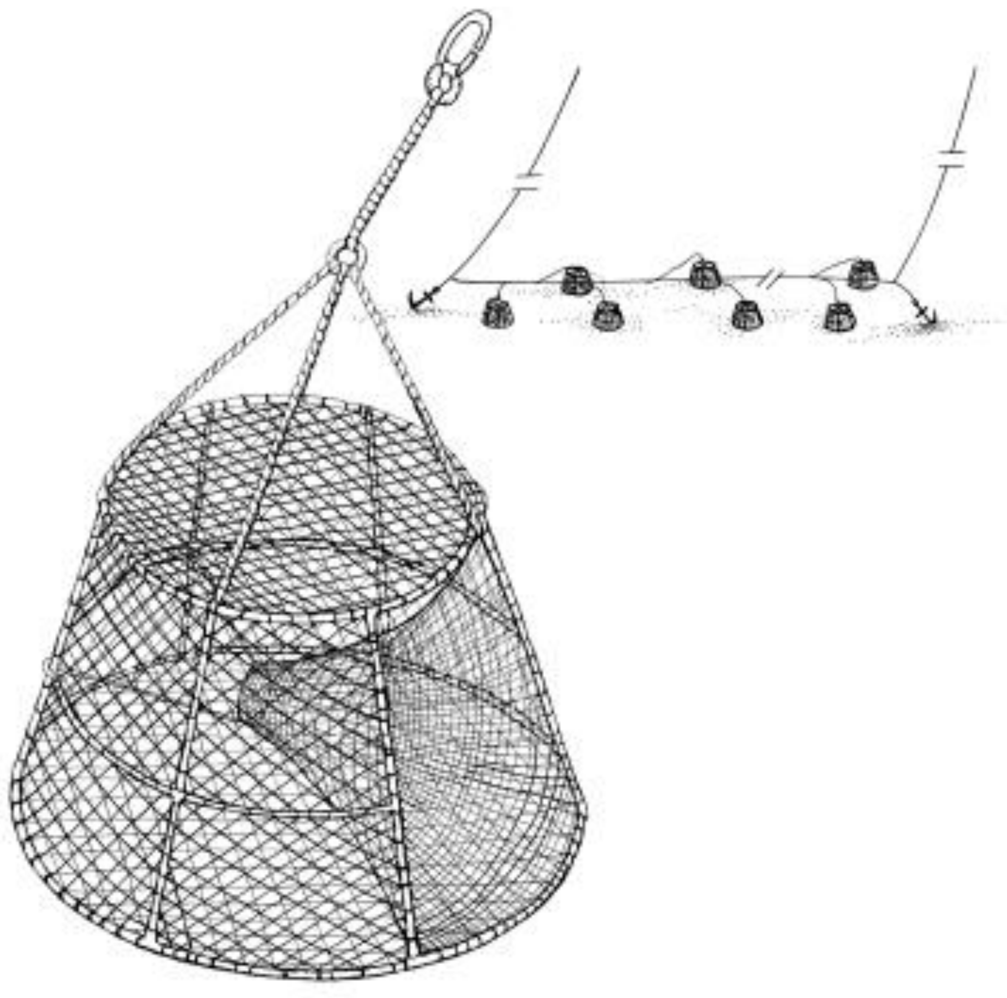

Figure 2.1 Holding cages for bycaught Tanner crab

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.

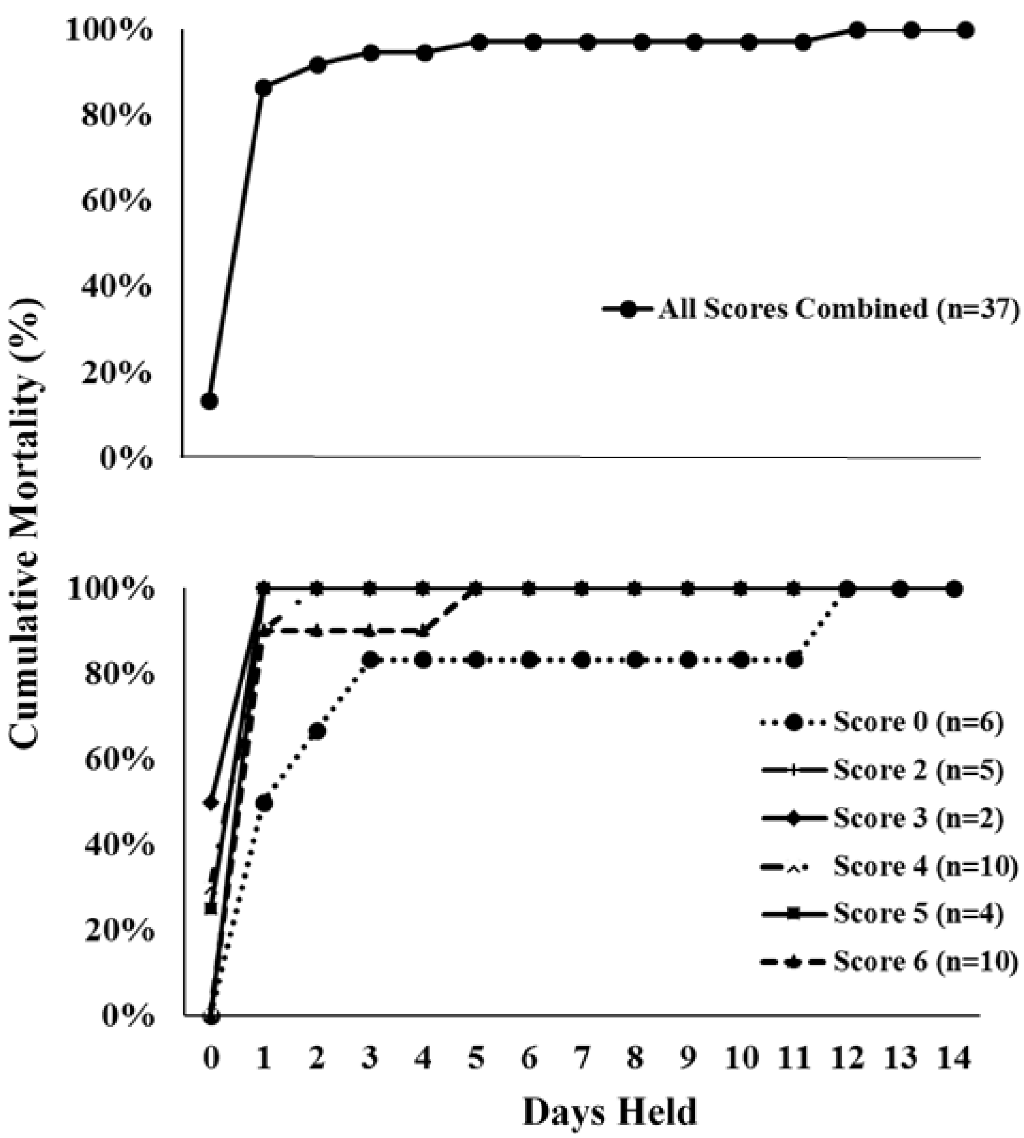

Figure 2.2 Cumulative mortality curves for held Tanner crab

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.

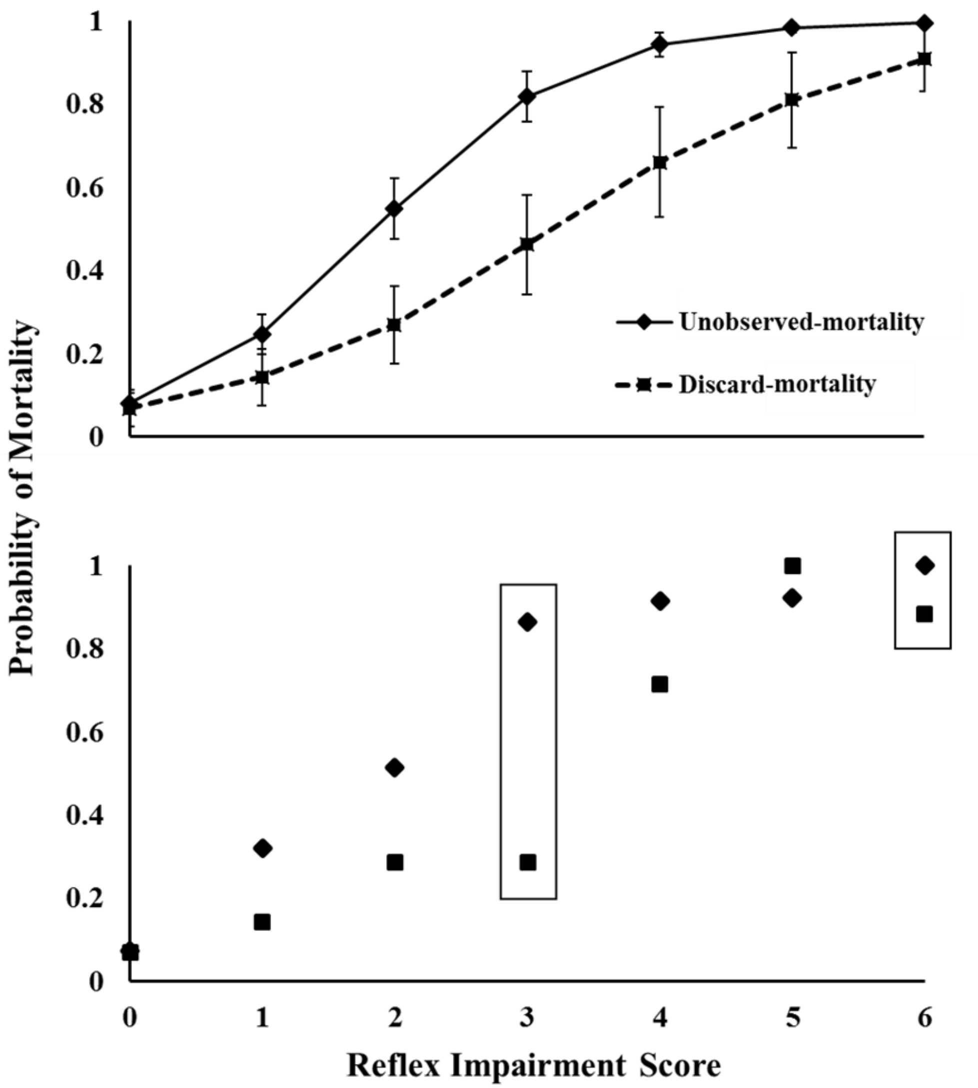

Figure 2.3 Reflex Action Mortality Predictors for Tanner crab

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).

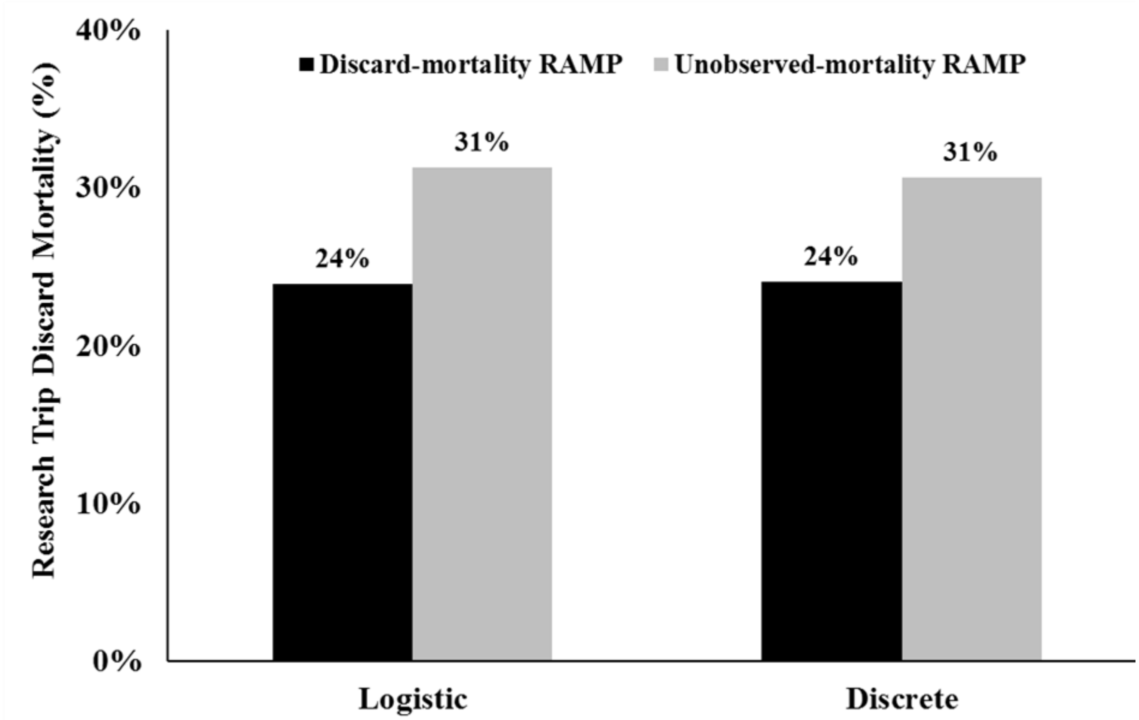

Figure 2.4 Gulf of Alaska trip mortality rate estimates

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 modelling) from both the Discard- and Unobserved-mortality studies.

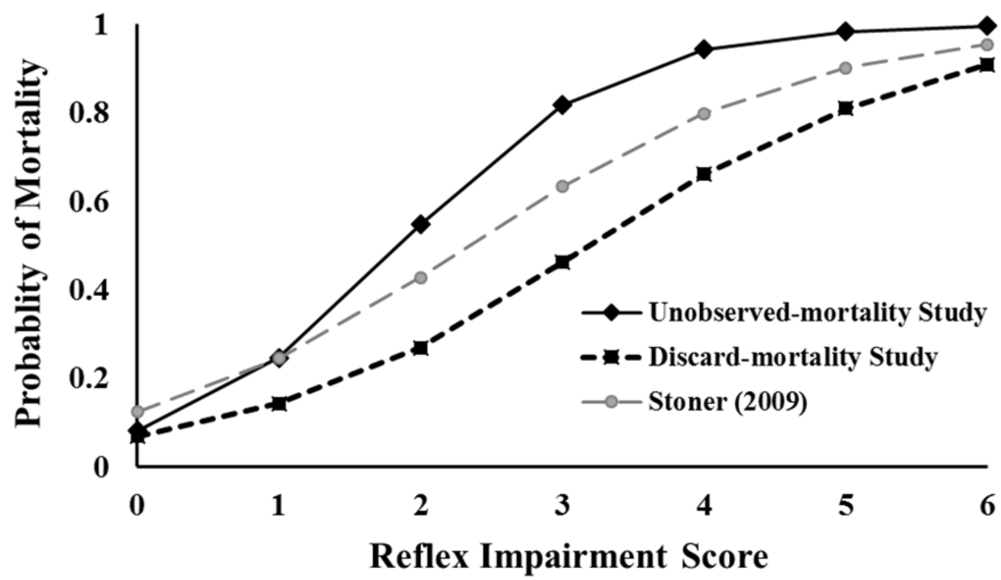

Figure 2.5 Comparison of Reflex Action Mortality Predictors by stressor

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

2.10 Tables

Table 2.1 Frequency of RAMP reflexes lost for Tanner crab

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

Table 2.2 Logistic model results

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	P-value
Discard Study Parameters				
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
Combined Study Parameters				
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

Table 2.3 Comparison of holding types for determining mortality

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	• Can be used during fishing operations • Is relatively inexpensive • Allows for easy monitoring	• Cannot easily regulate water temperature or quality • Subjects animals to vessel's motion • Requires deck space and plumbing • Requires monitoring and maintenance
Laboratory Tanks	• Can regulate water temperature and quality • Can control the environment • Allows for easy monitoring	• Requires transport and additional handling of the animals • Cannot be used during fishing operations • Requires a wet-laboratory near a fishing port • Requires monitoring and maintenance
At-Sea Cages	• Can be used during fishing operations • Provides more natural holding conditions • Does not require monitoring, feeding, or maintenance	• Requires additional handling of the animals • Prohibits knowing when or how an animal died (unless cameras are used) • Can become lost • Must be retrieved • Makes animals vulnerable to predation

CHAPTER 3

UTILIZING REFLEX IMPAIRMENT TO ASSESS THE ROLE OF DISCARD MORTALITY IN ‘SIZE, SEX, AND SEASON’ MANAGEMENT FOR OREGON DUNGENESS CRAB (*CANCER MAGISTER*) FISHERIES

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Alan Pazar, and Robert Eder

Yochum, N., A.W. Stoner, D.B. Sampson, C.S. Rose, A. Pazar, and R. Eder. Utilizing reflex impairment to assess the role of discard mortality in ‘Size, Sex, and Season’ management for Oregon Dungeness crab (*Cancer magister*) fisheries. Canadian Journal of Fisheries and Aquatic Sciences. *Accepted pending revisions*.

3.1 Abstract

We found that crab discarded from Oregon (U.S.A.) commercial and recreational fisheries for Dungeness crab (*Cancer magister*) have lower post-release mortality than previously estimated. This aligns with the goals of the '3-S' management strategy currently employed for these fisheries to protect discarded sub-legal male (Size), female (Sex), and soft-shell (Season) crab. We found that, for the commercial ocean Dungeness fishery, overall discard mortality rates (five days after release) were 0.08 (95% Confidence Interval 0.06-0.10) for females; 0.01 (95% Confidence Interval 0-0.02) for hard-shell males; and 0.09 (95% Confidence Interval 0.03-0.16) for soft-shell males. The overall discard mortality rate for the recreational bay fishery (from a boat) was estimated to be 0.01 (95% Confidence Interval 0-0.02). A Reflex Action Mortality Predictor (RAMP) relationship, which relates reflex impairment to mortality probability, was created and utilized to estimate mortality rates. Our study highlights the importance of looking not only at discard and mortality rates to evaluate '3-S' fishery management, but also the mortality- and bycatch- per retained ratios, and temporal trends relative to changes in effort, animal condition, and catch composition.

3.2 Introduction

Dungeness crab (*Cancer magister*) is currently the most valuable crab fishery in the United States, yielding nearly 25 thousand metric tons and $211 million in 2014 (National Marine Fisheries Service 2015), and has an over $40 million dollar (CAD 2008) ex-vessel value in Canada (Yonis 2010). In Oregon (U.S.A.), the ocean fishery for these crab is the most valuable single-species commercial fishery, with 300-350 vessels landing 5-15 thousand metric tons each season (Ainsworth et al. 2012), generating up to $50.2 million (ex-vessel; ODFW 2015a). In addition to the commercial fishery, Dungeness also contribute to local economies as a draw for tourism and recreational fishing (Ainsworth et al. 2012). Despite the economic significance, in the United States there is neither a stock assessment nor a Fishery Management Plan (FMP) for the commercial or recreational Dungeness crab fisheries along the Pacific coast nor in adjacent estuaries. Since 1947, Dungeness fisheries have been managed by state agencies (Demory 1985) that employ, along with effort controls and gear restrictions, a predominately '3-S' management strategy: Size, Sex, and Season.

The size and sex of harvestable crab are regulated within a specified season for commercial and recreational fishing, in the ocean and adjacent bays. In Oregon, commercial and recreational harvest is currently restricted to males with a minimum carapace width of 6¼ inches (159 mm) and 5¾ inches (146 mm), respectively (ODFW 2015b). Because males are mature by 137 mm (MacKay 1942), these size restrictions ensure crab are able to reproduce for one or two seasons before recruiting into the fisheries (Rasmuson 2013). In addition, male-only harvest protects breeding females and increases meat yield (Northrup 1975) given that females produce 42% less meat than male crab (PSMFC 1978).

The 'Season' component of the '3-S' management regulates the timing of the fishery to avoid capture of recently moulted, soft-shell crab (PSMFC 1978). In Oregon, male crab typically moult from spring to fall (Demory 1985, Rasmuson 2013), with an increasing abundance of moulting crab from April to July, and a substantial number of soft-shell crab in October and November (Spears et al. 1983). Timing of the moult, however, varies geographically, annually, and by sex (Robinson et al. 1977, Demory 1985). Unlike Washington, in both California and Oregon it is lawful to land soft-shell crab (PSMFC 1978), but they are seldom retained because of the poor meat quality (Stewart 1974). For the two to three months that it takes post-moult crab to harden and fill in muscle tissue (Dunham et al. 2011, Rasmuson 2013), the meat yield is approximately 13-14% compared to 25-30% for hard-shell crab (Robinson et al. 1977). Harder crab are preferable in meat quality and value for consumers and processors (Barry 1983, Demory 1985, Kruse et al. 1994), and so yield a higher price (Waldron 1958, PSMFC 1978). While fishermen have little incentive to harvest soft-shell crab, there is a period of time when hard-and soft-shell crab co-occur, resulting in incidental capture and discard of both sub-legal and legal-size soft-shell crab.

Temporal restrictions on harvest were put in place during the approximate moulting period to increase profitability and mitigate handling mortality of soft-shell crab and, therefore, increase the abundance and quality of legal, hard-shell males in the subsequent season (Waldron 1958). The annual season opener for the Oregon commercial ocean crab fishery is December 1st, but is delayed if the crab do not meet the minimum meat weight threshold of 25% (Figure 3.1; Didier 2002, ODFW 2009). Beginning the second Monday in June, as soft-shell crab increase in abundance, fishermen are restricted to landing 1,200 pounds (544 kg) of crab per week. This regulation remains in effect until the season closes on August 15th (ODFW 2009). Despite the regulated season, the majority of the effort and landings currently occur shortly after the season opens (Didier 2002), and during the first two months of the season. Post-winter, as catch rate decreases, fishermen often switch to alternative, concurrent fisheries (Youde et al. 1967, PSMFC 1978, Oregon Sea Grant 2008).

Fishing seasons vary across the commercial and recreational fisheries in the ocean and bays (Figure 3.1). The commercial bay fishery occurs from the Labor Day holiday (the first Monday in September) to December 31st, except on holidays and weekends (ODFW 2009). The recreational bay fishery is open year-round, both from a boat and shoreside. Recreational fishing in the ocean occurs from December 1st to October 15th (Ainsworth et al. 2012). Unlike the commercial ocean fishery, the majority of recreational fishing occurs in the summer and fall (June - October), with effort depending mostly on weather conditions (when fishing is safer and more enjoyable), catch rates, and timing of vacations. In addition, crabbing in the bay is influenced by rain and river run-off, which decreases water salinity and reduces catch (Ainsworth et al. 2012).

The objective of this study was to quantify discard rates (i.e., proportion of the total catch that is discarded) and discard mortality rates (i.e., proportion of the discarded animals that die as a result of the capture and release process) in the commercial ocean and recreational bay by boat Dungeness fisheries along Oregon's coast and in the Yaquina Bay. The '3-S' management relies upon these rates being low given that this strategy is largely based on discarding females, and sub-legal and soft-shell males. To further assess '3-S' management for Dungeness, we evaluated variation in the mortality- and bycatch- per retained ratios (MPRR, BPRR) over the fishing season. In addition, we make recommendations toward the goal of reducing both bycatch and discard mortality rates.

The impetus for this project was from commercial Oregon Dungeness crab fishermen and their interest in knowing discard mortality rates for the fishery. This aligns with historic efforts by Dungeness crab fishermen to instigate changes in fishing regulations to protect the crab population (Waldron 1958, Wild and Tasto 1983). This research benefited from a collaboration among industry, science, and management partners.

3.3 Materials and Methods

3.3.1 Dungeness Crab RAMP

To quantify discard mortality rates for the Dungeness crab fisheries we utilized the Reflex Action Mortality Predictor (RAMP) approach. This methodology relates vitality to mortality probability attributed to a stressor(s) through quantifying reflex impairment (Davis and Ottmar 2006, Davis 2007). Ideally, observations on impaired reflexes (or lack of impairment) can be used to estimate the probability of short-term delayed mortality. While this approach has not previously been utilized for Dungeness crab, it has effectively been used to determine bycatch mortality rates and to evaluate mortality attributed to individual fishing stressors (e.g., air exposure, injury from fishing gear) for several fish (Davis and Ottmar 2006, Raby et al. 2012, Barkley and Cadrin 2012, Nguyen et al. 2014) and crustacean species (Stoner et al. 2008, Stoner 2012a, 2012b, Hammond et al. 2013, Rose et al. 2013, Yochum et al. 2015, Urban 2015).

To apply the RAMP approach to discarded crab from theses fisheries, we first established a set of reflexes specific to Dungeness, then assessed reflex impairment in crab that endured the stressors specific to the fisheries, determined delayed mortality for crab with varying levels of reflex impairment through captive holding, and, finally, created a reflex action mortality predictor to model the relationship between reflex impairment and probability of delayed mortality.

3.3.1.1 *Establishing a Set of Reflexes*

To create a RAMP relationship for Dungeness crab, we first established a set of reflex actions (‘reflexes’; e.g., eye retraction when an eye is tapped) that could reliably be used for evaluating vitality. To accomplish this, we captured male and female crab of varying sizes using recreational crab gear in the Yaquina Bay. After being captured, the test crab were placed in an ice chest with wet burlap sacks to reduce stress from air exposure and captivity (Simonson and Hochberg 1986), and were carried less than 1 km to the Alaska Fisheries Science Center (AFSC) laboratory in Newport, Oregon.

