Determining the age-size relationship of *Panulirus argus* in the southwest area of Puerto Rico

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ABSTRACT

The Caribbean Spiny Lobster, alongside the queen conch, holds the highest economic value in Puerto Rico's fisheries. Given its considerable importance in commercial fishing, there are concerns regarding its potential overexploitation. Accurate information regarding the Caribbean Spiny Lobster population is crucial for conducting stock assessments. Parameters such as size, weight, age, and growth are vital for comprehending the dynamics of a population. This study aimed to estimate the age structure of *P. argus* in southwest Puerto Rico using ossicles and to establish length-age and weight-age relationships critical for stock assessment. A total of 100 lobsters were collected across southwest Puerto Rico and processed for aging using ossicles, but only 35 lobsters yielded consistent ages with high confidence between readers. Carapace lengths ranged from 60 to 140 mm, weights ranged from 191 to 1945 g, and ages ranged from 1.5 to 6.5 years across all lobsters with no detectable differences between male and female lobsters. Despite the uncertainty around the estimates of age with only 35 samples, this study shows great potential to determine age in *P. argus* and contribute valuable data for stock assessments and the management of this population. Results showed positive relationships between age and carapace length, age and weight, and carapace length and weight. Von Bertalanffy growth models indicated varying growth rates and maximum lengths among different populations around the Caribbean. While this method benefits higher latitude populations, its application in the Caribbean warrant's further investigation. The study's findings contribute valuable data for stock assessments and management of *P. argus* in Puerto Rico, highlighting the need for continued research and collaboration for sustainable fisheries management.

RESUMEN

La langosta espinosa caribeña, junto con el Carrucho, tiene el mayor valor económico en las pesquerías de Puerto Rico. Dada su importancia en la pesca comercial, existen preocupaciones sobre su posible sobrepesca. La información sobre la población de langosta espinosa caribeña es crucial para realizar evaluaciones de poblaciones. Parámetros como el tamaño, el peso, la edad y el crecimiento son esenciales para comprender la dinámica de una población. Este estudio tuvo como objetivo estimar la estructura de edades de *P. argus* en el suroeste de Puerto Rico usando osículos para establecer relaciones longitud-edad y peso-edad. Se recolectaron un total de 100 langostas en todo el suroeste de Puerto Rico y se procesaron para determinar su edad utilizando osículos, pero solo 35 langostas arrojaron edades consistentes con alta confianza entre los lectores. Las longitudes del caparazón oscilaron entre 60 y 140 mm, los pesos oscilaron entre 191 y 1945 g, y las edades oscilaron entre 1.5 y 6.5 años en todas las langostas sin diferencias detectables entre langostas macho y hembra. A pesar de la incertidumbre en torno a las estimaciones de edad con solo 35 muestras, este estudio muestra un gran potencial para determinar la edad en *P. argus* y contribuir con datos valiosos para evaluaciones de existencias y el manejo de esta población. Los análisis preliminares revelaron discrepancias en las estimaciones de edad entre los lectores y las calidades de las imágenes, lo que requirió un filtrado riguroso de los datos. Los resultados mostraron relaciones positivas entre la edad y la longitud del carapacho, la edad y el peso, y la longitud del carapacho y el peso. Los modelos de crecimiento de Von Bertalanffy indicaron diferentes tasas de crecimiento y longitudes máximas entre diferentes poblaciones alrededor del Caribe. Si bien este método beneficia a las poblaciones de latitudes más altas, su aplicación en el Caribe justifica una mayor investigación. Los hallazgos del estudio aportan datos valiosos para las evaluaciones y el manejo de las poblaciones de *P. argus* en Puerto Rico, destacando la necesidad de investigación y colaboración continuas para el manejo pesquero sustentable.

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I extend my sincere gratitude to Juan Cruz Motta, Travis Courtney, and Thomas Mathews for their invaluable guidance and encouragement throughout this process. I am also deeply appreciative of Emily Hutchinson for her exceptional training and generosity in sharing her expertise with me. For assistance in the field and laboratory I would like to thank: Veronica Seda, Benigno Rodriguez, Jorge Morales, Fabian Chaparro and Noemi Pena. And many thanks to Aida Rosario, Leysa Lopez, Idalmis Santos, Raymond Infante, Roy Marenco and Darimar Davila for their help on the methodology and the computer programs. Thanks to Michael for this loving encouragement, and thanks to my parents, Ireliz Martinez and Marco Medina, for their unconditional love and support.

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1. INTRODUCTION.

The Caribbean Spiny Lobster (*Panulirus argus*) represents up to 30% of the total commercial catch in Puerto Rico (Valle et al. 2016) and is one of the main commercially valuable species in Puerto Rico alongside the Queen Conch (*Aliger gigas*) and reef fish (i.e. snappers and groupers) (Tonioli et al. 2011). While the spotted spiny lobster, *P. guttatus*, is also reported in the catch, *P. argus* is the most abundant lobster in the archipelago of Puerto Rico (SEDAR, 2019). Due to its commercial importance, some studies have proposed that *P. argus* is a highly exploited crustacean in Puerto Rico (Mateo, 2004), where most of its catch (45%) came from the south coast of Puerto Rico, followed by the west coast (35%), and 15% from the east coast (Mateo, 2004). Catch rates have varied regionally in Puerto Rico with the largest amount of spiny lobster pounds caught between 1998 and 2003 along the south and between 2004 and 2013 along the west coast (Matos-Caraballo D. et al. 2019).

