REPRODUCTIVE BIOLOGY OF THE FEMALE RED DEEP-SEA CRAB, *CHACEON QUINQUEDENS* (SMITH, 1879), IN THE MID-ATLANTIC BIGHT

By

Stephanie Martínez Rivera

Dissertation submitted to the Faculty of the Graduate School of the

University of Maryland Eastern Shore in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

December 2018

Dissertation Committee

Dr. Bradley G. Stevens, Chair

Dr. J. Sook Chung

Dr. Andrea Johnson

Dr. William Christopher Long

Dr. Thomas Miller

ABSTRACT

The crabs of the Family Geryonidae are widely distributed on the continental slopes of the world (Hastie, 1995). Deep-water geryonid crabs have been described as slow-growing, with late maturation and possible infrequent recruitment (Hastie, 1995). Some geryonid crab have been commercially targeted around the world, however the previous characteristics imply that it is unlikely that high yields are sustainable (Hastie, 1995). The red deep-sea crab (RDSC), *Chaceon quinquedens* Smith, 1879, is an epi-benthic brachyuran that can be found along the continental shelf and slope in the Western Atlantic Ocean, ranging from Nova Scotia to Florida (including the Gulf of Maine and the Gulf of Mexico) at water depths of 200–1800+ m and temperatures of 5–8 °C (Haefner & Musick, 1974; Wigley *et al.*, 1975; Steimle *et al.*, 2001). Red deep-sea crabs support a small, male-only and market-driven fishery since the 1970s New England and the Mid-Atlantic (Wigley *et al.*, 1975; Wahle *et al.*, 2008). To this day, there is still little information about *C. quinquedens* biology, growth, natural mortality, abundance and reproduction. Therefore, RDSC are considered a data-poor stock. Although a fishery management plan was implemented in 2002 by the New England Fishery Management Council (Council), the lack of essential information makes it challenging to conduct stock assessment in order to determine the status of the stock. Consequently, research focus on RDSC biology is vital to provide information to improve management strategies to preserve the fishery resource. The understanding on the reproductive biology of a species is important for developing a sustainable management for the fishery (Zairion *et al.*, 2015). The overall goal of this dissertation research is to describe the reproductive biology of the present population of *C. quinquedens*.

DEDICATION

I want to dedicate my dissertation to my parents, Maribel Rivera Vega and Tomás Martínez González, for their unconditional love and support.

To my sister, Karina Marie Martínez Rivera, for the words of wisdom and encouragement to pursue my dreams.

ACKNOWLEDGEMENTS

I am thankful to my advisor Dr. Bradley G. Stevens for giving me the opportunity to work in this project and guidance throughout my degree. I would like to express my deep gratitude to my dissertation committee, Dr. J. Sook Chung, Dr. Andrea Johnson, Dr. William Christopher Long and Dr. Thomas Miller for their advice and insights.

Financial support for this research was provided by National Oceanic and Atmospheric Administration (NOAA) Grant NA11SEC4810002 and NA16SEC4810007 as part of the NOAA Living Marine Resources Cooperative Science Center.

I am extremely grateful to The Atlantic Red Crab Company, Jon Williams (CEO), Bruce Medeiros (Manager), Johnny (Captain) and the crew for allowing us on their fishing trips and assistance during sampling. I want to thank the Red Crab Research Group including Shadaesha Green, Dr. Indu Sharma and Justin Wilson for helping me in the sample collection. My summer intern Samantha Peart for the help with the histology analysis. I would like to acknowledge Dr. Andrés G. Morales-Núñez for the assistance at the lab and letting me use the microscopes in his laboratory.

I am very thankful to Audy J. Peoples for his review and comments to my dissertation. My friends and peers Wilmelie Cruz Marrero and Laura C. Almodóvar Acevedo for their support, advise and help throughout this process. I want to acknowledge all my other friends and fellow students for the support and good times during all the years of graduate school.

I will always be grateful for my family, they have always believed in me and encouraged me to work hard to achieve my goals. Los amo.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION

RED DEEP-SEA CRAB LIFE HISTORY

The red deep-sea crab (RDSC), *Chaceon quinquedens* Smith, 1879, is a deep-water brachyuran inhabiting the continental shelf and slope in the Atlantic Ocean from the Gulf of Maine to the Gulf of Mexico at water depths of 200–1800+ m and temperatures of 5–8 °C (Haefner & Musick, 1974; Wigley *et al.*, 1975; Stevens & Guida, 2016). Hastie (1995) described the life history of *C. quinquedens* as typical of most brachyurans (Fig. 1.1). Observations of RDSC mating were described by Elner *et al.* (1987) and consisted of a prolonged courtship followed by copulation shortly after female ecdysis. The main characteristics of mating for *C. quinquedens* are long duration of copulatory embrace, absence of pre-molt carrying embrace and no post-copulatory pairing phase (Elner *et al.*, 1987). Mating starts when males form a cage around females until they molt; this pre-copulatory phase can last 12–13 days (Elner *et al.*, 1987). During copulation the pair is clasped sternum to sternum, the duration of this embrace for *C. quinquedens* was from 7 to 11.5 days and varied among the three pairs in the study (Elner *et al.*, 1987). Elner *et al.* (1987) suggested that the male protects the soft-shelled female for an extended period of time to secure his genetic investment by ensuring the survival of the female and the likelihood that his sperm is not displaced by that of another male. Elner *et al.* (1987) reported that egg extrusion did not occur immediately after copulation and sperm masses were found in both spermathecae but no presence of sperm plugs was externally visible in the genital pores. Like other brachyurans, RDSC showed adaptive mating, where males and females may mate with several partners (Elner *et al.*, 1987).

After ovulation and fertilization, females carry the eggs attached to the abdomen for approximately 9 months (Erdman *et al.*, 1991). Ovigerous females are commonly found on the continental slope at water depths of 200–700 m, at water temperatures between 4 and 10 °C and a migration to shallower waters after extrusion has been suggested (Wigley *et al.*, 1975; Haefner, 1977, 1978). Mature eggs are large and yolky, ranging from 484 to 846 µm in diameter (Haefner 1977; Hines 1982, 1988). Embryo development has not been described for RDSC. However, a relationship between embryo color and stage of development has been reported, like other brachyurans (Haefner, 1978; Wigley *et al.*, 1975; Martínez-Rivera & Stevens, manuscript in preparation). Hatching of *C. quinquedens* has been suggested to occur at water depths of 200–400 m (Haefner, 1978 & Wigley *et al.*, 1975), water temperatures of 6–10 °C and it is located within the permanent thermocline of the continental slope (Beardsley & Flagg, 1976, as cited in Kelly *et al.*, 1982). Hatching duration for RDSC has been estimated at approximately 18 days (Pérez-Pérez, 2017). After hatching, larvae are released into the water column (Haefner, 1978). Larval development consists of a prezoeal stage, four zoeal stages and a final megalopa stage based on laboratory experiments (Perkins, 1973). The larvae require 23–125 days from hatching until the megalopa settles depending on water temperatures and food availability (Kelly *et al.*, 1982). Pérez-Pérez (2017) observed development from first zoeal stage to fourth zoeal stage at water temperatures of 15–20 ºC with diets of rotifers, *Artemia spp.* and combination of both. Kelly *et al.* (1982) reported that megalopae developed faster at surface water temperatures (>20 ºC) than at the hatching site (6–10 ºC). The first stage larvae of RDSC exhibited negative geotaxis, high barokinesis, high thermokinesis and the ability to go across thermoclines suggesting

ontogenetic upward vertical migration (Kelly *et al.*, 1982). Previous studies have determined that larvae of RDSC are planktotrophic (Haefner, 1978; Hines, 1988; Kelly *et al.*, 1982; Sulkin & van Heukelem, 1980). Sulkin & van Heukelem (1980) reported a nutritional flexibility of the larvae and speculated that this evolutionary adaptation was necessary to inhabit the deep-sea environment and retain the planktotrophic larvae with vertical migration. This nutritional flexibility may be an outcome of having large yolky eggs (Hines, 1988). However, the larvae of RDSC have rarely been collected in surface waters; only Serchuk & Wigley (1982) and Roff *et al.* (1986) have reported collections.

Megalopae descend looking for suitable habitat to settle, and recruitment of juveniles takes place in deep waters (~1000 m) (Wigley *et al.*, 1975; Wahle *et al.*, 2008; Stevens & Guida, 2016). Wigley *et al.* (1975) reported juveniles from 8 mm to 18 mm at depths of 740–1000 m, and Stevens & Guida (2016) indicated juveniles recruit in waters deeper than 850 m. The first crab instar after settlement was reported to be 4 mm carapace width (CW) (van Heukelem *et al.*, 1983, as cited in Steimle *et al.*, 2001), which is relatively large compared to most crab species (Hines, 1990) and a large size at settlement will reduce number of instars and time to reach maturity (Hines, 1986, 1990). Absence of juvenile RDSC in shallow waters may indicate that recruitment occurs primarily in deep waters or may be due to non-specific settlement followed by higher mortality in shallow waters (Hines, 1990).

Adult crabs reach a maximum size of about 150–180 mm in carapace width (CW), live for 15 years or more and exhibit slow growth and maturation rates (Wigley *et al.*, 1975; Gerrior, 1981; Serchuk & Wigley, 1982; Hastie, 1995). A total of 18–20 molts are needed for an individual RDSC to reach maximum size (Haefner, 1978; van

Heukelem *et al.*, 1983). Several studies have documented that males grow larger and heavier than females (Wigley *et al.*, 1975; Gerrior, 1981; van Heukelem *et al.*, 1983; Weinberg & Keith, 2003, Stevens & Guida, 2016). Males may reach a maximum size of 150–178 mm CW and females may reach a slightly smaller size of ~120–140 mm CW, which can be achieved in 7–8 years (Gerrior, 1981; van Heukelem *et al.*, 1983). Shell condition of RDSC showed evidence of slow growth; old-shell condition predominates in males from 75 to 100 mm in carapace length (CL) and in females above 60 mm CL (Stevens & Guida, 2016). Attempts have been made to estimate age using a new technique based on growth rings in the gastric mills, but age bands were not clear enough to interpret (Wilson, 2016). Furthermore, information on growth curves for *C. quinquedens* is not available, though a tagging study reported intermolt period of 6–7 years for large adult crabs (>100 mm CW) (Gerrior, 1981; Lux *et al.*, 1982).

Variations in the abundance of females, males, and juveniles at different water depths provided evidence of a size-related up-slope migration for *C. quinquedens* (Wigley *et al.*, 1975; Haefner, 1978; Stevens & Guida, 2016). Females tend to migrate to shallower water (< 500 m) than males (Hastie, 1995). The trend of females inhabiting shallower waters (200–650 m) and males uniformly predominating in all depths except near the upper slope has been observed in several studies (Wigley *et al.*, 1975; Haefner, 1978; Stevens & Guida, 2016). Haefner (1978) observed that crab size was inversely correlated with water depth in females inhabiting 200–1500 m and only in the shallow waters (200–500 m) for males. Hence, as crabs become older and larger they migrate to shallow waters on the continental slope where they fully mature, after which they undergo an ontogenetic migration back to intermediate depths (Wigley *et al.*, 1975;

Haefner, 1978; Stevens & Guida, 2016). The differences in location by sex suggest a possible seasonal migration related to reproduction, in which females migrate down-slope and males move up-slope during mating season (Wigley *et al.*, 1975). However, mating grounds have not been observed or discovered for *C. quinquedens*. To date, no evidence of restricted seasonality in breeding activity or female terminal molt has been found. The sex ratio of the RDSC population showed that females are considerably more numerous than males (Wigley *et al.*, 1975, Stevens & Guida, 2016). Sex ratios reported by Stevens & Guida (2016) suggested a depletion of large males relative to the abundance of females. The changes in the sex ratios were associated with water depths and geographical zone (Wigley *et al.*, 1975).

RED DEEP-SEA CRAB SYSTEMATICS

C. quinquedens is a decapod crustacean in the Superfamily Portunoidea, Rafinesque, 1815, characterized by the carapace being usually wider than long and of hexagonal, sub-hexagonal, rectangular, or transversely ovate shape (WoRMS, 2018). Other distinctive characteristics include chelipeds that are usually robust, short antennules and antennae, orbits broad and eyes conspicuous, and a quadrate buccal frame (WoRMS, 2018). Red deep-sea crab is in the family Geryonidae, Colosi, 1923, and the main characteristics of this group are a hexagonal carapace, antero-lateral margins with three or five teeth, a suborbital margin with only one inner tooth, and the absence of swimming legs (WoRMS, 2018). The original genus of *C. quinquedens* was *Geryon* (Smith, 1879) but was reclassified to *Chaceon* by Manning & Holthuis (1989). The characteristics of the Genus *Chaceon* include carapace length half to two-thirds of its width, semiquadrate shape, dorsal surface with the branchial regions not markedly

inflated, and the antero-lateral margins with five teeth (Manning & Holthuis, 1989). *C. quinquedens* can be identified by the five spines in the carapace, depressed dactyli on the walking legs, a sharp outer spine on the carpus, a distal dorsal spine on the propodus of the cheliped, each walking leg has a distinct distal dorsal spine, merus of the fifth leg is three-fourths the carapace width, and propodus of the fifth leg is 5-7 times as long as high (Manning & Holthuis, 1989). The complete systematics of *C. quinquedens* are described in Table 1.1.

RED DEEP-SEA CRAB ECOLOGY

The continental shelf and slope of the Mid-Atlantic Bight (MAB) are part of the Northeast U.S. Continental Shelf Large Marine Ecosystem. The MAB resides in the area from Cape Hatteras, North Carolina to Cape Cod, Massachusetts. It has a broad continental shelf extending up to 150 km to the shelf edge in water depths of 200 m that descends along the continental slope to a depth of 3000 m (Stenhouse *et al.*, 2015). Terrigenous sediments dominate the continental shelf including the shelf valleys leading to the submarine canyons (Pratt, 1968). The oceanography of the MAB is characterized by water flow from north to south by three major freshwater inputs, the Hudson River, Delaware Bay, and Chesapeake Bay (MAFMC, 2016). The predominant current is the Gulf Stream, an intense poleward warm current in the western North Atlantic with a structure, transport and water mass properties that vary geographically and temporally (Taylor & Stephens; 1998). Secondary is the deep western boundary current (DWBC), a cold water equatorward current that comes from the Labrador current in the Gulf of Maine and Georges Bank and enters the northern MAB (Pickart & Smethie, 1993). Seasonal stratification of water masses on the shelf influence phytoplankton blooms, with

the largest observed in the late fall and winter (Schofield *et al.*, 2008; Yoder *et al.*, 2001, as cited in Stenhouse *et al.*, 2015). These hydrographic characteristics shape the ecology of the system, influencing the distribution patterns and biology of species, food web and dispersal and migration pathways (MAFMC, 2016). Species found in the MAB include apex predators (e.g. sharks (Unc.) and bluefin tuna (*Thunnus thynnus*)), piscivores (e.g. spiny dogfish (*Squalus acanthias*), summer flounder (*Paralichthys dentatus*), Atlantic cod (*Gadus morhua*) and monkfish (*Lophius spp.*)), planktivores (e.g. Atlantic herring (*Clupea harengus*), alewife (*Alosa pseudoharengus*) and Atlantic mackerel (*Scomber scombrus*)), benthivores (e.g. RDSC and black sea bass (*Centropristis striata*)) and filter feeders (e.g. scallops, sand dollars, whelks and quahogs) (NEFSC, 2018). Red deep-sea crabs have been reported in the Gulf of Maine, Georges Bank, MAB and Gulf of Mexico (Wigley *et al.*, 1975) and have been associated with canyons including, but not limited to, Block Canyon, Hudson Canyon, Baltimore Canyon and Norfolk Canyon (Stevens & Guida, 2016).

Scarce information about the larvae of RDSC suggests that after hatching they undergo a vertical migration to surface waters (Haefner, 1978; Kelly *et al.*, 1982). Therefore, larvae experience drastic changes in temperature and salinity and important behavioral adaptations are required to survive, including nutritional flexibility. For example, larvae fed with rotifers and artemia+rotifers survived longer than larvae fed algae and unfed (Pérez-Pérez, 2017). Other behavioral adaptations of the larvae include negative geotaxis and tolerance to a wide range of temperature (6–25ºC) and pressure (Kelly *et al.*, 1982). The current annual temperature of the surface waters in the MAB range from 5 ºC to 30°C (MAFMC, 2016). Larval development time decreased and

swimming rate increased with warmer temperatures (>20 ºC) (Kelly *et al.*, 1982). Therefore, changes in surface water temperatures due to climate change may be a concern for recruitment and research in this area is vital. Kelly *et al.* (1982) developed a recruitment model for *C. quinquedens* larvae that may explain the ontogenic migration to increase dispersal. Larvae in the hatching site will be transported toward the southwest until entrainment in the Gulf Stream prior to settlement. The warm waters of the Gulf Stream may accelerate development and dispersal toward the northeast. However, the larvae at the northeastern geographical area will settle as megalopa without entrainment in the Gulf Stream. Few studies have been able to collect the larvae in surface waters, therefore, a lack of recruitment information is still a concern.

Red deep-sea crabs inhabit the mesopelagic to the bathypelagic zone. Wigley *et al.* (1975) stated that RDSC have been found in waters shallower than 200 m but only in the Gulf of Maine. Previous studies have reported small juveniles in mid-slope waters (~1000 m) and larger juveniles at >750 m. Wigley *et al.* (1975) provided the first evidence of the up-slope migration. Growth in crustacean increases with water temperature so would be enhanced by vertical migration (Wigley *et al.*, 1975; Haefner, 1978). Additionally, recruiting at deeper waters than the adults might provide protection from cannibalism (Linberg & Wenner, 1990). There is limited information about the food habits and settlement preferences of juveniles. Farlow (1980) reported that small crabs prey on sponges, hydroids, mollusks, small polychaetes, crustaceans, and possibly tunicates. The juveniles have been observed at temperatures from 4.4 ºC to 5.5 ºC outside the “warm band” in the continental slope (Wigley *et al.*, 1975, Steimle *et al.*, 2001), however, information on optimal temperature for survival is not available. The juveniles’

role in the ecosystem has not been defined, but they probably serve as prey for other organisms.

Bathymetric differences in the distribution of adult crabs have been observed; crabs were sparse in shallow waters, common at intermediate depths, and moderately sparse to absent in deep waters (Wigley *et al.*, 1975; Stevens & Guida, 2016). Geographical variations were also observed; the southern zone was inhabited by smaller crabs than those in the northeastern region (Wigley *et al.*, 1975). However, Stevens & Guida (2016) observed larger crabs in the Norfolk Canyon area. The biomass of crab was reported to be substantially larger in southern New England than in the other regions (Wigley *et al.*, 1975). Nonetheless, new surveys are needed to describe the current biomass by geographic regions. Up-slope migration was apparent in all geographic zones (Wigley *et al.*, 1975; Stevens & Guida, 2016). Previous studies suggest the population of RDSC in the Gulf of Mexico exhibit different distribution patterns, behaviors, and physiology from the population in the MAB (Lockhart *et al.*, 1990; Kilgour & Shirley, 2008). In the northeastern U.S., including the MAB, crabs were often found at 5–8 ºC, with some at temperatures above 9 ºC (Wigley *et al.*, 1975, Stevens & Guida, 2016). Wigley *et al.* (1975) observed variations in crab size by temperature; large males and females inhabited warmer waters, and intermediate size crabs of both sexes were present in moderate temperatures. Stevens & Guida (2016) showed no pronounced annual variation in temperature suggesting that RDSC live within a range of preferred temperatures year-round. The bottom sediment type of the habitat at all water depths (i.e. 200–1000+) has been described as soft, olive-green, silt-clays (Wigley *et al.*, 1975). The small-scale topography of the sea bottom surface was flat and smooth with irregularities

in the form of cones, craters, holes, burrows, and mounds of sediments, and some areas appeared to be impacted by fishing activities (Wigley *et al.*, 1975). Hines (1990) found that RDSC were patchily distributed on both soft and hard substrates. The RDSC have been described as scavengers, opportunistic, and benthivores. They are considered a predominant predator in the continental slope communities (Farlow, 1980; Hines, 1990). Adult crabs were observed eating benthic fauna and larger prey, such as demersal and mid-water fish, squid, and quill worm, but remains of RDSC shell were also found in their stomachs suggesting cannibalism (Farlow, 1980). The impact of RDSC activities (e.g. excavation) in the ecosystem include affecting the abundance of infaunal species, altering sedimentary texture and fabric and accelerating rates of sediment mixing. Therefore, RDSC are considered biotic modifiers of the seafloor that can alter the physical structure of the seafloor and the seabed hydrodynamic regime (Whitlatch *et al.*, 1990). Whitlatch *et al.* (1990) reported the most abundant epifaunal for the southern New England community to be RDSC, eelfish (*Aldrovandia affinis*), galatheid crabs (*Munida spp.*), Gulf Stream flounder (*Citharichthvs arctifrons*), rat-tail fishes (*Coryphaenoides carapinus*) and white hake (*Urophycis tenuis*). There is a lack of information about predation on RDSC, considering the absence of strong defensive mechanisms (Steimle *et al.*, 2001). The only competitive interactions with other species have been suggested by Hines (1990) and include golden crabs (*Chaceon fenneri*), Jonah crabs (*Cancer borealis*) and American lobster (*Homarus americanus*).

REPRODUCTIVE BIOLOGY

Reproductive cycle

Crustaceans have developed physiological and behavioral adaptations in order to survive in their particular environments (Sastry, 1983). The reproductive cycle is a sequence of complex physiological and behavioral events during the life history of a species, that includes gametogenesis (oogenesis in females), copulation (mating), ovulation, oviposition and fertilization, incubation of eggs, and hatching of the larvae (Sastry, 1983). Hence, the reproductive cycle is described by the chronological and temporal patterns of these events, usually using seasons or months, which are influenced by the environment inhabited by the species. Sastry (1983) indicated that the reproductive cycle can be considered a phenotypic expression in order to achieve reproductive success. Therefore, the reproductive strategy of a species is considered an evolutionary adaptation to ensure the survival of the offspring (Hartnoll & Gould, 1988). The reproductive cycle of brachyurans can be classified as seasonal reproduction when it is restricted to a few months or a single season; seasonal-continuous, when a peak occurs over multiple months or seasons; or continuous, when it takes place year round (Pinheiro & Fransozo., 2002; Zairion *et al.*, 2015). In brachyurans, a reproductive cycle can have an annual or multi-year pattern. Sainte-Marie (1993) indicated the importance of understanding the relationship between oceanography, demographics, and the reproductive cycle with annual and biennial patterns to elucidate why they are preferred or successful for any given species. Geryonid crabs are expected to have a complex reproductive cycle given the migration pattern, slow growth and maturation rates, and prolonged embryo development. Previous studies have described an annual reproductive cycle for *C. fenneri*

from southeastern Florida (Erdman & Blake, 1988), *Geryon trispinosus* (Attrill *et al.*, 1991) and *C. affinis* from the Azores (Pinho *et al.*, 2001), whereas others have suggested a continuous reproductive cycle for *C. maritae* (Meville-Smith, 1987), *C. quinquedens* (Haefner, 1977,1978) and *C. bicolor* (Smith, 2006). For this study the focus was on elucidating the reproductive biology of *C. quinquedens*.

Factors that regulate reproduction

The reproductive cycle in brachyurans is usually regulated by endogenous factors related to the anatomy and physiology of a species like molt cycle, energy allocation, maternal care and embryo development, and by exogenous factors related to the environment such as habitat, temperature and food supply (Sastry, 1983; Raviv *et al.*, 2008). The deep-sea environment does not exhibit seasonal variation except for food supply, hence, biotic interactions, such as competition for resources, avoidance of predators, behaviors of locating food and mates, are expected to have a greater impact on the reproductive strategy of the species (Sastry, 1983).

Endogenous factors such as age, growth, biochemical composition, metabolism, molt cycle and energy allocation are considered important regulators of reproduction (Sastry, 1983). In brachyurans, maturity and the activation and development of gametes are correlated with a certain age and/or size, thus the life span of a species regulates the reproductive strategy (Sastry, 1983). The major nutrients involved in biochemical processes related to ovarian maturation are lipids and proteins, and availability of these nutrients influences the reproductive cycle (Sastry, 1983; Cuzon *et al.*, 2008). The time of reproduction of a species can be influenced by metabolic activity in relation to the ability

of the animal to regulate metabolism under stressful conditions (Sastry, 1983). The endocrine system is a major internal regulator of reproduction and has been a priority of research related to aquaculture. In crustaceans, multiple hormones control the timing and duration of molting and reproduction, hence these events can occur sequentially or overlap (Subramoniam, 2011). Energy allocation influences the reproductive cycle due to energy-demanding processes like ovary maturation and ecdysis (Raviv *et al.*, 2008). The amount of energy invested in oocyte production may determine if the reproductive output will consist of many small eggs or fewer large eggs (Sastry, 1983).

Exogenous factors that regulate reproduction include temperature, salinity, food supply, and other physical characteristics of the environment (Sastry, 1983). However, deep-sea species lack environmental cues related to changes in temperature or salinity. Therefore, temporal patterns in quality and quantity of the food supply in the environment may influence the time of reproduction (Sastry, 1983; Cuzon *et at.*, 2008). Geographically separated populations of a species may have different reproductive patterns, as well as different size at sexual maturity (Sastry, 1983). Variations in size at sexual maturity and occurrence of ovigerous females were observed in two populations of *C. affinis* collected at different geographical locations (Biscoito *et al.*, 2015). Variations in migration patterns to suitable locations for mating and breeding may be influenced by geographic location of a population (Sastry, 1983). Elner *et al.* (1987) suggested that variations in female molt cycle due to geographical differences may influence variations in the mating season. Therefore, due to the lack of seasonal changes in the deep-sea environment, it is likely that food availability and geographic location are the predominant exogenous factors influencing RDSC reproductive seasonal pattern.

Ovary morphology

In brachyurans, the typical reproductive system in females consists of paired ovaries connected in an H-shaped form located either dorsal or dorsolateral to the gut (Sastry, 1983; Mclay & Becker, 2015). The ovary extends into a pair of oviducts that connect to the gonopores, also known as genital pore or vulvae, and the paired spermathecae (seminal receptacles) (Sastry, 1983). Females are inseminated through the external openings (i.e. gonopores) and store sperm in the spermathecae. The vagina is defined as the portion of the oviduct that connects the gonopore to the spermathecae (Mclay & Becker, 2015). The location of the gonopores and the connection of oviduct-spermathecae varied among species. In geryonid crabs, the gonopores are located in the sixth thoracic sternite (Haefner, 1977; Erdman & Blake, 1988).

Size at sexual maturity

In brachyurans, internal and external maturity indicators can be used to estimate size at sexual maturity (Mente, 2008). Conan *et al.* (2001) described three types of maturity for crustaceans: gonadal maturity, the ability to produce mature gametes; morphometric maturity, when secondary sexual characters are developed; and behavioral maturity, the ability to mate or produce eggs efficiently. Gonadal maturity is also referred to as physiological maturity and in females is defined by ovary maturation. Morphometric maturity, also known as functional maturity, is defined as the development or appearance of morphological characteristics needed for reproduction. Morphological and physiological maturation may be synchronized in some species but asynchrony has been reported in geryonid crabs (*C. affinis*, Biscoito *et al.*, 2015; *C. maritae*, Meville-

Smith, 1987). Female brachyurans can be classified into four phases of reproduction: immature (prepubertal), pubescent, primiparous, and multiparous (Sainte-Marie, 1993). Immature females have not developed the secondary sexual characters and their ovaries have not reached maturity. Females that are committed to oviposition the next molt are considered pubescent (Waddy & Aiken, 2005). The pubertal molt indicates the onset of sexual maturity, which includes morphological structures for reproduction and other secondary sexual characters (Sastry, 1983). Variations in the pubertal molt among species have been observed; for example, pubescent female snow crab (*Chionoecetes opilio*) have mature ovaries and will become morphometrically and functionally mature following the next molt (Sainte-Marie, 1993). However, in other species the secondary phase of oogenesis and mating occurs after the pubertal molt (Sastry, 1983). Primiparous females are mature carrying an egg mass for the first time (Sainte-Marie, 1993; Waddy & Aiken, 2005). Mature females that have produced a second or more egg brood are considered multiparous (Sainte-Marie, 1993). In brachyurans, two common estimates of size at maturity used are: the morphological size at maturity and the physiological size at maturity. Haefner (1977) estimated the size at sexual maturity for female *C. quinquedens* based on ovary and embryo condition and histology of 20 individuals to be 65–75 mm CL.

Morphological maturation

In brachyurans, separate sexes are common and the females' abdomen is usually broader than the males' (Mente, 2008). Therefore, the shape of the females' abdomen and the presence of the gonopore structures define the sexual dimorphism in brachyurans (Mclay & Becker, 2015). In order to estimate size at maturity the allometric growth of the

abdomen in relation to carapace length or width is used. Another common method is the use of gonopore condition, including signs of copulation such as sperm plugs or blackened areas surrounding the gonopore. Stevens & Guida (2016) could not determine size at maturity for females of *C. quinquedens* using abdomen allometry. However, estimates of size at maturity using allometric growth of the abdomen have been successful for *C. affinis* (Biscoito *et al.*, 2015).

Ovary maturation

The process of gamete maturation in females is called oogenesis and consists of a series of development stages, from the oogonium to oocyte maturation (Brown, 2009). Sastry (1983) described the process for crabs: oogenesis begins with primary oogonia originating from the germinal zone of the ovary and displacing to the lumen as new oogonia are formed. When the secondary oogonia in the lumen undergo the first meiotic division it is considered an oocyte. This primary oocyte phase is characterized by the nucleus swelling into a germinal vesicle and oocyte growth. After completion of the meiotic division they are considered secondary oocytes. The subsequent growth phases include previtellogenesis, which is characterized by an increase in size, followed by vitellogenesis growth phase during which the cytoplasmic region increases as yolk globules begin to appear. In crustaceans, yolk deposition occurs in two phases, primary and secondary vitellogenesis. Generally, physiological size at maturity is estimated using histological analysis of the ovary in order to classify the ovary stage as immature or mature.

Mating behavior

Hartnoll (1969) described two patterns of mating for brachyurans, both consisting of the hard-shell male forming a protective cage around the female. The first mating pattern involves females in soft-shell condition and copulation occur after the female has molted. This mating pattern was observed in *C. quinquedens* by Elner *et al*. (1987). The second mating pattern occurs when the female has not recently molting and copulation is between a hard-shell male and female. This second mating pattern was not observed in *C. quinquedens*, however the possibility of hard-shell female mating should be investigated (Elner *et al.*, 1987). Mating behavior includes pre-copulatory and post-copulatory male guarding and time and duration varies across species (Hartnoll, 1969). Elner *et al.* (1987) observed pre-copulatory male courtship but not post-copulatory guarding for RDSC. The pre-copulatory and post-copulatory behavior are considered male competitive strategies, the first one to monopolize a females before copulation and the second to avoid sperm competition with other males (Barki, 2008). In most brachyurans, males are larger than females due to mating strategy, this is observed in *Chionoecetes opilio*, (Sainte-Marie *et al.*, 1999) and *C. quinquedens* (Elner *et al.*, 1987; Wahle *et al.*, 2008).

Fecundity

In brachyurans, oviposition refers to the extrusion of eggs by the female and fertilization occurs simultaneously with the time of oviposition (Mclay & Becker, 2015). Like most crustaceans, *C. quinquedens* carries the extruded eggs attached to the pleopods of the abdomen until embryo development is completed and larvae hatch (Sastry, 1983; Elner *et al.*, 1987). Female *C. quinquedens* are considered iteroparous, meaning they can

reproduce multiple times. The reproductive output of a species depends on their life span, and may differ due to temporal and spatial variations (Sastry, 1983; Sainte-Marie, 1993; Swiney *et al.*, 2010). Fecundity refers to the number of offspring produced by a female. Different methods for estimating size-specific fecundity include: potential fecundity, the number of oocytes in mature ovaries; real fecundity, the number of eggs in the pleopods of the abdomen; effective fecundity, the number of eggs in the abdomen during hatching (Mente, 2008). It is important to account for egg loss in fecundity estimates, therefore females with a recently extruded brood mass are preferred to use for the estimate (Mente, 2008). Hines (1982) indicated that the principal determinant of size-specific fecundity is the body size of the female. Furthermore, energetic and mechanical or physical constraints may regulate reproductive output of a species, thus brood size is determined by the energy allocation and the volume of the body cavity of the females (Hines, 1982). Sainte-Marie (1993) found a positive relationship between brood weight and carapace width, and brood weight with weight of ovaries. Fecundity for *C. quinquedens* was estimated for 17 individuals and increased linearly with body size (Hines, 1988). In females, the volume of the body cavity limits the space available of yolk accumulation in the ovary (Hine, 1982). In general, larger females will produce greater number of offspring than small females, hence there is an advantage for males in selecting the largest female (Stevens *et al.*, 1993).