After being ‘burped’ to remove air that might be trapped under the carapace (Snow and Wagner 1965), the crab were placed in temperature-regulated (approximately 6 ˚C) flow-through sea water tanks (2 m diameter, filled to 1 m depth). In the field and over several days in captivity, the crab were assessed several times to identify reflexes that responded consistently to a stimulus. We began by testing RAMP reflexes established for two *Chionoecetes* species (Stoner et al. 2008), and consulted with fishermen who often use vitality assessments to determine whether or not to retain or sell a crab. The crab were allowed to recover in the holding tanks for a week and were then reassessed. Subsequent assessments were completed after exposing the crab to air and mimicking handling stressors (e.g., dropping on the ground).

Through this process, we established a series of six reflexes to test Dungeness crab vitality, which gave consistent, involuntary responses to stimulation, and a protocol for assessment. The reflexes include: (*i*) eye retraction; (*ii*) mouth defense; (*iii*) chela closure; (*iv*) leg wrap; (*v*) leg curl; and (*vi*) abdomen response (in this order; Table 3.1). The reflexes are tested by holding the crab vertically (dorsal side facing away), with the left hand, and assessing the right side of the crab (assessment can be completed on either side). A reflex is considered absent only if there is no response to stimulation. Similar to Stoner et al. (2008), we found that it is too ambiguous to include additional impairment categories (e.g., strong, moderate, weak). An overall reflex impairment score (‘Score’) is calculated by first assessing each reflex and assigning a ‘0’ to present reflexes (including weak responses) and ‘1’ to those absent, then summing over all reflexes. Davis and Ottmar (2006) calculated Score as a proportion (one minus the ratio of the total number of impaired reflexes to the total reflexes). This approach is advantageous if there are reflexes or individuals that cannot be tested due to missing or damaged body parts. This is seldom the case for Dungeness; therefore, Score was calculated as the sum of missing reflexes. In addition, reflex impairment is evaluated for live crab only. In some previous RAMP studies, immediate mortalities (i.e., crab that were dead in the fishing gear before assessment) were given a Score indicating maximum impairment (Hammond et al.

2013, Rose et al. 2013, Yochum et al. 2015). This is an advantageous approach if it is difficult to differentiate between dead and moribund individuals (Stevens 1990). This is not the case with Dungeness crab; therefore, the contributions to total bycatch mortality by both immediate and delayed mortality were evaluated separately.

3.3.1.2 *Assessing Crab*

We focused on the commercial ocean and recreational bay by boat (here forward referred to as 'commercial' and 'recreational') fisheries because they account for the vast majority of landed Dungeness crab catch (94-98% and 2-6% respectively; Ainsworth et al. 2012, data from 2007-2011). For the recreational fishery we focused on crab in the Yaquina Bay, as opposed to the ocean, because approximately 60% of annual recreational landings are from Oregon bays, and the Yaquina is both a heavily fished site (Ainsworth et al. 2012) and is the nearest bay to the research facility. Moreover, we focused on fishing in the bay by boat instead of from shore because of the low catch-per-angler-day rates for the latter fishery. Regardless, some data were gathered to evaluate bycatch and discard mortality in these additional fisheries, which are described in Appendices A-E.

Given that a model for predicting mortality from an assessment of reflex actions can be specific to a set of stressors (Yochum et al. 2015), we were careful to both collect bycatch data that were representative of actual fishing practices and to describe the methods and likely stressors associated with crabbing (e.g., soak duration- the duration of time between when a pot was set and retrieved; Musyl et al. 2009). By doing this we endeavored to establish RAMP relationships that can be utilized in future research. To this end, commercial fishery data were collected during 'ride-along' trips aboard fishing vessels, which also allowed us to gain feedback from fishermen on project methodology and insight into the fishery. We were unable, though, to dictate depth strata, location, and other sampling logistics. To evaluate differences among captains and crew members we aimed to complete trips on multiple fishing vessels and out of several ports. Obtaining ride-along opportunities for recreational fishing was difficult due to small vessel size and research permit restrictions prohibiting crab retention. Therefore, approximately half of the recreational sampling was completed on a research vessel by scientists with recreational crab fishing experience. To incorporate intra-annual variability in stressors (e.g., air temperature), we aimed to conduct at least one sampling trip for each calendar month when the fisheries were open.

3.3.1.2.1 *Commercial Ocean*

Between February 2012 and January 2014, we sampled all strings (a "continuous line of individual crab pots spaced a given distance apart from each other", Hicks 1987) during ride-along trips. Within each string, the selection of the first crab pot to sample was randomized and, subsequently, every fifth pot was assessed. This systematic sampling protocol was put in place to maintain consistency of sampling between strings, while not slowing down or interfering with fishing operations, and minimizing handling and air exposure for the crab beyond typical fishing processes. Modifications to this protocol were allowed as necessitated by sampling logistics (e.g., poor weather), under the constraint that each sampled pot be selected before it landed on deck.

For each assessed pot, data were recorded on both the conditions under which the pot was fished, and on the retained and discarded crab within. The following information was recorded per pot: (*i*) soak duration (days); (*ii*) sea state at the time the pot was brought onto the boat (Beaufort wind force scale); (*iii*) whether or not the crab were removed from the pot using a 'slam bar' (a bar on which a pot is thrown in order to push crab towards the pot opening); (*iv*) how many crab were retained; (*v*) the location of the pot within the string; and (*vi*) the depth where the pot was fished. In addition, retained crab were counted. All crab intended for discard were measured (carapace width, to the nearest millimeter), and sex and shell condition were noted. 'Soft' crab were described as those with little or no hardening (the crab recently moulted) to moderate hardening post-moult (carapace and legs flexible and soft).

Crab designated as 'hard' were those with carapace and legs nearly fully hard to near moulting (i.e., the shell condition that would be acceptable to most fishermen for retention). Crab intended for discard were also evaluated for the presence of any new injuries, including: broken, injured, or missing legs/chela, spines, dactyli, maxillipeds, or abdominal flap; autotomized legs/ chela; smashed carapace (ventral and dorsal); holes or cracks in carapace; and damage to an eye. Warrenchuck and Shirley (2002) found that old injuries did not affect mortality in snow crab (*Chionoecetes opilio)*, thus only new injuries were recorded. After one or two days post injury a 'sheath' or scab is visible at the site of injury (Durkin et al. 1984). We therefore considered 'new injuries' those without scabbing. For each crab, we noted total air exposure duration and tested each of the established RAMP reflexes to generate a reflex impairment score. Assessments took approximately 30 seconds per crab.

3.3.1.2.2 Recreational Bay by Boat

During sampling trips completed between April 2012 and April 2014, all pots and rings were assessed. Recorded information was similar to the commercial fishing trips; however, there were no 'slam bars', soak duration was measured in minutes, and legal crab were 5 ¾ inches (146 mm) or larger. All legal-sized males were marked as 'retained' if they were considered hard-shell by the definition of this study. Also the trips were executed, when possible, according to advice for maximizing catch from the Oregon Department of Fish and Wildlife (ODFW; ODFW 2015c).

3.3.1.3 *Measuring Mortality*

To relate reflex impairment to delayed mortality probabilities for the commercial and recreational fisheries, a total of 655 and 321 crab (respectively) were held in laboratory tanks (described previously) to determine survival. In selecting crab, we aimed to hold as many crab as possible that had impaired reflexes and to fill the remaining tank space with unimpaired crab. Regardless, given the catch composition, the majority of held crab for the commercial and recreational fisheries (77% and 88% respectively) were Score-zero (Appendix D). We also attempted to hold crab of varying combinations of sex, size, injury, and shell condition over the temporal extent of the fisheries to look at the potential influence on mortality of and interactions among various biological and environmental variables. For the commercial fishery, 54% of held crab were hard-shell females, and 67% were hard-shell males for the recreational fishery. These percentages similarly reflect catch composition.

For identification purposes, all held crab were tagged with a double ‘t-bar’ anchor tag (TBA-LEVO, Hallprint Fish Tags). This tag type was selected because it has successfully been used and has been proven to last through ecdysis for Dungeness crab (Smith and Jamieson 1989, Swiney et al. 2003, Barber and Cobb 2007), and because it can be used for a large range of sizes and cannot be lost during leg autotomization. Necropsies were performed on over 90% of crab that died in holding, which verified that mortality was likely not tag induced.

While crab were held in the laboratory for up to one month, cumulative mortality was evaluated to determine if holding conditions or tagging were influencing survival over time. To this end, at the beginning of the study we held minimally stressed crab for a month to monitor survival. For these crab and those held for this study, we observed that cumulative mortality stabilized by the second day of holding, but began increasing again after day five, even for Score-zero crab. Therefore, crab were only considered discard mortalities if they died within the first five days of holding to avoid confounding discard mortality with a captivity effect. This threshold holding duration was also based on findings by Yochum et al. (2015) that five days was an optimal holding duration for Tanner crab when determining mortality. In previous studies estimating Dungeness crab discard mortality, crab were held for four (Tegelberg 1972) and five (Barry 1984) days. Tegelberg and Magoon (1970) found that a captivity effect for Dungeness was evident after four days of holding. While survival in the laboratory can be improved with cold water temperatures, a pattern of increased mortality over time remains (Kondzela and Shirley 1993). For discarded Dungeness crab, there may be more long-term mortality, but it cannot be accurately determined in a laboratory given the unnatural setting and potential for a captivity effect to confound results.

Given our finding of a captivity effect and evidence from other studies that Dungeness crab can be difficult to keep alive in captivity (Barnett et al. 1973), we determined ways to reduce stress and injury attributed to captivity and transport. We found that the captivity effect was ameliorated by holding crab in individual containers. Similar to Jacoby (1983), we found agonistic behaviour primarily between females. Therefore, the majority of crab were held in individual compartments. We also cleaned the holding tanks weekly, maintained cold water temperatures to reduce stress (approximately 6 °C; Burton 2001, Bellchambers et al. 2005), and periodically checked the oxygen and ammonia levels (Barrento et al. 2008). We also fed the crab weekly and performed daily checks to monitor for (and remove) dead crab. To reduce impact from at-sea holding and during transfer, crab from commercial fishing trips were placed, after assessment, into an insulated fishing tote (interior dimensions: 91 cm x 53 cm x 53 cm) equipped with flow-through seawater during fishing operations (Basti et al. 2010). They were transported in ice chests with wet burlap sacks approximately 3.5 km to the holding facility. Crab from recreational trips, following assessment, were placed directly into ice chests with wet burlap sacks that were periodically re-soaked with seawater before taking the crab to the same holding facility (<1 km away).

3.3.1.4 *Predicting Mortality from Impaired Reflexes*

Binary logistic regression was used to determine if there was a relationship between the number of impaired reflexes (Score) and mortality, measured as the proportion of the 655 crab that died in holding for the commercial ocean fishery, and 321 for the recreational fishery. Model coefficients were estimated using maximum likelihood (Ramsey and Schafer 2002) based on the fate (mortality or survival) of individual crab that were held, as shown in Appendix D. ‘Score’ was treated as a continuous and categorical variable (in separate analyses), and with individual reflexes as predictors.

We also included fishing, environmental, and biological explanatory variables in the model to determine their role in predicting mortality.

Equation 3.1 $Log_e\left(\frac{p}{1-p}\right) = \alpha + \beta_o Score + \beta_i x_i$, where:

p = probability that a crab died during the holding period

α = intercept

β_o = model coefficient for reflex impairment score ('Score')

β_i = model coefficients for the explanatory variables (x_i) tested in the model

To determine the most parsimonious logistic model for the data, we performed forward stepwise model selection in R (R Development Core Team 2011) using a function (addterm) that allowed us to determine significance of individual predictors based on Akaike Information Criteria, and through drop-in-deviance tests. Model selection drew from a rich model that included a large number of possible explanatory variables: (*i*) reflex impairment score; (*ii*) sex; (*iii*) shell condition; (*iv*) carapace width (continuous); (*v*) fishery type; (*vi*) month; (*vii*) air exposure duration; (*viii*) number of crab retained; (*ix*) presence of new injuries; and interactions among these variables. Model selection was completed with several categories of injury, as well as the presence (non-specific) or absence of injuries. When analyzing the fisheries separately, (*i*) use of the slam bar; (*ii*) soak duration (days), (*iii*) depth (fathoms), and (*iv*) Beaufort wind force scale were included for the commercial analysis; and (*i*) soak duration (min), (*ii*) depth (meters), and (*iii*) gear type (ring or pot) for the recreational analysis.

3.3.2 Quantifying Discard Mortality Rates in the Fisheries

While only held crab were utilized to create the logistic model for predicting the probability of mortality, to quantify the overall fishery discard mortality rates we utilized data on all assessed crab from the sampling trips in the following equation.

Equation 3.3 $\mathrm{P}(m) = \sum_{k=0}^{6} P(m|s=k) * P(s=k)$

We summed over all Scores (s), k=0-6, the product of the probability of mortality, given Score, ($P(m|s = k)$), by the probability of catching a crab with that Score (from ride-along data; Appendix C) using the following.

Equation 3.4 $P(s = k) = \frac{n_k}{n_{total}}$

$P(m|s = k)$ was predicted by the regression model and its prediction variance was estimated using the predict() function in R (R Development Core Team 2011). For each Score, k, we calculated the prediction variance of the product $P(m|s = k) * P(s = k)$ using the delta method (Rice 1988). The prediction variance of $\mathrm{P}(m)$ was then estimated as the sum of variances of these products across all Scores, assuming independence among Scores. Finally, the 95% Confidence Interval for $\mathrm{P}(m)$ was estimated as $\mathrm{P}(m) \pm 1.96\sqrt{(variance\ of\ \mathrm{P}(m))}$.

For both fisheries, delayed discard mortality rates were calculated by averaging values by string (commercial only), trip, and month. Rates were also calculated by combining the data over all trips. Differences were observed in these estimated rates for soft-shell males. This was attributed to uneven sample sizes in the different data groupings. We therefore constructed 95% Confidence Intervals for estimates by string, trip, and month to see if there were trends in mortality rates that were overlooked in the logistic regression analysis. This analysis indicated that there were no significant differences by these data groupings, with the exception of grouping female data by month. During one trip in December only four female crab were caught, which heavily influenced mortality rates. No other significant patterns were determined; therefore, we calculated final rates with all data combined. Bycatch mortality rates (i.e., proportion of bycaught animals- non-target crab and immediate mortalities- that die) were calculated similarly, but included crab that died prior to assessment (immediate mortality). Estimates of mortality-per-retained ratios (MPRR) and bycatch-per-retained-ratios (BPRR) were calculated by dividing the number of mortalities (both immediate and predicted delayed) and bycaught crab (discarded alive or dead) by the number of crab retained.

3.4 Results

3.4.1 Dungeness Crab RAMP

3.4.1.1 *Establishing a Set of Reflexes*

We looked for patterns in reflex impairment to determine if fewer reflexes could be used for assessment (i.e., if some reflexes were seldom lost or linked, or others were primarily lost). We found that, for the majority of assessed males, if only one reflex was lost (Score-one) it was Chela Closure (64%), followed by Leg Wrap (26%). Similarly, for females, Chela Closure was most frequently the first reflex to be lost (49%); however, this was followed by Abdomen Response (20%) then Leg Wrap (18%). Of all lost reflexes, for males, 56% were Chela Closure, followed by Leg Wrap (28%), and Mouth Defense (10%). For females, Chela Closure (39%) was followed by Abdomen Response (23%), Leg Wrap (18%), and Mouth Defense (14%). For both sexes, the Leg Curl and Eye Retraction reflexes were seldom lost. Despite patterns in reflex loss, we could not determine reflexes that could be linked or eliminated given the low numbers of impaired crab.

3.4.1.2 *Assessing Crab*

We completed 26 sampling trips for the recreational and 22 for the commercial Dungeness crab fisheries, assessing 7,685 total crab. More information on sampling trips, catch composition, and size distributions can be found in Appendices B, C, and E. Catch size and composition of the commercial and recreational fisheries varied by time from fishery opening and trip, respectively (Figure 3.2 and Figure 3.3). Over all sampling trips (not factoring in sampling frequency by month), 57% of discarded crab from the commercial fishery were hard-shell females, and 28% were hard-shell males. Conversely, for the recreational trips, 53% were sub-legal, hard-shell males and 26% were hard-shell females. There were few soft-shell females caught during trips for either the commercial or recreational fishery (1% and 8% respectively), nor soft-shell males (11% and 12%).

3.4.1.2.1 Commercial Ocean

Two ride-along trips were completed for each calendar month of the fishing season except for December (one trip) and August (no trips). Sampling was completed aboard four different vessels from two fishing ports (Newport and Florence, Oregon). One trip was completed on the opening day of the fishing season, and in another season on the second day. Soak duration ranged from 1.5 to 30 days (6 days on average), and sea state from 1 to 6 (3 on average; Beaufort wind force scale). Pots were fished in depths ranging from 5.5 to 150 meters. Of all assessed crab, 83% were Score-zero, 10% were Score-one, 3% had Scores greater than one, and 3% were immediate mortalities (Appendix C).

The data revealed temporal trends in catch composition. This included that the number of immediate mortalities and soft-shell males per pot increased towards the end of the fishing season (Figure 3.2). While few soft-shell females were caught throughout the season, the percentage of legal-size males (both discarded and retained) that were considered soft-shell ranged from 0-2% for the majority of the season, then increased in June to 23% and up to 87% in July. Similarly, 0-10% of caught sub-legal males were soft until July, when the percent increased to 50%. In addition, females were uncommon during the trip taken on opening day and were approximately a quarter or less of the discarded catch during the first week of the season and in July. For trips completed during the middle of the fishing season, however, females comprised the majority of discards. Moreover, the portion of the catch retained decreased over the fishing season. When sampling was completed on the first trip of the season, 92% of the catch was retained (25 retained per pot), 74% two days after the opening in the previous season (9 retained per pot), 55% two weeks after the opening (five retained per pot), then from four weeks to 28 weeks after the season opened, the range of retention was between 11-76% (1-5 retained per pot). In

July, 29 weeks after fishing began, only 6-7% of the catch was retained (1-2 per pot). Additionally, 32% of discarded hard-shell males were legal size in July, indicating high-grading for crab with minimal superficial damage and both chela was potentially occurring coincident with when the pound limit was in effect.

3.4.1.2.2 Recreational Bay by Boat

Two recreational trips were completed during each calendar month with the exception of April (five trips), August (no trips), and October (three trips). During these trips, on average, there were 14.8 pot/ring pulls per trip (range: 8-33). Of assessed crab, on average by trip, there were 6.0 Score-zero (range 0.1-17.2) and 0.5 (range: 0-1.8) crab with Scores greater than zero per pot/ring. There were, on average by trip, 0.2 (range: 0-1.1) crab retained and 5.9 (range: 0.3-15.9) crab discarded per pot/ring. Of all assessed crab, 92% were Score-zero, 6% were Score-one, 2% were Score-two, 109 (5%) had new injuries, and there were no immediate mortalities. There were no clear patterns in the number of crab retained or discarded over time; variation was greater among trips (Figure 3.3). There were, however, slightly more crab discarded per gear for rings than pots (6.61 and 5.25 respectively) and slightly more crab retained per gear for rings than pots (1.98 and 1.56 respectively) when all data were combined.

3.4.1.3 *Predicting Mortality from Impaired Reflexes*

Preliminary model selection on all data combined indicated a significant difference (significance in this paper tested at an alpha value of 0.05) between fishery types, namely that recreational had lower mortality probability than commercial. We therefore analyzed the data discretely by fishery type to allow for fishery-specific variables in the analysis (e.g., use of the slam bar).

For the commercial crabbing data, preliminary analyses indicated that Score, sex, and shell hardness were variables that influenced mortality. The data indicated that there were differences between females and males and, within males, soft- and hard- shell. We therefore grouped the data using a sex-shell condition variable: female, hard-shell male, and soft-shell male. Model selection using Aikike Information Criteria and drop in deviance tests indicated that the most parsimonious model included only the ‘sex-shell’ variable in addition to Score (Figure 3.4). Overall, hard-shell males had the lowest mortality probability for a given Score and those with soft shells had the highest. Alternative models including (*i*) interactions, and (*ii*) the presence of new injuries did not significantly improve model fit (p-values 0.08 and 0.20 respectively; Table 3.2). Moreover, Score best predicted mortality probability when it represented the summation of all six reflexes as a continuous variable rather than as a categorical Score (p-value 0.05), or modeling the reflexes discretely (p-value 0.24). Model selection for the recreational fishery indicated that the most parsimonious model included only one variable: whether or not the crab was Score-zero. While the presence of new injuries appeared to increase the probability of mortality, it did not significantly improve model fit (p-value 0.12). Resultant mortality probabilities, from the data and model, were 0% for Score-zero crab, and 8% for those with higher Scores.

3.4.2 Quantifying Discard Mortality Rates in the Fisheries

3.4.2.1 *Commercial Ocean*

Predicted discard mortality rates (five days after release, integrated over Scores) were 0.08 (95% Confidence Interval 0.06-0.10) for females; 0.01 (95% Confidence Interval 0-0.02) for hard-shell males; and 0.09 (95% Confidence Interval 0.03-0.16) for soft-shell males. While discard mortality rates did not vary by month, the mortality- and bycatch-per retained ratios (MPRR, BPRR) increased over the fishing season (Figure 3.5). During a ride-along trip on opening day of the 2013-14 season, MPRR was 0.001 (688 crab were retained per mortality) given high catch of legal crab and low discard rates. On the second day (in the previous season) MPRR was 0.01 (73 crab retained per mortality). MPRR increased through the season until July when the value, at its highest, was 1.5 (range 0.2-1.5). Likewise, the BPRR increased through the fishing season until July when 14 crab were bycaught (range 2-14) for each retained crab.

3.4.2.2 *Recreational Bay by Boat*

The discard mortality rate was estimated to be 0.01 (95% Confidence Interval 0-0.02). There were no clear spatial or temporal trends in mortalities. With respect to MPRR and BPRR, not including trips when no crab were retained (n=6), there were 39.9 crab, on average, bycaught per retained crab (range 5.5-127.0). Meaning that, on average, only 4% of the catch was retained (range 0-15%). Moreover, there were, on average, 0.26 predicted mortalities per retained crab (range: 0-1.6; i.e., for every 3.8 crab that were retained, a discarded crab was predicted to die).

3.5 Discussion

3.5.1 Dungeness Crab RAMP

The RAMP approach was effective in determining the primary influences on discard mortality and quantifying discard mortality rates for Dungeness crab. An advantage of RAMP is that it eliminated bias linked with selecting animals for captive observation. If bycaught crab are held in captivity to determine discard mortality without using RAMP and only the healthiest or most impaired animals are unknowingly selected for evaluation, accurate estimates cannot be determined (Musyl et al. 2009). In addition, by applying the regression models relating mortality with Score to ride-along data, our mortality rates were estimated over a broader scale and range of impairment than if the former approach had been applied. In this way, RAMP allows for mortality estimates that are more representative of the fishery. Moreover, the reflex impairment Score incorporated the effects of injury. This was similar to findings by Stevens (1990) that vitality scoring is a better predictor of survival than presence of injuries. Without needing to score for injury, there is a reduction in subjectivity bias in assessment given that it is easy to overlook an injury. Also, it is time-consuming to do a thorough assessment of injuries for each individual crab and all injuries are not necessarily external.

While we felt the RAMP approach was effective, we acknowledge that there were limitations in data analysis and scope. When mortality is determined by holding animals in captivity, long-term survival and mortality attributed to increased susceptibility to predation or inability to eat cannot be assessed. Therefore, the discard mortality rates from this study should be viewed as minimum values that do not include possible long-term mortality resulting from capture and discard. With respect to limitations in analysis, low numbers of impaired crab (i.e., crab with Scores greater than zero) prevented a thorough assessment of some of the variables and interactions among them (e.g., mortality rates for soft- vs. hard-shell females). The infrequency of impaired crab also required us to extrapolate and interpolate mortality rates for Scores with limited to no data using the logistic curve. In addition, consistent with findings by Yochum et al. (2015) that a RAMP relationship can be specific to a set of stressors, we determined that separate RAMP relationships are required for the commercial and recreational fisheries. This result could have been influenced, however, by the fact that 67% of crab held for the recreational fishery were hard-shell males, and only 19% had soft-shells. Given that hard-shell males have the highest survival rate this could have influenced the difference in mortality probabilities between the fisheries, making the recreational mortality rate sensitive to the composition of held animals. For RAMP information related to recreational shoreside and ocean fishing see Appendices A, C, and D.

3.5.2 Quantifying Discard Mortality Rates in the Fisheries

Discard mortality rates of sub-legal, soft-shell male, and female Dungeness crab from this study are similar to, yet lower than previous estimates. Barry (1984) found that the handling mortality rate for soft-shell crab was 12.9% (and as low as 11.3%), and 0% for hard-shell crab after 3 days of holding. Tagging studies by Cleaver (1949), Waldron (1958), and Kruse et al. (1994) similarly indicated reduced discard survival for soft-shell crab. Likewise, a study by Tegelberg and Magoon (1970) found that hard-shell crab had a handling mortality rate of 4%, and 16% for soft-shell mortality. Tegelberg (1972) also found that when tagged with Peterson disc tags (but not with epimeral suture line dart tags that were more similar to those used in our study) these rates increased (23-41%, the latter with increased holding and handling), and that when soft-shell crab were dropped mortality increased to 57%. Alverson et al. (1994) reported that mortality estimates for the coast-wide pot fishery ranged from 22-25% for soft-shell crab and 2-4% for hard-shell sub-legal crab.

