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Source: Journal of Shellfish Research, 35(2) : 519-537

Published By: National Shellfisheries Association

URL: https://doi.org/10.2983/035.035.0225

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Journal of Shellfish Research, Vol. 35, No. 2, 519–537, 2016.

REPRODUCTION IN FEMALE STONE CRABS (GENUS *MENIPPE*) FROM TAMPA BAY, FLORIDA: INTERANNUAL, SEASONAL, AND TEMPERATURE-RELATED VARIATION

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ABSTRACT Knowledge of short- and long-term reproductive patterns is basic for numerous types of biological, ecological, evolutionary, and fisheries studies. Using data from more than 22 y of sampling commercially valuable stone crabs (genus *Menippe* (Say, 1819)) in Tampa Bay, FL, we defined size-specific interannual and intra-annual patterns in ovarian development and spawning; explored relationships between the molting cycle and intra-annual patterns; and identified the influence of water temperature on those interrelationships. Frequency of gravid females (frequency-gravid) seemed to shift in multiyear cycles but frequency of ovigerous females (frequency-ovigerous) did not. Frequency-gravid and frequency-ovigerous increased with size class except in the largest females. Interannual variation in frequency-ovigerous was the product of multiple patterns differing principally between size-class-70 and -80 females and size-class-90 and -100+ females. Ovarian development, spawning, and molting in females of size classes 20 through 50 occurred principally during peak temperatures in August and September. In females of size classes 70 through 100+, ovarian development, which was tightly and inversely linked to molting, occurred principally from February through August and spawning, which was influenced by both water temperature and molting, occurred principally from April through September. Size-class-60 females exhibited a mosaic of juvenile and adult patterns. Ovarian development and spawning ceased in all size classes when temperature decreased to approximately 25°C during October. Other specific temperatures or narrow temperature ranges were associated with additional shifts in reproduction and molting. Decreases through the years in frequency-ovigerous in females in size classes greater than or equal to 70, and particularly in those in size class 100+, concomitant with increases in frequency-ovigerous in females in size classes 50 and 60, and coupled with possible decreases in size at maturity and minimum spawning size, suggest fisheries selection. Shortening harvesting season by 6 wk during spring would protect females during the first spawning pulse in spring.

KEY WORDS: fishery management, fishery selection, Florida, interannual, *Menippe*, molting, ovarian development, reproduction, seasonality, spawning, stone crab, Tampa Bay, temperature

INTRODUCTION

Knowledge of the size-specific proportions of reproductively active females, timing of reproductive states and relationships between them, and long-term patterns of variation in reproduction is important for numerous applications. These population biology components are basic for studies of life history strategy (Fielding & Haley 1976, Sloan 1985, Fukui 1988, Liu & Li 2000, Hartnoll 2009); ecology (Salmon & Hyatt 1983, Hench et al. 2004); evolution (Wada et al. 2000); conservation issues (Lipcius et al. 2003); resource and fisheries management (García Flórez & Fernández-Rueda 2000, Yosho 2000, Lipcius & Stockhausen 2002, Sato & Yoseda 2008); zoogeographic variation (Somerton 1981, Hines 1989); aquaculture (Quinitio et al. 2007); and many other aspects of biology [e.g., endocrinology (Chaix & De Reggi 1982, Wada et al. 2000, Nagaraju et al. 2011); parasitology (Van Wyk 1982, Brockerhoff 2004)]. Detailed understanding of female reproduction is best obtained by long-term studies, which can reveal long-term cycles and trends, provide perspective for temporal variation, and elucidate reproductive patterns over ecological time. For crabs, long-term, population-level studies of female reproduction are rare, but are essential because reproductive cycles vary considerably among years (Pillai & Subramoniam 1984, Ituarte et al. 2004), and both general patterns and interannual variation are important. This is particularly true for intensively harvested species (Lipcius & Stockhausen 2002).

