THE EFFECT OF CLAW REMOVAL METHODS AND TEMPERATURE ON THE POST-RELEASE SURVIVAL AND CRITICAL THERMAL MAXIMUM OF STONE CRAB (MENIPPE MERCENARIA)

By

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ABSTRACT

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Florida stone crab (*Menippe mercenaria*) is an emerging commercial fishery in the Bahamas with its main export to the United States of America. This fishery capitalizes on the oversized claws of the crab, which are harvested, before the crab is returned to the sea where it can potentially regrow its claws. While it is often assumed that the crab will regrow its claws and re-enter the fishery, only 13% of harvested crabs in the fishery have regrown claws, and an estimated 2-81% of crabs survive post-claw removal and release. In addition, the Caribbean region is considered one of the most vulnerable areas with respect to climate change. Therefore, because most aquatic organisms cannot regulate their body temperature, they are directly influenced by environmental temperature stress, and when combined with the stress of claw removal may further decrease the capacity of the crab to survive warming temperatures.

The purpose of my thesis was to: 1) determine a method of claw removal that maximizes survival for stone crab, 2) determine the effect of rapidly warming water temperatures on the reflex behavior of crabs post-release, and 3) determine the effect of claw removal on the critical thermal maximum (CTMax) of stone crab.

Overall, the tool required to conduct the induced-autotomy method of claw removal is simple and easily purchased or constructed and can easily be taught to recreational and commercial harvesters as a way to improve survival and thus sustainability of this important fishery.

This thesis is dedicated to my friends and family, from Michigan to Eleuthera.

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## THESIS INTRODUCTION

In every significant body of water around the world, there inevitably lies a fishery. Fisheries are the livelihoods of millions of people worldwide. It is estimated that 4.5 billion people benefit from global fisheries, whether it is for food, cosmetics, livelihoods, and so much more. (FAO, 1983). Stone crab is an emerging commercial fishery in the Bahamas and estimated to be worth 2.8 million dollars in landings annually (Moultrie et al., 2016). This fishery provides income to many harvesters and their families, especially on the Bahamian islands where little tourism exists such as Eleuthera.

Stone crab (genus *Menippe*) inhabit the waters of the southeastern seaboard of the United States, including North Carolina, Florida and the Caribbean (Florida stone crab, *Menippe mercenaria*, Say 1818), along with the Gulf of Mexico (Gulf stone crab, *Menippe adina*, Williams and Felder, 1986). There are two species of stone crab, *Menippe mercenaria* and *Menippe adina*. *M. mercenaria* is light tan or grey, with uniform spots on the carapace and dorsal surface of the claws, and distinct brown and white banding on the legs, whereas *M. adina* is dark brown, with no markings on the dorsal surface and no banding on the legs (Bert, 1986). Additionally, there is a zone of "intermediates" between the two that are phenotypically similar. As a result, both species are managed as a single species in the fishery .

Stone crab inhabit nearshore and offshore habitats consisting of rock, sea grass flats and oyster reefs (Krimsky and Epifanio, 2008). Stone crabs are predators consuming mainly mollusks, making them essential to the tropical coastal ecosystem's nutrient cycling.

Stone crabs begin their life cycle as zoea, where they remain in the planktonic larval stage for 2-3 weeks. After 2-3 weeks the zoea metamorphosize to the megalopae stage, and settle to the benthos where they become juvenile stone crab (< 30mm CW) where it takes approximately 2 additional years to reach maturity. The average life span of the stone crab is 7-9 years.

Male stone crabs become reproductively mature at 63.1mm, whereas females become reproductively mature at 66.3mm. Copulation occurs only when the female is newly molted (Cheung, 1968) and as size increases, molt frequency decreases (Gerhart and Bert, 2008). Spawning occurs from June through October and is correlated with temperature (Gerhart and Bert, 2008).

The commercial stone crab fishery is one of the largest fisheries in the Bahamas and Florida. Landings are worth an estimated 30.4 million USD annually in Florida and 2.8 million USD in the Bahamas (Moultrie et al., 2016). In 2015, 54 million tons were harvested and harvest rates have steadily increased since 1990 (Moultrie & Deleveaux, 2016). Researchers suggest that stone crab populations in the Bahamas are less than 1% of Florida's population (FAO, 1986), although no formal population assessment has been completed. Stone crabs are caught in rectangular polypropylene traps with slotted panels and a funnel-shaped entrance on the top of the trap for crabs to enter. Traps are often baited with pigs feet and has a biodegradable wood panel to ensure animals can escape should the trap be lost. The traps a set to soak for several days to weeks across several kilometers. The stone crab fishery is unique because only the chelae (claws) are removed from the crab, and the crab is released alive. Studies have shown that claw removal resulting in large wounds can increase post-release mortality (Duermit et al., 2015). Crustaceans can regrow limbs, and this capability is the basis for the fishery. Male stone crab are more susceptible to the fishery due to their larger size (Bert et al. 1986) but females are often caught. Claw regeneration is dependent on many factors, including wound size, temperature, and food availability (Gandy et al., 2016). Under favorable conditions, it takes 1-2 molt cycles (2-3 years) for a crab to regrow a claw back to the size at which it was removed. However, this is unlikely given the lifespan of the crab and the time it takes for the crab to reach a harvestable size. Best practice suggests that only a single claw be removed so the crab can feed adequately and defend itself (Bert et al. 2016) once released, however this varies by region. For example it is illegal to harvest both claws in the Bahamas, but not in Florida. Post claw removal and release, it is suggested that crabs become scavengers as opposed to predators and spend less time feeding after claw removal (Duermit et al. 2015; Orrell et al., 2019). The legal size in Florida is 2 7/8 inches and 4 inches in the Bahamas. Bahamian stone crab are much larger because the population is largely unexploited, while stock sizes are much lower in Florida (Muller et al., 2011). In Florida, is has been suggested that stone crab stocks are largely over-exploited, with many stocks in decline (Florida Fish and Wildlife Conservation Commission, 2016).

There are some other important factors that impact management of stone crab fisheries. First, researchers can tell if a stone crab has previously had a claw removed based on stridulation marks on the regrown claw (Simonson 1985). Scientists and fishers found that less than 13% of crabs that have had a claw removed regrow the claw and are re-entering the fishery (Duermit et al., 2015). Second, large males have the highest mortality rate when claws are removed because such a high proportion of their body weight has been lost (Florida Fish and Wildlife Conservation Commission, 2016). Finally, managers can impact harvest by opening and closing seasons. Currently, the closed season for stone crab in the Bahamas is June 1st to October $15^{th}$.

Stone crabs inhabit the waters along the coast of Florida, the Carolinas, the gulf coast, and the Bahamas which are vulnerable areas to climate change. The general water temperature for this area ranges between 22 and 32 degrees Celsius. Between the years 2030 and 2052, global temperatures are expected to rise by 1.5 degrees Celsius (IPCC, 2018). The ocean absorbs heat from greenhouse gas emissions, which leads to increasing water temperatures. By 2100, the mean global ocean temperature is expected to rise 1.5 degrees Celsius (IPCC, 2013). Rising ocean temperatures can lead to a decrease in oxygen in the ocean, which can significantly impact marine life. Additionally, ocean acidification, which is the decrease in pH of the ocean due to increased uptake of carbon dioxide, can be highly detrimental to marine species and ecosystems. The potential impacts of climate change on stone crabs may be that their species distribution changes as they move to deeper, cooler water, and that shell and larval growth may decrease as ocean pH rises.

The purpose of my thesis was to: 1) determine a method of claw removal that minimizes mortality in stone crabs, 2) determine the effect of rapidly warming water temperatures on the reflex behavior of crabs post-release, and 3) determine the effect of claw removal on the critical thermal maximum (CTMax) of stone crab.

# CHAPTER 1: A COMPARISON OF CLAW REMOVAL METHODS ON THE POST-RELEASE SURVIVAL AND CLAW REGENERATION OF THE STONE CRAB (*MENIPPE MERCENARIA*)

## INTRODUCTION

Commercial and recreational stone crab (Menippe mercenaria) fisheries primarily occur along the Gulf of Mexico and Atlantic coasts of the southeastern United States, but smaller commercial fisheries, are also gaining popularity in Belize (Bert & Hochberg 1992) and The Bahamas (Moultrie et al. 2016). In The Bahamas, the stone crab fishery is currently the third largest export valued at approximately 2.4 million US dollars. These emerging fisheries primarily supplement the demand for stone crab in the United States of America (USA). Typically, crabs are captured in baited traps with fisheries regulations mandating a minimum size for claw harvest and prohibiting the claw removal from egg-bearing females. Legal claw-size, whether one claw or both claws can be removed, and seasonality of fisheries can vary by region and country, but best practice recommends that only a single (larger) claw be removed (Bert et al. 2016); nevertheless, both claws are harvested in many areas (Gandy et al. 2016, Sullivan 1979).

While the stone crab fishery is probably the most recognizable of the claw-only fisheries, others do exist. In the United Kingdom, the edible or brown crab (Cancer pagurus) fishery removes both claws before returning the crab to the sea (Patterson et al. 2009). In southern Portugal, the major claw is removed from the fiddler crab (Uca tangeri) and is considered a local delicacy (Patterson et al. 2009). In the Northwest Atlantic an emerging claw-only fishery for Jonah crab (Cancer borealis) is being studied (Goldstein & Carloni, 2021). Claw-only fisheries are often regarded as sustainable because they are assumed to exploit the natural ability of crabs to voluntarily drop (autotomize) and regenerate their claws when damaged or threatened. Because the animal is returned alive, it is assumed to re-enter the fishery once the claws regrow and in the meantime the ability to reproduce and provide a legacy of recruits to the fishery. However, post-release survival of de-clawed stone crabs is variable ranging between 2% and 81% in controlled laboratory conditions (Table 1; Davis et al. 1978, Duermit et al. 2015, Simonson & Hochberg, 1986) and between 37% and 59% in the wild (Table 1; Gandy et al. 2016). The large variation in survival is due to a myriad of factors that influence survival after claw removal and release. These include, but are not limited to, water temperature (Gandy et al. 2016), break location (Gandy et al. 2016), wound size (Duermit et al. 2015, 2017), and whether both claws are removed (Davis et al. 1978, Duermit et al. 2015, Gandy et al. 2016; Orrell et al. 2019). Previous studies also suggest that re-entry of de-clawed stone crabs into the fishery is relatively uncommon given the number of required molts for claws to reach harvestable size (~3 to 4 years; Savage & Sullivan 1978) and the life span of the stone crab (~8 years; Cheung 1973). Analysis of long-term trends in stridulation ridges (used to assess whether a claw has been regenerated or not; Simonson 1985) in commercial fisheries of Florida and South Carolina, USA, suggests only 3% to 13% of total landings consist of regenerated claws (Duermit et al. 2017, Gandy et al. 2016, Muller et al. 2011, Simonson & Hochberg 1986, Wilber 1995).