Previously estimated mortality rates are higher than those estimated from this study, likely due to differences in study methodology. For example, Tegelberg (1972) held crab together in groups of 25. Given that he estimated cannibalism rates to be 6.8% on soft-shell crab, depredation could have contributed to mortality rates from that study. Mortality attributed to tagging could have also influenced estimated rates from previous studies. We were able to improve upon prior methodologies. We also generated discard mortality rates that are more representative of Oregon fisheries by incorporating representative composition of the levels of reflex impairment, and by detecting differences in mortality rates not only by shell condition, but by sex and fishery We acknowledge, however, that our estimates and confidence limits may not be representative of fishermen that are less careful with handling than those with whom we sampled crab.

We note that in comparing soft-shell mortality rates among studies, there is the concern of having a consistent definition of ‘soft’ (Appendix F). For Dungeness, soft-shell crab have been defined as such based on meat weight (Robinson et al. 1977, ODFW 2009); physical appearance (encrustation, color, etc.) and flexibility of carapace and legs (Waldron 1958, Tegelberg 1972, Barry 1983, Hicks 1987, Fisheries and Oceans Canada 2014); and time relative to moulting (Reilly 1983, Dunham et al. 2011). Other studies have utilized a combination of these descriptors to define shell condition (Spears 1983, Penson JR and Tetty 1988 from Somerton and Macintosh 1983, Hicks and Johnson 1999, Lippert et al. 2002), and others used durometer measurement (Hicks and Johnson 1999, Fisheries and Oceans Canada 2014). The durometer is a spring driven device that measures, in durometer units 0-100, the pressure required to indent the exoskeleton (Hicks and Johnson 1999). While the durometer has the advantage of generating an objective, measured value for shell hardness, there are limitations to this method. These include that the measurement (*i*) is subjective to the body part measured as there is variation in how quickly different parts of the crab harden; (*ii*) cannot be repeated because the device softens and cracks the shell; (*iii*) varies with how quickly the operation is completed; and (*iv*) does not factor in decreases in shell hardness with old age (Foyle et al. 1989). In addition to the concern of accurate readings, the terms ‘hard’, ‘intermediate’, and ‘soft’ for some studies were largely undefined (e.g., Northrup 1975), and vary in practice. During our ride-along trips we noted that what was considered ‘too soft’ for retention varied by fisherman, typically by the amount of experience handling crab and the target market for the product, and was influenced by whether or not the crab was caught when the pound limit was in effect (starting the second Monday in June). We therefore highlight the importance of clarifying what is meant by ‘soft’ and the importance for consistency in designation of shell condition, including how ‘soft’ is defined, what part of the crab is assessed, and how much pressure is exerted when testing. Also, dividing ‘soft’ into two categories (very recent moult or ‘jelly crab’, and soft with some hardening) might provide more information on discard mortality. Moreover, we recommend measuring an area of the crab that hardens last, namely the ventral surface of the carapace, halfway between the 10th anterolateral spine and the coxa of the second walking leg (Hicks and Johnson 1999).

3.6 Conclusions

3.6.1 3-S Management

Previous research has deemed the current management practices for the Oregon Dungeness crab fishery to be conservative “relative to what the population can sustain” (Heppell 2011). Moreover, the commercial fishery was awarded a certification for sustainability by the Marine Stewardship Council (Daume and DeAlteris 2014). In accordance with these findings, we determined that discard mortality rates are relatively low for the commercial and recreational Dungeness crab fisheries. This finding supports the goals of the ‘3-S’ management strategy for Dungeness fisheries to protect sub-legal, female, and soft-shell crab. However, it is important to consider MPRR, BPRR, and occurrence of soft-shell crab when evaluating management for this fishery, especially with respect to temporal trends.

In addition, while Dungeness discard mortality rates are relatively low, the potential suite of stressors experienced by discarded crab could be reduced and future research to determine optimal fishing locations and ways to conduct fishing operations would benefit bycatch and discard mortality mitigation (Figure 3.6). To determine best practices for reducing discard mortality rates we recommend utilizing the RAMP approach and, where applicable, the RAMP relationships created from this study.

3.6.1.1 *Size*

Given the low discard- and immediate- mortality rates for sub-legal males, the “size” component of the “3-S”s benefits the population and fishery by allowing male crab to reproduce for an additional reproductive cycle(s), and to grow, yielding more meat weight per individual in future seasons. This study, however, did not evaluate the minimum size nor the potential benefits of adjusting this regulation.

3.6.1.2 *Sex*

While females were the majority of discard and discard mortality for the commercial fishery, and discard- and immediate- mortality rates were higher than that for hard-shell males, the relatively low discard mortality rates indicates that it is advantageous to release females. This allows for protection of reproductive females and avoidance of harvesting crab with inferior meat yield. Moreover, current management regulations with the estimated discard mortality rates allow the population to maintain high levels of eggs-per-recruit (Heppell 2011).

3.6.1.3 *Season*

The most evident pattern in discard and discard mortality was in the temporal variation for the commercial fishery. For one, the percent of captured legal-size males that were soft increased from 0-2% from December to May, to 23-87% in June and July (Figure 3.2). These latter values exceed the 10% threshold used by the Fish Commission of Oregon in 1948 to determine when to close the fishery (Waldron 1958). We note, however, that the percentages from our study were calculated from a limited number of sampling trips that were not stratified by depth or location.

In addition, MPRR and BPRR increased as the season progressed. As available legal-size crab abundance decreased, each retained crab came at an increasing cost in terms of discards and discard mortality. Zhang et al. (2004) found that, for Dungeness, with a handling mortality rate of 5% or 10%, above a BPRR of 40 or 20 (respectively; discarded sub-legal male only to legal-sized crab), there is net loss in long-term yield. The ratio for the commercial fishery (including females, and sub-legal and legal size males) is below these thresholds and, while BPRR is near these levels for the recreational fishery, the mortality rate is lower. We note that our estimates of MPRR and BPRR are based on a limited number of sampling trips, and could be influenced by fisherman skill level and definition of a 'soft' crab, and high-grading when the pound limit is in effect. Regardless, the trend of increasing MPRR and BPRR is apparent and reflects a decrease in catch of legal crab and an increase in non-target catch as the season progresses. In contrast, when monthly values of MPRR and BPRR (average of trips by month) were speculatively applied to ODFW commercial landings data for the 2011/12 – 2013/14 fishing seasons (converting pounds to individual landed crab by approximating each crab to weigh two pounds), we estimated that approximately half of the total discards and bycatch mortality took place in the first three months of the season. While MPRR and BPRR were lowest for the commercial fishery at the beginning of the season, the fleet-wide effort was highest at this time resulting in higher total mortality and discard than in the subsequent months.

To determine whether the commercial fishery closure is appropriately set on August 15th an in-depth assessment is required of the trade-offs between discard, and discard- and natural- mortality rates, while factoring in socioeconomic considerations and fleet dynamics. It should also be considered, given that mortality is a function of effort, how the impact on soft-shell crab would be affected if effort in the spring and summer were to increase in the future due to increased price per pound of crab, or low prices or catch in concurrent fisheries. Moreover, in evaluating efficacy with the current management strategy, high effort with low bycatch and discard mortality rates in the beginning of the season should be weighed against the increase in soft-shell crab, and hence higher mortality rates, and in MPRR and BPRR later in the season.

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3.9 Figures

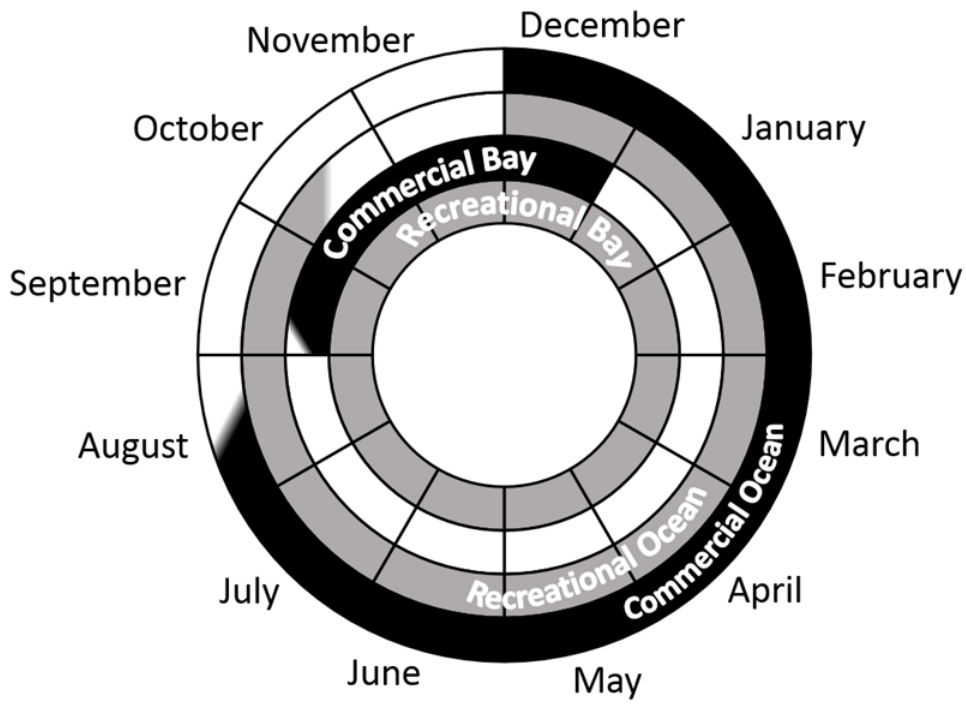

Figure 3.1 Fishing season for Oregon Dungeness crab

The fishing season for Oregon's recreational (grey) and commercial (black) fisheries for Dungeness crab (*Cancer magister*) in both the Pacific Ocean and adjacent bays.

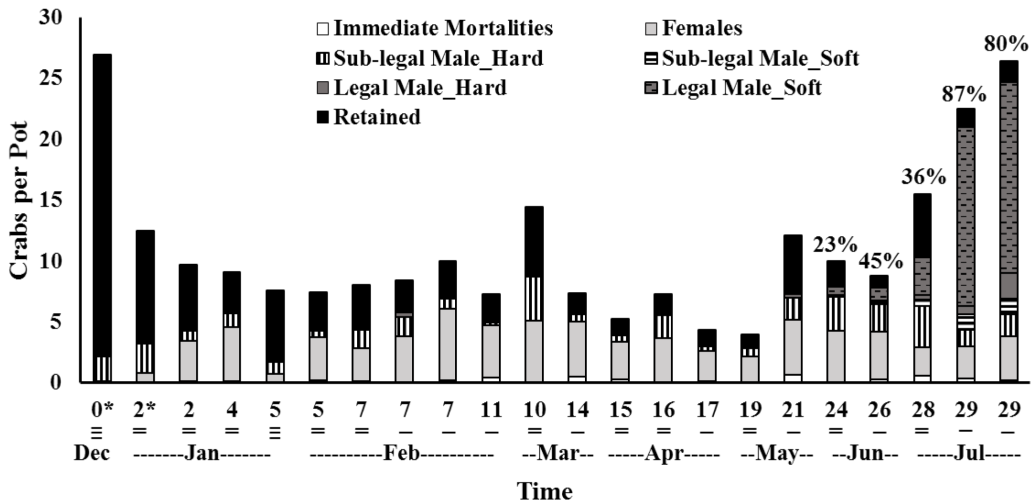

Figure 3.2 Catch composition for commercial ocean sampling

Catch composition for each commercial ocean sampling trip (n=22), including the number of sub-legal (<159 mm) and legal male (hard- and soft-shell), female (all sizes and shell conditions combined), and dead ("immediate mortalities", including all sex and shell condition categories) crab intended for discard per pot, and number of retained crab per pot. Males without a specified shell condition were not included (n=15). Trips are listed by number of weeks past the opening of the fishing season (*: the first two trips are listed by days from the fishery opening). Indicated are the calendar months that the trips took place and the sampling year: first (2011/12), second (2012/13), and third (2013/14) sampled fishing seasons correspond to number of bars. For trips in June and July, the numbers above the bars indicate the percent of legal-size male crab (those retained and discarded) that were soft. For the remaining trips, the percentage ranged from 0-2 by trip.

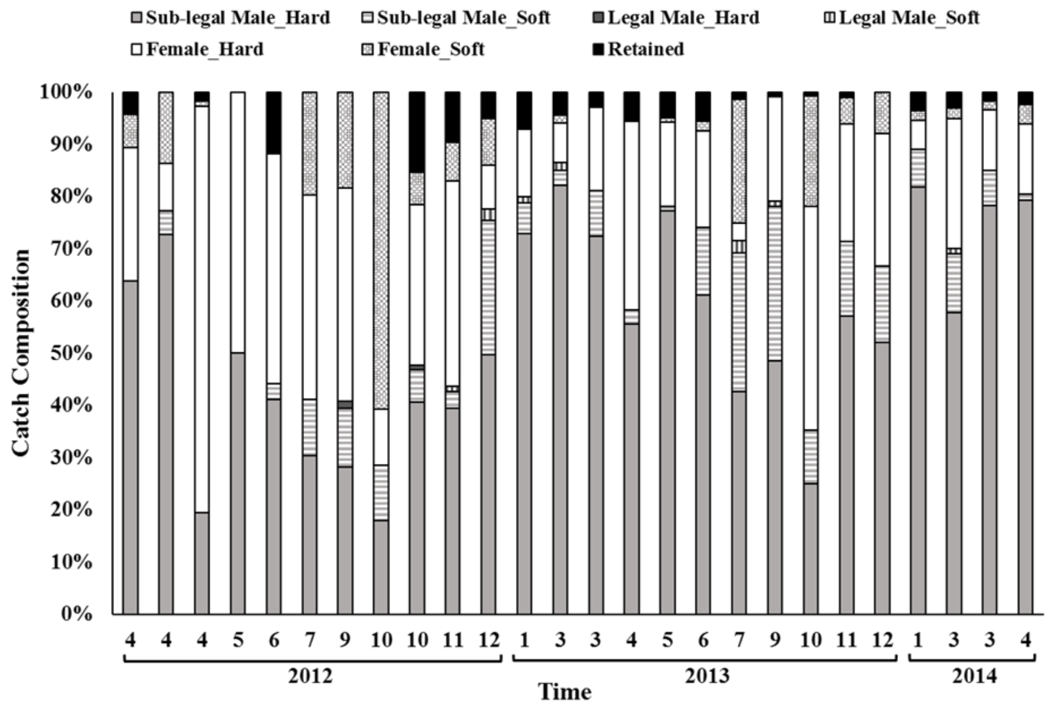

Figure 3.3 Catch composition for recreational bay by boat sampling

Catch composition for each recreational bay by boat sampling trip (n=26), shown by the numeric calendar month and year sampling took place, of crab intended for discard (hard- and soft-shell sub-legal, <146 mm, and legal male, and female crab) and those retained.

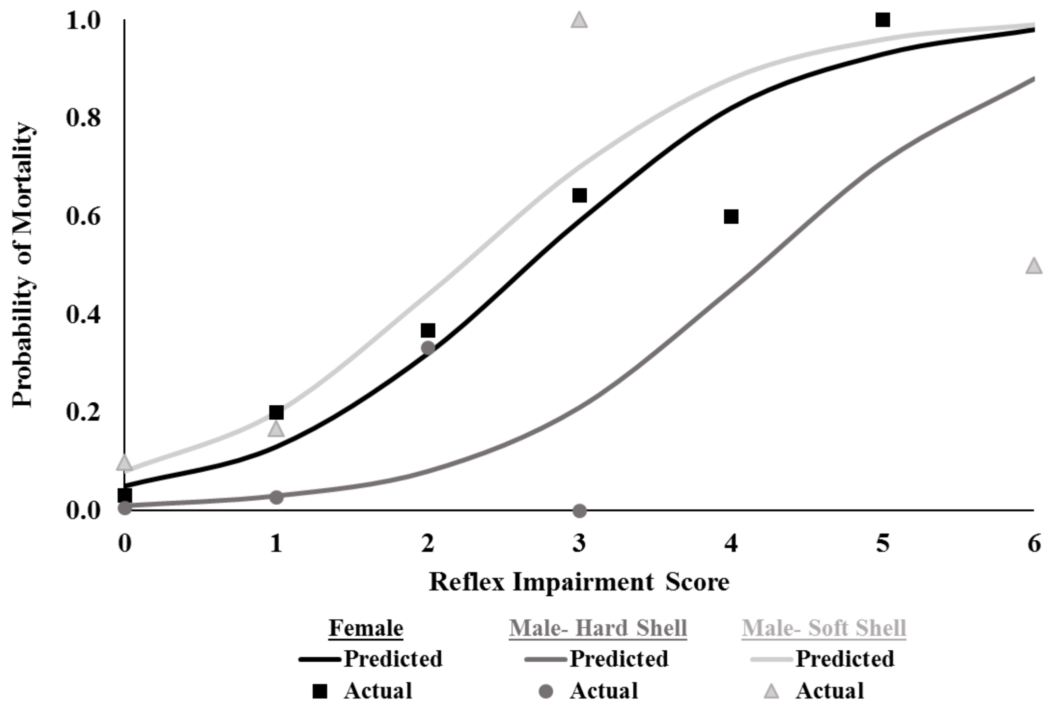

Figure 3.4 Commercial Reflex Action Mortality Predictors for Dungeness crab

Logistic model predictions of the probability of mortality by reflex impairment score for Dungeness crab (*Cancer magister*) discarded from the commercial ocean crab fishery for three categories of crab; and the actual proportions of crab that died during laboratory holding (five days of observation).

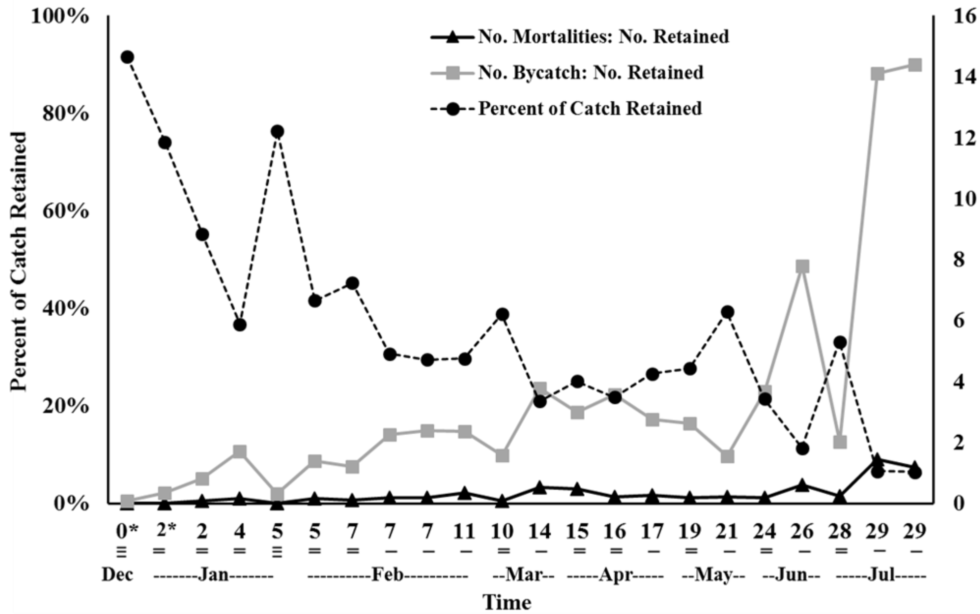

Figure 3.5 Catch composition relative to crab retained for commercial ocean sampling

By commercial ocean sampling trip (n=22), the number of mortalities (discard mortality and immediate mortality, those dead in the pot), and the number of bycaught crab (discard and immediate mortality) per retained Dungeness crab (*Cancer magister*; MPRR and BPRR), and the percent of the total catch retained listed by number of weeks past the opening of the fishing season (*: the first two trips are listed by days from the fishery opening). Indicated are the numeric months that the trips took place and the sampling year: first (2011/12), second (2012/13), and third (2013/14) sampled fishing seasons correspond to number of bars.

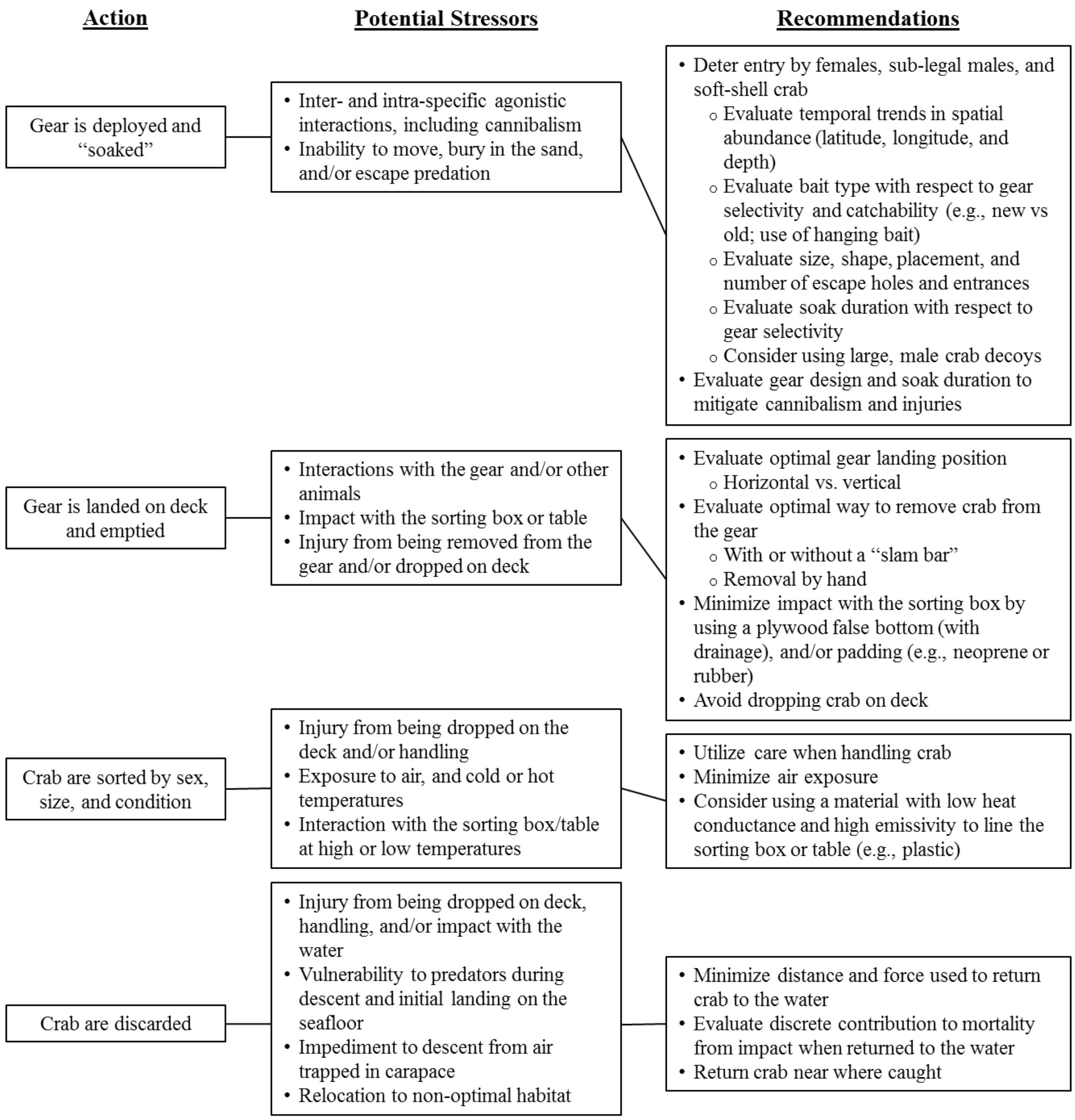

Figure 3.6 Fishing stressors and recommendations for mitigation and future research

The potential stressors experienced by Dungeness crab (*Cancer magister*) in directed recreational and commercial crab fisheries, and recommendations for future research and ways to reduce these stressors.

3.10 Tables

Table 3.1 Established reflexes for assessing Dungeness crab vitality

The established reflexes used to assess Dungeness crab (*Cancer magister*) vitality to create a Reflex Action Mortality Predictor (RAMP) relationship, along with the method for assessment and metrics for determining if a given reflex is ‘present’ or ‘absent’.