Embryo development

Sastry (1983) indicated that the size of the eggs will determine the rate of embryo development and size of larvae hatching, which have important consequences for recruitment. Hines (1982) indicated that in addition to constraints by the body size of the

female, the egg size influenced the number of eggs produced. Sainte-Marie (1993) found a negative relationship between brood weight and egg diameter for *Chionoecetes opilio*. An adaptation in crustaceans is that larger eggs produce larger offspring that provide greater feeding and competition abilities, but large eggs typically means fewer offspring and more maternal care (Sastry, 1983; Mclay & Becker, 2015). The duration of embryo development varies across species and is influenced mostly by the water temperatures (Kelly *et al.*, 1983; Moriyasu & Lanteigne, 1998).

FISHERY

The fishery for *C. quinquedens* was developed in the 1970s in New England and the Mid-Atlantic (Wigley *et al.*, 1975; Wahle *et al.*, 2008), and is one of the largest and oldest geryonid fisheries in the Atlantic (Hastie & Saunders, 1992). Red deep-sea crabs support a small, male-only and market-driven fishery. Presently, the only company fishing for RDSC is The Atlantic Red Crab Company owned by Jon Williams and established in 1996. The company has five fishing vessels of approximately 20–34 m (F/V Hannah Boden, F/V Krystle James, F/V Benthic Mariner, F/V Diamond Girl, and F/V Sea King). Fishing trips last between seven and nine days and catch approximately 70,000 pounds of live crabs. The fishing grounds include Baltimore Canyon and Norfolk Canyon and fishing occurs year-round at water depths >600 m. The main processing plant is located in New Bedford, Massachusetts. The standard trap used in the fishery is a conical pot (120 cm diameter × 60 cm height) with ~7.6 cm nylon mesh and a top round entry (25 cm) (Tallack *et al.*, 2007). The fishing gear consists of baited traps tied to a string in groups from 70 to 100 traps. Fishery selectivity is approximately 0% at <80 mm CW, 50% at 92 mm CW and near 100% at 120 mm CW (Wahle *et al.*, 2008). Stevens &

Guida (2016) reported the equivalent size of the crabs captured in carapace length to be 63.4–96.5 mm CL with a medium crab size of 73.8 mm CL. The acceptable marketable size changed from 114 mm CW in 1974 to less than 94 mm CW since 2008 (Chute *et al.*, 2008).

In 2002 the New England Fishery Management Council (Council) implemented the fishery management plan based mostly on quota restrictions and fishing effort controls, due to scarce information on *C. quinquedens*. The RDSC is managed as a single stock and the management unit in US Atlantic waters ranges from Cape Hatteras, North Carolina (35º 15.3' N. lat.) to the US–Canada border (NEFMC, 2016). The management unit is divided into three statistical area groups: 1) Georges Bank/ southern New England, 2) New Jersey and 3) Delmarva (Fig. 1.2) (NEFMC, 2016). The RDSC fishery in the US has been operating nearly equally in all regions of the statistical area areas in recent years (NEFMC, 2016). In 2015, the Mid-Atlantic Fishery Management Council (MAFMC) approved an amendment to protect deep-sea corals from the impacts of bottom-tending fishing gear. Following the amendment in 2016, NOAA Fisheries announced a proposed rule to designate deep-sea coral protection zones in the Mid-Atlantic and designation of the first marine national monument in the Atlantic Ocean, the Northeast Canyons and Seamounts Marine National Monument, in September 2016. This monument will provide critical protection for important ecological resources and marine species. The Atlantic Red Crab Company earned an exemption by the MAFMC to continue fishing in the canyons and seamounts under the deep-sea coral amendment (Fig. 1.3). Additionally, NOAA Fisheries allowed them seven years of fishing in the monument. The Canadian RDSC fishery was inactive until 2011 and recent fishing activity started in 2014 when

RDSC licenses were acquired by new owners (NEFMC, 2016). At present, RDSC are considered a data-poor stock because little is known about the biology, growth, natural mortality, abundance or reproduction. The lack of this essential information makes it challenging to develop appropriate management strategies, such as biological reference points (e.g. maximum sustainable yield (MSY) and overfishing limit (OFL)). In addition, minimum legal size (MLS) has not been set due to lack of information on biological parameters such as size at 50% sexual maturity (SM_{50}), fecundity, or timing of reproduction. The last peer-reviewed assessment on *C. quinquedens* was conducted by the Data Poor Stocks Working Group (DPSWG) in 2009, however landings have been increasing since 2015 (Fig. 1.4) (NEFMC, 2016). The Fishing Years Specifications since 2011 have remained the same, using average long-term (1974-2008) landings as Total Allowable Landings (TAL) (i.e. 1775 mt) (NEFMC, 2016). The Council believes the TAL is safely below an undetermined overfishing threshold (NEFMC, 2016) and concluded that the fishery appears to be sustainable based on the historical landings (NEFMC, 2013). However, it is important to take into consideration the possible negative impacts of a male-only fishery, in which the primary discards are females (NEFMC, 2013). Up to 85% of the catch consists of females and undersized crabs, which are discarded, with a possible mortality of approximately 5% (Tallack, 2007). The Council (2013) found that discard rates show an increasing trend, possibly due to truncated size structure of the male population caused by a male-only fishery. Additionally, the male-only fishing strategy may have an effect on egg production and sperm-limitation and create problems with spawning behavior (NEFMC, 2013).

Two surveys have estimated abundance of RDSC populations using towed camera systems; the first (Wigley *et al.*, 1975) was conducted in 1974 prior to the onset of commercial fishing, whereas the second (Wahle *et al.*, 2008), showed a 250% increase in overall biomass (mostly due to juveniles) in 2003-2005, after three decades of targeted harvesting on males. However, the biomass of large males (>114 mm carapace width (CW)) declined by 42% (Wahle *et al.*, 2008). Weinberg & Keith (2003) also reported a decline in large males in the 350-500 m depth zone, where fishing occurs, as well as a decline in body condition factors (carapace length:weight ratios). For another geryonid crab, *C. notialis*, the population structure was negatively affected due to long term harvesting that resulted in a decrease of individual weight (Masello & Defeo, 2016). The reduction in biomass of large males has caused concerns about sperm limitation for *C. quinquedens* (Wahle *et al.*, 2008). The most recent survey conducted by Stevens & Guida (2016) reported density of RDSC similar to Wigley *et al.* (1975) in similar geographic zones, and a sex ratio including mature females to be <0.5, indicating depletion of large males. Sex ratios are an important determinant of the effect of fishing in a crab population (Stevens & Guida, 2016). Removing large males from a population may have an impact on reproductive success and output. The abrupt decline of large males may have implications for reproduction of *C. quinquedens* as females reach maturity at 65–75 mm CL (Haefner, 1977) and ovigerous females are found primarily at sizes between 63–105 mm CL (Wigley *et al.*, 1975 (converted to CL using equation in Stevens & Guida, 2016); Haefner, 1978), hence appropriate male partners for mating have to be above 90 mm CL. For fisheries management, it is essential to understand the mating behavior of a

species, because mating of females with smaller males due to lack of large males may reduce reproductive output (Weinberg & Keith, 2003).

At this moment the Mid-Atlantic RDSC fishery is certified by the Marine Stewardship Council, as a sustainable fishery, but they remain a data-poor fishery, hence research remains essential to determine whether or not it is in fact truly sustainable. Previous studies on reproductive biology of *C. quinquedens* were conducted ~30 years ago and may not represent the currently fished population. The NOAA Red Crab Working Group recommended a variety of high priority research needs for RDSC, including a better understanding of the reproductive cycle, maturity schedule, and fecundity of female *C. quinquedens*, the potential reproductive consequences of removing large males from the population, and more (Miller *et al.*, 2009). Information on size at sexual maturity, the size-specific fecundity, and reproductive cycle of a species have important implications on fisheries management. Deep-water species need priority for stock assessment because possible infrequent recruitment may be a barrier to achieving a long-term sustainable fishery (Pinho *et al.*, 2001). The information about the reproductive biology of *C. quinquedens* presented in this study is extremely useful to improve management strategies and merits further investigation. This project was committed to NOAA's mission of providing scientific data to improve, supplement, and enhance management.

RESEARCH OBJECTIVES AND HYPOTHESES

We investigated the size at 50% sexual maturity (SM_{50}), size-specific fecundity, embryo development, and timing of reproduction for *C. quinquedens* in the Mid-Atlantic Bight. The objectives and hypotheses of this research were to:

1. Estimate size at SM_{50} for female *C. quinquedens* using two methods:

    1.1. Examination of oocyte and ovarian development

    1.2. Examination of the vulvae condition

        H1. Size at SM_{50} does not change across collections and locations.

2. Estimate the size-specific fecundity and the stages of embryo development.

        H2.1. Fecundity is positively correlated with body size.

        H2.2. Size-specific fecundity does not change across collections.

3. Determine timing of reproduction.

        H3.1. *C. quinquedens* have a biennial reproductive cycle.

SAMPLE COLLECTION

Red deep-sea crabs for this dissertation project were collected in the Mid-Atlantic Bight aboard National Oceanic and Atmospheric Administration (NOAA) research vessels in 2011–2013 at depths >250 m by trawling (Stevens & Guida, 2016), and commercial vessels via collaboration with The Atlantic Red Crab Company in 2014–2016 at depths >600 m by baited traps (Fig. 1.5). The sampling stations of the NOAA surveys were located in Block Island Canyon, Hudson Canyon, Baltimore and

Washington Canyons, and Norfolk Canyon. The fishing sites of The Atlantic Red Crab Company were located along the Norfolk Canyon and Baltimore Canyon. All dissected crabs were sexed, and measured for carapace length (CL, from the rostral teeth to the center of the edge of the carapace), carapace width (CW, including lateral spines), and abdomen width (AW, at the 3rd segment). The gross morphology of each crab was recorded including the presence, size and color of ovaries, the presence and color of external eggs, vulvae condition and exoskeleton (shell) condition.

REFERENCES

Attrill, M. J., R. G. Hartnoll, and A. L. Rice. 1991. Aspects of the biology of the deep-sea crab *Geryon trispinosus* from the Porcupine Seabight. Journal of the Marine Biological Association of the United Kingdom. 71:311–328.

Barki, A. 2008. Mating behavior. In, Reproductive Biology of Crustaceans, Mente, E. (eds.), 223–265, Boca Raton: CRC Press.

Beardsley, R. C., and C. N. Flagg. 1976. The water structure, mean currents, and shelf-water/slope-water front on the New England continental shelf. Mémoires de la Société royale des sciences de Liège. 6:206–225.

Biscoito, M., M. Freitas, J. G. Pajuelo, R. Triay-Portella, J. I. Santana, A. L. Costa, J. Delgado, and J. A. González. 2015. Sex structure, depth distribution, intermoult period and reproductive pattern of the deep-sea red crab *Chaceon affinis* (Brachyura, Geryonidae) in two populations in the north-eastern Atlantic. Deep-Sea Research I. 95:99–114.

Brown, C. E. 2009. Ovarian morphology, oogenesis, and changes through the annual reproductive cycle of the female blue crab, *Callinectes sapidus* Rathbun, in Tampa Bay. Graduate Theses and Dissertations. M.S. Thesis. University of South Florida. http://scholarcommons.usf.edu/etd/1877

Chute, A. 2006. Deep sea red crab. Northeast Fisheries Science Center. https://www.nefsc.noaa.gov/sos/spsyn/iv/redcrab/archives/37_DeepSeaRedCrab_2006.pdf

Chute, A., L. Jacobson, and P. Rago. 2008. Deep sea red crab. NMFS/NOAA Northeast Fisheries Science Center, Woods Hole, MA.

Cuzon, G., G. Gaxiola, and C. Rosas. 2008. In, Reproductive Biology of Crustaceans, Mente, E. (eds.), 319-364, Boca Raton: CRC Press.

Conan, G. Y., M. Comeau and M. Moriyasu. 2001. Are morphometrical approaches appropriate to establish size at maturity for male American lobster, *Homarus americanus*? Journal of Crustacean Biology. 21(4):937–947.

Elner, R. W., S. Koshio, and G. V. Hurley. 1987. Mating behavior of the deep-sea red crab, *Geryon quinquedens* Smith (Decapod, Brachyura, Geryonidae). Crustaceana. 52:194–201.

Erdman, R. B., and N. J. Blake. 1988. Reproductive biology of female golden crabs *Geryon fenneri* Manning and Holthuis, from southeastern Florida. Journal of Crustacean Biology. 8:392–400.

Erdman, R. B., N. J. Blake, F. D. Lockhart, W. J. Lindberg, H. M. Perry, and R. S. Waller. 1991. Comparative reproduction of the deep-sea crabs *Chaceon fenneri* and *C. quinquedens* (Brachyura: Geryonidae) from the northeast Gulf of Mexico. Invertebrate Reproduction & Development. 19:175–184.

Gerrior, P. 1981. The distribution and effects of fishing on the deep sea red crab, *Geryon quinquedens* Smith, off southern New England. M.S. thesis, Southeastern Massachusetts Univ., North Dartmouth, MA. 130 p.

Haefner, P. A., and J. A. Musick. 1974. Observations on distribution and abundance of red crabs in Norfolk Canyon and adjacent continental slope. Marine Fisheries Review. 36(1):31–34.

Haefner, Jr., P. A. 1977. Reproductive biology of the female deep-sea red crab, *Geryon quinquedens*, from the Chesapeake Bight. Fishery Bulletin. 75:91–102.

Haefner, Jr., P. A. 1978. Seasonal aspects of the biology, distribution and relative abundance of the deep-sea red crab *Geryon quinquedens* Smith, in the vicinity of the Norfolk Canyon, Western North Atlantic. Proceedings of the National Shellfisheries Association. 68:49–62.

Hartnoll, R. G. 1969. Mating in the Brachyura. Crustaceana. 16(2):161–181.

Hartnoll, R. G., and P. Gould. 1988. Brachyuran life history strategies and the optimization of egg production. Symposia of the Zoological Society of London. 59:1–9.

Hastie, L. C. 1995. Deep-water geryonid crabs: a continental slope resource. In A. D. Ansell, R. N. Gibson, and M. Barnes eds. p. 561–584. Oceanography and Marine Biology: an Annual Review. 33. 665 p.

Hines, A. H. 1982. Allometric constraints and variable of reproductive effort in brachyuran crabs (Crustacea, Decapoda, Brachyura). Marine Biology. 69:309–320.

Hines, A. H. 1986. Larval patterns in the life histories of brachyuran crabs (Crustacea, Decapoda, Brachyura). Bulletin of Marine Science. 39(2):444–466.

Hines, A. H. 1988. Fecundity and reproductive output in two species of deep-sea crabs, *Geryon fenneri* and *G. quinquedens* (Decapoda: Brachyura). Journal of Crustacean Biology. 8(4):557–562.

Hines, A. H. 1990. Commentary on life history and ecology of deep-sea crabs of the family Geryonidae. In geryonid crabs and associated continental slope fauna: a research workshop report. W. J. Lindberg and E. L. Wenner (eds.), 30–28, Technical Report 58, Florida Sea Grant College.

Kelly, P., S. D. Sulkin, and W. F. Van Heukelem. 1982. A dispersal model for larvae of the deep sea red crab *Geryon quinquedens* based on behavioral regulation of vertical migration in the hatching stage. Marine Biology. 72:35–43.

Kilgour, M. J., and T. C. Shirley. 2008. Distribution of red deepsea crab (*Chaceon quinquedens*) by size and sex in the Gulf of Mexico. Fishery Bulletin. 106(3):318–320.

Lindberg, W. J. and E. L. Wenner, eds. 1990. Geryonid crabs and associated continental slope fauna: a research workshop report. Technical Report 58, Florida Sea Grant College. 61 p.

Lockhart, F. D., W. J. Lindberg, N. J. Blake, R. B. Erdrnan, H. M. Perry and R. S. Waller. 1990. Distributional differences and population similarities for two deep-sea crabs (Famili Geryonidae) in the Northeastern Gulf of Mexico. 47:2112–2122.

Lux, F. E., A. R. Ganz, and W. F. Rathjen. 1982. Marking studies on the red crab *Geryon quinquedens* Smith off southern New England. Journal of Shellfish Research. 2(1):71–80.

Manning R. B., and L. B. Holthuis. 1989. Two new genera and nine new species of geryonid crabs (Crustacea, Decapoda, Geryonidae). Proceedings of the Biological Society of Washington. 102:50–77.

Martínez-Rivera, S. and B. G. Stevens. Manuscript in preparation. Fecundity of red deep-sea crab, *Chaceon quinquedens* Smith, 1879, in the Mid-Atlantic Bight.

Masello, A., and O. Defeo. 2016. The deep-sea red crab *Chaceon notialis* (Geryonidae) in the southwestern Atlantic Ocean: Spatial patterns and long-term effects of fishing. Fisheries Research. 183:254–262.

Mclay, C. L., and C. Becker. 2015. Reproduction in Brachyura. In, Treatise on Zoology - Anatomy, Taxonomy, Biology. The Crustacea: P. Castro, P. F. J. Davie, D. Guinot, F. R. Schram, J. C. von Vaupel Klein (eds.), Volume 9C, Chapter 7.

Melville-Smith, R. 1987. The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off South WestAfrica/Namibia. Crustaceana. 53(3):259–275.

Mente, E. (eds.). 2008. Reproductive Biology of Crustaceans. Boca Raton: CRC Press.

Mid-Atlantic Fishery Management Council (MAFMC). 2016. Ecosystem Approach to Fisheries Management Guidance Document.

Miller, T., R. Muller, B. O'Boyle, and A. Rosenberg. 2009. Report by the Peer Review Panel for the Northeast Data Poor Stocks Working Group. Northeast Fisheries Science Center, NOAA/NMFS, Woods Hole, MA.

Moriyasu, M., and C. Lanteigne. 1998. Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. Canadian Journal of Zoology. 76(11):2040–2048.

New England Fishery Management Council (NEFMC). 2013. Scientific and Statistical Committee Report, September 3, 2013. Review of red crab interim acceptable biological catch (ABC) control rule and development of 2014–2016 ABC recommendations.

New England Fishery Management Council (NEFMC). 2016. Atlantic deep-sea red crab fishing years 2017-2019 specifications, including Supplemental Information Report (SIR), and Regulatory Flexibility Analysis (IRFA). Newburyport, MA.

Northeast Fisheries Science Center (NFSC). 2018. State of the Ecosystem - Mid-Atlantic Bight. March 01, 2018.

Pérez-Pérez, N. 2017. Hatching periodicity, survival and development of the early life stages of red deepsea crab (*Chaceon quinquedens*) in laboratory conditions: effects of diet and temperature. M.S. Thesis. Delaware State University.

Perkins, H. C. 1973. The larval stages of the deep sea red crab, *Geryon quinquedens* Smith, reared under laboratory conditions (Decapoda: Brachyrhyncha). Fishery Bulletin. 71:69–82.

Pickart, R. S., and W. M. Smethie Jr. 1993. How does the Deep Western Boundary Current cross the Gulf Stream? Journal of Physical Oceanography. 23:2602–2616.

Pinheiro M. A. A., and A. Fransozo. 2002. Reproduction of the speckled swimming crab *Arenaeus cribrarius* (Brachyura: Portunidae) on the Brazilian Coast near 23°30'S. Journal of Crustacean Biology. 22(2):416–28.

Pinho, M. R., J. M. Goncalves, H. R. Martins, and G. M. Menezes. 2001. Some aspects of the biology of the deep-water crab, *Chaceon affinis* (Milne-Edwards and Bouvier, 1894) off the Azores. Fisheries Research. 51(2-3):283–295.

Pratt, R. M. 1968. Atlantic Continental Shelf and Slope of the United States: Physiography and Sediments of the Deep-Sea Basin. Geological Survey Professional Paper 529-B.

Raviv, S., S. Parnes, and A. Sagi. 2008. In, Reproductive Biology of Crustaceans, Mente, E. (eds.), 365–390, Boca Raton: CRC Press.

Roff, J. C., L. P. Fanning, and A. B. Stasks. 1986. Distribution and association of larval crabs (Decapoda: Brachyura) on the Scotian Shelf. Canadian Journal of Fisheries and Aquatic Sciences. 43:587–599.

Sainte-Marie, B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, *Chionoecetes opilio*, in the Northwest Gulf of Saint Lawrence. Canadian Journal of Fisheries and Aquatic Sciences. 50:2147–2156.

Sainte-Marie, B., N. Urbani, J. M. Sévigny, F. Hazel, and U. Kuhnlein. 1999. Multiple choice criteria and the dynamics of assortative mating during the first breeding season of female snow crab, *Chionoecetes opilio* (Brachyura, Majidae). Marine Ecology Progress Series. 181:141–153.

Sastry, A. N. 1983. Ecological aspects of reproduction. In, W. B. Vernberg, (ed.), The Biology of Crustacea: Environment adaptations. Academic Press Inc., New York. 8:179–270.

Serchuk, F. M., and R. L. Wigley. 1982. Deep-sea red crab, *Geryon quinquedens*. In M.D. Grosslein and T.R. Azarovitz eds. Fish distribution. p. 125–129. MESA New York Bight Atlas Monograph 15. N.Y. Sea Grant Institute, Albany, NY.

Schofield, O., R. Chant, B. Cahill, R. Castelao, D. Gong, A. Kahl, J. Kohut, M. Montes-Hugo, R. Ramadurai, P. Ramey, X. Yi, and S. Glenn. 2008. The decadal view of the Mid-Atlantic Bight from the COOLroom: Is our coastal system changing? Oceanography. 21:108–117.

Smith, S. I. 1879. The stalk-eyed crustaceans of the Atlantic coast of North America north of Cape Cod. Transactions of the Connecticut Academy of Arts and Sciences 5(1):27–136.

Smith, K. D. 2006. Distributions, relative abundances and reproductive biology of the deep-water crabs *Hypothalassia acerba* and *Chaceon bicolor* in Southwestern Australia. Ph.D. dissertation. Murdoch University, School of Biological Sciences.

Steimle, F. W., C. A. Zetlin, and S. Chang. 2001. Essential Fish Habitat Source Document: red deepsea crab, *Chaceon (Geryon) quinquedens*, life history and habitat characteristics. NOAA Tech. Memo. NMFSNE-163, 27 p.

Stenhouse, I. J., K. A. Williams, E. E. Connelly, S. M. Johnson, A. T. Gilbert, H. F. Goyert, and M. W. Goodale. 2015. Ecosystem background and project activities. In: Wildlife Densities and Habitat Use Across Temporal and Spatial Scales on the Mid-Atlantic Outer Continental Shelf: Final Report to the Department of Energy EERE Wind & Water Power Technologies Office. In, K. A. Williams, E. E. Connelly, S. M. Johnson, and I. J. Stenhouse (eds.) Award Number: DE-

EE0005362. Report BRI 2015-11, Biodiversity Research Institute, Portland, Maine. 21 p.

Stevens, B. G., W. E. Donaldson, J. A. Haaga, and J. E. Munk. 1993. Morphometry and maturity of male Tanner crabs, *Chionoecetes bairdi*, grasping pubescent and multiparous females in shallow and deepwater environments. Canadian Journal of Fisheries and Aquatic Sciences. 50:1504–1516.

Stevens, B. G., and V. Guida. 2016. Demographics, morphometrics, depth, and temperature distribution of red deepsea crabs *Chaceon quinquedens* at four sites in the Mid-Atlantic Bight. Fishery Bulletin. 114: 343–359.

Subramoniam, T. 2011. Mechanisms and control of vitellogenesis in crustaceans. Fisheries Science. 77:1–21.

Sulkin S. D., and W. F. van Heukelem. 1980. Ecological and evolutionary significance of nutritional flexibility in planktotrophic larvae of the deep sea red crab *Geryon quinquedens* and the stone crab *Menippe mercenaria*. 2:91–95.

Swiney, K. M., J. B. Webb, G. H. Bishop, and G. L. Eckert. 2010. Temporal and spatial variability of Alaska red king crab fecundity, and accuracy of clutch fullness indices in estimating fecundity. In: G. H. Kruse, G. L. Eckert, R. J. Foy, R. N. Lipcius, B. Sainte-Marie, D. L. Stram, and D. Woodby (eds.), Biology and management of exploited crab populations under climate change. Alaska Sea Grant, University of Alaska Fairbanks.

Tallack, S. M. L. 2007. Escape ring selectivity, bycatch, and discard survivability in the New England fishery for deep-water red crab, *Chaceon quinquedens*. ICES Journal of Marine Science. 64: 1579–1586.

Taylor, A. H., and J. A. Stephens. 1998. The North Atlantic Oscillation and the latitude of the Gulf Stream. Tellus. 50A:134–142.

van Heukelem, W., M. C. Christman, C. E. Epifanio, and S. D. Sulkin. 1983. Growth of *Geryon quinquedens* (Brachyura: Geryonidae) juveniles in the laboratory. Fishery Bulletin. (U.S.) 81:903–905.

Waddy, S. L., and D. E. Aiken. 2005. Impact of invalid biological assumptions and misapplication of maturity criteria on size-at-maturity estimates for American lobster. Transactions of the American Fisheries Society. 134:1075–1090.

Wahle, R. A., C. E. Bergeron, A. S. Chute, L. D. Jacobson, and Y. Chen. 2008. The Northwest Atlantic deep-sea red crab (*Chaceon quinquedens*) population before and after the onset of harvesting. ICES Journal of Marine Science. 65:862–872.

Weinberg, J. R., and C. Keith. 2003. Population size-structure of harvested deep-sea red crabs (*Chaceon quinquedens*) in the northwest Atlantic Ocean. Crustaceana. 76:819–833.

Whitlatch, R. B., J. F. Grassle, L. F. Boyer, and R. N. Zajac. 1990. Animal-sediment relationships involving red crabs (*Chaceon quinquedens*) on the southern New England upper continental slope. In Geryonid crabs and associated continental slope fauna: a research workshop report. W. J. Lindberg and E. L. Wenner (eds.), 14–15, Technical Report 58, Florida Sea Grant College.

Wigley, R. L., R. B. Theroux, and H. E. Murray. 1975. Deep sea red crab, *Geryon quinquedens*, survey off northeastern United States. Marine Fisheries Review. 37(8):1–21.

Wilson, J. L. 2017. Age determination of red deep-sea crabs (*Chaceon quinquedens*) by growth ring analysis. M.S. Thesis. University of Maryland Eastern Shore.

WoRMS Editorial Board. 2018. World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ.

Yoder, J.A., J. E. O'Reilly, A. H. Barnard, T. S. Moore, and C. M. Ruhsam. 2001. Variability in coastal zone color scanner (CZCS) Chlorophyll imagery of ocean margin waters off the US East Coast. Continental Shelf Research. 21:1191–1218.

Zairion, Z., Y. Wardiatno, and A. Fahrudin. 2015. Sexual maturity, reproductive pattern and spawning female population of the blue swimming crab, *Portunus pelagicus* (Brachyura: Portunidae) in East Lampung coastal waters, Indonesia. 8(7):596–607.

Table 1.1. Description of the systematics of *Chaceon quinquedens*.

Red deep-sea crab
Phylum: Arthropoda
Subphylum: Crustacea, Brünnich, 1772
Class: Malacostraca, Latreille, 1802
Order: Decapoda, Latreille, 1802
Infraorder: Brachyura, Latreille, 1802
Superfamily: Portunoidea, Rafinesque, 1815
Family: Geryonidae, Colosi, 1923
Genus: Chaceon, Manning & Holthuis, 1989
Species: *Chaceon quinquedens* Smith, 1879
Original name: *Geryon quinquedens*

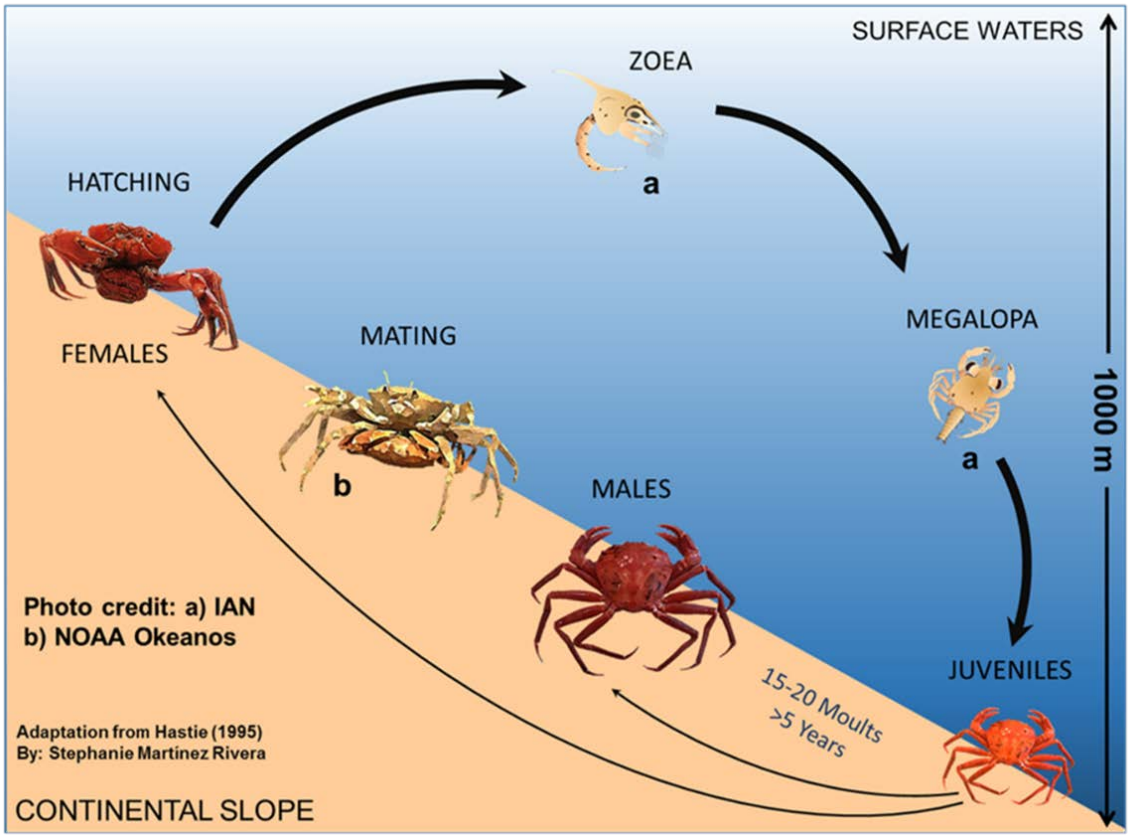

Figure 1.1. Life history of red deep-sea crab, *Chaceon quinquedens*. An adaptation from Hastie (1995).