In the U.S., fishery management actions, plans, and rules of commercially important species are mostly based on stock assessments of individual species or closely related groups of species (NMFS 2021). The way stock assessments are conducted are very heterogeneous, but all models are based on basic population parameters such as age-length structure, age-weight structure, sex structure, size frequencies, densities, distribution, mortality (natural and fishing), and growth, among others (NMFS 2021). In particular, the question of age is very important to a stock assessment for estimating or constructing age-size or age-weight relationships (Maxwell et al. 2007). In Puerto Rico, signs of lobster overfishing were recognized in the Lobster Fishery Management plan in territorial and federal waters in 1985 (Matos-Caraballo, 1999). However, the maximum sustainable yield (MSY) couldn’t be estimated for Caribbean Spiny Lobster stocks because of the lack of a spawner-recruit relationship for the panmictic Caribbean wide stock (SEDAR 2019). Since then, various studies have suggested that Puerto Rico’s population is close to overfished (SEDAR 2019, Matos 2001) owing to declining lobster catches and mean carapace length decreasing through time (Mateo 2004, Matos-Caraballo 1999). However, during the last SouthEast Data Assessment and Review process (SEDAR 57) of *P. argus*, it was determined that the resource was not subject to overfishing; yet due to lack of data, there is a high degree of uncertainty around this conclusion (NMFS, 2023).

The larval stage of *P. argus* is relatively long as it lasts between 6 to 12 months (Monterrosa 1991, Goldstein et al. 2008) which has resulted in a single, fully genetically mixed pan-Caribbean population connected through larval dispersal (Ehrhardt 2005, Truelove et al. 2017). Through ontogenic changes, the juvenile stages move further from shore to offshore reef systems and grow to maturity in structurally complex habitats such as undercut coral heads, rocks, and other types of hiding shelters (Childress and Herrnkind 1994, Ramos-Aguilar et al. 2003, Lozano-Álvarez et al. 2009). Adult lobsters primarily inhabit reefs and hard bottom habitats at depths determined by their thermal tolerances (Tonioli et al., 2011). However, various factors could potentially influence the migration patterns of *P. argus* (Saul, 2004). Typically, the planktonic phyllosomes are retained in the currents to coastal areas, where they metamorphose to pueruli (Wolfe and Felgenhauer 1991, Yeung and Lee 2002, Briones-Fourzán 2008, Segura-García et al. 2019). As postlarval lobsters (pueruli) they distribute themselves in seagrass meadows (*Thalassia testudinum*), mangrove prop roots (*Rhizophora mangle*), and macroalgae habitats (Booth et al. 1994, Tonioli et al. 2011). After a few months, these early-stage benthic juvenile lobsters look for crevice shelters such rocks, holes, ledges, and undercut coral heads (CFMC 2004). After a year of benthic life, they transition into sub-adults and can be found in mangrove, seagrass, reef, and hard bottom environments (Childress & Herrnkind, 2001, Behringer et al. 2009). Finally, adults typically gather in groups in dens and shelter settings, such as burrows and rocky outcrops, to form communal living arrangements (Saul 2004). Despite a deep understating of the life cycle of *P. argus*, some basic biological information is still elusive such as age structure (Leland et al. 2011, Kilada et al. 2012).

Several authors (Mateo, 2004; Kilada et al., 2012; Leland et al., 2017) have emphasized the importance of understanding the age of animals for crustacean stock assessment. However, determining the age of lobsters has proven to be challenging (Leland et al., 2011; Kilada et al., 2012). While the age determination technique based on lipofuscin accumulation was successful in limited circumstances (Matthews et al. 2009) this technique was based on physiological age and likely not suitable across the broad geographic range of the population. In this sense, it has been proposed that age determination using the ossicle of lobsters, may be a viable technique to determine lobster age and support stock assessment (Gnanalingam et al. 2018). The spiny lobster together with five other crustaceans (Leland et al. 2011) appear to record annual growth bands in the ossicle, which is a calcareous structure inside their stomach (Gnanalingam et al. 2018). This structure is inside the gastric mill, located within the anterior chamber of the foregut and is used to grind food (Wolfe et al. 1991, Mikami & Takashima 2000). The gastric mill consists of four calcified ossicles that have bands with broad translucent zones bordered by narrow opaque zones that appear to represent annual age (Leland et al. 2011). Before the structural bands in the ossicles were discovered, it was assumed that crustaceans didn't have any hard structures that stored the growth information because of their molting (Becker et al. 2018). Gnanalingam et al. (2018) reared *P. argus* spiny lobsters from capture at age 0 (as pueruli) to 10 years of age in their laboratories and successfully related the number of bands in the ossicles with the actual known age of the lobsters. As such, the Gnanalingam et al. (2018) approach has been used in other studies around the world to determine lobster age in different species (Kilada et al. 2012; Leland et al. 2011; Sheridan et al. 2016; Huntsberger et al. 2020). However, some critiques were raised because the age-size relation is only applicable to the studied population as it has been shown that environmental factors exert an important influence on the growth rate of lobsters (Kilada et al. 2012). In addition, Sheridan et al. (2016) concluded that the ossicle bands of the Norway lobster (*Nephrops norvegicus*) are not annual but are a result of the post molt calcification processes. Therefore, potentially not all the gastric mill ossicles of different species of lobster can be used to determine annual growth. Similarly, other authors have shown that growth information is not reliably stored in ossicles for all crustaceans, as bands are lost through molting in the white clawed crayfish (*Austropotamobius pallipes*, Sheridan et al. 2016) and the shore crab (*Carcinus maeans*, Sheridan & O'Connor 2018, Becker et al. 2018). To identify if the lobsters retained the ossicle thought molting, juvenile lobster were marked with fluorescent calcine, before or during molting (Kilada et al. 2012). This chemical tagging confirmed that any mineral features of the cuticle are perpetuated through molting; suggesting that regardless of the specific mechanism for the creation of the bands (i.e. still unknown), annual growth bands were maintained for the specific case of the Caribbean spiny lobster (*P. argus*) (Huntsberger et al. 2020). While the mechanism of age-band formation and retention remains unknown, the verification of the correlation between bands in ossicles and age in known age lobsters creates an important fishery research and management opportunity (Huntsberger et al. 2020).

