Life history and trophic ecology of the Atlantic brief squid, Lolliguncula brevis.

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Introduction

Developing a holistic understanding of estuarine ecology requires the investigation of the life history and trophic ecology of individual species. Throughout the marine biosphere, few habitats undergo the degree of daily change observed in estuaries. Diurnal and even semi-diurnal tidal changes structure a dynamic ecosystem with constantly changing salinity, temperature, and nutrient flow (Islam *et al.*, 2005). The organisms that occupy these estuarine habitats possess a wide range of physiological adaptations to tolerate their environmental stressors (Bornatowski *et al*., 2014). A predator's ability to utilize special adaptations to exploit more resources can have large impacts on its life history and trophic ecology and also the organisms with which it interacts (Hussey *et al*., 2014). One such predator is the Atlantic brief squid, *Lolliguncula brevis*, one of few squid species that can tolerate salinities from 35.5 ppt down to 17 ppt, and even as low as 8.5 ppt (Hendrix *et al.*, 1981; Lauchlin & Livingston, 1982). This broad salinity tolerance enables this species to inhabit a wide range of habitats. From the Chesapeake Bay in North America, to as far south as Argentina, *L. brevis* occupies open estuaries of marine salinity to low salinity tidal creeks.

Lolliguncula brevis has adapted to feed on larval and benthic crustaceans and larger fish and shrimp (and other squid) depending on its developmental stage (Dragovich & Kelly, 1964; Hanlon *et al*., 1983; Ogburn-Matthews & Allen, 1993). As *L. brevis* increases in size, adult squid with mantle lengths typically less than 12 cm in females and less than 8 cm in males shift to feeding on larger prey items toward the end of their 100-200 day lifespan (Hanlon *et al.*, 1983; Jackson *et al.*, 1997). In addition, *L. brevis* is a common prey item found in the gut contents of many recreationally-important finfish species, such as black sea bass (*Centropristis striata*), and have been found to consist of 16.6% of the diet of endangered juvenile hammerhead sharks (*Sphyrna gilberti* and *Sphyrna lewini*) (Sedberry, 1988; Bornatowski *et al.*, 2014). Furthermore, *L. brevis* has also been documented as the most abundant prey item found in the gut contents of 63 female bottlenose dolphin (*Tursiops truncatus*) stomachs; 19 of these females were mature and/or lactating, thus highlighting the nutritional importance of *L. brevis* to higher trophic level organisms within coastal habitats (Pate, 2008).

In Charleston Harbor, an estuarine watershed of South Carolina, *L. brevis* comprises a considerable portion of the benthic animal biomass component of trawl catches. The South Carolina Department of Natural Resources (SCDNR)'s Crustacean Research and Monitoring Section's long-term trawl survey regularly catches *L. brevis* in otter trawl samples at stations within the Charleston Harbor-Ashley River estuarine system spanning average salinities from 12 ppt to 24.4 ppt (Whitaker, 1978a; Whitaker & Kingsley-Smith, 2015). Previous data collected by the SCDNR CRMS trawl survey (Whitaker, 1982, unpubl.) suggest that brief squid inhabit different parts of the Charleston Harbor estuary at different stages in their life history in varying abundances. For example, there were higher abundances of squid in spring and fall months than compared to other months in 1983 and 2018 (Peyla, 2018, unpubl.) suggesting seasonality to abundance. According to past CRMS trawl surveys, smaller squid were more frequently caught in 12 ppt salinity and larger squid more frequently caught in 25 ppt salinity. This apparent downstream migration between habitats in Charleston Harbor as *L. brevis* age implies an ontogenetic shift in diet, likely switching from benthic prey to more pelagic organisms. The age and developmental stage at which an ontogenetic diet shift occurs in the life history of *L. brevis* is currently undescribed.

Other estuarine organisms with spatially-complex life cycles that utilize different environments at different stages of their life cycle, like the bull shark, have well understood trophic interactions in estuarine habitats (Matich & Heithaus, 2014); however, the lack of such detailed information for *L. brevis* warrants further investigation. By furthering knowledge of the life history and trophic ecology of this species, a more detailed understanding of the Charleston Harbor food web, which can likely be applied to other estuarine systems within the geographic range of *L. brevis*, can be achieved.