Both internal and external factors influence the size and seasonality of reproductively active female crabs. The most important internal factor is ecdysis (molting). Molting and spawning are regulated by hormonal activity (Ituarte et al. 2004) such that they are opposed in many crab species. Water temperature is the most common, and usually most important, external factor influencing crab reproduction. Water temperature, either alone or together with other factors such as, salinity, rainfall, lunar periodicity, photoperiod, or (for semi-terrestrial crabs) air temperature, has been identified as a principal driver of gonad development, spawning initiation, or spawning seasonality in numerous female crab species inhabiting shallow waters (Pillay & Ono 1978, Wild 1980, Zimmerman & Felder 1991, Dayakar & Rao 1992, Emmerson 1994, Flores & Negreiros-Fransozo 1998, Negreiros-Fransozo et al. 2002, Cobo & Fransozo 2003, Brante et al. 2004, Hamasaki et al. 2004, Ituarte et al. 2004, Darnell et al. 2009).

In this study, interannual and intra-annual patterns in ovarian development and spawning of stone crabs (genus *Menippe*) from the Tampa Bay, FL, area (Fig. 1) are explored, as are the influences of molting and water temperature (henceforth, "temperature") on those reproductive attributes. This study builds on the work of Gerhart and Bert (2008), who used a subset of the data set used in this study to estimate size-at-(sexual)-maturity and size-at-age, to generally characterize reproductive activity, and to define size-class-specific intra-annual variation in molting for female stone crabs from Tampa Bay.

Reproduction in female stone crabs is similar to that of many crab species. Female stone crabs mate after molting, while the

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DOI: 10.2983/035.035.0225

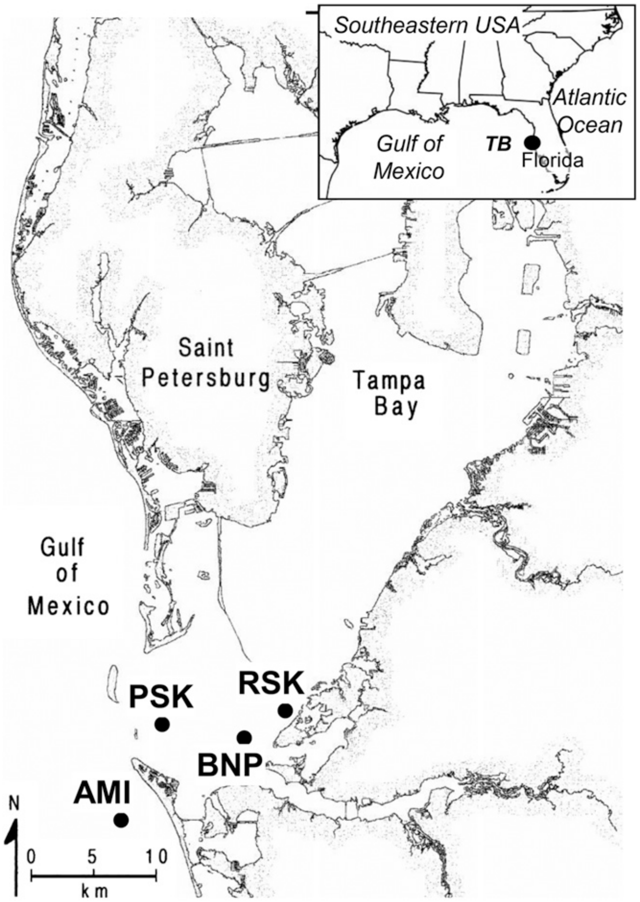

Figure 1. Sampling locations for study of stone crab (genus *Menippe*) reproduction. Inset, location of Florida and Tampa Bay (TB) in the southeastern USA. RSK, Rattlesnake Key; BNP, Bean Point; PSK, Passage Key; AMI, Anna Maria Island.

carapace is still soft (Binford 1913, Savage 1971). They retain sperm in spermathecae and can spawn multiple times after a single mating (Cheung 1969, Wilber 1992). The stages of ovarian development have been histologically defined for females from waters off northwest Florida (Noe 1967); and ovarian color has been used to qualitatively stage ovaries in female stone crabs from northwest Florida and South Carolina waters (Noe 1967, Caldwell 1986). Females extrude large numbers of eggs during each spawning event; the average number of eggs in an egg mass is exponentially related to female size (Bert et al. 2016). The eggs are attached to fine hairs on the abdominal pleopods and are carried for approximately 7–18 days (Binford 1913, Porter 1960, McConaugha et al. 1980). During that time, the coloration of the egg mass changes and some zoeal body parts become discernible (McRae 1950, Field 1989, Wilber 1989). Stone crabs from Florida Gulf of Mexico waters (the ‘‘Florida Gulf’’) spawn principally from April or May through September or October, depending on the area (Bert et al. 1986). Spawning season lengthens with movement southward, and spawning at low levels occurs throughout the year from the west-central Florida Gulf southward. Nearly all females from Tampa Bay mature when they are between 56- and 65-mm carapace width (CW); and size at 50% maturity has been estimated at 60-mm CW using allometric, physical (frequency of ovigerous females), and behavioral (size at mating) methods (Gerhart & Bert 2008). Although the timing of spawning and size at maturity are generally known for stone crabs, size-specific information is lacking, and nothing is known about the timing of ovarian development.