	Reflex	Method	Present	Absent
1	**Eye Retraction**	A probe is used to lightly tap the top of an eye	Crab retracts the eye downward	Crab does not react, leaving the eye in place
2	**Mouth Defense**	A probe is used to attempt to pull forward the 3rd maxillipeds	Crab defends its mouthparts with its chela making it difficult to access the maxillipeds	Crab allows it's maxillipeds to be manipulated
3	**Chela Closure**	A probe is placed below the chela dactyl	Crab reacts by closing the chela tightly, then opening it again without manipulation	Crab does not open and close its chela without manipulation
4	**Leg Wrap**	A probe is used to pull pereopods 2-4 to a 180 degree angle	Crab draws the pereopods back in (i.e., joints at less than a 180 degree angle)	Crab pereopods do not move without manipulation
5	**Leg Curl**	Pereopod 5 is straightened and pulled downward	Crab pulls up and curls its pereopod in a controlled manner	Crab does not move the pereopod without manipulation
6	**Abdomen Response**	A probe is used to attempt to pull the top of the abdominal flap away from the crab's body	Crab exhibits a strong, agitated reaction	Crab does not react

Table 3.2 Logistic modelling results

Results for the most parsimonious logistic model (in bold) and alternatives (including standard errors, SE, and Aikike Information Criteria, AIC) indicating that mortality is best predicted using reflex impairment (Score; continuous from 0 to 6) and sex-shell hardness (female soft- and hard-shell combined: reference category) for the commercial ocean Dungeness crab (*Cancer magister*) fishery, and using whether or not a crab has impaired reflexes (reference category) or not (Score-zero) for the recreational bay by boat fishery. Alternative models that were considered included the presence of injuries (commercial and recreational), and treating Score as a continuous variable (recreational).

	Parameter	Coefficient	SE	P-value	AIC
Commercial Ocean	**Intercept**	**-2.98**	**0.24**	**< 2 e-16**	**277.8**
	Score	**1.12**	**0.15**	**1.3 e-13**	
	Male- Hard Shell	**-1.71**	**0.62**	**0.006**	
	Male- Soft Shell	**0.49**	**0.54**	**0.36**	
	Intercept	-3.06	0.25	< 2 e-16	278.08
	Score	1.13	0.15	1.9 e-13	
	Injury	0.73	0.53	0.17	
	Male- Hard Shell	-1.70	0.62	0.006	
	Male- Soft Shell	0.49	0.54	0.36	
Recreational Bay by Boat	**Intercept**	**-2.48**	**0.60**	**3.6 e-5**	**38.5**
	Score-Zero	**-3.17**	**1.17**	**0.007**	
	Intercept	-3.16	0.87	0.0003	38.08
	Injury	1.77	1.11	0.112	
	Score-Zero	-2.69	1.24	0.03	
	Intercept	-5.07	0.70	5.2 e-13	40.79
	Score	1.52	0.53	0.004	

CHAPTER 4

COMPARING CAPTIVE HOLDING AND MARK-RECAPTURE STUDIES TO EVALUATE DISCARD MORTALITY FOR OREGON DUNGENESS CRAB (*CANCER MAGISTER*) FISHERIES: FIELD VALIDATION OF THE REFLEX ACTION MORTALITY PREDICTOR APPROACH

4.1 Abstract

This field validation study evaluated the accuracy of results generated using laboratory holding with the Reflex Action Mortality Predictor (RAMP) approach. The RAMP methodology, which relates reflex impairment to probability of mortality due to fishing stressors, requires that mortality of animals with varying levels of impairment be determined. Given the unnatural conditions and short-term duration for determining mortality in laboratory captivity, it is unknown if this approach under- or over-estimates mortality. To assess this, we compared discard mortality rates of Dungeness crab (*Cancer magister*) in Oregon crab fisheries that were previously estimated using a laboratory-based RAMP approach with mortality rates inferred from a mark-recapture study described here. Similarities between the studies lends support for the validity of RAMP to efficaciously estimate mortality rates using laboratory holding. Trade-offs between the two approaches are dictated by the overall objectives of the study, logistic constraints, and the level of reflex impairment by the fishing and handling process. For the Oregon Dungeness crab fisheries, the two approaches, used together, provided a more comprehensive evaluation of what affects survival of discarded crab than either alone. Between the two, however, the laboratory-based RAMP approach was superior in its ability to quantify discard mortality rates. However, the mark-recapture study was also able to determine important influences on mortality and allowed for increased collaboration and outreach in the fishing community.

4.2 Introduction

The reflex action mortality predictor (RAMP) approach has increasingly been utilized to evaluate and quantify mortality rates attributed to fishing stressors (e.g., handling and discarding) since its introduction in 2006 by Davis and Ottmar. RAMP relates impairment in reflex actions to mortality probability. This is accomplished by first establishing a set of reflexes (i.e., involuntary responses to a stimulus) that are present in a minimally stressed animal and that give a consistent response to stimulation. Animals enduring the stressor(s) of interest (either directly during fishing operations or through laboratory simulation) can then be evaluated by determining whether each of these reflexes is present or absent. To relate the levels of impairment to mortality probability, survival is determined through captive holding in on-board holding tanks (Stoner et al. 2008, Hammond et al. 2013, Rose et al. 2013, Depestele et al. 2014, Humborstad et al. 2016) or laboratory tanks (Davis and Ottmar 2006, Davis 2007, Humborstad et al. 2009, Stoner 2009, 2012a, Braccini et al. 2012, Barkley and Cadrin 2012, McArley and Herbert 2014, Hendrick-Hopper et al. 2015, Uhlmann et al. 2015). Survival has also been determined through telemetry, including radio (Raby et al. 2012, Nguyen et al. 2014), acoustic (Donaldson et al. 2012), and satellite (Gallagher et al. 2014), through the use of in-situ net pens or cages (Diamond and Campbell 2009, Brownscombe et al. 2015, Bower et al. 2016), and through visual monitoring (Campbell et al. 2010c, Hochhalter 2012, Danylchuk et al. 2014, Brownscombe et al. 2014). The relationship between reflex impairment level and mortality probability is then explained with a RAMP relationship, a predictor of mortality.

The increasing use of RAMP application in research studies reflects its efficacy; however, this has also revealed the limitation that results generated using RAMP could be influenced by the method used to measure mortality. Resultant mortality rates could be influenced by human error in observation, mortality attributed to tagging for identification (Tegelberg and Magoon 1970, Wassenberg and Hill 1993) or telemetry studies, or a captivity effect (i.e., due to captive holding conditions).

Mortality caused or influenced by holding can be the result of agonistic interactions or predation among captive animals, sub-optimal temperature or water quality, holding density, or failure to meet other biological or environmental requirements of the animal (Tegelberg 1972, Simonson and Hochberg 1986, Kondzela and Shirley 1993, Wassenberg and Hill 1993, Spanoghe and Bourne 1997, Portz et al. 2006, Weltersbach and Strehlow 2013). RAMP studies that utilize laboratory holding are also limited in that they are confined to assessing short-term mortality given the absence of natural conditions in the long-term. Specifically, impairment attributed to fishing stressors (e.g., missing or regenerating legs for crab) that affects an animal's ability to feed, grow, and/or avoid predation are not considered (Durkin et al. 1984, Stoner 2009, Uhlmann et al. 2009, Benoît et al. 2010, Urban 2015). Displacement from suitable habitat and the impact from the return to water are similarly not considered. In captivity, these indirect and delayed effects are not contributing to the mortality rates being quantified, thereby potentially overestimating survival. Short-term captive holding is also limited in its ability to capture chronic mortality that results in differential mortality rates between impaired and unimpaired animals over a long period of time (Wassenberg and Hill 1993, Bergmann and Moore 2001). Despite the aforementioned limitations, captive holding is a frequently used technique for determining mortality given the advantages over alternative methods. Unlike for mark-recapture, telemetry, and visual monitoring studies, controlled laboratory holding allows scientists to differentiate mortality causes, observe degradation in health and changes in behaviour, and know the time of death (Davis and Ryer 2003).

We conducted a field validation study to assess whether a relationship between mortality probability and reflex impairment estimated in the laboratory is under- or over-estimating mortality rates given the limitations associated with captive holding. This was done by evaluating mortality rates for Dungeness crab (*Cancer magister*) discarded from Oregon (U.S.A) commercial and recreational crab fisheries using mark-recapture and comparing the results with those from a laboratory captive holding RAMP study. The former study and comparison are described here and the latter is described in Chapter 3. These Dungeness fisheries were selected for this field-validation because of their high level of discard and because Oregon fishermen have experience with tagging studies for these crab, which yielded high tag return rates (Jow 1963, Snow and Wagner 1965, Demory 1971, Hildenbrand et al. 2011).

The impetus for evaluating Dungeness discard mortality rates was to assess the “3-S” management (Size, Sex, and Season) strategy utilized to regulate these fisheries. The commercial ocean fishery only allows permit holders to retain male crab at or above 6 ¼ inches (159 mm) from December 1st to August 14th. The estuarine recreational fishery (from a boat or shoreside) is open all year, and only 12 male crab at or above 5 ¾ in (146 mm) may be retained per permit (open access) per day (PSMFC 1978, ODFW 2015b). Temporal restrictions on the commercial fishery are imposed to minimize capture of recently moulted, ‘soft-shell’ crab. Regardless, a number of legal-size, soft-shell males are captured and discarded. These soft-shell crab are similarly discarded in the recreational fishery (See Chapter 3). Overall mortality rates for the commercial ocean fishery were estimated from the laboratory study to be 0.08 (95% Confidence Interval 0.06-0.10) for females; 0.01 (95% Confidence Interval 0-0.02) for hard-shell males; and 0.09 (95% Confidence Interval 0.03-0.16) for soft-shell males. The discard mortality rate for the recreational fishery from a boat was estimated to be 0.01 (95% Confidence Interval 0-0.02).

To evaluate whether or not the discard mortality rates estimated through captive holding were under- or over-estimated, we compared results from the laboratory study to those from the mark-recapture study described here. One differential source of mortality experienced by crab from the mark-recapture study and not by those held in the laboratory is the impact of the return to water after release. An assessment of this mortality source was evaluated discretely to determine if it influenced differences in mortality rates between studies. The aim of this research was to assess the accuracy of mortality rates estimated using a laboratory-based RAMP approach, and to evaluate mark-recapture as a potential alternative method.

4.3 Materials and Methods

4.3.1 Mark-Recapture Study

The mark-recapture study was conducted in parallel with the laboratory captive holding RAMP research described in Chapter 3. For the latter study, between February 2012 and April 2014, crab intended for discard were assessed during 22 commercial fishing trips ('ride-alongs'), and 26 recreational fishing trips were completed on a boat in Yaquina Bay (Oregon). In addition, on 15 occasions, sampling of the recreational shoreside fishery was completed at the Port of Newport Public Fishing Pier (on Yaquina Bay). During these sampling events, crab from all pots or rings were assessed of two to six willing individuals or groups of people on the pier that were crabbing for the pre-determined sampling period or until they stopped fishing. For all crab intended for discard, assessments included noting the sex and shell condition ('soft', those with little or no hardening post-moult, or 'hard', what would be generally considered acceptable for retention by fishermen), along with carapace width, presence of new injuries, and amount of time spent out of water prior to assessment. In addition, each crab was evaluated for presence or absence of the six reflexes established for assessing Dungeness vitality, and was given a reflex impairment score ('Score') equal to the number of absent reflexes (0-6, 'weak' reflexes were considered 'present'). Environmental and fishing variables were also recorded, including soak duration (i.e., the amount of time from gear set to retrieval), and depth where the pot or ring was set. See Chapter 3 for additional details. Data recorded during these trips provided information on catch composition over the fishing season with respect to sex and shell condition, as well as composition by reflex impairment score.

During the sampling trips, some crab intended for discard were transported to a laboratory and were held there to measure mortality. Due to an observed captivity effect after five days of holding, a crab was considered a 'mortality' only if it did not survive during the first five days. Through logistic regression modeling, relationships were established between reflex impairment score and mortality probability. It was determined that mortality for the commercial ocean fishery was dependent upon Score, sex, and shell condition. For the recreational bay fishery (from a boat), it was determined that the most important predictor of mortality was whether or not the crab had a Score of zero or greater. The mortality rates for crabs with Scores greater than zero were statistically indistinguishable by Score, but were measurably higher than Score-zero crab. However, the high proportion of hard-shell male crab held for this fishery could have influenced modeling outcomes. In addition, small sample sizes precluded estimating a relationship between Score and probability of mortality for the shoreside recreational fishery; however, results indicated that the relationship for the commercial ocean fishery can cautiously be applied to this fishery.

Beginning with sampling trips in October 2012, in addition to assessing and holding crab in captivity, a subset was also tagged and released at the location of capture. Care was taken to balance the number of impaired crab that were returned to the laboratory for the holding study, and the number that were tagged and released over different combinations of Score, sex, shell condition, and injury. Similar data were collected for tagged and released crab as those held in the laboratory. We tagged, in addition to those intended for discard, 38 ‘experimental’ crab that were Score-zero, legal size hard-shell males (i.e., those that would have been retained) to see how their return rate compared to the discarded crab. This was done to evaluate the potential for the tag return rates to be biased by size given that fishermen spend more time handling larger male crab to determine if they will be retained, thereby increasing the chance that the tag is noticed. The number of experimental crab was limited for the commercial fishery by permission to release crab that could have been retained, and, for the recreational fishery, by the number caught. Tag returns were accepted until August 15, 2014.

4.3.1.1 *Tag Selection and Application*

Crab were tagged with a lime green double ‘t-bar’ anchor tag (TBA-LEVO, Hallprint Fish Tags; Figure 4.1), the same that was utilized for the laboratory holding study for identification purposes. The tag is polyethylene molded to a polypropylene filament, has a space of 1.5 cm between ‘t-bars’, and is 6 cm long following the second ‘t-bar’. Tag selection and placement were determined through a pilot study. Several tag types were tried on captive Dungeness, including spaghetti tags. These tags have been used on Dungeness crab in previous research, both by inserting the tag into the suture line (Snow and Wagner 1965, Lehman and Osborn 1970, Demory 1971, Collier 1983, Kruse et al. 1994) and tying the tag around the crabs leg (Hildenbrand et al. 2011). For the latter approach, it was anecdotally reported that tag retention was linked with the knot tying ability of the person attaching the tag. We also noted higher tag loss rates with the spaghetti tag than the TBA-LEVO during pre-experiment tag trials (See Chapter 3). We similarly tried a single t-bar anchor tag. This tag type was often drawn into the crabs’ bronchial chamber and thus was found unsuitable for Dungeness. The t-bar style was advantageous, however, over tags that attach externally to the carapace, given the tendency for crab to crawl on top of each other, and, because the t-bar tag is external, it is relatively inexpensive, easy to apply and detect, applicable to a large range of crab sizes, and able to hold information (e.g., identification number; Thorsteinsson 2002). This tag was also selected because Dungeness can retain it through ecdysis (Swiney et al. 2003) and because of its longevity. In one case, the TBA-LEVO tag was retained by a giant crab (*Pseudocarcinus gigas*) for 17.5 years and showed no deterioration in print legibility on the tag’s notation barrel (Andrew Levings, personal communication).

Suitable tag placement and application practices were determined by dissecting a number of Dungeness crab and evaluating the internal morphology. The resultant protocol was to insert tags using a tagging gun with a stainless steel needle at an upward angle in the epimeral ‘suture line’ (where the crab splits during ecdysis; MacKay 1942), dorsal to the right posterior third walking leg, and near the bronchial chamber. This location was selected with the intention of inserting the tag into an empty cavity to prevent damage to the gills or other internal organs, and to avoid hindering the moulting process. The tagging needle was inserted only deeply enough to release the tag into the body cavity. While other studies have dipped the needle in 100% isopropyl alcohol between each tag application (McPherson 2002), we did not do this given the small, moving work space. After insertion, the tag was pulled gently to ensure that it did not get snagged on any internal structure, was inserted correctly, and would not fall out (Levings 2008).

4.3.1.2 *Outreach Program to Solicit Tag Returns*

An extensive outreach campaign began prior to the commencement of the mark-recapture study and was a focal part of the project throughout its duration. To encourage fishermen participation, for each tag returned (either the physical tag or a picture of the tag was required) fishermen were given $20, a hat, or a shirt, and an entry ticket for two cash prize raffles that took place in October 2013 and August 2014. Outreach efforts to make fishermen aware of the rewards and project (entitled 'Oregon C.R.A.B. Project', Collaborative Research to Assess Bycatch), and to encourage participation included: (*i*) frequently talking with fishermen at the docks; (*ii*) regularly posting flyers at local docks and in fishing and marine supply stores; (*iii*) providing flyers and information to be distributed by the Oregon Department of Fish and Wildlife, the Oregon Dungeness Crab Commission (a commodity commission), charter fishing operations coast-wide, and two state troopers who regularly inspected catch; (*iv*) posting information on fishing websites; (*v*) maintaining a project website (www.oregoncrabproject.org); (*vi*) speaking at meetings attended by fishermen; (*vii*) distributing stickers, magnets, crab measurers, etc. with the project logo (Figure 4.2); and (*viii*) distributing 'tag return packets' that included tag return forms, a pen, tape, and information on the project and where to return tags all inside a waterproof envelope. We also solicited the help of Oregon Sea Grant (Newport, OR) to be a location where fishermen could return tags and collect rewards. We hoped that this arrangement would encourage tag returns given that this organization and location is well-known and frequented by fishermen, and because this was the place where fishermen returned tags for a previous Dungeness crab tagging study. In addition, efforts were made by participating in interviews through news media, radio and magazine advertisements, and by hosting booths at relevant events (e.g., Saltwater Sportsmen's Show in Salem, OR and the Hatfield Marine Science Center Marine Science Day in Newport, OR).

4.3.2 Evaluating the Impact of the Return to Water

We conducted two laboratory based experiments to evaluate the potential contribution to discard mortality from returning crab to the water, which is a potential cause of differential mortality between boat fisheries, in which released crab drop a short distance when returned to the water, and the recreational shoreside fishery, in which crab were dropped up to 7 meters.. We collected Score-zero (i.e., unimpaired) crab using recreational fishing gear. We noted sex, size, and shell condition, tagged them similarly to those released for the mark-recapture study, and held them for two weeks in the same tanks used for the laboratory study to allow for recovery before experimentation. For the first experiment, conducted in November 2013, 64 crab (20 at a time) were taken from the holding tanks and placed in a large ice chest with wet burlap sacks. Three at a time, the crab were lifted in a bucket with wet burlap sacks to one of three drop heights, and released one by one into a tank of sea water. After the three crab were dropped, they were removed from the tank and placed in an ice chest filled with sea water and burlap sacks. The crab were grouped such that the first third were dropped from eight meters ("high"), the next three meters ("medium"), and the last from one meter ("low"). Once all 20 crab were dropped for a given height treatment, they were returned to the holding tanks, placed in individual compartments, and the experiment was repeated on the next group of 20 crab. In April 2014, the second experiment was conducted with similar protocols as the first, except 64 crab were dropped (20 each) from the medium and high distances, and, instead of the low distance, were dropped from six meters. The drop distances attempted to mimic the free-board from recreational and commercial vessels ("low" and "medium", respectively), and an approximate range of maximum distances that a crab would be thrown from a pier or dock during shoreside recreational fishing at low tide (6 and 8 meters, "high"). The second experiment also differed in that crab were kept in water until they were lifted to the dropping point. Also, for the first experiment we attempted to drop half of the crab, for each distance, such that they would land on their dorsal side, and the other half ventrally. For the second experiment, however, we did not force the side on which the crab landed; however, it was noted. Subsequent to the first and second experiments, crab were held in captivity for 18 and 34 days, respectively, to determine mortality. However, for data analysis mortality was only considered for the first five days of holding to be consistent with the laboratory study.

4.3.3 Data Analysis

4.3.3.1 *Relative Short-Term Survival*

To evaluate relative survival rates between unimpaired crab (i.e., Score-zero) and impaired crab (i.e., Scores greater than zero) from the mark-recapture study, we employed an approach described by Hueter et al. (2006). This analysis estimates relative survival between two conditions of animals (e.g., good versus poor) under the assumption that there is differential survival between two conditions during a short-term 'recovery period', and subsequently crab with both conditions experience the same survival rate (i.e., there are no differences in chronic long-term survival after this period). Because the laboratory RAMP study only considered mortalities in the first five days of holding, this was considered the 'recovery period'. If both groups experience the same short-term survival rate, then they will have the same tag return rates after the recovery period and the ratio of tag recaptures will be one.

$$\hat{R}_{tag} = \frac{C_{t>5,2}/C_{t>5,1}}{T_2/T_1} \qquad \textit{Equation 4.1}$$

where

$\hat{R}_{tag}$: Relative survival rate between Conditions 1 (Score 0) and 2 (Score > 0) after the five day 'recovery period'

t: Days-at-large

C_j: Number of tags for the *jth* Condition recaptured after five days-at-large ($t > 5$); and

T_j: Number of tagged crab for the *jth* Condition

For the analysis, 'Condition 1' was assigned to crab with a reflex impairment score equal to zero, and 'Condition 2' to crab with Scores greater than zero. Scores 1-6 were combined for this analysis due to low sample size of tagged impaired crab. This was done by sex and shell condition combination (i.e., male hard-shell, male soft-shell, female hard-shell, and female soft-shell) given that differential mortality rates were determined for these variables in the laboratory study. This analysis was done for each release event (i.e., sampling trip) to control for the influence on return rate of days-at-large, natural and fishing mortality, and temporal variability in catchability, fishing effort, tag loss, and reporting rate.

Ratios of relative short-term survival from the tagging study were then compared to similar ratios estimated for crab held in the laboratory to provide an indirect validation of the laboratory study results.

$$\hat{R}_{lab} = \frac{S_2/S_1}{H_2/H_1} \qquad \textit{Equation 4.2}$$

where

$\hat{R}_{lab}$: Relative survival rate between Conditions 1 (Score 0) and 2 (Score > 0) after five days of captive holding

S_j: Number of crab with the *jth* Condition that survived after five days of captive holding; and

H_j: Number of crab with the *jth* Condition that were held to determine mortality following capture, handling, and transport to the laboratory

Two sided confidence intervals for the relative survival rates were utilized to compare the mark-recapture and laboratory studies. The intervals were calculated as (Hueter et al. 2006)

$$\left(\hat{R}e^{-Z_{1-\alpha/2}\sqrt{v}},\ \hat{R}e^{Z_{1-\alpha/2}\sqrt{v}}\right),$$ *Equation 4.3*

where $Z_{1-\alpha/2}$ is the 100 (1-α/2)th percentile of the standard normal distribution and

$v_{tag} = \frac{T_1 - C_1}{T_1 C_1} + \frac{T_2 - C_2}{T_2 C_2}$ and $v_{lab} = \frac{H_1 - S_1}{H_1 S_1} + \frac{H_2 - S_2}{H_2 S_2}$.

4.3.3.2 *Relative Long-Term Survival*

If there is a more chronic effect from the capture, handling, and discard process a difference in survival between Conditions will continue beyond the five day recovery period. To evaluate the potential change in relative survival over time, by sex and shell hardness, we utilized logistic regression modeling.

$$Logit\ (\pi) = \beta_o + \beta_1 t\ + \beta_2 X_1 + \beta_3 X_2 + \beta_4 X_3 + \beta_5 t X_1 + \beta_6 t X_2 + \beta_7 t X_3$$

Equation 4.4

where

π: Probability that a recaptured tag was from a Condition 2 (Score > 0) crab

β_o: Intercept

β_n: Model coefficients for n variables

$$X_1 = \begin{cases} 1 \text{ if male soft} - \text{shell} \\ 0 \text{ otherwise} \end{cases}$$

$$X_2 = \begin{cases} 1 \text{ if female hard} - \text{shell} \\ 0 \text{ otherwise} \end{cases}$$

$$X_3 = \begin{cases} 1 \text{ if female soft} - \text{shell} \\ 0 \text{ otherwise} \end{cases}$$

This analysis allows for the determination of whether the odds of return between the two Conditions changes over time. A non-zero slope coefficient (β_1) indicates differential long-term survival between the two Conditions. The intercept (β_o) will be zero if the same number of tagged crabs were released in the two Conditions and they suffered the same rate of short-term mortality at *t*=0. If the two conditions have the same short-term survival then a non-zero intercept will reflect the ratio of the number of tagged animals in the two conditions at t=0. By comparing crab within a release event the results reflect relative survival given that relative natural mortality, catchability, and reporting probability are assumed to be the same for a given condition over time.

4.3.3.3 *Evaluating the Impact of the Return to Water*

Logistic regression modeling was used to determine if there was a relationship between drop height and the probability of mortality, and if any other variables influenced the likelihood of survival, including (*i*) whether or not the crab was kept in water before being dropped; (*ii*) the side on which the crab landed (dorsal, ventral, or side); (*iii*) carapace width (mm; continuous); (*iv*) sex; (*v*) shell condition (soft or hard); and (*vi*) whether or not the carapace cracked as a result of the drop.

$$Log_e\left(\frac{p}{1-p}\right) = \alpha + \beta_o(Drop\ Height) + \beta_i x_i \qquad \textit{Equation 4.5}$$

where:

p = Probability that a crab died within five days of captive holding

α = Intercept

β_o = Model coefficient for the height from which the crab were dropped

β_i = Model coefficients for the explanatory variables (x_i) tested in the model

Model coefficients were estimated using maximum likelihood (Ramsey and Schafer 2002) based on the fate (mortality or survival) of individual crab that were held following the drop experiments. Akaike Information Criteria (AIC) was used to determine the most parsimonious logistic model for the data and to determine which variables had the greatest influence on mortality.