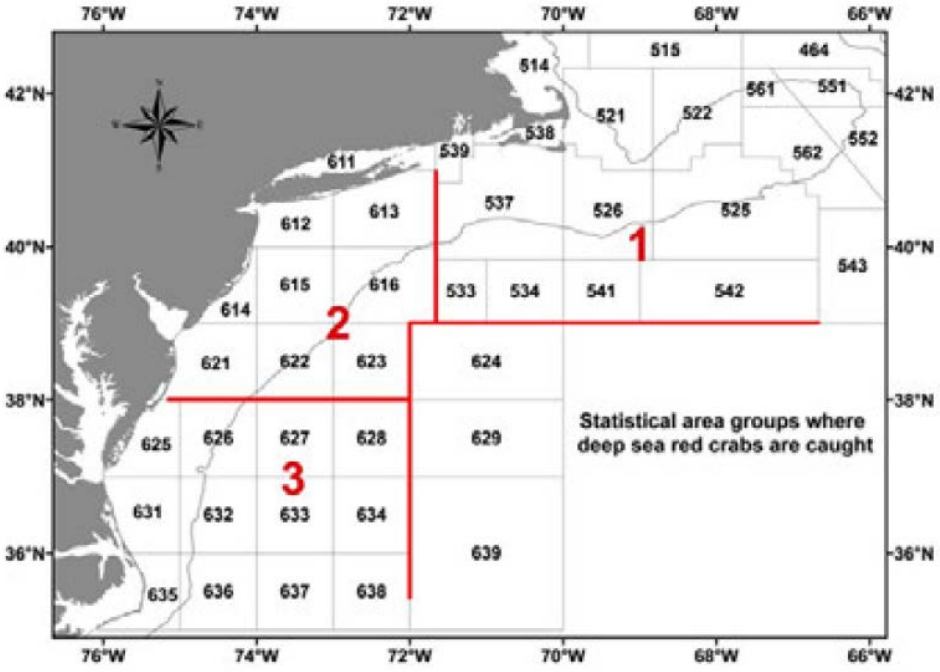

Figure 1.2. Statistical areas used in *Chaceon quinquedens* fishery management plan.

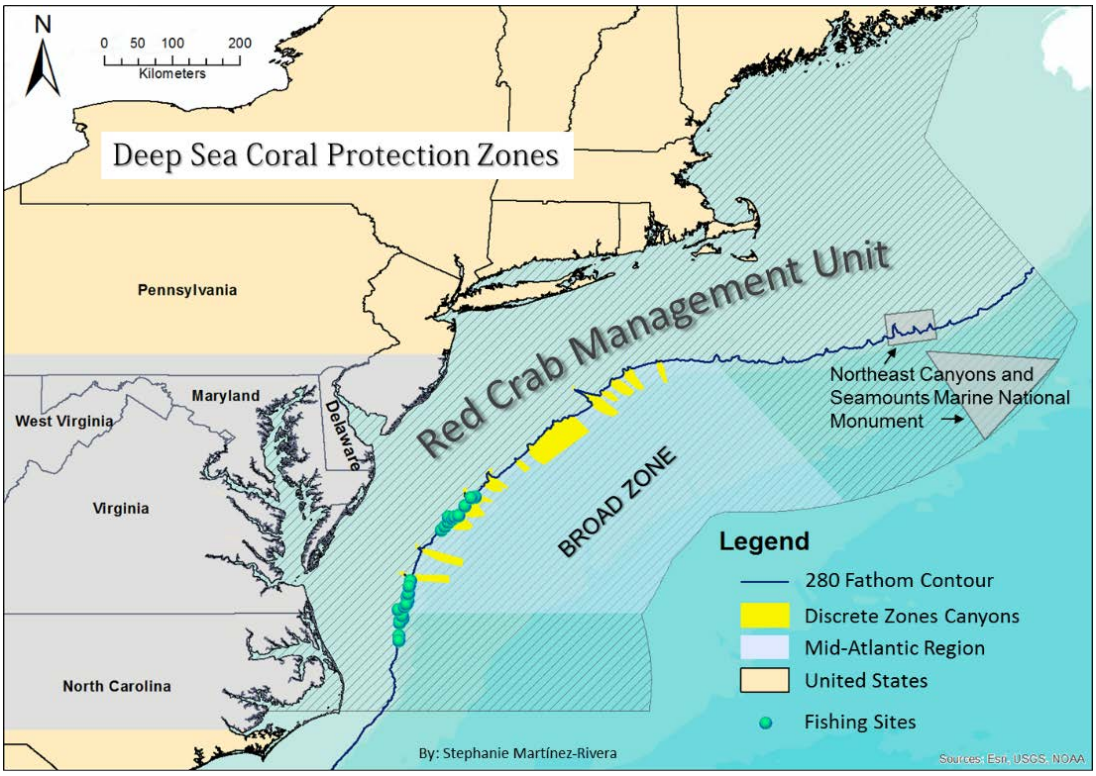

Figure 1.3. Location of The Atlantic Red Crab Company fishing sites in the Mid-Atlantic Bight, relative to the MAFMC deep-sea coral protection zones (broad and discrete). The coordinates of the fishing sites were recorded during our sampling trips (2014–2016) aboard the *F/V* Hannah Boden. Sources: Esri, USGS and NOAA.

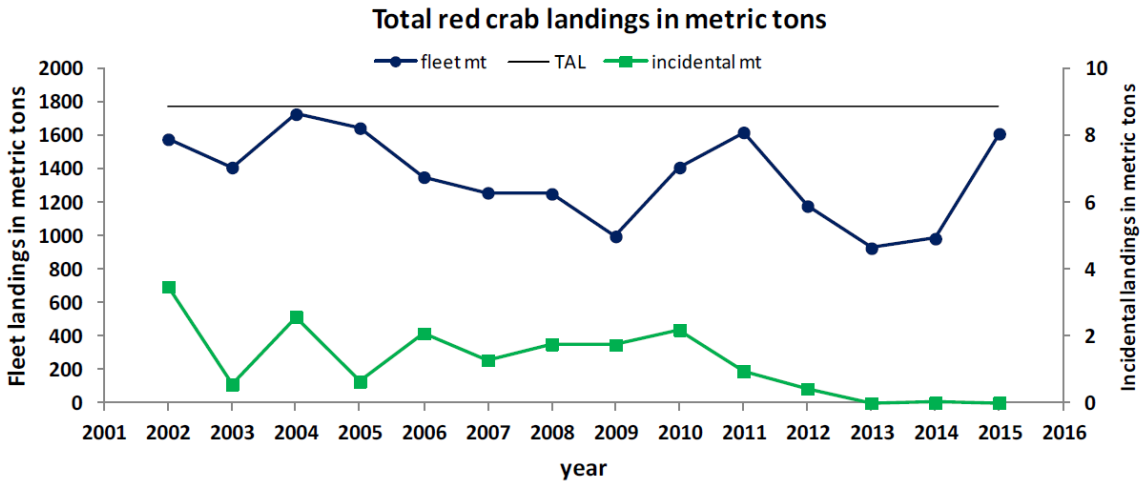

Figure 1.4. Total landings of *Chaceon quinquedens* (NEFMC, 2016).

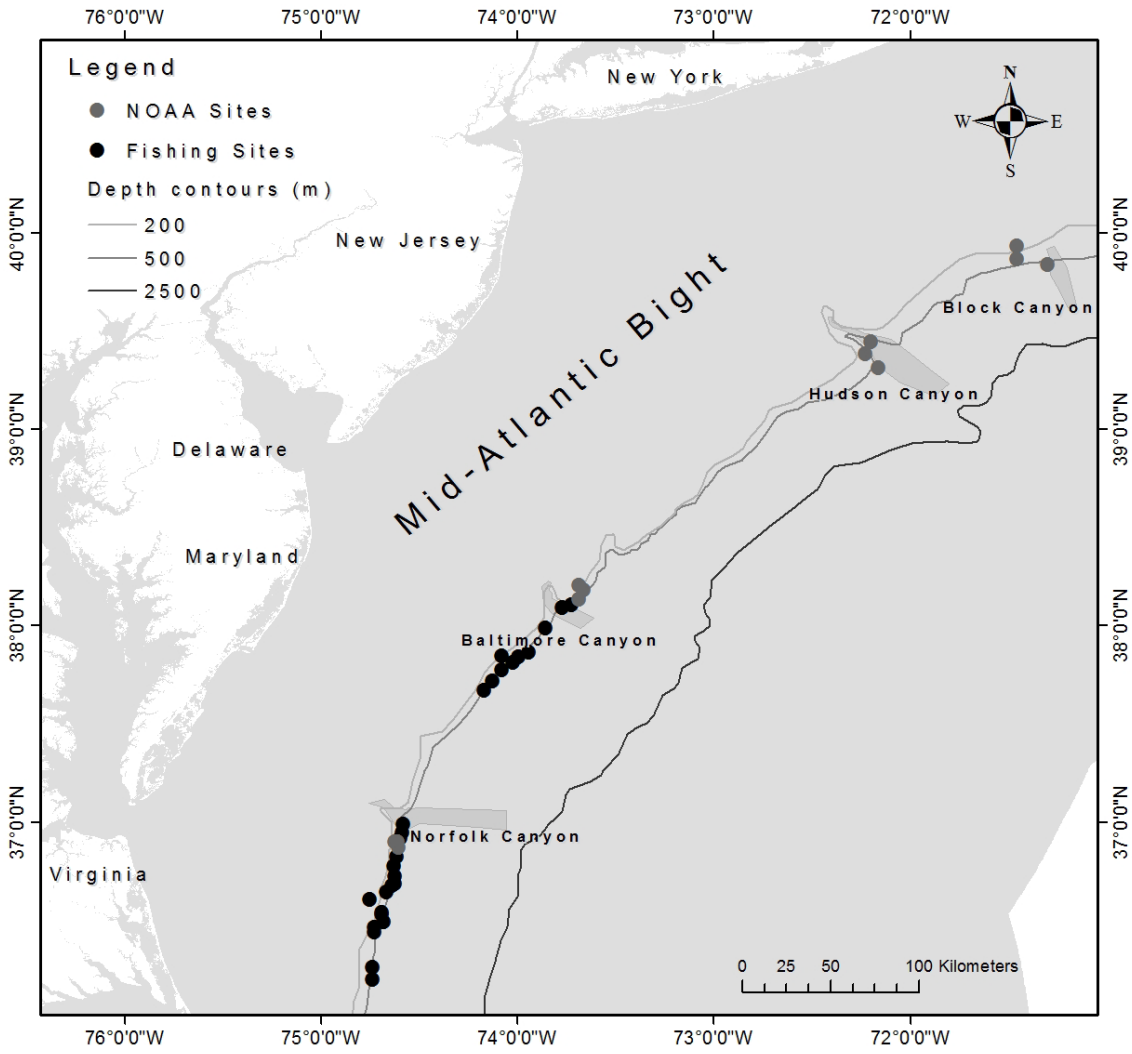

Figure 1.5. Location of sampling sites for red deep-sea crab, *Chaceon quinquedens*, during cruises aboard NOAA research vessels (2011–2013) and Atlantic Red Crab Co. F/V Hannah Boden (2014–2016).

CHAPTER 2: OVARIAN DEVELOPMENT, OOGENESIS AND SIZE AT SEXUAL MATURITY OF THE FEMALE RED DEEP-SEA CRAB, *CHACEON QUINQUEDENS* SMITH, 1879, IN THE MID-ATLANTIC BIGHT

ABSTRACT

Red deep-sea crabs, *Chaceon quinquedens* Smith, 1879, inhabit the continental shelf and slope of the western Atlantic, and range from Nova Scotia to the Gulf of Mexico in water depths of 200–1800 m and water temperatures of 5–8 °C. The objective of this study was to describe ovary and oocyte development and estimate the size at 50% sexual maturity (SM_{50}) using morphological and physiological features for females in the Mid-Atlantic Bight. Samples were collected by trawling aboard NOAA research vessels in 2011–2013 and by traps aboard a commercial fishing vessel in 2014–2016. Female carapace length (CL) and morphological (i.e. abdomen width and vulva condition) and physiological (i.e. ovarian development and the presence or absence of embryos) reproductive indicators were recorded in order to estimate the size at maturity. Histological analysis was used to describe ovarian and oocyte development stages. Five stages of oocyte development were described: 1) proliferation, 2) previtellogenesis, 3) vitellogenesis, 4) fully developed, and 5) oosorption. Five stages of ovarian development were also described: 1) immature, 2) early maturing, 3) advanced, 4) mature, and 5) redeveloping. A non-linear model was used to estimate the SM_{50} using maximum likelihood methods. Morphological SM_{50} decreased with latitude, and was estimated at 53.8 mm CL, 62.5 mm CL and 65.5 mm CL for crabs collected near the Hudson, Baltimore, and Norfolk canyons, respectively. Physiological SM_{50} varied depending on season of collection and was estimated at 63.6 mm CL for winter and spring, and 74.8

mm CL for summer and fall. Results implied asynchrony between morphological and physiological sexual maturity, suggesting that mating occurs prior to completion of ovarian development.

INTRODUCTION

Red deep-sea crabs, *Chaceon quinquedens* Smith, 1879, are found along the continental shelf and slope of the western Atlantic, ranging from Nova Scotia to the Gulf of Mexico in water depths of 200–1800 m and water temperatures of 5–8 °C (Haefner & Musick, 1974; Wigley *et al.*, 1975; Steimle *et al.*, 2001). Red deep-sea crabs (RDSC) have been economically important since the 1970s, sustaining a small fishery in New England and the Mid-Atlantic (Wigley *et al.*, 1975; Wahle *et al.*, 2008). *C. quinquedens* is considered a data-poor stock and research recommendations include estimation of reproductive parameters (Chute *et al.*, 2008). Studies of sexual maturity are needed to provide essential information for fisheries management and reproductive parameters for stock assessment models.

Sexual maturity for crustaceans can generally be divided into three categories: morphological maturity physiological maturity (also referred to as gonadal maturity) and behavioral maturity (also referred to as functional maturity) (Conan *et al.*, 2001). This study focuses on the morphological maturity and physiological maturity of *C. quinquedens* females. Morphological maturity indicates the presence of external secondary sexual characters necessary for reproduction. Size at morphological maturity in females is commonly determined by using allometric relationships (e.g. abdomen width vs. carapace size) or other external characteristics (e.g. vulva condition). Morphological maturity has been estimated for brachyurans such as *Chaceon notialis* (Delgado & Defeo, 2004; Sant'Ana & Pezzuto, 2009), *Chaceon ramosae* (Pezzuto & Sant'Ana, 2009), *Chaceon affinis* (Fernández-Vergaz *et al.*, 2000; Pinho *et al.*, 2001), *Chionoecetes opilio* (Comeau & Conan, 1992) and *Chionoecetes bairdi* (Stevens *et al.*,

1993). Efforts to determine size at sexual maturity for female *C. quinquedens* using allometric growth have thus far been unsuccessful. Stevens & Guida (2016) concluded that SM_{50} for female *C. quinquedens* could not be determined with abdomen allometry but was estimated at 61.6 mm in carapace length (CL) based on vulva condition.

Physiological maturity can be reached when the gonads are completely developed and ready to produce offspring. Size at physiological maturity in females is commonly determined by the examination of the ovary and oocyte development. The macroscopic condition of the ovary is an important element to characterize the stages of ovarian development, but for an adequate comprehension of the female reproductive cycle, it is necessary to describe oogenesis (Sharifian *et al.*, 2014). Ovarian development has been studied for other geryonid crabs including *Chaceon notialis* (Delgado & Defeo, 2004), *Chaceon fenneri* (Erdman & Blake, 1988; Hinsch, 1988), and *Chaceon maritae* (Meville-Smith, 1987). Haefner (1977) was the first to estimate physiological size at sexual maturity of *C. quinquedens* females to be between 65–75 mm CL and described five ovarian development stages. However, a detailed description of the maturation of germ cells in the ovary of the current population of *C. quinquedens* is necessary to understand the reproductive cycle. Understanding reproductive cycles and strategies is critical for developing population models and improving management. In recognition of this, we decided to study the stages of ovary and oocyte development and to estimate SM_{50} using the stages of ovarian and oocyte development and morphological conditions.

MATERIALS AND METHODS

Red deep-sea crabs for this study were collected in the Mid-Atlantic Bight during January of 2011 and 2012, and July of 2013 aboard NOAA research vessels at depths >250 m by trawling (Stevens & Guida, 2016). During July and September of 2014, and July of 2015, sampling was conducted off Newport News, VA, along the Norfolk Canyon at depths >600 m aboard commercial vessels using traps, via collaboration with The Atlantic Red Crab Company. In August of 2016 crabs were collected off New Bedford, MA, along the Baltimore Canyon at depths >600 m by traps. During 2016, samples were collected bimonthly at the fishing port in Newport News and Hampton, VA from vessels that were fishing along the Norfolk Canyon at depths >600 m using traps. A total of 706 crabs was collected for the study across all years and months, ranging from 30 to 140 mm CL. All females caught were dissected, sexed, and measured for carapace length (CL, from the rostral teeth to the center of the edge of the carapace), carapace width (CW, including lateral spines), and abdomen width (AW, 3rd segment). Gross morphology was recorded including the presence, size, and color of ovaries, external eggs, and vulvae condition.

Morphological Maturity

Morphological maturity for 646 female crabs was defined based on condition of their vulvae (Fig. 2.1). Females with clean, closed vulvae were defined as immature, and those with blackened areas around the vulva, indicative of prior copulation, were defined as mature (Fig. 2.2).

Physiological Maturity

Physiological maturity was defined by histological examination of 458 female crabs (Fig. 2.1). Ovary samples (~1 cm^3) were fixed in 10% formalin-sea water, transported to the laboratory at the Paul S. Sarbanes Coastal Ecology Center (Berlin, MD), where histological analyses of ovary samples were performed, and stored in 70% EtOH until processed. Tissues were dehydrated in a tissue processor, embedded in paraffin, sectioned at 5–10 µm (depending on oocyte dimensions), stained with hematoxylin and eosin, and mounted in Permount. Ovary sections were examined for the presence of oocytes, and digitally photographed with a Leica M125 (Leica Microsystems, Wetzlar, Germany) stereo-zoom microscope and Olympus DP73 digital camera mounted on a compound microscope Olympus BX41 and stereomicroscope Olympus SXZ16 (Olympus Corporation, Tokyo, Japan). Diameters of 30 oocytes per female were measured from photographs using ImageJ Software (Schneider *et al.*, 2012). We preferred to measure oocytes with the full nucleus visible, but if those were scarce, we measured oocytes with part of the nucleus visible. The stages of oocyte development were described based on microscopic characters such as size and condition of oocytes. After determination of the stages of oocyte development, the percentage of each oocyte stage in the ovary was calculated using oocyte diameter. The stages of ovarian development were described based on macroscopic characters such as size and color of the ovary, and incorporating the stages of oocyte development. Females with ovary stages 1 to 3 and no evidence of external eggs were defined as immature, and females with ovary stages 4 or 5, maximum oocyte diameter ≥350 µm and/or presence of external eggs were defined as mature.

Size at Sexual Maturity

The SM_{50} of female RDSC was estimated using a non-linear logistic model to describe the proportion of mature females, shown in Equation 1:

$$P_{CL} = \frac{1}{1+\left(\frac{CL}{CL_{50}}\right)^{s}} \quad (1)$$

where P_{CL} is the proportion of mature individuals as a function of carapace length (CL), and *s* is the slope parameter. Equation 1 was used for morphological and physiological criteria separately. Different models with varying parameters were fitted in R 3.5.0 assuming a binomial distribution of errors using the maximum likelihood function *mle*. Data were tested for differences between seasons, geographic location and years. However, models that grouped data by years were not successful. Post hoc models included multiple slope parameters grouped by seasons and geographic location, separately. The best fit model for SM_{50} was determined using the AIC_c (i. e. Akaike's Information Criterion corrected for sample size). For morphological maturity, post hoc we noted the model with the lowest AICc was the one with data grouped by geographic location (Hudson Canyon, Baltimore Canyon, and Norfolk Canyon). Therefore, sites were also regrouped as North (Hudson Canyons) or South (Baltimore and Norfolk Canyon) but the AICc was higher than the previous model, therefore we chose to separate all geographic location. Models grouped data by geographic area were also analyzed with and without separate slope parameters. For physiological maturity, post hoc we noted the model with the lowest AICc was the one with data grouped by season. Therefore, the data

were grouped as Early (winter and spring seasons) and Late (summer and fall seasons). All statistical results in this study are described as (mean ± standard error).

RESULTS

Morphology of the female reproductive system

The reproductive system of the female *C. quinquedens* consisted of an ovary, oviducts, sperm reservoirs or spermathecae, and gonopores or vulvae. Like most brachyurans, the ovary is H-shaped and is located in the dorsal portion of the cephalothorax above the hepatopancreas (Haefner, 1977). The anterior horns of the ovary extend to each side of the cardiac stomach. The posterior horns connect to the spermathecae via oviducts. The spermatheca is a subcylindrical-shaped organ where the females store sperm. The vagina is the connection between the spermatheca and the gonopore. The gonopores are sternal openings located on the sixth thoracic segment. The ovarian wall is formed by a thin epithelium and connective tissue. The ovary is formed by lobular areas where oogenesis occurs. The center of the ovary lobes is the germinal zone where new oogonia and oocytes develop, and upon maturation, they move from the center to the periphery. Our descriptions of oocyte and ovarian development follow those of Smija & Sudha Devi (2015), Ravi *et al.* (2012) and Brown (2009).

Stages of oocyte development

We described six stages of oocyte development for *C. quinquedens* based on oocyte diameter, nuclear appearance and yolk accumulation (Table 2.1).

Stage 1: Proliferation – Germ cells, called oogonia (Fig. 2.4A), are diploid cells located in the center of the ovary known as the germinal zone (Fig. 2.3). The germ cells are small with an oval shape and the nucleus is large in relation to the ooplasmic area. At this phase the oogonial cells are basophilic (Fig. 2.6C). Germ cells develop into primary and secondary oocytes during the primary growth phase (Fig. 2.4B). The ovary wall is thick, and we could distinguish the three layers of epithelium and the underlying thick connective tissue. Only germ cells, primary and secondary oocytes, and agglomerations of follicle cells were present in the ovary in this phase. We considered oocyte diameters up to 50 µm to be in the proliferation stage but we were able to measure oocytes with a mean diameter of 32 µm from one crab only.

Stage 2: Previtellogenesis – In this phase the oocytes undergo late primary growth, increase in size, and become previtellogenic oocytes. Oocytes developed from early previtellogenic oocytes (Fig. 2.4C, D) to late previtellogenic oocytes (Fig. 2.5A). The early previtellogenic oocytes have gradual basophilia of the ooplasm, smaller nuclei than primary oocytes, and large nucleoli. A clear space between the nucleus and ooplasm was observed in the oocytes. The early previtellogenic oocytes start moving from the germinal zone to the periphery with follicle cells nearby. Oocytes in late previtellogenic phase exhibited vacuolated globules and hemolymph between the oocytes (Fig. 2.6D). The ovary wall was not as evident, with a thin connective tissue, and the central lumen in the lobes was distinguishable. Oocytes with diameters of 50–250 µm (mean 185 ± 2.2 µm) were considered previtellogenic.

Stage 3: Vitellogenesis – Secondary growth of the oocyte occurs during this phase, which defines the start of yolk accumulation. The ooplasm of the vitellogenic

oocytes was gradually eosinophilic, and the presence of yolk platelets and lipid droplets are evident (Fig. 2.5B). The ovary wall is very thin and the germinal zone and central lumen are linear extensions amongst the larger oocytes. At this time, we observed the perinuclear zone in the oocytes, the presence of a chorion, and follicle cells enclosing the oocytes (Fig. 2.5B). By the end of this phase oocytes are completely eosinophilic with a condensed nucleus that is also eosinophilic, a granular ooplasm, and an increased number of tubular follicle cells (Fig. 2.6B) surrounding the oocytes. Yolk platelets, lipid droplets (Fig. 2.6A) and vacuolated globules increased in number and extended to the perinuclear zone. Larger yolk platelets were observed at the periphery of the oocyte. A reduction in nucleus and ooplasm ratio was observed. Oocyte diameters were 250–350 µm (mean 278 ± 3 µm).

Stage 4: Fully developed – Prior to ovulation, the oocytes complete maturation and become ready to be extruded and fertilized. The ovary wall at this stage is very thin with nearly visible germinal zone and central lumen. Mature oocytes are filled with yolk platelets and lipid droplets (Fig. 2.5C). The yolk platelets in the oocyte were all of similar size and eosinophilic. The lipid droplets in the oocytes are clear and round-shaped. The nucleus and perinuclear zone become less visible as the oocyte increases in size, eventually the perinuclear zone disappear. Consequently, the areal ratio of nucleus to ooplasm is reduced. The ooplasm of the oocyte is very granular and eosinophilic. Oocytes with a diameter larger than 350 µm (mean 472 ± 7.9 µm) were considered fully developed.

Stage 5: Oosorption – During this phase oocytes that were not extruded from the ovary are resorbed, a process known as atresia. Atretic oocytes had a degenerating

ooplasm, thick surrounding membrane, amorphic shape and were located in the ovarian stroma (Fig. 2.5D). In some cases, empty circular spaces were found in the ovarian stroma. Advanced atretic oocytes turned a light brown color. The atretic oocytes observed can be classified as fully developed and had a mean diameter of 551 ± 16.8 µm.

Stages of ovarian development

Ovarian development stages were characterized by different degrees of oocyte development, and changes in color and size of the ovary (Table 2.1). One-way analysis of variance indicated a significant difference in the mean diameters of the oocytes between crabs with stage 2 through stage 5 ovaries ($F = 49.0$; $P < 0.01$). We described five stages of ovarian development for female *C. quinquedens* (Fig. 2.7).

Stage 1: Immature (Proliferation) – Very early development (Fig. 2.7A), ovary very thin, small and colorless, white or ivory (Fig. 2.8A). The ovary was difficult to distinguish in small females. The stage of oocyte development exhibited in the ovary was proliferation with oogonia (100%) (Fig. 2.9). Female sizes ranged from 34.6 to 68.3 mm CL (mean 48.3 ± 7.4 mm CL, $N = 4$) (Fig. 2.10).

Stage 2: Early maturing (Previtellogenesis) – Early development (Fig. 2.7B), ovary relatively small but larger than Stage 1, the color ranged from white to beige, yellow and light orange (Fig. 2.8B, C). At this stage, oocytes increased in size due to primary growth and entered the previtellogenesis phase. Ovaries were dominated by previtellogenic oocytes (92.6%) but also exhibited vitellogenic oocytes (7.4%) usually distributed in the peripheral zone (Fig. 2.9). Female sizes ranged from 47.3 to 110.7 mm CL (mean 78.6 ± 0.9 mm CL, $N = 196$) (Fig. 2.10).

Stage 3: Advanced (Vitellogenesis) – In this intermediate development stage, ovaries mature prior to forming an external clutch (Fig. 2.7C). Ovary size ranges from medium to large, and color is typically bright orange or variations of orange and brown (Fig. 2.8D-F). The ovary increased in size due to yolk accumulation. The ovary was composed of oocytes in three developmental stages: previtellogenic oocytes (29.8%), vitellogenic oocytes (63.1%) and fully developed oocytes (7.1%) (Fig. 2.9), suggesting the start of a new cohort in the ovary. Female sizes ranged from 61.8 to 109.2 mm CL (mean 84.2 ± 1.3 mm CL, $N = 70$) (Fig. 2.10).

Stage 4: Mature (Fully developed) – The ovary is very large, ranging in color from red-orange to purple, dark brown and variaptions in between (Fig. 2.7D and Fig. 2.8G, H). The ovary is entirely mature with fully developed oocytes (81.5%) shortly before ovulation. Previtellogenic oocytes (14.9%) and vitellogenic oocytes (3.6%) were found in the ovary indicating the development of a new cohort (Fig. 2.9). Female sizes ranged from 66.4 to 110.9 mm CL (mean 89 ± 0.7 mm CL, $N = 140$) (Fig. 2.10).

Stage 5: Redeveloping (Oosorption) – After egg extrusion, the ovary undergoes severe reduction in size, and becomes beige in color (Fig. 2.7E and Fig. 2.8I). At this stage atresia has occurred, as un-extruded oocytes were being resorbed by the ovary. Atretic oocytes (7.9%) at different degrees of oosorption were found in the ovary. Although the ovary was dominated by previtellogenic oocytes (84.5%) and some vitellogenic oocytes (7.6%) (Fig. 2.9). Female sizes ranged from 70.3 to 109.9 mm CL (mean 90.5 ± 1.1 mm CL, $N = 48$) (Fig. 2.10).

Seasonality of ovarian maturation

Analysis of the mean oocyte diameter across months showed seasonal differences ($F = 7.8$; $P < 0.01$) (Fig. 2.11). We observed a gradual increase of the mean oocyte diameter from January to July and then a gradual decrease until September. This is consistent with the seasonality observed in the stages of ovarian development (Fig. 2.12). The results suggest that ovarian development starts in the summer and maturity is reached next spring through summer.

Size at morphological sexual maturity

A total of 646 female *C. quinquedens* were analyzed to estimate size at morphological maturity using condition of the vulvae. The best fit model showed geographical differences in the estimated SM_{50} (Table 2.2). Female morphological SM_{50} was estimated at 53.9 ± 3.6 mm CL for Hudson Canyon, 62.5 ± 1.5 mm CL for Baltimore Canyon and 65.5 ± 1.5 mm CL for Norfolk Canyon (Fig. 2.13A). Morphologically immature females (MIF) ranged from 34.6 to 74.4 mm CL (mean 56.5 ± 1.3 mm CL) (Fig. 2.14A). Morphologically mature females (MMF) ranged from 53.7 to 123.4 mm CL (mean 85.9 ± 0.4 mm CL) (Fig. 2.14A). One-way analysis of variance indicated a significant difference between the mean lengths of morphologically immature and mature females ($F = 424.9$; $P < 0.01$).

Size at physiological sexual matu^{r}*ity*

Histological preparations from a total of 449 female *C. quinquedens* were analyzed in order to estimate size at physiological sexual maturity. The best fit model showed seasonal differences in the estimation of physiological SM_{50} (Table 2.3).

Physiological SM_{50} was estimated at 63.6 ± 1.5 mm CL for early seasons and 74.8 ± 1 mm CL for late seasons (Fig. 2.13B). Physiologically immature females (PIF) ranged from 34.6 to 100.7 mm CL (mean 69.8 ± 1.5 mm CL) (Fig. 2.14B). Physiologically mature females (PMF) ranged from 59.7 to 110.9 mm CL (mean 86.7 ± 0.5 mm CL) (Fig. 2.14B). One-way analysis of variance indicated a significant difference between the mean lengths of immature and mature females ($F = 161.2$; $P < 0.01$).

DISCUSION

Morphology of the female reproductive system of female *C. quinquedens* as described in this study was very similar to the description by Haefner (1977) and to other geryonid crabs including *C. notialis* (Delgado & Defeo, 2004), *C. fenneri* (Erdman & Blake, 1988; Hinsch, 1988), and *C. maritae* (Meville-Smith, 1987). Ovarian development for brachyurans is usually assessed by macroscopic analyses (i.e. the color and relative size of the gonads in the cephalothorax) (Mollemberg *et al.*, 2017). However, studies on oocyte development are necessary to understand the reproductive cycle and maturation of a species. Thus, we described five stages of oocyte development: 1) proliferation, 2) previtellogenesis, 3) vitellogenesis, 4) fully developed and 5) oosorption, and five stages of ovarian development: 1) immature, 2) early maturing, 3) advanced, 4) mature and 5) redeveloping. Understanding ovarian development is crucial to adequately understand the population dynamics of a species (Sharifian *et al.*, 2014). The stages of ovarian development are similar to those described for other species of geryonid crabs (Meville-Smith, 1987; Delgado & Defeo, 2004; Fernández-Vergaz *et al.*, 2000) but differ in the number of stages defined. Although Haefner (1977) also described five stages of ovarian development using oocyte diameter, we classified five stages of oocyte development of

C. quinquedens for the first time. We observed oogonia and previtellogenic oocytes with a basophilic ooplasm, whereas the oocytes in the vitellogenesis stage became gradually eosinophilic, similar to Brown (2009), Ravi *et al.* (2012) and Mollemberg *et al.* (2017). The reason for this change is mainly attributed to yolk accumulation in the oocytes (Smija & Sudha Devi, 2015). As oocyte development progressed the ovary increased in size and changed in color. By examining the stages of oocyte development we were able to identify the production of a new cohort in the ovary during stage 3 and 4, demonstrating that oogenesis restarts before ovulation. After ovulation, the ovary drastically reduces in size, becomes beige in color, and oogenesis continues, indicating the start of stage 5. After stage 5, ovarian development does not restart at stage 1 but at stage 2 instead. This study lacked the ability to determine autosynthesis and heterosynthesis of yolk during oocyte development or to distinguish between early and late vitellogenesis; for this purpose, histochemical study of the oocyte ultrastructure is necessary. Oocyte diameters progressively increased from January to July with a peak in March. Furthermore, stage 5 ovaries occurred mostly during summer and fall. This suggests that mating and egg extrusion occur in the summer, and that ovaries begin redeveloping in the fall. However, the reproductive season for *C. quinquedens* is not strongly defined. Reproductive seasonality has been reported for *C. fenneri* (Erdman & Blake, 1988), and *C. affinis* (Pinho *et al.*, 2001), whereas Meville-Smith (1987) indicated *C. maritae* has no seasonal fluctuation.