In this study, the Gnanalingam et al. (2018) methodology was used to estimate the age of Caribbean spiny lobsters (*P. argus*) in southwest Puerto Rico using their ossicles. The main objective of this study was to use the ossicle growth bands to estimate and construct the age structure of *P. argus* population of the southwest coast of Puerto Rico and compare this to the respective carapace length and age to construct length-age and with the weight to construct weight-age relationships, which are fundamental for stock assessment. This study therefore fills critical knowledge gaps to support determination of the status of *P. argus* population in Puerto Rico (Matos 2001, SEDAR 2019, NMFS 2023) to inform decision making for the Lobster Fishery Management Plan.

2. MATERIALS AND METHODS

2.1 Field sampling

Samples used in this study were collected between September 2021 and July 2022 near the Coast of Cabo Rojo, southwest Puerto Rico (Figure 1). This area is an extended and shallow shelf, which facilitates the use of diving for fishing (Tonioli et.al 2011). Lobsters were collected in 123 different diving sites, stratified by depth: Shallow (0-10 m), Medium (10-20 m), and Deep (20-30 m), however this stratification was not used for further analyses. The amount of lobster captured was 301, from those 140 were selected for ossicle reading, but only 100 were selected based on stratified sex and size categories for age verification processing. The other 40 lobsters served as replacements in case of problems with ossicle processing. For each lobster, the following information was recorded: i) specific localization (GPS coordinates), ii) time of collection, iii) depth, iv) water temperature, and v) the moon phase. Every animal captured was tagged with an identification number and the sex of the animal was determined in the field, and whether it had a spermatophore and/or eggs.

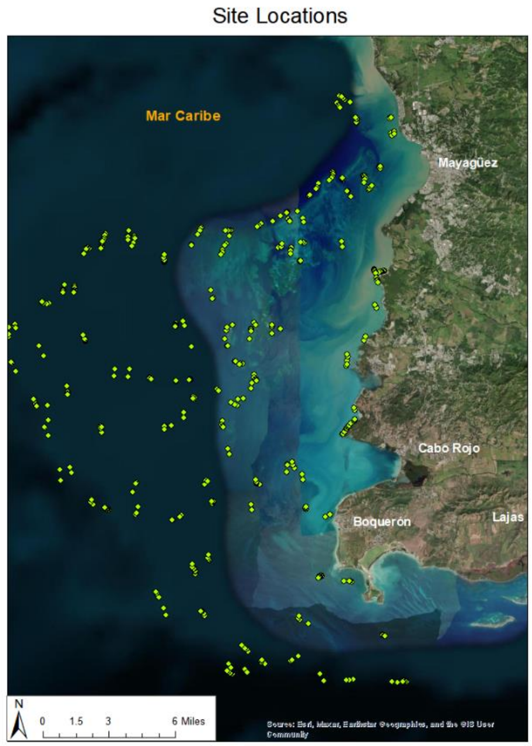

Figure 1. Area of study in Southwest Puerto Rico. Each green dot represents a diving site where Caribbean Spiny Lobsters (P. argus) were collected for analysis in this study.

2.2 Laboratory Work

In this study, the age of *P. argus* was estimated following Gnanalingam et al. (2018). Captured lobsters were stored in a freezer (-4 C^{o}) until final processing. All captured lobsters were processed in the Fisheries Laboratory of the Department of Natural Resources of Puerto Rico (DRNA) in Cabo Rojo, Puerto Rico. Animals were weighed to the nearest 0.1 g with a bench scale and the carapace length were measured to the nearest 0.1 mm with a caliper. Lobsters were then dissected, and gonads and stomach were removed. The gonads were weighed and stored in a preservation solution of glycerol, alcohol, and distilled water and kept for further studies not considered in this article. The stomachs were preserved in a solution of 74% alcohol, 22% distilled water, and 4% glycerol until extraction of the ossicles. A total of 100 lobsters were measured, dissected, and their stomachs fixed and preserved. Stomachs were opened and cleaned with water, to obtain the ossicles. The main piece was cut to divide the pterocardiac into two pieces, mesocardiac in one piece, and the two zygocardiac ossicles, resulting in a total of five pieces. These pieces were stored in the preservation solution with its animal tag number.

The pterocardiac was embedded in a 2-part epoxy with Araldite 502 and a hardener HY 956 solution to efficiently manage the small, delicate samples for further study. A silicon ice cube tray was used as an embedding container. For the first round of epoxy, the ice cube trays slot was partially filled without any samples and put in the oven at 60°C for 3 hours. While the trays were baking, the samples were prepared, dried, and labelled. After the first round of epoxy was ready, the ossicles were placed on the prepared epoxy and covered with a second layer of epoxy and baked for another 3 hours at 60°C.

Once the samples were baked, each sample was stored in a labelled coin envelope with its tag number, date processed, and identifier for the specific part of the ossicle. After all the embedding process was completed, all samples were cut using paired diamond blades with a 1 mm space between the two blades to create a thin section of each ossicle. The final cut was measured with a caliper and then polished using fine sandpaper. The polishing process was an interactive process in which samples were sanded and evaluated under the microscope until an adequate sample was obtained for reading. The image was saved and then a second image was annotated with the annual bands and saved as a digital archive. The annual bands were then counted and entered into a database with the animal's information.

