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Size at maturity, reproductive cycle and fecundity of the southern California brown box crab, *Lopholithodes foraminatus*, and implications for developing a new targeted fishery

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Arts in Ecology, Evolution and Marine Biology

by

Ashley Marie Stroud

Committee in charge:
Professor Armand M. Kuris, Co-Chair
Professor Douglas J. McCauley, Co-Chair
Doctor Carolynn S. Culver, Researcher
Doctor Henry M. Page, Lecturer

March 2022

The thesis of Ashley Marie Stroud is approved.

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Carolynn S. Culver

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Henry M. Page

_____

Douglas J. McCauley, Committee Co-Chair

_____

Armand M. Kuris, Committee Co-Chair

March 2022

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Ashley Marie Stroud

DEDICATION

This thesis is dedicated to my parents, Tom and Diana Stroud, and my brother Brian Stroud and sister Natalie Zepeda, for their endless support, love, patience and encouragement. And to Aaron Weaver, because partners of graduate students deserve their own special award, and you deserve the highest of them all.

ACKNOWLEDGEMENTS

I want to thank my committee, Drs Carolynn Culver, Mark Page, Armand Kuris and Doug McCauley for their guidance, encouragement and recommendations. I grew tremendously as an academic through this experience with each of you. Armand, it was such a privilege to talk all things crab with you and thank you for access to the most extensive library. Mark, thank you for your regular enthusiasm and guidance on this project. Doug, thank you for always challenging my thought process, encouraging me to think outside the box. And lastly, the biggest thank you to Carrie. I am so grateful to have worked so closely with you on this project. Thank you for the countless hours you devoted to this project and my work. Your expertise in this field is a gift I tried to soak up as much as I could, but there is truly not enough time in the day. Your mentorship before and during this this project, pushed me to be the best researcher I can be, I am forever indebted. Thank you for always believing in me.

This work could not have been done without the incredible team of undergraduates and lab technicians that assisted both in the lab and in the field: Lauren Enright, Ally Aplin, Lauren Jennings, Kimberly Krotine, Christina Patricia, Kristen Huffman, Chloe Jenniches, Charin Parks, Emily Hascall, Cassiel Nortier-Tilly and Victoria Torres. I cannot imagine a better team to process 1600+ crabs and count thousands of eggs with, truly the best, thank you all so much. A special thank you to Lauren Enright. You joined our team at a time when undergraduates were not allowed on campus due to the pandemic. This project would not have processed nearly as many samples and photographs had you not been there during that crazy restrictive time with your incredible work ethic and spirit. I can’t thank you enough

for working all day alone double masked until restrictions were lifted, not an easy feat. Graduate school during a pandemic was not nearly as bad as it could have been because of co-working with you and the friendship we built during it, thank you.

Thank you to California Department of Fish and Wildlife for all of your assistance, specifically Julia Coates, Lindsay Orisini, and Tom Mason. And most importantly, this could not have happened without the efforts of the fishermen - each of you were such a pleasure to work with, and I am grateful for the time and knowledge you shared with me. I have chosen to leave these names out for confidentiality reasons

I would also like to thank our San Diego team, Theresa Sinicrope-Talley, Catherine Courtier, and Kendra Struffenegger for their assistance with the San Diego work, including pick-ups and animal care. Thank you to Carrie Pomeroy for all the time and effort you devoted to the survey work as well as your constant mentorship and friendship. A special thank you to Allison, Lauren G., Briana, Liz, and Kristy. There are no words for the support you provided me during this time. Without each of you I would not be where I am. And last, but definitely not least, a huge thank you to all my friends and extended family, whom I consistently ditched for box crabs, for sticking by me, loving me and supporting me through it all.

Project funding was awarded by the Saltonstall-Kennedy Fund under Grant Number NA18NMF4270219. Supplemental in-kind support was provided through the California Sea Grant Program (Grant Number NA18OAR4170073), fishing partners and CDFW staff. The reviews herein do not necessarily reflect the views of any of those organizations or partners.

ABSTRACT

Size at maturity, reproductive cycle and fecundity of the southern California brown box crab, *Lopholithodes foraminatus*, and implications for developing a new targeted fishery

By

Ashley Marie Stroud

The brown box crab, *Lopholithodes foraminatus*, is a deepwater crab that is found along the eastern Pacific Coast. Historically, landings in California have been low for this species, but an increase in fishing pressure prompted the state to designate it as an emerging fishery and implement an experimental fishery program for it. With no known biological studies on brown box crab in California, gathering essential fisheries information to evaluate the feasibility of a targeted fishery is necessary. We investigated the reproductive capacity of the brown box crab in southern California. From 2018 – 2021 brown box crabs were collected monthly from fishermen. Size at sexual maturity was determined by presence/absence of mature gonads (physiological), relative growth of the chela for males and abdomen width for females (morphometric), and presence/absence of eggs on females (functional). We found that females reach physiological maturity at a carapace width between 50.8 mm and 71.7 mm, and males between 43.3 mm and 66.3 mm. Morphometric maturity analysis showed a clear inflection point of abdomen width between immature and mature females. Females were 50% functionally mature at 75 mm carapace width. Morphometric and functional maturity was not detected for males, albeit samples of small

male crabs were extremely limited thus warranting further study. Females followed a biennial reproduction pattern, mating in the fall followed by an 18-month brooding period with hatching the following spring. Fecundity was positively related to size and ranged from 8,352 and 62,181 eggs per female for crabs measuring 67.8 to 130.5 mm carapace width, respectively. These findings provide biological information that can inform the evaluation of a fishery for the brown box crab, including potential management strategies and models for assessing stock condition.

INTRODUCTION

In recent years, fishermen have expanded fishing effort for the brown box crab, *Lopholithodes foraminatus,* in southern California to diversify their businesses and take advantage of new marketing opportunities. Commercial fishing for the brown box crab is not new, it has been commercially landed throughout its distribution along the eastern Pacific Coast of North America for decades. Historically, coastwide landings have been small and incidental to other fisheries. Spikes in brown box crab landings, while rare, typically resulted when effort shifted towards deeper fishing grounds in search of target species, as occurred during the 1983 El Niño in Oregon (Kato., 1992). A similar dramatic increase in California landings came at a time when rock crab landings were down (CDFW, 2020), in part due to the warm water blob and domoic acid events of 2015 and 2016. California brown box crab landings increased from 13,000 pounds in 2016 to nearly 70,000 pounds in 2017; a fivefold increase (Fig. 1). During this time, the price per pound reached a high of seven dollars as new markets were developed for this species. The newly developed markets undoubtedly were fueled by increased direct to consumer marketing efforts and the local food movement.

The new market demand and increased landings of the brown box crab, however, raised some concerns. A fishery for this species was not designated and thus only incidental, not targeted take, was permitted. To address this issue, in 2018, California Department of Fish and Wildlife (CDFW) designated the brown box crab as an emerging fishery and limited incidental take to 25 pounds per day (Coates, 2018). Then, in 2019, CDFW implemented an experimental fishery program (EFP) for the brown box crab (Coates, 2018).

The experimental program provides four years to evaluate and determine whether a commercial fishery is feasible. Making this determination requires having the necessary information to evaluate the sustainability of the resource. This includes, but is not limited to, biomass estimates and sustainable yield, basic biology, and reproduction.

To help inform this evaluation, we examined reproductive capacity of the southern California brown box crab. Although several studies have reported on distribution and catch rates (Colomy, 1989; ODFW, 1997; Zhang, 2001), there have been very few studies of the biology of *L. foraminatus*. These studies were conducted in Oregon and British Columbia and reported limited information on size at maturity and fecundity (McCrae and Hoover, 1997; Goddard, 1997; Zhang, 2001). Most recently, Duguid and Page (2011), investigated the egg development and seasonality of brown box crab in British Columbia. To expand on the limited information, we conducted a study of southern California brown box crab reproduction with the following objectives: 1) identify size at maturity, 2) assess fecundity and 3) determine reproductive cycle, brood duration and seasonality.

Size at maturity is defined in three ways: physiological maturity, morphometric maturity, and functional maturity (Hartnoll, 1969; Pinheiro & Fransozo, 1998). The presence of mature gonads where the reproductive organs are producing gametes, taken to indicate physiological maturity, alone does not infer a crab is ready to mate (Waiho, 2017). Morphometric maturity is the development of secondary sexual characteristics that enable an animal to be physically capable to mate (Hartnoll, 1974, 1978). In many species of Anomura and Brachyura, this is denoted by an increase in abdominal flap width in females and a change in claw characteristics in males (length, height, gape, etc.) (Jewett et al., 1985; Haefner, 1990; Culver, 1991; Alunno-Bruscia, 1998). A successful mating event (functional

maturity), while often harder to determine in males (e.g. mating marks) than females (presence of eggs), is the most conclusive sign of sexual maturity (Boolootian et al., 1959; Butler, 1960). Taken together the three parameters present a clear picture of size at maturity.

Determining reproductive capacity includes assessments of fecundity, brood duration and mortality, and reproductive seasonality. Fecundity, the number of eggs per brood, varies among species, as well as within species (Haynes, 1968; Hartnoll, 1985; Otto, 1990). In general, fecundity increases with increasing size (Otto, 1990; Shields, 1991). In addition to size, regional difference in environmental conditions, such as water temperature, can influence brooding time and seasonality (Wear, 1974; Shields, 1991). Duguid & Page (2011) found that brown box crab in British Columbia underwent a 12-month diapause, with an ~18 month overall brooding period and biannual reproduction. Whether California brown box crab have a similar reproductive cycle is unknown. Brood mortality, particularly due to egg predators, has impacted egg production for other lithodid crab fisheries (Kuris et al. 1991; Kuris & Wickham, 1997). This has not been investigated for *L. foraminatus*. Together, these reproductive parameters contribute to estimates of annual egg production for a population.

Our findings provide information on the reproductive capacity of brown box crab needed for evaluating the feasibility of a targeted fishery for this species. Determining at what size brown box crabs mature, how many eggs they produce, and when they reproduce, is critical for determining the potential sustainability of the population in the face of fishing pressure. Study results will inform discussions about potential management options, such as size limits, quotas and seasonal closures, and may be used to develop models (e.g., eggs per recruit, spawning potential ratios) for assessing stock condition.

MATERIALS AND METHODS

Sampling and Measurements

Crab samples were obtained from and in collaboration with commercial fishermen participating in the brown box crab EFP. Samples were collected from the Southern California Bight, south of Point Conception and north of the Mexican border, including both island and coastal mainland areas at depths ranging from 120 to 245 meters. Data were collected over the course of 3 years (March 10, 2018 – August 31, 2021), excluding a five-month period (March 2020 – July 2020) impacted by research travel restrictions associated with the COVID-19 pandemic. Each fisherman provided a monthly sample of 15 males and 15 females as weather permitted (Coates, 2018). Monthly samples were supplemented with samples of small crabs (<75 mm CW) and molting crabs as available. Additional crabs were also provided by the CDFW. Sample crabs were caught using commercial lobster traps (5 x 10 cm mesh) and custom research or prawn traps – traps with smaller mesh size (2.5 x 2.5 cm mesh) and closed or no escape ports. The research and prawn traps (referred to as experimental traps) were designed to collect a wider size range of crabs that were representative of the brown box crab population as compared to crabs captured and retained in the commercial traps.

Size at Maturity

Carapace width (the widest point not including spines, CW), abdominal width (at the suture line near the widest part), left and right propodus length (posterior surface from tip to joint), and left and right propodus height (posterior surface from joint to base) were measured to investigate morphometric maturity. Measurements were recorded using a digital

caliper (0.01 mm). Although maximum carapace width (including spines) is used for the fishery, CW is used in this study to avoid the inherent issues caused by worn and damaged spines. Additional measurements were taken and used for comparisons with other studies as well as fisheries data. Conversion equations are provided in Appendix A.