There was a significant latitudinal trend in morphological SM_{50} for female *C. quinquedens*, which increased from south (Norfolk Canyon) to north (Hudson Canyon). Stevens & Guida (2016) estimated morphological SM_{50} for female *C. quinquedens* to be

61.6 mm CL by using logistic regression, including crabs collected from all sites in 2011–2013 (but not from commercial catches). Moreover, Stevens & Guida (2016) reported mean CL of female crab to be significantly greater at Norfolk Canyon than any of the other sites. Our SM_{50} estimate for Norfolk site was greater than the rest, which is consistent with larger mean size of crabs at this site as reported by Stevens & Guida (2016). Therefore, our estimates of morphological SM_{50} suggest there are differences in the population dynamics for each geographical site. Haefner (1977) estimated that size at maturity ranged from 65–75 mm CL using the relationship between abdomen width and carapace length to distinguish morphologically mature and immature females. Our estimate of morphological SM_{50} is smaller than that of Haefner (1977) suggesting that females in the current population may be mating at smaller sizes. Furthermore, we observed vulval indications of copulation in 100% of 567 females ≥70 mm CL, and 43% of 79 females <70 mm CL. In contrast, all females <70 mm CL examined by Haefner (1977) had vulvae with intact margins (i.e. were MIF), which further supports the conclusion that size of MMF has decreased. This could be the consequence of fishing activity, by removing large males during the 40 years between Haefner's study and ours. Estimates of SM_{50} for other geryonid species lie between 108 and 113 mm CW for *C. affinis* (Fernández-Vergaz *et al.*, 2000); 107 and 123 mm CW for *C. ramosae* (Pezzuto & Sant'Ana, 2009); 70 and 71.7 mm CW for *C. notialis* (Delgado & Defeo, 2004); 85 and 100 mm CW for *C. fenneri* (Erdman & Blake, 1988). Although these estimates were made using CW instead of CL, they can be converted to CL using equations in Stevens & Guida (2016); the species most similar to *C. quinquedens* was *C. fenneri*.

The estimate of physiological SM_{50} for *C. quinquedens* was lower for early seasons than the estimate for late seasons. This suggests females start redeveloping their ovaries in the summer, explaining why we observed large stage 2 females in the fall. However we were unable to differentiate stage 2 mature from immature females. Size at sexual maturity for *C. quinquedens* showed seasonality, and asynchrony between morphological and physiological maturity, implying that mating may occur prior to completion of ovarian development. We observed a high proportion of MMF (38%, N = 379) and all of MIF with ovaries at early development stages, suggesting females reach morphological maturity prior to physiological maturity. Melville-Smith (1987) also reported asynchrony for *C. maritae* from South West Africa, however, Delgado & Defeo (2004) suggested synchrony for *C. notialis* from the Southwestern Atlantic Ocean. Moreover, Fernández-Vergaz *et al.* (2000) described a sequence of maturation for *C. affinis* from the Canary Islands and concluded that females need to be morphometrically mature before completing physiological maturation. Lastly, we did not observe any evidence of a terminal molt for *C. quinquedens*.

Fisheries management relies on biological information such as sexual maturity to incorporate the population dynamics of a species into stock assessment. We recommend that special attention be given to the geographical differences in SM_{50} within the *C. quinquedens* population, in order to improve management. Studies of sexual maturity have provided essential information such as reproductive timing, size at maturity, and the interplay between morphological and physiological maturity. Updated estimates of SM_{50} for the current population of RDSC suggest that size at maturity has declined over time, possibly as a result of fishing. It also implies the existence of different stocks defined by

geographic range, and a subtle reproductive seasonality. This information should be considered in the development of appropriate management strategies for *C. quinquedens*.

ACKNOWLEDGEMENTS

We thank The Atlantic Red Crab Company for the allowing us to sample during fishing trips and the assistance provided aboard the vessel. We are thankful to Shadaesha Green, Dr. Indu Sharma and Justin Wilson for their participation during the sample collection. We wish to acknowledge the help provided by Samantha Peart with the histological analysis. Special thanks to Dr. Andrés G. Morales-Núñez for permitting us use of his laboratory equipment. Funding for this research was provided by NOAA grant NA11SEC4810002 and grant NA16SEC4810007.

REFERENCES

Brown, C. E. 2009. Ovarian morphology, oogenesis, and changes through the annual reproductive cycle of the female blue crab, *Callinectes sapidus* Rathbun, in Tampa Bay. Graduate Theses and Dissertations. M.S. Thesis. University of South Florida. http://scholarcommons.usf.edu/etd/1877

Chute, A., L. Jacobson, and P. Rago. 2008. Deep sea red crab. NMFS/NOAA Northeast Fisheries Science Center, Woods Hole, MA.

Comeau, M., and G. Y. Conan. 1992. Morphometry and gonad maturity of male snow crab, *Chionoecetes opilio*. Canadian Journal of Fisheries and Aquatic Science. 49:2460–2468.

Conan, G. Y., M. Comeau and M. Moriyasu. 2001. Are morphometrical approaches appropriate to establish size at maturity for male American lobster, *Homarus americanus*? Journal of Crustacean Biology. 21(4):937–947.

Delgado, E. and O. Defeo. 2004. Sexual maturity in females of deep-sea red crab *Chaceon notialis* (Brachyura, Geryonidae) in the southwestern Atlantic Ocean. Invertebrate Reproduction and Development. 46(1):55–62.

Erdman, R. B., and N. J. Blake. 1988. Reproductive biology of female golden crabs *Geryon fenneri* Manning and Holthuis, from southeastern Florida. Journal of Crustacean Biology. 8:392–400.

Fernández-Vergaz, V., L. J. López Abellán and E. Balguerías. 2000. Morphometric, functional and sexual maturity of the deep-sea red crab *Chaceon affinis* inhabiting

Canary Island waters: chronology of maturation. Marine Ecology Progress Series. 204:169–178.

Haefner, P. A., and J. A. Musick. 1974. Observations on distribution and abundance of red crabs in Norfolk Canyon and adjacent continental slope. Marine Fisheries Review. 36(1):31–34.

Haefner, Jr., P. A. 1977. Reproductive biology of the female deep-sea red crab, *Geryon quinquedens*, from the Chesapeake Bight. Fishery Bulletin 75:91–102.

Hinsch, G. W. 1988. Morphology of the reproductive tract and seasonality of reproduction in the golden crab *Geryon fenneri* from the Eastern Gulf of Mexico. Journal of Crustacean Biology. 8(2):254–261.

Melville-Smith, R. 1987. The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off South WestAfrica/Namibia. Crustaceana. 53(3):259–275.

Mollemberg, M., F. J. Zara and W. Santana. 2017. Morphology and ultrastructure of the adult ovarian cycle in Mithracidae (Crustacea, Decapoda, Brachyura, Majoidea). Helgoland Marine Research. 71:14.

Pezzuto, P. R. and R. Sant'Ana. 2009. Sexual maturity of the deep-sea royal crab *Chaceon ramosae* Manning, Tavares & Albuquerque, 1989 (Brachyura: Geryonidae) in southern Brazil Latin American Journal of Aquatic Research., 37(3).

Pinho, M. R., J. M. Goncalves, H. R. Martins, and G. M. Menezes. 2001. Some aspects of the biology of the deep-water crab, *Chaceon affinis* (Milne-Edwards and Bouvier, 1894) off the Azores. Fisheries Research. 51(2-3):283–295.

Ravi, R., M. K. Manisseri and N. K. Sanil. 2012. Ovarian maturation and oogenesis in the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae). Acta Zoologica, xx: 1–9. doi:10.1111/j.1463-6395.20

Sant'Ana, R. and P. R. Pezzuto. 2009. Sexual maturity of the deep-sea red crab *Chaceon notialis* Manning & Holthuis, 1989 (Brachyura: Geryonidae) in southern Brazil. Latin American Journal of Aquatic Research. 37(3):429–442.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of Image Analysis. Nature methods 9:671–675.

Sharifian, S., E. Kamrani, M. Safaie and S. Sharifian. 2015. Oogenesis and ovarian development in the freshwater crab *Sodhiana iranica* from south of Iran. Tissue & Cell. 47:213–220.

Smija, M. K. and A. R. Sudha Devi. 2015. Oocyte development and female reproductive cycle in the freshwater crab *Travancoriana schirnerae*. Turkish Journal of Fisheries and Aquatic Sciences. 15:691–702.

Smith, S. I. 1879. The stalk-eyed crustaceans of the Atlantic coast of North America north of Cape Cod. Transactions of the Connecticut Academy of Arts and Sciences 5(1):27–136.

Steimle, F. W., C. A. Zetlin, and S. Chang. 2001. Essential Fish Habitat Source Document: red deepsea crab, *Chaceon (Geryon) quinquedens*, life history and habitat characteristics. NOAA Technical Memorandum NMFSNE-163, 27 p.

Stevens, B. G., W. E. Donaldson, J. A. Haaga, and J. E. Munk. 1993. Morphometry and maturity of male Tanner crabs, *Chionoecetes bairdi*, grasping pubescent and

multiparous females in shallow and deepwater environments. Canadian Journal of Fisheries and Aquatic Sciences. 50:1504–1516.

Stevens, B. G., and V. Guida. 2016. Demographics, morphometrics, depth, and temperature distribution of red deepsea crabs *Chaceon quinquedens* at four sites in the Mid-Atlantic Bight. Fishery Bulletin. 114:343–359.

Wahle, R. A., C. E. Bergeron, A. S. Chute, L. D. Jacobson, and Y. Chen. 2008. The Northwest Atlantic deep-sea red crab (*Chaceon quinquedens*) population before and after the onset of harvesting. ICES Journal of Marine Science. 65:862–872.

Wigley, R. L., R. B. Theroux, and H. E. Murray. 1975. Deep sea red crab, *Geryon quinquedens*, survey off northeastern United States. Marine Fisheries Review. 37(8):1–21.

Table 2.1. Description of the microscopic characteristics of the oocyte development stages and the macroscopic characteristics of the ovarian development stages in *Chaceon quinquedens*.

Oocyte Development Stages					
	Stage 1 Proliferation	Stage 2 Previtellogenesis	Stage 3 Vitellogenesis	Stage 4 Fully developed	Stage 5 Oosorption
Mean Diameter (µm)	32	185	278	472	551
N	1	196	70	140	48
SE	-	2.2	3.0	7.9	16.8
Ovarian Development Stages					
	Stage 1 Immature	Stage 2 Early maturing	Stage 3 Advanced	Stage 4 Mature	Stage 5 Redeveloping
Ovary Color	Colorless, white or ivory	White-beige or yellow-light orange	Bright orange or orange-brown	Red-orange, dark-brown or purple	Beige
Ovary size	Small	Small	Medium	Large	Small
Mean size (mm CL)	48.3	78.6	84.2	89.0	90.5
N	4	196	70	140	48
SE	7.4	0.9	1.3	0.7	1.1

Table 2.2. Model selection table for size at morphological sexual maturity of *Chaceon quinquedens*. Akaike's information criterion corrected for sample size (AICc) was used to determine the best fit model.

Model	K	AIC_c	ΔAIC_c	Likelihood	AIC_c weight
CL_{50}, *s*	2	85.0	7.4	0.0	0.0
CL_{50}(N), CL_{50}(S), *s*	3	78.1	0.5	0.8	0.4
CL_{50}(N), CL_{50}(H), CL_{50}(B), *s*	4	77.5	0.0	1.0	0.6
CL_{50}(E), CL_{50}(L), *s*	3	86.5	8.9	0.0	0.0

Model indicates the parameters used in the model, where CL_{50} is the carapace length at 50% maturity and *s* is the slope. N and S refers to northern and southern sites, respectively. N, H and B refers to Norfolk Canyon, Hudson Canyon and Baltimore Canyon. E and L refers to early and late seasons, winter and spring were considered early while summer and fall where classified as late. K is the number of parameters for each model.

Table 2.3. Model selection table for size at physiological sexual maturity of *Chaceon quinquedens*. Akaike's information criterion corrected for sample size (AICc) was used to determine the best fit model.

Model	K	AIC_c	ΔAIC_c	Likelihood	AIC_c weight
CL_{50}, *s*	2	300.4	30.7	0.0	0.0
CL_{50}(E), CL_{50}(L), *s*	3	269.6	0.0	1.0	0.3
CL_{50}(WSp), CL_{50}(S), CL_{50}(F), *s*	4	269.9	0.3	0.9	0.3
CL_{50}(N), CL_{50}(H), CL_{50}(B), *s*	4	287.8	18.1	0.0	0.0

Model indicates the parameters used in the model, where CL_{50} is the carapace length at 50% maturity and *s* is the slope. E and L refers to early and late seasons, winter and spring were considered early while summer and fall where classified as late. W, Sp, S and F refers to winter, spring, summer and fall seasons, respectively. N, H and B refers to Norfolk Canyon, Hudson Canyon and Baltimore Canyon. K is the number of parameters for each model.

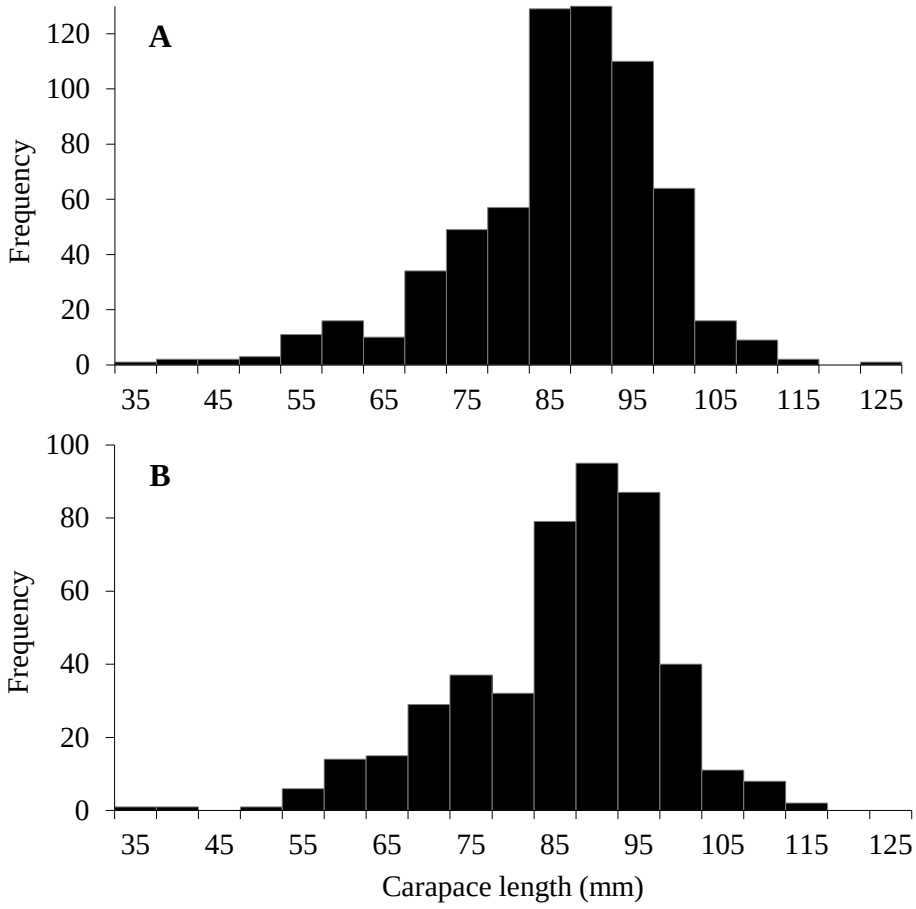

Figure 2.1. Length-frequency distributions of female red deep-sea crab, *Chaceon quinquedens*, in the Mid-Atlantic Bight used to estimate SM_{50} with morphological data (N = 646) (2012–2016) (**A**) and physiological data (N = 449) (2011–2016) (**B**).

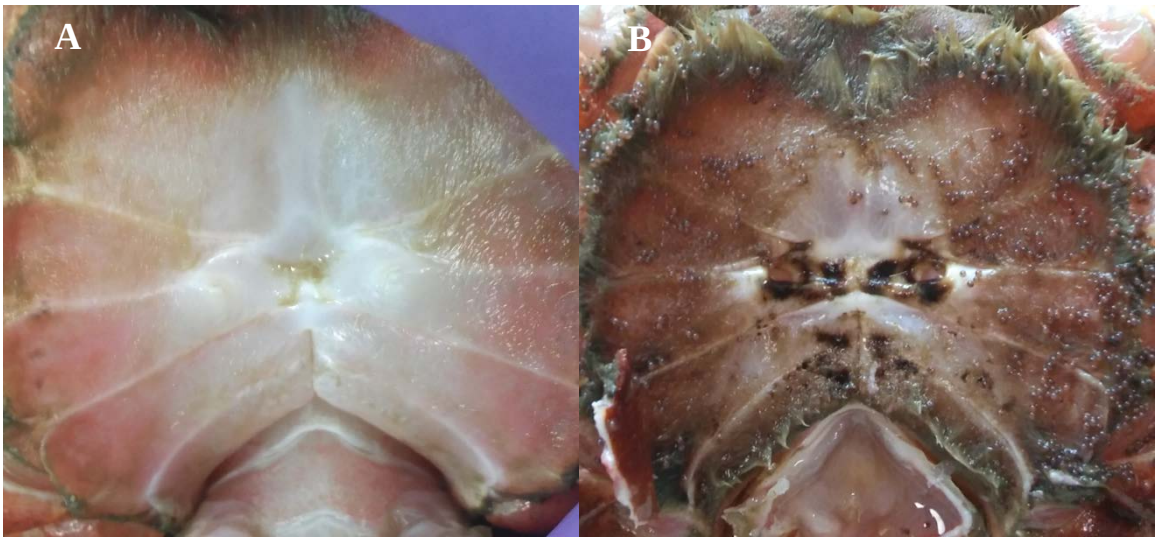

Figure 2.2. Vulvae of immature (**A**) and mature (**B**) *Chaceon quinquedens* females.

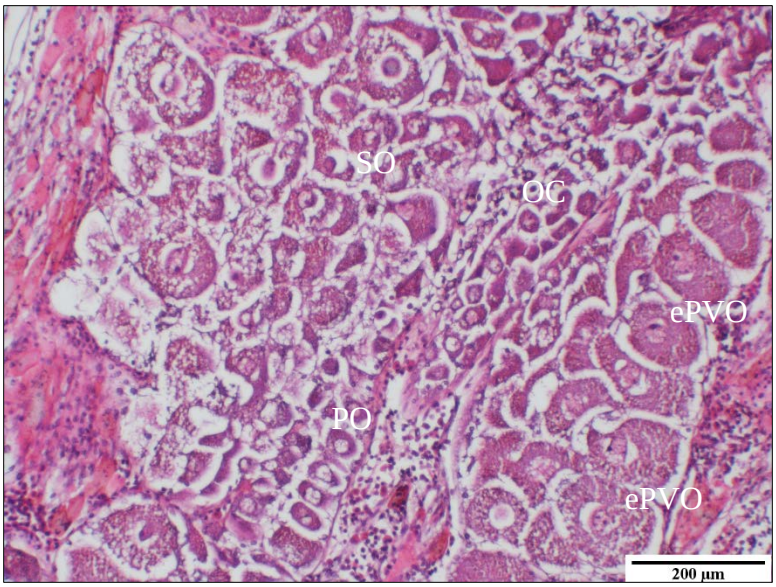

Figure 2.3. Germinal zone in the ovary of *Chaceon quinquedens*. OC, oogonial cells; PO, primary oocytes; SO, secondary oocytes; ePVO, early previtellogenic oocytes.

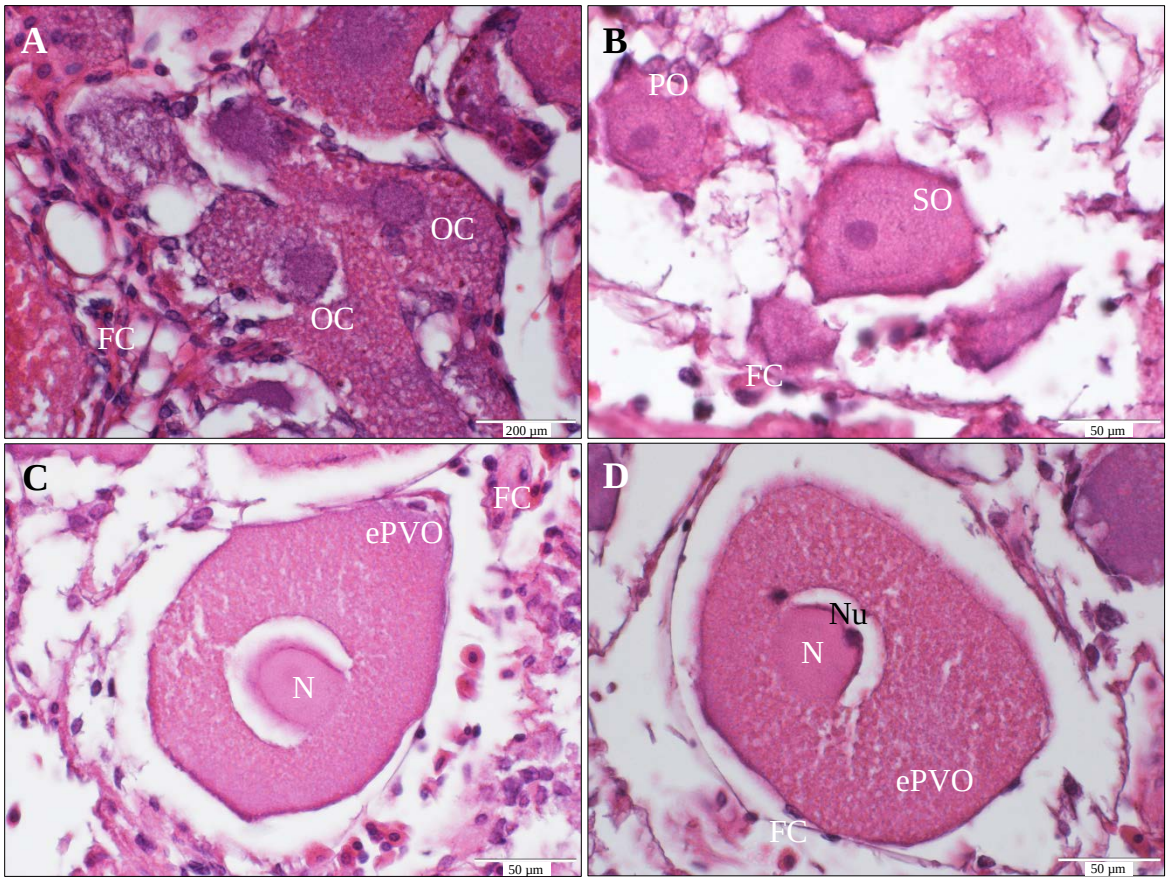

Figure 2.4. First two stages of oocyte development of *Chaceon quinquedens* females. Stage 1 proliferation (**A, B**). Stage 2 previtellogenesis (**C, D**). OC, oogonial cells; FC, follicle cells; PO, primary oocytes; SO, secondary oocytes; ePVO, early previtellogenic oocytes; N, nucleus; Nu, nucleolus.

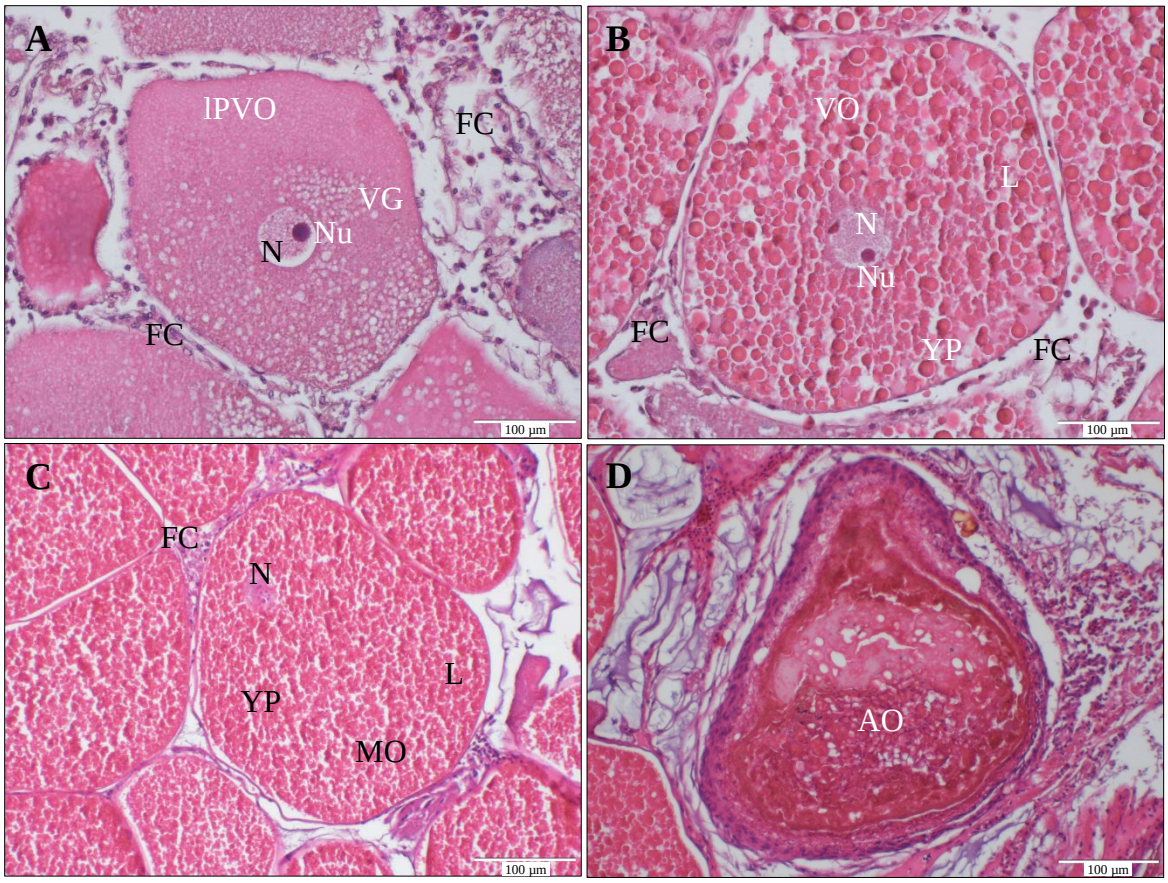

Figure 2.5. Stages (2–5) of oocyte development of *Chaceon quinquedens* females. Stage 2 previtellogenesis (**A**). Stage 3 vitellogenesis (**B**). Stage 4 fully developed (**C**). Stage 5 oosorption (**D**). lPVO, late previtellogenic oocytes; VG, vacuolated globules; N, nucleus; Nu, nucleolus; FC, follicle cells; VO, vitellogenic oocyte; L, lipid droplets; YP, yolk platelets; MO, mature oocytes; AO, atretic oocytes; chorion (black arrow).

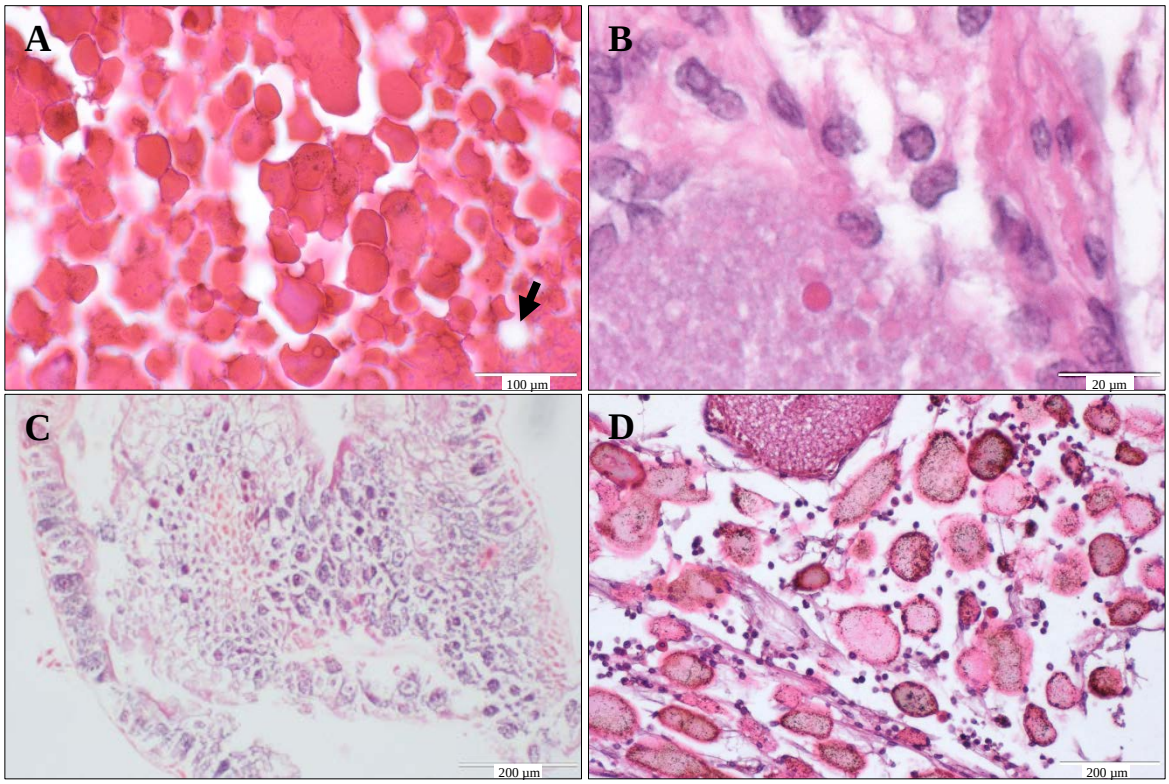

Figure 2.6. Different type of cells found in the ovary of *Chaceon quinquedens* females. Yolk platelets (**A**) Follicle cells, purple (**B**). Oogonial cells in the germinal zone of a Stage 1 ovary (**C**). Hemolymph cells (**D**). Lipid droplets (black arrow).