All ossicles were read by a total of 4 readers to determine the number of bands and verify band counting precision between readers. Each reader made two estimates of the maximum and the minimum bands that the animal showed in the image. Then the average of those two estimates were calculated to determine the age. All images were categorized by quality of the picture and each age determination was labeled with the level of confidence of the reader. A comparison of the age estimation by readers showed a high degree of discrepancy between them, as demonstrated by the relationship between age and carapace length, which was not the same for the four readers. Estimations made by 2 of the four readers were removed from the database as they resulted in a negative age-length relationship and poor agreement with the ages of the other two readers. Lacking any knowledge of accuracy to the actual lobster age, we chose to remove all readings provided by readers 2 and 4 reported because they reported a negative age/length relationship, whereas readers 1 and 3 showed the expected positive relationship. Based on these results, only readers 1 and 3 were considered for the analyses. Similarly, the quality of the image (i.e. “Excellent”, “Good”, “Regular”, and “Bad”) also had an influence on the estimation of the age-length relationship and as a result only images considered “Excellent” or “Good” were considered for further processing. Furthermore, the confidence around each reading (“High”, “Medium”, and “Low”), regardless of the quality of the image, was also considered in this preliminary selection. Since the “Low” confidence category showed a negative relationship between age and length, it was not considered for further analysis. Based on all these preliminary analyses, the data used for final analysis was filtered by i) reader: only two of the four readers were considered; ii) quality of the images: only images classified as “Excellent” and “Good” quality were used, and iii) confidence of the reading: only those reading classified as “High”, and “Medium” were used. Out of the 100 processed ossicles, this filtering for sample quality assurance and quality controlled resulted in a total of 35 ossicles used for the final analyses presented below.

2.3 Statistics

All statistical analyses were conducted using *R* (R Core Team, 2023). A two-sided Student's t-Test (function *t*.test) was used to test for differences in mean carapace length, weight, and age between male and female lobsters. A two-sided Kolmogorov-Smirnov test (function *ks*.test) was used to test for differences in the distribution of carapace length, weight, and age between male and female lobsters. Relationships between carapace length, weight, and age can be fit by either linear, logarithmic, or von Bertalanffy relationships (Matthews and Maxwell 2007). However, there was a high degree of scatter in the carapace length vs age and weight vs age models in this study so models assessing carapace length vs age, the log of weight vs age, and the log of weight vs carapace length models were selected a priori and constructed individually for male and female lobsters using the function *lm*. A von Bertalanffy growth model was constructed for male lobsters, female lobsters, and all lobsters in this study using the package *FSA* (Ogle, 2023). The von

Bertalanffy K and L_{inf} values from this study were compared to Table 1 of de Leon et al. 2005 to compare these parameters for *P. argus* populations across the Caribbean.

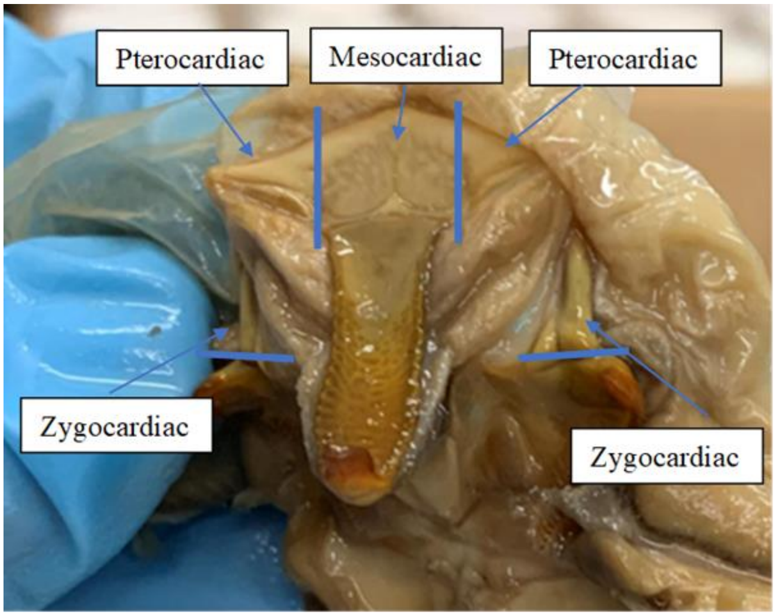

Figure 2. Ossicle exposed and labeled by parts.

3. RESULTS

3.1 Prelimnary analyses

A comparison of the age estimation by readers showed a high degree of discrepancy between them, as demonstrated by the relationship between age and carapace length, which was not the same for the four readers **(Figure 3)**. Readers 2 and 4 reported a negative age/length relationship, whereas readers 1 and 3 showed the expected positive relationship. Based on these results, only readers 1 and 3 were considered for further analyses.

Similarly, and comparison of the age/length relationship between the four quality categories (i.e. “Excellent”, “Good”, “Regular” and “Bad”) used for the images, showed some discrepancy; as the relationship for the “Bad” category showed a negative trend, the “Regular” category showed no relationship, and the other two categories (“Good” and “Excellent”) showed a positive relationship **(Figure 4)**. Based on these results image quality “Bad” and “Regular” were filtered out of the data, and final analyses only considered the “Good” and “Excellent” quality images.

Furthermore, not only the quality of the image was considered in these preliminary analyses, but also the confidence around each reading regardless of the quality of the image ("High", "Medium", and "Low"). As per the other results, positive relationships between age and CL were only found for the "High" and "Medium" confidence, whereas the "Low" confidence readings showed a negative relationship (**Figure 5).**

Based on all these preliminary analyses, the data to be used for final analysis was filtered by i) reader: estimations of age of only two (1 and 3) of the four readers were used; ii) quality of the images: only images classified as "Excellent" and "Good" quality were used, and iii) confidence of the reading: only those reading classified as "High", and "Medium" were used.