For determining morphometric and functional maturity, female crabs were categorized as mature when eggs or a mat of remnant brood setae, filaments to which eggs were attached and remain with empty egg cases, also referred to as "moss" per Zhang et al. (1999), were present. When such was not the case, the crab was dissected and the gonads were examined. Those with colored (yellow, orange, or red) gonads were categorized as mature. Translucent or white gonads were categorized as immature. For males whose vasa deferentia were not obviously white and filled, gonad tissue was removed and examined microscopically at 100x magnification to determine presence/absence of spermatophores, and further confirmed by pressing on the gonopore to see if sperm was released.

Egg Development

Egg development of captive brooding females was assessed from September 2019 through March 2020 at which time research restrictions associated with the COVID-19 pandemic precluded further observations. Cohorts of eleven crabs were held in chilled (8 - 10° C) filtered seawater tanks at UC Santa Barbara and Scripps Institution of Oceanography, UC San Diego. Each individual was assigned a unique ID by affixing vinyl tags to their carapace with ethyl cyanoacrylate gel (bSi Insta-Cure). Crabs were fed a variety of food, including mussels, squid, shrimp, scallop, brittle star and an occasional fish carcass every 7 to 10 days. Samples of approximately 30 eggs or greater were retrieved from each crab by

separating two of their limbs and inserting forceps under the abdominal flap. [Retrieving eggs by pulling back the abdominal flap was more difficult and lead to damage of the flap.] Because Duguid and Page (2011) reported a lengthy diapause stage (up to 12 months of no development), eggs were sampled once a month until egg development was initiated and then twice a month when eggs were developing. Eggs were observed through a 100x magnified lens on a dissecting microscope, development stage determined and recorded based off Duguid and Page (2011) and then further grouped into six categories for analyses (Table 1).

Egg stage observations were also recorded for all monthly samples to assess reproductive status in nature. Observations were made visually (rather than using a microscope) to mimic field techniques and included four categories: bright orange (diapause), dark orange/eye spot (developing), moss (hatched), and empty (absence of eggs or moss, clean pleopods).

Fecundity

To assess fecundity, crabs were measured as described above and abdominal flaps containing full clutches of undeveloped eggs (no eyespots visible by eye) were removed and frozen for processing at a later date. Full clutches were defined as egg masses that extended close to, or at the edge of abdominal flap. Seven small clutches were also assessed for reference. Upon processing, the abdominal flap with the intact clutch was defrosted, the un-eyed diapause stage was confirmed via microscopic (100x) examination. Pleopods were then detached, and eggs were removed from each pleopod by gently scraping the setae with a spatula in a petri dish of shallow seawater and subsequently separating clusters with forceps.

In preparation for manual and digital counts of the eggs, the eggs were spread in a single layer covering 75% of a petri dish avoiding the edges. Multiple dishes were used as needed based on the amount of eggs removed per pleopod. Photographs of the separated eggs in the petri dishes were taken using a digital camera (Olympus stylus) magnified to 3.0x and mounted on a frame with a sheet of blue paper and tracing light underneath, methods modified from Bohenek 2017. Counts were then made from the photographs using ImageJ software v. 1.52q (https://imagej.nih.gov/ij/) following a validation period when manual and digital counts were compared for 45 petri dishes totaling 23,000 eggs. Because the manual and digital counts were found to be nearly identical – averaging 1.08 percent error (median, 0.29%) – only digital counts (Appendix B) were done for this analysis (n= 100). A relationship was found between the number of eggs on the third pleopod and total fecundity leading to the development of a calibration equation for fecundity estimations: total number of eggs = (number of eggs on pleopod three) * 4.255. For 13 crabs, only eggs attached to pleopod 3 were counted and the equation was applied to calculate total number of eggs.

Analysis

Statistical analyses were performed using R software (R Core Team, 2020) and Microsoft Excel. The significance level used for all analyses was $p = 0.05$. Data was log transformed and analysis of covariance (ANCOVA) was used to analyze morphometric maturity and the influence of location on fecundity. Morphometric relationships and the relationship between size (CW) and fecundity were determined using linear regression. We also explored a quadratic fit for fecundity, with further data needed on both extremes to resolve the relationship. The size at 50% maturity was calculated and illustrated with the

Aquatic Life History Package in R (Smart, 2016). Due to limitations on receiving a full-size range of crabs, the effect of location on size at maturity could not be analyzed.

RESULTS

Size at Maturity

In total, 1637 brown box crabs from the Southern California Bight were examined with 767 females and 870 males. Females ranged from 45.3 mm to 134.0 mm CW. Males ranged from 43.7 to 188.5 mm CW.

Physiological

Thirty-three females were classified as immature. The largest immature female was 71.7 mm CW, while the smallest female with mature gonads was 50.8 mm CW, and the smallest ovigerous female was 67.8 mm CW.

We identified only four immature males with measurements of 43.7, 47.0, 60.5, and 63.6 mm CW. The smallest mature male was 47.3 mm CW, with all males at or above 66.3 mm CW physiologically mature.

Morphometric

A significant allometric shift in abdomen width was identified for immature and mature females (Fig. 2) ($F(1, 728) = 47.685$, $p < .001$). Immature and mature crabs overlapped in size between 50 mm and 72 mm CW, with crabs below 50 mm CW immature and those above 72 mm CW mature.

No allometric shift denoting maturity was identified for male brown box crabs. The relationships between size (CW) and male propodus length and height were similar for immature and mature crabs (Figs. 3, 4).

Functional

Females were 50% and 95% functionally mature (i.e. presence of eggs or moss) at 75.0 ± 1.01 mm CW and 88.9 ± 1.26 mm CW respectively (Fig. 5). Functional maturity could not be estimated for males due to the lack of immature specimens.

Reproductive Seasonality and Egg Development

Mating events occurred in the fall as evident from observations of grasping pairs, ovigerous females with newly extruded (bright orange) eggs, spent ovaries and soft shells. The bulk of the mating occurred between September and October. There was a shift in the ratio of moss females and egg bearing females in September to nearly all females bearing eggs in October (Fig. 6).

Egg bearing females occurred year-round, but were most prevalent (> 90% of samples) from October through March (Fig. 6). Females with eggs in diapause occurred year-round, and those with developing eggs were observed from October through May with fully hatched broods (moss) evident from June through September.

For crabs held in the laboratory, eggs began developing in October. Eggs adjacent to one another developed at differing rates. Asynchronous development and hatching were not localized to areas within a clutch but rather dispersed throughout. Broods developed over an

approximately six to seven-month period, with the first signs of hatching observed in February (Fig. 7).

Fecundity

Fecundity ranged from 8,352 to 62,181 eggs per clutch for brown box crabs (n=93) ranging in size from 67.8 to 130.5 mm CW (Fig. 8). The slopes of the size-fecundity relationship did not significantly differ between locations ($F(1, 96) = 0.0185$, $p > 0.05$). There was a positive relationship between clutch size and carapace width that was generally linear ($R^2 = 0.83$), although a similar quadratic fit was also detected (Fig. 9, $R^2 = 0.81$). There was high variation in fecundity within size classes (Table 2). Fecundity of abnormal small broods (n=7) ranged from 7,813 to 40,787 for crabs 94.4 to 125.5 mm CW (Fig. 8).

DISCUSSION

Our results provide essential fishery information to evaluate the potential of a California fishery for the brown box crab, *Lopholithodes foraminatus*. Female size at maturity was smaller in southern California compared to studies further north. In British Columbia, 50% functional maturity was reported at 80 mm CL, and 95% at 90 mm, as compared to 68 mm CL (75 mm CW) and 79 mm CL (89 mm CW), respectively, in our study. The smallest gravid female reported in British Columbia was between 65 and 75 mm CL (specific size not cited) (Zhang, 2001) and in Oregon was 68 mm CL, whereas in our study it was 62.3 CL (67.8 CW). These comparisons follow the general pattern of size at maturity decreasing with lower latitude (Hines, 1989; Dugan et al., 1991), a pattern also found in North Pacific populations of some king crab species (Olsen et al., 2018). However,

obtaining small immature crabs required fishing with smaller mesh traps (2.5” x 2.5” cm) and in deeper waters (~250 m) than where legal-sized crabs were fished, which may have influenced the size at maturity we identified as compared to others.

We report the first evidence for female morphometric maturity in the brown box crab. Previously, coverage of the coxa by the abdominal flap and the presence of setae on the pleopods has been used to distinguish juvenile from adult female king crab (Donaldson & Byersdorfer, 2005). Our examination of these features did not coincide with maturation as determined by observation of gonad status. Although the change in female abdomen width relative to carapace width was not readily evident as it is with Brachyuran crabs, a clear relative width inflection between the two groups was consistent with assigned physiologically based categories of mature and immature female box crabs. Since the morphometric differences are subtle, presence of eggs will still be the most readily available technique to identify mature female crabs in the field, despite the overlap in size for juvenile and adult female crabs.

Male size at maturity could only be recognized physiologically, not morphometrically or functionally. In agreement with the 1997 Oregon study, no inflection point was detected when evaluating the relationship between crab size and the two right (dominant) claw morphology parameters (PL, PH) for immature and mature male crabs. Additionally, we examined the male genital pore for setae abrasion to determine functional maturity as suggested by Goddard (1997), but it was not a useful indicator. Setae were observed over a wide range of sizes with no distinguishable differences of wear. The large size range of physiologically mature males (47 mm – 189 mm CW) and small sample size of

immature males (n=4), necessitates further investigations of morphometric indicators and functional maturity to determine the size at which male crabs can successfully mate.

Our study provides the most comprehensive fecundity analysis of *L. foraminatus*. Through it, we documented the highest reported egg count (62,000 eggs) for this species, and demonstrated the similarity in fecundity for crabs throughout the Southern California Bight. As expected, the number of eggs increased with increasing size, with no indication of senescence as seen with other species of crabs (Swiney et al., 2012; Danielsen et al., 2019). Oregon's fecundity estimates (n=4) were similar to ours for large (~120mm CW) crabs, 48,100 eggs and 51,200 eggs respectively. However, their estimates were a bit higher for small (~75 mm CW) crabs, 20,100 and 16,000 eggs respectively. Given variation within a size class, fecundity-size relationships may be similar for crabs from these regions, but further fecundity studies in Oregon are required. With this, we conclude that the resulting size/fecundity relationship can be used to evaluate reproductive capacity of box crabs throughout southern California, and potentially elsewhere.

Brood mortality can have a significant effect on reproductive output (Kuris, 1991). In particular, the red king crab has experienced high brood mortality, losing the majority of broods due to a nemertean egg predator (Shields et al., 1990; Kuris et al., 1991). Even though we did not directly study brood mortality, during our fecundity assessments we did not observe empty egg cases, nor egg predators, even for broods that were further along in diapause (~8-10 months brooding time) and presumably had longer exposure time to potential mortality sources. The egg counts of these older broods were similar to those that were fresh broods, suggesting negligible brood mortality. On occasion we observed small clutches or barren females. It is unclear if this was the result of brood mortality or a less

successful mating event. Although we encountered this rarely, suggesting it likely has little impact on reproductive capacity of the population, we recommend future monitoring of brood size and, if warranted, potential factors influencing it.

Our field and captive observations of the Southern California brown box crab support an ~18-month biennial reproductive cycle (Duguid & Page, 2011) (Fig. 10). The majority of time (~12 months) the eggs are in diapause, therefore females with eggs can be found year around. During winter and spring, eggs will be either in diapause or developing and hatching depending on when the female mated. The duration of the reproductive cycle in California matches that of British Columbia (Duguid & Page, 2011). Studies have shown that temperature can affect duration of embryonic development (Stevens et al., 2008). The similarity in the duration of the reproductive cycle between California and British Columbia may be partially explained by the minimal difference in thermal temperatures of deepwater habitat in the two locations (Culver et al., unpubl.data; Duguid & Page; 2011), far less a difference than what occurs in shallow waters. While the duration of the cycle is similar, the timing differs. In British Columbia, mating and egg development commence in late summer, whereas in California these events take place largely in the fall. Investigating other environmental parameters (e.g., ocean productivity, day length, swings in temperature; REFs) that differ between the two locations and can influence timing of mating and reproduction may reveal why the cycles commence at different times.