Despite a number of factors contributing to post-release survival of stone crab following claw removal, the role of harvester experience should not be underestimated (Duermit et al. 2017). This is because the typical claw removal procedure involves the harvester being able to induce the crab to voluntarily drop (autotomize) the claw, rather than to break it off with force. Typically this is done by the harvester exerting enough downward force to the fully extended cheliped to cause the crab to release the claw cleanly along the autotomy plane at the basi-ischium, located between the coxa at the base of the leg and merus. This is not without risk to the crab and harvester. Too much force by the harvester will cause the claw to incorrectly break and often result in a fatal wound for the crab. However, if done correctly, the crab will voluntary drop the claw which allows for the formation of a hypodermal diaphragm (Savage & Sullivan, 1978) which seals off hemolymph loss at the release site (Savage & Sullivan, 1978) and results in high survival of the crab following release (Duermit et al. 2015, 2017). Therefore, modifications to the typical procedure or alternative procedures, to limit the role of harvester experience level and minimize the number of incorrect breaks and wounds should increase post-release survival following claw removal and would be beneficial in the sustainability of claw only fisheries for stone crab and potentially other claw removal fisheries.

In this study, a controlled laboratory experiment was used to compare a new autotomy-inducing technique to the typical method of claw removal. For the two different claw removal methods, I compared the survival and start time for claw regeneration as a function of harvester experience and whether one claw or both claws were removed. Finally the claws that were removed were inspected by independent observers to see if any differences using the two methods could be identified.

## METHODS

One hundred and eighteen stone crabs (mean mass ± SD = 276.2 ± 65.6 g; mean carapace width ± SD = 95.1 ± 9.3 mm; right propodus length mean ± SD = 74 ± 9.5 mm; left propodus length mean ± SD = 69.57 ± 8.7 mm) with both claws intact were purchased from a stone crab harvester in Hatchet Bay, Eleuthera, The Bahamas. The crabs were transported (covered in seawater-soaked towels in large coolers) to the wet lab facility at the Cape Eleuthera Institute, Eleuthera, The Bahamas. The crabs were acclimated for 21 days prior to experimentation in two large circular aerated tanks (3.7m diameter, 1m water height) supplied with flow through seawater at a temperature of 26.3 (± 1.2 SD) °C and a salinity of 36 (± 1 SD) psu. The crabs were fed pieces of conch and fish offal *ad libitum* every other day. Pieces of PVC pipe were added to the tanks to provide enrichment and shelter and to help minimize agonistic behaviors among crabs. Crabs were checked daily and tanks were cleaned bi-weekly.

Five days prior to claw removal trials, all crabs (female = 58, male = 60) were tagged using a small piece of numbered waterproof paper (Rite-In-The-Rain; JL Darling LLC, Washington, USA) that was glued (Krazy Glue; Ohio, USA) to the carapace, allowing identification of individual crabs throughout the experiment. To determine the effect of claw removal method, harvester experience, and whether one claw or both claws were removed on the post-release survival and start of claw regeneration time, crabs were randomly assigned to one of nine treatment groups (Table 2). Briefly, these included two people, a novice and an experienced commercial fisher that removed one or two claws using either the typical method (herein referred to as forced break) or our new proposed method (herein referred to as induced-autotomy). The experienced harvester was a commercial stone crab fisher on the island of Eleuthera with > 10 years' experience, whereas the novice harvester was a researcher with limited stone crab fishing experience but was familiar with handling and claw removal procedures for stone crab. Forced break was conducted using the typical method of applying a downward force applied to the crabs fully extended cheliped until the claw was released or broken along the autotomy plane at the basi-ischium, located between the coxa at the base of the leg and merus (Figure 1). Our new induced-autotomy method was conducted by puncturing the arthrodial membrane (the soft joint, or unsclerotized cuticle) between the carpus and the merus with a marlin spike (5 cm long X 2mm diameter metal spike often used to splice rope and repair sails) and slowly rotating the spike. This resulted in the induced release of the claw (autotomy) distal to the coxa (Figure 2). Following claw removal all crabs were haphazardly released into one of three flow-through circular tanks (3.7m diameter, 1m water height) and held under the same conditions and feeding regime described above for acclimation. Data recorded included: left and right claw length, left and right claw weight (for the claw removal group and depending on which claw was removed), carapace width, incorrect break (claw removals that resulted in visible signs of damage to the coxa), sex, and the total amount of time it took harvesters to remove claws per claw-removal treatments.

Following claw removal, crabs were checked daily for 35 days for the survival analysis portion of the experiment and every 7 days for a total time period of 60 days thereafter until all crabs showed signs of claw regeneration. If a mortality occurred, the individual tag identification number and the number of days to the mortality event from the time of claw removal was recorded and the individual was removed from the experiment. The start of claw re-growth was assessed as the presence or absence of a small appendage forming at the removal site of the coxa where the claw (cheliped) was removed (Figure 3). If a crab was observed to have started claw regeneration, the individual tag identification number, and the number of days to the start of claw regeneration from the time of claw removal was recorded.

To determine whether a claw removed using the typical forced break or our new induced-autotomy methods could be identified (perhaps for enforcement purposes if our induced-autotomy method were ever mandated), a survey involving five independent observers with no prior claw removal experience was conducted. All observers were given a short 5-10 minute lesson on identifying features of the two methods (Figure 4) and then given a bag of 50 stone crab claws that were removed using the two methods (25 forced break and 25 induced autotomy) during the experiment and frozen. Observers were then asked to identify which claws were removed using forced break or induced-autotomy. Claws were numbered using a random-number generator so that observers and administrators of the survey were blind to the removal method. Participants were not told how many claws were in each removal category.

Following experiments all remaining crabs were released back into the wild. All work was carried out under The Bahamas Department of Marine Resources permit number MAMR/FIS/2/12A/17/17B.

A Cox proportional hazards (CPH) analysis using the R packages “survival” (Therneau 2015) and “survminer” (Kassambara 2021) was used to examine potential effects of removal method (forced break or induced-autotomy), harvester experience level (novice or experienced), whether one claw or both claws were removed, sex and carapace width on the post-release survival of stone crabs. Additionally, a Kaplan-Meier (KM) analysis using the same R packages as above, and days to the event (dead or alive) was used to determine survival rate estimates over the course of the study (1 to 35 days) across removal methods, harvester experience level, and claw removal treatments. To compare the cumulative effects of removal methods (i.e. differences among individual treatments; Table 2), harvester experience and claw removal treatments on survival (i.e. 35 days following claw removal), I used a Kruskal Wallis test to compare survival among individual treatments. To determine the effect of incorrect claw breaks on survival and to compare the number of incorrect breaks that occurred among treatments, I used a Spearman’s-Rank correlation test and a Kruskal Wallis test, respectively. Procedure times of the various treatments was calculated by dividing the number of crabs that were de-clawed in a treatment by the total claw removal time for that treatment. While I fully recognize that procedure times for individual crabs per treatment would have been ideal, the addition of this data was opportunistic but nevertheless important enough to be presented as a summary statistic in Figure 6A for context in highlighting potential differences in procedure times between the typical removal method of forced break and our new induced-autotomy method. This is an important consideration in the feasibility and adoption with any proposed modification to a commercial fishery procedure, where excess time often results in greater costs and thus low support for the adoption of the proposed change. Lastly, to examine the effect of claw removal method, harvester experience and whether one claw or both claws were removed on the time to the start of claw regeneration we again used a Kruskal Wallis test. Finally, the number of correct and incorrect claw removal method identifications by observers were tallied and the results presented in Figure 6B. All statistical significance was assessed at $p < 0.05$.

## RESULTS

There was a significant effect of claw removal method (CPH = -1.90, $z = -3.40$, $p < 0.001$; Table 3; Figure 5C), whether one claw or both claws were removed (CPH = 0.96, $z = 2.14$, $p = 0.03$; Table 3; Figure 5A), and harvester experience level (CPH = 0.90, $z = 2.16$ , $p = 0.03$; Table 3; Figure 5B), on the post-release survival of crabs. However, there was no significant effect of sex (CPH = 0.31, $z = 0.63$, $p = 0.53$) or carapace width (CPH = 0.003, $z = 0.14$, $p = 0.89$). Overall, survival for the control treatment (handled but no claw removal) was 93% and crabs from the one claw removal treatments had higher survival (84%) than crabs from the two claw removal treatments (70%) (Table 3; Figure 5A). Survival of all crabs with claws removed via both methods by an experienced harvester was 84% whereas survival of all crabs with claws removed via both methods by a novice harvester was 67% (Table 3; Figure 5B). Survival across our new induced-autotomy method treatments was 92% whereas those removed across the typical forced break method treatments was 63% (Table 3; Figure 5C). Therefore, our new induced-autotomy method resulted in a 29% increase in survival relative to the typical forced break method and only a 1% decrease in survival relative to the control. Finally, there was a significant cumulative effect of removal method (i.e. treatment effect), harvester experience level and whether one claw or both claws were removed on the survival of crabs (Kruskal Wallis = 31.45, df = 8, $p < 0.001$; Table 3; Figure 5D). Crabs with both claws removed by a novice harvester using a forced break (N-2-F) had the lowest survival at 43% (6 / 14), while crabs with one claw removed by an experienced harvester using induced-autotomy (E-1-A) had the highest survival at 100% (14 / 14; Table 3; Figure 5D).

The number of incorrect claw breaks that occurred among treatments was negatively correlated with post-release survival ($\rho_7 = -0.83$, $p = 0.005$; Table 4; Figure 6). Crabs with both claws removed by the novice harvester via the typical forced break method (N-2-F) resulted in the greatest number of incorrect claw breaks with 64% (9 / 14; Kruskal Wallis = 32.42, df = 7, $p < 0.001$; Table 4; Figure 6) of claw removals resulting in incorrect breaks and ultimately the lowest survival of all treatments at 43% (6 / 14). However, the E-1-F, E-1-A and N-1-A treatments all resulted in clean claw breaks during all removals.