4.4 Results

4.4.1 Mark-Recapture Study

Between October 2012 and April 2014, 4,093 live crab intended for discard were tagged and 430 tags were returned by August 15, 2014 (11%). Six tags were returned after the study period, but they were not included in the analysis. There were 13 release events for the commercial ocean fishery, and 19 and eight for the recreational bay fisheries by boat and shoreside, respectively. 207 different fishermen returned tags, ranging from 1 to 60 per fisherman (2.18 tags on average, mode: one). Returns were made by fishermen from Oregon as well as those vacationing from California, Idaho, Nevada, South Dakota, Utah, and Washington. Of returned tags (including 'experimental' crab that were legal, hard-shell, Score-zero) that were exchanged for a reward, 78% were exchanged for cash ($20), 13% for a hat (a beanie or two baseball style hats), and 9% for a t-shirt (multiple styles). Of fishermen who returned the physical tag (as opposed to sending a picture via email), 71% turned the tag in directly either to a project scientist or to the Sea Grant office, and 29% were sent via the mail. When the date of recapture was known, three were recaptured the same day on which they were released, 53 within the first week at large (12% of the returns), 142 within the first 30 days (33%), 295 within the first 100 days (69%), and 415 within a year (97%). The longest time at large was 468 days; there were 135 returned 100 days or more after release, and 15 more than a year after release. On average, returned tags were at large for 97.9 days (SD: 108.5; mode: 6; median: 55).

For the recreational bay fishery by boat and shoreside, 13% (122 of 911) and 10% (30 of 298) of tags, respectively, were returned; and 10% (278 of 2,884) for the commercial ocean fishery (Table 4.1). 18% (7 of 38) of tags applied to crab that would have been retained ('experimental' crab) were returned when all fisheries were combined (11%, 2 of 18, for commercial; 26%, 5 of 19, for recreational by boat; and 0%, 0 of 1, for recreational shoreside). Ignoring all other variables, the highest return rate was for male hard-shell crab (16%, 289 of 1,832), followed by male soft-shell crab (11%, 26 of 236), female hard-shell crab (6%, 112 of 1,930), and female soft-shell crab (3%, 3 of 95). When looking at returns only of Score-zero crab, the proportions of returns did not change from the values listed above with the exception of male soft-shell crab (10%). All fisheries combined, 187 injured crab were tagged and released and only nine were returned (5%). However, for all sex- shell condition combinations, the average Score for non-injured crab was lower than for injured crab (all crab combined: 0.18 for non-injured; 0.49 for injured), which is consistent with the idea that injury is reflected in the reflex impairment score. There was a difference in percent of tags returned by year released. In 2012, 9% of tags were returned (all other variables combined), and 11% for those released both in 2013 and 2014. However, in 2012 26% of released crab were soft-shell compared to 7% in 2013 and 8% in 2014, and only crab discarded from the recreational fisheries were released in 2012.

Differences were detected between tagged crab that were recaptured and those that were not. First, returned crab had lower Scores, on average, than those not returned, and tags on crab with Scores greater than three were not returned (Table 4.2), which is consistent with the idea that crabs with higher Scores had higher rates of mortality. The maximum number of days at large for returned tags did not exceed 468 days, while the maximum possible days at large was 674, indicating a potential limit to the tag's longevity. Also, non-returned tags were on smaller crab, on average, than those returned, and the minimum size was smaller, possibly indicating that fishermen preferentially inspected crab that were closer to legal size. With respect to cross-over among the three fisheries, of tags released in the commercial fishery, 90% were recaptured by commercial fishing operations. For those tagged during recreational fishing by boat, 94% were recaptured by recreational fishing, 2% by recreational charter fishing, and 3% by commercial fishing. For those released at the Newport Pier, 93% were recaptured by recreational fishing, 3% by charter fishing, and 4% other. For tags recovered by recreational fishing gear, 66% were caught in pots and 25% in rings. With respect to returns by Score, the commercial and shoreside fisheries had decreased returns as Score increased, while there was no clear pattern for the recreational bay by boat fishery (Figure 4.3), suggesting that reflex impairment score is a weaker predictor for the latter fishery. In evaluating returns, fishery specific patterns were observed, warranting evaluation at this level of data grouping.

4.4.1.1 *Patterns in Tag Returns for the Commercial Fishery*

Patterns in the tag return data suggest that probability of return for the commercial fishery is influenced by Score, sex, shell condition, carapace width, and time of release relative to the opening of the fishing season. Males had higher tag return rates than females (16% versus 6%), and, for both sexes, hard-shell crab had higher return rates than soft-shell (10% versus 5%). For female-hard and -soft, and male-hard and -soft crab, the percentages of tags applied to Score-zero crab were 81%, 86%, 89%, and 92%, respectively (Figure 4.4). This suggests that differences in returns were not influenced by variation in composition of tagged crab by Score. Rather, differences were likely due to differences in survival. Limited returns of tagged crab with Scores greater than zero (36 returned tags), prevented a clear assessment of tag return trends by Score. However, there were decreasing returns as Score increased for females and soft-shell males (Figure 4.5), which is consistent with the relationship observed in the laboratory study.

Patterns in the tag return rates also indicated potential sources of bias in the data. For both female and male crab, the frequency of returns was higher for larger crab relative to size composition of tagged crab (Figure 4.6), possibly indicating that the size of crab affected the likelihood of return. In addition, the proportion of tags returned was highest in December and decreased over the months of the fishing season, regardless of the composition of tagged crab by sex-shell condition combination, or proportion of tagged crab that were Score-zero (Figure 4.7). Similarly, an evaluation of the cumulative proportion of tags returned over time, by release event, revealed higher overall return rates when tags were released closer to the beginning of the season, and that days-at-large influenced overall returns less than this temporal variable (Figure 4.8).

4.4.1.2 *Patterns in Tag Returns for the Recreational Bay by Boat Fishery*

Patterns in the tag return data for the recreational bay fishery from a boat suggest that probability of return, while not clearly linked with reflex impairment score or shell condition (Figure 4.3 and Figure 4.5), varied by sex and carapace width (Figure 4.6) and was lower than the other fisheries (Figure 4.3). Male and female crab with hard- and soft- shells had similar return rates (15% to 16% for males; 6% and 5% for females). However, return rates for females were lower than for males (15% versus 6%). Also, similar to the commercial ocean fishery, the frequency of returns was higher for crab with larger carapaces. With respect to injury, 3% of injured crab were returned compared with 14% of non-injured crab (all other variables combined), consistent with the laboratory study. However, significance of the relationship between tag return probability and injury was difficult to measure given the small number (35) of injured crabs that were tagged and released. Also, when return rates based on injury were evaluated by sex, this pattern was inconsistent, likely due to the small number (8) of tagged injured females. Similarly, there were no clear temporal patterns, suggesting that tag returns were not strongly influenced by days-at-large or month tagged (Figure 4.8 and Figure 4.9).

4.4.1.3 *Patterns in Tag Returns for the Recreational Bay Shoreside Fishery*

Patterns in the tag return data suggest that, similar to the commercial ocean fishery, probability of return for the recreational bay shoreside fishery is influenced by Score, sex, shell condition, and carapace width. Tag return rates for were higher for larger crab (Figure 4.6), males than females (11% versus 6%), and, within sex, for hard-shell crab than soft-shell (8% versus 0% for females; 7% versus 11% for males), regardless of the proportion that were Score-zero (Figure 4.4 and Figure 4.5). There was no clear temporal pattern in returns by release event; however, sample sizes were small for each event. There were higher returns for non-injured crab than injured (all combined: 11% not-injured, 3% injured). This was consistent for both sexes (males 12% vs 4%, females 7% vs 0%); however, there were only 25 injured tagged crab and the average Score was higher for injured animals, suggesting that the decrease in probability or return for injured crab was likely represented by the reflex impairment score.

4.4.2 Relative Short-Term Survival

The ratios of short-term survival rates between Condition 2 (Score greater than zero) and Condition 1 (Score-zero) crab were highly variable among release events for the mark-recapture study, but suggested minimal differences in survival between Conditions. Ratios of relative tag returns and relative survival in captive holding were similar for females and hard-shell males (Figure 4.10); some release events indicated that Condition 1 crab had higher survival than 2, and others the opposite. Regardless, for all release events, the overlapping confidence intervals indicated no statistical difference between Conditions, and rates for the laboratory study were close to one or included one in the 95% confidence interval (i.e., no detectable differences). For both females and hard-shell males, the rate for the shoreside fishery laboratory study was higher than that for the commercial and recreational by boat fisheries. For soft-shell males, there was only one release event with enough data to calculate a relative survival rate. That one event, though, indicated that survival was significantly higher for crab with Scores greater than zero than Score-zero, which is inconsistent with the expectation of higher survival for crab with no impaired reflexes. In contrast, for the laboratory study, survival was higher for the Score-zero crab.

4.4.3 Relative Long-Term Survival

Model results indicated that, for all release events where there were adequate sample sizes to complete the analysis, the intercept and variable coefficients were not significantly different from zero. When back-transformed from the logit scale and plotted out, however, the intercepts were consistently at or above 0.5 (Figure 4.11). This indicates that the proportion of tag returns from Condition 2 crab (i.e., Score greater than zero) were 50% or greater, even though there were higher numbers of tags released for Condition 1 crab. In addition, the estimated slope coefficients for the majority of the release events were negative, indicating that the log-odds of a returned tag being from a Condition 2 crab decreased over time. This suggests that there is a chronic difference in survival between conditions, namely that the probability of a tag return, and therefore survival, for Condition 2 crab decreases over time. There were not consistent patterns, however, among release events that would signify the duration of the effects beyond the five day 'recovery period', and therefore an optimal monitoring duration. Despite these observed patterns, the lack of significance in the estimated model coefficients challenges the notion that there were measurable changes over time in the relative probability of a tag being returned from a Condition 1 or 2 crab.

4.4.4 Impact of the Return to Water

For the first and second experiments evaluating mortality attributed to the impact of the return to water, a total of 63 and 58 crab, respectively, were included in the data analysis. Seven crab were excluded because they hit either the ground or tank during the experiment and died as a result (Table 4.3). For the first experiment, 21 crab were successfully dropped from 1-meter, 22 from 3-meters, and 20 from 8 meters (81%, 73%, and 70%, respectively, were male; and there were only seven soft-shell males, and six soft-shell females dropped for all heights combined). By drop height, 0%, 5%, and 45%, respectively, died within five days of holding. The one crab that died within the five day observation period for the 3-meter drop was a soft-shell female that had both a broken leg and an autotomized leg as a result of the drop. For the 8-meter drop, six of the nine mortalities were male, and three were female (43% of males died, and 50% of females). All of the crab that died for this experiment, except one, had a cracked carapace as a result of the drop. While the aim was to have equal numbers of crab land dorsally and ventrally, this was not possible to control. Because of this, 48% landed dorsally, 44% ventrally, and 8% landed on their side. Of the ten that died during this experiment, six landed dorsally, one ventrally, and three on their side.

For the second experiment, a total of 18 crab were successfully dropped from 3-meters, 18 from 6-meters, and 22 from 8 meters (83%, 89%, and 82%, respectively, were male; and only one soft-shell male, and three soft-shell females were dropped for all heights combined). By drop height, 0%, 0%, and 14%, respectively, died within five days of holding. Only male crab died during the five day observation period for the 8-meter drop (3 of 18 males compared with no dead females out of the four from the experiment), and all of the crab that died had major cracks in the carapace. For this study, without attempting to control the side on which the crab entered the water, 7% landed dorsally, 26% ventrally, and 7% either on their side or it was uncertain. Of the three that died as a result of this experiment, within five days of holding, two landed dorsally and one ventrally.

Mortality was only considered for the first five days of holding to be consistent with the laboratory study. However, if all crab that died over the 18 days of holding for the first study are included, the percent of crab that died for the 1-, 3-, and 8- meter drops changed from 0%, 5%, and 45%, respectively, to 0%, 14%, and 55%. Similarly, for the second study, if all mortalities over the 34 days of holding are included for the 3-, 6-, and 8- meter drops, the percent of crab that died changed from 0%, 0%, and 14%, respectively, to 27%, 22%, and 18%. Increasing mortality rates over time, especially for crab from the lower drop height treatment, are suggestive of a captivity effect. This finding corroborates a similar effect detected in the laboratory study and supports the rationale for determining mortality only over a five day period.

Logistic model results indicate that mortality attributed to the release back into the water is primarily influenced by whether or not the carapace cracks as a result of the drop, in addition to the height from which the crab is thrown, the side on which the crab lands (dorsal, ventral, or side), and the shell condition (soft or hard) (Table 4.4). Whether a crab’s carapace cracked was closely linked with drop height and whether or not the crab was in water before being dropped. For the first experiment, 0%, 0%, and 55% of crab dropped from 1-, 3-, and 8-meters had cracked carapaces. This corresponded closely with the 0%, 5%, and 45% of crab that died after being dropped from those heights. Similarly, for the second experiment, 0%, 6%, and 14% of crab dropped from 3-, 6-, and 8-meters had cracked carapaces, and 0%, 0%, and 14% died. In addition, with both experiments combined, 0%, 0%, 6%, and 33% of crab dropped from 1-, 3-, 6-, and 8-meters had cracked carapaces, indicating increased probability of cracking as drop height increases. Considering whether or not the crab was left out of water before it was dropped, for just the 8-meter drop, 14% of those left in water, and 55% of those left out of water cracked. In combining drop height, 7% of those left in water, and 17% of those out of water cracked. These results indicate that mortality is linked with whether or not a crab's carapace cracks, which is heavily influenced by both drop height and whether or not a crab is left out of water before being returned. With respect to the side on which the crab lands, for those dropped from 8-meters, 38% of those that landed dorsally cracked, 33% ventrally, and 25% on the side, suggesting this variable may also influence mortality probability. Further research is required, however, to evaluate this variable.

Overall, these experiments indicated that freeboard drop distance is likely not contributing to mortality in operations aboard commercial and recreational vessels, but long drop distances potentially contribute to mortality for shoreside fishing (particularly at low tide). Future research is needed to determine at what height the drop into water begins to affect survival, and how mortality is influenced by time out of water, the side on which a crab lands, the force used to throw back the crab and distance travelled horizontally, and whether the crab is returned while the vessel is underway. Additional research would also inform how mortality is influenced by shell condition and sex.

4.5 Discussion

4.5.1 Reliability of Estimating Mortality using Laboratory Captive Holding

While limitations with respect to sample size of impaired crab in the mark-recapture study precluded a thorough comparison of discard mortality rates estimated in the laboratory and mark-recapture studies, similarities in the results support those estimated in Chapter 3. Similar to mortality rates estimated through laboratory holding, for the commercial ocean and recreational shoreside fisheries, tag return rates varied by reflex impairment score, sex, and shell condition. Results from the tagging study indicated lower returns for crab with higher Scores, females compared to males, and soft- rather than hard-shell animals. While the laboratory study was unable to differentiate survival for females by shell condition, the mark-recapture study detected differences in returns for these categories of crab. This suggests that the mortality rate estimated for females from the laboratory-based RAMP study could be underestimated for soft-shell females. For the recreational bay fishery by boat the two studies were similar in that Score did not have a strong relationship with mortality in the laboratory or tag return rates, and that injury could be linked with mortality. However, low numbers of impaired and injured animals prevented a more thorough analysis of the role of injury for both studies. Similarly, limited number of tag returns obscured our ability to detect statistical differences in relative short- and long-term survival rates. This was made worse by the need to combine Scores 1-6, which combined crab that had mortality probabilities close to those for unimpaired crab with crab that were moribund. These issues reflect a limitation of the RAMP approach in that it can be difficult to apply to a low-impact fishery (i.e., one where the majority of animals have unimpaired reflexes).

With respect to differences between recreational bay fishing by boat and shoreside, lower tag return rates for the latter fishery align with higher mortality rates estimated from the laboratory study. Differences in tag return rates, however, could also be attributed to the impact experienced when returned to water, as indicated by our drop experiments. Where tagged crab were released for the shoreside fishery, the distance from the rail of the pier to the water at mean lower low water was 6.3 meters, and there were commonly California sea lions (*Zalophus californianus*) present where crab were returned. Also, while we did not include crab from the drop experiments that hit the side of the tank or ground, during actual discard practices from the shore this is a realistic additional source of mortality. If a crab were to hit a piling, for example, the mortality rate would likely be high. The additional mortality from the return to water for the shoreside fishery indicates that the laboratory-based RAMP estimates are likely underestimated for this fishery.

4.5.2 Efficacy of Mark-Recapture for Discard Mortality Research

Trade-offs between laboratory holding and mark-recapture for estimating discard mortality rates depend on the overall objectives of the study, logistic constraints, and the level of reflex impairment caused by the stressor(s) being studied. For the Oregon Dungeness crab fisheries, the two approaches, used together, provided a more comprehensive evaluation of what affects survival of discarded crab than either alone. Between the two, however, the laboratory-based RAMP approach was superior in its ability to quantify mortality rates. However, the mark-recapture study was also able to identify important potential influences on mortality and allowed for increased collaboration and outreach in the fishing community.

Disadvantages of applying a mark-recapture approach are linked with the extensive list of factors that determine whether or not a tag will be returned. For this study, these include natural mortality, fishing mortality (both retention and handling mortality if a tagged animal is recaptured and released without the tag being observed), catchability, moult failure attributed to the tag, tag loss, tag induced mortality, and reporting rate. Each of these is a potential source of bias. In the case of Dungeness, natural mortality differs for crab that are and are not moulting (Zhang et al. 2004). In addition, fishing mortality must be considered when evaluating soft-shell crab that, after a period of time, become hard and recruit back into the fishery, or sub-legal crab that become legal after moulting during the study period. Moreover, differential catchabilities could apply to recently moulted male crab as evidenced by variation in temporal rates of catch per unit effort (Taggart et al. 2004), and it has been shown that pots preferentially catch females that are non-ovigerous (Swiney et al. 2003). Also, catchability potentially could be influenced by reflex impairment score if a reduction in vitality affects a crab's inclination or ability to eat. Also, for the commercial fishery, spatial patterns in where fishermen set traps varies over the fishing season (Gotshall 1978, Barry 1983), meaning that return rates could be affected by where a tag is released at different times of the year. The physical presence of the tag may also affect return rates given its potential to impede moulting, or for the tag to be lost or contribute to mortality. This could vary by size or health of the animal. Crab with higher reflex impairment scores may be more susceptible to infection caused by the tag (for example).

The biggest source of uncertainty associated with returns is potentially tag reporting. A major difficulty in conducting a mark-recapture study on discarded animals is that the animals are less likely to be inspected than if the tag was on a retained animal. As indicated by this study, reporting rate could be influenced by the sex of the crab if only male crab may be retained. Size and shell condition could also affect returns if crab more similar to those retained get preferential inspection. Anecdotally, fishermen reported that, because female and sub-legal male crab are not allowed to be retained, they were not sure if they were supposed to remove the tag or leave it in place. Differential levels of fishing effort over the season is another possible influence on tag return rate that could bias mortality rates. The majority of the effort and landings for the commercial fishery occur shortly after the season opens and during the first two months, after which point fishermen often switch to an alternative fishery (Didier 2002, Oregon Sea Grant 2008). Because of this, crab tagged and released near the beginning of the season (or crab at large during multiple season openings) had a higher chance of being observed than those tagged toward the end of the season when effort is lower. Timing of the release was a bigger influence on cumulative tag return rate than days-at-large. In addition, tag reporting is potentially dependent on successful and extensive outreach, and the willingness and ability of fishermen to participate. However, if tag return analysis assesses relative survival of different conditions of crab, as done in this study, many of these aforementioned variables of concern become irrelevant.

Despite the disadvantages, there are benefits to employing a mark-recapture approach. Advantages over a laboratory holding study include: (*i*) the lack of necessity for holding facilities and of husbandry requirements; (*ii*) that the conditions more closely mimic actual fishing stressors (assuming the tag does not contribute to mortality) and a more natural environment post-release; (*iii*) the increased involvement of the fishing community; (*iv*) reduced handling (transporting the animals from the fishing vessel to the laboratory and maintaining them in tanks); (*v*) the potential ability to capture long-term impacts; (*vi*) the lack of constraint on sample size by holding capacity; and (*vii*) increased opportunities for 'ride-alongs' if collaborators are not limited by their ability to hold crab at sea.

4.5.3 Recommendations for Future Mark-Recapture Research

To successfully apply the methods described here in future research, we highlight the successes of this study and lessons learned. With respect to successes, we feel that our outreach campaign was effective in encouraging fishermen to look for and return recaptured tags. This is evidenced by the number of participating fishermen, both local and visiting from other states, and the number of fishermen who returned multiple tags. This was attributed in part to our reward system (Pollock et al. 2001). To encourage fishermen to report multiple tags if found, we provided multiple options for the reward. While a fisherman likely does not want more than one project hat, we hoped to encourage fishermen to not only report additional tags, but to want to find them based on having several hat and shirt options, and by offering cash rewards and entry to cash prize raffles. Along these lines, we had feedback that fishermen were encouraged to return tags because we required minimal paperwork and were able to hand (or mail) fishermen cash. On several occasions we were told that interest in finding tags was based on the logo, clothing options available, and cash prize raffles. With respect to outreach, the most effective approach, particularly for the commercial fishery, was ‘walking the docks’ and talking to fishermen about the project (especially crew members who would be looking for the tags), and asking them if they had tags to trade for a reward at that time. At the height of the fishing season, when fishermen are very busy and profits are high, there is less incentive to slow operations to look for tags. We attempted to counteract this by making the project highly visible and by making the tag return process simple through ‘tag return packets’ and providing multiple ways to exchange the tag for a reward.

We felt that the tag selected for this study was also a success of the project. Given that 135 tags were returned after 100 days, 15 over a year, and that the proportion of tags returned increased at the start of the second season after going through the moult we feel confident that this tag type and application were successful in allowing this study to be conducted over the long term and through moulting. However, tags were not returned over the full duration of the study, which may indicate a potential maximum time at large for Dungeness. In addition, this tag method was advantageous over using acoustic tags and telemetry given reduced costs and because with this tag it is not possible to falsely conclude that a dead animal is alive (Yergey et al. 2012).

In addition to these successes, future studies could benefit from lessons learned from our study. Of significant importance is the need for tagging a sufficient number of animals as to be able to detect differences in recapture rates. When this study began influences by fishery, sex, and shell condition on mortality were unknown, and the influence on return rates of differential fishing effort over the commercial season was not well understood. Because of this, sample sizes for analysis were low. To counteract that in future, if a similar study is done on a low-impact fishery, it would be beneficial to also tag, at depth, animals that did not experience the stressor (i.e., control animals). In addition to allowing for an assessment of absolute, rather than relative, survival (Hueter et al. 2006, Rudershausen et al. 2014), this also provides a way to quantify mortality of Score-zero animals. This also suggests the importance of analyzing some data early on to look for variables that might be influencing returns beyond survival (e.g., effort in the fishery or shell condition). In doing this, sample sizes potentially can be increased by releasing additional animals while controlling for these variables.

Despite improvements that can be made on future studies based on our recommendations, our inability to quantify discard mortality rates using a mark-recapture based RAMP suggests that this method for measuring mortality may be ineffective for low-impairment fisheries such as those for Dungeness. It may not be possible to tag a sufficiently large number of impaired animals to be able to analyze the data by Score as opposed to unimpaired (i.e., Score-zero) compared to impaired (i.e., Scores greater than zero). For the case study to which Hueter et al. (2006) apply the data analysis methods utilized here to determine relative short- and long-term survival, the minimum number of animals tagged for a given Condition was 365. For the commercial ocean fishery, that which had the highest number of impaired crab, of the 5,594 crab assessed, only 202 had reflex impairment scores greater than one (129 Score-two; 46 Score-three; 14 Score-four; five Score-5; and eight Score-6; Appendix C). These sample sizes are even smaller when sex and shell-condition are considered, and for the recreational bay by boat fishery. The requirement of assessing a large number of crab to tag sufficient numbers to quantify mortality by Score, sex, and shell condition, becomes logistically impractical when the fact that the highest return rates are linked with release events early in the fishing season, a time when there are few soft-shell crab. This constraint should be considered when mark-recapture is considered for future discard mortality studies.

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4.8 Figures

Figure 4.1 Tagged Dungeness crab

An example of a Dungeness crab (*Cancer magister*) from the mark-recapture discard mortality study, tagged with a double t-bar anchor tag.

Figure 4.2 The 'Oregon C.R.A.B. Project' logo

The logo designed for the mark-recapture study that was printed on t-shirts, hats, flyers, and other project materials. For outreach in the fishing community, the project was entitled 'Oregon C.R.A.B. (Collaborative Research to Assess Bycatch) Project'.

