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A reflex action mortality predictor (RAMP) for commercially fished blue crab *Callinectes sapidus* in Florida

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Abstract

The paucity of data on discard mortality in the commercial blue crab fishery contributes significant uncertainty to stock assessments. The reflex action mortality predictor (RAMP) method is used to predict mortality of commercially fished species by establishing a relationship between reflex impairment and the likelihood of mortality (RAMP curve). The present study adapted the RAMP method to commercially fished blue crabs in two unique habitats in Florida, the Tampa Bay estuary and the St. Johns River. The euryhaline nature of blue crabs permits the fishery to operate in myriad habitats, which adds complexity to cumulative effects that influence discard mortality. A total of 697 crabs were assessed, and a RAMP curve was generated. Logistic regression analyses determined that reflex impairment score, injury score, and salinity are strong predictors of discard mortality, with 91% of predictions correct. For every 1-unit increase in reflex impairment and injury score, mortality was 2.71 and 1.65 times more likely, respectively. Additionally, for every 1-SD (11.35 ppt) increase in salinity, mortality was 2.17 times more likely. Crab size, sex, water temperature, and emersion time minimally influenced mortality. The present study is the first crustacean RAMP research that included water quality and demonstrated that its inclusion increases the predictability of discard mortality. The RAMP curve can be used as a tool to estimate discard mortality aboard commercial vessels and discard mortality in future stock assessments.

Previous Next

Keywords

Callinectes sapidus; Blue crab; Discard mortality; Reflex impairment

1. Introduction

Florida's blue crab fishery landed 2580.6 metric tons of whole crab valued at $11.4 million (USD) in 2020 (Florida Fish and Wildlife Fisheries Information System, 2020). Landings of blue crabs in Florida are highly variable from year to year and exhibit an overall decline from high levels in the late 20th century (Cooper et al., 2011, VanderKooy, 2013). The high variability in blue crab abundance is due to seasonal fluctuations in climate, habitat quality, and freshwater input to coastal estuaries (Cooper et al., 2011, Guillory, 2001, Perry and VanderKooy, 2015). Habitat loss and degradation negatively impact blue crab abundance throughout its U.S. range. This impact is apparent where coastal communities are developing rapidly (Cooper et al., 2011, Perry and VanderKooy, 2015, Wilber, 1994). Stock assessments for blue crab in Florida have determined that the population is not overfished nor undergoing overfishing. However, the abundance remains below historic levels (Cooper et al., 2011, VanderKooy, 2013). Improving the accuracy of the Florida assessments is essential for better stock status estimates under these lower population densities. Future data needs include improving age estimates, quantifying recreational harvest, and improving estimates of fishery-related discard mortality (Cooper et al., 2011, VanderKooy, 2013).

Predicting delayed mortality in exploited fishery species is essential to understanding the effects of commercial fishing, integrating discard mortality into stock assessments, and providing advice to fishery managers about the effects of fisheries practices (Stoner, 2012). Crabs discarded from the blue crab fishery in Florida include those that do not meet the requirements for legal take (under the legal-size limit and egg-bearing females) and those that are deemed non-marketable to retailers and wholesalers. Criterion for discard of non-marketable crabs can include light weight or recently molted crabs, those with disease (i.e., cotton crab or bitter crab disease), too many injuries, or lethargy. Fishery-induced physiological stress begins when the crab enters a commercial trap and is subjected to containment stress, physical injury, and disease transmission because of confined proximity with other crabs (Guillory, 2001). Stress increases when the trap is hauled aboard and crabs are shaken into a cull box where lateral spines can be broken, limbs lost, and carapace damage sustained by physical contact with other crabs. Depending on the fishers' culling procedure, which is generally dependent on the presence of a deckhand, crabs can spend minutes to hours onboard. While on board they are susceptible to dehydration and thermal stress from prolonged air exposure prior to discard (Giomi et al., 2008; Stoner, 2012). The stress of commercial fishing practices is not conducive to the survival of discarded blue crabs (Guillory, 2001). Despite this potential source of mortality, no research has sought to determine the relationship between Florida fishing practices and the delayed mortality of discarded blue crabs.

The reflex action mortality predictor (RAMP) is a method to determine the relationship between the number of reflexes lost and the probability of discard mortality. This method was initially developed for fish species (Davis and Ottmar, 2006, Davis, 2010) but has been adapted for numerous commercial crab species such as Tanner crab *Chionoecetes bairdi*, Snow crab *Chionoecetes opolio*, Red King Crab *Paralithodes camtschaticus*, Stone crab *Menippe mercenaria*, and Dungeness crab *Cancer magister* (Stoner et al., 2008, Hammond et al., 2013, Rose et al., 2013, Yochum et al., 2017; Kronstadt et al., 2018). The RAMP method is a rapid and noninvasive assessment of reflex actions (i.e., eye retraction, leg retraction, mouth closure) that are quantifiable and highly effective in predicting delayed mortality (Stoner, 2012). In the present study, commercially discarded crabs were reflex tested using a predetermined set of reliable reflexes and monitored for 48 h following discard. Our objective was to develop a RAMP index for commercially discarded blue crabs in Florida and apply this method during fishery operations to assess discard mortality. The application of the RAMP method to blue crabs in Florida can provide an estimate of unknown discard mortality that was previously unavailable for stock assessment and will be useful in providing advice for improving fisheries' culling practices.

2. Methods

2.1. Identifying reliable reflexes

A preliminary study was conducted to determine which reflexes consistently responded to stimuli and could be reliably used in a RAMP assessment. A total of 22 crabs (11 males and

11 females) ranging in carapace width (CW) from 109 to 176 mm, with no injuries nor signs of molting (intermolt), were purchased from a local fish market in St. Petersburg, Florida, in April 2019. Crabs were brought to the Fish and Wildlife Research Institute in St. Petersburg, Florida, and immediately placed in individual cylinder-shaped cages (V=763.41 m^3), constructed of 6.35-mm-square plastic mesh. The cages were equally distributed inside two 284-liter flow- through seawater tanks. Prior to reflex testing, crabs were acclimated in tanks for two days to lessen the effects of capture and transport. On day two, crabs were fed ~20 g of sardines and reflexes were tested on day three through five to determine their repeatability. Each day, eye retraction, leg retraction, mouth closure (3rd maxilliped), abdomen turgor, abdomen retraction, antennule reaction, antennae reaction, joint reaction, appendage turgor, chela wave, and chela closure were tested. Every crab was tested on both the right and left sides of the body to determine if there was any significant difference (Kronstadt et al., 2018). McNemars' test determined there was no significant difference in the response of reflexes tested on the right side of the crab versus the left side (p value=0.42). Therefore, all subsequent testing was conducted on the left side of the crab (i.e., left eye, left leg, left side of mouth). If a crab was missing a left chela or swimmeret, the right appendage was tested instead. We chose to use 9 of the 11 reflexes in subsequent testing because they responded rapidly and consistently to stimuli (Table 1). The reflexes of abdomen turgor and abdomen reaction were excluded because the aprons of immature female crabs are sealed to the abdomen (Engel, 1958), which would exclude crabs in this life stage.