Our findings can inform the evaluation of management strategies for a fishery targeting *L. foraminatus*. Currently, the experimental fishery is managed primarily through the use of a size limit and a quota. The minimum legal size for brown box crab is set at 146 mm CW including spines (i.e., maximum carapace width) which was based largely on

market demand. As female brown box crab typically are smaller than the legal size, they are not currently being harvested and likely won't be considered in the future. As a result, they are able to contribute reproductively to the population throughout their lives. For males, our current research supports this size limit as they mature at a much smaller size allowing them to contribute reproductively prior to harvest. Further, our preliminary analyses of growth rates (Culver et al., unpubl. data), albeit limited, suggest they may reproduce for many years prior to harvest. Molt increments decreased from around 16% for males between 64 and 100 mm CW to less than half that (~7%) for males above 120 mm CW. Additionally, results from our monthly samples indicated that males likely molt only once per year. Based on this slow growth rate, if functional maturity is close to physiological maturity, a 64 mm CW male brown box crab would have about six years to contribute to the population before reaching legal size.

While the catch size limit seems appropriate, size selective fisheries using a minimum size can have drawbacks (Conover & Munch, 2002; Fenberg & Roy, 2008; Varisco et al., 2019). By selecting for large males, there is a long-term potential to drive the population towards a smaller size at maturity and slow growing individuals, evolving towards small body size and lower fecundity. Some fisheries have implemented a slot limit (Ahrens et al., 2019; Pellowe & Leslie, 2020), restricting harvest to intermediate sizes, a measure to continue to allow reproduction prior to harvest while also protecting the genetics and reproductive potential of the larger individuals. Future studies on growth rates and mating success would help inform the determination of the most appropriate size limits for a sustainable fishery.

Another strategy that might be considered is seasonal closure. Many fisheries use seasonal closures to protect stocks when crabs are molting and/or reproducing (mating, eggs hatching). Because female brown box crabs are not currently harvested, and not likely to be harvested due to their small size, protection of hatching season is not needed. Instead, closing the fishery during the molting and mating season might be considered. Because molting and mating are basically synchronous and quite seasonal, a short-term closure during September and October would protect the majority of the mating/molting population should additional measures be required. The specific timing and duration of the closure would need to be periodically reassessed in light of ongoing environmental changes.

Lastly, if California brown box crabs do indeed have limited movement as reported (Colomy, 1989; Kato, 1992), rotational zone management could be an option. Density can play a large role in reproductive potential of marine organisms. Populations with a low density of reproductive males can result in lower reproductive success due to limited sperm resulting from high mating frequency, female waiting time for a mate or the inability to find a mate (Powell et al., 1974; Kendall et al., 2001; Sato et al., 2005). Use of rotating area closures could help to slow reductions of localized densities as seen with the sea cucumber fishery (Plagányi et al. 2015). Ongoing investigations of brown box crab movement by resource managers, together with investigations of density-dependent reproductive success (e.g. tracking number of barren mature females) is needed to better understand the potential utility of this management strategy for the brown box crab fishery.

Although the EFP provided a unique opportunity to collect information prior to implementing a large-scale brown box crab fishery, research was limited for several reasons. In particular, due to the structure of the EFP, the limited number, varying

experience, and varying desire to partake in scientific studies of participating fishermen created inconsistencies in the quantity and quality of samples. The regulatory nature of the program also resulted in unanticipated constraints that affected collection of research samples, especially small crabs caught incidentally in other fisheries. Additionally, the COVID-19 pandemic restricted our ability to conduct laboratory work and pick-up samples while simultaneously disrupting seafood supply chains, limiting ability of fishermen to harvest crabs. Overall, while essential fishery information was gathered, further research is needed to inform development of a large-scale commercial fishery for *L. foraminatus.*

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Table 1. Comparison of Duguid and Page (2011) egg development images to categories used in this study.

Duguid Figure	**Egg Development Category**
C/D	Diapause, no development occurring
E/F/G	Development initiated
H	Eye spots present, >80% yolk
I	Eye spots with ~75% yolk
-	Eye spots with ~50% yolk (not pictured in Duguid figure)
K	Eye spots with ≤ 25% yolk

Table 2. Fecundity of southern California brown box crab for 10 mm interval size classes. Note, for comparison to other studies, conversions were made to rostrum carapace length.

Size class of CW (mm)	n()	Min	Max	Mean	SD
60.0 - 69.9	1	8,352	8,352	8,352	NA
70.0 - 79.9	7	12,023	16,492	13,974	1778
80.0 - 89.9	12	16,318	27,247	20,449	3606
90.0 - 99.9	33	16,791	41,588	26,966	5260
100.0 - 109.9	19	24,895	51,587	36,864	7414
110.0 - 119.9	15	33,694	50,202	42,266	6138
120.0 - 129.9	4	42,362	51,245	46,918	4178
130.0 - 139.9	1	62,181	62,181	62,181	NA

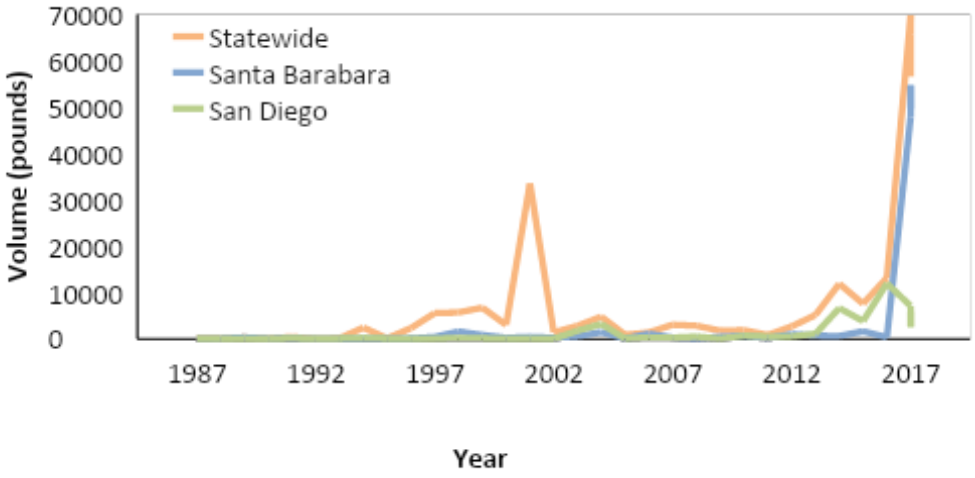

Figure 1. Statewide fisheries landing data of *Lopholithodes foraminatus* in California, 1987 – 2017. San Diego and Santa Barbara included for reference.

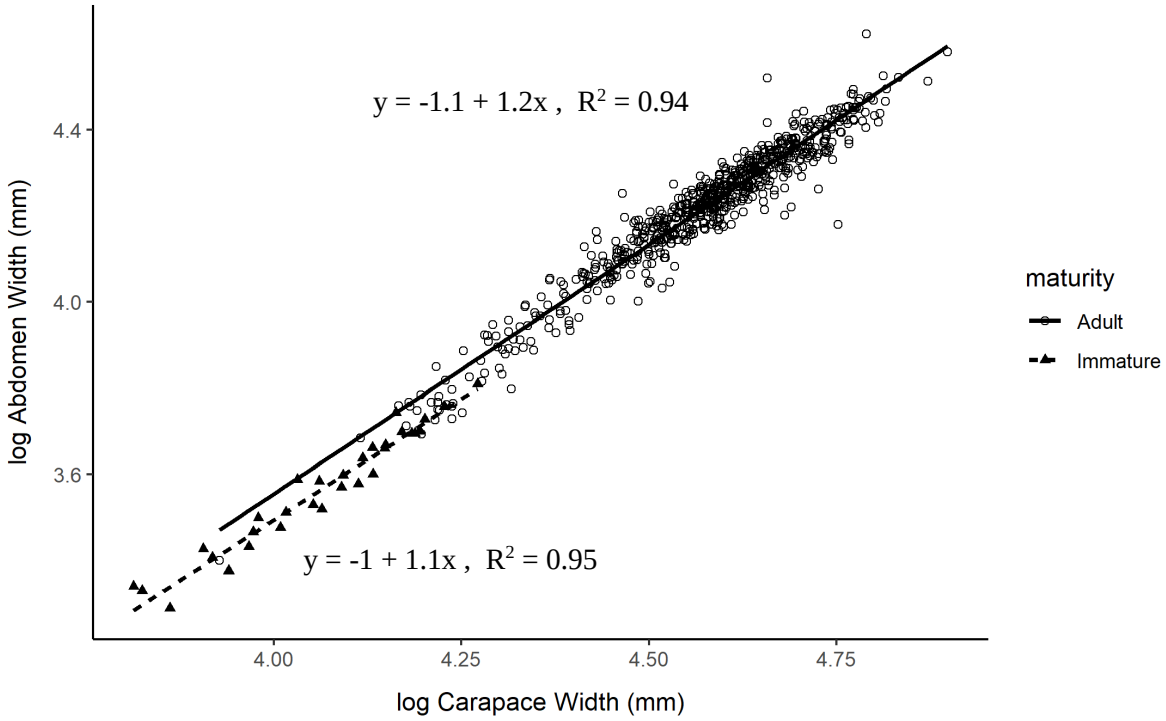

Figure 2. The relationship between abdomen width and carapace width for female box crabs, *Lopholithodes foraminatus*. Separate allometric equations have been fitted for adults (open circle and solid line) and juveniles (closed triangle and dashed line).

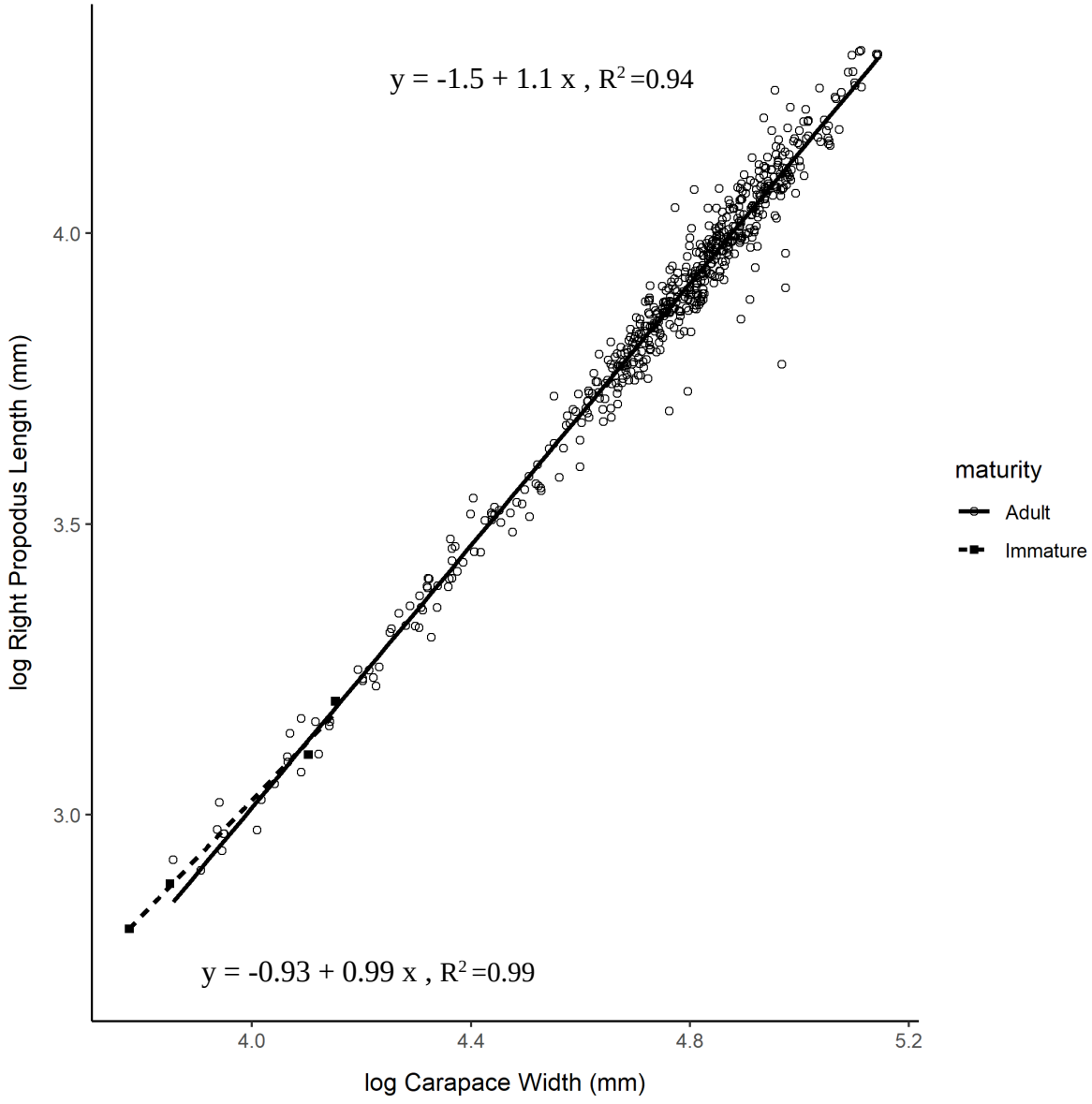

Figure 3. Relative growth of log carapace width to log propodus length for male *Lopholithodes foraminatus* by maturity group. Separate allometric equations have been fitted for adults (open circle and solid line) and juveniles (closed square and dashed line).