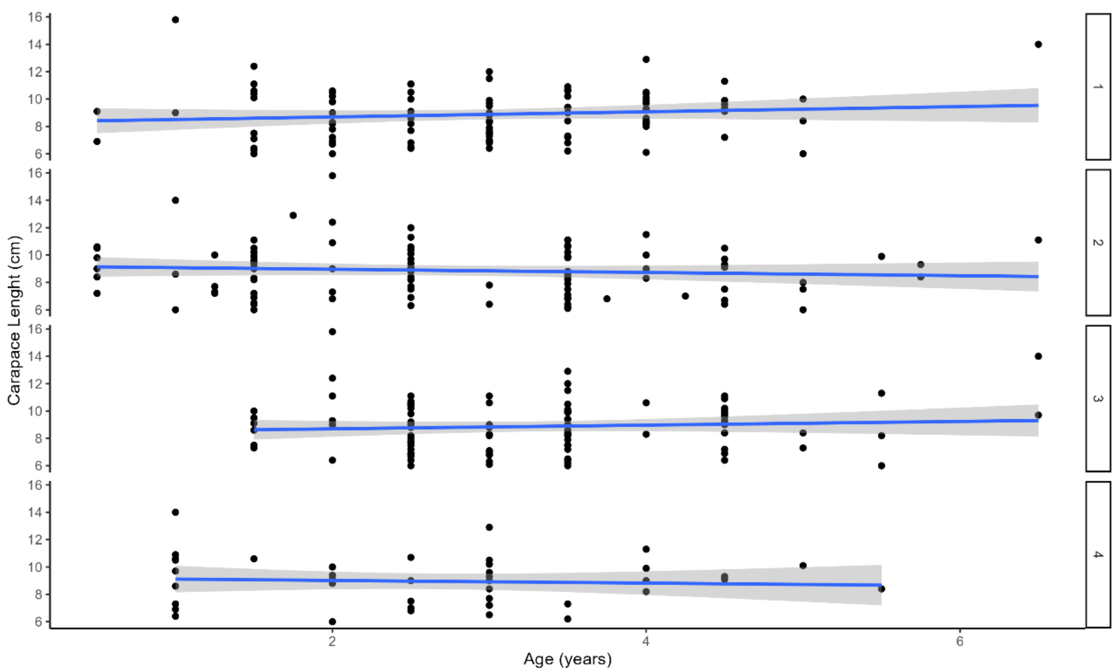

Figure 3. Relationship between the age determined by Reader (1,2,3,4) and carapace length (CL).

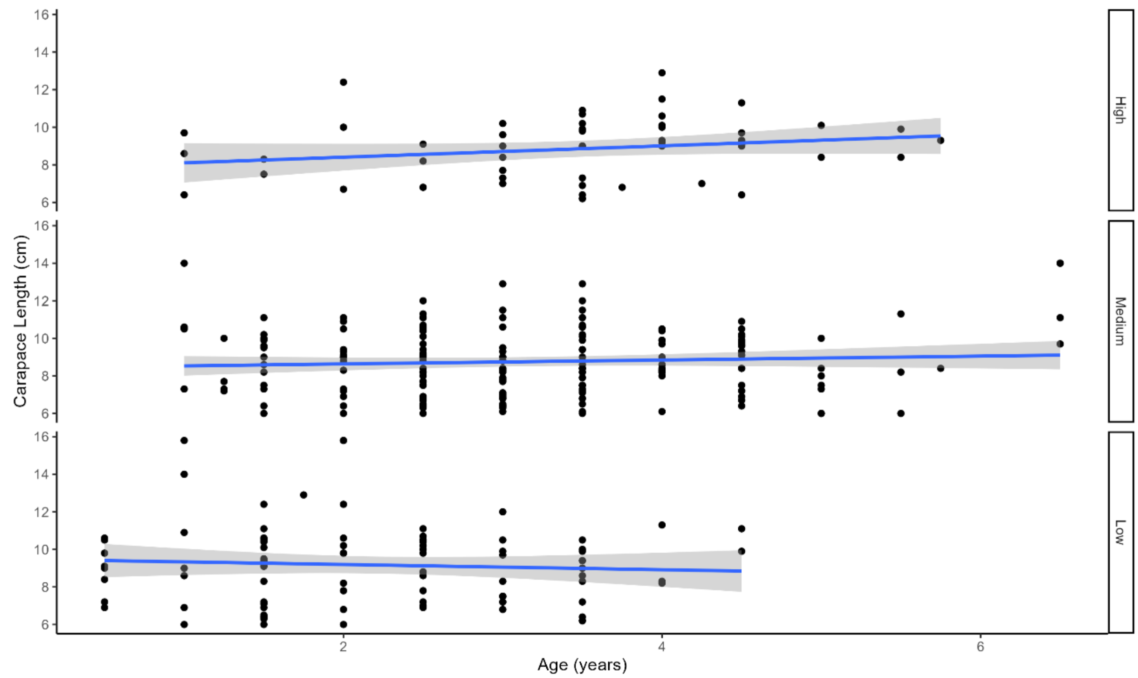

Figure 4. The determined age based on the level of confidence of each reader (High, Medium, Low) correlated with carapace length (CL).