Table 1. Reflexes tested on *Callinectes sapidus*. Individual reflexes were considered absent if no response was observed during testing. Reflexes were always tested in the order listed below and while the crab was in ventrum-down position. Stimulus was applied with a stainless steel 177.8-mm Cushing forceps.

Reflex	Test	Reflex present	Reflex absent
Eye retraction	Touch eye stalk with forceps end. If eye is retracted, wait for eye to move out from under carapace hood.	Eye retracts below the carapace hood or moves away from the probe.	Eye does not move and remains out from under the carapace hood.
Leg retraction	Gently squeeze dactyl of first walking leg with forceps. Do not pull.	Leg is pulled away.	Leg does not pull away.
Mouth closure	If closed, attempt to open 3rd maxillipeds with forceps. If	3rd maxillipeds retract to cover the smaller	Maxillipeds droop open and do not close or

Reflex	Test	Reflex present	Reflex absent
	open, touch inside mouth parts with forceps.	mouth parts. Smaller mouther parts are moving.	slightly close upon probing. Smaller mouth parts are not moving.
AntennuleReaction	Observe antennule position. If out of carapace hood, lightly brush with forceps to elicit retracted position under carapace hood. Once, retracted, pull out using forceps.	Antennule is pulled back under carapace hood.	Antennule is not pulled back under carapace hood.
Antennae Reaction	Brush or gently squeeze antennae and observe movement.	Antennae move in response to contact with the forceps.	Antennae do not move.
JointReaction	Softly lay the side of forceps across the joint membrane between the coxa and basi-ischium of the swimmeret.	Swimmeret is quickly pulled up in response.	Swimmeret does not move at all.
Chela Closure	Place forceps in between the dactylus and fixed finger to initiate a closure response.	Chela closes in defensive behavior.	Chela does not respond and elicit open or closure of dactyl.
AppendageTurgor	Observe orientation and movement of limbs during reflex testing.	Limbs are either moving in attempt to escape manipulation and are not limp in response to environment.	Limbs are drooping and limp while manipulation elicits no response.
Chela Wave	Observe chela defensive movement during reflex test.	Chela respond defensively during handling and manipulation.	Chela do not respond during handling or manipulation and elicit no response.

2.2. Reflex testing protocol

Reflexes were always tested in the same order: eye retraction, leg retraction, mouth closure, antennule reaction, antennae reaction, joint reaction, and chela closure. Chela wave and appendage turgor were assessed throughout the manipulation and testing of the other seven reflexes (i.e., crab waved extended chela in defense of manipulation). The procedure started with a crab placed on a flat surface. Stimulus was applied using a stainless steel 177.8-mm Cushing forceps to assess reflex actions. Reflex response was evaluated as a binary score (presence or absence) to reduce observer bias and provide a thorough index of crab condition (Stoner, 2012). Thus, an individual reflex was scored as present (zero) or absent (one) and reflex impairment score (RIS) calculated as the sum of lost reflexes (Stoner et al., 2008, Stoner, 2012). On average, each reflex test took 30 s to conduct.

2.3. Field experiment

To determine the relationship between loss of reflexes and discard mortality, crabs discarded from commercial blue crab traps were examined for loss of reflexes followed by containment in situ in cages for 48 h. Field experiments were conducted aboard commercial blue crab vessels in the Tampa Bay estuary in August of 2019 and February of 2020 and in the St. Johns River in August, September, and December of 2019 (Fig. 1). Blue crab collection was designed to reduce interruption of fishing operations and minimize excess air exposure and handling of animals beyond normal fishing conditions. This was achieved by allowing the fishers to follow their normal culling practices and immediately hand all discarded crabs to the researcher for assessment and placement in the cage. The researcher rapidly processed the discarded crabs, and they were held for no more than 15 min beyond typical discard by the fisher to reduce added air emersion and handling time.