As expected, the removal time for the one claw removal treatments and the experienced harvester treatments were the fastest and took an average of 4.6 and 8.4 seconds per crab, respectively. The two claw removal treatments and the novice harvester treatments were the slowest and on average took 10.7 and 8.4 seconds per crab, respectively. The new induced-autotomy treatments on an average were slower and took 11.4 seconds per crab to complete, whereas the typical forced break treatments took an average of 4.7 seconds to complete. Overall, the experienced harvester removing one claw using the typical forced break treatment (E-1-F) was the fastest (Figure 7A), with an average of 1.1 seconds per crab while the experienced harvester removing two claws using our new induced-autotomy (E-2-A) method was the slowest, with an average of 16.4 seconds per crab.

There was some variation in the number of correct identifications of removal types among observers (range = 68% to 92%; Figure 7B). However, on average observers correctly identified 88% of claws removed using the typical method of forced break (Figure 7B) and 79% of claws removed using the new induced-autotomy method (Figure 7B).

There was no significant effect of removal method (Kruskal-Wallis = 0.92, p = 0.33; Figure 8C), harvester experience level (Kruskal-Wallis = 0.0005, p = 0.98; Figure 8B), and whether one claw or both claws were removed (Kruskal-Wallis = 0.12, p = 0.73; Figure 8A), on the start of claw regeneration time. Among treatments, crabs took approximately 40 days for the start of claw regrowth to be visually observed.

## DISCUSSION

The results of my study showed that not only does the induced-autotomy method increase survival, but it does so irrespective of both harvester experience level and whether one or both claws are removed. Survival among our induced-autotomy treatments was 92% (99% relative to the control) and is among the highest reported in the literature (Table 1). For example, Gandy et al. (2016), in a field-based experiment, found that overall survival of crabs following claw removal was 48% (59% for one claw removals and 37% for two claw removals). In the laboratory, Davis et al. (1978), found an overall survival of 63% (72% survival for one claw removals and 53% survival for two claw removals), whereas Simonson & Hochberg (1986), found an overall survival of 80% for a single claw removal. While some differences exist in environmental and experimental conditions among studies (Table 1), the key difference between survival reported in the literature and our study is the method used to remove the claw. Our new induced-autotomy method produced a drop of the claw or clean break across the autotomy plane 96% of the time. In contrast, the typical forced break method only produced a drop of the claw or clean break 67% of the time with 33% of cases resulting in damage or hemolymph loss at the release site (Savage & Sullivan, 1978) and subsequent death of the crab one to five days post-release.

While the typical forced break method of claw-removal in the stone crab fishery is believed to exploit the natural ability of crabs to voluntarily drop (autotomy) their claw, it is clear that this is not always the case, and some damage can occur across the autotomy plane. This damage may occur even after accounting for harvester experience and whether both claws are removed. Common injuries include breaking the claw into the merus side of the basi-ischium segment, or breaking the claw and exposing the body cavity (Simonson & Hochberg 1986, Gandy et al. 2016). Both of these injuries have the potential for loss of hemolymph and also leave an open surface for bacterial infection to occur many days post-release (Simonson & Hochberg 1986, Gandy et al. 2016). The higher incidence of damage that occurred during removal of the second claw likely occurs because stone crab are reliant on their claws for protection and feeding (Gunter 1955, Menzel & Nichy 1958). Therefore, once a claw has already been removed the resulting level of perceived impairment by the crab increases and it requires more force to remove subsequent limbs (Robinson et al. 1970), which further increases likelihood of an incorrect break.

Interestingly, the effect of claw removals across treatments was for the most part only observed when comparing among the typical forced break method treatments (i.e. experienced harvester-1 claw removal-forced break (E-1-F) and experienced harvester-2 claw removal-forced break treatments (E-2-F) to the novice harvester-1 claw removal-forced break (N-1-F) and novice harvester-2 claw removal-forced break treatments (N-2-F)) and not among the new induced-autotomy treatments (i.e. experienced harvester-1 claw removal-induced autotomy (E-1-A) and experienced harvester-2 claw removal-induced autotomy treatments (E-2-A) to the novice harvester-1 claw removal-induced autotomy (E-1-A) and novice harvester-2 claw removal-induced autotomy treatments (N-2-A)). This result further suggests that the induced-autotomy method is likely what resulted in the higher survival as result of the lower incidence of damage across the break plane that occurred among these treatments. In fact, the novice harvester-2 claw removal-induced autotomy treatment (N-2-A) had a 7% higher mean survival than the experienced harvester-2 claw removal-induced autotomy treatment (E-2-A) and although the experienced harvester-1 claw removal-induced autotomy treatment (E-1-A) had a slightly higher mean survival than the experienced harvester-2 claw removal-induced autotomy treatment (E-2-A), the novice harvester-2 claw removal-induced autotomy treatment (N-2-A) had a slightly higher survival than the novice harvester-1 claw removal-induced autotomy treatment (N-1-A). While it's difficult to determine the number of claw removals required before maximum survival of the forced break method is achieved by a harvester, it is clear that there is a learning curve to conducting the typical forced break method. For example, survival following a single claw removal by an experienced harvester using the typical forced break method was 36 % higher (93 % over the course of the study) than the same treatment conducted by a novice harvester (57 % over the course of the study). However, both the experienced harvester and novice harvester took similar times to remove claws using the induced-autotomy method. This is not unexpected, as both harvesters were new to this procedure. While the time to perform the new induced-autotomy method appears longer than the typical method, we expect with practice, that the time would be reduced and fishing efficiency improved. Even if the new method is not as rapid, crab survival rates would increase and likely improve the future catch rates and sustainability of the fishery. However this experiment was carried out under controlled laboratory conditions and further studies employing this method in the field in combination with perhaps mark and recapture should be carried out to quantify the accuracy of the laboratory study presented here.

There was no difference in the start time for claw regeneration when comparing the new induced-autotomy method to the typical forced break method. This is not unexpected because the crabs with damaged breaks subsequently died and thus the crabs that regenerated claws were predominately those with clean breaks or dropped their claws, irrespective of the method used to remove the claws. In the present study, crabs took approximately 40 days to show the first signs of claw regrowth, but it usually takes an additional 3 to 4 years for these claws to reach harvestable size (Savage & Sullivan 1978). Therefore animals that can be legally harvested are estimated to be between 3 to 4 years old (Savage & Sullivan 1978), so for an animal that has an average lifespan of approximately 8 years, they may not be able to regenerate harvestable claws again to re-enter the fishery (Savage & Sullivan 1978). However, one could argue that because the crab is still alive and returned to the water they could still contribute to the breeding pool. However, claw loss and regeneration not only significantly slows growth, it also impedes spawning fitness including fecundity, and mating success (Bender 1971, Davis et al. 1978, Savage & Sullivan 1978, Juanes & Smith 1995, Wilber, 1995, Hogan & Griffen 2014, Duermit et al. 2015), so this is an additional factor that must be considered for crabs that do survive the removal of the claws.

During a “correct” drop (autotomy) or clean break of the claw, the resulting end of the claw was observed to have smooth edges and a distinct triangle remaining on the proximal end of the claw (Figure 4). This observation led to claws removed using the induced-autotomy method correctly identified 79% of the time by independent observers versus 88% of claws removed using the typical method of forced break. While this may appear counter-intuitive the discrepancy in the identification type is a result of incorrectly identified claws removed using the typical forced break method. This occurred because not all claws removed using the typical forced break method resulted in incorrect breaks that could be distinguished from those resulting from the induced-autotomy method (i.e. the forced break had it's intended effect of eliciting a autotomy or clean break response). Therefore, despite the discrepency between identifying claws removed using the two methods, this result is promissing and suggest that compliance in the fishery to the induced-autotomy methodology may have the potential to be assesed dockside during off-loads and likely enforceable, especially with some additional added experience provided to the observer.

The tool required to conduct the induced-autotomy method of claw removal is simple and easily purchased or constructed, thus it easily can be taught to recreational and commercial harvesters as a way to improve survival and thus sustainability of this important fishery.

# APPENDIX

**Table 1. Published data from studies investigating the post-release survival of crabs following claw removal.** Forced break is the typical method of claw removal used in the fishery, whereby downward force is applied to the crabs fully extended cheliped until the claw is released or broken along the autotomy plane at the basi-ischium. Autotomy is the proposed method of claw removal from this study and was conducted by puncturing the articular membrane (the soft joint, or unsclerotized cuticle between the hard sclerotized parts of the exoskeleton) between the carpus and the merus with a sharp pointed implement and rotating until the claw is released along the autotomy plane at the basi-ischium. Mechanical refers to removal using tin straight pattern snips to severe the claw between the merus and coxa. *Asterisks denotes data from this study for comparison.

| Species | Common name | Location | Removal Method | Mean Water Temperature (°C) | Mean Salinity (psu) | Duration | Sample Size / Treatment | Survival (Control) | Survival (1 claw removal) | Survival (2 claw removal) | Purpose | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Menippe mercenaria* | Stone Crab | Lab | Autotomy | 26.3 | 35 | 35 days | 14-28 | 93% | 93% | 93% | Assess mortality based on claw removal techniques | Walus et al. (current study) |
| **Menippe mercenaria* | Stone Crab | Lab | Forced Break | 26.3 | 35 | 35 days | 14-28 | 93% | 75% | 50% | Assess mortality based on claw removal techniques | Walus et al. (current study) |
| *Menippe mercenaria* | Stone Crab | Field | Forced Break | 20.9 | 34.5 | Not reported | 384-927 | 87% | 59% | 37% | Effect of temperature on mortality | Gandy et al. 2016 |
| *Menippe mercenaria* | Stone Crab | Lab | Forced Break | 23.3 | 33.5 | 10 days | 100 | N/A | 72% | 53% | Assess mortality from claw removal | Davis et al. 1978 |
| *Menippe mercenaria* | Stone Crab | Lab | Forced Break | 27 | Not reported | 14 days | 28-41 | 81% | 47% | 58% | Evaluating mortality and feeding post claw removal | Duermit 2015 |
| *Menippe mercenaria* | Stone Crab | Lab | Forced Break | Not reported | Not reported | 13 days | 58-639 | N/A | 2-81% | N/A | Effects of claw breaks and air exposure on mortality | Simonson & Hochberg 1986 |
| *Menippe mercenaria* | Stone Crab | Lab | Forced Break | 27.4 | 37 | 48h | 7-13 | 75% | 75% | 69% | Examine physiological and behavorial effects post claw removal | Orrell et al. 2019 |
| *Cancer borealis* | Jonah Crab | Lab | Forced Break | 3-12 | Not reported | 27 days | 40-100 | 84% | 49% | 30% | Assess mortality post manual claw removal | Goldstein & Carloni 2021 |
| *Cancer borealis* | Jonah Crab | Lab | Forced Break | 3-12 | Not reported | 27 days | 10-15 | 90% | 13% | N/A | Assess mortality based on manual and mechanical claw removal | Goldstein & Carloni 2021 |
| *Cancer borealis* | Jonah Crab | Lab | Mechanical | 3-12 | Not reported | 27 days | 10-15 | 90% | 60% | N/A | Assess mortality based on manual and mechanical claw removal | Goldstein & Carloni 2021 |
| *Cancer pagurus* | Edible crab | Lab | Forced Break | 8-13 | Not reported | 24h | 14 | N/A | 82% | N/A | Physiological stress responses of claw removal | Patterson et al. 2007 |
| *Cancer pagurus* | Edible crab | Lab | Autotomy | 8-13 | Not reported | 24h | 14 | N/A | 100% | N/A | Physiological stress responses of claw removal | Patterson et al. 2007 |