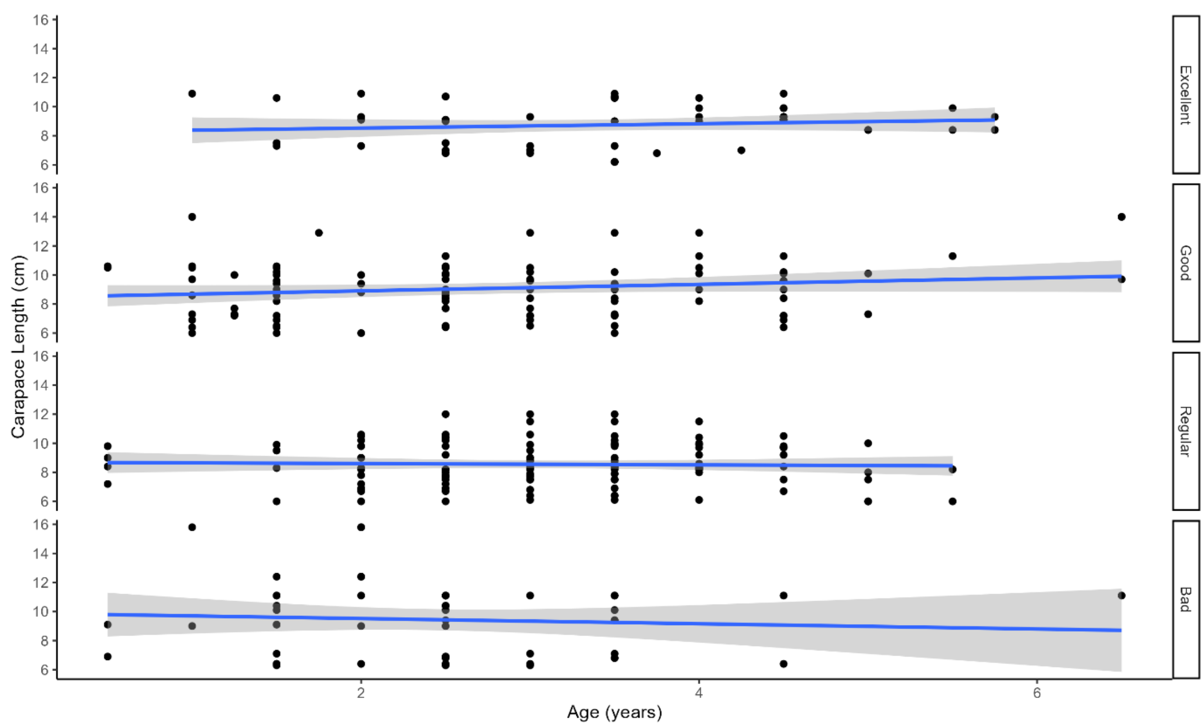

Figure 5. Age determined with the different image quality correlated with carapace length (CL).

3.2 Age, length, and weight structure

The mean (range) carapace length in the samples was 8.8 cm (range = 6.2 to 14.0 cm) for female and 9.4 cm (6.0 to 12.9 cm) for male lobsters. The mean (range) weight registered in this study was 686 g (236 to 1945 g) for the female lobsters and 775 g (191 to 1556 g) for the male lobsters. The mean (range) age registered in this study was 3.4 years (1.5 to 6.5 years) for female lobsters and 3.7 years (2.25 to 5 years) for male lobsters. There were no detectable differences between the mean carapace length (Student's T-Test: $p=0.35$), weight (Student's T-Test: $p=0.49$), or age (Student's T-Test: $p=0.54$) between male and female lobsters. Similarly, there were no detectable differences between the distribution of carapace length (Kolmogorov-Smirnov test: $p=0.312$), weight (Kolmogorov-Smirnov test: $p=0.48$), or age (Kolmogorov-Smirnov test: $p=0.60$) between male and female lobsters.

3.3 Age vs. length

There was a positive relationship ($p=0.07$) between age and carapace length for female lobsters with a mean ± SE slope of 0.6±0.3 cm/year, mean ± SE intercept of 6.9±1.1 cm, and a p-value of 0.07 (Figure 7a). Similarly, age and carapace length were positively correlated for male lobsters with a mean ± SE slope of 1.0±0.5 cm/year, mean ± SE intercept of 5.6±1.8 cm, and a p-value of 0.05 (Figure 7a).

3.4 Age vs. weight

There was a positive relationship between age and weight for female lobsters with a mean ± SE slope of 129.3 ±61.0 g/year, mean ± SE intercept of 240.4±227.3 g, and a p-value of 0.05 (Figure 7b). Similarly, age and length were positively correlated for male lobsters with a mean ± SE slope of 188.0±99.1 g/year, mean ± SE intercept of 81.1±335.0 g, and a p-value of 0.08 (Figure 7b).

3.5 Weight vs. length

There was a positive relationship between carapace length and log(weight) for female lobsters with a mean ± SE slope of 0.28±0.18 log(g)/cm, mean ± SE intercept of 3.9±0.16 log(g), and a p-value of <0.001 (Figure 7c). Similarly, carapace length and log(weight) were positively correlated for male lobsters with a mean ± SE slope of 0.30±0.18 g/year, mean ± SE intercept of 3.7±0.17 log(g), and a p-value of <0.001 (Figure 7c).

3.6 Growth Structure von Bertalanffy

Female lobsters had a von Bertalanffy growth rate k of 2±4 and maximum length L_{inf} of 88±5 mm. Male lobsters had a von Bertalanffy growth rate k of 0.5±0.2 and maximum length L_{inf} of 120±20 mm. All lobsters had a von Bertalanffy growth rate k of 0.8±0.3 and maximum length L_{inf} of 97±6 mm (Figure 8). The von Bertalanffy growth parameters for all lobsters in this study were then compared to the data from Leon (2005) (Figure 8a). Growth rates k from Puerto Rico were greater than every other region (Figure 8a) and maximum length L_{inf} from Puerto Rico were smaller than every other region (Figure 8b).

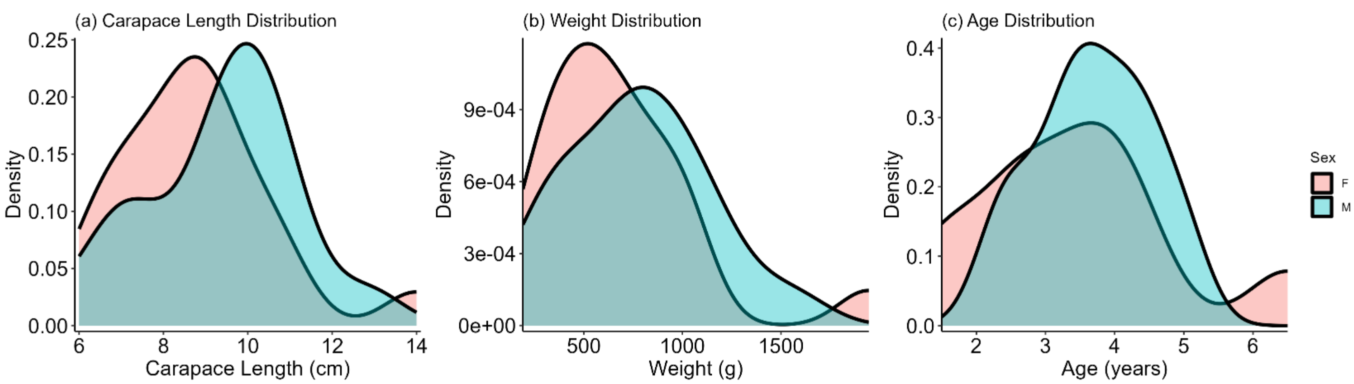

Figure 7. Plots showing the distribution of (a) carapace length, (b) weight, and (c) age with pink shading for female lobsters (F) and blue shading for male lobsters (M).