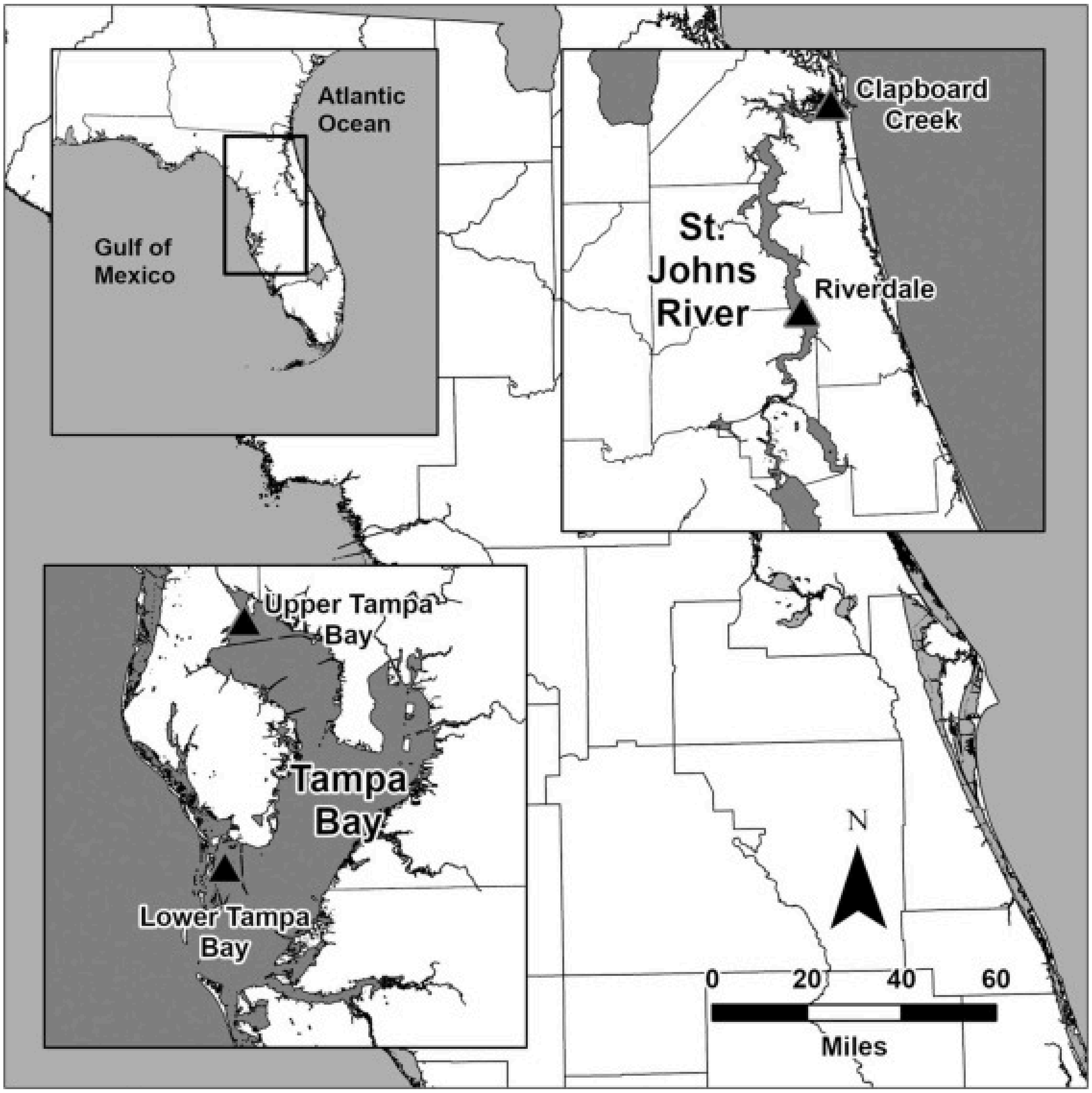

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Fig. 1. Map of field experiment locations in Florida (triangles). Field experiments were conducted in two locations in the St. Johns River (Clapboard Creek, and Riverdale) and two locations in Tampa Bay (Upper Tampa Bay, and Lower Tampa Bay).

Discarded crabs were immediately evaluated by the researcher, for CW (measured in mm), sex, number of new apparent external injuries (i.e., one missing walking leg and cracked carapace equals two injuries) obtained from culling (injury score), total time the crab was out of water (time), and a RIS. Only intermolt crabs were included in the current study because compromised mobility during post-molt recovery interferes with accurate RIS. Intermolt crabs were retained, fitted with a tag with a unique identification number (9.53 × 50.8 mm oval Floy Tag Inc., Seattle, WA) wired to the dorsal carapace using the lateral carapace spines, their reflexes tested, and placed into a wire holding pen (101.6 cm × 60.9

cm×15.2 cm). Holding pens were constructed out of 1-inch-square plastic-coated 16-gauge (1.3 mm) galvanized wire and partitioned into 15 cells, each cell measuring 20.3 cm × 20.3 cm × 15.2 cm (Fig. 2). Holding pens confined ten discarded crabs and five control crabs contained individually in each holding pen. Control crabs were selected from crabs retained by the fisher (those deemed marketable) and had an RIS ≤ 3. Control crabs were held in each holding pen to confirm that the holding method did not contribute to mortality. A HOBO data logger pendant (Onset Computer Corporation, Cape Cod, MA) was affixed to one of the holding pens during each field experiment and recorded water temperature every five minutes while the cages were deployed. Once a holding pen was filled with crabs, it was closed, GPS coordinates recorded, and the pen was immediately deployed to minimize the amount of time the crabs were out of water, beyond normal onboard handling. Holding pens were retrieved after 24 and 48 h to record mortality of discarded animals. If a crab was found dead, the tag number was recorded, the animal removed, and the holding pen redeployed at the same fixed location where commercial fishing occurred. After the 48-hour retrieval all live crabs were released. Additionally, during each retrieval, salinity was recorded with a YSI 650 multiparameter display system (YSI Inc., Yellow Springs, Ohio).

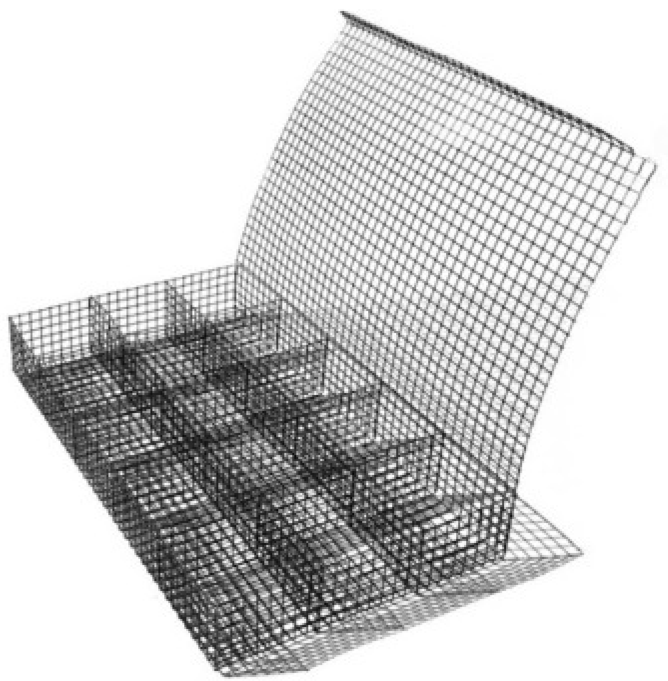

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Fig. 2. Custom holding pens used in field experiments to monitor discard mortality of *Callinectes sapidus*. Each holding pen held 15 crabs separately, each cell measuring 20.32 cm L x 20.32 cm W x 15.24 cm H.