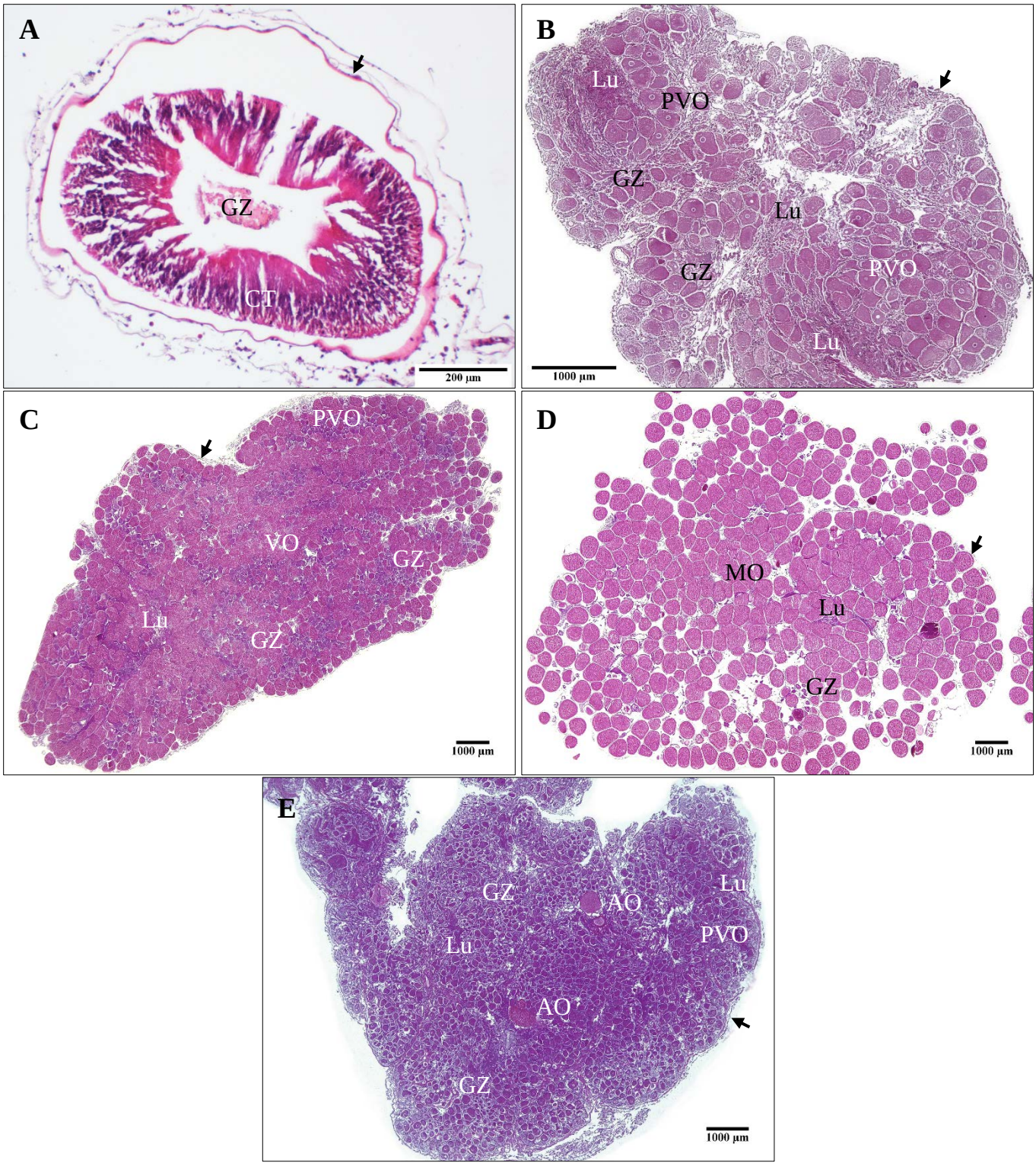

Figure 2.7. Stages of ovarian development of *Chaceon quinquedens* females. Cross section of a stage 1 immature ovary showing the germinal zone and the connective tissue of the ovary wall (**A**). Stage 2 early maturing ovary (**B**). Stage 3 advanced ovary (**C**).

Stage 4 mature ovary (**D**). GZ, germinal zone; CT, connective tissue; Lu, lumen; PVO, previtellogenic oocytes; VO, vitellogenic; AO, atretic oocyte; ovary wall (black arrow).

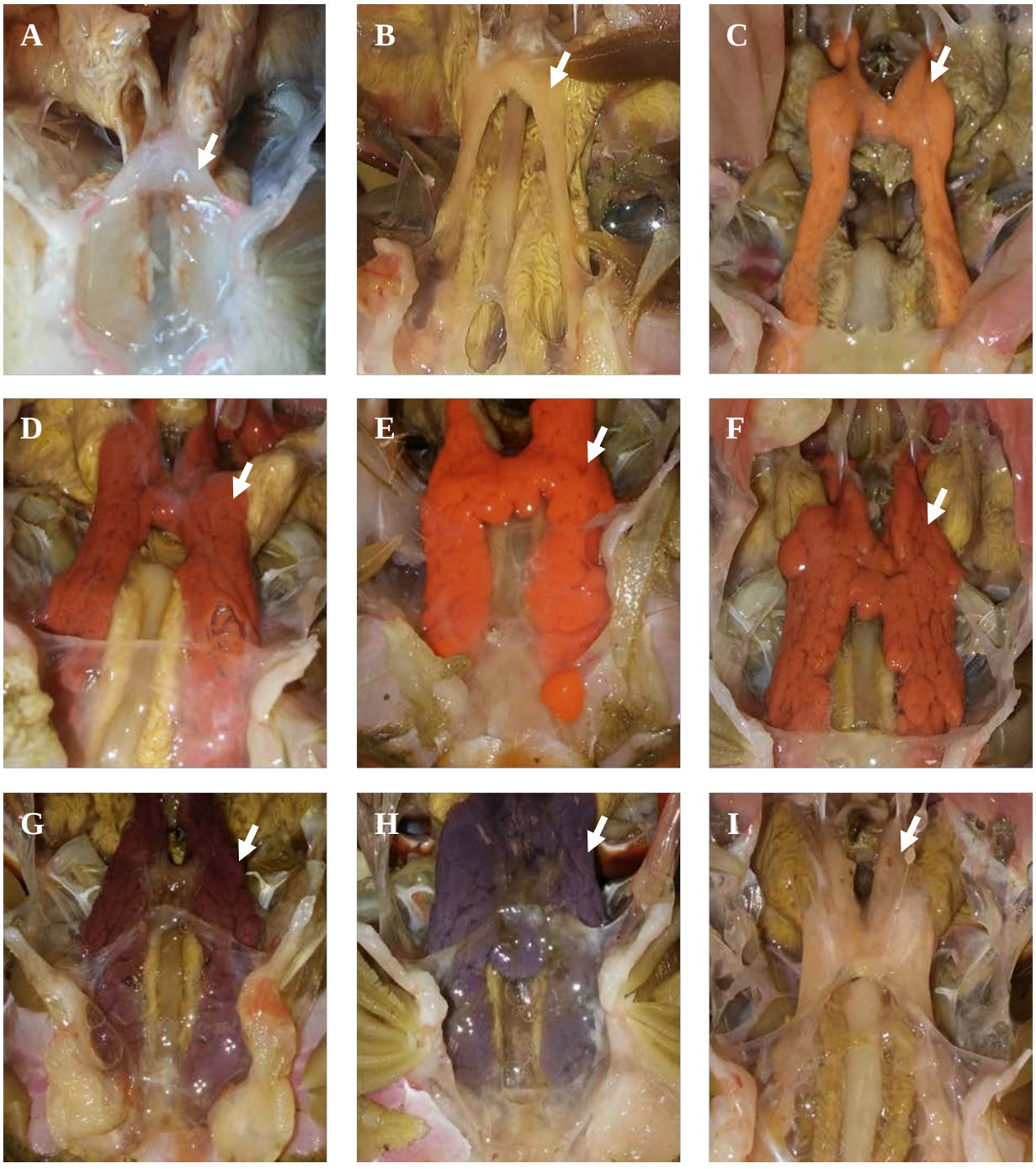

Figure 2.8. Morphology of the ovary in stages of ovarian development of *Chaceon quinquedens* females. Stage 1 immature (**A**). Stage 2 early maturing (**B, C**). Stage 3 advanced (**D, F**). Stage 4 mature (**G, H**). Stage 5 redeveloping (**I**).

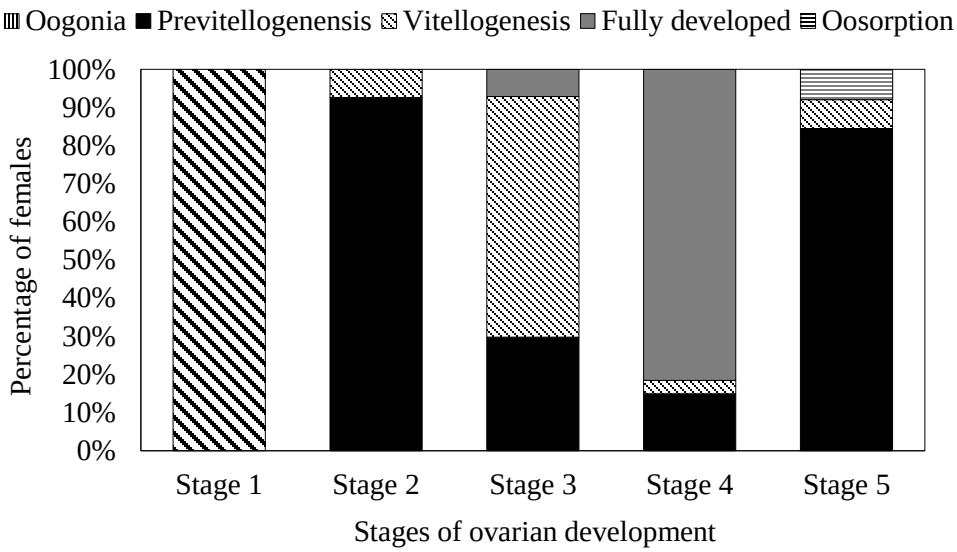

Figure 2.9. Oocyte development stages in the ovarian development stages of *Chaceon quinquedens*.

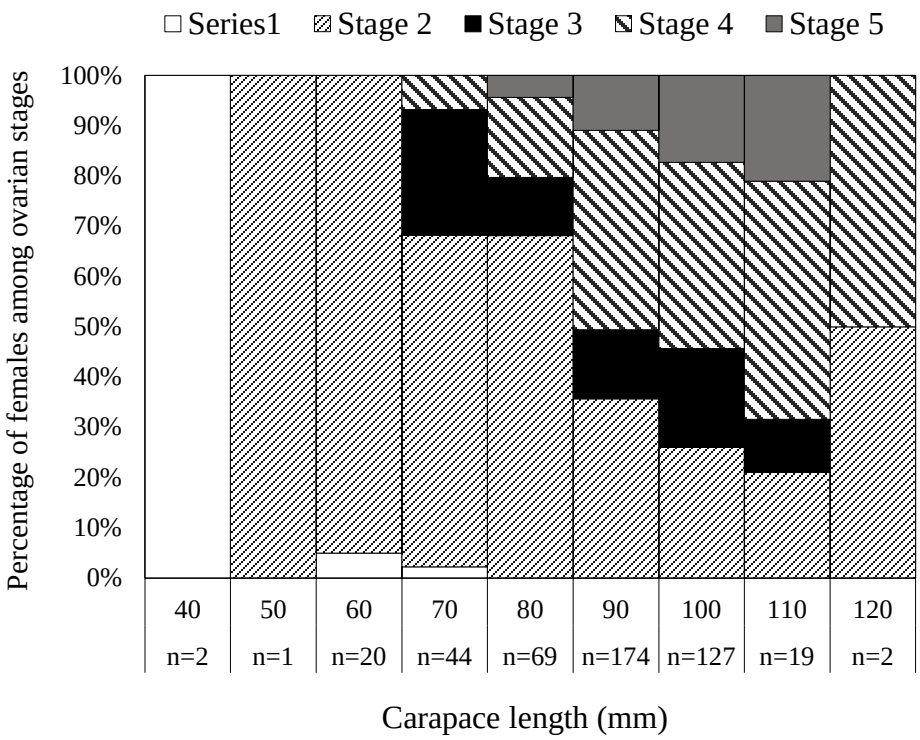

Figure 2.10. Percentages of female *Chaceon quinquedens* in each ovarian stage as a function of carapace length.

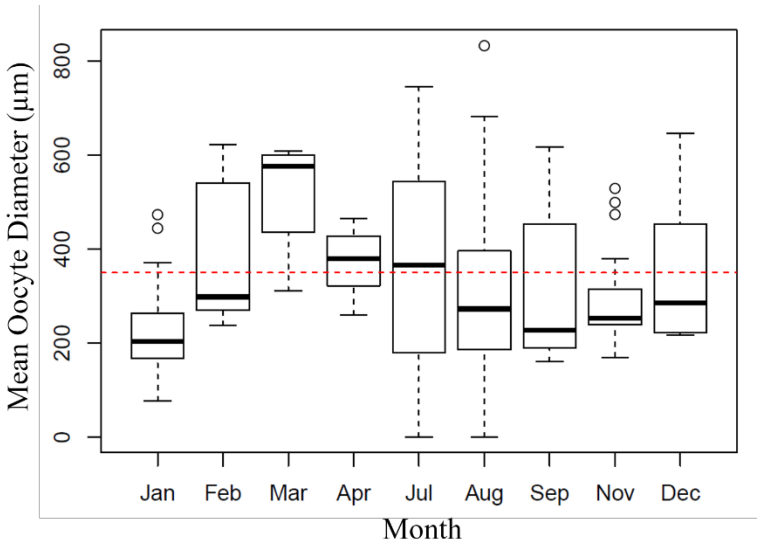

Figure 2.11. Mean oocyte diameter (µm) of *Chaceon quinquedens* by month of sample (*N* = 458). Boxes enclose central 50% of data; horizontal line is median; vertical bars delimit observations within 1.5 box lengths, whereas circles represent outliers, dashed red line is the mean size of fully developed oocytes.

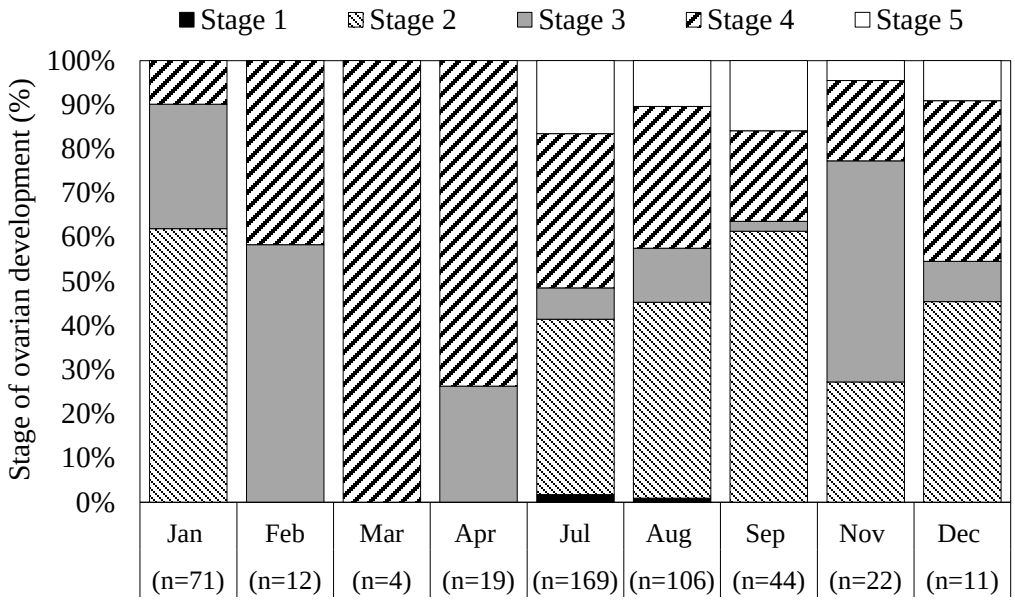

Figure 2.12. Percentage of ovarian development stages of *Chaceon quinquedens* in each month (N = 458).

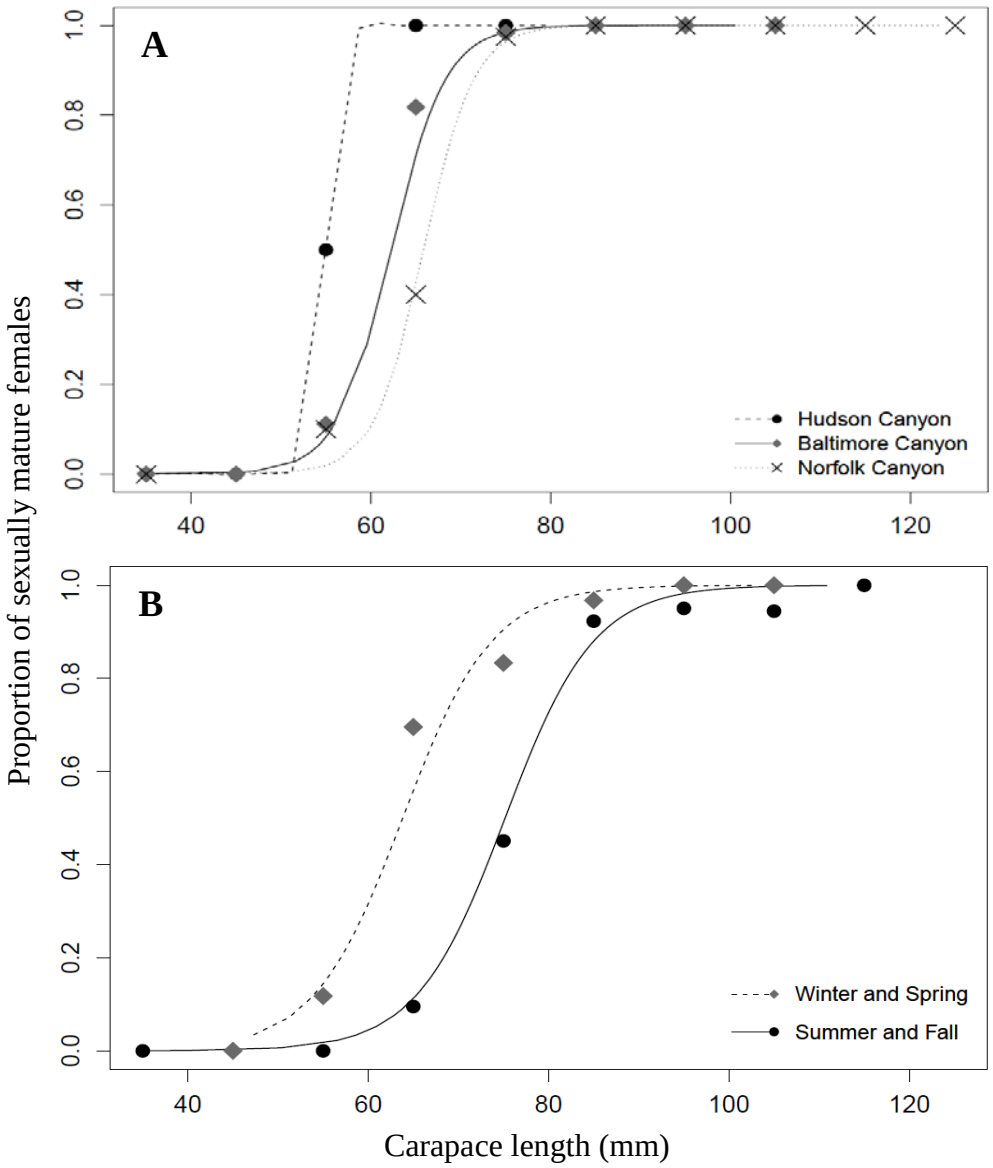

Figure 2.13. Logistic curve fitted to the morphological (**A**) and physiological (**B**) sexual maturity of *Chaceon quinquedens* females.

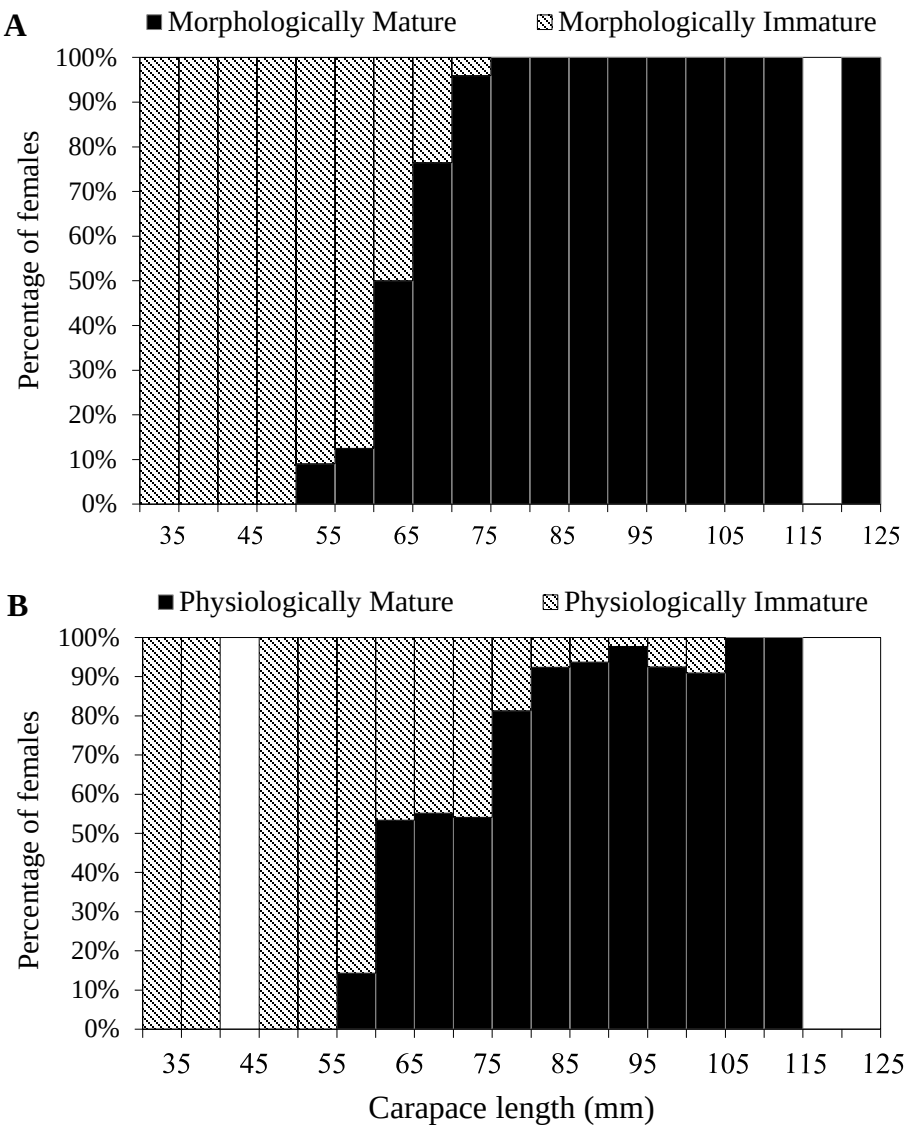

Figure 2.14. Size-frequency distributions of morphologically (**A**) and physiologically (**B**) mature and immature females of *Chaceon quinquedens*.

CHAPTER 3: FECUNDITY OF RED DEEP-SEA CRAB, *CHACEON QUINQUEDENS* SMITH, 1879, IN THE MID-ATLANTIC BIGHT

ABSTRACT

The red deep-sea crab, *Chaceon quinquedens* Smith, 1879, has been harvested from the US Atlantic continental shelf since the 1970's but knowledge about their biology is extremely limited. The objective of this study was to determine size-specific fecundity for *C. quinquedens* in the Mid-Atlantic Bight. Samples were collected by trawling aboard NOAA research vessels in 2012–2013, and from traps aboard commercial fishing vessels in 2014–2016. Fecundity was estimated by using an automated imaging method for egg samples during early embryo development. We observed a relationship between stages of embryo development and color of the egg mass. Fecundity, the number of eggs per brood, of *C. quinquedens* ranged from 34,691–324,729 for females between 62.6 and 106.2 mm in carapace length (CL). A positive correlation between fecundity and female size in CL ($R^2 = 0.636$) was observed. A breeding cycle was proposed after analyzing seasonal changes in the stages of embryo development.

INTRODUCTION

The red deep-sea crab (RDSC), *Chaceon quinquedens* Smith, 1879, is a large brachyuran that inhabits the benthos of the Western Atlantic continental shelf and slope, and live at water temperatures of 5–8 ºC (Wigley *et al.*, 1975). In the Mid-Atlantic Bight of the US Atlantic continental shelf, *C. quinquedens* have been commercially important since the 1970's. A management plan was implemented in 2002, consisting primarily of effort controls due to major uncertainties about their biology such as natural mortality, growth and reproduction (Chute *et al.*, 2008). Stock assessment models can be improved by including biological parameters such as fecundity. Integration of reproductive data may help elucidate the impact of removing large males from the population of *C. quinquedens* for 40+ years (Wahle *et al.*, 2008).

An essential component of reproductive biology is fecundity, the number of offspring produced by an individual, which provides information about the reproductive potential and possible recruitment of a population. The principal determinant of fecundity and reproductive output in female brachyuran crabs is body size (Hines, 1982, 1988). Hines (1982) reported that egg size increased with increasing body size, and concluded that the main constraint on brood size is the allometric restriction on space available for yolk accumulation in the body cavity. Hines (1988) reported fecundity for *C. quinquedens* and *C. fenneri* at 160,000–275,000 eggs per brood, which is unusually low for large crabs. A possible explanation for low fecundity is the size of the egg; *C. quinquedens* have relatively large yolky eggs ranging in diameter from 484 to 846 µm (Haefner, 1977, 1978; Hines, 1982, 1988). Reduced fecundity is the result of a trade-off between egg size and the number of eggs per brood in brachyurans (Hines, 1982). The

yolky eggs of *C. quinquedens* could be an adaptation to living in the deep temperate and boreal waters of the northwest Atlantic in order to provide nutritional flexibility to the larvae (Hines, 1988).

Embryo development of a congener, *C. affinis*, was described by Tuset *et al*. (2011), who suggested that the changes in embryo color during development vary for all geryonid crabs, and that individual descriptions should be made for every species. Hence, a detailed description of the stages of embryo development for *C. quinqedens* was necessary. The goals of this study were to determine the seasonal progression of embryo development for *C. quinquedens*, estimate size-specific fecundity, and determine if it varies with season or geographic location.

MATERIALS AND METHODS

Female *C. quinquedens* were collected in the Mid-Atlantic Bight during January 2012, and July of 2013 aboard NOAA research vessels at depths >250 m by trawling (Stevens & Guida, 2016). In July and September of 2014, and July of 2015 sampling was conducted aboard commercial vessels, via collaboration with The Atlantic Red Crab Company (ARCC), off Newport News, VA, along the Norfolk Canyon at depths >600 m using baited traps. In August of 2016 crabs were collected off New Bedford, MA, along the Baltimore Canyon at depths >600 m by baited traps. Throughout 2016, additional samples were collected bimonthly at the fishing port located in Newport News and Hampton, VA from ARCC vessels that were fishing near the Norfolk Canyon using baited traps at depths >600 m. However, we specifically requested hatching (black) egg masses from the fishermen during February, April and May (2016). All females caught

were sexed and measured for carapace length (CL, from the rostral teeth to the center of the edge of the carapace), carapace width (CW, including lateral spines), and abdomen width (AW, 3rd segment) with a digital caliper to the nearest 0.01 mm. Abdominal flaps with attached embryos were removed from female crabs and preserved in 10% formalin. The stage of embryo development was recorded according to color and condition of the egg mass (Wigley *et al.*, 1975; Haefner, 1978) (Table 3.1). Approximately 20 eggs from each female crab were preserved in Bouin's solution for determination of stages of embryo development.

Samples were transported to the laboratory at the Paul S. Sarbanes Coastal Ecology Center (Berlin, MD) and stored in 70% EtOH until they were processed. Egg clutches stored in 70% EtOH were rinsed with distilled water (DW) and drained prior to detachment. The pleopods of abdominal flaps with attached eggs were cut off at the base, and eggs were detached from the pleopods using disposable finger scalpels and placed in a pre-numbered pan prior to oven-drying at 60 °C for 24 h. Eggs were separated from the remaining pleopods, setae, and clusters of eggs by sifting them through a sieve with mesh size of 1000 µm and 750 µm. Sieving was complete when very few to no setae remained in the sample. After sieving, eggs were placed into pre-numbered aluminum weighing dishes and oven-dried at 60 °C for another 24 h. Dried egg clutches were weighed and three subsamples of 4 mg from each clutch were photographed using Canon EOS 50D (Tokyo, Japan) camera and counted using an automated imaging method (ImageJ 1.48v Software). The eggs preserved in Bouin's solution were placed in a microscope well depression-slide with DW under a Leica M125 (Leica Microsystems, Wetzlar, Germany) stereo-zoom microscope with reflected light and a transmitted light base. Embryos were

classified into one of six developmental stages as defined by Tuset *et al.* (2011). Only egg masses in stage 1 to 3 were used for the estimation of fecundity due to the potential loss of eggs in late stages of embryo development.

In order to determine seasonality, we divided the sample collections into winter (December and February), spring (April and May), summer (July and August) and fall (September and November). Data were $\log_{10}$ transformed to conduct the allometric analysis between reproductive output and body size. Linear regression was used to determine the relationship between fecundity and body size (CL). Analysis of covariance (ANCOVA) was used to test the effects of CL, years, and embryo stage on size-specific fecundity. For the ANCOVA, embryo development stages 1 and 2 were grouped together. One-way ANOVA was used to test the effects of embryo stage, season and geographic location on fecundity, separately. Post hoc linear regression was used to determine the relationship between fecundity and embryo stage, season and geographic location on fecundity, separately. Descriptive data are reported as the mean and standard error (mean ± SE).

RESULTS

Seasonal changes in stages of embryo development

A total of 309 ovigerous females of *C. quinquedens* was collected from 2012 through 2016 (Fig. 3.1). The stages of embryo development were related to color of the egg mass for *C. quinquedens* (Fig. 3.2). Recently extruded (new) and early development eggs ranged in color from bright orange to light orange and orange and were classified as stage 1 and 2, respectively (Fig. 3.2A, B). Eggs at mid-development, stage 3, are

distinguished by the presence of eyes in the embryos, and color ranged from red orange to red brown (Fig. 3.2C, D). Embryos at the prehatching stage of development (stage 4) were dark brown and become black as they reached the hatching stage (5) (Fig. 3.2E, F). Females with empty egg cases were classified as stage 6. We observed undivided eggs in stage 1, and the free region of yolk in stage 2, but the slight pigmentation of the eyes in stage 3 described for *C. affinis* was not visible in our study (Fig. 3.3) (Tuset *et al.*, 2011). In stage 4, prehatching, enlargement of the eyes and the segmented appendages of the embryos were observed (Fig. 3.3C). The last stage of embryo development observed was hatching, characterized by fully grown embryos inside the egg and broken egg cases starting to hatch (Fig. 3.3D).

We observed seasonality in the stages of embryo development in *C. quinquedens* in the Mid-Atlantic Bight (Fig. 3.4). In winter, the predominant stages of embryo development were stage 2 and 3 (78%), however females with stage 4 eggs were also observed. It is important to note that the females with stage 4 egg masses were collected in late February. The stages of embryo development increased progressively from winter to spring. In spring, we observed females with embryos from stage 3 (despite collections biased towards late stage eggs) to stage 6, but the majority of females had brood masses at stage 4 (86%). Summer was the only season where ovigerous females with stage 1 embryos (8%), and all stages of embryo development were observed at the same time. However, the predominant stages of embryo development in summer were stage 2 (49%) and stage 5 (17%). During fall, females with egg masses at stage 2 (52%) and 3 (48%) were observed. These data suggest that *C. quinquedens* females in the Mid-Atlantic Bight exhibit a seasonal reproductive cycle; egg clutches are produced in summer, early

development takes place in summer, fall and winter, late development occurs in winter through spring, and hatching starts in spring and peaks in summer (Fig. 3.4).

Size-specific fecundity

Fecundity estimates for *C. quinquedens* females ranged from 34,691 (66.0 mm CL) to 324,729 (102.3 mm CL) with a mean of 188,356 ± 4,478. Ovigerous females (*n*=171) used to estimate fecundity ranged from 62.6 to 106.2 mm CL (mean 86.2 ± 0.7 mm CL), but a few were excluded from the analysis due to unusually small broods (*n*=8). A positive relationship was observed between fecundity and CL (logFec = – 0.57 + 3.01 logCL; $R^2 = 0.64$; $P < 0.001$) (Fig. 3.5A) using a linear regression (Table 3.2). The ANCOVA analysis showed that the effect on fecundity caused by year of collection ($F < 0.1$; $P = 0.9$) was not significant, but effects of embryo stage ($F = 3.9$; $P = 0.05$) and CL were significant ($F = 116.3$; $P < 0.001$) (Table 3.3). One-way ANOVA showed significant differences in fecundity between embryo stages ($F = 4.8$; $P = 0.03$) seasons ($F = 35.7$; $P < 0.001$), and geographic location ($F = 12.3$; $P < 0.001$) (Table 3.4). Therefore, separate linear regressions (Table 3.2) were fitted for each group of embryo stages: stage 1 and 2 ($R^2 = 0.42$; $P < 0.001$) and stage 3 ($R^2 = 0.48$; $p < 0.001$) (Fig. 3.5B), for each season (Figure 6): winter-spring ($R^2 = 0.704$; $p < 0.001$), summer ($R^2 = 0.538$; $p < 0.00$), and fall ($R^2 = 0.282$; $p < 0.002$), and for each geographic location (Fig. 3.5C): Baltimore Canyon ($R^2 = 0.608$; $p < 0.001$) and Norfolk Canyon ($R^2 = 0.278$; $p < 0.001$) (Fig. 3.5D). These data suggest that extrusion of eggs by *C. quinquedens* occurs over a prolonged period, that size-specific fecundity might be affected by differences in CL among seasonal collections, and that populations at different latitudes may differ in size-specific fecundity.