The objective of this project is to investigate the underlying life history of *L. brevis* in the Charleston Harbor in order to lay the foundation of further research into the squid's trophic ecology and growth. Examining the details of *L. brevis'* life history across space and time will develop a better understanding of its trophic ecology. In turn, examining the details of trophic ecology, *L. brevis*' size-at-age determinations, growth rates, and life history can be better understood. This comprehensive examination of *L. brevis* will lead to a more holistic understanding of estuarine ecology in Charleston Harbor. Ultimately, the project seeks to provide newly collected data and information to natural resources managers, primarily to the SCDNR's Office of Fisheries Management, responsible for species closely trophically-connected to *L. brevis*.

Methods

Squid Collection and Preservation

Samples of *L. brevis* were collected monthly from Charleston Harbor by the R/V *Silver Crescent* as part of the SCDNR's Crustacean Research and Monitoring Section (CRMS) trawl survey. Four stations (Figure 1) were sampled each month from January through October of 2019 with a 6.10 m-wide otter trawl with a 1.27 cm bar mesh for 15 minutes (nets set until retrieval begins). Anchorage and Fort Johnson stations are representative of salinities of approximately 25 ppt and Lower Ashley and Upper Ashley stations are representative of salinities of approximately 12 ppt. These sampling stations were chosen to be utilized for this project because of the successful and consistent past captures of *L. brevis* each month. Tide stage, weather conditions, and water quality measurements (salinity (ppt), pH, temperature (°C), and dissolved oxygen (mg/L) recorded via YSI) were recorded at the time of collection at each station. Specimens of *L. brevis* collected were bagged, put on ice, and placed in a -20˚C freezer for preservation upon return to the laboratory.