2.4. Statistical analysis

A logistic regression was used to model the relationship between crab mortality and predictor variables (RIS, CW, sex, injury score, and time) and local water quality (average salinity and average water temperature). Mortality was a binary response variable represented by mortality (1) or survival (0) over the 2-day confinement period. To facilitate model-fitting, the continuous predictor variables CW, temperature, and salinity were standardized with a mean of zero and a standard deviation of one. Additionally, to account for spatial dependence among crabs in the same holding pen, Holding Pen ID (a unique holding pen identifier) was included as a random effect associated with the model intercept (Gelman and Hill, 2007). A total of 20 candidate logistic regression models were fitted, with each model representing a unique combination of the crab-specific and environmental predictor variables described above. Following model-fitting, the most plausible models were identified by calculating Akaike's Information Criterion (AIC; Akaike, 1973) with a small sample bias adjustment (AICc; Hurvich and Tsai, 1989) and relative AICc weights (Burnham and Anderson, 2002). Because AIC can perform poorly if there are too many parameters in relation to sample size (Burnham and Anderson, 2002), we used AICc, which adds a small penalty for adding additional terms to the model, where the size of the additional penalty is determined by both sample size and model complexity. We based all inferences on effect sizes of parameter estimates from candidate models with AICc weights that were at least 10% of the AICc weight of the best approximating model, which is similar to Royall's 1/8 or 12% threshold for evaluating strength of evidence (Royall, 1997). We considered there to be strong evidence of an effect if the 95% confidence interval of a parameter did not overlap zero. All logistic regression models were fit using the statistical software R version 3.6.3 (R Core Team, 2020) using the package 'glmmTMB' (Brooks et al., 2017). Goodness of fit of the best-approximating model was assessed by conducting the le Cessie–van Houwelingen–Copas–Hosmer goodness-of-fit test, implemented in the R package 'rms' (Harrell, 2020). Lastly, classification accuracy of each candidate model was assessed using an area under the curve (AUC) statistic, as implemented in the R package 'ROCR' (Sing et al., 2005), where AUC ranged from 0 to 1, and where higher AUC values indicated greater classification accuracy.

3. Results

3.1. Field experiments

Six field experiments were conducted during the summer months of August and September 2019, four in the Tampa Bay estuary and two in the St. Johns River, and three were conducted in the winter months of December and February, two in the Tampa Bay estuary and one in the St. Johns River (Table 2). To maintain consistency of culling procedures, the same commercial fisherman and crew were used for each field experiment in the Tampa Bay estuary and St. Johns River.

Table 2. Location, date, average water temperature (°C), average salinity (ppt), number of discarded crabs (D*n*), average reflex impairment scores (RIS) of discarded crabs, range of RIS scores of discarded crabs, discard mortality (% discard crabs that died), number of control crabs (C*n*), and control mortality (% discard crabs that died), for each 2-day observation period.

Location	Date	Average water temperature (°C)	Average salinity	(D*n*)	Discard RIS average	Discard RIS range	Discard mortality	(C*n*)	Control mortality
Tampa Bay	8/5/2019	31.2	31.4	50	2	0–7	30%	25	0%
Tampa Bay	8/7/2019	31.3	30.4	50	2	0–8	30%	25	4%
Tampa Bay	8/12/2019	31.3	29.9	50	1	0–8	22%	25	4%
Tampa Bay	8/26/2019	31.6	27.9	50	2	0–7	26%	25	0%
Tampa Bay	2/10/2020	19.2	20.8	60	0	0–5	5%	30	3%
Tampa Bay	2/12/2020	22.8	20.9	50	1	0–5	16%	25	0%
St. Johns River	8/19/2019	28.7	0.6	50	1	0–4	6%	25	0%
St. Johns River	9/9/2019	29.7	0.45	50	1	0–5	4%	25	0%
St. Johns River	12/18/2019	15.7	26.6	56	1	0–4	1.80%	26	0%
	Total N			466				231	

Location	Date	Average water temperature (°C)	Average salinity	(D*n*)	Discard RIS average	Discard RIS range	Discard mortality	(C*n*)	Control mortality
	Average mortality						15%		1%

3.2. Relationship between mortality and reflex impairment score

A total of 697 crabs (232 from the St. Johns River and 465 from the Tampa Bay estuary) were assessed to establish the relationship between RIS and discard mortality. Discarded legal and sublegal males, mature and immature females were used in both control and experimental groups (Fig. 3). Over the entire study period, 231 crabs served as the control group and only three (1%) died, which confirmed that our holding methods did not significantly contribute to mortality during the holding period (Table 2). Thus, data collected from the discard and control crabs (N = 697) were combined to relate RIS to mortality (Table 2). Throughout the study, 71 discarded crabs died within the 48 h, and 94% of those died within the first 24 h of the holding period. Discard mortality ranged from 2% to 30%, with the highest mortality seen during the summer months in the Tampa Bay estuary (Table 2). The widest range of RIS was present in the summer in Tampa Bay estuary, which produced mortality rates exceeding 20%. The lowest discard mortality (<7%) was in the St. Johns River in both the summer and winter and in one winter Tampa Bay sampling (Table 2). Crab mortality increased with increasing RIS and reached 100% with a RIS of 8 (Table 3). The majority of crabs (N = 573) had a RIS ≤ 2 and less than 10% mortality (Table 3). Crabs with a RIS of 4 reached 50% mortality and those with a RIS of 6 reached 75% mortality (Table 3).