DISCUSSION

We observed ovigerous females of *C. quinquedens* in all seasons, and a progression of embryo development that indicated a seasonal reproductive cycle similar to that reported by Haefner (1978). However, although we observed females with hatching eggs from spring to summer, we did not observe them as early as January, as reported by Haefner (1978), or late winter as reported by Wigley *et al.* (1975). We only observed females with empty egg cases in spring (N = 2) and summer (N = 10), whereas, during winter, we observed mostly early-stage eggs and some prehatching (late-stage) eggs. These data suggest a prolonged breeding season for *C. quinquedens*. Wigley *et al.* (1975) stated that oviposition took place in water depths >640 m, with the greatest proportion of ovigerous females at depths of 412–503 m. Furthermore, Wigley *et al.* (1975) reported that oviposition and embryo development was influenced by water temperature and/or depth. An inverse relationship between depth and female size has been reported by Haefner (1978), Wigley *et al.* (1975), and Stevens & Guida (2016). Differences between previous studies and ours in seasonality of the breeding season and the scarcity of females with empty egg cases may be due to the limited depth distribution of crabs collected by commercial traps.

We observed a progressive relationship between stages of embryo development and color of the egg mass for *C. quinquedens*, which has been reported for other brachyurans (Comeau *et al.*, 1999; Tuset *et al.*, 2011). Wigley *et al.* (1975) reported that *C. quinquedens* embryos at early development ranged from light orange to red orange, and became gradually darker as they matured. However, examination of preserved eggs was necessary to accurately assess developmental stage. This enabled us to distinguish

between eggs in early development (stage 1 to 3), late development (stage 4) and hatching (stage 5) stages (Fig. 3.3).

Fecundity estimates for geryonid crabs reported by other authors had similar ranges. Our estimates of fecundity were in the same range as those reported for *C. bicolor* in Southwestern Australia (15,592 – 288,512 eggs; Smith, 2006), slightly higher (and lower) than reported for *C. quinquedens* and *C. fenneri* (160,000 – 275,000 eggs per brood; Hines, 1988) and lower than that reported for *C. affinis* from the Canary Islands (199,690 –566,956 eggs; Tuset *et al.*, 2011). Differences in fecundity between geryonid species may be due to differences in egg size for each species, as reported by Hines (1988) for *C. quinquedens* and *C. fenneri*. Our maximum fecundity estimate for *C. quinquedens* belongs to a crab from Norfolk Canyon (324,729 ± 4,448), and was greater than the maximum fecundity in Baltimore Canyon (294,096 ± 6,706). These results are consistent with the body-size restriction hypothesis of Hines (1982), and the conclusions of Stevens & Guida (2016) who showed that mean size of females was greater in the Norfolk Canyon area than other sites further north. Differences in size at sexual maturity of RDSC from different geographic locations may lead to variations in size-specific fecundity in the populations (Martínez-Rivera & Stevens, manuscript in preparation 1). Comparisons of fecundity between populations of RDSC at different latitudes can provide information for delineating stocks (Davidson *et al.* 1984). Therefore, estimates of fecundity can be used to understand the recruitment of RDSC population.

Reproductive output of brachyurans can be regulated by energetic, mechanical, or physical constraints, maternal condition and characteristics, and external factors like food supply (Hine, 1982, 1988, 1991; Webb *et al.*, 2016). The reproductive output for *C.*

quinquedens increased progressively with increasing female size, as shown by previous studies on *C. fenneri*, *Chionoecetes opilio*, *C. affinis* and *Paralithodes camtschaticus* (Hines, 1988; Sainte-Marie, 1993; Comeau *et al.*, 1999; Tuset *et al.*, 2011; Swiney *et al.*, 2012). The positive relationship between fecundity and CL explained 64% of the total variance. Eggs of *C. quinquedens* are relatively large and yolky, and ranged in diameter from 484 to 846 µm (Haefner, 1977, 1978; Hines, 1982, 1988). Hines (1988) suggested a possible evolutionary adaptation of *C. quinquedens* was to increase the cephalothorax area in order to accumulate yolk for a significantly larger eggs than most brachyurans. Hence, allocating energy toward egg size instead of quantity may be a mechanism to ensure larvae survival for those geryonid crabs that undergo an upward vertical migration.

We were unable to classify females as primiparous or multiparous. Previous studies have reported differences between primiparous and multiparous females; primiparous females were 70% as fecund as same size multiparous females for *Chionoecetes bairdi* (Somerton & Meyers, 1983), approximately 80% for *Chionoecetes opilio* (Sainte-Marie, 1993), and around 28%–30% for *Paralithodes camtschaticus* (Swiney & Long, 2015). Sainte-Marie (1993) suggested that primiparous and multiparous females may differ in fecundity but also in quality of progeny. Stevens *et al.* (1993) suggested differences in fecundity between primiparous and multiparous females are related to the volume of the body cavity, thus no real advantage for males when selecting a multiparous females over a pubescent female of the same size. We observed large females (>95 mm CL) that could be considered multiparous with low fecundity. Previous studies in brachyurans reported that egg diameter increased with female size, whereas

size-specific fecundity decreased with increasing egg diameter (Hines, 1982, 1988; Graham *et al.*, 2012). In crustaceans, very old females may have low fecundity due to reproductive senescence (Sastry, 1983; Koopman *et al.*, 2014; Webb *et al.*, 2016). Lastly, multiparous females that fertilize a brood using sperm reserves, i.e. which undergo oviposition without mating and molting, can have low fecundity (Webb *et al.*, 2016). Hence, observation of *C. quinquedens* multiparous females with low fecundity can be explained by larger egg size, reproductive senescence or oviposition without mating. However, primiparous female *Callinectes sapidus* with low fecundity due to egg loss have been observed by Graham *et al.* (2012). Another factor that influences fecundity is the stored sperm in the spermatheae of the multiparous females, Paul (1984) showed a decrease in fecundity of multiparous females that used the stored sperm to produce the subsequent clutches in comparison with similar females that mated before extrusion of eggs. Furthermore, Sainte-Marie *et al.* (2002) reported that stored sperm of primiparous female *Chionoecetes opilio* may be insufficient to produce a brood. Webb *et al.* (2016) reported an increase in size-specific fecundity as females transition from primiparous to multiparous, but a decrease from old multiparous to very old multiparous females, based on shell condition. Therefore, a study of the differences between primiparous and multiparous *C. quinquedens* females is needed.

Size-specific fecundity of in crab populations has implications for fishery management. Swiney & Long (2015) suggested that including fecundity in stock assessment models without accounting for differences between primiparous and multiparous females may lead to overestimating population fecundity if size-fecundity relationships of multiparous females are used in the calculations. Another concern for

fishery management is the impact of removing large males on the reproductive output of a species. Sainte-Marie (1993) suggested that fishery removals of large male snow crabs could lead to more participation of small males in reproduction. Comeau *et al.* (1999) added that a decline in the availability of mature males due to fishing would lead multiparous females to rely on sperm stored in the spermathecae for egg fertilization, which may reduce their fecundity. Sainte-Marie *et al.* (2002) indicated that variability in sperm reserves of females caused by the scarcity of large males was greater than that caused by small males. Stevens & Guida (2016) reported sex-ratios that indicated a decrease in male mating partners for females as they grow, and this can have a negative impact on recruitment. We recommend that further studies focusing on the connection between removal of males, sperm storage, and fecundity are necessary to improve our understanding of the reproduction and population dynamics of *C. quinquedens*.

ACKNOWLEDGEMENTS

We are grateful to The Atlantic Red Crab Company, their assistance was an integral part of the sample collection. Special thanks to Shadaesha Green, Dr. Indu Sharma and Justin Wilson that assisted with the sample collection. This research was funded by NOAA grant NA11SEC4810002 and grant NA16SEC4810007.

REFERENCES

Chute, A., L. Jacobson, and P. Rago. 2008. Deep sea red crab. NMFS/NOAA Northeast Fisheries Science Center, Woods Hole, MA.

Comeau, M., M. Starr, G. Y. Conan, G. Robichaud, and J. C. Therriault. 1999. Fecundity and duration of egg incubation for multiparous female snow crabs (*Chionoecetes opilio*) in the fjord of Bonne Bay, Newfoundland. Canadian Journal of Fisheries and Aquatic Sciences. 56(6):1088–1095.

Davidson, K., J. C. Roff, and R. W. Welner. 1984. Morphological, electrophoretic, and fecundity characteristics of Atlantic snow crab, *Chionoecetes opilio*, and implications for fisheries management. Canadian Journal of Fisheries and Aquatic Sciences. 42:474–482.

Graham, D. J., H. Perry, P. Biesiot, and R. Fulford. 2012. Fecundity and egg diameter of primiparous and multiparous blue crab *Callinectes sapidus* (Brachyura: Portunidae) in Mississippi Waters. 32(1):49–56.

Haefner, Jr., P. A. 1977. Reproductive biology of the female deep-sea red crab, *Geryon quinquedens*, from the Chesapeake Bight. Fishery Bulletin. 75:91–102.

Haefner, Jr., P. A. 1978. Seasonal aspects of the biology, distribution and relative abundance of the deep-sea red crab *Geryon quinquedens* Smith, in the vicinity of the Norfolk Canyon, Western North Atlantic. Proceedings of the National Shellfisheries Association. 68:49–62.

Hines, A. H. 1982. Allometric constraints and variable of reproductive effort in brachyuran crabs (Crustacea, Decapoda, Brachyura). Marine Biology. 69:309–320.

Hines, A. H. 1988. Fecundity and reproductive output in two species of deep-sea crabs, *Geryon fenneri* and *G. quinquedens* (Decapoda: Brachyura). Journal of Crustacean Biology. 8(4):557–562.

Hines, A. H. 1991. Fecundity and reproductive output in nine species of *Cancer* crabs (Crustacea, Brachyura, Cancridae). Canadian Journal of Fisheries and Aquatic Sciences. 48:267–275.

Koopman, H. N., A. J. Westgate, and Z. A. Siders. 2015. Declining fecundity and factors affecting embryo quality in the American lobster (*Homarus americanus*) from the Bay of Fundy. Canadian Journal of Fisheries and Aquatic Sciences. 72:352–363.

Paul, A. J. 1984. Mating frequency and viability of stored sperm in the tanner crab *Chionoecetes bairdi* (Becapoda, Majidae). Journal of Crustacean Biology. 4:375–381.

Sainte-Marie, B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, *Chionoecetes opilio*, in the Northwest Gulf of Saint Lawrence. Canadian Journal of Fisheries and Aquatic Sciences. 50:2147–2156.

Sainte-Marie B., J. M. Sévigny, and M. Carpentier. 2002. Inter-annual variability of sperm reserves and fecundity of primiparous females of the snow crab (*Chionoecetes opilio*) in relation to sex ratio. Canadian Journal of Fisheries and Aquatic Sciences. 59:1932−1940.

Sastry, A. N. 1983. Ecological aspects of reproduction. In, W. B. Vernberg, (ed.), The Biology of Crustacea: Environment adaptations. Academic Press Inc., New York. 8:179–270.

Smith, K. D. 2006. Distributions, relative abundances and reproductive biology of the deep-water crabs *Hypothalassia acerba* and *Chaceon bicolor* in Southwestern Australia. Ph.D. dissertation. Murdoch University, School of Biological Sciences.

Somerton, D. A. and W. S. Meyers. 1983. Fecundity differences between primiparous and multiparous female Alaskan tanner crab (*Chionoecetes bairdi*). Journal of Crustacean Biology. 3(2):183–186.

Stevens, B. G., W. E. Donaldson, J. A. Haaga, and J. E. Munk. 1993. Morphometry and maturity of male Tanner crabs, *Chionoecetes bairdi*, grasping pubescent and multiparous females in shallow and deepwater environments. Canadian Journal of Fisheries and Aquatic Sciences. 50:1504–1516.

Stevens, B. G., and V. Guida. 2016. Demographics, morphometrics, depth, and temperature distribution of red deepsea crabs *Chaceon quinquedens* at four sites in the Mid-Atlantic Bight. Fishery Bulletin. 114:343–359.

Swiney, K. M., W. C. Long, G. L. Eckert and G. H. Kruse. 2012. Red king crab, *Paralithodes camtschaticus*, size-fecundity relationship, and interannual and seasonal variability in fecundity. Journal of Shellfish Research. 31(4):925–933.

Swiney, K. M. and W. C. Long. 2015. Primiparous red king crab *Paralithodes camtschaticus* are less fecund than multiparous crab. Journal of Shellfish Research. 34(2):493–498.

Tuset, V. M., D. I. Espinosa, A. García-Mederos, J. I. Santana, and J. A. González. 2011. Egg development and fecundity estimation in deep-sea red crab, *Chaceon affinis* (Geryonidae), off the Canary Islands (NE Atlantic). Fisheries Research. 109:373–378.

Wahle, R. A., C. E. Bergeron, A. S. Chute, L. D. Jacobson, and Y. Chen. 2008. The Northwest Atlantic deep-sea red crab (*Chaceon quinquedens*) population before and after the onset of harvesting. ICES Journal of Marine Science. 65:862–872.

Webb, J. B., L. M. Slater, G. L. Eckert, and G. H. Kruse. 2016. The contribution of fecundity and embryo quality to reproductive potential of eastern Bering Sea snow crab (*Chionoecetes opilio*). 73:1800–1814.

Wigley, R. L., R. B. Theroux, and H. E. Murray. 1975. Deep sea red crab, *Geryon quinquedens*, survey off northeastern United States. Marine Fisheries Review. 37(8):1–21.

Table 3.1. Code used to classify the stages of embryo development of *Chaceon quinquedens*. Modified from Wigley *et al.* (1975) and Haefner (1978).

Stage	Embryo stage
0	Absent
1	New, pre-blastula, bright orange
2	Early develop, blastula, orange
3	Eyed, late develop, red orange to red brown
4	Eyed, prehatching, dark brown to purple
5	Hatching, black
6	Empty egg cases

Table 3.2. Results of linear regressions for *Chaceon quinquedens*: log-transformed fecundity, logFec; log-transformed carapace length, logCL; winter and spring, W-S; summer, S; fall, F; Baltimore Canyon, BC; Norfolk Canyon, NC; embryo stages 1 and 2, St 1–2; embryo stage 3, St 3.

Description	**Group**	***N***	**Linearized equation Log transformed**	R^2	***P*-value**
All	–	171	logFec = – 0.57 + 3.01 logCL	0.64	>0.001
Embryo stages	St 1–2	119	logFec = 1.56 + 1.92 logCL	0.42	>0.001
	St 3	32	logFec = 1.03 + 2.21 logCL	0.48	>0.001
Seasons	W-S	30	logFec = – 1.40 + 3.40 logCL	0.70	>0.001
	S	108	logFec = 0.76 + 2.33 logCL	0.54	>0.001
	F	33	logFec = 2.31 + 1.54 logCL	0.28	>0.005
Geographical location	BC	50	logFec = 0.53 + 2.44 logCL	0.61	>0.001
	NC	101	logFec = 2.26 + 1.57 logCL	0.28	>0.001

Table 3.3. Results of the analysis of covariance for *Chaceon quinquedens*: log-transformed fecundity, logFec; log-transformed carapace length, logCL.

Variable	**Factor**	***df***	**Sum squares**	**Mean squares**	***F*-value**	***P*-value**
logFec	logCL	1	0.75	0.75	116.33	>0.001
	Year	1	0.00	0.00	0.00	0.98
	Embryo Stage	1	0.03	0.03	3.92	0.05
	Residuals	147	0.95	0.01		

Table 3.4. Results of the analysis of variance for *Chaceon quinquedens*.

Factor	*df*	Sum squares	Mean squares	*F*-value	*P*-value
Embryo stage	1	0.05	0.05	4.83	0.03
Seasons	2	1.47	0.73	35.68	>0.001
Geographical location	1	0.13	0.13	12.29	>0.001

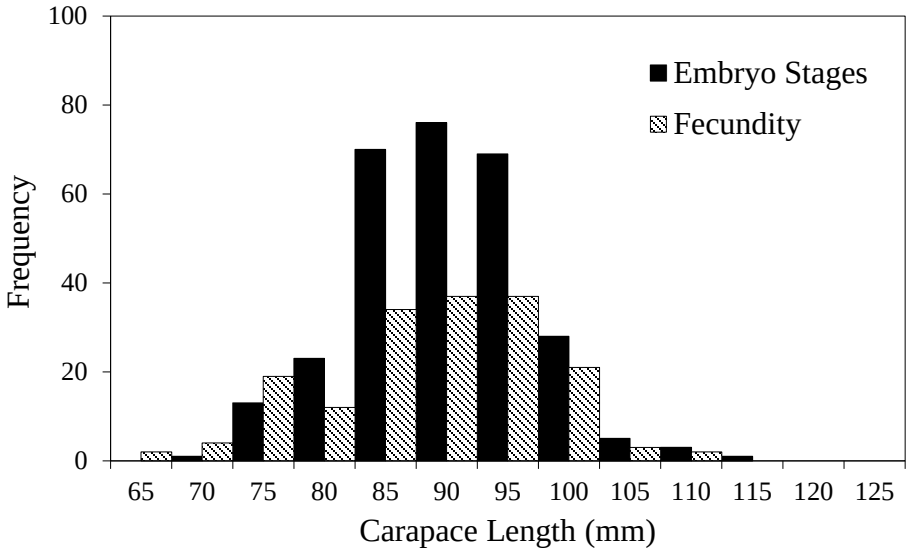

Figure 3.1. Length-frequency distributions of female red deep-sea crab, *Chaceon quinquedens*, in the Mid-Atlantic Bight used to determine stages of embryo development (black) and size-specific fecundity (dashed).

Figure 3.2. Color variation of egg mass at different stages of embryo development of *Chaceon quinquedens*: bright orange (**A**), orange (**B**), red orange (**C**), red brown (**D**), dark brown (**E**), and black (**F**).

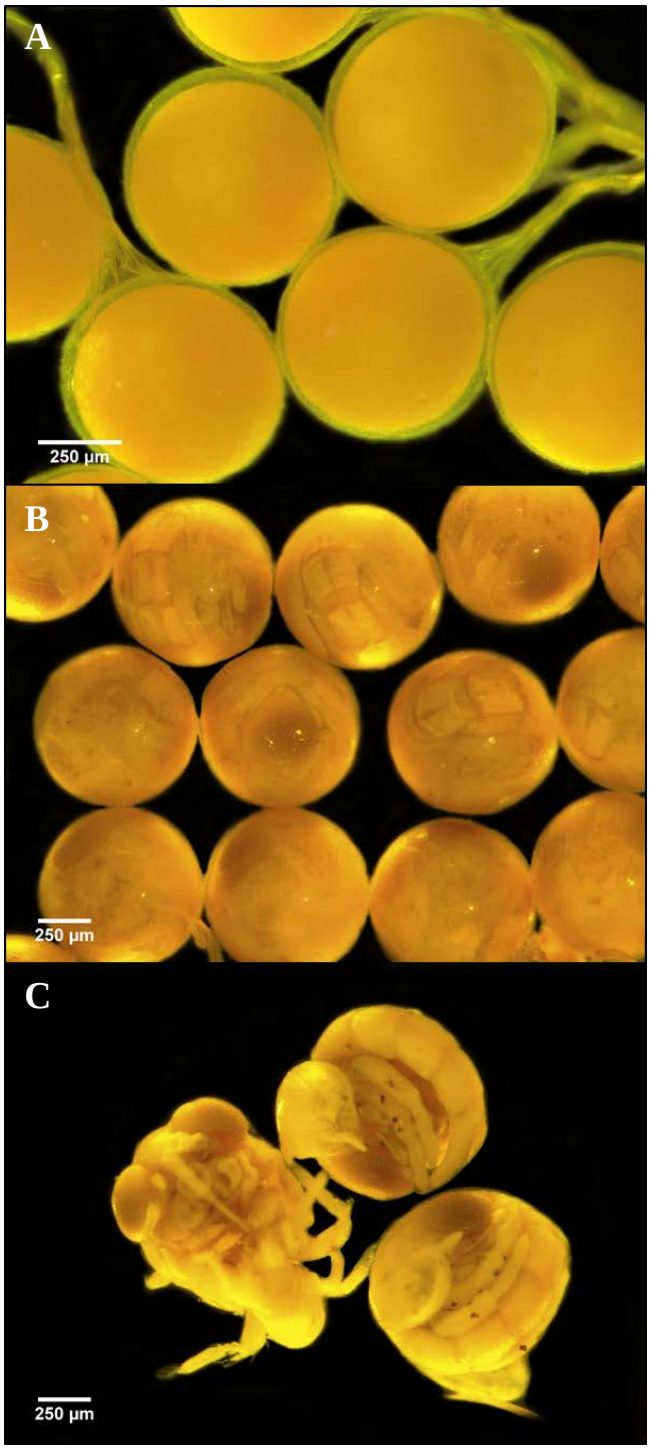

Figure 3.3. Stages of embryo development of *Chaceon quinquedens* preserved in Bouin's solution: early development (stage 1 to 3) (**A**), late development (stage 4) (**B**), and hatching (stage 5) (**C**).

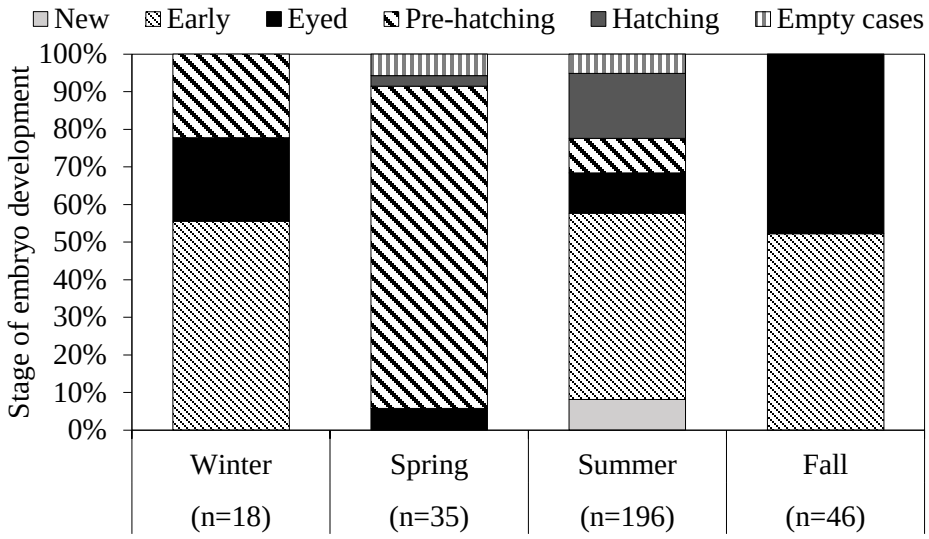

Figure 3.4. Stages of embryo development of *Chaceon quinquedens* across seasons of sample collection.

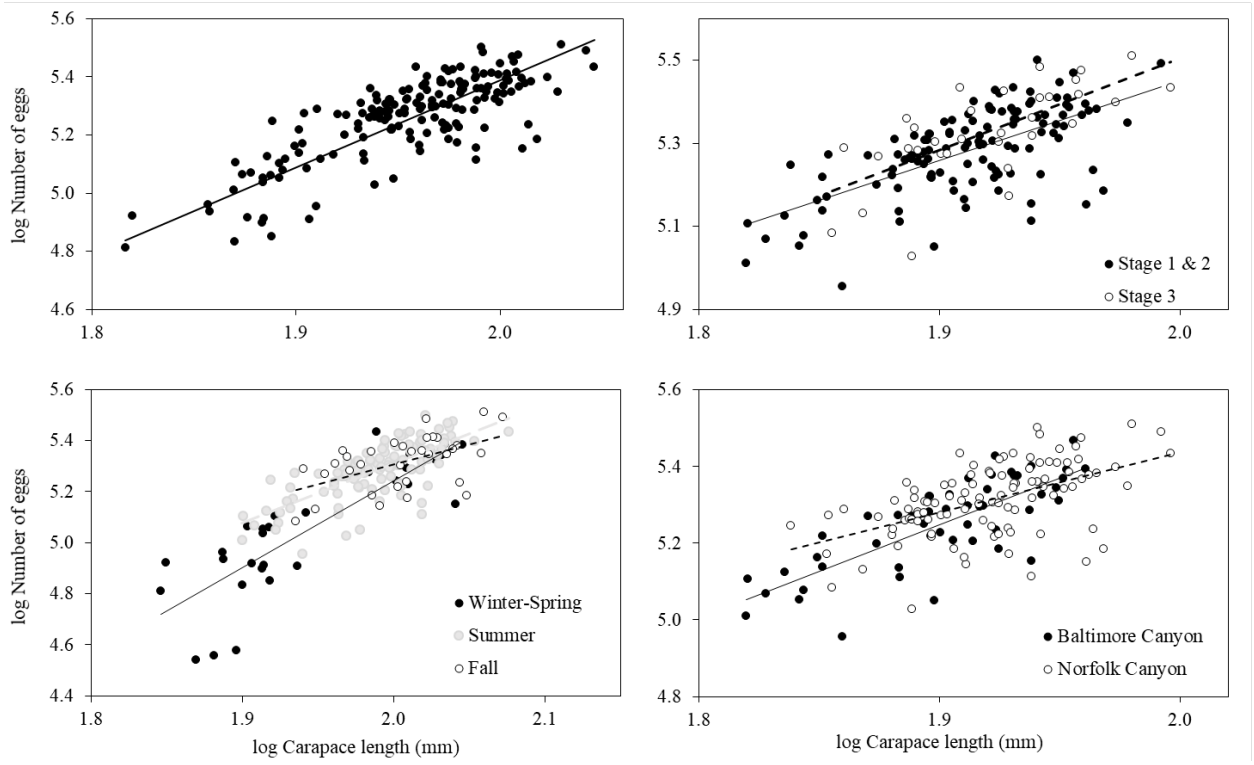

Figure 3.5. Linear regression of fecundity as a function of carapace length for female *Chaceon quinquedens* with log_{10}-transformed axes (**A**). Comparisons of relationship between fecundity and carapace length by: embryo stages (**B**), seasons (**C**), and geographic location (**D**).

CHAPTER 4: BIENNIAL FEMALE REPRODUCTIVE CYCLE IN A TEMPERATE CRAB: THE RED DEEP-SEA CRAB, *CHACEON QUINQUEDENS* SMITH, 1879, IN THE MID-ATLANTIC BIGHT

ABSTRACT

Reproductive cycles of crabs tend to vary inversely with environmental temperature. Crabs living in warm water (hence, tropical or shallow) tend towards cycles of weeks or months, whereas species living in temperate water (or on continental shelves) tend towards cycles of a year or more, and those living at temperatures < 2 °C (or in the deep ocean) tend to have biennial or aseasonal reproduction. Biennial reproductive cycles are common among crabs living at extremely low temperatures, but are uncommon for crabs living at temperatures above 5 °C. Red deep-sea crabs, *Chaceon quinquedens* Smith, 1879, are found along the continental shelf and slope of the western Atlantic, from Nova Scotia to the Gulf of Mexico, in water depths of 200–1800 m and constant water temperatures of 5–8 °C, and thus would be expected to have annual or aseasonal reproduction. We studied the reproductive cycle of female red deep-sea crabs collected during targeted research cruises and commercial fishing trips. Timing of reproduction was determined using ovarian maturation, embryonic development, occurrence of ovigerous and non-ovigerous females, and shell condition across collections. Results indicate that female *C. quinquedens* have a reproductive cycle that is at least two years in length, and that hatching is seasonal but semi-continuous. Red deep-sea crab support a small fishery that is classified as data-poor due to lack of information on biology, life history, and reproduction, and this information will be useful for development of improved management strategies.

INTRODUCTION

In crustaceans, the reproductive cycle comprises a series of events including gametogenesis (oogenesis in females), copulation (mating), ovulation, oviposition and fertilization, incubation of eggs and hatching of the larvae (Sastry, 1983). In order to understand the reproductive cycle of a species it is critical to incorporate the peak periods of each reproductive event (Tallack, 2007). The timing of reproduction is commonly determined by examination of the frequency of ovigerous females over time (Negreiros-Fransozo *et al.*, 2002). The reproductive periodicity can be classified as seasonal when restricted to months or seasons; seasonal-continuous, when a peak occurs in some months or seasons; or continuous, when taking place year-round (Pinheiro *et al.*, 2002; Zairion *et al.*, 2015). The reproductive cycle of a species is affected by the environmental conditions (e.g. temperature), which is reflected in the diversity of reproductive patterns in crustaceans (Sastry, 1983). Reproductive cycles of crabs tend to vary inversely with environmental temperature. Crabs living in warm water (hence, tropical or shallow) tend towards cycles of weeks or months; for example the blue crab, *Callinectes sapidus*, may produce three to eight broods of eggs per year (Hines *et al.*, 2003). Species living in temperate water (or on continental shelves), such as Jonah crabs, *Cancer borealis* and snow crabs, *Chionoecetes opilio* tend towards cycles of a year (Olsen, 2018; Watson, 1969, as cited in Sainte-Marie, 1993). In contrast, crabs living at temperatures < 2 °C (or in the deep ocean) such as snow crabs in eastern Canada (Kuhn & Choi, 2011) and golden king crabs (Paul & Paul, 2001) tend to have biennial or aseasonal reproduction. Biennial reproductive cycles are common among crabs living at extremely low temperatures (e.g. Gulf of St. Lawrence snow crabs living at < 1.0 °C), which have only

two broods over their reproductive lifespan (Moriyasu & Lanteigne, 1998; Comeau *et al.*, 1999), whereas snow crabs living at higher temperatures reproduce annually, potentially producing 4 lifetime broods (Kuhn & Choi, 2011).

Deep-sea crabs in the family Geryonidae are commonly found on the continental slopes and some have sustained valuable fisheries for a long time (Hines, 1990; Hastie, 1995; Masello & Defeo, 2016). Red deep-sea crabs (RDSC), *Chaceon quinquedens* Smith, 1879, are found along the continental shelf and slope of the western Atlantic, ranging from Nova Scotia to the Gulf of Mexico in water depths of 200–1800 m and water temperatures of 5–8 °C (Haefner & Musick, 1974; Wigley *et al.*, 1975; Steimle *et al.*, 2001). The RDSC have been harvested for over 40 years in the Mid-Atlantic Bight of the US east coast. The crab fisheries target only males which raises concerns about their impact on crab reproduction (Weinberg & Keith, 2003). Masello & Defeo (2016) suggested that larger reproductive female crabs may have difficulty finding partners of appropriate size, due to selective harvesting of large males. Sainte-Marie (1993) suggested that primiparous and multiparous female snow crab, *Chionoecetes opilio,* may produce genetically different progeny due to the differences in the size of males that mate with them.