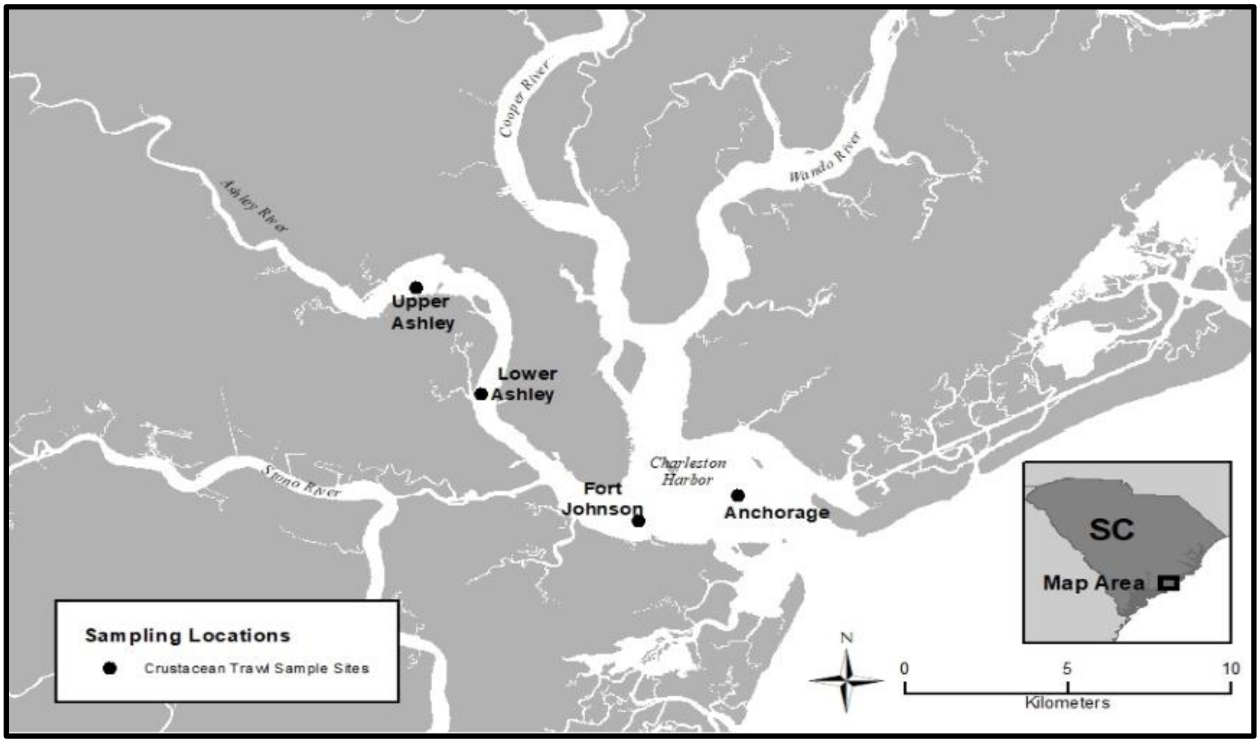

Figure 1. Four sampling stations of the SCDNR's Crustacean Research and Monitoring Section (CRMS) trawl survey in the Charleston Harbor, South Carolina.

Life History

Total number of squid caught (abundance) was recorded for each month and station. Mantle length (cm), length overall (cm), and gladius length (cm) were measured for each squid (Figure 2). Gladius length was chosen for the final size measurement for brief squid due to the a more reliable, firm structure to base length measurements in comparison to the variable flexibility of mantle tissue. Using a regression analysis in R between mantle length and gladius length of sampled brief squid, gladius length can be justified as comparable size measurement than the traditionally used mantle length (Figure 3).

Squid sex and maturity are determined via the presence of hectocotylus/spermatophores in males and nidamental glands/ovaries in females (Figure 2). The spermatophores of male *L. brevis* and nidamental glands of female *L. brevis* remain differentially unstained when methylene blue dye is added to the dissected anterior mantle cavity, allowing these glands to be distinguished from the rest of the visceral mass. This allows a qualitative assessment of maturity as described by Whitaker (1978b) assigning increasing size of sex organs to a 1 to 5 scale. Squid lacking in these sexual organ developments were labeled as juveniles.

Statistical Analyses

Squid abundance was plotted for month and station (Figure 4) and analyzed for significance with temperature and salinity. Average squid gladius sizes (interquartile ranges) were calculated for each station and each month using R software (Figure 5). The patterns in gladius sizes over month and station were analyzed for significance in respect to the salinity and temperature data associated with the sample's collections using an analysis of variance (ANOVA) in R. Counts of female male and juvenile squid per station and month were tallied using R software to assess changes in male/female ratios in the population (Figure 6). Ratios of male, female and juvenile squids were analyzed with salinity and temperature data using an ANOVA in R.