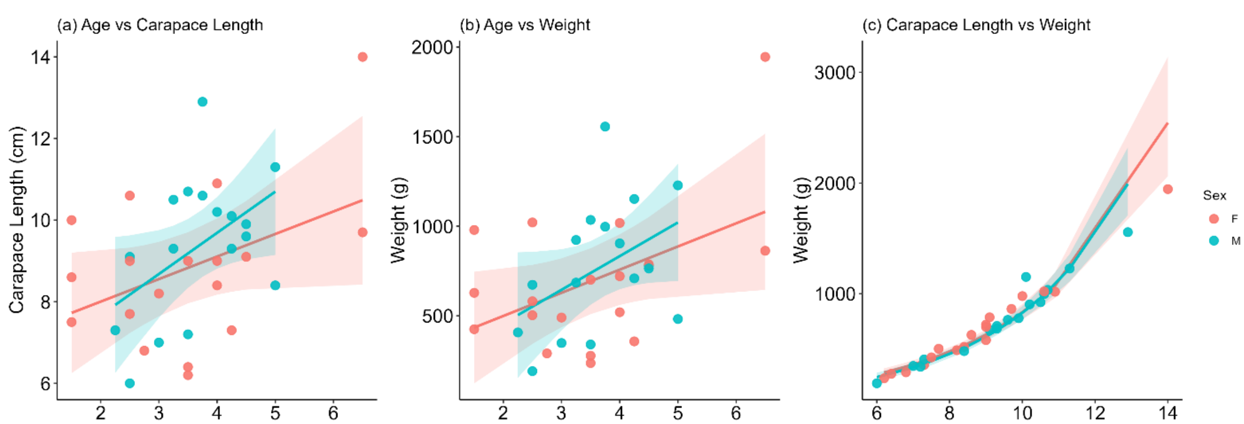

Figure 6. Relationships between (a) carapace length and age, (b) weight and age, and (c) carapace length and weight are reported for female lobsters (F, pink) and male lobsters (M, blue).

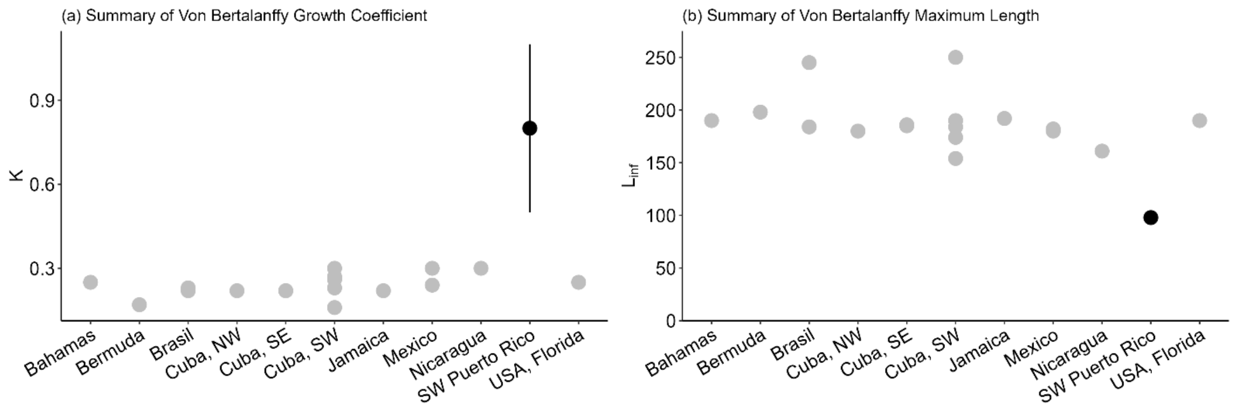

Figure 8.Comparison of von Bertalanffy (a) growth rate k values and (b) maximum length (Linf) for Caribbean Spiny Lobster P. argus populations across the Caribbean. The gray points are from Leon (2005) and labeled by their respective region and the black points are the mean estimates (±standard error) for all lobsters from this study.

4. DISCUSSION

The age, carapace length, and weight of Caribbean Spiny Lobster (*P. argus*) has been determined for a total of 35 individuals from southwest Puerto Rico. Despite the verification to directly age lobsters from their ossicles (Gnanalingam et al. 2018), there was considerable uncertainty in ossicle aging between readers in this study and the quality of the images of the ossicles developed. Moreover, the quality of the images significantly impacted the outcomes, which indicates that the technique can be further enhanced through continuous training of new readers and continuous practice of the more experienced. Even though some filtering of the images and readers used in this study was necessary due to readers experience and image quality, the age-length and age-weight relationship can be considered in further analysis of the status of the lobster population in the study area. Consequently, there was considerable variability in the age-length, age-weight, and von Bertalanffy relationships from this study (Figure 6, 7, 8), especially for female von Bertalanffy growth parameters. Nonetheless, this study provides estimations of age, length, and weight structure as well as the derived growth rates that can be used in stock assessments of the species and inform SEDAR (Southeast Data, Assessment, and Review and Atlantic Highly Migratory Species) processes.