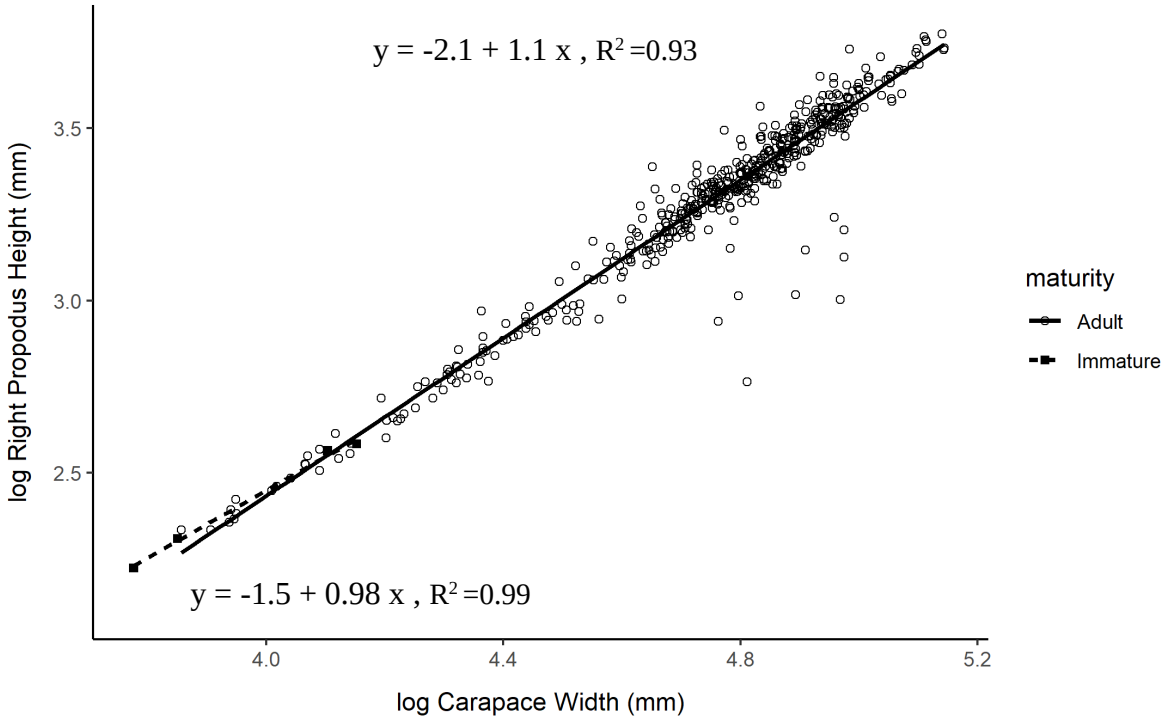

Figure 4. Relative growth of log carapace width to log propodus height for male *Lopholithodes foraminatus* by maturity group. Separate allometric equations have been fitted for adults (open circle and solid line) and juveniles (closed square and dashed line).

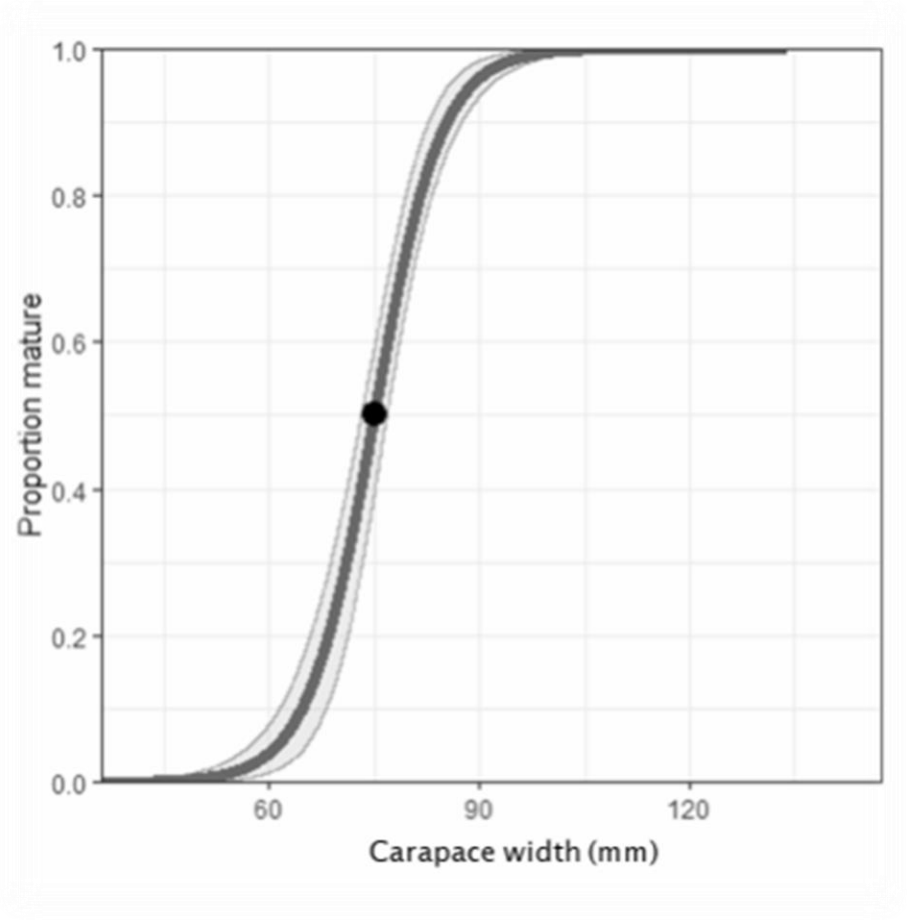

Figure 5. A curve fitting the proportion of females classified as mature by carapace width. The size at 50% maturity is denoted by the black filled circle.

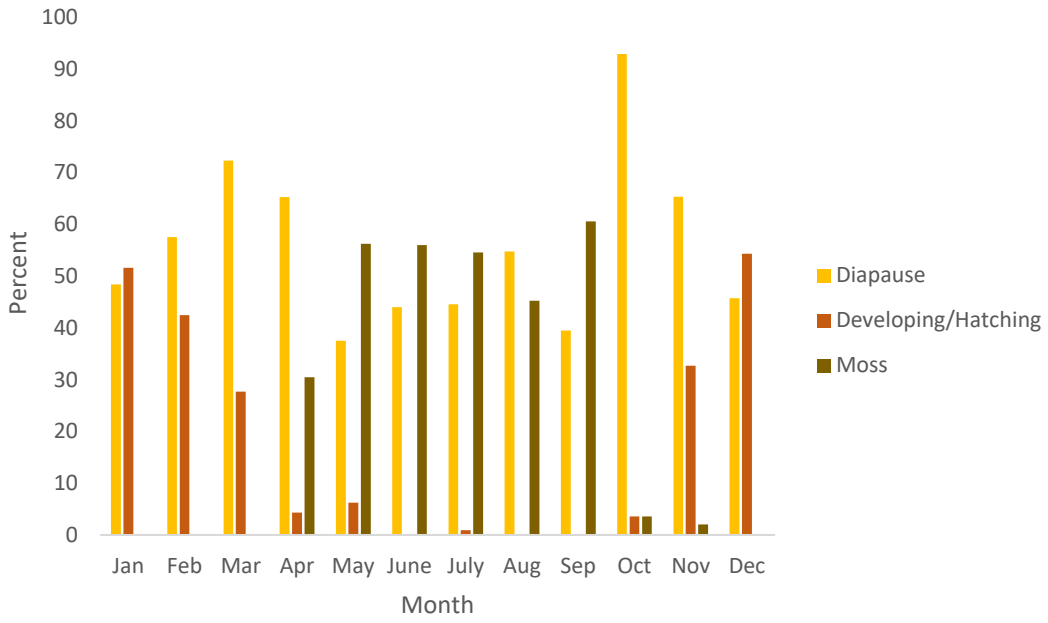

Figure 6. Seasonality of southern California brown box crab egg development.

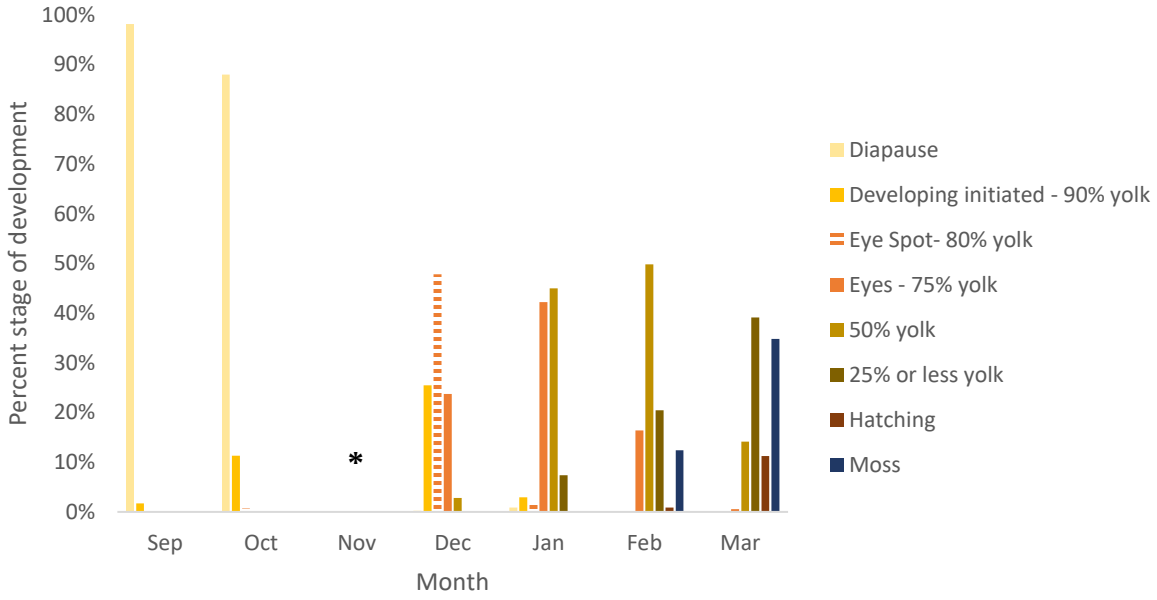

Figure 7. Asynchronous clutch development over time for laboratory held brown box crab. *Samples not taken in November.