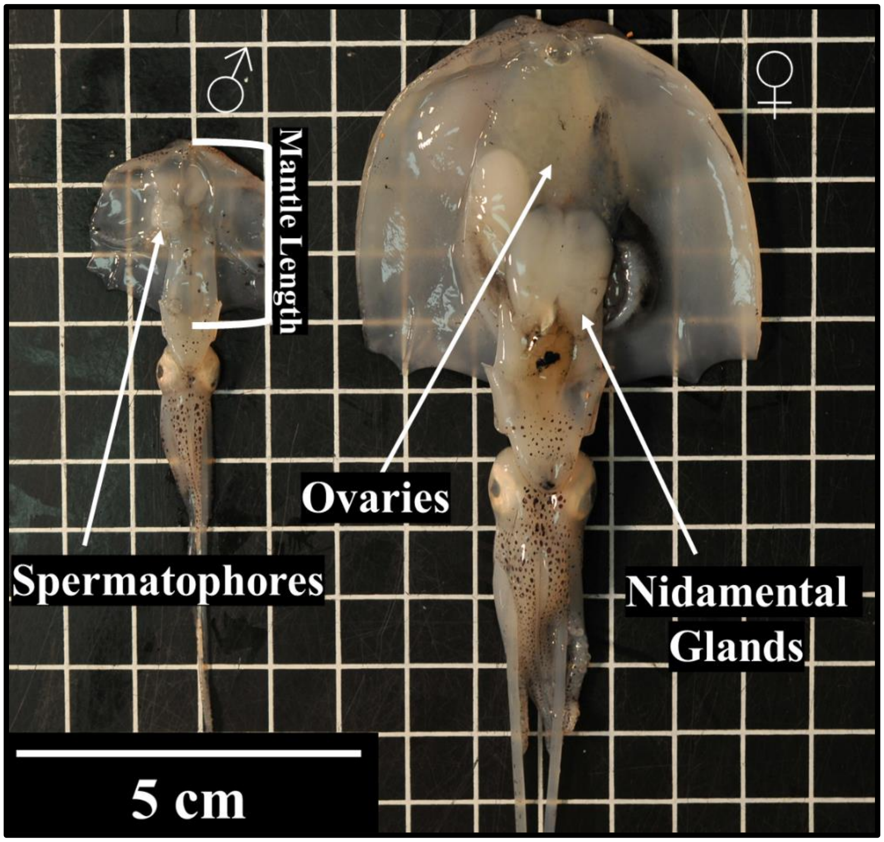

Figure 2. *Left*: A male *L. brevis* with visible spermatophores present. *Right*: Female *L. brevis* exhibiting nidamental glands and ovaries.

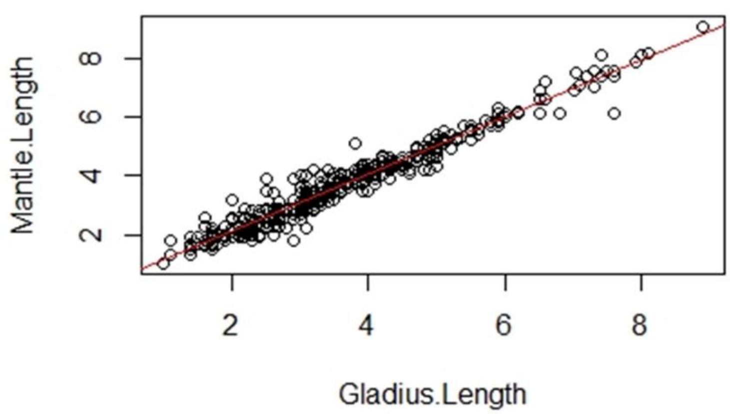

Figure 3. Linear regression analysis between mantle length (cm) and gladius length (cm) of each sampled squid showing the relative similarity of these two life history parameters. ($R^2 = 0.954$).

Results

A total of 598 squid were processed for this project. Abundance of *L. brevis* samples collected from January to October of 2019 was highest during the spring months of April and August. With regards to stations, Fort Johnson contained the greatest number of squid (242 individuals) followed by Anchorage (197 individuals), Lower Ashley (109 individuals), and Upper Ashley (50 individuals) (Figure 4). When this abundance distribution across month and station was compared to abiotic factors of temperature and salinity, temperature was seen to have a significant influence on abundance (ANOVA: $p < 0.001$).

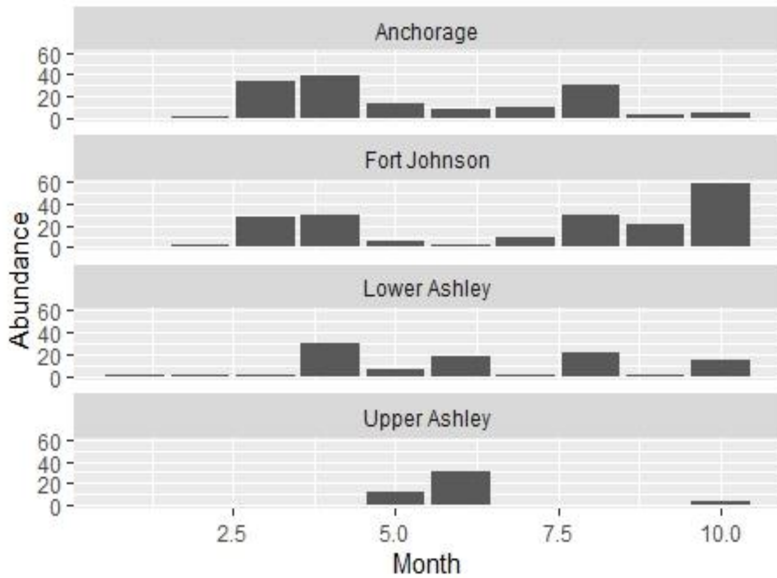

Figure 4. Abundance of *L. brevis* caught from January (Month 1) to October (Month 10) at each of the four stations: Anchorage, Fort Johnson, Lower Ashley, Upper Ashley.