Information about the reproductive biology of female RDSC is scarce; few studies about red crab biology and reproduction have been conducted in the past 30 years. Previous studies have indicated that RDSC may reach a maximum size of 150–180 mm carapace width and live for 15 years or more (Wigley *et al.*, 1975; Gerrior, 1981; Serchuk & Wigley, 1982). Ovarian maturation for *C. quinquedens* using histological analysis was described by Haefner (1977) and Martínez-Rivera & Stevens (manuscript in preparation

1). Stages of ovarian development were classified as: 1) immature, 2) early maturing, 3) advanced, 4) mature and 5) redeveloping, based on oogenesis and color and size of the ovary (Martínez-Rivera & Stevens, manuscript in preparation 1). Haefner (1977) determined that females become sexually mature within the intermolt size of 65-75 mm carapace length (CL). Stevens & Guida (2016) reported that estimation of size at maturity using abdomen allometry was unsuccessful, but morphological maturity was estimated at 61.6 mm CL. Lastly, Martínez-Rivera & Stevens (manuscript in preparation 1) estimated size at morphological maturity at 53.8–65.5 mm CL, and physiological maturity at 63.6–74.8 mm CL. Mating behavior for *C. quinqedens* is considered typical of most brachyurans (Elner *et al.*, 1987; Hastie, 1995). Ovigerous females have been collected in all seasons and found primarily at sizes between 71-113 mm CL (Haefner, 1978). Martínez-Rivera & Stevens (manuscript in preparation 2) estimated fecundity of RDSC to range from 34,691 to 324,729 number of eggs, which is greater than Hines' (1988) estimate. Embryo development has not been described for RDSC but several studies reported the correlation between stage and egg mass color (Wigley *et al.*, 1975; Haefner, 1978; Martínez-Rivera & Stevens, manuscript in preparation 2). Six stages of embryo development were identified based on color of egg mass and observations of embryonic development: 1) new, 2) early development, 3) eyed, 4) prehatching, 5) hatching and 6) empty cases (Martínez-Rivera & Stevens, manuscript in preparation 2). Females carry eggs attached to the abdomen for approximately 9 months (Erdman *et al.*, 1991). No definite seasonal fluctuations in reproductive patterns have been observed for RDSC (Wigley *et al.*, 1975; Haefner, 1978; Hastie, 1995). However, Wigley *et al.* (1975) reported that oviposition extends at least from late winter through early summer and is

influenced by water depth and/or water temperature. Haefner (1978) reported a peak incidence of ovigerous females with recently extruded eggs and with late stage eggs in November. Gerrior (1981) suggested hatching occurred between July and October, based on the ratio of ovigerous to non-ovigerous females. Although seasonality of the events varied in the previous studies, the occurrence of ovigerous females with broods at different embryo development stages support the prolonged egg incubation period of RDSC. The combination of slow-growth, late maturation, long intermolt period of females, and the assumption that fertilization only occurs at molting suggests that red crabs may not reproduce annually, although sperm storage for intermolt fertilization without mating may be occurring (Hines, 1982; Hastie, 1995; Lux *et al.*, 1982; Van Huekelem *et al.*, 1983; Erdman *et al.*, 1991; Biesiot and Perry, 1995; Steimle *et al.*, 2001). The presence of multi-year reproductive cycles among crabs of the Family Geryonidae, which live in water temperatures above 1.0 °C (Hines, 1990; Hastie, 1995), is unusual, and raises questions about the adaptive value of this reproductive strategy.Biennial reproductive cycles have been suggested for some geryonid crabs, including *C. maritae, C. ramosae* and *C. affinis* (Meville-Smith, 1987; Pezzuto & Sant'Ana, 2009; López-Abellán *et al.*, 2002). In contrast, few studies indicated that *C. fenneri* from the southeastern coast of Florida and *C. quinquedens* from the Gulf of Mexico have an annual reproductive cycle (Erdman & Blake, 1988; Erdman *et al.*, 1990) and several studies have proposed a continuous reproductive cycle for *C. quinquedens* (Haefner 1977, 1978; Hines, 1988). A necessary step for improving management of the RDSC is to obtain a better understanding of female reproductive biology, and its adaptive value. In the present study, we examined the reproductive cycle of *C. quinquedens* by

examining seasonal changes in ovarian maturation and embryo development, the ratio of ovigerous to non-ovigerous females, and exoskeleton condition of female crabs.

MATERIALS AND METHODS

The RDSC were collected in the Mid-Atlantic Bight in January of 2011 and 2012, and in July of 2013 aboard NOAA research vessels at depths >250 m by trawling (Stevens & Guida, 2016). Samples from July and September of 2014, and July of 2015 were collected near the Norfolk Canyon at depths >600 m aboard commercial vessels using traps, in collaboration with The Atlantic Red Crab Company. In August of 2016 crabs were collected along the Baltimore Canyon at depths >600 m by traps. Samples collected bimonthly in 2016 were obtained at Newport News and Hampton, VA from vessels that were fishing along the Norfolk Canyon at depths >600 m with traps. Crabs were sexed, and measured for carapace length (CL, from the rostral teeth to the center of the edge of the carapace), carapace width (CW, including lateral spines), and abdomen width (AW, 3rd segment). We used CL instead of CW as our standard dimension, because it is a more accurate measurement of size than CW, which includes spines that wear down over time (Stevens & Guida, 2016). Gross morphology was recorded including exoskeleton (shell) condition. Exoskeleton condition of females was classified as soft, new, hard or old, based on the degree of coloration, shell degradation, and biofouling, as described by Jadamec *et al.* (1999) (Table 4.1). The ovarian development stages (OS) used were OS1 (immature), OS2 (early maturing), OS3 (advanced), OS4 (mature) and OS5 (redeveloping) (Martínez-Rivera & Stevens, manuscript in preparation 1) (Table 4.2). The embryonic development stages (ES) used were ES0 (absent), ES1 (new), ES2 (early development), ES3 (eyed), ES4 (prehatching), ES5 (hatching) and ES6

(empty egg cases) (Martínez-Rivera & Stevens, manuscript in preparation 2) (Table 4.2). Females were classified as non-ovigerous or ovigerous based on the presence of external eggs attached to the pleopods on the abdomen. Timing of reproduction was determined by describing the stages of embryo and ovarian development in ovigerous females, occurrence of ovigerous and non-ovigerous females, and shell condition of females across collections. Descriptive statistics are reported as mean and standard error (mean ± SE).

RESULTS

Ovary maturation and brood development

A total of 176 ovigerous females were examined to compare stages of ovarian development and stages of embryo development (Fig. 4.1). Females with ES1 broods (*n*=8) had ovaries in OS2 (63%), OS3 (13%) and OS5 (25%). Only one female had OS4 ovary, which was small and bright orange. Females with ES2 broods (*N* = 77) had ovaries in OS2 (68%), OS3 (1%), OS4 (1%) and OS5 (30%), and ES3 brood females (*N* = 32) had OS2 (47%), OS3 (9%), OS4 (6%) and OS5 (38%). Females with external eggs at early development stages had early developing or redeveloping ovaries. However, we observed a change in the ovarian development in ES4 to ES6 females. Most females with ES4 broods (*N* = 35) had OS3 (37%) and OS4 (54%) ovaries. Likewise, females with ES5 broods (*N* = 21) had ovaries at OS3 (29%) and OS4 (57%). Lastly, all females with ES6 broods had OS4 ovary, but the sample size was small (*N* = 3). Results suggest that the ovary is maturing while females are carrying eggs.

In order to examine seasonal changes, females were separated into groups (I–VI) depending on stages of ovary development and embryonic development (Table 4.3; Fig. 4.2). Non-ovigerous females (ES0) were classified in Group I, if they had immature or redeveloping ovaries (OS1, OS2 and OS5), or Group II, if they had mature ovaries (OS3 and OS4). Ovigerous females with early embryonic development broods (ES1–ES3) were classified as Group III if they had immature or redeveloping ovaries, or Group IV if they and mature ovaries. Finally, oigerous females with late embryonic development broods (ES4 –ES6) were classified in Group V, if they had immature or redeveloping ovaries, or Group VI, if they had mature ovaries. In winter we observed mostly females in Group I (43%) and II (39%), and some Group III, IV and VI (late winter). Spring was dominated by Group VI (83%) and some Group II. In summer all groups were present, Group II (32%) prevailed, and Group V (2%) crabs were rare. In fall Group III predominated (48%), but Group I, II and IV were also present. Groups II and VI were common in spring during the start of the breeding season and hatching period, respectively. Likewise, Group VI females in late winter had prehatching broods and intermediate ovaries, which were developing and maturing prior to the hatching period and breeding season. Group VI females found in summer were nearing the end of the hatching period and breeding season. The presence of Group III females in summer marked the start of the oviposition season that continues in fall and winter, when the ovaries are redeveloping. Females in Group V (i.e. brood at late embryonic development and immature or redeveloping ovaries) suggest that they can only produce one brood during the breeding season occurring every 2 years.

Occurrence of ovigerous and non-ovigerous females

A total of 672 females were examined for the presence of external eggs. Ovigerous females (*N* = 399) ranged from 59.7–123.4 mm CL (mean 85.2 ± 0.4 mm CL) and non-ovigerous females (*N* = 273) ranged from 65.7–110.9 mm CL (mean 85.2 ± 0.6 mm CL) (Fig. 4.3). In winter, 50% of females were ovigerous (*N* = 101). In spring, 90% of females were ovigerous but this is due to low sampling size (*N* = 39) and only 4 females were non-ovigerous. In summer and fall, 61% and 53% of females were ovigerous, respectively. The majority of ovigerous females (65%) were found in summer, but this season also had the highest sampling size (*N* = 259). However, ovigerous females were observed in all seasons. Including all females throughout the year, 59% were ovigerous and 41% were non-ovigerous. The ratio of non-ovigerous to ovigerous females was 1:1.5 across all seasons and sizes, and strongly suggests that females produce a single brood every 2 years.

Shell condition

A total of 754 females were examined for exoskeleton condition analysis, ranging in size from 34.6 to 123.4 mm CL (mean 82.2 ± 0.5 mm CL) (Fig. 4.4). We found soft-shell females (36.0–100.7 mm CL, mean 75.3 ± 1.6 mm CL) and new-shell females (34.6–123.4 mm CL, mean 73.4 ± 1.4 mm CL) that were larger than the size at sexual maturity (Martínez-Rivera & Stevens, manuscript in preparation 1), indicating that *C. quinquedens* undergoes ecdysis after sexual maturity, i.e. there was no evidence of terminal molt. Females between 50.9 and 110.7 mm CL (mean 82.9 ± 0.6 mm CL) had hard shells with dirty exoskeletons. Similarly, old-shell females occurred in the size range

55.5–110.9 mm CL (mean 89.3 ± 7.9 mm CL). The increase in body size of females with hard and old exoskeletons suggests that the occurrence of ecdysis decreases and the intermolt period increases in larger females.

Females with soft shells, found soon after ecdysis, were captured in the summer (94%) at 600–750 m and some were caught in the winter (6%) at ~300 m (Fig. 4.5). New, clean-shell females were observed across all seasons: winter (34%, at ~400–800 m), spring (1%, at 600–750 m), summer (47%, at ~300–750 m) and fall (18%, at 600–750 m). Females with hard shells covered with black spots occur year-round: winter (26%, ~400–800 m), spring (7%, 600–750 m), summer (55%, ~300–800 m) and fall (13%, 600–750 m). Lastly, old-shell females also were found in all seasons: winter (12%, 500–750 m), spring (7%, 600–750 m), summer (64%, 600–750 m) and fall (18%, 600–750 m). Females with hard to very old exoskeleton condition were found throughout the year suggesting the intermolt period is extensive.

Females were analyzed for embryonic development and exoskeleton condition (Fig. 4.6). As expected, all females in soft-shell condition were non-ovigerous (ES0). New, clean-shell ovigerous females presented brood masses from ES1 to ES5. Ovigerous females with hard and old shells had ES1 to ES6 broods. The presence of old-shell ovigerous females with early-stage eggs indicates that female red crabs can mate in the hard-shell condition. It is important to mention that non-ovigerous females were found with all the exoskeleton conditions suggesting a prolonged period time without carrying eggs. Our findings provide evidence that female *C. quinquedens* do not have a terminal molt, that intermolt period increases with body size, and that hard-shell mating occurs.

Biennial reproductive cycle

We propose that female *C. quinquedens* have a biennial reproductive cycle (Fig. 4.7) based on the timing of ovarian and embryonic development, occurrence of ovigerous and non-ovigerous females, and exoskeleton conditions. The cycle starts in the summer with immature females with OS1 ovaries, which only occur once in their life-span. During the first year, females are barren and the ovary undergoes development from OS1 through OS4, which consists of oocyte proliferation due to yolk deposition (Martínez-Rivera & Stevens, manuscript in preparation 1). Females reach morphometric maturity at the pubertal molt, becoming pubescent females when they are able to mate but the ovary is not mature to produce the first brood mass. Pubescent females developed their ovaries at the beginning of the second year, in spring and summer. The start of the breeding season occurs during the transition from spring to summer. Oviposition takes place at the end of summer, and early embryonic development lasts until winter. Elner *et al.* (1987) reported that oviposition did not occur immediately following copulation and sperm masses were found in both spermathecae, suggesting that fertilization occurred later. This could explain our observation of females with old shell conditions and ES1 brood masses as well as the occurrence of hard-shell mating. After oviposition, the ovary redevelops from fall through winter as eggs develop from ES2 through ES5. In late winter through spring, mature females have prehatching external eggs (ES4) and an advanced ovary (OS3). Then, from spring to summer, the external eggs start hatching (ES5) and by the end of summer, females will have egg remnants (ES6). Erdman *et al.* (1991), reported that *C. quinquedens* females carry the egg mass for approximately 9 months, which agrees with our observations. The observed proportions of ovigerous and non-ovigerous

females, and the fact that approximately 50% of females carry eggs simultaneously, implies that, after the larvae hatch, females with advanced (OS3) or mature (OS4) ovaries will not produce another clutch until the next year. Therefore, females do not extrude eggs annually. Examination of the stages of oocyte development by Martínez-Rivera & Stevens (manuscript in preparation 1) demonstrated that it takes 12 months for the ovary to fully mature from OS2 to OS4. The biennial reproductive cycle we proposed for *C. quinquedenes* can be summarized as 1 year of ovarian development followed by 1 year of embryonic development.

DISCUSSION

This study provided compelling evidence that female *C. quinquedens* have a biennial reproductive cycle and allowed us to create the first conceptual model of the reproductive cycle for RDSC (Fig. 4.7). The results are consistent with previous studies of RDSC reproduction (Wigley *et al.* 1975; Haefner, 1977, 1978; Stevens & Guida, 2016). Based on the proportion of ovigerous females, Wigley *et al.* (1975) suggested that females have a prolonged breeding season. Haefner (1977, 1978) suggested a continuous reproductive cycle using ovary maturation stages and the presence of ovigerous females. Stevens & Guida (2016) reported that <50% of mature females in their samples were ovigerous, providing a strong indicator of biennial reproduction. This study adds significant details to the findings of Stevens & Guida (2016). In contrast, Erdman *et al.* (1990) stated that *C. fenneri*, from southeastern Florida and the eastern Gulf of Mexico, and *C. quinquedens*, from the Gulf of Mexico, exhibited an annual reproductive pattern, although the reproduction cycle of *C. quinquedens* was more protracted than that of *C. fenneri*. The combination of biennial reproduction and planktotrophic larvae in crabs

living at warm temperatures >6ºC seems unusual (Stevens & Guida, 2016). Therefore, this reproductive strategy for RDSC may be the result of adaptation to aspects of their environment other than temperature alone. In the absence of environmental temperature changes, reproductive patterns of deep-sea species are expected to be continuous (i.e. no seasonality) (Erdman *et al.*, 1990). However, we observed seasonality in oviposition and hatching of *C. quinquedens*. The reproductive cycle of a species is a result of adaptations required to survive in a particular environment (Sastry, 1983; Hilário & Cunha, 2013). Therefore, if temperature is not a major regulator of reproduction then other external factors like quality and quantity of the food available and resource competition may have an impact on reproductive pattern (Sastry, 1983). This may explain seasonality and biennial reproductive cycles in *Chaceon* crabs including *C. quinquedens*, *C. ramosae* and *C. affinis* (Pezzuto & Sant'Ana, 2009; López-Abellán *et al.*, 2002; Biscoito *et al.*, 2015).

The presence of ovigerous females year-round and ovigerous females with ovaries at early development suggest a lengthy period of time between oviposition and hatching of the larvae. Our observations showed that oviposition occurs in summer and potentially through fall, and hatching occurs from spring to summer. Wigley *et al.* (1975) reported that oviposition ranged from late winter to early summer. Haefner (1977, 1978) stated that the peak of ovigerous females with late stage eggs and recently extruded eggs was in November. In November, we observed females with broods at early embryonic development (ES2 and ES3) but did not observe any in late embryonic development stages. Stevens & Guida (2016) found 80% of females in July 2013 carried eggs at prehatching (ES4) or hatching (ES5) stages, and only 20% were in early embryonic development. However, we found 26% of females with eggs at prehatching (ES4) or

hatching (ES5) stages in summer (July and August), 69% of crabs with eggs at early embryonic development, and 5% of females with egg remnants (ES6). These differences could be attributed to differences in water depths of the locations sampled, as most of our samples are from commercial grounds >600 m. Similar to our findings, Wigley *et al.* (1975) found *C. quinquedens* in deep waters off the southern New England shelf had a relatively large proportion of ovigerous females with newly deposited eggs and none with late development eggs. Likewise, Haefner (1978) found females with external eggs in early development within the 401–700 m depth range, and only crabs with late development eggs were present in the 201–300 m depth range. Females with egg remnants were more prevalent in shallower water ranging from 201 to 400 m (Haefner, 1978), potentially explaining the lack of females with egg remnants in our samples. Hence, there is evidence that *C. quinquedens* ovigerous females exhibit a vertical up-slope migration pattern, in which females mate and produce clutches in deep water, then migrate to shallow water as their eggs develop, and finally hatch at the shallowest end of their depth range. Hilário & Cunha (2013) suggested a migration to deep water related to mating for *C. affinis* in the Gorringe Bank as well as for other geryonid crabs (Hastie, 1995). This pattern could be a mechanism whereby crabs utilize warmer water to enhance embryonic development, hatching and upward larval vertical migration (Haefner, 1978; Kelly *et al*. 1982). Warmer temperature in shallower water decreases the development period of embryos and larvae (Haefner, 1978; Wigley *et al.*, 1975; Kelly *et al.*, 1982). Additionally, previous studies suggested that females inhabiting shallow water may be carried to deeper water by males during copulation (Lindberg and Lockhart, 1988, 1993, as cited by Hastie, 1995). Lastly, the simultaneous presence of ovigerous females with

broods at early embryonic development and those carrying late development eggs support the indication made by Stevens & Guida (2016) that hatching and oviposition are separated by a protracted period of time, potentially a year in RDSC. This prolonged embryonic development period and the biennial reproductive pattern of *C. quinquedens* may reflect the greater geographical range and deep slope distribution of this species (Erdman & Blake, 1988). Some species may compensate for the low frequency of reproduction by other adaptations such as relatively large eggs and relatively long life spans (Somerton & MacIntosh, 1985), which are observed in *C. quinquedens* (Hines, 1988; Wigley *et al.*, 1975; Gerrior, 1981; Serchuk & Wigley, 1982). Furthermore, species with low reproductive frequency may have an accessory reproductive activity, such as the vertical migration patter of *C. quinquedens* (Somerton & MacIntosh, 1985). Such low frequency reproduction makes *C. quinquedens* vulnerable to overfishing, hence it is important to understand the impact of fishing practices on the population. Sex ratios reported by Stevens & Guida (2016) indicated that male mating partners for females became scarce as females grew. Weinberg & Keith (2008) detected a reduction over time in the proportion and body weight of large male *C. quinquedens* off southern New England, indicating that smaller males may have to mate with females. Observations made in situ indicate that males in mating pairs are approximately 50% larger than females (Wahle *et al.*, 2008). Additionally, Comeau *et al.*, (1999) suggested that fishing may lead to fewer mature males becoming available for mating, and therefore multiparous females would have to rely on old stored sperm in their spermathecae for egg fertilization. Similarly, Sainte-Marie (1993) indicated that, due to the lack of suitable male mates, large primiparous females may produce nonviable broods and multiparous

females may use stored sperm acquired from small male mates. Masello & Defeo (2016) reported a decrease in individual weight indicating that fishing negatively affected the population structure of *C. notialis*. Hence, the reduction of the number of mature males by the fishery can reduce the fecundity of females and increase egg mortality (Comeau *et al.*, 1999).

Shell conditions varied greatly across seasons and body size. Females carrying eggs at early embryonic stages were in both hard and old shell condition suggesting hard-shell mating, although Elner *et al.* (1987) did not describe it. Hilário & Cunha (2013) reported hard-shell mating for *C. affinis* in the Gorringe Bank by video survey. The largest female with a soft shell was 100.66 mm CL and the largest crab with a new clean shell was 123.4 mm CL. Haefner (1978) suggested that females continue to molt until they reach at least 116.0 mm CL. Female *C. notialis* in Uruguayan waters, that were larger than the size at maturity, exhibited signs of recent molting (Delgado & Defeo, 2004). Previous studies have reported molting occurring in crabs after reaching the size at maturity and increasing intermolt periods up to 3–7 years (Lux *et al.*, 1982; Melville-Smith, 1989; Hastie, 1995; Pinho *et al*., 2001; Pezzuto & Sant'Ana, 2009). We propose that the pubertal molt of *C. quinquedens* females is not a terminal molt, and continuous molting is typical of other geryonid crabs as well.

Sastry (1983) indicated that populations of a species living at different latitudes and depths may have distinctive reproductive cycles. Variations in size at sexual maturity and time of occurrence of ovigerous females among two populations of *C. affinis* at different latitudes have been reported (Biscoito *et al.*, 2015). Martínez-Rivera & Stevens (manuscript in preparation 1) observed a decrease in morphological size at sexual

maturity with latitude for *C. quinquedens*. Haefner (1977) did not find any crab <70 mm CL with external eggs in Norfolk Canyon, but we observed ovigerous females less than 59 mm CL in Hudson Canyon. Research conducted in the eastern Gulf of Mexico suggested that differences in the reproductive cycles between *C. fenneri* and *C. quinquedens* were due to bathymetrical segregation (Erdman *et al.*, 1990). Differences in the reproductive cycles of *C. quinquedens* populations due to bathymetrical and geographical location are unknown and should be studied.

We concluded that female *C. quinquedens* in the Mid-Atlantic Bight have a biennial reproductive cycle, as suggested for *C. fenneri* and *C. affinis* (Erdman & Blake, 1988; Hilário & Cunha, 2013). The presence of biennial reproduction in a shelf-slope crab living at moderate temperatures is not only unusual, but makes them vulnerable to climate change and fishing impacts. Furthermore, the frequency of ovigerous females is not useful to determine if sufficient males for reproduction remain available in population that exhibit a biennial reproductive cycle (Somerton & McIntosh, 1985). The implications of this study for fisheries management include extremely useful information to improve strategies to optimize the reproductive potential of *C. quinquedens*. We recommend that future research should focus on elucidating information about *C. quinquedens* reproductive strategy in order to conserve the fishery resource.

ACKNOWLEDGEMENTS

Special thanks to The Atlantic Red Crab Company, Shadaesha Green, Dr. Indu Sharma and Justin Wilson for assisting with the sample collection. This research was funded by NOAA grant NA11SEC4810002 and grant NA16SEC4810007.

REFERENCES

Biesiot, P. M., and H. M. Perry. 1995. Biochemical composition of the deep-sea red crab *Chaceon quinquedens* (Geryonidae): organic reserves of developing embryos and adults. Marine Biology. 124:407–416.

Biscoito, M., M. Freitas, J.G. Pajuelo, R. Triay-Portella, J.I. Santana, A.L. Costa, J. Delgado, and J.A. González. 2015. Sex structure, depth distribution, intermoult period and reproductive pattern of the deep-sea red crab *Chaceon affinis* (Brachyura, Geryonidae) in two populations in the north-eastern Atlantic. Deep-Sea Research I. 95:99–114.

Comeau, M., M. Starr, G. Y. Conan, G. Robichaud, and J. C. Therriault. 1999. Fecundity and duration of egg incubation for multiparous female snow crabs (*Chionoecetes opilio*) in the fjord of Bonne Bay, Newfoundland. Canadian Journal of Fisheries and Aquatic Science. 56(6):1088–1095.

Delgado, E. and O. Defeo. 2004. Sexual maturity in females of deep-sea red crab *Chaceon notialis* (Brachyura, Geryonidae) in the southwestern Atlantic Ocean. Invertebrate Reproduction and Development. 46(1):55–62.

Elner, R. W., S. Koshio, and G. V. Hurley. 1987. Mating behavior of the deep-sea red crab, *Geryon quinquedens* Smith (Decapod, Brachyura, Geryonidae). Crustaceana. 52:194–201.

Erdman, R. B., and N. J. Blake. 1988. Reproductive biology of female golden crabs *Geryon fenneri* Manning and Holthuis, from southeastern Florida. Journal of Crustacean Biology. 8:392–400.

Erdman, R. B., N. J. Blake, H. M. Perry, R. S. Waller, W. J. Lindberg, and F. D. Lockhart. 1990. Annual reproduction in deep-sea brachyuran crabs (*Chaceon spp.*) from the southeastern United Stated. In Geryonid crabs and associated continental slope fauna: a research workshop report. W. J. Lindberg and E. L. Wenner (eds.), 14-15, Technical Report 58, Florida Sea Grant College.

Erdman, R. B., N. J. Blake, F. D. Lockhart, W. J. Lindberg, H. M. Perry, and R. S. Waller. 1991. Comparative reproduction of the deep-sea crabs *Chaceon fenneri* and *C. quinquedens* (Brachyura: Geryonidae) from the northeast Gulf of Mexico. Invertebrate Reproduction and Development. 19:175–184.

Gerrior, P. 1981. The distribution and effects of fishing on the deep sea red crab, *Geryon quinquedens* Smith, off southern New England. M.S. thesis, Southeastern Massachusetts Univ., North Dartmouth, MA. 130 p.

Haefner, Jr., P. A. 1977. Reproductive biology of the female deep-sea red crab, *Geryon quinquedens*, from the Chesapeake Bight. Fisheries Bulletin. 75:91–102.

Haefner, Jr., P. A. 1978. Seasonal aspects of the biology, distribution and relative abundance of the deep-sea red crab *Geryon quinquedens* Smith, in the vicinity of the Norfolk Canyon, Western North Atlantic. Proceedings of the National Shellfisheries Association. 68:49–62.

Hastie, L. C. 1995. Deep-water geryonid crabs: a continental slope resource. In A. D. Ansell, R. N. Gibson, and M. Barnes eds. p. 561-584. Oceanography and Marine Biology: an Annual Review. 33. 665 p.

Hines, A. H. 1982. Allometric constraints and variable of reproductive effort in brachyuran crabs (Crustacea, Decapoda, Brachyura). Marine Biology. 69:309–320.

Hines, A. H. 1988. Fecundity and reproductive output in two species of deep-sea crabs, *Geryon fenneri* and *G. quinquedens* (Decapoda: Brachyura). Journal of Crustacean Biology. 8(4):557–562.

Hines, A. H. 1990. Commentary on life history and ecology of deep-sea crabs of the family Geryonidae. In Geryonid crabs and associated continental slope fauna: a research workshop report. W. J. Lindberg and E. L. Wenner (eds.), 30-28, Technical Report 58, Florida Sea Grant College.

Hines, A. H., P. R. Jivoff, P. J. Bushmann, J. van Montfrans, S. A. Reed, D. L. Wolcott, and T. G. Wolcott. 2003. Evidence for sperm limitation in the blue crab, *Callinectes sapidus*. Bulletin of Marine Science. 72(2):287–310.

Hinsch, G. W. 1988. Morphology of the reproductive tract and seasonality of reproduction in the golden crab *Geryon fenneri* from the Eastern Gulf of Mexico. Journal of Crustacean Biology. 8(2):254–261.

Kelly, P., S. D. Sulkin, and W. F. Van Heukelem. 1982. A dispersal model for larvae of the deep sea red crab *Geryon quinquedens* based on behavioral regulation of vertical migration in the hatching stage. Marine Biology. 72:35–43.

Kuhn, P. S., and J. S. Choi. 2011. Influence of temperature on embryo developmental cycles and mortality of female *Chionoecetes opilio* (snow crab) on the Scotian Shelf, Canada. Fisheries Research. 107(1–3):245–252.

Lindberg, W. J., and F. D. Lockhart. 1988. Fisheries development for deep-sea golden crabs (*Geryon fenneri*): Direct measurements for stock size and population structure. Final Project Report R/Lr-B-17, Florida Sea Grant College Program.

Lindberg, W. J., and F. D. Lockhart. 1993. Depth-stratified population structure of geryonid crabsin the eastern Gulf of Mexico. Journal of Crustacean Biology. 13(4):713–722.

Lopez-Abellan, L. J., E. Balguerias, and V. Fernandez-Vergaz. 2002. Life history characteristics of the deep-sea crab *Chaceon affinis* population off Tenerife (Canary Islands). Fisheries Research. 58(2):231–239.

Lux, F. E., A. R. Ganz, and W. F. Rathjen. 1982. Marking studies on the red crab *Geryon quinquedens* Smith off southern New England. Journal of Shellfish Research. 2(1):71–80.

Masello, A., and O. Defeo. 2016. The deep-sea red crab *Chaceon notialis* (Geryonidae) in the southwestern Atlantic Ocean: Spatial patterns and long-term effects of fishing. Fisheries Research. 183:254–262.

Martínez-Rivera, S. and B. G. Stevens. Manuscript in preparation 1. Ovarian development, oogenesis and size at sexual maturity of the female red deep-sea crab, *Chaceon quinquedens* Smith, 1879, in the Mid-Atlantic Bight.

Martínez-Rivera, S. and B. G. Stevens. Manuscript in preparation 2. Fecundity of red deep-sea crab, *Chaceon quinquedens* Smith, 1879, in the Mid-Atlantic Bight.

Melville-Smith, R. 1987. The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off South WestAfrica/Namibia. Crustaceana. 53(3):259–275.

Moriyasu, M., and C. Lanteigne. 1998. Embryo development and reproductive cycle in the snow crab, *Chionoecetes opilio* (Crustacea: Majidae), in the southern Gulf of St. Lawrence, Canada. Canadian Journal of Zoology. 76(11):2040–2048.

Negreiros-Fransozo, M. L., A. Fransozo, and G. Bertini. 2002. Reproductive cycle and recruitment period of *Ocypode quadrata* (Decapoda, Ocypodidae) at a sandy beach in southeastern Brazil. Journal of Crustacean Biology. 22(1):157–161.

Olsen, N. A. 2018. Reproductive biology and size at sexual maturity of Jonah crabs (*Cancer borealis*) in the Mid-Atlantic Bight. M.S. Thesis. University of Maryland Eastern Shore.

Paul, A. J. and J. M. Paul. 2001. The reproductive cycle of golden king crab *Lithodes aequispinus* (Anomura: Lithodidae). Journal of Shellfish Research. 20(1):369–371.

Pezzuto, P. R. and R. Sant'Ana. 2009. Sexual maturity of the deep-sea royal crab *Chaceon ramosae* Manning, Tavares & Albuquerque, 1989 (Brachyura: Geryonidae) in southern Brazil Latin American Journal of Aquatic Research. 37(3).

Pinheiro M. A. A., and A. Fransozo. 2002. Reproduction of the speckled swimming crab *Arenaeus cribrarius* (Brachyura: Portunidae) on the Brazilian Coast near 23°30'S. Journal of Crustacean Biology. 22(2):416–28.

Pinho, M. R., J. M. Goncalves, H. R. Martins, and G. M. Menezes. 2001. Some aspects of the biology of the deep-water crab, *Chaceon affinis* (Milne-Edwards and Bouvier, 1894) off the Azores. Fisheries Research. 51(2-3):283–295.

Sainte-Marie, B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, *Chionoecetes opilio*, in the Northwest Gulf of Saint Lawrence. Canadian Journal of Fisheries and Aquatic Sciences. 50:2147–2156.

Sastry, A. N. 1983. Ecological aspects of reproduction. In, W. B. Vernberg, (ed.), The Biology of Crustacea: Environment adaptations. Academic Press Inc., New York. 8:179–270.

Somerton, D. A., and R. A. MacIntosh. 1985. Reproductive biology of the female blue king crab *Paralithodes platypus* near the Pribilof Islands, Alaska. Journal of Crustacean Biology. 5(3):365–376.

Steimle, F. W., C. A. Zetlin, and S. Chang. 2001. Essential Fish Habitat Source Document: red deepsea crab, *Chaceon* (*Geryon*) *quinquedens*, life history and habitat characteristics. NOAA Technical Memorandum. NMFSNE–163, 27 p.

Stevens, B. G., and V. Guida. 2016. Demographics, morphometrics, depth, and temperature distribution of red deepsea crabs *Chaceon quinquedens* at four sites in the Mid-Atlantic Bight. Fisheries Bulletin. 114:343–359.

Tallack, S. M. L. 2007. The reproductive cycle and size at maturity observed in *Cancer pagurus* in the Shetland Islands, Scotland. Journal of the Marine Biological Association of the United Kingdom. 87:1181–1189.

van Heukelem, W., M. C. Christman, C. E. Epifanio, and S. D. Sulkin. 1983. Growth of *Geryon quinquedens* (Brachyura: Geryonidae) juveniles in the laboratory. Fisheries Bulletin. (U.S.) 81:903–905.