This method of aging is still relatively novel and has not yet been applied to many of the *P. argus* sub-populations found in the Caribbean. While the mechanisms driving annual banding remain unknown, seasonal environmental variability is a likely driver (Gnanalingam et al. 2018). It therefore remains to be tested if the ossicle aging technique is only applicable to P. *argus* populations in higher latitudes, such as the Florida Keys, Bahamas, Cuba, and Mexico, and not for populations in lower latitudes where environmental conditions remain more stable, such as the in Puerto Rico (Gnanalingam et al. 2018).

The results presented here apply to the population of the southwestern coast of Puerto Rico, which is the region from where most landings are reported in Puerto Rico (Matos-Caraballo et al., 2019). Size is considered a poor measure of the age of crustaceans living in the field because growth is dependent on many environmental factors (Maxwell et al., 2007). Factors such as water temperature, salinity, nutrient availability, and oceanographic features play crucial roles in influencing growth rates, reproduction, and overall productivity of lobsters (Ross & Behringer, 2019). For instance, regions with higher inherent productivity may see lobsters reaching larger sizes at younger ages due to favorable environmental conditions and abundant food resources (Maxwell et al., 2009; Maxwell et al. 2013. Fishing pressure can also influence length and growth of *P. argus* (Maxwell et al., 2009), since it has been shown that larger lobsters are consistently found in non-catch areas when compared to open areas (Bertelsen et al., 2004; Maxwell et al., 2007). For example, lobsters harvested from the Dry Tortugas (lower fishing pressure) were significantly larger than those caught in the Florida Keys (higher fishing pressure) (Bertelsen et al., 2004; Maxwell et al., 2007). For the case of Puerto Rico, there are no reliable estimations of fishing effort; furthermore, there is a lack of restrictions on fishing activities, and many marine protected areas in Puerto Rico, such as the Nature Reserve of La Parguera (LPNR), lack an active management plan or its effective implementation (Jiusto, S. 2005).Unfortunately, that is why it has been proposed that the fishing pressure is equal in protected and non-protected areas in Puerto Rico (Jiusto, S. 2005). Regardless, there are likely gradients of environmental conditions and fishing pressure around the islands of Puerto Rico, suggesting that growth rates and maximum length may also vary by region. Importantly, *P. argus* from the south and east fisheries of Puerto Rico, which represent 51.4% of the total catch in 2013 (Matos-Caraballo D. et al. 2019), were not considered in this study. It is recommended that this study be repeated for the rest of the island, especially the south and east to account for these potential differences in growth rates and population age structure under varying environmental conditions and fishing pressure.

The fishing pressure on *P. argus* likely varies between Puerto Rico and other parts of the Caribbean due to differences in fishing regulations (e.g. larger size limit and no closed season), population densities, fishing practices, management strategies, and market demand (Cox & Hunt 2005; Chakalall et al. 1998). Different parts of the Caribbean also have distinct environmental conditions that can affect the productivity of spiny lobster populations (Ehrhardt 2005, Chavez 2009). Because there is a single, fully genetically mixed pan-Caribbean population connected through larval dispersal (Ehrhardt 2005), any differences in growth rates and maximum length between *P. argus* populations across the Caribbean are likely due to local-scale environmental conditions and fishing pressure and not genetic variability.

While von Bertalanffy growth rates (k) and maximum length (L_{inf}) varied significantly around the Caribbean (Figure 4, Leon et al. 2005), growth rates (k) from Puerto Rico were greater than every other region (Figure 8a) and maximum length (L_{inf}) from Puerto Rico were smaller than every other region (Figure 8b). L_{inf} in this study was likely artificially low based on the low sample size and limited scope of this project. The algorithms used do not allow independent estimation of the main parameters K and L_{inf}. A low L_{inf} affects the asymptote of the von Bertalanffy growth curve and increases the value of k inappropriately (Stamatopoulos and Caddy, 1989; Lee et al., 2020). We caution that the ossicle-based aging method used here differs from the growth-based methods to calculate age used elsewhere in the Caribbean. Specifically, von Bertalanffy growth curves for Caribbean spiny lobsters suffer from considerable tautological issues; that is, often tag recapture studies are used to calculate growth and then those growth curves are used to reverse calculate total age for studies elsewhere in the Caribbean (Muller et al., 1997). Conversely, while the ossicle-based methods here provide direct estimates of age, they were subject to considerable uncertainties (Figure 6, Figure 7). Aside from methodological differences and their inherent uncertainties, it is difficult to directly attribute any regional differences in growth rates and maximum length between Puerto Rico and elsewhere in the Caribbean to either environmental gradients or fishing pressure as local lobster populations are responding to both factors simultaneously. In a fishery like spiny lobster in Puerto Rico that has existed for decades at very high exploitation rates, the loss of large lobsters from the population impacts the determination of

L_{inf} (Ricker, 1975; Gulland, 1983; Pauly, 1984). Further research comparing growth rates, reproductive success, and population dynamics of spiny lobsters using directly comparable methods across the Caribbean would be essential for providing a more definitive comparison of growth rates between regions (Gnanalingam et al. 2018).

Long-term monitoring programs and assessments of lobster populations can also help evaluate changes in productivity over time and guide management efforts toward sustainable fisheries management (Mateo 2004, Kilada et al. 2012, Leland et al. 2017). By providing valuable data on population dynamics, growth rates, and ecosystem interactions, aging research contributes to the sustainable management and conservation of spiny lobster fisheries. This study estimated growth rates for the southwest Puerto Rico Caribbean spiny lobster (*P. argus*) study population but, importantly, these estimates are only for this population since age-size vary with environmental conditions and fishing pressure. Despite the limitations of this study (uncertainty), the information presented here can be directly used in the population assessment (stock assessment) and management of the southwest Puerto Rico *P. argus* fishery. Ongoing ossicle age training, extending this study to other areas of Puerto Rico, and collaborating with fishermen and restaurants who can donate the carapace (stomach) part to this type of studies instead of sacrificing wild lobsters can further increase growth rate data to support and reinforce regulations for a sustainable Caribbean Spiny Lobster fishery in Puerto Rico.

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