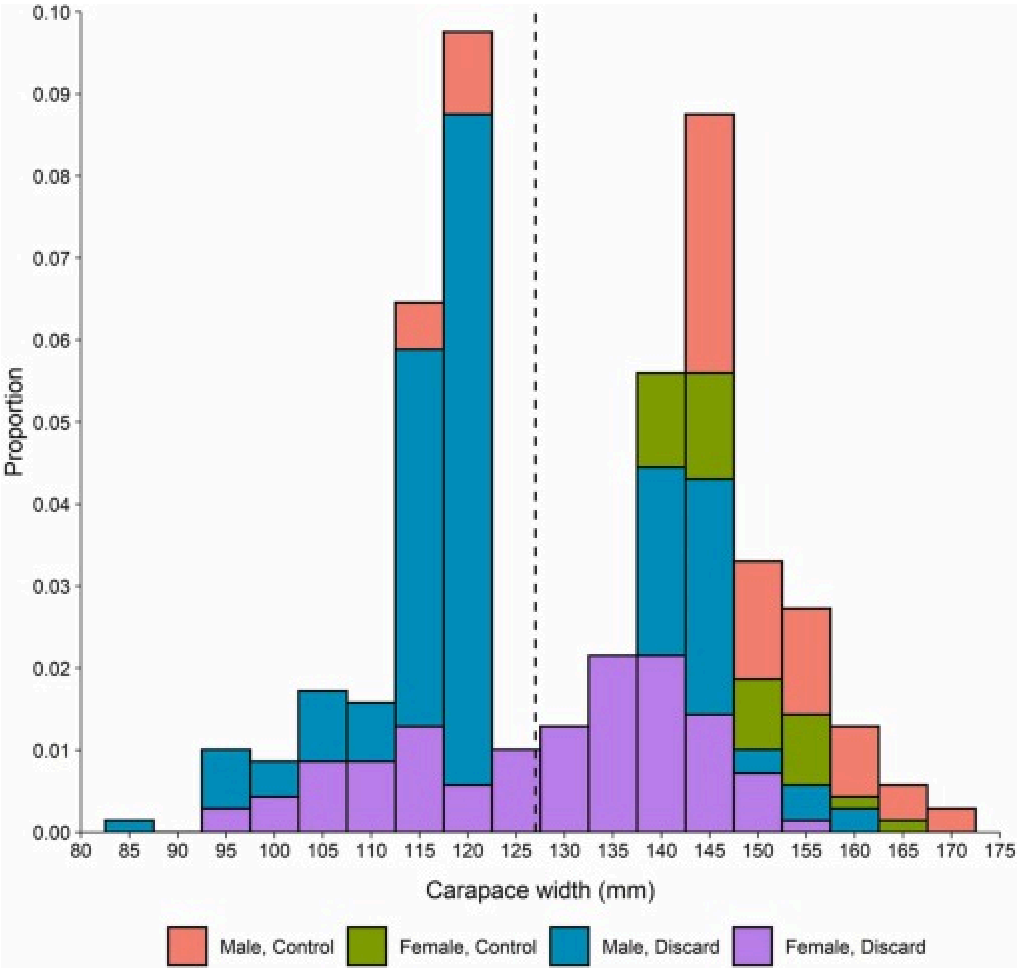

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Fig. 3. Proportion of blue crabs per 5 mm carapace width bin, by Sex and Treatment. Male, Control (red), Female, Control (green), Male, Discard (blue), and Female, Discard crabs from all field experiments. Vertical dashed line designates the split between legal crabs (right of the dashed line) and sublegal crabs (left of the dashed line). Minimum legal size is CW ≥ 127 mm.

Table 3. Reflex impairment scores and percent delayed mortality (% DM) for *Callinectes sapidus* over the 2-day holding period. Delayed mortality is reported as percent dead in each reflex impairment score (RIS) category.

RIS	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Total N	285	184	104	66	30	14	4	8	2
Dead N	4	6	7	20	15	10	3	7	2

RIS	0	1	2	3	4	5	6	7	8
% DM	1%	3%	7%	30%	50%	71%	75%	88%	100%

Appendage turgor (N = 302) and chela wave (N = 216) reflexes were most frequently lost, while the mouth closure reflex (N = 11) was rarely lost. However, 100% of crabs that lost the mouth closure reflex died, and only 19.5% of crabs that lost the appendage turgor reflex died (Table 4). The mortality associated with the remaining reflexes examined ranged from 21% to 62% (Table 4). When only one reflex was lost, that reflex was most commonly appendage turgor.

Table 4. Number of crabs that lost each reflex. Values are presented as the number of each reflex lost, the number of crabs that died for each reflex lost, and the percentage of total reflexes lost.

Reflex	No. of each reflex lost	No. of crab dead	% dead
Eye retraction	34	21	61.8%
Leg retraction	67	31	46.3%
Mouth Closure	11	11	100.0%
Antennule reaction	76	27	35.5%
Antennae reaction	55	21	38.2%
Joint reaction	57	12	21.1%
Appendage turgor	302	59	19.5%
Chela closure	58	30	51.7%
Chela wave	216	61	28.2%

3.3. Logistic regression analysis

The best-approximating model relating crab-specific and environmental factors to discard mortality included RIS, injury score, and salinity. This model was 1.38, 1.38, 2.64, 3.63, and 7.25 times more plausible than the second through sixth best-approximating models, respectively. Based on the AIC there was less support for the remaining 14 models (Table 5). The Le Cessie test for global goodness of fit indicated that the best-approximating logistic regression model provided an adequate fit to the data. Except for the intercept-only model

(AUC = 0.81), the AUC statistic was greater than 0.91 for all candidate models, indicating that they all predicted the observed data well (Table 5). Parameter estimates from the best-approximating model indicated that discard mortality was strongly and positively related to RIS, injury score, and salinity (Table 6; Fig. 4). Odds ratios (OR) indicated that for every 1-unit increase in RIS and injury scores, mortality over the 2-day observation period was 2.71 and 1.65 times more likely, respectively. Similarly, the odds ratio associated with salinity indicated that blue crab discard mortality was 2.17 times more likely for every 1-SD (11.35 ppt) increase in salinity (Table 6). Parameter estimates associated with the remaining predictor variables in the top four candidate models, water temperature and CW, were considered imprecise as their 95% confidence intervals overlapped zero (Table 6).

Table 5. Model parameters (Model), number of parameters (K), AICc, ΔAICc, Akaike weights (w), and AUC for the candidate set of logistic regression models relating crab specific (carapace width, sex, injury score, RIS) and local environmental factors (salinity, water temperature, time crab was out of water) to blue crab mortality.

Model	K	AICc	ΔAICc	*w*	AUC
Intercept, RIS, injury score, salinity	5	288.51	0.00	0.29	0.916
Intercept, RIS, injury score, salinity, sex	6	289.20	0.68	0.21	0.912
Intercept, RIS, injury score, salinity, water temperature	6	289.21	0.70	0.21	0.915
Intercept, RIS, injury score, salinity, carapace width	6	290.46	1.95	0.11	0.917
Intercept, RIS, injury score, salinity, water temperature, carapace width	7	291.17	2.66	0.08	0.914
Intercept, RIS, injury score, carapace width, salinity, water temperature, sex	8	292.74	4.22	0.04	0.911
Intercept, RIS, salinity, water temperature	5	293.63	5.11	0.02	0.914
Intercept, RIS, salinity, time	5	294.07	5.56	0.02	0.910
Intercept, RIS, carapace width, salinity	5	294.89	6.37	0.01	0.914
Intercept RIS, carapace width, salinity, water temperature	6	295.63	7.11	0.01	0.914
Intercept, RIS, injury score, water temperature	5	298.34	9.83	0.00	0.917
Intercept, RIS, injury score, carapace width, water temperature	6	300.36	11.85	0.00	0.917
Intercept, RIS, injury score, carapace width, time	6	301.08	12.56	0.00	0.925

Model	K	AICc	ΔAICc	*w*	AUC
Intercept, RIS	3	301.99	13.47	0.00	0.914
Intercept, RIS, sex	4	302.71	14.20	0.00	0.91
Intercept, RIS, time	4	303.10	14.59	0.00	0.917
Intercept, RIS, carapace width, water temperature	5	303.92	15.41	0.00	0.912
Intercept, RIS, carapace width	4	304.01	15.50	0.00	0.915
Intercept, RIS, carapace width, sex	5	304.73	16.21	0.00	0.91
Intercept	2	456.28	167.77	0.00	0.81

Table 6. Parameter estimates, standard errors (SE), lower and upper 95% confidence limits (lower and upper), and odds ratios (OR) from the four best-approximating mixed effects logistic regression models relating crab-specific and environmental variables to blue crab mortality. Accurate predictors of crab mortality were parameters with confidence intervals that did not overlap zero.