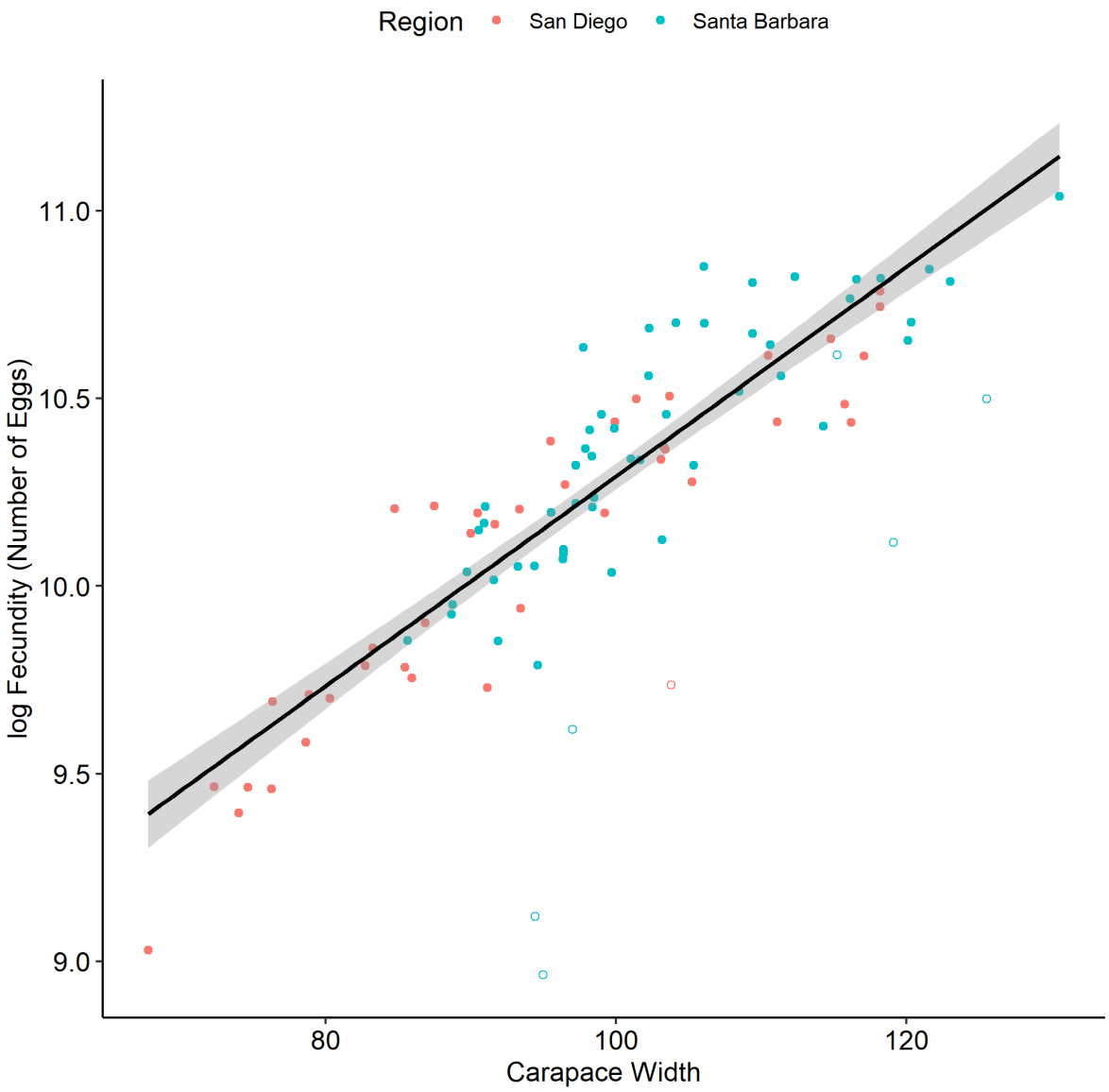

Figure 8. Relationship between log fecundity (F) and carapace width (CW). The straight line represents the linear relationship (n=93), F = 0.03 (CW) +7.49 (R^2= 0.83, p < 0.001). The shaded area indicates the 95% confidence interval. Small clutches (n=7) represented as open circles.

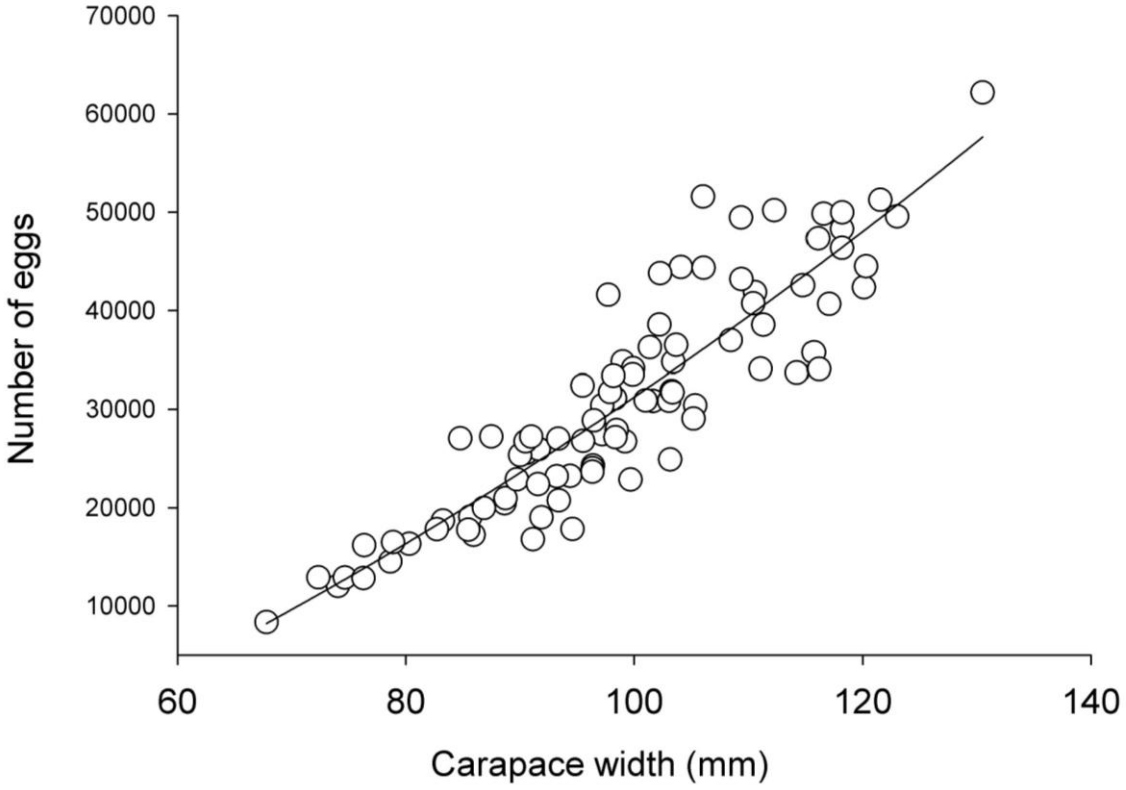

Figure 9. Quadratic relationship between fecundity and carapace width of the female brown box crab. $F = -23{,}707 + 306.4x + 2.4x^2$ ($R^2 = 0.81$, $p < 0.001$).

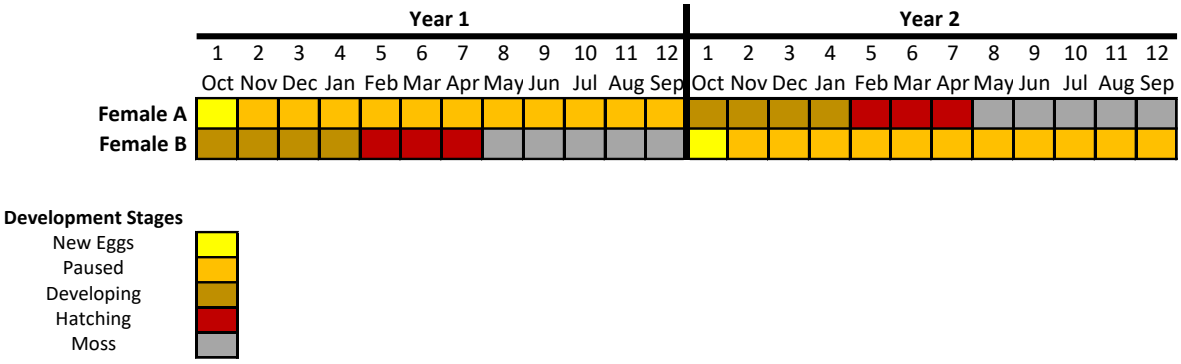

Figure 10. Timeline depicting general duration of egg development phases during biannual reproduction of the brown box crab. Female A mated and extruded new eggs in October of Year 1. Female B mated October the prior year (year 0) to the depicted Year 1.

Appendix A

Conversion Equations

for Box Crab Measurements

Studies of *Lopholithodes foraminatus* use a variety of measurements to define crab size and associated anatomical attributes. To address this issue and enable comparisons across studies, we took additional measurements and developed calibration equations using linear regression. Measurements were recorded using a digital caliper (0.01 mm) as described and shown in Table 1 and Figure 1. All measurements were compared to carapace width (CW), our standard measurement for this study, with males and females reported separately. We used these equations to calculate measurements that enabled comparisons across studies for our manuscript. Our equations complement those provided by Zhang et al. (1999), which represent female and male crab measurements combined.

Table 1. Definition of measurements for the brown box crab. Number corresponds to measurements in Figure 1.

Attribute (abbreviation)	**Number**	**Measurement**
Carapace length (CL)	1	Rostral teeth to posterior carapace edge
Orbit length (OL)	2	Right eye orbit to posterior carapace edge
Mid carapace width (mid CW)	3	Midsection not including spines
Carapace width (CW)	4	Widest point not including spines
Max carapace width (max CW):	5	Widest point including spine
Propodus length (PL)	6	Posterior surface from tip to joint
Propodus height (PH)	7	Posterior surface from joint to base
Total body wet weight (wgt)	8	Measured to the nearest 0.5 gram

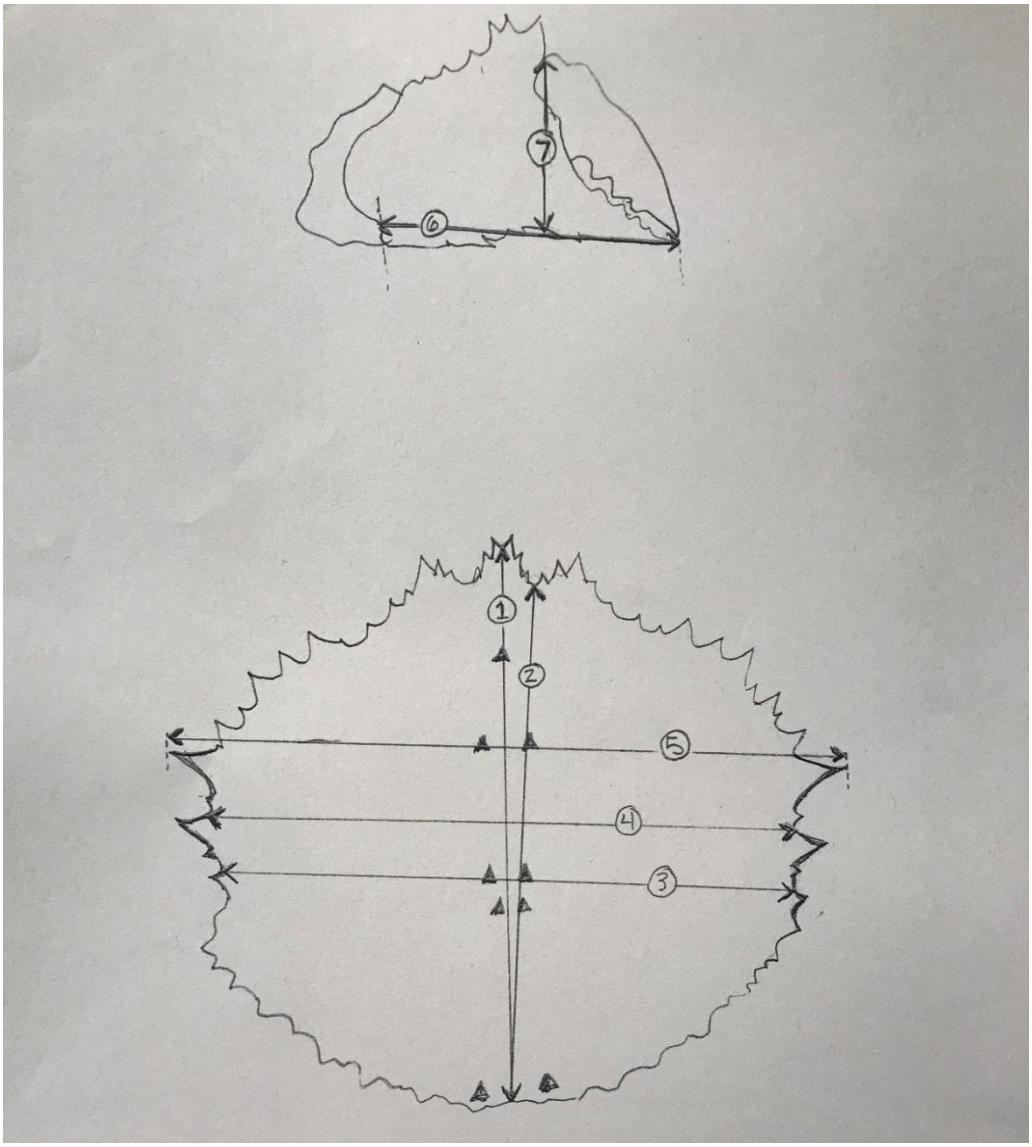

Figure 1. Illustration of brown box crab measurements. Number corresponds to measurements defined in Table 1. Drawing adapted from Goddard (1997).