**Table 2. Breakdown of treatments, treatment abbreviations and sample size of crabs used in the experiment to investigate the effect of harvester experience, number of claws removed and claw removal method on the survival and start of claw regeneration time.**

| Treatment | Abbreviation | Sample size (n) |
|---|---|---|
| Experienced harvester-1 claw removal-forced break | E-1-F | 14 |
| Experienced harvester-2 claw removal-forced break | E-2-F | 13 |
| Experienced harvester-1 claw removal-induced autotomy | E-1-A | 14 |
| Experienced harvester-2 claw removal-induced autotomy | E-2-A | 14 |
| Novice harvester-1 claw removal-forced break | N-1-F | 14 |
| Novice harvester-2 claw removal-forced break | N-2-F | 14 |
| Novice harvester-1 claw removal-induced autotomy | N-1-A | 7 |
| Novice harvester-2 claw removal-induced autotomy | N-2-A | 14 |
| Handled but no claw removal | Control | 14 |

**Table 3. Kaplan-Meier survival estimates (mean ± 95% CI) for stone crab following claw removal.** Tables are presented to illustrate the effect across (A) claw removal treatments, (B) experience level treatments, (C) removal method treatments and (D) individual treatments [(Expert (E) or Novice (N) - 1 claw removed (1) or 2 claws removed (2) – forced break (F) or autotomy (A)] and correspond to figure 1A - D. Day refers to the duration individuals were held in tanks post-release, whereas N.risk and N.event refer to the number of individuals alive at a given duration of the study and the number of mortalities that occurred between the day duration of the study. Mean water temperature and salinity for the study ± SD was 26.3 ± 1.2°C and 36 ± 1 psu.

| | | | | Survival probability | | |
|---|---|---|---|---|---|---|
| **Figure 2A** | Day | N.risk | N.event | Lower 95% CI | Mean | Upper 95% CI |
| **Claw removal** | | | | | | |
| 0 Claws removed | 5 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| 1 Claw removed | 5 | 42 | 7 | 0.77 | 0.86 | 0.96 |
| 2 Claws removed | 5 | 41 | 15 | 0.63 | 0.73 | 0.86 |
| 0 Claws removed | 15 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| 1 Claw removed | 15 | 42 | 0 | 0.77 | 0.86 | 0.96 |
| 2 Claws removed | 15 | 41 | 0 | 0.63 | 0.73 | 0.86 |
| 0 Claws removed | 35 | 13 | 1 | 0.80 | 0.93 | 1.00 |
| 1 Claw removed | 35 | 41 | 1 | 0.74 | 0.84 | 0.95 |
| 2 Claws removed | 35 | 39 | 2 | 0.59 | 0.70 | 0.83 |

| | | | | Survival probability | | |
|---|---|---|---|---|---|---|
| **Figure 2B** | Day | N.risk | N.event | Lower 95% CI | Mean | Upper 95% CI |
| **Experience level** | | | | | | |
| Control | 5 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| Expert | 5 | 56 | 8 | 0.77 | 0.86 | 0.95 |
| Novice | 5 | 49 | 14 | 1.00 | 0.71 | 1.00 |
| Control | 15 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| Expert | 15 | 48 | 0 | 0.77 | 0.86 | 0.95 |
| Novice | 15 | 35 | 0 | 0.60 | 0.71 | 0.85 |
| Control | 35 | 13 | 1 | 0.80 | 0.93 | 1.00 |
| Expert | 35 | 47 | 1 | 0.75 | 0.84 | 0.94 |
| Novice | 35 | 33 | 2 | 0.55 | 0.67 | 0.82 |

| | | | | Survival probability | | |
|---|---|---|---|---|---|---|
| **Figure 2C** | Day | N.risk | N.event | Lower 95% CI | Mean | Upper 95% CI |
| **Removal method** | | | | | | |
| Control | 5 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| Autotomy | 5 | 47 | 2 | 0.91 | 0.96 | 1.00 |
| Forced break | 5 | 36 | 20 | 0.53 | 0.64 | 0.78 |
| Control | 15 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| Autotomy | 15 | 47 | 0 | 0.91 | 0.96 | 1.00 |
| Forced break | 15 | 36 | 0 | 0.53 | 0.64 | 0.78 |
| Control | 35 | 13 | 1 | 0.80 | 0.93 | 1.00 |
| Autotomy | 35 | 45 | 2 | 0.85 | 0.92 | 1.00 |
| Forced break | 35 | 35 | 1 | 0.51 | 0.63 | 0.76 |

| | | | | Survival probability | | |
|---|---|---|---|---|---|---|
| **Figure 2D** | Day | N.risk | N.event | Lower 95% CI | Mean | Upper 95% CI |
| **Overall model** | | | | | | |
| Control | 5 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| E-1-F | 5 | 13 | 1 | 0.80 | 0.93 | 1.00 |
| E-1-A | 5 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| E-2-F | 5 | 9 | 5 | 0.44 | 0.64 | 0.95 |
| E-2-A | 5 | 12 | 2 | 0.69 | 0.86 | 1.00 |
| N-1-F | 5 | 8 | 6 | 0.36 | 0.57 | 0.90 |
| N-1-A | 5 | 7 | 0 | 1.00 | 1.00 | 1.00 |
| N-2-F | 5 | 6 | 8 | 0.23 | 0.43 | 0.79 |
| N-2-A | 5 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| Control | 15 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| E-1-F | 15 | 13 | 0 | 0.80 | 0.93 | 1.00 |
| E-1-A | 15 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| E-2-F | 15 | 9 | 0 | 0.44 | 0.64 | 0.95 |
| E-2-A | 15 | 12 | 0 | 0.69 | 0.86 | 1.00 |
| N-1-F | 15 | 8 | 0 | 0.36 | 0.57 | 0.90 |
| N-1-A | 15 | 7 | 0 | 1.00 | 1.00 | 1.00 |
| N-2-F | 15 | 6 | 0 | 0.23 | 0.43 | 0.79 |
| N-2-A | 15 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| Control | 35 | 13 | 1 | 0.80 | 0.93 | 1.00 |
| E-1-F | 35 | 13 | 0 | 0.80 | 0.93 | 1.00 |
| E-1-A | 35 | 14 | 0 | 1.00 | 1.00 | 1.00 |
| E-2-F | 35 | 8 | 1 | 0.36 | 0.57 | 0.90 |
| E-2-A | 35 | 12 | 0 | 0.69 | 0.86 | 1.00 |
| N-1-F | 35 | 8 | 0 | 0.36 | 0.57 | 0.90 |
| N-1-A | 35 | 6 | 1 | 0.63 | 0.86 | 1.00 |
| N-2-F | 35 | 6 | 0 | 0.23 | 0.43 | 0.79 |
| N-2-A | 35 | 13 | 1 | 0.80 | 0.93 | 1.00 |

**Table 4**. **The relationship between mean survival and the number and percent of claw breaks for stone crab across the claw removal treatments, experience level treatments, removal method treatments and individual treatments.** [(Expert (E) or Novice (N) - 1 claw removed (1) or 2 claws removed (2) – forced break (F) or autotomy (A)].

| | Sample size | Mean survival % (35 days) | Number of bad breaks | Number of breaks (%) |
|---|---|---|---|---|
| **Claw removal** | | | | |
| 0 Claws removed | 14 | 93 | 0 | 0 |
| 1 Claw removed | 49 | 84 | 4 | 8 |
| 2 Claws removed | 55 | 70 | 16 | 29 |
| **Experience level** | | | | |
| Control | 14 | 93 | 0 | 0 |
| Expert | 55 | 84 | 6 | 11 |
| Novice | 49 | 67 | 14 | 29 |
| **Removal method** | | | | |
| Control | 14 | 93 | 0 | 0 |
| Autotomy | 49 | 92 | 2 | 4 |
| Forced break | 55 | 63 | 18 | 33 |
| **Overall** | | | | |
| Control | 14 | 93 | 0 | 0 |
| E-1-F | 14 | 93 | 0 | 0 |
| E-1-A | 14 | 100 | 0 | 0 |
| E-2-F | 13 | 57 | 5 | 38 |
| E-2-A | 14 | 86 | 1 | 7 |
| N-1-F | 14 | 57 | 4 | 29 |
| N-1-A | 7 | 86 | 0 | 0 |
| N-2-F | 14 | 43 | 9 | 64 |
| N-2-A | 14 | 93 | 1 | 7 |

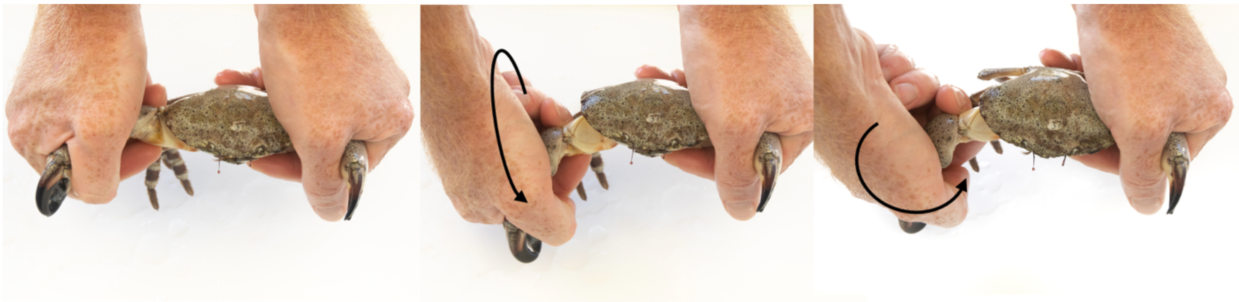

**Figure 1. A photo series of the typical recommended method of claw removal for stone crabs (*Menippe* spp.) and other claw-only fisheries (herein referred to as forced break).** This method uses (a) two hands to hold the crabs claws, (b) a downward force applied to the crabs fully extended cheliped to (c) release or in some cases break the claw along the autotomy plane at the basi-ischium, located between the coxa at the base of the leg and merus.