The largest, and therefore assumed to be the oldest, average gladius lengths of squid were collected from during the month of May at the Fort Johnson Station. Overall, smaller squid were found during the fall and winter months in the upstream stations of Lower and Upper Ashley (Figure 5). When compared to the abiotic variables of these collections, temperature had the greatest effect on average gladius length across months and stations (ANOVA: $p = 0.01$).

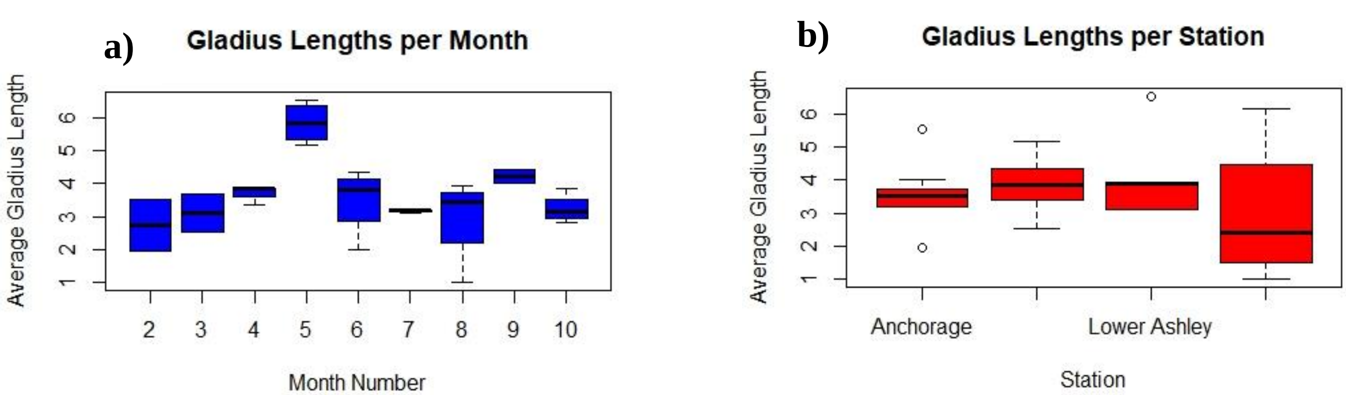

Figure 5. Average gladius length (cm) of *L. brevis* samples collected from (a) January to October and (b) stations Anchorage, Fort Johnson, Lower Ashley, and Upper Ashley.

When comparing sexual distribution of females, males, and juvenile brief squid across month and time, juvenile squid are observed to dominate the brackish water stations of Lower Ashley and Upper Ashley. The marine stations of Anchorage and Fort Johnson have more abundant counts of mature females and males. Likewise, adult squid, both male and females are more prevalent in spring and fall months (Figure 6). Also, when analyzing the abiotic effects on sex, salinity had a significant effect on only male gladius lengths over months and stations (ANOVA: $p = 0.05$).