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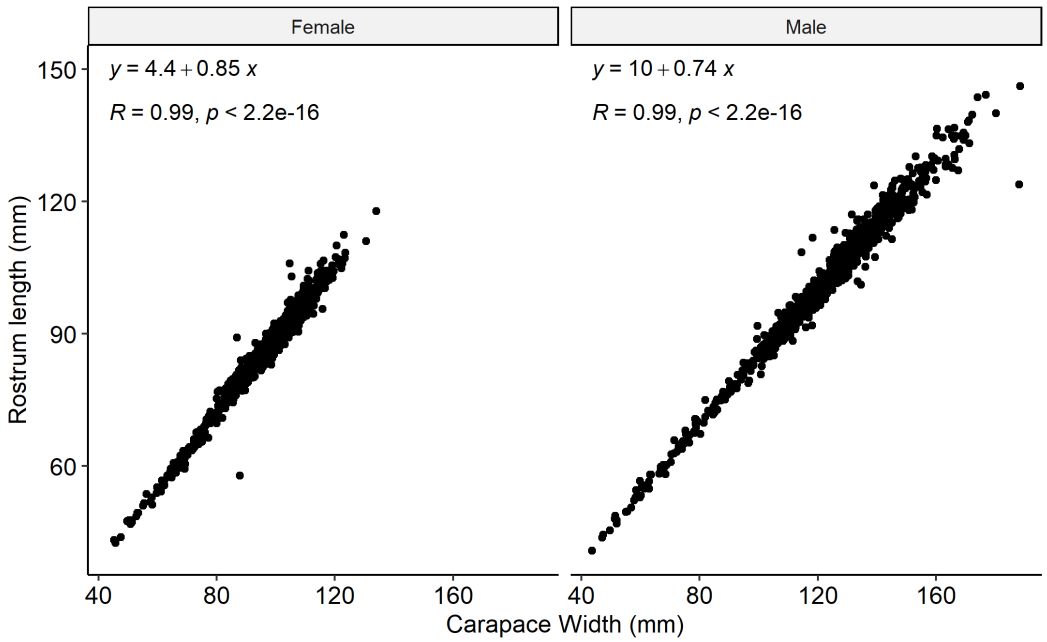


Figure 2. The relationship between carapace width and carapace length for female and male brown box crabs, *Lopholithodes foraminatus*

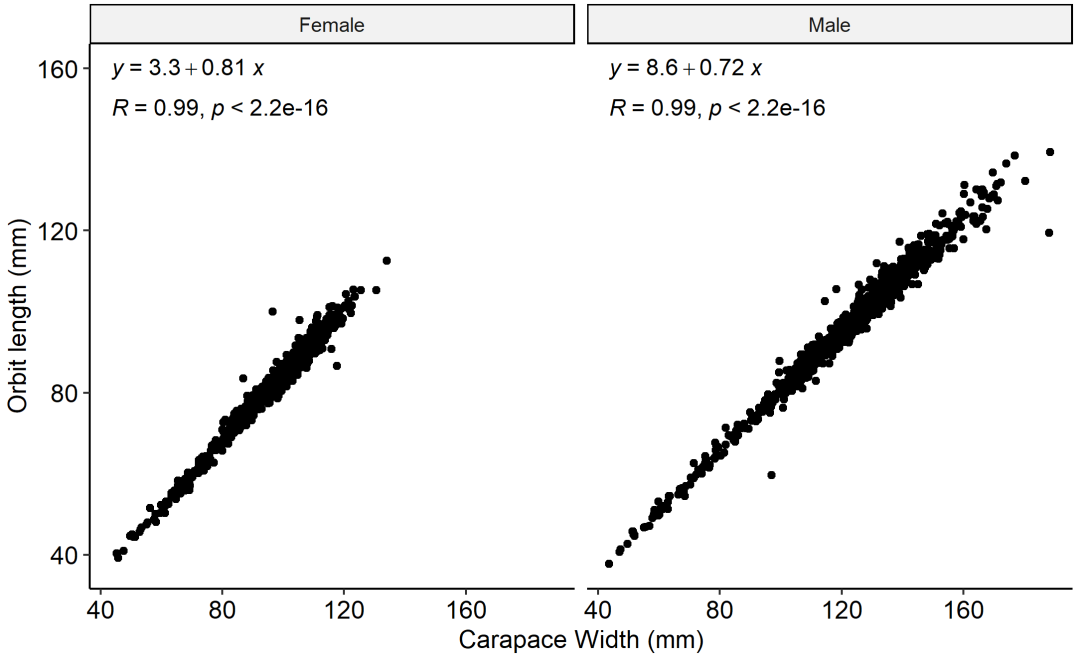


Figure 3. The relationship between carapace width and orbit length for female and male brown box crabs, *Lopholithodes foraminatus*

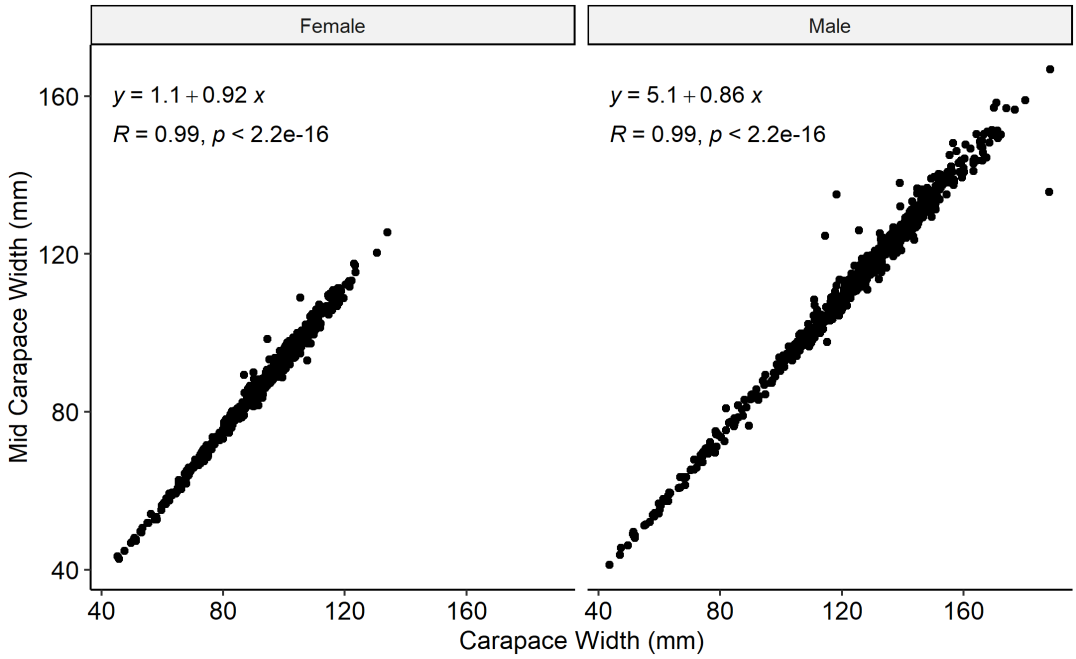


Figure 4. The relationship between carapace width and mid carapace width for female and male brown box crabs, *Lopholithodes foraminatus*

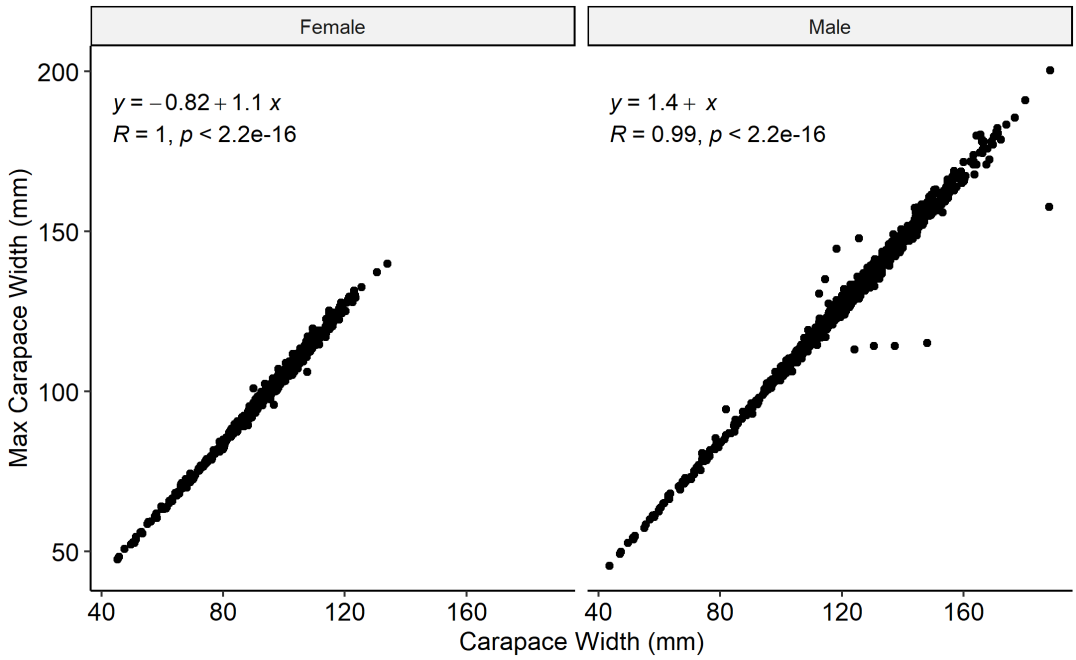


Figure 5. The relationship between carapace width and mid carapace width for female and male brown box crabs, *Lopholithodes foraminatus*