Parameter	Estimate	SE	Lower	Upper	OR
Best-approximating model					
Fixed effects					
Intercept	-4.754	0.414	-5.677	-4.029	
reflex score	0.995	0.116	0.783	1.242	2.705
injury score	0.502	0.193	0.116	0.881	1.652
salinity	0.775	0.243	0.336	1.316	2.171
Random effect					
Intercept (Holding Pen ID)	0.5				
Second best-approximating model					
Fixed effects					
Intercept	-5.206	0.596	-6.513	-4.148	
reflex score	0.982	0.115	0.771	1.226	2.67
injury score	0.516	0.190	0.136	0.890	1.675

Parameter	Estimate	SE	Lower	Upper	OR
Best-approximating model					
Fixed effects					
salinity	0.715	0.234	0.296	1.244	2.044
sex	0.608	0.537	-0.406	1.742	1.837
Random effect					
Intercept (Holding Pen ID)	0.419				
Third best-approximating model					
Fixed effects					
Intercept	-4.705	0.409	-5.625	-3.989	
reflex score	0.971	0.117	0.757	1.219	2.641
injury score	0.495	0.190	0.116	0.869	1.64
salinity	0.706	0.233	0.289	1.236	2.026
temperature	0.232	0.202	-0.167	0.663	1.261
Random effect					
Intercept (Holding Pen ID)	0.508				
Fourth best-approximating model					
Fixed effects					
Intercept	-4.753	0.415	-5.677	-4.027	
reflex score	0.992	0.117	0.778	1.239	2.697
Injury score	0.506	0.194	0.119	0.887	1.659
salinity	0.781	0.244	0.339	1.327	2.184
carapace width	0.059	0.197	-0.324	0.451	1.061
Random effect					
Intercept (Holding Pen ID)	0.43				

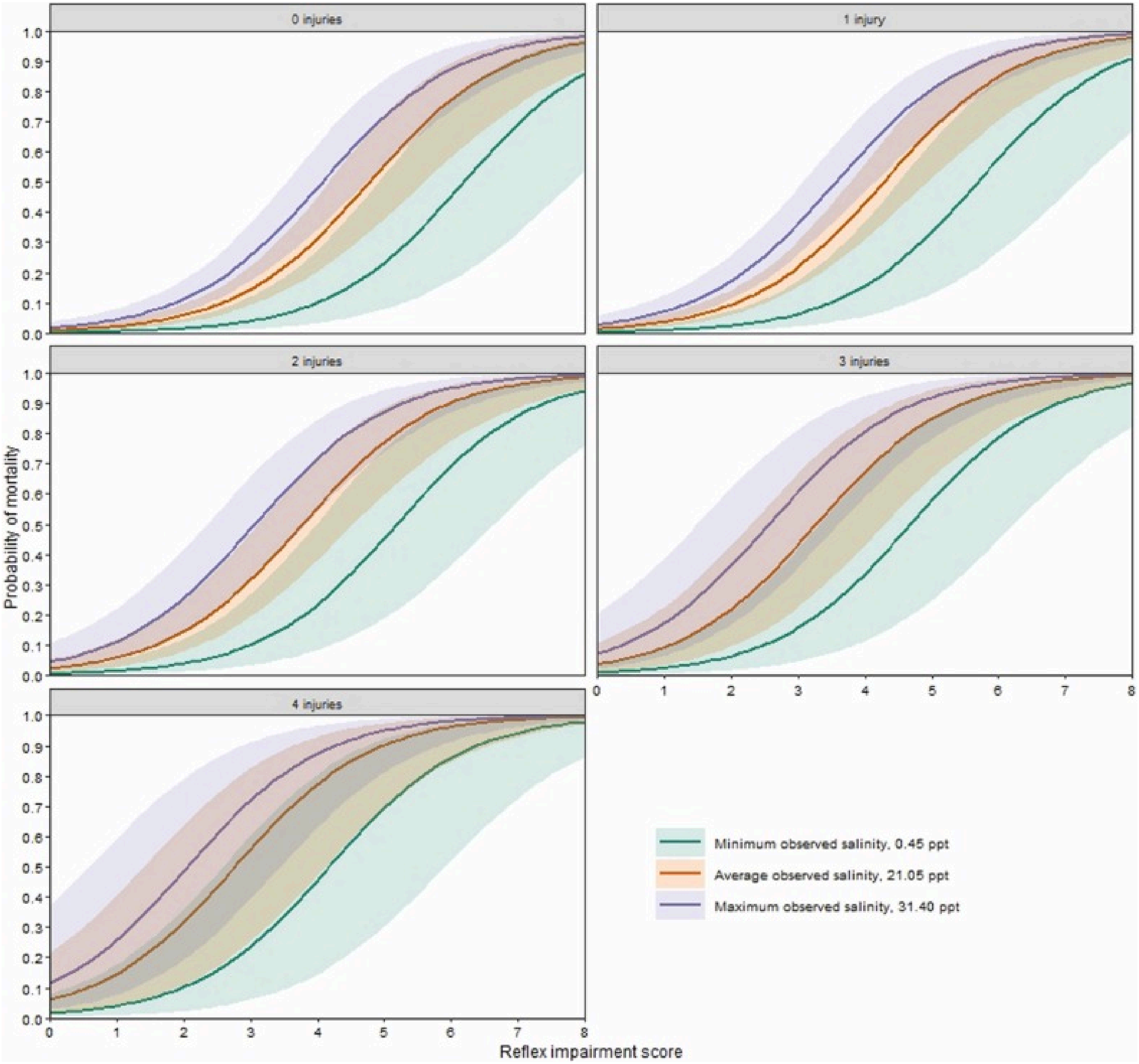

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Fig. 4. Predicted mortality of blue crab, *Callinectes sapidus,* as a function of reflex impairment score (RIS), injury score (0–4 injuries), and salinity. Each line represents minimum, average, and maximum observed salinity, 0.45 ppt (green), 21.05 ppt (orange), and 31.40 ppt (purple), respectively.