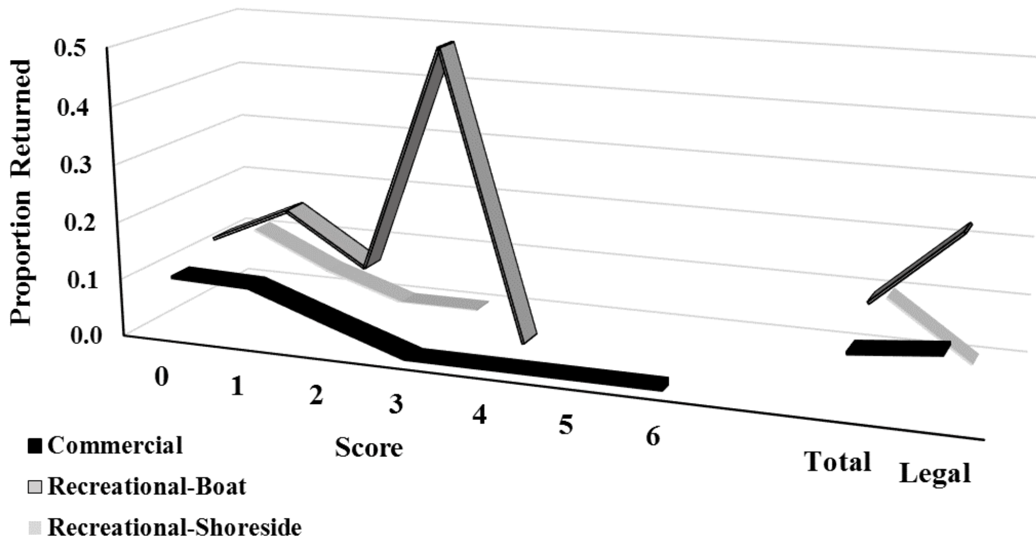

Figure 4.3 Proportion of tags returned by fishery and 'Score'

The proportion of tags returned for Dungeness crab (*Cancer magister*) by fishery (commercial ocean, and recreational bay by boat and shoreside) and reflex impairment score ('Score'). Also shown are the overall proportion of tags returned by fishery ('Total') and the proportion of tags returned for those attached to legal-size Score-zero hard-shell male crab (i.e., those that would have been retained; 'Legal').

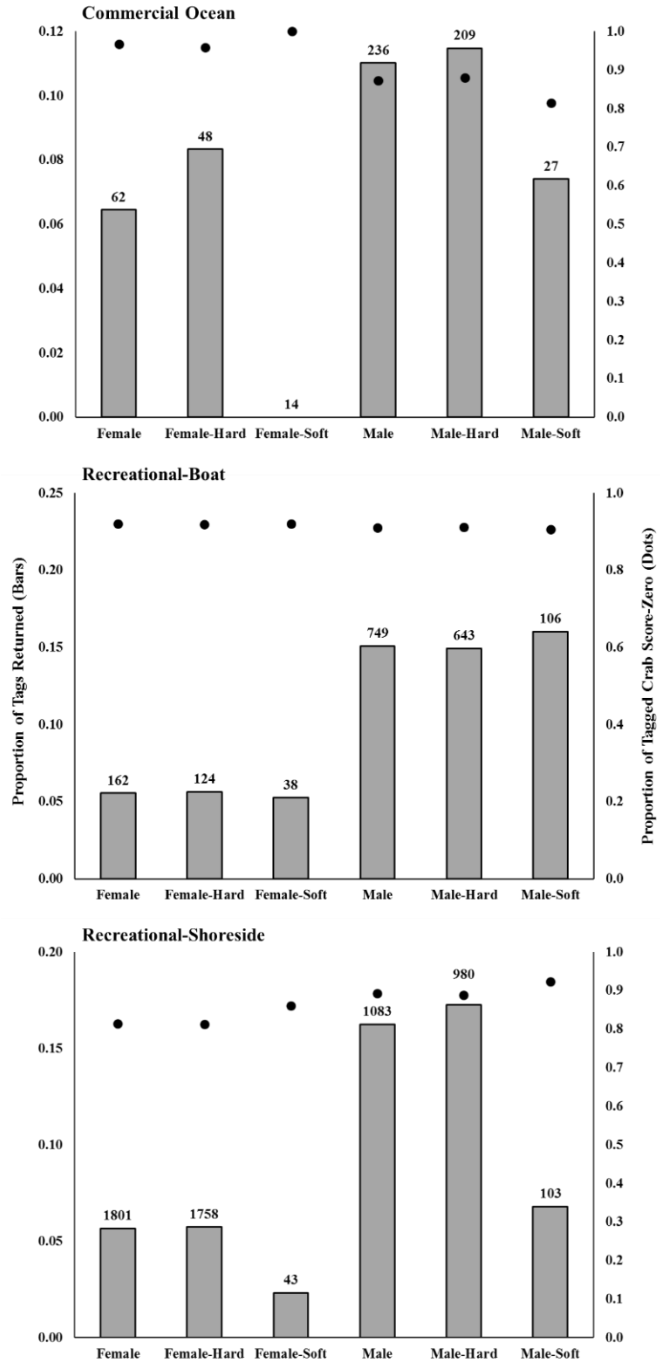

Figure 4.4 Proportion of tags applied to Score-zero crab and proportion of tags returned by sex-shell condition and fishery

The proportion of tags returned (bars) and the proportion of tags applied to Score-zero (i.e., no impaired reflexes) Dungeness crab (*Cancer magister*; dots) by fishery (commercial ocean, and recreational bay by boat and shoreside; vertical bars), and sex-shell condition combination.

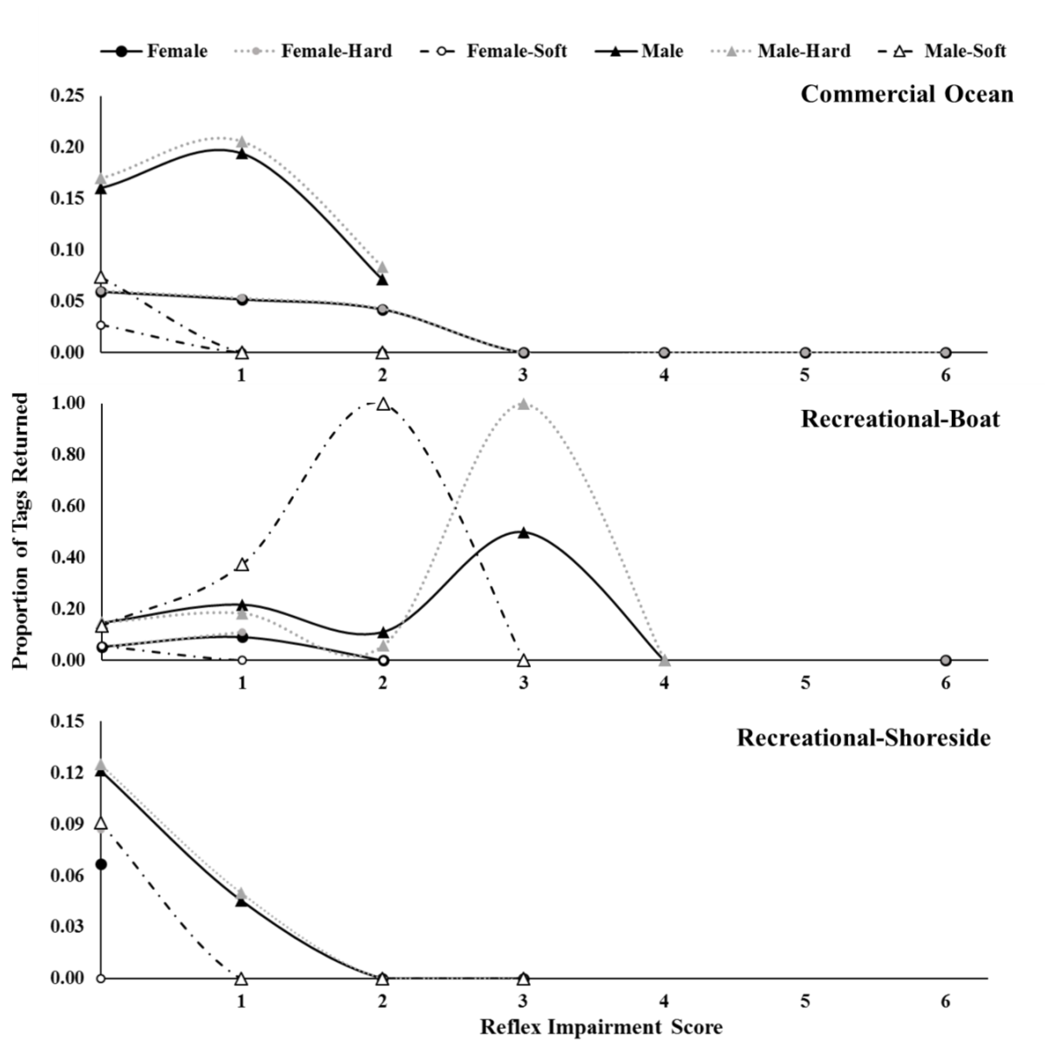

Figure 4.5 Proportion of tags returned by sex-shell condition, fishery, and 'Score'

The proportion of tags returned for Dungeness crab (*Cancer magister*) by fishery (commercial ocean, and recreational bay by boat and shoreside), reflex impairment score ('Score'), and sex-shell condition combination.

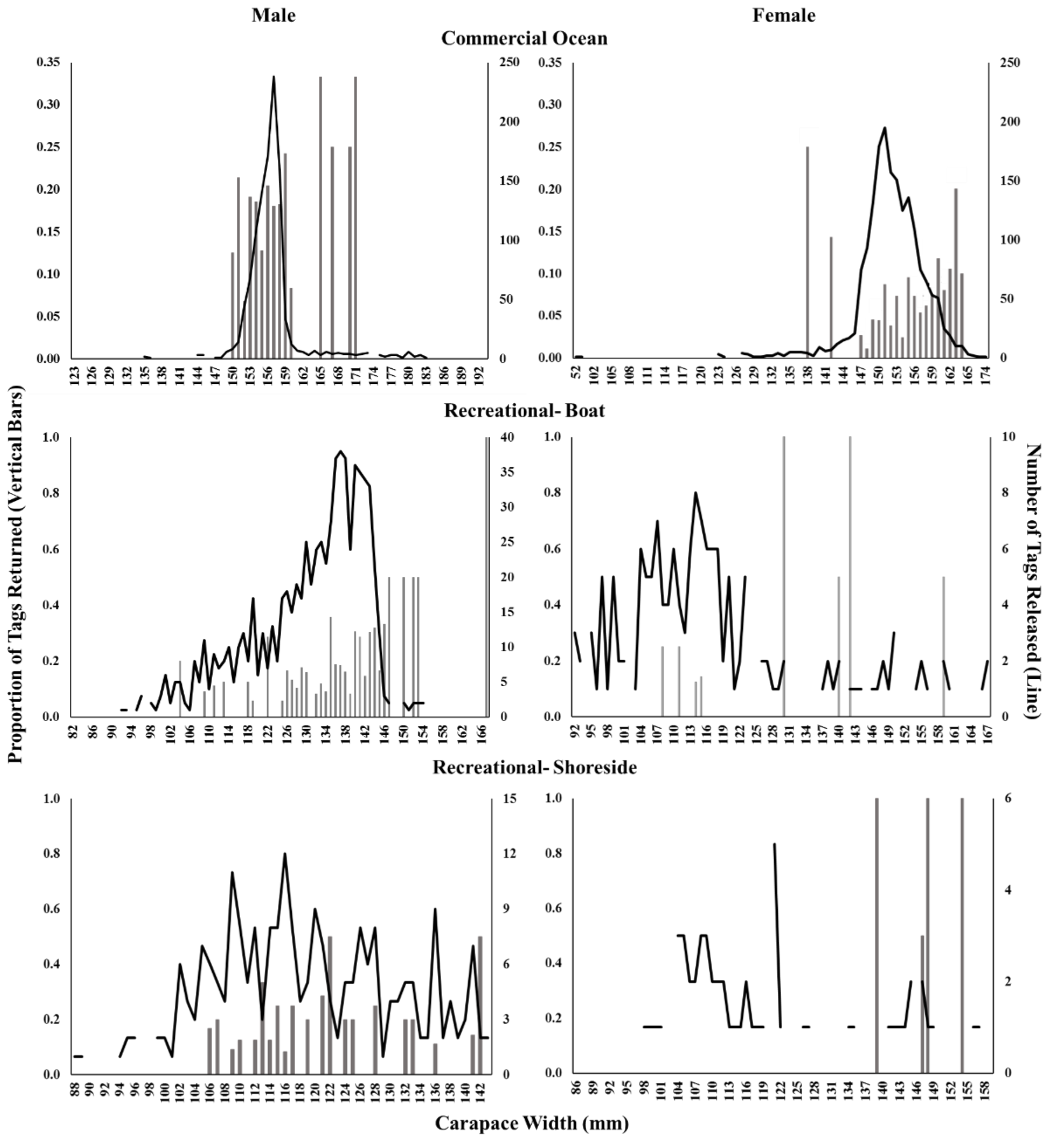

Figure 4.6 Tags released and returned by carapace width

The number of tags released on male and female Dungeness crab (*Cancer magister*; black line) and the proportion of tags returned by carapace width (mm; grey bars) for the commercial ocean, and recreational bay by boat and shoreside fisheries. These figures include crab intended for discard as well as some crab that would have been retained (i.e., Score-zero, hard-shell legal-size males) but were tagged and released. The minimum size of male crab that can be retained in the commercial fishery is 159 mm, and 146 for the recreational fisheries.

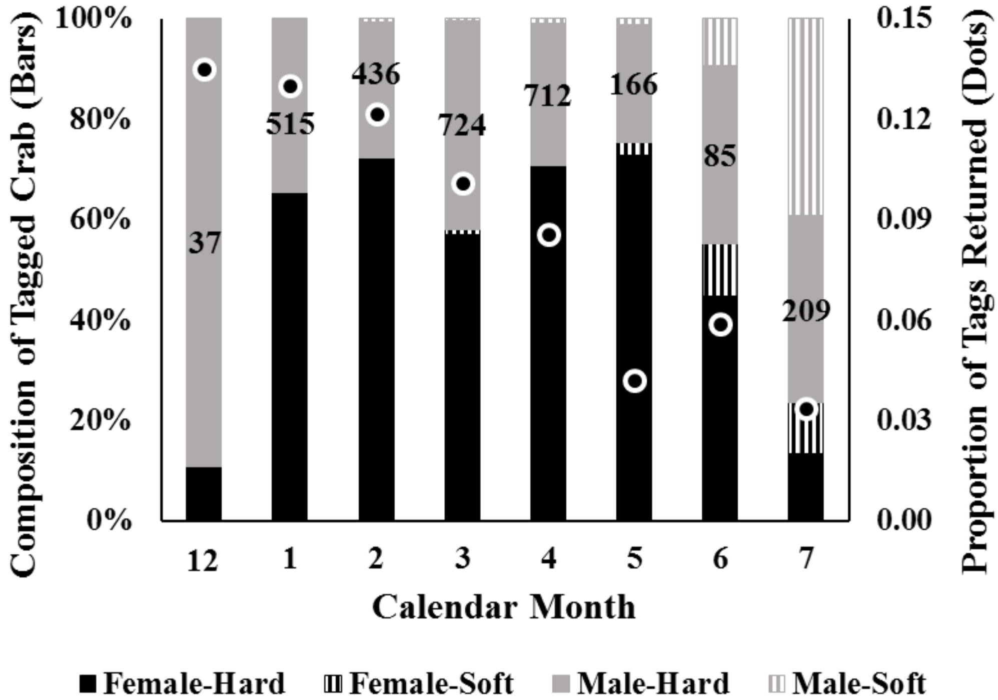

Figure 4.7 Composition of tags released and proportion of tags returned for the commercial fishery by month

Composition of tags released from the commercial ocean fishery for Dungeness crab (*Cancer magister*) by sex-shell condition combination (bars), and proportion of tags returned (dots) by the calendar month in which the tags were released. Total number of tags released by month are included in the bars.

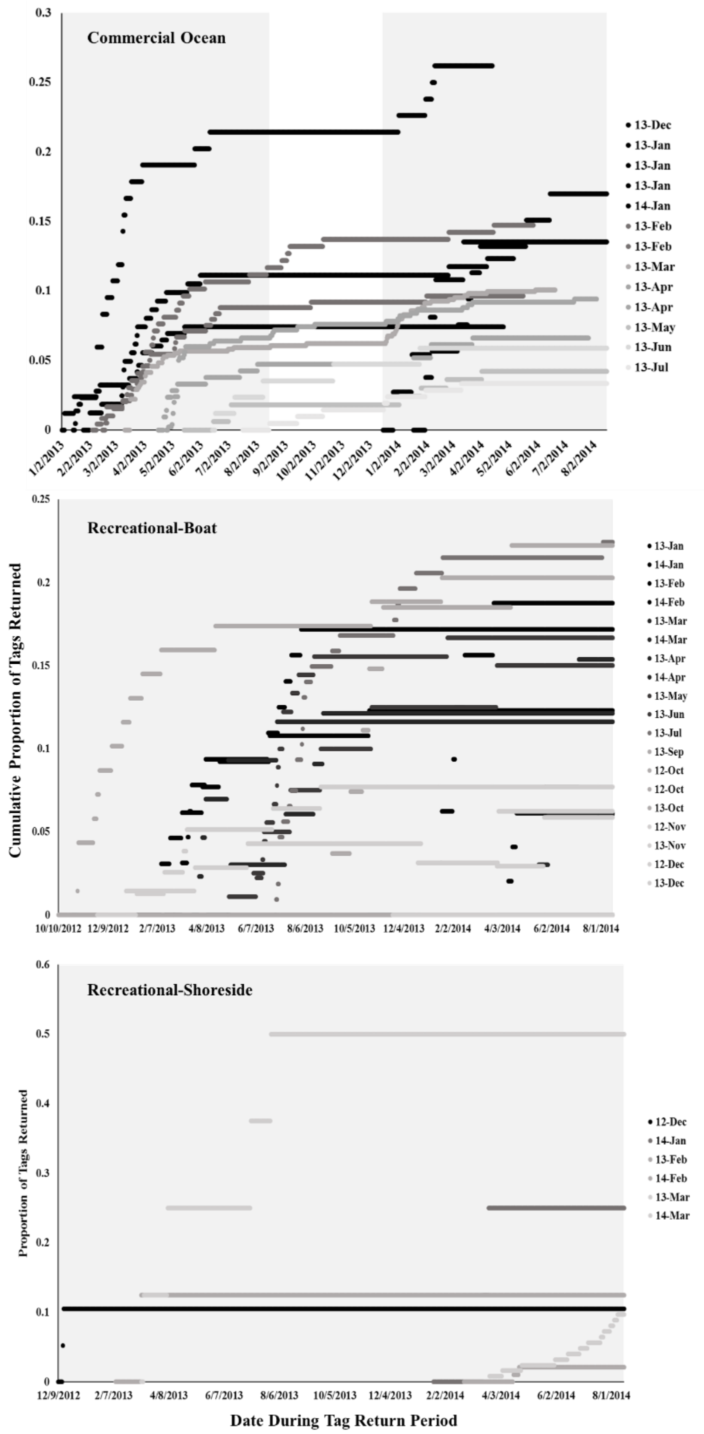

Figure 4.8 Cumulative proportion of tags returned by fishery and release event over the extent of the return period

The cumulative proportion of tags returned for Dungeness crab (*Cancer magister*) by release event (year-month released) and fishery (commercial ocean, and recreational bay by boat and shoreside).

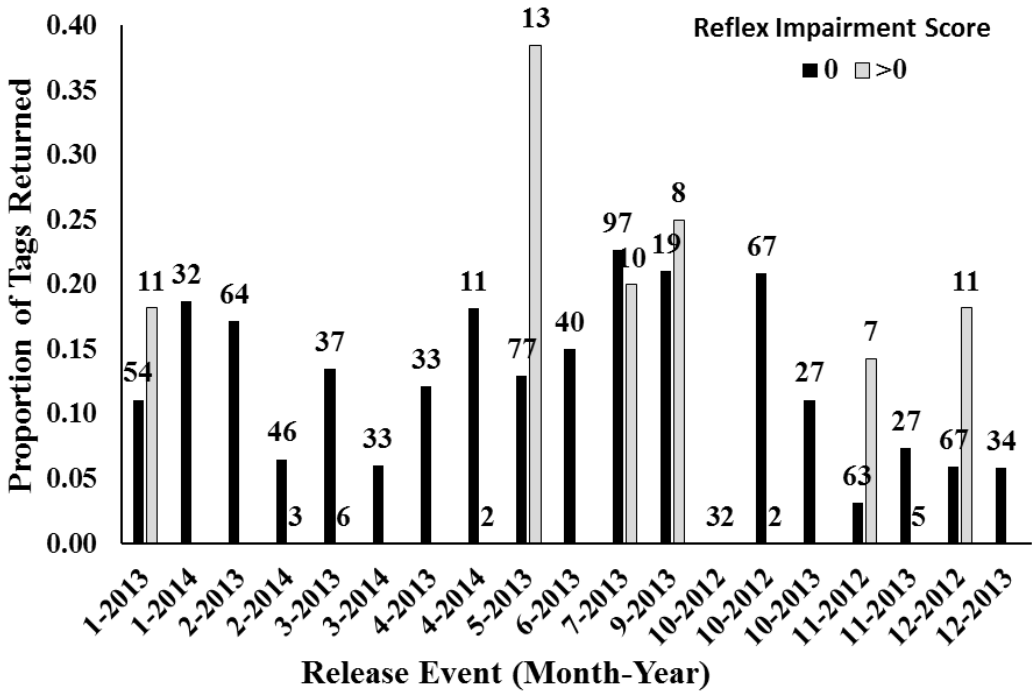

Figure 4.9 Proportion of recreational bay by boat tag returns by release event and 'Score'

Proportion of tags returned from the recreational bay by boat fishery for Dungeness crab (*Cancer magister*) for crab with a reflex impairment score ('Score') of zero (no impaired reflexes) and those greater than zero (Scores 1-6 combined). Indicated above each bar are the number of tags released for each category.

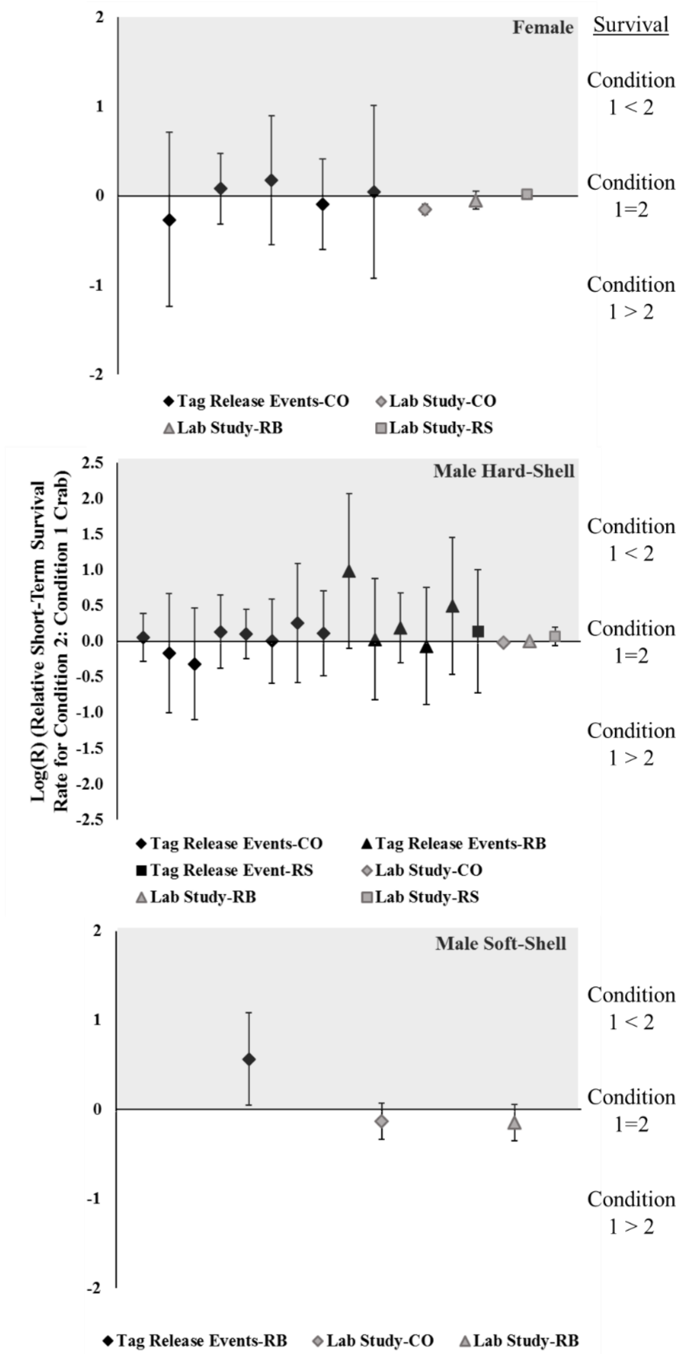

Figure 4.10 Relative short-term survival rates for Score-zero and greater than zero crab

The relative short-term survival rates for Condition 2 (reflex impairment score, 'Score', greater than zero) to Condition 1 (Score-zero) Dungeness crab (*Cancer magister*) for individual tag release events and laboratory holding study by fishery (CO: commercial ocean; RB: recreational bay by boat; and RS: recreational bay shoreside).