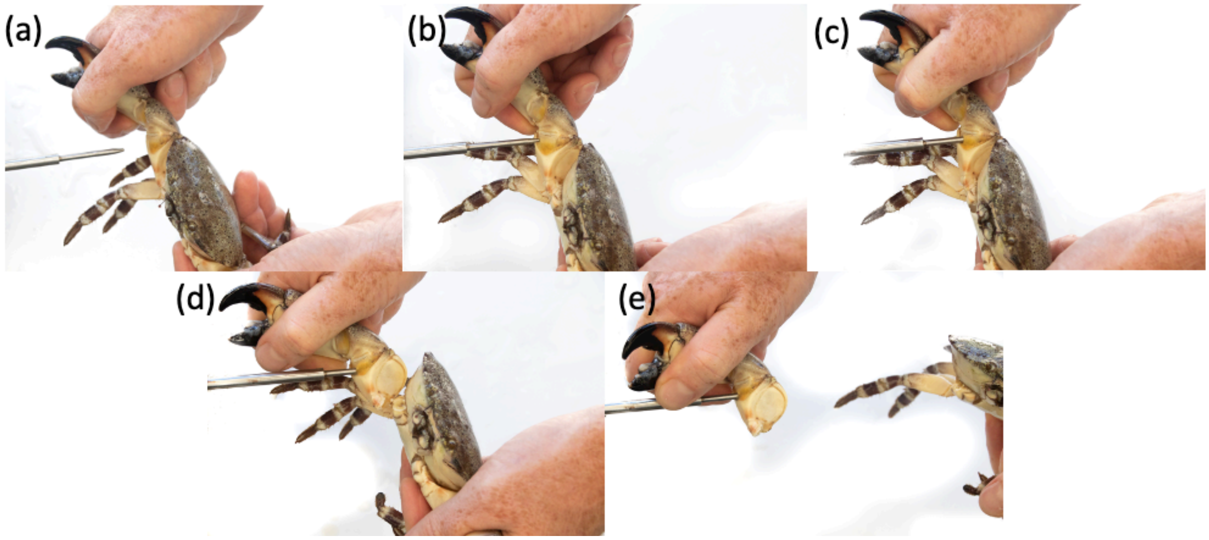


**Figure 2. A photo series of our proposed method of claw removal for stone crabs (Menippe spp.) and potentially other claw-only fisheries (herein referred to as induced-autotomy).** This method uses a marlin spike (a) to puncture the articular membrane (the soft joint, or unsclerotized cuticle between hard sclerotized parts of the exoskeleton) between (b, c) the carpus and the merus and (d) rotating the implement towards the crab's body and inducing (e) the voluntary drop (autotomy) of the claw distal to the coxa.

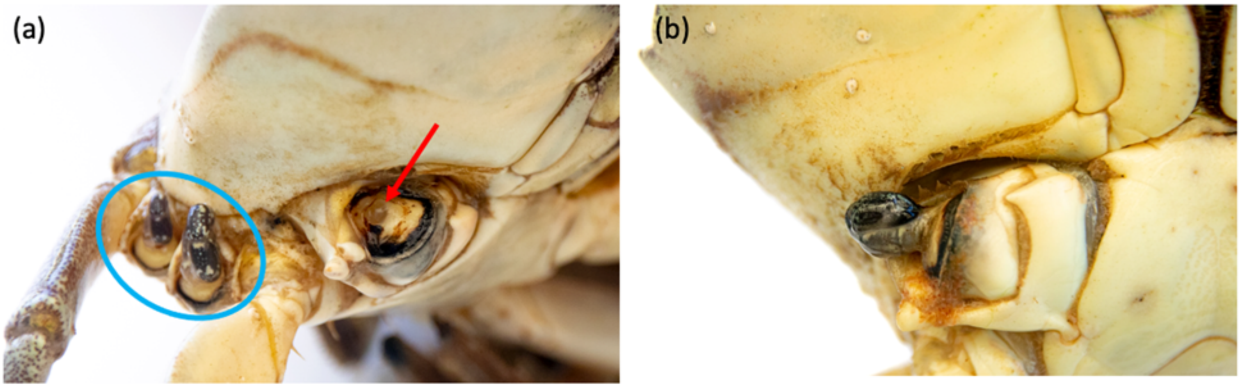


**Figure 3. Beginning stages of limb regeneration in the stone crab Menippe mercenaria following removal.** (a) A small appendage (indicated by the red arrow) at the site of the coxa where the cheliped was removed and walking legs that are in later stages of regeneration (blue circle). (b) A later stage of regeneration by which a small cheliped has begun to form on the coxa.

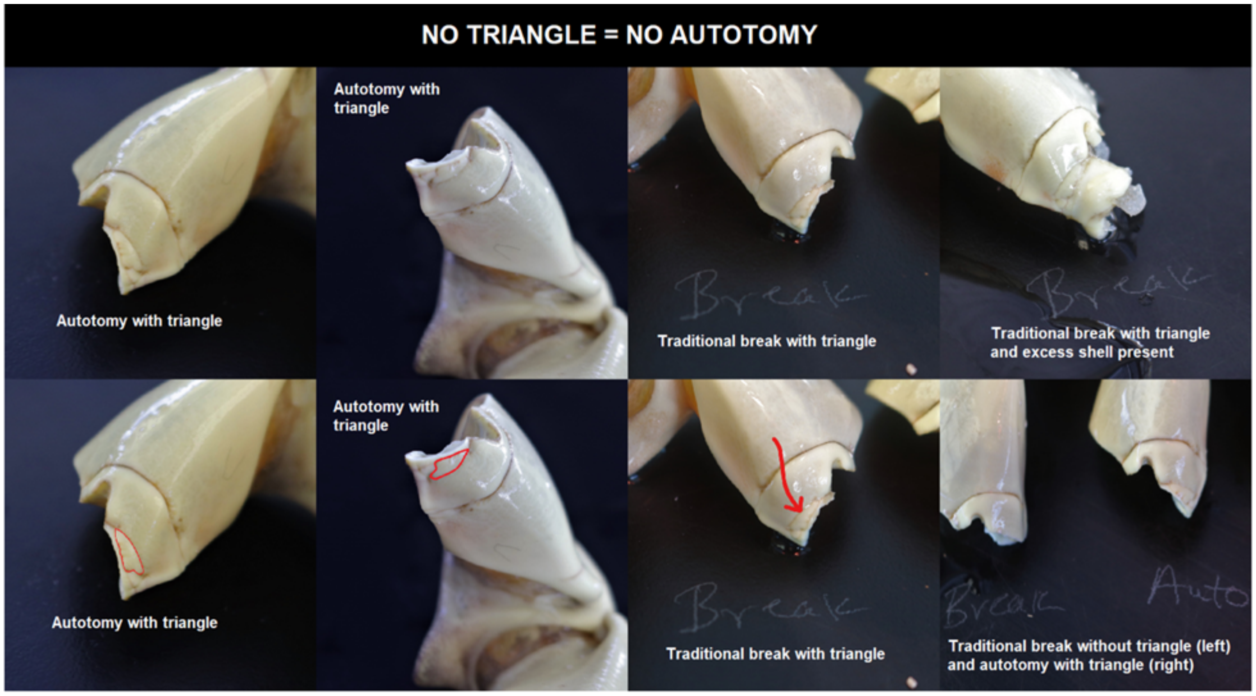


**Figure 4. Various images of stone crab claws removed using the typical recommended method of claw removal (herein referred to as forced break or traditional break) or our proposed method of claw removal (herein referred to as induced-autotomy or autotomy).** Note: the distinct triangle remaining on the shell of all the claws removed using the induced-autotomy method.

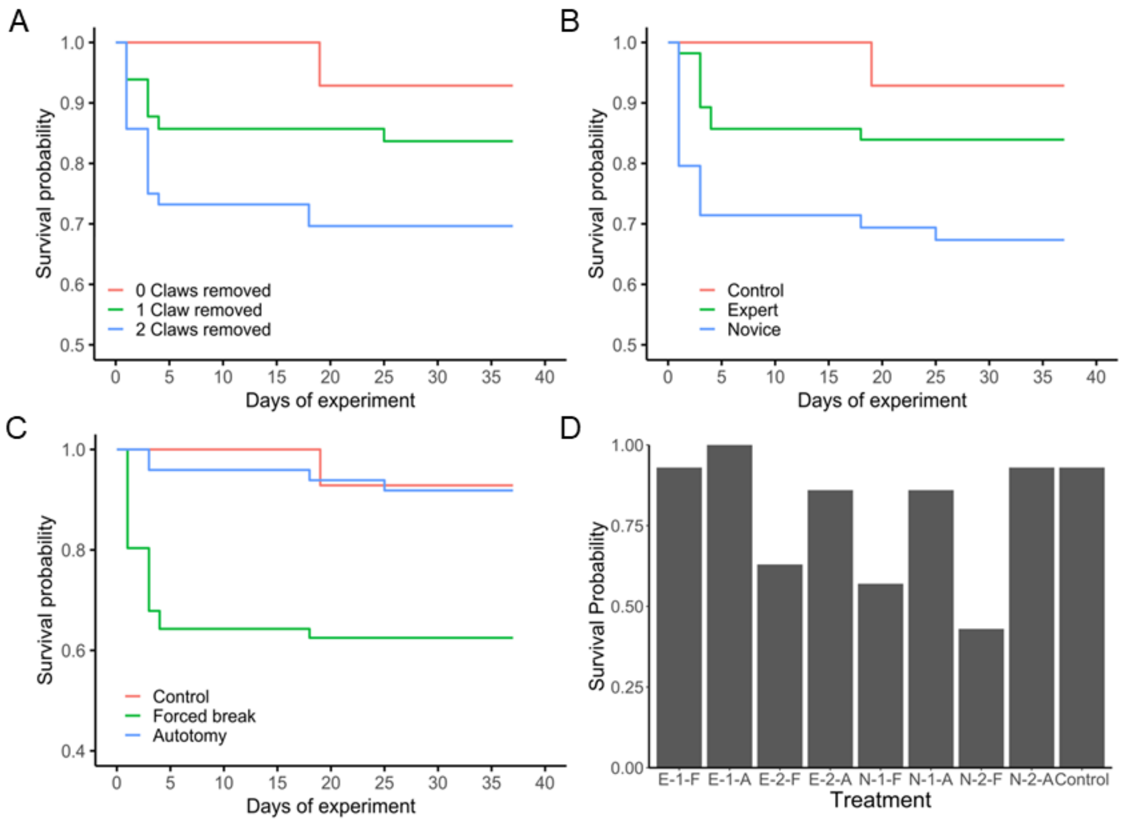


**Figure 5. (A - C) Kaplan-Meier survival curves (mean ± 95% CI) and (D) Survival probability of stone crab following claw removal.** Panels are presented to illustrate the effect across (A) claw removal treatments, (B) experience level treatments, (C) removal method treatments and (D) individual treatments [(Expert (E) or Novice (N) - 1 claw removed (1) or 2 claws removed (2) – forced break (F) or autotomy (A)] and correspond to Table 3A - D. Days of the experiment refers to the duration individuals were held in tanks. Mean water temperature and salinity for the study ± SD was 26.3 ± 1.2°C and 36 ± 1 psu.