4. Discussion

The RAMP method was successfully adapted and provides the first estimation of short-term (2-day) discard mortality for the blue crab fishery in Florida. The successful development of the RAMP index (RAMP curve) for the fishery in Florida allows for the further development of estimates of discard mortality directly from commercial fishing trips in different regions. Regional specificity may be necessary in Florida because the blue crab and the fishery that relies on it occur in numerous habitats throughout Florida where environmental conditions (i.e., salinity and water temperature) and culling processes may vary. The RAMP index adapted in this work provides a standard by which the effect of various physiological stresses induced by environmental and culling conditions can be assessed and discard mortality estimated on regional and statewide scales.

A well-designed RAMP assessment considers how well the RIS captures the collective stress and injury of an animal and generates a relationship between reflex impairment and discard mortality (Stoner et al., 2008, Stoner, 2012). The nine reflex actions selected for testing in the present study are commonly used for vitality assessments in commercially fished crustaceans (Stoner, 2012). For blue crabs in Florida, the most reliable predictors of discard mortality (within 48 h) were the loss of the mouth closure, followed by the loss of eye retraction. The reliability of these reflexes to predict mortality has been previously documented in other crabs. Stoner et al. (2008) found the loss of mouth closure and eye retraction in Tanner crabs was associated with mortality because these reflex actions are essential for ventilating the gills, require minimal energy expenditure, are the last involuntary movement maintained in crabs near death, and the final loss of motion before death. While appendage turgor and chela wave were the most common reflexes lost, often simultaneously, they were associated with the lowest mortality percentages (Table 4). It is notable that these two reactions did not always require a specific stimulus to elicit a response. These responses are commonly linked with defense and escape and are likely lost early on when stress or lack of energy overwhelms them, while other reflex actions tested are involuntary movements associated with physiological maintenance that are the last to be lost.

The RIS explained a significant amount of variation in all models and was the most influential variable in determining the probability of discard mortality for blue crabs in Florida. For every 1- unit increase in RIS, blue crab discard mortality was 2.71 times more likely over the 2-day observation period. The relationship between RIS and discard mortality was also significant in RAMP indices for Dungeness crab, Tanner crab, and Stone crab (Yochum et al., 2015, Yochum et al., 2017; Kronstadt et al., 2018). While the top model included RIS, salinity, and injury score, singularly, RIS explains enough variability to warrant estimation of discard mortality solely from this parameter. In application, measuring RIS alone is less tedious or time consuming than collecting injury data and salinity data. Previous RAMP research has suggested the collection of only reflex data to measure unobserved mortality is sufficient (Stoner et al., 2008, Hammond et al., 2013). However, if feasible the collection of injury score and/or salinity during field operations is useful to further explain the variability in effect for a species and fishery that occur in multiple environments in Florida.

Blue crabs spend time in different habitats with different salinity ranges, temperatures, dissolved oxygen, coastal nutrient inputs, and overall system productivity that causes variability in physiological condition and ambient stress in fished blue crabs throughout Florida. In the present study, two habitat types were assessed, a river system (St. Johns River) and an estuary (Tamp Bay estuary), which produced different salinities, temperatures, and mortality percentages during different seasons for discarded crabs following commercial fishing (Table 2). The design of this study allowed us to test several factors to determine which influenced discard mortality. Logistic regression analysis determined that salinity and injury score were strong predictors of discard mortality while water temperature, time, sex, and size of crab appeared to minimally influence discard mortality.

In the best-approximating model, increased salinity and RIS also increased the probability of discard mortality (Fig. 4). This was most evident in summer sampling within the lower Tampa Bay estuary where average salinity exceeded 27.9 ppt. Although water temperature was a variable in the third-best-approximating model and received reasonable support, it was not well estimated, perhaps because the temperature range sampled during the study was only 16 °C. The highest average water temperature encountered was in Tampa Bay during the summer, averaging about 31 °C. While there was weak evidence to support temperature in the models, anecdotal evidence suggests that when crabs are harvested from waters with temperature > 30 °C, discarded crabs are most likely to die.

The effect of temperature and salinity on metabolism and osmoregulation can lead to increased physiological stress that likely increases discard mortality. Crustaceans are ectotherms; thus, when water temperature is increased, metabolic rate also increases and produces stress. This stress then negatively affects immunological and physiological processes (Shields, 2019). Blue crabs have been shown to be less tolerant to experimental temperature extremes at low and high salinities, 6.8 ppt and 34 ppt, respectively, because they produce near lethal shock (Tagatz, 1969). Additionally, research conducted by Tan, Engel, W.A (1966) suggests that male crabs are less efficient at osmoregulation in high salinity (> 30 ppt). In the present study, the highest discard mortality percentages (> 20%) were seen in environments where salinity was near or greater than 30 ppt and average water temperature exceeded 31 °C (Table 2). During these high- temperature and high-salinity conditions, catch was dominated by males (98% male). Under those conditions, 56 males died but no female crabs (N = 6) died during the 2-day holding period. The elevated discard mortality of male crabs during these environmental conditions suggests that the threshold for efficient osmoregulation in male crabs was surpassed under the added stress of the fishing process. The high physiological demands at low temperature and salinity extremes have been well documented as causes of mortality in blue crabs in their northern ranges (Rome et al., 2005, Bauer and Miller, 2010). Blue crabs were most vulnerable to mortality in the lowest temperature and salinity treatments depending on life stage (Rome et al., 2005). It is apparent that higher salinity may increase physiological demand, reduce osmoregulation efficiency, and become more lethal when combined with other stress (i.e., culling, handling, air exposure, elevated water temperatures). Little research has been done to assess the physiological effects of high salinity and water temperature in blue crabs. However, results of the present study highlight the need to assess physiological stress in elevated temperatures under various salinity regimes.