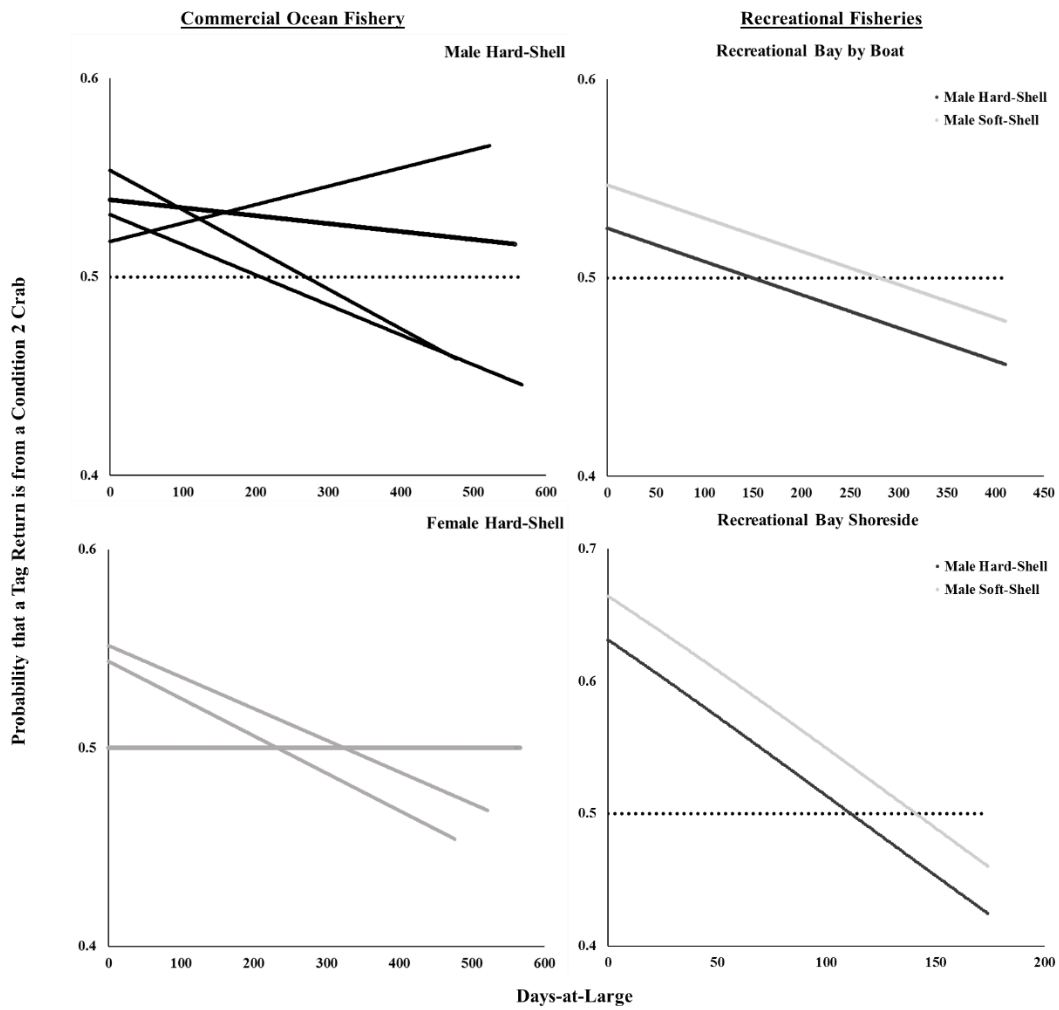

Figure 4.11 Results from relative long-term survival modeling

Predicted probability that a tag return is from a Condition 2 Dungeness crab (*Cancer magister*; i.e., reflex impairment score greater than zero) from long-term modeling, by fishery and sex- shell condition for all release events where there were at least 100 tagged crab released and at least 20 returns. A dashed black line indicates equal probability of return between Condition 1 and 2 crab.

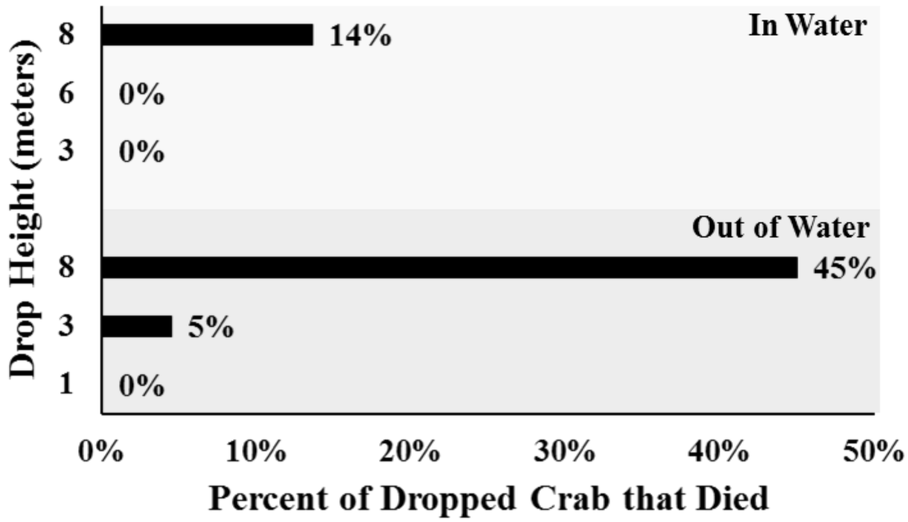

Figure 4.12 Percent of dropped crab that died

Percent of Dungeness crab (*Cancer magister*) that died after five days of holding as a result of the two drop experiments (one where the crab were left in the water before being dropped, the other out of water), all sex and shell conditions combined.

4.9 Tables

Table 4.1 Total number of released tags and returns

Total number of tagged Dungeness crab (*Cancer magister*) over all release events and number of tags returned by fishery (commercial ocean, and recreational bay by boat and shoreside), by sex-shell condition combination, and by reflex impairment score.

		Reflex Impairment Score							
		0	1	2	3	4	5	6	Total
Commercial									
Total	Tagged	2432	334	85	20	5	2	6	2884
	Returned	242	32	4	0	0	0	0	278
Female Hard-Shell	Tagged	1429	226	70	20	5	2	6	1758
	Returned	86	12	3					101
Female Soft-Shell	Tagged	37	5	1					43
	Returned	1							1
Male Hard-Shell	Tagged	871	97	12					980
	Returned	148	20	1					169
Male Soft-Shell	Tagged	95	6	2					103
	Returned	7							7
Recreational-Boat									
Total	Tagged	831	57	19	2	1	0	1	911
	Returned	108	11	2	1	0	0	0	122
Female Hard-Shell	Tagged	114	9					1	124
	Returned	6	1						7
Female Soft-Shell	Tagged	35	2	1					38
	Returned	2							2
Male Hard-Shell	Tagged	586	38	17	1	1			643
	Returned	87	7	1	1				96
Male Soft-Shell	Tagged	96	8	1	1				106
	Returned	13	3	1					17
Recreational-Shoreside									
Total	Tagged	266	22	7	3	0	0	0	298
	Returned	29	1	0	0	0	0	0	30
Female Hard-Shell	Tagged	46		1	1				48
	Returned	4							4
Female Soft-Shell	Tagged	14							14
	Returned								
Male Hard-Shell	Tagged	184	20	4	1				209
	Returned	23	1						24
Male Soft-Shell	Tagged	22	2	2	1				27
	Returned	2							2
Total	Tagged	3529	413	111	25	6	2	7	4093
	Returned	379	44	6	1	0	0	0	430

Table 4.2 Information on returned and non-returned tags

Information about tagged Dungeness crab (*Cancer magister*) and differences between those that were and were not recaptured and returned (all release events combined) for the commercial ocean, and recreational bay by boat and shoreside fisheries, including the number of days at large (time between release and recapture for returned tags and between release and end of the study for non-returned tags), carapace width, reflex impairment score ('Score'), depth at the location the tag was released, and the number of days from the opening of the fishery that the tag was released (commercial only).

	Returned		Not Returned	
	Mean	Range	Mean	Range
Commercial				
Days at Large	107	2-468	499	209-590
Carapace Width (mm)	155	138-171	154	52-193
Score	0.14	0-2	0.25	0-6
Depth at Release (fathom)	26.8	3-82	28.0	3-82
Days from Fishery Opening	66	0-198	83	0-198
Recreational-Boat				
Days at Large	78	0-449	448	136-674
Carapace Width (mm)	137	104-183	127	82-167
Score	0.15	0-3	0.12	0-6
Depth at Release (meters)	14.2	4-28	14.1046	4-28
Recreational-Shoreside				
Days at Large	79	0-163	268	146-672
Carapace Width (mm)	124	106-154	119	86-159
Score	0.03	0-1	0.16	0-3

Table 4.3 Composition of crab and mortalities for the drop experiments

For the two Dungeness crab (*Cancer magister*) experiments evaluating the impact of the return to water, listed are the number of crab for each drop height that survived after five days of holding by sex and shell condition (soft and hard), and by whether or not the crab were kept out of water or not before being dropped.

Out of Water	1-Meter		3-Meter		8-Meter		
	Lived	Died	Lived	Died	Lived	Died	Total
Males	17	0	16	0	8	6	47
Soft	3	0	1	0	1	2	7
Hard	14	0	15	0	7	4	40
Females	4	0	5	1	3	3	16
Soft	2	0	1	1	1	1	6
Hard	2	0	4	0	2	2	10
Total	21	0	21	1	11	9	63

In Water	3-Meter		6-Meter		8-Meter		
	Lived	Died	Lived	Died	Lived	Died	Total
Males	15	0	16	0	15	3	49
Soft					0	1	1
Hard	15	0	16	0	15	2	48
Females	3	0	2	0	4	0	9
Soft	1	0	1	0	1	0	3
Hard	2	0	1	0	3	0	6
Total	18	0	18	0	19	3	58

Table 4.4 Logistic regression model results for the drop study

Modeling results for the two drop experiments conducted to determine the most significant influences on mortality for Dungeness crab (*Cancer magister*) dropped into sea water, including whether or not the carapace cracked as a result of the drop ('Carapace'), the height from which the crab was dropped, whether or not the crab was kept in water before being dropped ('Water'), which side the crab landed on as it entered the water (dorsally, ventrally, side, or unknown), sex and shell condition (hard or soft), carapace width (mm), and a combination variable that combines the height and 'Water' variables ("Ht-Wtr'). Listed are the Akaike Information Criteria (AIC) for models with different combinations of variables (designated with an 'X') and change in AIC between all models and that with the lowest AIC value (highlighted in dark grey). All models with a difference in AIC less than one are highlighted in light grey.

Δ AIC	AIC	Carapace	Height	Water	Side	Sex	Shell	Width	Ht-Wtr
4.1	51.6	X	X	X	X	X	X	X	
2.7	50.2	X			X	X	X	X	X
2.9	50.4	X							
19.9	67.4		X						
35.3	82.7			X					
37.9	85.4				X				
38.2	85.7					X			
33.5	81.0						X		
38.9	86.4							X	
17.7	65.2								X
2.4	49.9	X	X						
3.9	51.4	X		X					
0.8	48.3	X			X				
4.7	52.2	X				X			
1.1	48.6	X					X		
4.8	52.3	X						X	
5.5	53.0	X							X
0.2	47.7	X	X		X				
2.7	50.2	X		X	X				
2.1	49.5	X			X	X			
0.5	48.0	X			X		X		
2.1	49.6	X			X			X	
2.2	49.7	X	X	X	X				
1.6	49.1	X	X		X	X			
	47.5	X	X		X		X		
1.4	48.9	X	X		X			X	
2.0	49.5	X	X	X	X		X		
0.5	47.9	X	X		X	X	X		
0.6	48.1	X	X		X		X	X	
2.4	49.9	X	X	X	X	X	X		

CHAPTER 5

AN ASSESSMENT OF AND RECCOMMENDATIONS FOR USING THE REFLEX ACTION MORTALITY PREDICTOR

5.1 Research Overview

Through the studies described in this dissertation and previous research conducted by others, the RAMP approach has proven to be an inexpensive, minimally invasive, quick, simple, and effective approach for assessing mortality attributed to fishing stressors. Whereas similar approaches rely on the assumption that evaluated animals are representative of a fishery, or on subjective categorization of impairment (e.g., active, moribund, and dead, Stevens 1990; or excellent, poor, and dead, Williams 2013), by determining presence or absence of a set of predefined reflexes the RAMP approach reduces the potential for subjective bias in mortality estimation. Similarly, subjectivity is reduced because reflexes are assessed rather than injuries, which can be difficult to detect and subjective to measure (Stewart 1974, Neilson et al. 1989). In addition, RAMP can be performed both at sea and in a laboratory setting, and can be utilized for a variety of applications, including conservation engineering and fisheries management evaluation. Most importantly, once a relationship has been established between reflex impairment and probability of mortality, the method can provide a mechanism to increase the scope, in time and space, of mortality estimates to inform fisheries impact assessments.

Advantages of RAMP were identified over the course of this dissertation research; however, limitations were also recognized. For the study on Tanner crab in Alaska (Chapter 2), it was determined that an established relationship between reflex impairment score (‘Score’) and probability of mortality will produce more accurate mortality estimates when applied to animals experiencing similar stressors as those evaluated to create the relationship. This includes the methods utilized to assess the animals, including air exposure beyond what is experienced in the fishery and time in water before assessment. Air exposure has the potential to superficially inflate reflex impairment scores and time in water can facilitate recovery. Both variables can bias the reflex impairment score, especially for animals, like Tanner crab, that demonstrated the ability to recover quickly after a short amount of time in water. These findings highlight the importance of documenting in detail the stressors experienced by the study animals and establishing consistent methods for how the animals will be treated before and during assessment. It is also important that the stressors be representative of what the animals experience in the fishery and to be conscious of steps in the assessment methodology that could potentially affect the relationship between Score and mortality probability. The latter should be considered with the biology of the animal in mind.

The laboratory holding study evaluating Dungeness crab discard mortality (Chapter 3) highlighted additional limitations with using RAMP. When mortality is determined by holding animals in captivity, long-term survival and mortality attributed to increased susceptibility to predation or inability to eat cannot be assessed. Mortality attributed to the stressor can also be confounded with a captivity effect if the animals are sensitive to being held in captivity. In addition, because the Dungeness fisheries have low impact on discarded crab in terms of producing impaired reflexes, the field sampling resulted in small sample sizes for impaired crab (i.e., crab with Scores greater than zero). This led to difficulties in analyzing the relationship between potential explanatory variables and mortality probability. This limitation was amplified by the fact that the relationship between reflex impairment score and mortality varied by fishery, sex, and shell condition, which reduced the effective sample size. These findings highlight the importance of having accurate estimates of mortality rates for ‘unimpaired’ animals. To accomplish this, control animals can be utilized to evaluate the potential for handling and scientific operations to contribute to mortality (e.g., captivity effect or tag induced mortality). Moreover, for the recreational bay fishery by boat, crab with Scores greater than zero were encountered so infrequently that a relationship between reflex impairment and mortality was difficult to ascertain. In addition to highlighting a limitation of RAMP with respect to use on low-impact fisheries, results from this research reveal a strong resilience in Dungeness crab and their ability to withstand stressors and recover from these fixed gear fisheries. Likewise, results from Chapter 2 revealed lower discard mortality rates than previously estimated, and that the rates vary by fishery, sex, and shell condition. This highlights the importance of assessing environmental, biological, and fishing variables in addition and relative to the reflex impairment score.

The mark-recapture study described in Chapter 4 gave some credibility to the mortality rates generated using laboratory captive holding as described in Chapter 3, and also highlighted potential issues with using laboratory holding and mark-recapture to measure mortality. For the mark-recapture study, because of the relatively low numbers of recaptured crab and high variability in tag return rates, it was difficult to disentangle effects of the handling and discard process from other variables (e.g., sex, effort in the fishery) or to quantify discard mortality rates. Also, because tags were applied to discarded animals rather than those retained, the study may have suffered from non-reporting due to fishermen spending minimal amount of time handling the tagged crab. This could also have led to bias in reporting if males and larger crab were more likely to be inspected. Results from this study primarily highlight the importance of considering the advantages and disadvantages of the method utilized to determine mortality for a RAMP study, and how stressors might be obscured from the mortality rate estimates based on the method selected.

5.2 Recommendations for RAMP Application

Lessons learned from my dissertation research and studies by others informed the following recommendations on how to effectively execute each methodological step of a RAMP study. In providing this guidance I hope to make the approach easier to apply in future research, to create a foundation on which to build a body of knowledge on how to effectively use RAMP, and to help standardize the RAMP methodology.

5.2.1 Step 1: Determine a Set of Reflex Actions and a Protocol for Assessment

Once it has been decided what species and stressors are going to be evaluated through a RAMP study, the first step is to establish a set of reflex actions ('reflexes') that will be assessed and, ultimately, related to mortality. To do this, test animals should be collected and evaluated for reflexes that are exhibited consistently with stimulation. These should be tested when the animal is experiencing minimal stress as well as after a stressor is applied. Candidate reflexes can be drawn from previous RAMP studies, knowledge of the animal's physiology, and from fishermen and/or processors who have extensive experience in handling the animal and may use reflexes as a way of determining vitality for sales purposes. If it is difficult to determine if a reflex is present in an unimpaired animal, that reflex should not be selected. A reflex should also be excluded if it is physiologically linked to another reflex, is influenced by other reflexes, is difficult to ascertain presence/ absence, or if it responds differentially depending on an uncontrolled variable. For example, temperature could play a role in reflex scoring for crab given that, for some species, different body parts cool more quickly than others (van Tamelen 2005). Similarly, reflexes should not be selected if they are linked with the stressor being studied (e.g., tail grab reflex to test swimming exhaustion; Szekeres et al. 2014). RAMP success relies on the selection of reflexes that are not subjective and give consistent response to stimulation or bias may be introduced (Depestele et al. 2014). In addition, multiple reflexes should be selected given that using only one reflex is less effective (Gallagher et al. 2014). Moreover, the reflexes should be assessed to determine if some are better predictors of mortality than others, and if fewer can be used in the assessment.

Once reflexes are selected, a detailed protocol should be written to describe how to conduct the assessments. Information should include: (*i*) a clear description of the movement(s) that must be demonstrated by the animal to be considered 'present' or 'absent'; (*ii*) the time duration under which a determination must be made; (*iii*) the order in which the reflexes must be tested (this should be standardized to prevent bias from reflexes that may be physiologically linked); (*iv*) how many attempts are allowed for determining presence or absence; (*v*) how immediate mortalities (i.e., dead before assessment) are categorized (are they considered to have all reflexes absent or are they classified separately?); and (*vi*) how a reflex will be classified if it presents weakly. Based on anecdotal evidence from the research described in this dissertation, combining weak reflexes with those present reduced subjectivity; however, a formal study on this has not been completed. With respect to reflex assessment, it would be useful for all scientists involved in scoring reflex impairment to verify that what is considered a 'present' reflex is consistent among the group of assessors. At this time, if a reflex shows variation among assessors the reflex should be removed or the descriptions of 'present' and 'absent' should be improved.

A decision must also be made as to how the reflex impairment score will be quantified. In some previous RAMP studies, impairment level was quantified by scoring present reflexes (i.e., unimpaired) as "0" and absent reflexes as "1", and summing over all reflexes tested (i.e., a Score of zero would be given to the most unimpaired animals; Stoner et al. 2008, Stoner 2009, 2012a, Hammond et al. 2013, Rose et al. 2013, Brownscombe et al. 2014, Urban 2015). Other researchers have calculated Score by summing the "0"s and "1"s over all reflexes, and dividing by the total number of reflexes (i.e., the proportion of the reflexes that were impaired; Raby et al. 2012, Barkley and Cadrin 2012, Donaldson et al. 2012, Depestele et al. 2014, Nguyen et al. 2014, Danylchuk et al. 2014, McArley and Herbert 2014, Bower et al. 2016). Additional methods include subtracting the proportion of impaired reflexes from one (Davis and Ottmar 2006, Humborstad et al. 2009, LeDain et al. 2013), and including a multiplier for replicates within a treatment group (Davis 2007). Other studies have included an impairment index in addition to reflex impairment (e.g., buoyancy status, barotrauma, injury; Campbell et al.2010a, 2010b, Diamond and Campbell 2009, Hochhalter 2012, Humborstad et al. 2016). Calculating Score as a proportion is advantageous for animals that cannot be tested for all reflexes due to missing or damaged body parts. Regardless, for more in depth analysis of how impairment relates to mortality, Score should be analyzed categorically and continuously, and by individual missing reflex.

5.2.2 Step 2: Design a Study to Assess Animals for Reflex Impairment

In designing a RAMP study, it must be determined if the stressor will be experienced by the animal under natural conditions or through laboratory simulation. If the former, care must be taken to record data on methods and all possible stressors (including injury), and the environmental and fishing variables that the assessed animals are experiencing, and the extent to which the experiment is representing fishing operations and the broader scope of the fishery. If done in the laboratory, care must be taken to consider how the stressor differs from actual fishing conditions and how that might affect mortality estimates. Moreover, effort should be made (within logistic constraints of field and laboratory research) to minimize having the scientific procedures contribute to mortality, and to consider how an animal treated post-stressor and before evaluation can affect the relationship between Score and mortality (e.g., additional air exposure or recovery in water). In addition, the scope of the study should be considered and data should be gathered when possible over the extent to which the results intend to be applied (geographic area, animal size, season, etc.). Along these lines, effort must be taken to obtain sample sizes that will allow for meaningful results given that the relationship between reflex impairment and mortality can be difficult to determine if only a few animals die (McArley and Herbert 2014) or if the majority of mortality is immediate rather than delayed (Davis 2007). Also, it is important to create repeatable methods that are well documented and published to increase the likelihood that the estimated RAMP relationship will be applied efficaciously in future studies. This includes being clear in subjective descriptors (e.g., ‘soft’ or ‘hard’ with respect to shell hardness for crustaceans).

5.2.3 Step 3: Select a Method for Measuring Mortality

A variety of methods have been employed to measure mortality, including: captive holding in on-board holding tanks (Stoner et al. 2008, Hammond et al. 2013, Rose et al. 2013, Depestele et al. 2014, Humborstad et al. 2016) or laboratory tanks (Davis and Ottmar 2006, Davis 2007, Humborstad et al. 2009, Stoner 2009, 2012a, Braccini et al. 2012, Barkley and Cadrin 2012, McArley and Herbert 2014, Hendrick-Hopper et al. 2015, Uhlmann et al. 2015); telemetry, including radio (Raby et al. 2012, Nguyen et al. 2014), acoustic (Donaldson et al. 2012), and satellite (Gallagher et al. 2014); mark-recapture (Chapter 4); through the use of in-situ net pens or cages (Diamond and Campbell 2009, Brownscombe et al. 2015, Bower et al. 2016); and through visual monitoring (Campbell et al. 2010c, Hochhalter 2012, Danylchuk et al. 2014, Brownscombe et al. 2014). Trade-offs among these methods depend on project goals, logistics, timeframe, and budget. The duration of observation must also be determined, keeping in mind that the majority of mortality occurs within the first days. Considerations also must be made regarding the biology and behaviour of the study animal.

There are drawbacks associated with each method for measuring mortality, including captive holding. This method does not incorporate indirect effects on mortality such as an animal's ability to eat or avoid predation (Stoner 2009, 2012b, Uhlmann et al. 2009, Benoît et al. 2010, Urban 2015), or allow for the inclusion of chronic mortality that is experienced as the result of the stressor. Without knowing the period of time required to measure mortality to its full extent, there is the potential to underestimate mortality given a short holding duration. For this reason, care must be taken to observe mortality for a sufficiently long time. With respect to holding in on-board tanks, sample size is restricted by available space on the vessel, the need for which may limit participation by some fishing and research vessels. In addition, care must be taken to ensure that the water quality and holding conditions (e.g., movement of the ship) are not contributing to mortality, and it may be difficult to feed the captive animals or to create holding conditions that are conducive to the biological needs of the animal. Also, the duration of holding on board is limited to that of the trip. There are similar drawbacks for captive holding in laboratory tanks. Differences, though, are that sample size is limited by the tank capacity, and project and holding duration.

Also, water quality and holding conditions are potentially easier to control. However, the additional handling and transport required for this holding type can contribute to mortality. In addition, captivity effects can confound mortality attributed to the stressors being studies, as was observed for Tanner crab held in on-board and laboratory tanks (Chapter 2) and Dungeness crab held in the laboratory (Chapter 3). Despite these limitations, captive holding allows for more control over the experiment than for other methods, for observing degradation in health and changes in behaviour, and for the scientist to know time of death (Davis and Ryer 2003).

Tagging studies have different advantages and disadvantages (Pine et al. 2012). This method allows for observation over a longer duration and for the animals to experience natural conditions, and it does not require laboratory facilities, husbandry, or tank maintenance. Also, it avoids the concern that holding or transport is contributing to mortality. In contrast, disadvantages include the high cost of tags (depending on the type) and reward program (if offered) given that obtaining reasonably precise estimates of survival requires high tag return rates. The type of tag should be selected thoughtfully with respect to the duration that the tag must last, whether or not it needs to last through a moult or other physical change in the animal, and if the tag would affect the animal's behaviour. For mark-recapture studies, it can be difficult to differentiate between the influence of mortality attributed to the stressor of interest on tag return rates and to other causes (e.g., reporting rate). In addition, the success of a mark-recapture study can depend on participation of fishermen or frequency of scientific sampling.

In-situ cages and visual monitoring are alternative approaches for measuring mortality. While in-situ cages provide more natural conditions than holding tanks, they are subject to ocean conditions, could become lost, and may affect the animals' susceptibility to predation and ability to feed. In addition, as was observed for Tanner crab held in at-sea cages (Chapter 2), without using video cameras it can be difficult to know if mortality is attributed to the stressors being studied. Visual monitoring is advantageous in its simplicity, but results may be subject to the training, experience, or eyesight of the observer, and this method only provides information on mortality over the very short-term.