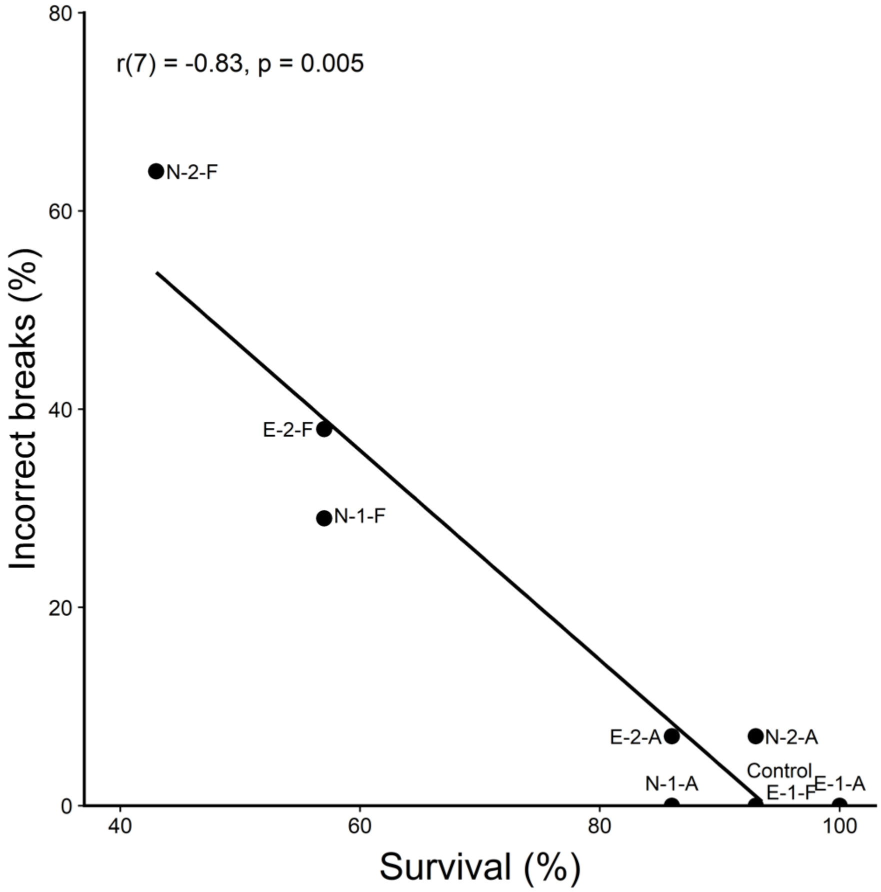


**Figure 6. The relationship between incorrect claw breaks (claw removals that resulted in a wound) and survival for the various treatments of the study [(Expert (E) or Novice (N) - 1 claw removed (1) or 2 claws removed (2) – forced break (F) or autotomy (A)].**

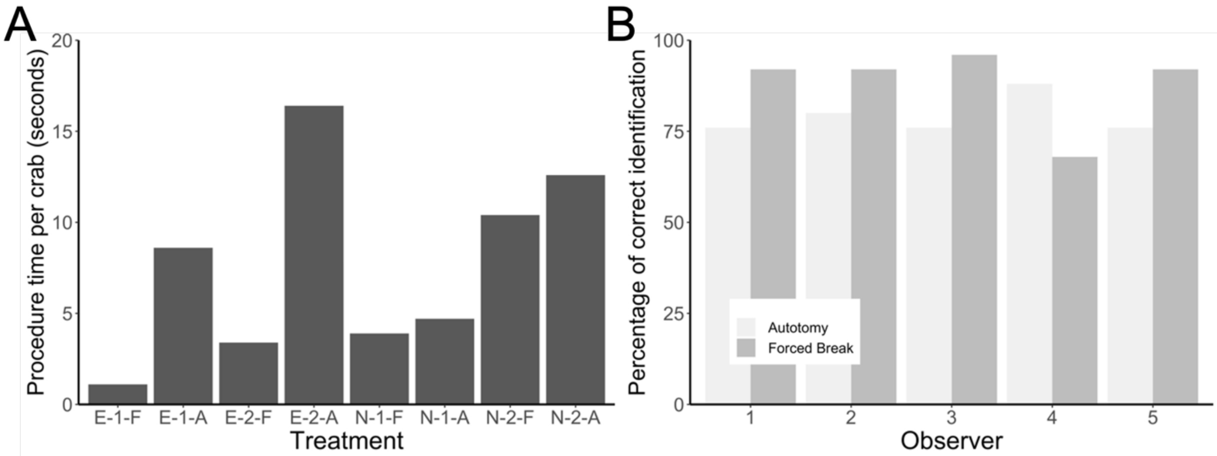


**Figure 7. (A) Stone crab claw removal procedure durations per crab for the various treatments [(Experienced (E) or Novice (N) - 1 claw removed (1) or 2 claws removed (2) – forced break (F) or induced-autotomy (A)].** Experienced refers to claws removed by the commercial harvester with >10 years' experience, whereas novice refers to a researcher with limited recreational stone crab fishing experience but was familiar with handling and claw removal procedures for stone crab. Forced break refers to the typical practice of claw removal whereby a firm downward motion is used to remove or in some cases break the claw along the autotomy plane at the basi-ischium, located between the coxa at the base of the leg and merus. Induced-autotomy refers to our proposed technique whereby individual crabs voluntarily release their claw following a propodus puncture with a marlin spike. (B) the percentage of correct claw removal method identifications (forced break or induced autotomy) by observers. Observer refers to a person who has been briefed on removal methods and the anatomy of the claw prior to completing a removal method indentification survey.

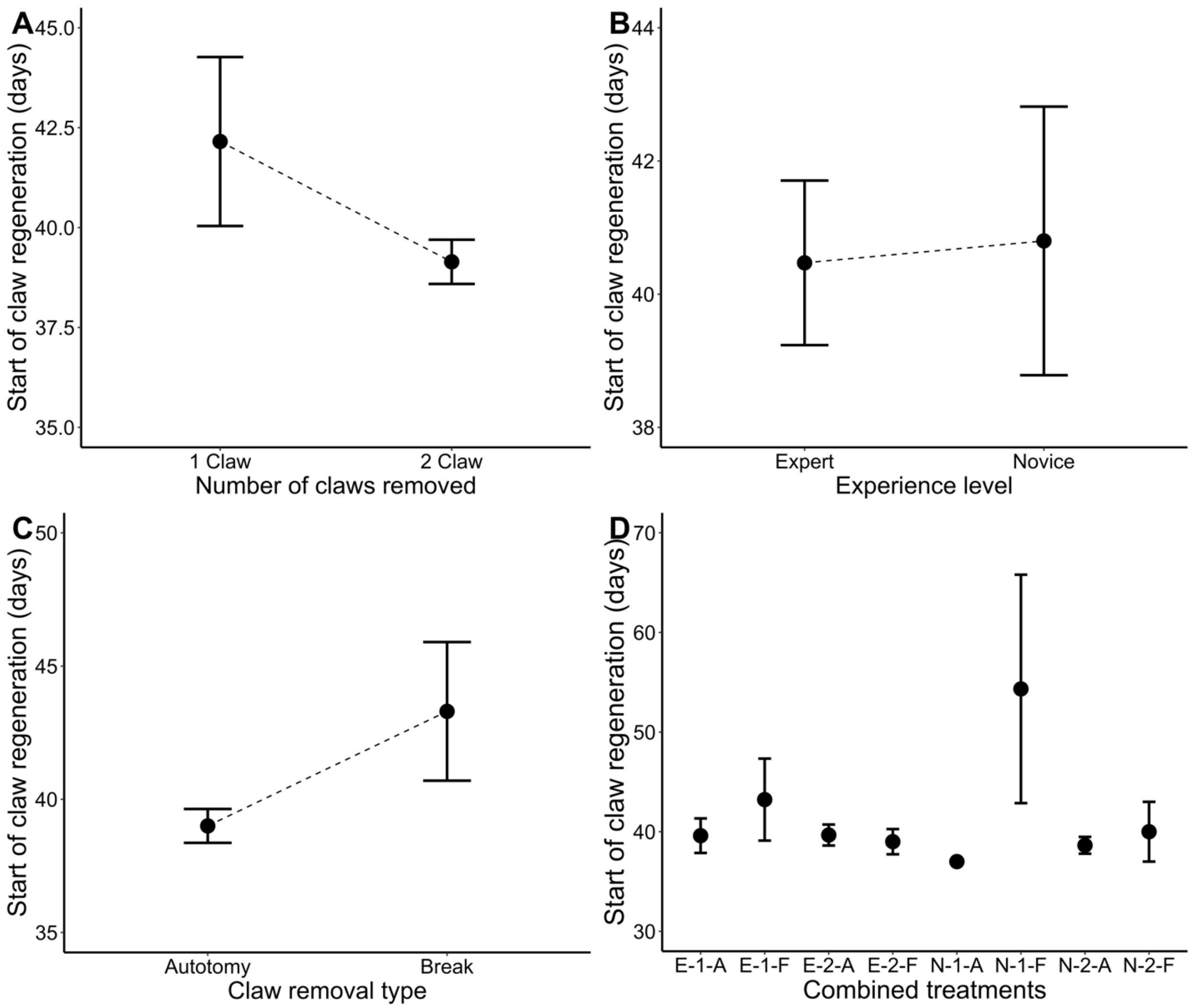


**Figure 8. (A - C) Mean claw regrowth time (mean ± S.E.) and (D) time to start of claw regrowth based across treatments.** Panels are presented to illustrate the effect across (A) claw removal treatments, (B) experience level treatments, (C) removal method treatments and (D) individual treatments [(Expert (E) or Novice (N) - 1 claw removed (1) or 2 claws removed (2) – forced break (F) or autotomy (A)]. Start of claw regeneration (days) refers to the days until signs of claw regrowth were seen. Mean water temperature and salinity for the study ± SD was 26.3 ± 1.2°C and 36 ± 1 psu.