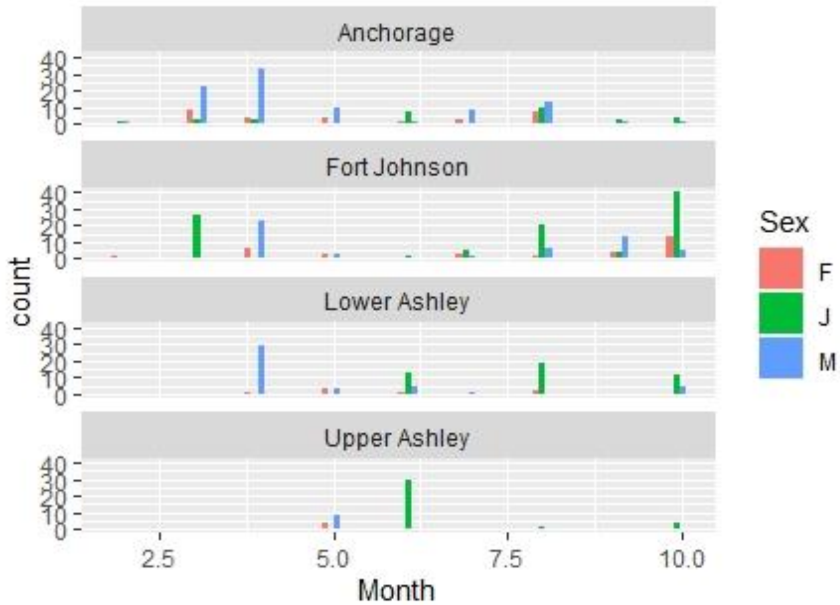

Figure 6. Distribution of female (F), juvenile (J) and male (M) *L. brevis* across the months and stations in which they were collected.

Discussion

One of the most important patterns observed in these analyses was the consistent influence of the abiotic variable temperature on squid abundance and gladius length over months and stations. The obvious interaction of temperature's effect on squid over the sampling months can easily be explained by the changing seasonal temperatures through the course of the year and temperature changes between the stations due to geological factors. However, temperatures significant interaction with gladius length over station begs the question of why salinity was not seen to be significantly influencing. Although the salinity varies greatly from the marine station of Anchorage up through the brackish station of Upper Ashley, *L. brevis* remains to be one of the only unique cephalopods to be an osmoconformer (Hendrix *et al.*, 1981). Their unique adaptation of physiological osmoregulation would explain why other abiotic variables such as temperature would be more conducive to their growth.

With this osmoconforming physiology comes the brief squid's broad range across a wide and dynamic estuarine habitat. The patterns of abundance observed in across stations through the year show that the majority of squid congregate in the marine areas of the harbor. When overlayed with the sexual distribution of the squid in the same stations, adult brief squid seem to control the abundance in these areas. The abundance and distirbution of the different sexes across the space and time presented in this project suggests that there are multiple breeding seasons during the the year. The fluctuating abundance of adult male and female squid in the marine stations suggest a spring and fall breeding season. Also, juvenile squid seem to exclusively occupy the brackish waters of the tidal creeks. This area may be used as a sheltered nursery in which juvenile brief squid have access to early sources of food to where they can mature and move out to marine habitats in search of larger prey. Although previous patterns along these lines have not been described in literature, the analyses here suggest the possibility of overlapping cohorts of brief squid in a signle year in the Charleston Harbor.

These unique artifacts of this animal's life history calls for the research of a possible ontogenetic diet shift with the likely change of habitat during the maturity of brief squid. Future research of this project will include the inculsion of stable isotope analyses of the collected sample's buccal tissue to determine what and where different aged squid are feeding in the estuary. Also, statolith age ring analyses (as suggested by Jackson *et al.* (1997)) will determine the approximate age (in days) of brief squid samples in order to backtrack birth days and originating habitats.

Due to interuptions in data collection due to the COVID-19 pandemic, solutions to investating these patterns of the *L.brevis* life history may lie in the past. Unpublished historical data, including similar life history parameters extending back to the early 1980s and 2018 (Whitaker, 1982, unpubl.; Peyla, 2018, unpubl.) are available for analysis. In the future, these data will be compared to present in order to assess differences in life histories of *L. brevis* from 1982, 2018 and this project's collections in 2019.

Presently, structural equation modeling still needs to be applied to fully comprehend the multitude of variables that may be influencing the growth and distribution of the brief squid in the Charleston Harbor. With so little literature investigating its life history, *L. brevis* represents a large gap in knowledge when investigating the estuarine ecosystems of South Carolina. In order to provide agencies like the SCDNR with the most accurate information to manage and protect the commercially important species, these analyses should be utilized and continued to be built upon. Future quantitative analyses, the foundations of which lie in R coding, will lead to the clarification of the role that *L. brevis* plays in the Charleston Harbor estuarine community.

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