Discard mortality in fished crustaceans is also influenced by physical injury sustained from intraspecific interactions in traps and handling onboard. These injuries include missing limbs and cracks to the carapace and abdomen, and pose high threat to survivability (Davis, 1980, Juanes and Smith, 1995). Injury score (the number of injuries) accounted for a large portion of variability associated with discard mortality in the present study. Blue crab mortality was 1.65 times more likely for each additional injury sustained. These findings are consistent in RAMP studies for snow crab (Hammond et al., 2013) and Tanner crab (Hammond et al., 2013, Stoner et al., 2008). While not all injuries result in mortality, the severity and quantity of injury can be influential to a crab's survival following discard. The portunid crab *Portunus pelagicus*, was most susceptible to mortality when recorded with a crushed body and missing claws (Wassenberg and Hill, 1989). Rock lobster *Jasus lalandi* were observed in situ for 10 months following the systematic removal of antennae, feeding limbs, and walking legs. This study determined that mortality was highest when antennae or feeding limbs were removed and lowest when walking limbs were removed (Brouwer et al., 2006). Multiple limb loss in crustaceans can also reduce growth rate (Smith, 1990a), ultimately delaying recruitment of legal-size animals to the fishery. Limb loss can also alter defensive and escape behaviors from predators (Smith, 1990b), thereby increasing discard mortality. Although long-term effects such as predator avoidance behaviors and reduced growth rates were not evaluated in this study, earlier research demonstrated that the indirect effects of the commercial fishing process can reduce survivability of sublegal animals and delay recruitment of legal-size animals to the fishery.

Results supported previous conclusions whereby crab-specific variables, CW and sex, do not explain significant variability seen in fishing-related discard mortality (Stoner et al., 2008, Hammond et al., 2013, Kronstadt et al., 2018). Only intermolt crabs were included in analyses to reduce variability associated with RIS from crabs undergoing post-molt recovery, which limits crab movement and reflexes. These crabs can be identified by their soft or paper-like exoskeleton. When exoskeleton condition was included in analyses for Tanner crab and snow crab, there was weak evidence to support its relationship to mortality (Stoner et al., 2008). However, our field observations suggest crabs that have recently molted are noted as lightweight and are more sensitive to the stress of the fishing process. These crabs can be identified by their translucent cuticle, absence of fouling, and low weight-to-CW ratio. When lightweight crabs were recorded, they were found to be weak during onboard handling and more likely to die during the 2-day observation period. However, the subjective nature of identifying a recent molt precluded its use in our analyses. If a recent molt condition can be accurately determined, it should be included in future research and may account for significant variability in estimating discard mortality. It should be noted that this light weight was observed almost entirely in crabs sampled during the month of February in the Tampa estuary and may have contributed to the higher discard mortality percentage during these cooler months.

The environmental conditions onboard a vessel during the culling of catch can add additional stress to the discarded catch. The physiological effects of air exposure (emersion) also contribute to mortality (Agodi et al., 2015). During onboard handling and sorting, crabs undergo a series of metabolic, circulatory, and respiratory impairments. In the present study, we found that the air emersion time minimally affected the likelihood of mortality. Similarly, Kronstadt et al. (2018) found that emersion did contribute to reflex impairment in *M. mercenaria* but did not predict mortality. Impairments from emersion may be exacerbated by high air temperatures as seen in portunid crabs *Liocarcinus depurator* (Giomi et al., 2008). These impairments may have the same impact on discard mortality that exposure to decreased air temperatures has on crabs fished in northern latitudes (Stoner, 2009). Crustaceans exposed to emersion often suffer collapsed gill tissue that terminates important physiological processes such as excretion and gas exchange (DeFur, 1988, Taylor and Whiteley, 1989). In the current study, the assistance of a deckhand to immediately cull and discard crabs reduced emersion time and negative physiological impacts. Conversely, the absence of a deckhand may force commercial captains to cull crabs after several traps have been hauled on board, extending emersion. While this method may be more efficient for the solo commercial operation, it can increase mortality following discard. Additionally, we have observed some onboard culling procedures that involve submerging boxed crabs in a slurry of fresh water and ice prior to culling discards. This procedure is thought to enhance the longevity of catch to market. However, it may add further cumulative stress to crabs by an added temperature and salinity shock prior to discard. The various onboard culling strategies deserve further study to determine how common they are and their influence on discard mortality. Their study was beyond the scope of this work.

The majority (94%) of discard mortality was incurred within the first 24 h of the 48-hour holding period. Therefore, we conclude that holding period did not impact the discard mortality found in this study. Our finding concurs with several similar studies that determined the holding period (observation time) needs to be sufficiently long to characterize most of the mortality (Stoner et al., 2008, Stoner, 2012, Wassenberg and Hill, 1993). However, the holding period should be limited. Research on Tanner and Dungeness crabs suggests that cumulative mortality stabilizes on day 2 and gradually increases after day 5; limiting the holding time avoids overlap of the prolonged captivity effect on mortality (Barry, 1983, Tegelberg, 1970). Our holding methods are therefore assumed to be adequate for capturing most of the mortality while excluding the effect of captivity. This finding suggests that most discard mortality occurs in the first 24 h for blue crabs in the commercial fishery in Florida.

The assessment of reflex actions to predict unobserved mortality in crustaceans is a valuable and accurate tool to understand the cryptic nature of discard mortality in the commercial blue crab fishery. Research has sought to understand the effects of capture, handling, and discard and their additive effects imposed on commercially fished species (Davis, 2002, Stoner et al., 2008, Stoner, 2012), however, the euryhaline nature and life history traits of blue crabs add additional factors that influence survival following discard. The present study is the first crustacean RAMP research that includes in situ salinity and water temperature to assess the relationship between RIS and crab discard mortality. Our results provide further evidence that the inclusion of water quality into RAMP analyses better predicts blue crab survival. Observations aboard commercial blue crab vessels highlighted the unique fishing practices and myriad of habitat types in which the Florida blue crab fishery operates. These dynamics were essential in generating a RAMP index that captures the complexity and cumulative effects of the blue crab fishing process. The RAMP index generated in the present study will be a useful tool to estimate discard mortality of discarded blue crabs aboard commercial vessels throughout Florida. These data will advance our understanding of discard mortality and aid in future stock assessments and management actions in the Florida blue crab fishery.

CRediT authorship contribution statement

Erin A. Walters: Conceptualizing, Methodology, Software, Validations, Formal analysis, Investigation, Resources, Data curation, Writing, Visualization, Project administration. **Claire E. Crowley:** Conceptualizing, Methodology, Software, Formal analysis, Writing – review & editing, Supervision. **Ryan L. Gandy:** Conceptualizing, Methodology, Software, Writing – review & editing, Supervision. **Donald C. Behringer:** Conceptualizing, Methodology, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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