# CHAPTER 2: THE EFFECT OF TEMPERATURE AND INDUCED AUTOTOMY CLAW REMOVAL ON THE CRITICAL THERMAL MAXIMUM OF STONE CRAB (*MENIPPE MERCENARIA*)

## INTRODUCTION

Average global air temperature has increased 0.74°C since 1906 with ten of the warmest years on record occurring since 2005 (NASA/GISS) and predictions of further increases to reach 1.8 to 4.0°C by the end of the century (Hein et al. 2012; Taylor et al. 2018).

The Caribbean region is considered one of the most vulnerable areas with respect to climate change (Taylor et al., 2018). Not only is the frequency and intensity of extreme weather predicted to increase (e.g. hurricanes), but the dry season is predicted to become wetter, and the wet season is predicted to become drier, especially during the early part (May–July) of hurricane season (Christensen et al., 2007; Campbell et al., 2011; Hall et al., 2013; Taylor et al., 2011, 2013). These changes will likely have a profound impact on oceanographic conditions and the small-island communities that are reliant on coastal fisheries and the distribution of aquatic organisms. For example, because most aquatic organisms cannot regulate their body temperature, they are directly influenced by environmental temperature fluctuations (Brett 1971). Therefore, changes in the distribution and migration timing of targeted species are expected, thus fishing efficiency, the number of fishing days available, and the number of targeted species for harvest is also likely to change. As a result, it is incumbent that studies, such as these, continue to evaluate fishery practices under various climate change scenarios to help predict how coastal fisheries will respond to climate warming and how certain fisheries practices may increase or decrease the capacity of species to survive warming temperatures.

Commercial and recreational stone crab fisheries primarily occur along the Gulf of Mexico and Atlantic coasts of the southeastern United States (US), but smaller commercial fisheries, are also gaining popularity in Belize (Bert & Hochberg 1992) and The Bahamas (Moultrie et al. 2016). In The Bahamas, the stone crab fishery is currently the third largest export valued at approximately $2.4 million annually. Legal claw-size, number of claws removed, and seasonality of fisheries can vary by region and country. In the Bahamas, the stone crab season is closed from June 1st to October 15th while in Florida it is closed from May 1st to October 15th. These closed seasons coincide with the mating season of the stone crab. While best practice recommends that only a single, larger claw be removed on each legally sized crab (Bert et al. 2016), both claws are harvested in many areas (Gandy et al. 2016, Sullivan 1979).

Claw-only fisheries are often regarded as sustainable because they are assumed to exploit the natural ability of crabs to autotomize (voluntarily drop) and regenerate their claws when damaged or threatened. However, post-release survival of de-clawed stone crabs is variable and depends on a myriad of factors that influence survival after claw removal and release. These include, but are not limited to, break location (Gandy et al., 2016), wound size (Duermit et al., 2015, 2017), whether both claws are removed (Davis et al., 1978, Duermit et al., 2015, Gandy et al., 2016; Orrell et al., 2019) and water temperature (Gandy et al., 2016). While some of these factors can be mandated by managers, the water temperature is likely to change under the current climate change scenario.

Studies to date have used the typical method of claw removal to assess post-release survival after claw removal and not the induced-autotomy method recently proposed by Walus et al (Chapter 1), which has been shown to increase survival independent of harvester experience and the number of claws removed. The typical method of claw removal involves the harvester exerting enough downward force to the fully extended cheliped to cause the crab to release the claw cleanly along the autotomy plane at the basi-ischium, located between the coxa at the base of the leg and merus. However, this method is prone to incorrect breaks and has been shown to cause significant mortality post-release (Chapter 1). Instead Walus et al. (Chapter 1), suggest using an induced-autotomy method. Nevertheless, this method has not been evaluated across temperature ranges or in the context of evaluating critical thermal maximum (the breadth of temperatures over which the organisms can tolerate at least short exposure) for the stone crab (*Menippe mercenaria*).

Recent studies on the Cuban stone crab (*Menippe nodifrons*) indicate a critical thermal maximum of 39 °C (Vinagre et al., 2016). Furthermore, the loss of self-righting, commonly used as an endpoint to determine critical thermal maximum previously, may be problematic for evaluating the effect of claw removal and temperature on the critical thermal maximum. This is because crabs, to some capacity, may use their large claws to self-right, and if so, crabs with two claws removed may become disadvantaged compared to those with one claw or two claws removed. Because of this potential, I compared the eight reflexes outlined by Krondstadt et al., (2018) and loss of equilibrium to determine the best indicator of critical thermal maximum in Florida stone crab.

To conduct this study, I conducted a series of laboratory experiments to measure the impact of claw removal using induced-autotomy and thermal response simultaneously on critical thermal maximum of stone crabs. To do this, I evaluated nine reflex action mortality predictor reflexes of crabs, eight of which have been previously in Kronstadt et al., 2018 and the ninth being loss of equilibrium. All reflexes were evaluated with and without claws removed using the induced-autotomy method at various increasing water temperatures.

## METHODS

Forty six stone crabs (mean mass = 460 ± 190 g; mean carapace width = 112 ± 20 mm; right propodus length mean = 94 ± 19 mm; left propodus length mean = 79 ± 17 mm) with both claws intact were purchased from stone crab harvesters in Hatchet Bay and Spanish Wells, Eleuthera, The Bahamas. During transport to the aquatics holding facility at the Cape Eleuthera Institute, crabs were placed in several large coolers and covered with seawater-moistened towels. Upon arrival, crabs were housed in two large circular aerated tanks which measured 3.7m in diameter and 1m water height. The tanks were supplied with flow through sea water at a temperature ± SD of 26.3 ± 1.2 °C and a salinity ± SD of 36 ± 1 psu. All crabs were fed pieces of conch and fish offal *ad libitum* every other day and were acclimated for 14 days prior to experimentation. Pieces of PVC pipe were added to the tank to provide enrichment and shelter to help minimize agonistic behaviors among crabs. Tanks were checked daily and cleaned biweekly.

Four crabs were randomly selected daily, starved for 24 hours, and acclimated to 28 °C sea water in individual tanks. The following day, crabs were reflex scored prior to claw removal. This assessment consisted of a series of nine tests (Table 2.) to check for the presence or absence of a reflex as outlined by Kronstadt et al., 2018. While reflexes are often used as a predictor of post-release mortality, the goal of my study was to use the various reflexes as a non-lethal evaluation of critical thermal maximum and to determine which reflexes would be suitable for evaluating the effect of induced-autotomy claw removal on critical thermal maximum. The typical method of using a self-righting reflex (loss of equilibrium) to evaluate critical thermal maximum in crabs may be confounded, as crabs to some capacity, may use their large claws to self-right, thus crabs with two claws removed may become disadvantaged compared to those with one claw or no claws removed.

Once baseline reflex scoring was complete, crabs were divided into three groups. One group had no claws removed (control) while the other two groups had one claw or two claws removed, respectively. Claw removal was conducted using induced-autotomy (Chapter 1) to ensure consistency. Induced-autotomy was conducted by puncturing the articular membrane (the soft joint, or unsclerotized cuticle between the hard sclerotized parts of the exoskeleton) between the carpus and the merus with a sharp pointed implement that was mounted to a base. This resulted in the induced release of the claw (autotomy) distal to the coxa. Following claw removal, crabs were again reflex scored and placed into individual plastic containers for critical thermal maximum trials. Water temperature was raised by 1 °C every 30 minutes until loss of equilibrium. Loss of equilibrium was determined by turning the crab onto the carapace and observing whether the crab loss their ability, or not, to self-right. If individual crabs could not self-right the crab was removed and placed in a separate holding tank to recover. In addition to evaluating the loss of equilibrium reflex, eight additional reflexes, commonly used in reflex action mortality predictor assessments (Table 5) were also evaluated every 30 minutes.

Following experiments all remaining crabs were released back into the wild. All work was carried out under The Bahamas Department of Marine Resources permit number MAMR/FIS/2/12A/17/17B.

Two crabs died following claw removal and were therefore removed from the experiment and subsequent analyses. Critical thermal maximum was calculated using the following equation:

$$CTM_{\text{stone crab}} = \sum(T_{end})/n$$

Where $T_{end}$ is the temperature at which the end-point was reached for individual 1, individual 2, individual n, divided by the n individuals that were in the sample. The effect of reflex, claw removal and the interaction between reflex and claw removal on CTmax was compared using a generalized linear model. If a significant effect was found, a Tukey post-hoc comparison was used to compare levels within a factor. A significance level of 0.05 was used in all tests. All statistical analysis was conducted using R Studio.

## RESULTS

Of the nine reflexes used to develop an endpoint necessary for calculating CTMax, three were determined to be suitable (Figure 9). These included: equilibrium, mouth closure and appendage turgor. Of those three reflexes, there was only a significant difference between mouth closure and equilibrium (Tukey post-hoc, $p = 0.02$), as no differences in CTMax were found between mouth closure and appendage turgor, and appendage turgor and equilibrium. Additionally, there was a significant effect of claw number (GLM, df = 94, f = 4.37, $p = 0.02$), and reflex (GLM, df = 96 f = 3.15 $p = 0.05$) on CTMax. Critical thermal maximum, using the equilibrium reflex, was 38.2°C ± 1.33 °C, whereas CTMax calculated using mouth closure and appendage turgor was 37.1°C ± 2.27 °C and 36.4 ± 3.5 °C, respectively. Therefore, mean CTMax, across suitable reflexes was 37.6 ± 2.1 °C (Table 6) and there was a significant difference in CTMax between crabs with two claws removed and the control (Tukey post-hoc = 0.16; Figure 9) and between crabs with one claw removed and the control (Tukey post-hoc, $p = 0.04$). Crabs with zero claws removed (control) had an overall CTMax of 36.6 °C ± 2.2 °C (Table 6), while crabs with one claw removed had an overall CTMax of 38.2°C ± 1.4 °C. Crabs with two claws removed had a CTMax of 38.0 °C ± 2.5 °C.

## DISCUSSION

The results of this study showed that not only do reflexes change as water temperature increases, but there is an effect of claw removal. Here, we discuss two new reflexes that could be used to determine CTMax that are independent of the claw and better suited for evaluating the effect of claw removal on CTMax.