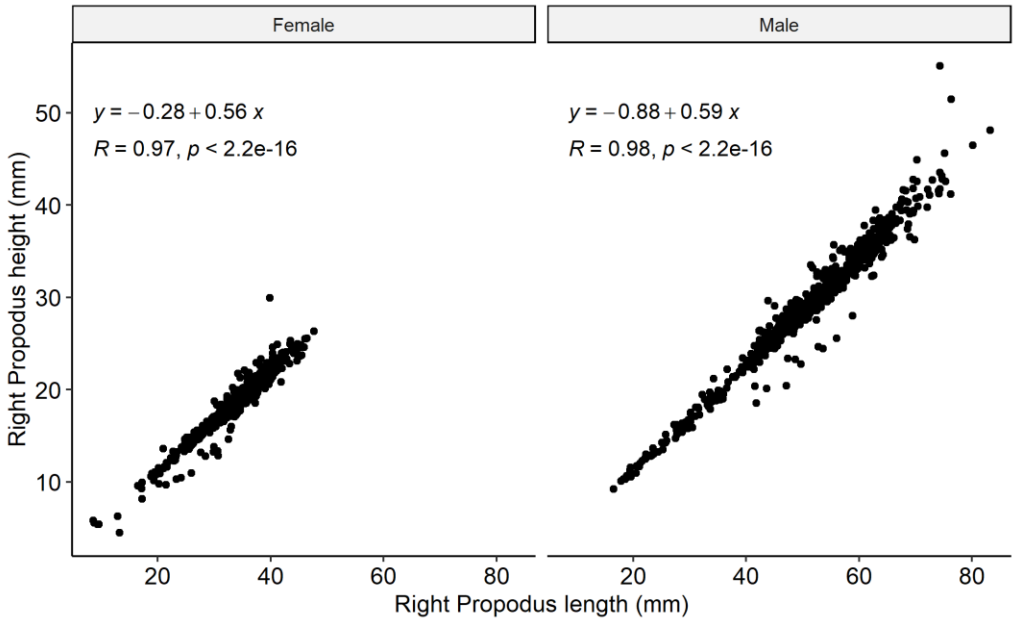


Figure 6. The relationship between right propodus length and right propodus height for female and male brown box crabs, *Lopholithodes foraminatus*

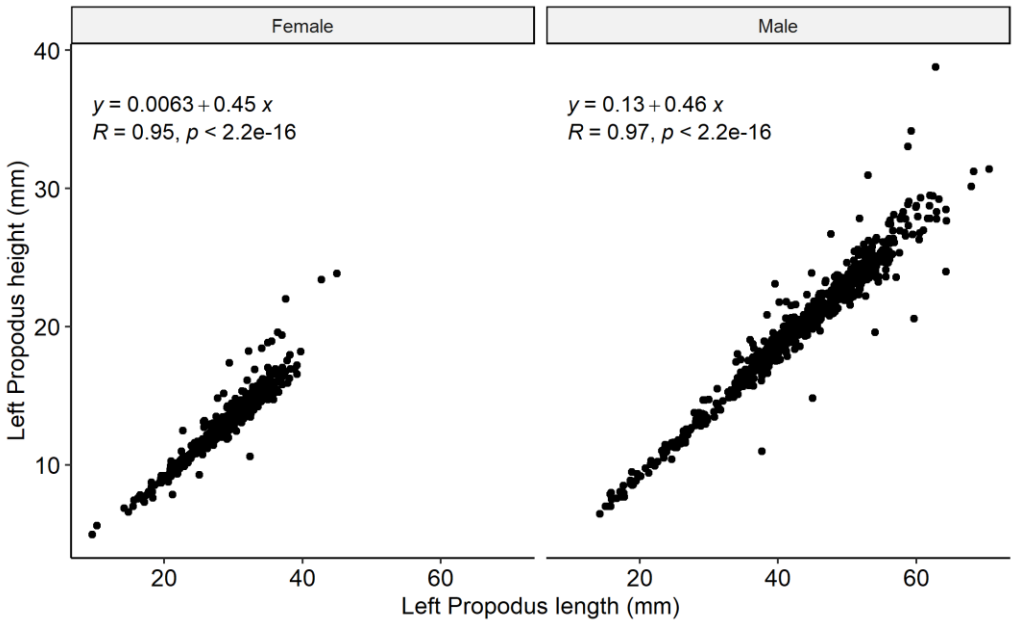


Figure 7. The relationship between left propodus length and left propodus height for female and male brown box crabs, *Lopholithodes foraminatus*

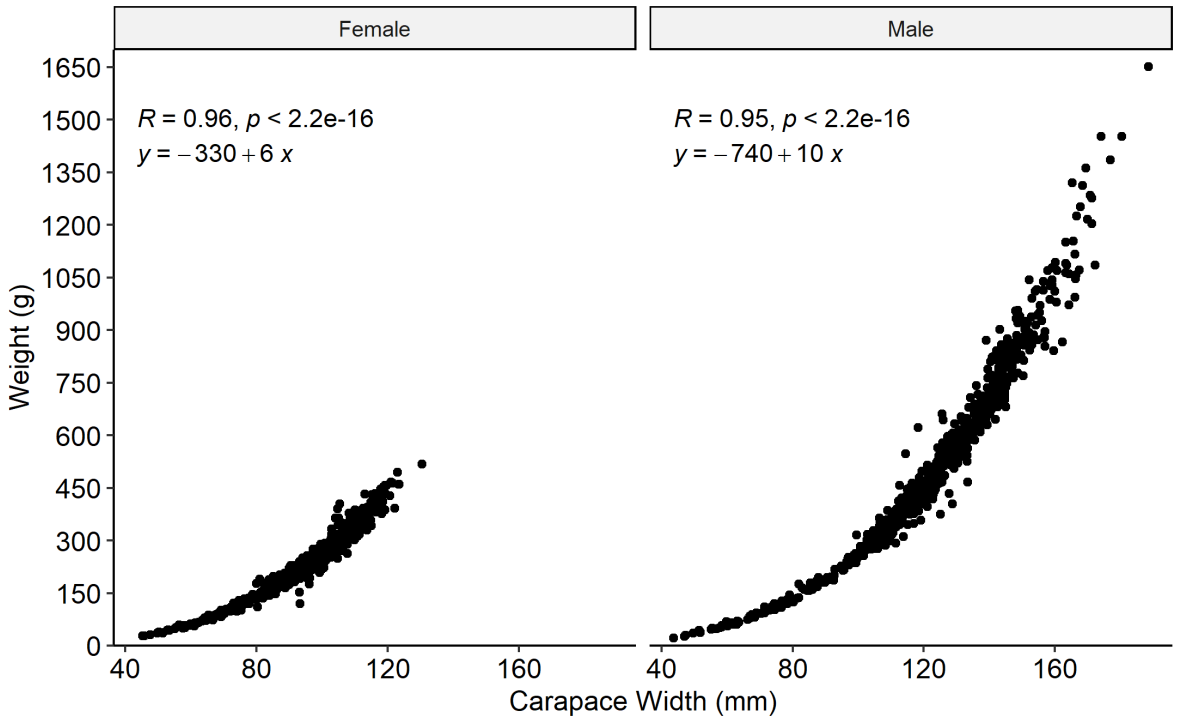


Figure 8. The relationship between carapace width and weight (g) for female and male brown box crabs, *Lopholithodes foraminatus*

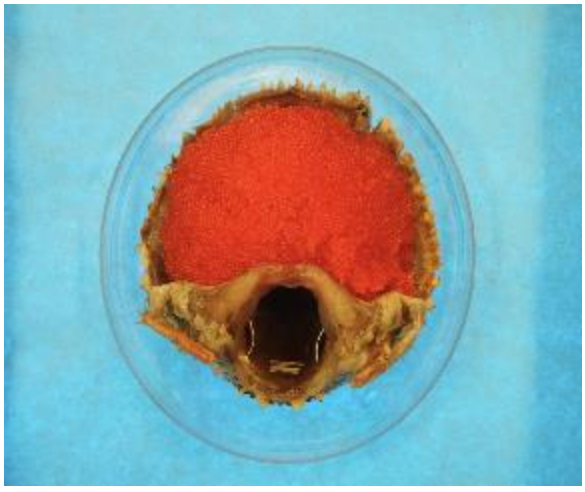
Appendix B

Egg Separation and imageJ egg count protocols

Modified from Bohenek 2017
Citation: Patrick, C., L. Enright, A. Stroud and C. Culver. 2020.
Software: ImageJ ver 1.8.0

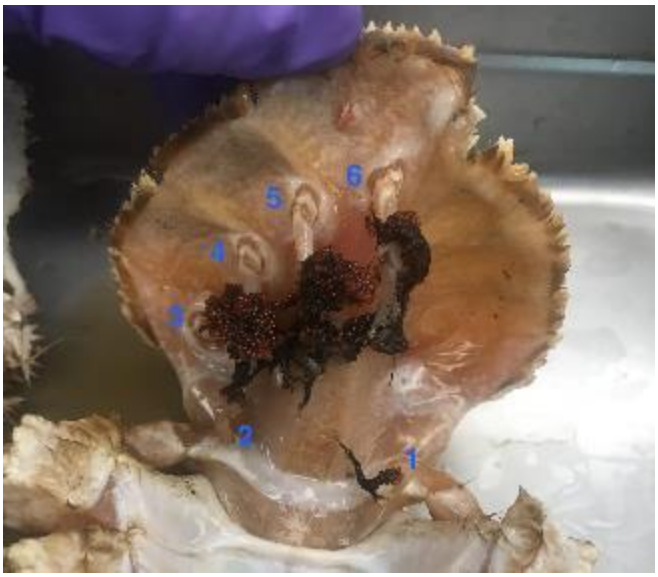
Defrosting the Pleopods

1. Place the frozen abdominal flap of the crab in a plastic container filled with warm water. Do not remove the abdominal flap from the plastic bag.
    a. TIP: Make sure the plastic bag is sealed all the way. Exposing the eggs to fresh water can make it difficult to separate the eggs & will lead to more breakages.
2. Allow the abdominal flap to defrost for 5-10 minutes.
3. Remove the plastic bag from the water and extract the abdominal flap from the bag.
4. Place the abdominal flap in a petri dish. Using the camera, take a “brood photo” before removing any pleopods or eggs. This is a reference photo that may be used at a later time



Pleopod numbering

1. Each abdominal flap should have six pleopods. These are appendages within the abdominal segment where the eggs are attached.
2. Pleopod number, as defined in the below picture, is to be recorded along with the affiliated egg count.



Pleopod Removal

1. Begin with Pleopod #1 (according to numbering above). Remove in numerical order from 1 through 6.
2. Gently grasp the base of the pleopod close to the abdominal flap with forceps. Be careful to not crush any of the eggs.
3. Pull the pleopod outwards from the abdominal flap. Do not rip the base of the pleopod off the abdomen. The pleopod should separate easily from the other

pleopods while maintaining all attached eggs and remaining connected to the abdominal flap.

4. Using dissecting scissors, carefully cut the base of the pleopod, separating it from the abdominal flap. Perform the cut as close to the base as possible, in an area where there are no eggs attached to the pleopod. This ensures that all eggs attached to the pleopod remain intact and accounted for.
5. Occasionally during defrost or pleopod removal, you may notice some eggs that are not attached to any pleopod, such as eggs that are loose in the ziploc bag. Be sure to COUNT and RECORD the quantity of these loose eggs.

Note: Often, you cannot finish an entire sample in one day. Samples can be refrozen but be sure there is no excess seawater in the ziploc. When eggs are refrozen with excess seawater, they more readily break and can be difficult to separate

Separation of Eggs

1. Place the pleopod into a plastic or glass petri dish.
2. Add seawater to the petri dish until the bottom of the dish is covered with water, filling the dish up to less than halfway with seawater.
3. Place the petri dish under the dissecting microscope and adjust the lens so that sections of the pleopod and the end of a spatula can be seen.
4. Grip the base of the pleopod with a pair of forceps, and gently scrape the eggs off the pleopod using a spatula. Use downward scraping motions, as well as motions parallel to the dish, to remove the eggs until all eggs have been separated from the main

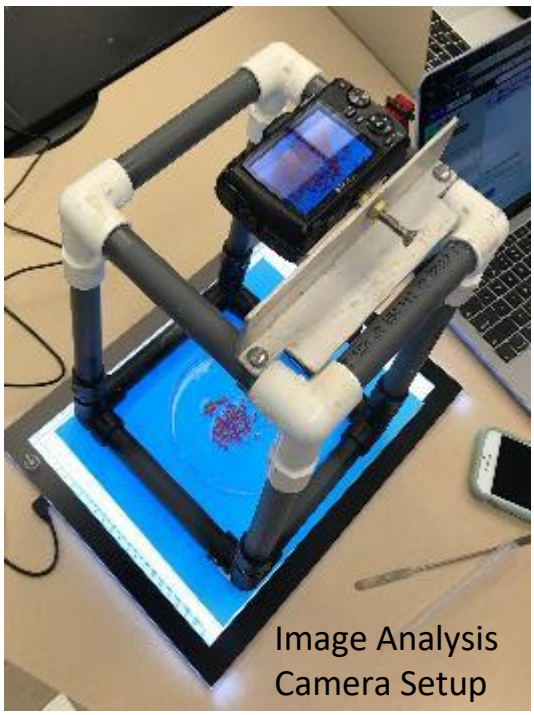
section of the pleopod. Once this has been completed, remove the eggless pleopod stalk from the petri dish.