Wahle, R. A., C. E. Bergeron, A. S. Chute, L. D. Jacobson, and Y. Chen. 2008. The Northwest Atlantic deep-sea red crab (*Chaceon quinquedens*) population before and after the onset of harvesting. ICES Journal of Marine Science. 65:862–872.

Watson, J. 1969. Biological investigations on the spider crab, *Chionoecetes opilio*. Canadian Fisheries Reports. 13:24–47.

Weinberg, J. R., and C. Keith. 2003. Population size-structure of harvested deep-sea red crabs (*Chaceon quinquedens*) in the northwest Atlantic Ocean. Crustaceana. 76:819–833.

Wigley, R. L., R. B. Theroux, and H. E. Murray. 1975. Deep sea red crab, *Geryon quinquedens*, survey off northeastern United States. Marine Fisheries Review. 37(8):1–21.

Zairion, Z., Y. Wardiatno, and A. Fahrudin. 2015. Sexual maturity, reproductive pattern and spawning female population of the blue swimming crab, *Portunus pelagicus* (Brachyura: Portunidae) in East Lampung coastal waters, Indonesia. Indian Journal of Science and Technology. 8(7):596–607.

Table 4.1. Criteria used for classifying exoskeleton condition of *Chaceon quinquedens* females in the Mid-Atlantic Bight.

Stage	Exoskeleton condition
Soft	Soft, light red color, immaculate, lateral and chela spines intact
New	New, hard, clean, bright red color, lateral and chela spines intact, no epifaunal organisms
Hard	Hard, red color, scratches and black spots in the carapace and legs, carapace with few epifaunal organisms, degradation of the lateral and chela spines
Old	Hard, dark red color, extensive scratches and black spots in the carapace and legs, carapace covered by epifaunal organisms

Table 4.2. Stages of ovarian development and embryo development of *Chaceon quinquedens* females in the Mid-Atlantic Bight (Martínez-Rivera & Stevens, manuscript in preparation 1, 2).

Stage	**Criteria**
Ovarian development	
Stage 1	Immature ovary, small and white
Stage 2	Early maturing ovary, small and beige or yellow
Stage 3	Advanced ovary, medium, and orange or brown
Stage 4	Mature ovary, large, and dark-brown or purple
Stage 5	Redeveloping ovary, small and beige
Embryo development	
Stage 0	No brood
Stage 1	New embryos and bright orange
Stage 2	Early developing embryos and orange
Stage 3	Eyed embryos and red-orange or red-brown
Stage 4	Prehatching embryos and dark-brown to purple
Stage 5	Hatching embryos and
Stage 6	Empty egg cases

Table 4.3. Groups based on the stages of ovary development and embryo development of female *Chaceon quinquedens*.

Group	**Criteria**	
	Ovarian development	Embryo development
I	Immature, early maturing and redeveloping	No brood
II	Advanced and Mature	No brood
III	Immature, early maturing and redeveloping	New embryos, early develop and eyed
IV	Advanced and mature	New embryos, early develop and eyed
V	Immature, early maturing and redeveloping	Prehatching, hatching and empty egg cases
VI	Advanced and mature	Prehatching, hatching and empty egg cases

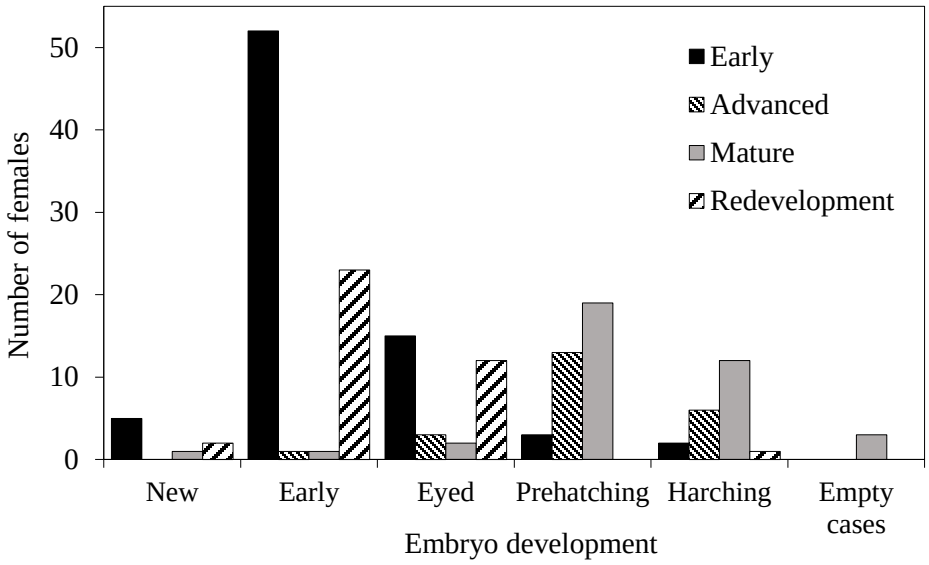

Figure 4.1. Frequency of stages of ovarian development in each stage of embryo development for *Chaceon quinquedens* females.

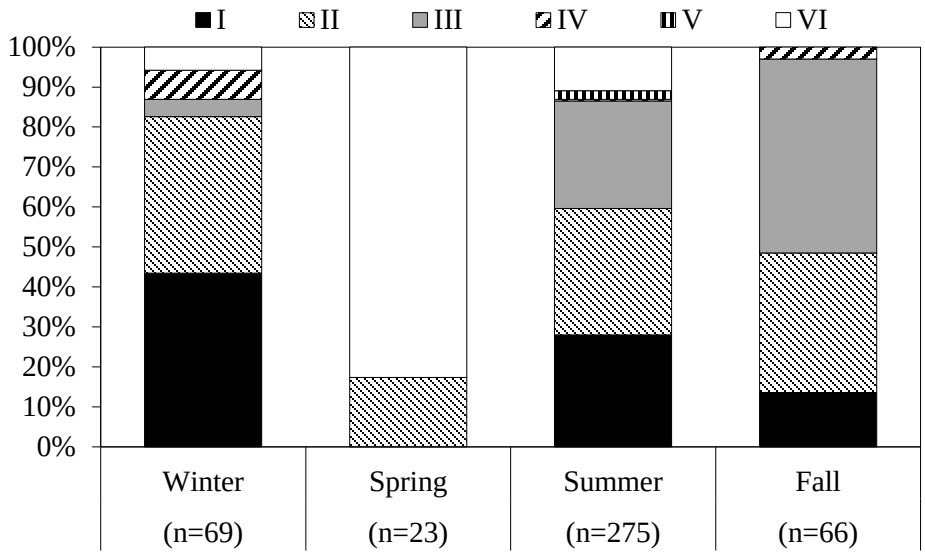

Figure 4.2. Percentages of ovarian and embryo development groups across seasons of *Chaceon quinquedens* in the Mid-Atlantic Bight.

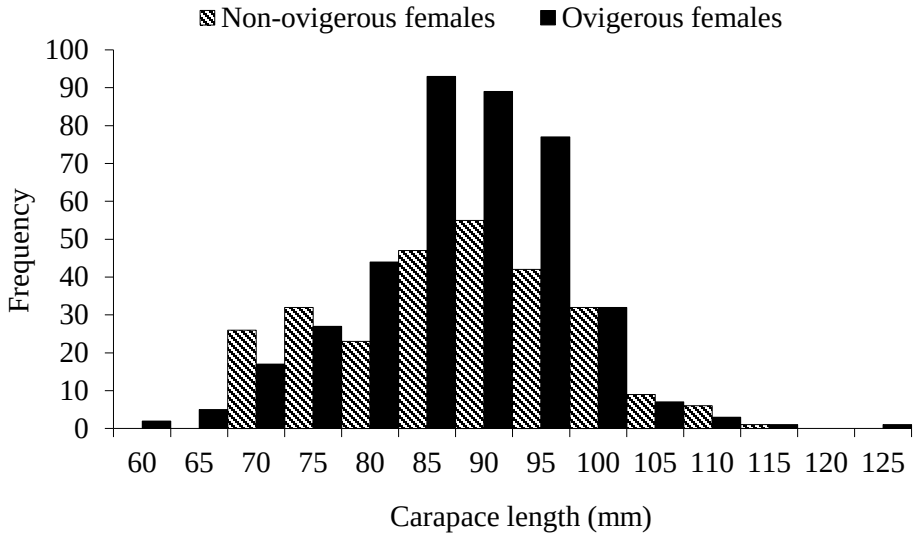

Figure 4.3. Length-frequency distributions of non-ovigerous (lines) and ovigerous (black) female red deep-sea crab, *Chaceon quinquedens*, in the Mid-Atlantic Bight.

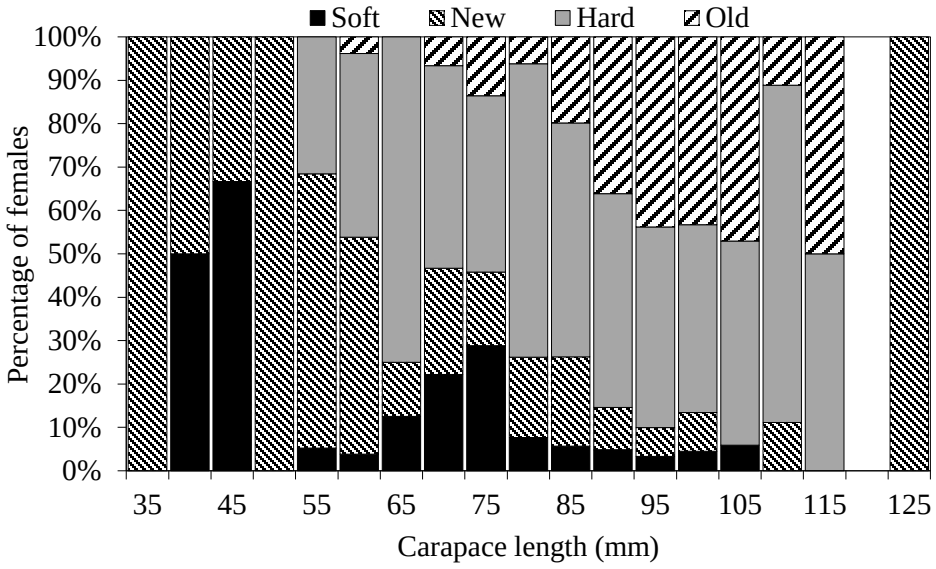

Figure 4.4. Length-frequency distributions of female red deep-sea crab, *Chaceon quinquedens*, across shell condition in the Mid-Atlantic Bight.

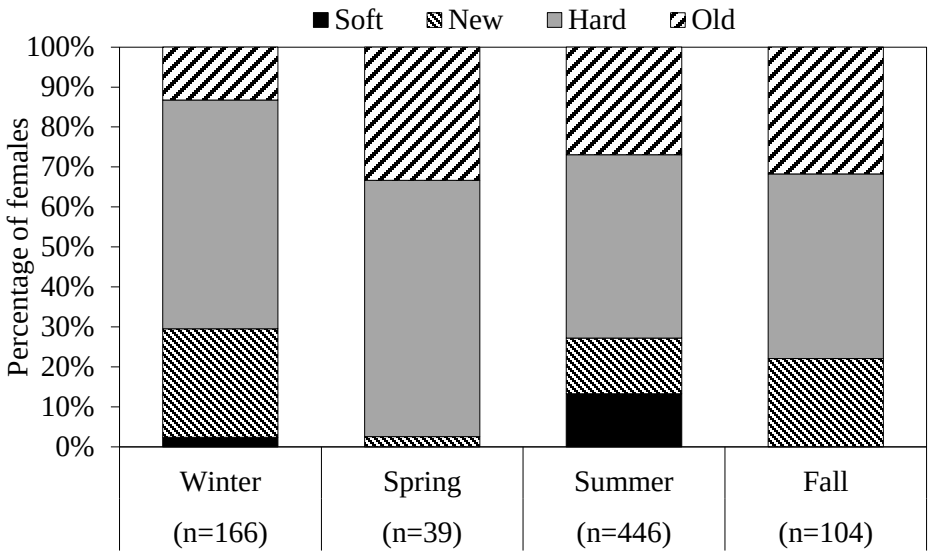

Figure 4.5. Percentages of shell condition across seasons in *Chaceon quinquedens* females.

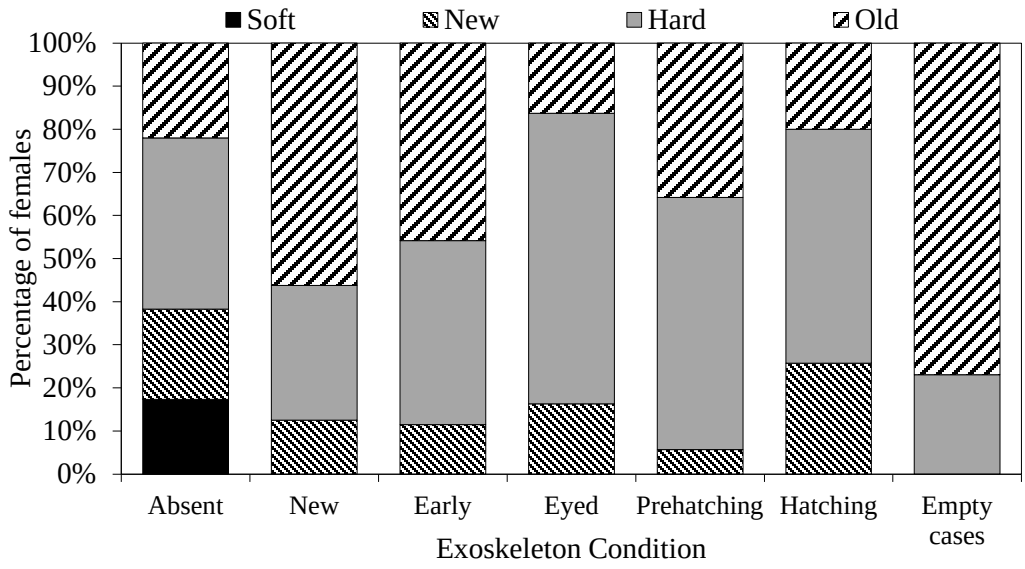

Figure 4.6. Percentages of shell condition of non-ovigerous and ovigerous females with broods at different stages of embryo development in *Chaceon quinquedens*.

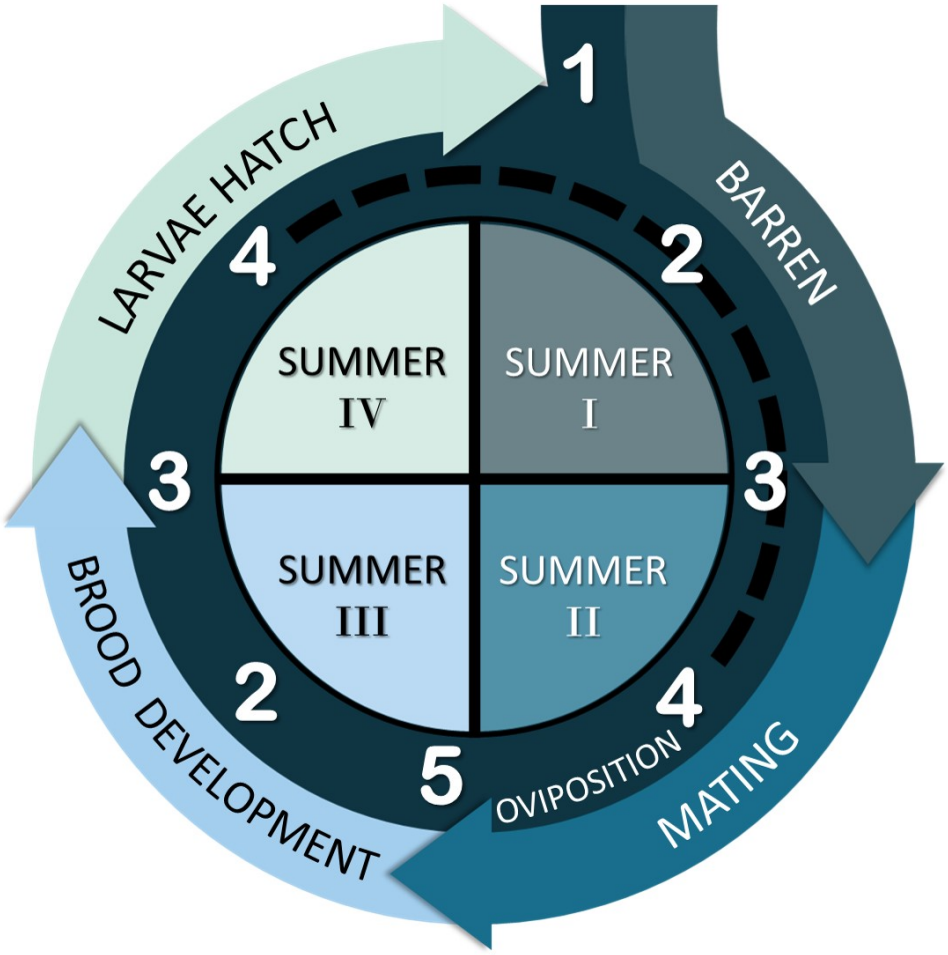

Figure 4.7. Diagram of the biennial reproductive cycle of *Chaceon quinquedens* females in the Mid-Atlantic Bight.

CHAPTER 5: GENERAL CONCLUSION

The primary goal of this research project was to elucidate the reproductive biology of the female *Chaceon quinquedens* in the Mid-Atlantic Bight in order to provide information for fisheries management. Three objectives were achieved, including 1) estimating size at sexual maturity (SM_{50}); 2) determining size-specific fecundity, and 3) defining the reproductive cycle of *C. quinquedens*. The SM_{50} was estimated by examination of the morphological (i.e. vulvae condition) and physiological characters (i.e. oocyte and ovarian development) of reproduction in females. The size-specific fecundity was estimated for ovigerous females with egg masses at early embryo development by an automated imaging method for egg samples. The reproductive cycle of red deep-sea crab (RDSC) was determined by analyzing the relationship between the ovarian maturation stages and embryonic development described in Chapter 2 and Chapter 3, the occurrence of ovigerous and non-ovigerous females, and shell condition across collections.

To determine the stages of oocyte and ovarian development separately, we described the microscopic and macroscopic characteristics of the ovary over time. Examination of ovarian development showed a continuous development of oocytes in the ovary, indicating a consecutive maturation of oocytes in two simultaneously overlapping cohorts that do not start only after the egg extrusion (i.e. oviposition). The non-linear models for the morphological and physiological data indicated asynchrony between morphometric maturity and gonadal maturity. These results suggest that after the pubertal molt (i.e. when they reach morphometric maturity) the pubescent females can mate but may take up to a year to produce their first brood mass. The SM_{50} is generally estimated

as a biological parameter used in fisheries management to establish size limits (Pardo *et al.*, 2009). The best-fit model for the physiological data showed differences in SM_{50} by seasons (i.e. winter/spring, summer and fall) suggesting seasonality of ovarian maturation. The best-fit model for the morphological data showed differences in SM_{50} of females in different geographic locations; specifically, SM_{50} decreases with increasing latitude. Stevens & Guida (2016) reported that females at the southern range of their distribution had a greater mean carapace length (CL), which agrees with our finding of greater SM_{50} among females at that location. This study provided the first evidence of variations in the reproductive biology of RDSC across latitude. From a fisheries management perspective, defining the intraspecific variation in the reproductive biology of RDSC can be an essential element for stock identification and establishing legal size controls.

Our estimates of size-specific fecundity were greater than previously recorded for *C. quinquedens* and had a positive relationship with female body size. We tested the relationship between fecundity and stages of embryo development (i.e. stage 1 & 2 and stage 3), seasons (i.e. winter/spring, summer and fall), geographic location (i.e. Baltimore Canyon and Norfolk Canyon), and shell condition (i.e. new, hard and old). The differences between stages of embryo development were statistically significant suggesting variations in fecundity due to embryo development. We observed that embryo development stage 3 had a steeper regression slope and some large females with egg broods at stage 1 & 2 had unusually small broods suggesting oviposition is a prolonged process. Fecundity differed between seasons, implying seasonality of breeding; this result may imply that smaller crabs extruded earlier than larger crabs, but might also be

partially due to differences in female size-frequency between season collections. Significant differences in fecundity between Baltimore Canyon and Norfolk Canyon may be associated with latitudinal decrease in size at maturity, and provide additional evidence of intraspecific variations in reproductive patterns for *C. quinquedens*. There was no significant relationship between shell condition and fecundity, which provides evidence for continuous molting in RDSC, i.e. the absence of a terminal molt. Stages of embryo development of *C. quinquedens* were correlated with the color of the egg mass. The corroboration of embryo development stages under the microscope indicated differences between embryo early development stages (stages 1–3), stage 4 and stage 5. However, we were not able to describe the stages of embryo development in detail like *C. affinis* (Tuset *et al.*, 2011). A pattern of embryo development proposed for *C. quinquedens* starts with oviposition (i.e. egg extrusion) in the summer, followed by early embryo development until winter, late embryo development between winter and spring, and hatching in spring until summer.

We proposed that female *C. quinquedens* have a biennial reproductive cycle. This conclusion was based on seasonal observations of ovarian and embryonic development, occurrence of ovigerous and non-ovigerous females, and shell condition. The comparisons between ovarian and embryonic development of ovigerous females indicated prolonged (9–12 months) ovarian maturation after egg extrusion. Seasonality of ovarian development was consistent with breeding season. Observation of females with recently extruded eggs and mature ovaries is unusual, and in most cases the ovary exhibited morphological characteristics of an early maturing ovary. Two possible explanations are: not all mature oocytes were extruded, or the piece of the ovary collected

for each female did not represent the entire ovary. Individual ovaries with more than one color were occasionally observed. Consequently, the ovary and egg brood analysis suggested that *C. quinquedens* reproduction is highly complex. The ratio of non-ovigerous to ovigerous females was 1:1.5 across all seasons and sizes. Large females without egg masses were observed throughout the year implying that individual females produce an egg brood every 2 years. The examination of female shell condition indicated females do not have a terminal molt and hard-shell mating may occur in *C. quinquedens*. We presented the first conceptual diagram of the biennial reproductive cycles for female *C. quinquedens*. Understanding the female reproductive cycle of a species is fundamental for developing improved management strategies. Hines (1990) emphasized that successful management of fisheries requires strategic decisions based upon accurate knowledge of the life history and ecology (e.g. reproductive cycles) of target species.

Recommendations for future research include collecting samples in a greater range of depths (e.g. 200–1200 m) and latitudes of the western Atlantic (e.g. from Florida to U.S.-Canada border). This will provide information about the reproductive biology of smaller crabs (i.e. juveniles) and populations of RDSC at different latitudes to help fill the gaps in our study. The description of the stages of oocyte development can be improved by an ultrastructural study that will be able to describe the changes in a cellular level. Further examination of terminal molt in RDSC can enhance knowledge about their mating strategy. Research on fecundity estimates, sperm loads, and egg mortality in primiparous and multiparous females are necessary to fully understand reproductive output. A study of the process of embryo development in *C. quinquedens* would provide useful information about the breeding cycle described in our study. Future research using

crabs maintained in a laboratory is essential to corroborate the biennial reproductive cycle we proposed. Lastly, research about the mechanisms (i.e. internal or external factors) that triggers or influence reproduction in *C. quinquedens* are recommended.

This study provided new information about the reproductive ecology of *C. quinquedens*, including ovarian maturation, egg production and reproductive pattern. The conceptual diagram presented below shows the connections between the events of reproductive biology for *C. quinquedens* (Fig. 5.1). Size at maturity is dependent on latitude, and the process of ovarian development, and may be influenced by fishery removal of male crabs. Fecundity of primiparous females is a function of size at maturity given the positive relationship with body size. Female body size influences fecundity because it delimits the space available for the ovary and yolk deposition. The duration and time of ovarian development and embryo development probably differs between primiparous and multiparous crabs, and will influence the occurrence of ovigerous females and brood production schedules. Population reproductive output is affected by the ratio of ovigerous to non-ovigerous mature females, which is a function of maturity and ovarian maturation schedules. Seasonal changes in ovarian development, time of oviposition and hatching observed imply seasonality of the reproductive cycle of *C. quinquedens*, which can be uncommon for a deep-sea organism. All of these factors combined exert selective evolutionary pressure towards biennial reproduction in this species. Other factors, such as food availability, larval biology, and oceanographic influences probably also had an impact on the evolution of this adaptative strategy.

Examination of these seasonal changes suggests that the reproductive cycle starts in summer with the development of the ovary (Fig. 5.2). The next summer the ovary

reaches maturation and females are capable of producing the first clutch (i.e. oviposition). After oviposition, early embryo development occurs in fall through winter while the ovary is redeveloping. Late embryo development takes place from winter to spring, eggs start hatching in spring and the ovary reaches maturity. Although females have a mature ovary, the ratio of non-ovigerous to ovigerous females suggested they will not produce a new clutch until the next year. The chronological and temporal patterns of these events (i.e. size at maturity, ovarian development, fecundity, embryo development and occurrence of ovigerous and non-ovigerous females) result in a biennial reproductive cycle for female *C. quinquedens* have a. The biennial reproductive cycle observed in RDSC is unusual due to the relative warm temperatures they inhabit. Hence, this species may serve as a model for temperate deep-sea crabs, and further investigation is warranted. Our study will provide fishery managers with new information about the reproductive biology of *C. quinquedens* that will be useful to for improving management strategies. The implications of this study for fisheries management include developing legal size limits, providing biological data for stock assessment models, and reconsidering the stock identification of *C. quinqquedens*.

REFERENCES

Hines, A. H. 1990. Commentary on life history and ecology of deep-sea crabs of the family Geryonidae. In Geryonid crabs and associated continental slope fauna: a research workshop report. W. J. Lindberg and E. L. Wenner (eds.), 30-28, Technical Report 58, Florida Sea Grant College.

Pardo, L. M., J. P. Fuentes, A. Olguín, and J. M. (Lobo) Orensanz. 2009. Reproductive maturity in the edible Chilean crab *Cancer edwardsii*: methodological and management considerations. 89(8):1627–1634.

Stevens, B. G., and V. Guida. 2016. Demographics, morphometrics, depth, and temperature distribution of red deepsea crabs *Chaceon quinquedens* at four sites in the Mid-Atlantic Bight. Fisheries Bulletin. 114:343–359.

Tuset, V. M., D. I. Espinosa, A. García-Mederos, J. I. Santana, and J. A. González. 2011. Egg development and fecundity estimation in deep-sea red crab, *Chaceon affinis* (Geryonidae), off the Canary Islands (NE Atlantic). Fisheries Research. 109:373–378.

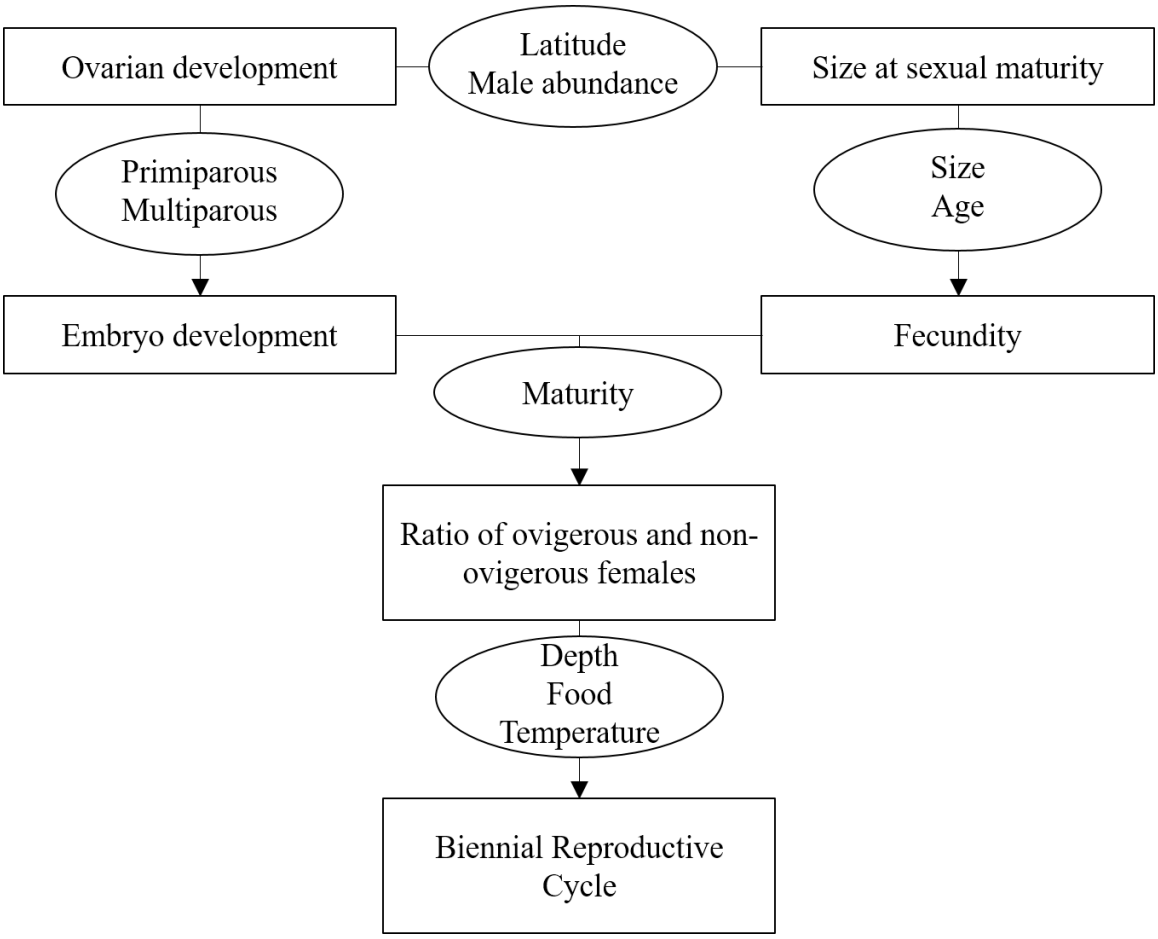

Figure 5.1. Intrinsic components of the reproductive biology (boxes) of female *Chaceon quinquedens*, and extrinsic factors (ovals) affecting them.

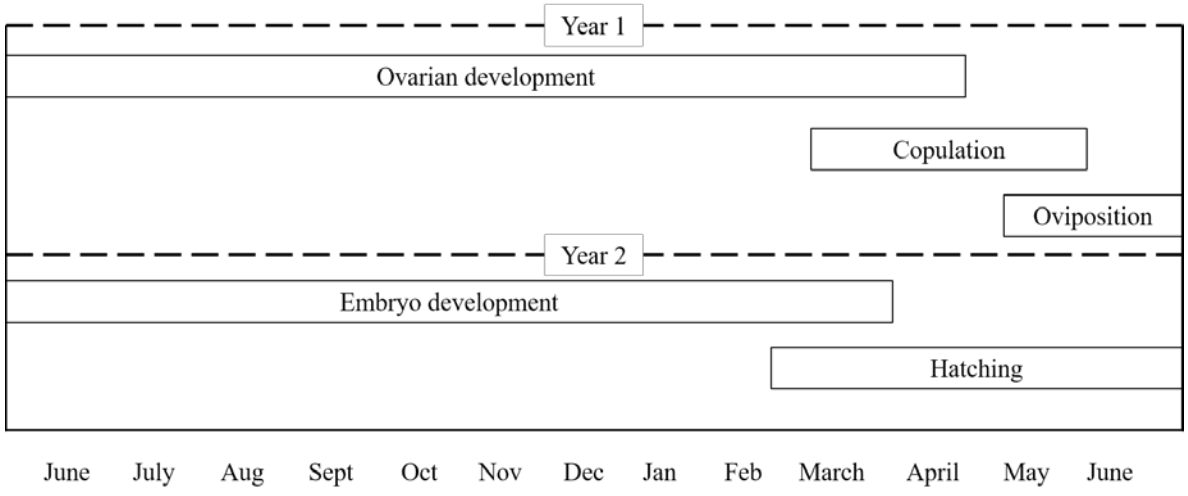

Figure 5.2. Timeline of reproduction for female *Chaceon quinquedens*.