Based on our data, 6 out of 9 reflexes showed a relationship with increasing water temperature compared to the effect on control crabs (Figure 9). Since there was no relationship with eye retraction and abdomen reaction, suggest that these will not be useful in determining critical thermal maximum within stone crab populations. However give the distinct endpoint it was determined that mouth closure and appendage turgor would be sutiable. Using mouth closure and appendage turgor as novel CTMax indicators, we calculated their critical thermal maximums to be 37.1 °C and 36.4 °C, respectively. Recent research on *M. nodifrons* suggests a CTMax of ~39 °C (Vinagre et al., 2016) which is higher than our suggested CTMax of 37.6 °C. Assessing the critical thermal maximum with different claw numbers is not "predictable", therefore we believe claw number does not have a significant impact on critical thermal maximum. In our study, appendage turgor and mouth closure are a more effective indicator of CTMax. When conducting thermal experiments that manipulate claw number, we suggest using a different CTMax indicator that is independent of claw usage.

Results showed that control crabs had a lower CTMax than crabs with one or both claws removed. A potential explanation for this could be that stone crabs use their chela to thermoregulate (Darnell and Munguia, 2011; Windsor et al., 2004). When organisms experience thermal stress, there is a change in energy allocation in the organism's activities such as growth, reproduction, and foraging. An organism's performance and fitness is negatively affected which may be confounded after claw removal. Studies on stone crab claw removal indicate that larger wounds increase mortality due to larger wounds and increased hemolymph loss (Duermit et al., 2015). This could be a potential explanation as to why control stone crabs had a lower CTMax than crabs with claws removed. Using a field based experiment, Gandy (2016) determined that post release mortality is dependent on temperature, wound and break type.

Declawing a crab can result in loss of haemolypmh, which decreases the alertness of the crab (Davis et al. 1978, Savage and Sullivan 1978, Patterson et al. 2007), while autotomy results in lower physiological stress (Savage and Sullivan, 1978). There have not been research into the changes on lactate and glucose levels post-claw removal in stone crab, but research on *C. pagurus* found that there is an increase in glucose and lactate levels immediately following claw removal (Patterson et al., 2007), indicating an increase in physiological stress. Research shows that *U. panacea* use their claws to thermoregulate, meaning they use the claw as a heat sink and can transfer the heat away from the body and into the water (Darnell and Munguia, 2011; Windsor et al., 2004). We believe this could be an explanation as to why control crabs have a lower CTMax value and further studies on stone crab should revolve around this potential physiological effect.

After claw removal, only 2/46 crabs died; this high survival rate can be attributed to the induced autotomy method tested here. Induced autotomy does not leave a wound (Walus, Chapter 1), so crabs are not as negatively impacted by potential loss of internal tissue and also do not have to put as much energy towards healing.

Temperature is known to have a significant impact on survival especially when claws are removed (Gandy et al. 2016). When water temperature increased above 25 °C in their study, the probability of mortality increased to more than 90%. These results may have been because of the way the claws were removed. Edible crabs with manually declawed claws showed much higher rates of stress as compared to induced autotomy (Patterson et al., 2007).

As the climate continues to change, we need to be aware of how best to minimize impacts of temperature change on stone crab populations. It seems unlikely that water temperature increases will significantly impact mortality in crabs with no claws removed, however, when crabs are harvested the stress of claw removal combined with thermal stress could lead to negative consequences for the organisms. Therefore, we suggest induced autonomy should be used for claw removal to minimize harvesting stress. We believe that survival differences associated with induced autotomy are likely greater than any effect of water temperature on claw removal and post release mortality. Also, in my experiment, we subjected to the crabs to high temperatures for a limited amount of time. Continual exposure to higher water temperatures could lead to different results compared to the short temperature challenge used here.

Currently, stone crab closed season is during the summer months, which are the hottest of the year and are when stone crabs reproduce. To prevent major climate induced changes from occurring, we suggest keeping the closed season during these months. Crabs that do not have to put significant energy into healing while also dealing with rising water temperatures and heat waves will give them a higher chance of survival overall in this unique claw-only fishery.

**APPENDIX**

**Table 5. Reflexes tested to assess critical function in *Menippe mercenaria*.** "Test" is the handling action needed to stimulate the desired reflex action. The reflex was considered absent when no action was observed after being tested. All tests were applied with the crab in the ventrum-down position. Modified from Kronstadt et al., 2018

| **Reflex** | **Test** |
|---|---|
| Equilibrium | Flip crab on to carapace |
| Eye retraction | Touch the eyestalk with a blunt probe. If it is already completely retracted, wait for eye to move at least slightly out from under carapace hood. |
| Leg retraction | Very gently squeeze dactyl of first walking leg with forceps. Do not pull. |
| Mouth closure | If closed, attempt to open 3rd maxillipeds with a sharp probe. If open, touch inside mouthparts with probe. |
| Abdomen reaction | Lift the abdominal flap away from the body with a sharp probe. May have to open completely (especially in females). |
| Abdominal turgor | Pull open the abdominal flap. |
| Antennule reaction | Brush probe across the antennules. If antennules are retracted, extend them using a sharp probe. |
| Joint reaction | Softly lay the side of a thin probe across the joint membrane between the coxa and basiischium of the last walking leg. |
| Appendage turgor | Observe orientation of limbs. |

**Table 6. The relationship between three different reflexes and number of claws removed on critical thermal maximum (CTMax).** Of the 44 crabs tested, 23 did not lose appendage turgor as a reflex, and 12 did not lose the ability to close their mouths. Overall refers to the combined claw removal groups.

| Reflex | Claws Removed | CTMax (°C) | STDEV | n |
|---|---|---|---|---|
| Equilibrium | Overall | 38.2 | 1.3 | 44 |
| Equilibrium | 2 | 38.9 | 1.3 | 13 |
| Equilibrium | 1 | 38.4 | 1.2 | 16 |
| Equilibrium | 0 (Control) | 37.4 | 1.1 | 15 |
| Mouth Closure | Overall | 37.1 | 2.3 | 33 |
| Mouth Closure | 2 | 37.5 | 2.8 | 13 |
| Mouth Closure | 1 | 38.4 | 1.6 | 11 |
| Mouth Closure | 0 (Control) | 36.1 | 2.0 | 9 |
| Appendage Turgor | Overall | 37.5 | 2.8 | 22 |
| Appendage Turgor | 2 | 37.6 | 3.3 | 11 |
| Appendage Turgor | 1 | 37.8 | 1.4 | 7 |
| Appendage Turgor | 0 (Control) | 36.4 | 3.5 | 4 |
| Combined | Overall | 37.6 | 2.1 | 99 |
| Combined | 2 | 38.0 | 2.5 | 37 |
| Combined | 1 | 38.2 | 1.4 | 34 |
| Combined | 0 (Control) | 36.6 | 2.2 | 28 |

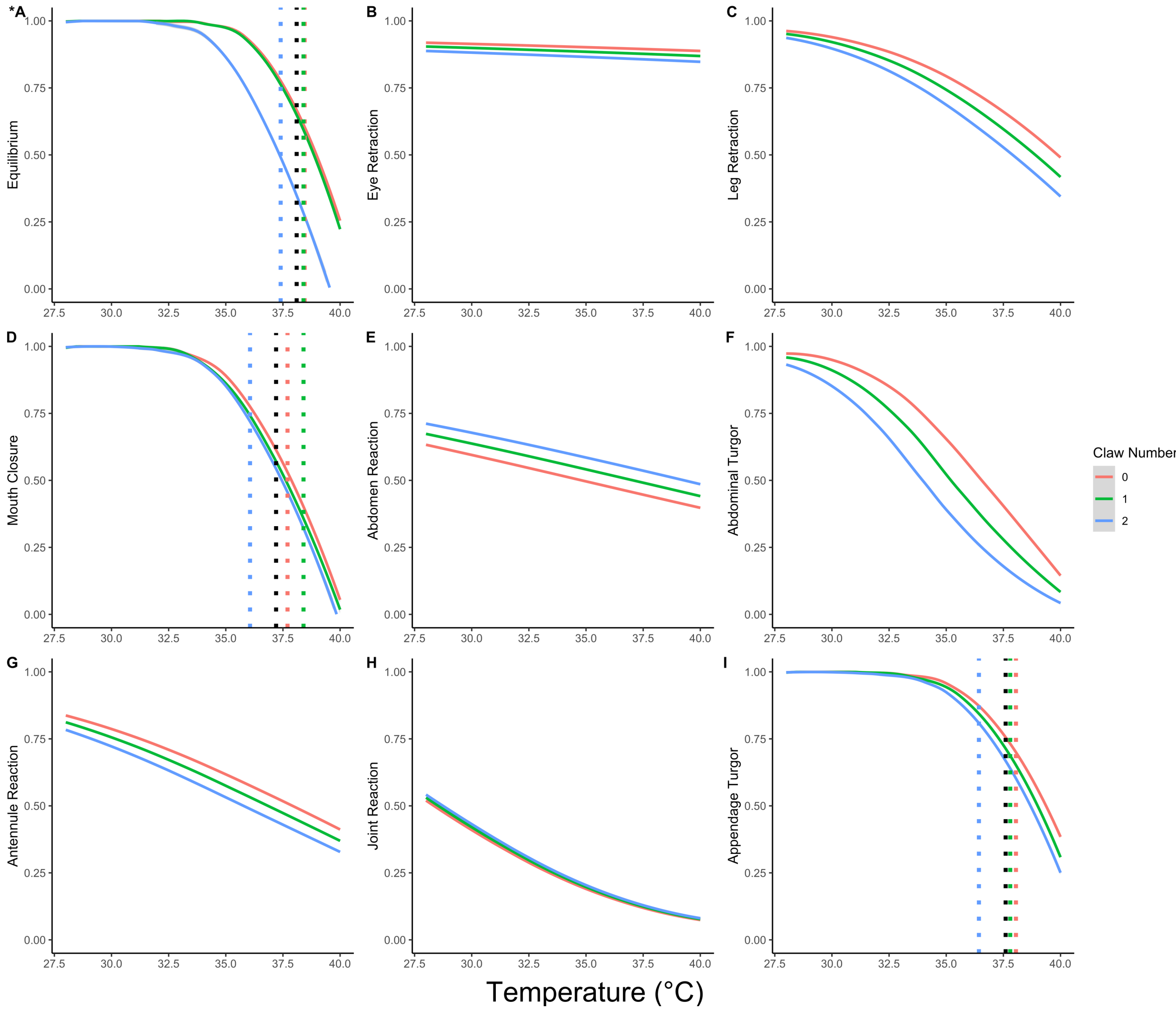


**Figure 9. Calculated CTMax per reflex (vertical lines) fitted against regression curves for 0 (pink), 1 (green), and 2 (blue) claws removed.**

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