5.2.4 Step 4: Conduct a Pilot Study to Evaluate Methods

A pilot study is recommended to assess potential influences on mortality, to evaluate the reflexes and period of time for measuring mortality, and to determine if study practices are contributing to mortality. To assess the selected reflexes, an evaluation can be made at this time to determine if fewer reflexes can be used or if alternative reflexes would improve the assessment. Patterns in reflex impairment can also be evaluated to ensure that absence or presence of reflexes is not influenced by environmental or biological variables (e.g., temperature or sex), to determine if some reflexes are too difficult to assess in the field, or if some are consistently lost first or not at all. Patterns may also become clear with respect to mortality by sex or other variable that would require a change in sampling design to ensure a sufficient sample size of impaired animals are being observed to reliably estimate mortality.

A pilot study also provides an opportunity to establish the requisite duration for determining survival and whether or not modifications need to be made to the study design to minimize impairment attributed to the study process (e.g., a captivity effect). A decision can be made regarding how long animals need to be observed if mortality of impaired and unimpaired animals stabilizes during this initial study. Mortality of unimpaired animals during this period may also indicate the need to modify the methods. This can similarly be addressed by including control animals in the study (Reilly 1983, Neilson et al. 1989). In addition, different tag types used for tagging studies and/or identification purposes can be evaluated for retention and the potential for tag induced mortality.

5.2.5 Step 5: Relate Reflex Impairment to Mortality Probabilities

Once mortality has been measured for various levels of reflex impairment a relationship can be estimated. It is possible to relate the two directly, equating probability of mortality with the proportion of animals that died by Score (Hammond et al. 2013, Rose et al. 2013). The relationship can also be determined through logistic regression (Stoner et al. 2008). The advantages of this approach are that it provides a mechanism for determining if other variables influence mortality in addition to Score. This also provides an opportunity to determine if Score should be treated as a continuous or categorical variable, or if the absence of individual reflexes reliably predicts mortality. Also, when sample sizes are small, the death of one animal can have an unduly large influence on estimated mortality rates. This may make it advantageous to use results from logistic modeling rather than the observed proportion of dead animals. The disadvantage of using logistic regression is that it makes assumptions about the relationship between Score and mortality (i.e., sigmoidal). Also, the ability to detect significant relationships and interactions among variables is limited by the sample size. Regardless of how mortality probabilities are estimated, regression analysis can be done as a preliminary step to determine the most significant predictors of mortality.

5.2.6 Step 6: Apply the RAMP Relationship to Assessment Data

By assessing the reflexes of a set of animals and then applying an established RAMP relationship between mortality and levels of impairment mortality rates can be estimated beyond the scope of the animals that were directly measured for mortality when the RAMP relationship was developed. However, careful consideration should be given to whether the RAMP relationship is based on comparable stressors and animals with similar traits. For example, mortality rate may depend on the size of the animal (Davis and Parker 2004, Rose et al. 2013) and therefore should only be applied to animals that are within a specified range. Also, caution should be used in applying the RAMP to animals caught with different fishing practices or gear or assessed using different protocols. In applying the RAMP relationship to estimate mortality rates, data should only be grouped if there is no statistical significances that can be detected within the sub-groups (e.g., differences by month or trip). Finally, when making conclusions about mortality rates it is important to be aware of how study limitations may have led to over- or under-estimation of mortality.

5.2.7 Future Research

This dissertation has provided a critique of the RAMP approach and suggestions for improving data generated using the method. Future research can build upon this foundation and should include studies to evaluate how subjectivity in scoring impairment might influence results (Benoît et al. 2010, Depestele et al. 2014, Uhlmann et al. 2015). Inter- and intra-observer variability may result from differences in reflex response due to the way a fish is handled, the interpretation of what is meant by ‘presence’ and ‘absence’, experience with fish handling and/or RAMP assessment, or expectation based on the stressors experienced by the animal. In addition, an observer may be more inclined to call a reflex ‘absent’ if previous reflexes were absent. Also, additional research is needed to clarify how ‘weak’ reflexes should be treated in order to minimize subjectivity.

There is potential for additional applications of RAMP, including involving citizen scientists or fishermen in collecting RAMP data, or incorporating RAMP assessments in observer programs or surveys. There is also the potential to conduct RAMP assessments through video footage in support of electronic monitoring, or to use RAMP to look at product quality for the live market, determining optimal shipping and handling procedures, animal husbandry and aquaculture practices, and evaluating previously unassessed fishing related stressors. In addition, there are potential applications for helping fishermen decide whether to retain, release, or facilitate recovery of animals that have been caught incidentally, and to decide how effective their fishing performance is relative to mitigating discard mortality.

While there is further evaluation that can be done to determine optimal ways of utilizing RAMP, the approach remains effective and superior to alternative methods. This research will hopefully serve as a foundation from which to continue assessing and improving upon this methodology to support fisheries science and management.

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Appendices

Appendix A Additional Dungeness Crab Fisheries Assessed

In addition to the research presented in Chapter 3 on bycatch and discard mortality rates in Oregon commercial ocean and recreational bay by boat fisheries for Dungeness crab (*Cancer magister*), we completed sampling for additional fisheries: recreational ocean and shoreside, and commercial bay. Information from these sampling trips are presented here.

Sampling methodology for the additional fisheries was similar to that described in Chapter 3 for the commercial ocean and recreational bay by boat fisheries, with some exceptions. To assess discard in the recreational shoreside fishery, on a given sampling 'trip', we went to the Port of Newport Public Fishing Pier (Yaquina Bay, Oregon) and sampled all pots or rings of two to six willing individuals or groups of people on the pier that were crabbing for the pre-determined sampling period or until they stopped fishing. Sampling methods for the commercial bay fishery differed from those for the commercial ocean fishery in that: (*i*) a slam bar was not used; (*ii*) rings were used instead of pots (per management regulations); (*iii*) soak duration was measured in minutes; and (*iv*) the first assessed ring was selected at random and the subsequent rings were sampled as soon as all crab from the previous ring were assessed and returned to the water. Fewer rings (15 maximum) are fished during a commercial bay trip and the above protocol maximized the opportunity for sampling. However, rings to be sampled were pre-determined before coming out of the water to avoid bias. In addition, sampling trips were completed in Alsea Bay (Oregon), which is approximately 25 km south of Yaquina Bay.

Appendix A.1 <u>Commercial Bay Fishery</u>

Four commercial bay ride-along trips were completed in the months of September and October, aboard one vessel and with one captain, in water ranging from 1.2-11.6 meters (3.6 meters on average), and soak duration from 10 to 46 minutes (17 minutes on average). It was difficult to obtain ride-along opportunities given the small vessel size, and limited number of active permit holders. Across all trips, 53 rings were sampled, containing 348 bycaught and 16 retained crab. The number of crab discarded per ring ranged from 2.9 to 10.5. The majority of crab assessed were Score-zero (range for rings: 92%-100%), and hard-shell females were predominant over other sex/shell hardness combinations (35%-92%). There were no immediate mortalities in the rings that were assessed and only 6 crab (2%) were injured. Crab caught during commercial bay ride-along trips were handled less than those from sampling trips for all other fisheries. Only crab that were close to legal size were removed from the rings and measured; all obviously sub-legal males and females were left in the gear as it was reset.

Appendix A.2 Recreational Shoreside Fishery

In the variety of fishing gears sampled during 15 sampling 'trips', over each calendar month, 508 crab were discarded, and 12 Dungeness crab were retained. The vast majority of the catch was discarded, and the majority of discards were hard-shell males (55%) and Score-zero crab (91%), with soft-shell males having the lowest relative percent of Score-zero individuals. Only 14% of crab assessed during shoreside sampling were soft-shell. We observed that care taken in removing crab from the fishing gear, and therefore amount of injury to the crab, often depended on the experience level of the fisherman (i.e., more experience, less injury). In addition, the distances for returning crab to water were higher for shoreside fishing than by boat. Where we sampled, the distance from the rail of the pier to the water at mean lower low water was 6.3 meters, and there were commonly California sea lions (*Zalophus californianus*) present where crab were returned to the water. The contribution to mortality from the return to water and increased susceptibility to predation were not considered in the discard mortality assessments.

Appendix A.3 Recreational Ocean Fishery

During the one recreational ocean sampling trip (in August), there were 40 crab that would have been retained, and 116 discarded; indicating that for every retained crab, there were 3 discarded.

Appendix A.4 RAMP Relationship for Additional Fisheries

Preliminary model selection to determine the relationship between probability of mortality and reflex impairment score and environmental, biological, or fishing variables, on all data combined (for all five assessed fisheries), indicated a significant difference (tested at an alpha value of 0.05) between fishery types, namely that recreational bay fishing by boat had lower mortality than the other fishery types (commercial ocean, and recreational ocean and shoreside). We therefore combined recreational crabbing in the ocean and shore-side with commercial ocean crabbing and determined that there were no significant differences among these fisheries. We repeated this analysis using only recreational data. Again, the bay fishery by boat was significantly different from the other recreational fisheries in that mortality was reduced. We therefore separated the data by fishery type. Captive holding datasets for shoreside and recreational ocean were not large enough to create separate RAMP relationships. We therefore only created them for the commercial ocean and recreational bay by boat fisheries. No crab caught during commercial bay trips were held because the small vessel size prevented retaining crab during the fishing trip.

While RAMP relationships for the additional fisheries were not created, the lack of significant difference for fishery type when the shoreside and recreational ocean fisheries were combined with the commercial ocean fishery, indicates that until future research is conducted to create RAMP relationships for these fisheries, that for the commercial ocean fishery could be applied with caution. For example, we applied this model to the single recreational ocean fishing trip. The resultant total delayed mortality for the trip was 6% for females, 2% for hard-shell males, and 14% for soft-shell males. For all categories combined, 6% of all discarded crab from this trip were expected to die after release; indicating that 17 crab would die for every 100 retained. These values should not be applied to the fishery, however, as this was only one trip and may not be representative for the fishery as a whole. With more data collected on catch composition for this fishery with respect to sex-shell condition and reflex impairment score, the RAMP application could be more useful in estimating discard mortality rates for this fishery.

Appendix B Dungeness Crab Sampling Trip Information

The number of sampling trips completed on a given number of fishing vessels, over specified months (listed numerically) and depths between 2012 and 2014, and the mean carapace widths (mm) of assessed crab (range in parentheses) for the indicated fisheries.

	Commercial		Recreational		
	Ocean	Bay	Bay by Boat	Bay Shoreside	Ocean
No. Trips	22	4	26	15	1
No. Vessels	4	1	3		1
Depth Range (m)	6-150	2-11	2-11		22
Calendar Month	1, 2, 3, 4, 5, 6, 7, 12	9, 10	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	8
Carapace Width (mm)					
Male	160 (93-193)	116 (80-160)	125 (61-167)	115 (35-147)	153 (131-173)
Female	152 (52-174)	131 (79-162)	114 (67-167)	127 (41-160)	139 (116-159)

Appendix C Dungeness Crab Sampling Trip Catch Composition

By fishery, the number of completed trips and pots sampled during those trips, and the number of dead Dungeness crab (*Cancer magister*) in the sampled pots (“immediate mortalities”), and those discarded alive per each reflex impairment score (0-6) by sex and shell hardness (<>: indicates crab that were thrown over before a full assessment could be completed).

Type	No. Trips/ Pots	Sex & Shell Condition	<>	0	1	2	3	4	5	6	Immediate Mortality	Total	% of Total
Commercial Bay	4 / 53	Female	2									2	1%
		FemaleHard	1	259	6	1						267	77%
		FemaleSoft		17		1						18	5%
		MaleHard		46			1					47	14%
		MaleSoft		13	1							14	4%
		Total	3	335	7	2	1	0	0	0	0	348	
		% of Total	1%	96%	2%	1%	0%	0%	0%	0%	0%		
Commercial Ocean	22 / 966		9	1							37	47	1%
		Female	18	23	4	1	2	1			18	67	1%
		FemaleHard	1	2606	355	104	42	11	5	6	69	3199	57%
		FemaleSoft		67	6	1					1	75	1%
		Male	11	4							3	18	0%
		MaleHard	1	1379	143	15	1	1			29	1569	28%
		MaleSoft	1	571	27	8	1	1		2	8	619	11%
		Total	41	4651	535	129	46	14	5	8	165	5594	
		% of Total	1%	83%	10%	2%	1%	0%	0%	0%	3%		
Recreational Bay_Boat	26 / 384	Female		1								1	0%
		FemaleHard		520	25	3				1		549	26%
		FemaleSoft		164	9	3		1				177	8%
		MaleHard		1017	63	24	2	2				1108	53%
		MaleSoft		232	20	3	1					256	12%
		Total	0	1934	117	33	3	3	0	1	0	2091	
		% of Total	0%	92%	6%	2%	0%	0%	0%	0%	0%		
Recreational Bay_Shoreside	15 / 436			1								1	0%
		Female	3									3	1%
		FemaleHard		139	6	3	1					149	29%
		FemaleSoft		26	1							27	5%
		Male	1	1								2	0%
		MaleHard		253	23	4	1					281	55%
		MaleSoft		39	3	2	1					45	9%
		Total	4	459	33	9	3	0	0	0	0	508	
		% of Total	1%	90%	6%	2%	1%	0%	0%	0%	0%		
Recreational Ocean	1 / 11	Female	1									1	1%
		FemaleHard		53	3							56	48%
		FemaleSoft		5	1							6	5%
		MaleHard		31	2			1				34	29%
		MaleSoft		17		1		1				19	16%
		Total	1	106	6	1	0	2	0	0	0	116	
		% of Total	1%	91%	5%	1%	0%	2%	0%	0%	0%		
All Combined	68 / 1850	Total	49	7485	698	174	53	19	5	9	165	8657	
		% of Total	1%	86%	8%	2%	1%	0%	0%	0%	2%		

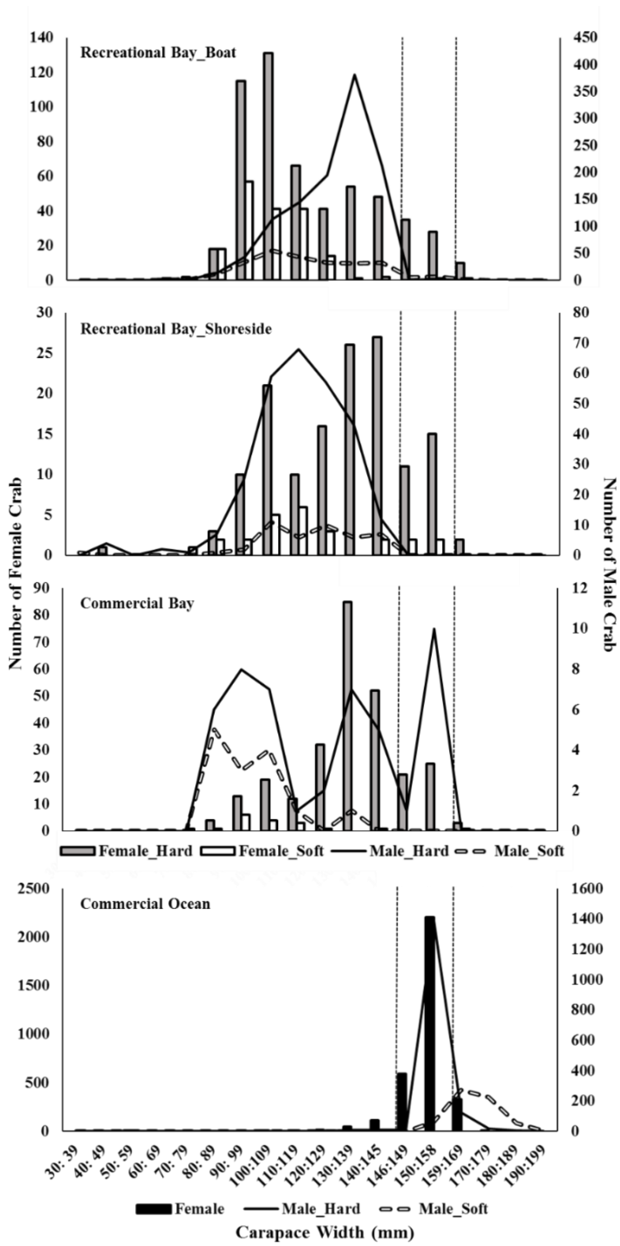
Appendix D Numbers of Dungeness Crab Held to Determine Mortality

The number of Dungeness crab (*Cancer magister*) from the sampled pots with each reflex impairment score (0-6) by sex and shell condition (i.e., hardness) that were assessed and held in the laboratory to determine discard mortality, by fishery (immediate mortalities were not held).

Fishery	Sex_Shell Condition	Reflex Impairment Score 0	1	2	3	4	5	6	Total	% of Total
Commercial Ocean	Female	13	2	1	2	1			19	3%
	Female_Hard	255	62	18	12	4	1		352	54%
	Female_Soft	20	1						21	3%
	Male_Hard	173	36	3	1				213	33%
	Male_Soft	41	6		1			2	50	8%
	Total	502	107	22	16	5	1	2	655	
	% of Total	77%	16%	3%	2%	1%	0%	0%		
Recreational Bay by Boat	Female_Hard	39	5	1					45	14%
	Female_Soft	20	2						22	7%
	Male_Hard	193	15	7	1				216	67%
	Male_Soft	31	7						38	12%
	Total	283	29	8	1	0	0	0	321	
	% of Total	88%	9%	2%	0%	0%	0%	0%		
Recreational Shoreside	Female_Hard	24	1						25	54%
	Female_Soft	7	1						8	17%
	Male_Hard	7	2						9	20%
	Male_Soft	4							4	9%
	Total	42	4	0	0	0	0	0	46	
	% of Total	91%	9%	0%	0%	0%	0%	0%		
Recreational Ocean	Female_Hard	16	2						18	42%
	Female_Soft	4							4	9%
	Male_Hard	11	1			1			13	30%
	Male_Soft	6		1		1			8	19%
	Total	37	3	1	0	2	0	0	43	
	% of Total	86%	7%	2%	0%	5%	0%	0%		
All Combined	Total	864	143	31	17	7	1	2	1065	
	% of Total	81%	13%	3%	2%	1%	0%	0%		

Appendix E Size Distributions of Dungeness Crab Caught During Sampling Trips

Number, by carapace width (10 mm bins), of captured and assessed male and female Dungeness crab (*Cancer magister*) intended for discard (alive and dead) by fishery, with breaks made at 146 and 159 mm (the minimum size required for retaining male crab for the recreational and commercial fisheries respectively).



Appendix F Definitions of 'Soft-Shell' for Dungeness Crab from the Literature

Shell condition descriptions ranging from hard- to soft-shell (left to right) from published studies evaluating Dungeness crab (*Cancer magister*; listed alphabetically).

Chapter 3	Hard: Carapace and legs mostly hard to near moult	Soft: Recently moulted (little to no hardening) to moderate hardening post-moult (carapace and legs flexible and soft)				
Barry 1983	Old shell (Impending moult): Grade I with encrusted exoskeleton and/or dark coloration	Grade I (Hard shell): Carapace inflexible when moderate pressure is applied	Grade I (New): Carapace slightly flexible and largest segment of first walking leg moderately flexible	Grade II (Intermediate): Carapace moderately flexible when moderate pressure is applied	Grade III (Soft shell): Carapace very flexible when moderate pressure is applied	

Dunham et al. 2011	Code 7: Hard, very old; > 24 months since moult	Code 6: Hard, old; 12-24 months since moult	Code 8: Hard, between new and old; 6-12 months since moult	Code 1: Hard, new; 3- 6 months since moult	Code 2: Soft, new; 1-3 months since moult	Code 3: Soft, very new; 6 days - 1 month since moult	Code 4: Soft, just moulted; 2-6 days since moult	
Fisheries and Oceans Canada 2014	Code 5: Moulting crab; the shell has split at the suture line at the back; however, the crab has not yet exited the old shell. Generally this stage lasts only one day. Shell conditions 4 and 5 indicate a moult is in progress….	Code 7: Very old hard shell. Much claw wear, fouling growth Males typically show old mating marks which have worn through claw; may have shell disease; tips of walking legs may be black or rotting off. Crab is lethargic and	Code 6: Old hard shell. Shows claw wear and often barnacle encrustation or other fouling growth. In exposed conditions the shell may appear clean and bright, but the claws will show signs of wear. Carapace spines will	Code 8: Between a new (Code 1) and old (Code 6) hard shell. Shell shows signs of wear, especially on teeth and tips of claws, but the crab is still relatively clean and vigorous. Typically the shell is hard, although prior to a moult the shell will soften slightly. Many crabs with this code	Code 1: New hard shell. No deflection on underside of carapace with heavy pressure from thumb. Very little claw wear and tips of claws are sharp and hooked. Few signs of wear or abrasions on carapace. May have barnacles,	Code 2: New springy soft shell. Evident by slight shell deflection with heavy pressure on underside of carapace. Little epiphytic growth, fouling, or abrasion. Barnacles, if present, will be small. Underside of carapace	Code 3: New crackly soft shell. Shell is easily deformed by finger pressure. Usually there is bright orange downy hair on underside of carapace.	Code 4: New plastic soft shell. Shell is extremely soft. Crab has moulted within the past few days.

		likely will not moult again or may soon die.	also be blunted as may be tips of walking legs.	indicate a moult is imminent.	but these may be small.	still has dense orange or yellowish hair.		
Fisheries and Oceans Canada 2014	Hard: Durometer measurement more than 70 units	Soft: Durometer measurement of 70 units or less						
Hicks 1987	Very old shell: The sternal surface inflexible when moderate pressure is applied, carapace and appendages heavily encrusted with epifauna, scars and abrasions present, dactyli worn,	Old shell: The sternal surface inflexible when moderate pressure is applied, carapace and appendages with epifauna present, scars and abrasions present on sternal surface and appendages	New shell: The sternal surface inflexible when moderate pressure is applied, carapace and appendages clean of epifauna and void of excessive wear	New soft: The sternal surface flexes considerably when moderate pressure is applied				

	obvious skip moult								
Hicks and Johnson 1999	New (soft) shell: Less than 66 durometer units	Old Shell: 66 durometer units and higher							
Lippert et al. 2002	Moulting "Old Shell" (1-1m): Crab appears as 1-1 but legs and/or area around mouthparts are softening, moult suture line may be cracked.	Pre-moult (1-1): The color of the ventral surface of carapace is now dark yellow or brown. Has mature barnacles, moult suture line intact, often many	Late hard shell (1-2): All crab parts hard, little or no epifaunal growth (ex. barnacles). The color of the entire exoskeleton is beginning to darken.	New hard shell (1-3): Carapace is hard. Mouth and/or walking leg won't flex under moderate pressure. Slight flex on merus of first walking leg under	Late intermediate (2-1): Top carapace is completely hard and underside has very slight flex under moderate pressure	Early intermediate (2-2): Top of carapace does not flex under moderate pressure. Crab light weight. Shell of ventral carapace, near	Recent moult (3-1): Top center carapace flexes but snaps back, margins soft to significantly flexible	New moult (3-2): Crab completely soft (like a beanbag), shell is like parchment

		scars or may be missing appendages.		moderate pressure		mouthparts, is fragile and flexes with light up to moderate pressure		
Northrup 1975	Old shell: Hard shell approaching a shell moult	I: Hard shell	II: Intermediate	III: Soft shell				
ODFW 2009	Hard shell: 20-30 percent meat by weight	Soft shell: As low as 12 percent meat by weight						
Penson JR 1988 (From Somerton and Macintosh 1983)	Skip-moult/ 4: Same as shell condition 3 except dactyl wear is more pronounced and barnacles are larger; Assumed age since last moult >24 months	Worn shell/ 3: Carapace firm, sternum with dark brown scratches and spots, dactyl tips worn or blunt, barnacles usually present; Assumed	New shell/ 2: Carapace firm, sternum without dark brown scratches or spots, little epifauna; Assumed age since last moult >2 weeks, <12 months	Soft shell (recent moult)/ 1: Carapace flexible, sternum without dark brown scratches or spots; Assumed age since last moult <2 weeks				

		age since last moult >12 months, <24 months			
Reilly 1983	Hard	Filling: Flesh/ muscle mass increases to fill out the new exoskeleton	Soft: Immediately after moulting		
Robinson et al. 1977	Prime hard shell: Potential meat yield of 25-30 percent	Recently moulted: 13-14% meat yield			

Spears 1983	Hard shell: Shell will not give when pinched; it will yield 25% of its weight in top quality meat	Soft shell: Recently moulted; new exoskeleton is bluish-white (instead of yellowish-brown) and free of barnacles and algae; crab will be lightweight in comparisons to its size; squeeze the edge of the shell near the lateral spines or pinch the large section of one of the walking legs (pinch with about the same	

		pressure you would use to burst a grape), if the shell gives easily it will yield less than 20 percent of its weight in soft, mushy meat; if the shell gives just a little when squeeze, it will yield about 20 percent of its weight in poor quality meat	

Tegelberg 1972	Grade I: Shell hard, little or no flexibility in carapace	Grade II: Shell intermediate between I and III	Grade III: Recently moulted soft shells, carapace and legs flexible, easily cracked by finger pressure	
Waldron 1958	Grade 1: Carapace very rigid; exoskeleton of legs rigid or slightly pliable	Grade 2: Carapace slightly to moderately flexible	Grade 3: Carapace very flexible, may be crushed in hand	

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