5. Zoom in on the eggs so that individual eggs can be clearly visualized, as well as the connective tissue between the eggs. Using two forceps, or a spatula and forcep, separate the eggs from each other. The latter method is better for separating larger clumps of eggs, while the former is better for separating small clumps of 2-3 eggs. One pair of forceps can be used to hold the connective pleopod tissue between the eggs while the other is used to pull the eggs apart, breaking the connective setae. Be careful using the spatula method, as it can result in more broken eggs. All broken eggs should be recorded.
6. Some eggs may be discolored (opaque white or grayish with a red spot in the center). These eggs are naturally deceased (often via bacteria). These eggs should not be counted in the main count, as they will not become larvae. However, the eggs should be separated, photographed and count recorded to help determine mortality.

Photographing the Eggs

1. After the eggs have been fully separated, divide the eggs into separate plastic or glass petri dishes. The number of petri dishes used will vary by pleopod and crab. Each petri dish should only be covered ½ to ⅔ in area by eggs so there is enough spacing for eggs to be recognized by ImageJ.
2. Set up the photography station (see image below). Use a lighted tracing board for the base, and place a blue sheet of paper on top to create a contrasting background for

the eggs. Center the photography apparatus on the paper, and screw on the digital camera pointing downwards per the stand set-up protocol.



3. Place a petri dish on the blue paper so that it is centered in the frame of the camera. Adjust the zoom of the camera to 3X so the petri dish fills the frame. Keep the level of zoom consistent for all photos.
4. Add a small amount of seawater to the petri dish so the eggs are slightly floating and movable. Use a spatula to move the eggs away from the edge of the dish, and separate the eggs from each other to minimize clumping. Eggs should not be overlapping and should be spread in the dish as a single layer.
5. Turn the lights off in the lab to maximize contrast between the eggs and the background in the photo. This is important for processing in ImageJ later.

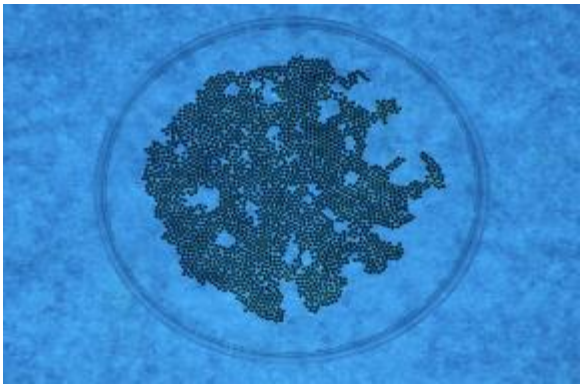
6. Take a photograph using the digital camera. Record the camera photograph number and the amount of seawater
7. Repeat the process using different amounts of seawater to create a photograph with as much distinction between the eggs as possible. Levels of seawater (with advantages and disadvantages) include:

    a. No seawater (minimizes mobility of eggs, can cause clumping)

    b. Some seawater (lessens mobility of eggs, allows for separation)

    c. Full of seawater (causes mobility of eggs, allows for more separation)

    Note: Avoid individually floating eggs; as they are easily excluded in imageJ.

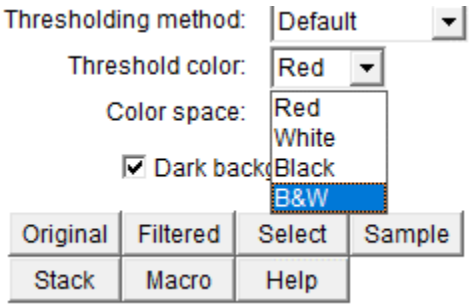


ImageJ Analysis (Automated Egg Counting, ImageJ ver 1.8.0)

1. Open ImageJ software and load the Image to analyze from File → Open
2. If necessary, crop the image by selecting the rectangular selection tool  , make a selection over the petri dish, and press Ctrl+Shift+X to crop the image.

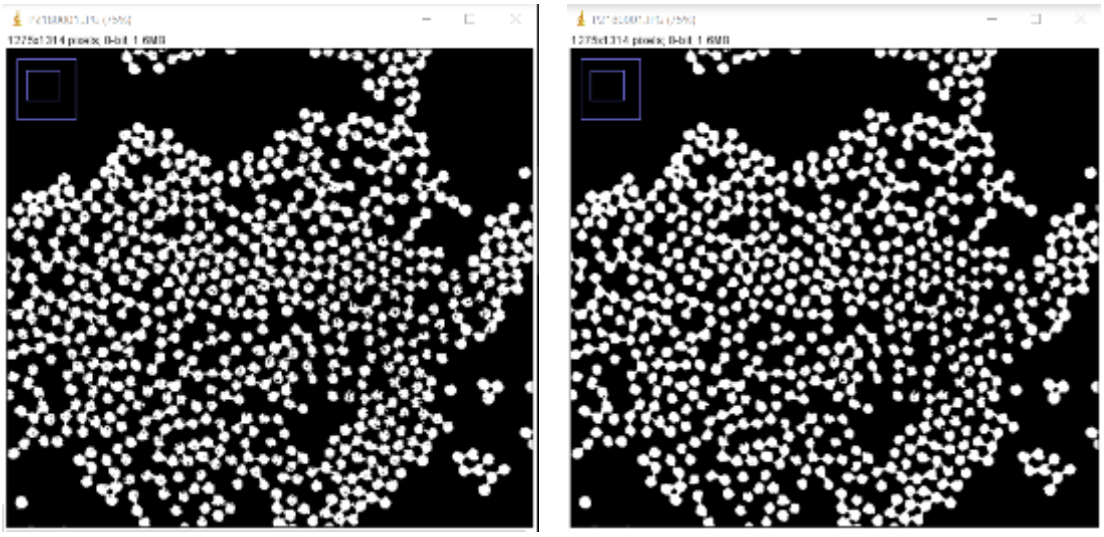
3. Go to Image → Adjust → Color Threshold to select the eggs apart from the background. Modify Threshold color from Red to B&W from the drop down box. Note that on older versions, the B&W is set to recognize black as objects and white as background. As per version 1.8.0, this is reversed, where white is the object and black is the background.

   (Note: If the eggs appear black and the background is white, just unclick "dark background")

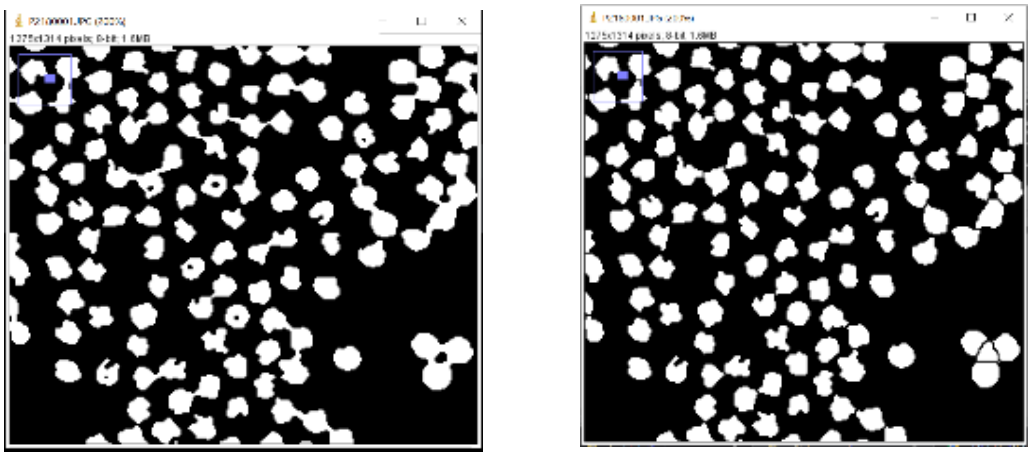


4. Adjust the brightness to include darker shades by moving the upper slider to the left to capture the darker edges of the eggs. The bottom slider value will vary depending on the photo. However, starting at a value of 121 (as pictured below) is a good baseline for making adjustments.

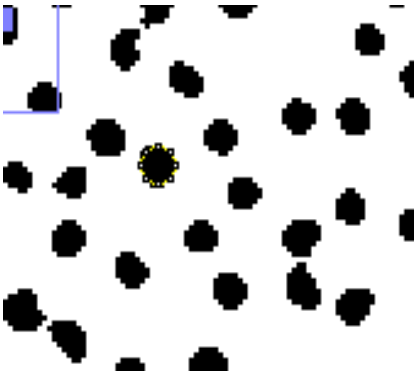
5. Close the Color Threshold window and go to Image → Type → 8-bit. This will make the image black and white.
6. Go to Process → Noise → Despeckle to make the edges of the eggs cleaner. This can be done multiple times as needed to get clean silhouettes of the egg.



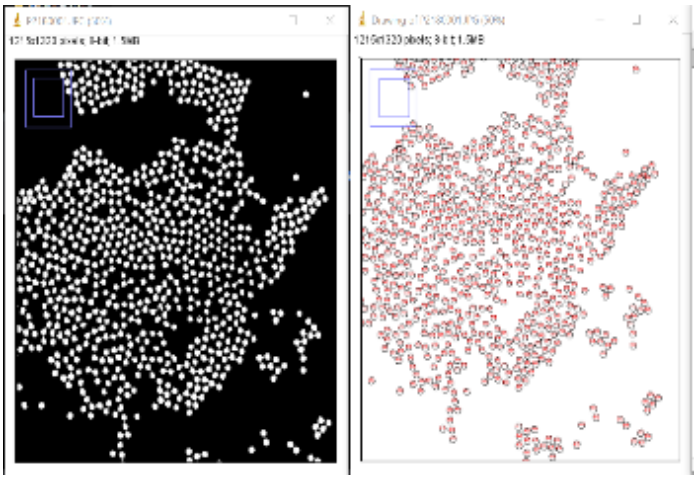
7. Go to Process → Binary → Watershed. This causes ImageJ to introduce a 1 pixel gap between shapes where the computer's algorithm thinks edges of objects are touching.

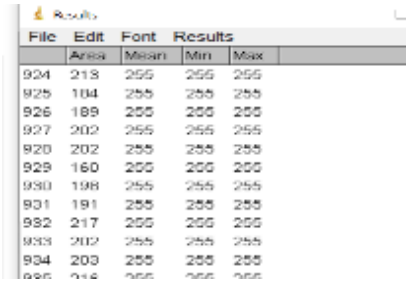


8. Click Analyze → Analyze Particles. A new window asking for analysis parameters will open. Set the size boundary to be 5 to infinity to exclude pixels that are noise. On the dropdown box for Show, choose Outlines. Make sure that Display results and Summarize are checked, and click OK.



9. A new window containing the outlines of the counted shapes, and a window summarizing the number of objects counted for the image will show. Record the results.





NOTE: Sometimes the photos taken in the lab are not compatible with ImageJ processing. For example, the eggs are too close together or there is not enough contrast. If this happens, retake the photo.

Manual Counting of Eggs on ImageJ

1. Open ImageJ software and load the Image to analyze from File → Open
2. If necessary, select the rectangular selection tool  , make a selection over the petri dish, and press Ctrl+Shift+X to crop the image.
3. Select the Multi-point tool 
4. Click on the eggs to count.