In stone crabs, molting is known to inhibit spawning (Cheung 1969); but precise relationships between ovarian development, spawning, and molting are not known. Impending molting can be identified in crabs missing major appendages (legs, claws) by the presence, size, and developmental state of appendage buds (Savage & Sullivan 1978, Somerton & MacIntosh 1985). Thus, the general relationship between molting and both ovarian development and spawning in stone crabs can be determined using crabs with missing appendages.

The effect of temperature on reproductive periodicity in crabs has been studied by comparing monthly or bimonthly values, averages, or ranges with mean frequencies of gravid females (frequency-gravid, i.e., the proportion of females with developing ovaries) or ovigerous females (frequency-ovigerous, the proportion of females carrying masses, or clutches, of eggs) collected on similar time scales (Lardies et al. 2004). In past studies of reproduction in *Menippe* inhabiting Florida (Noe 1967, Cheung 1969, Bender 1971, Sullivan 1979, Bert et al. 1986, Wilber 1989), researchers have used one of these approaches or have not considered temperature in the analyses. The only precise relationship between temperature and stone crab reproductive states was Noe’s (1967) speculation that 23°C was the lower temperature limit for spawning in females inhabiting northern Florida Gulf waters.

In marine waters off the southeastern United States, the two common *Menippe* species (*Menippe mercenaria* and *Menippe adina*) hybridize in two distinct areas (Bert 1986). The Gulf of Mexico hybrid zone in northwest Florida waters (Bert & Harrison 1988) extends southward into Tampa Bay, where the local population is composed principally of *M. mercenaria* and *M. mercenaria*-like hybrids (79% and 15%, respectively, Gerhart & Bert 2008). The two genotype classes are similar in multiple reproductive and physiological characteristics (Brown & Bert 1993, Duermit et al. 2016; T. M. Bert, unpublished data), and spawning frequencies and egg viability are similar among both species and their hybrids in northwest Florida waters (Wilber 1989). Thus overall, reproduction in Tampa Bay stone crabs probably has minimal or no variation related to hybridization.

In the Florida Gulf, stone crabs support a vigorous commercial fishery that is overcapitalized and overfished (Muller et al. 2011). The harvesting season (October 15 to May 15) and minimum legal size (70-mm propodus length; claws only are harvested, declawed crabs are returned to the sea) were established, in part, to protect female stone crabs during the principal reproductive season and to minimize harvest of immature stone crabs. Gerhart and Bert (2008) established that female stone crabs have at least 1 y to reproduce before attaining legal size, but potential effects of the fishery on stone crabs have not been well explored.

In this study, size-specific patterns of variation in ovarian development and spawning through years and within a year are described. Then the relationships between the ovarian development, spawning, and molting cycles are established and the effects of water temperature on intra-annual patterns of ovarian development and spawning are identified. This study utilizes nearly 23 y of data; that is, 7–10 stone crab generations, counting from initiation of reproduction from one generation to the next; or three generations, counting from one maximal life span to the next (Gerhart & Bert 2008). Sample sizes were large enough to define the interrelationships between temperature, ovarian development, spawning, and molting in unique detail. In addition, long-term, size-specific changes in spawning that could be attributable to harvesting are documented for the first time and the effectiveness of the harvesting season in protecting females during the reproductive season is discussed.

MATERIALS AND METHODS

Field

From May 1988 through November 2010, female stone crabs (''females'') were examined for evidence of ovarian development and spawning. The females were captured by trapping at four stations (five traps/station, arrayed linearly, approximately 100 m apart) in and offshore from the mouth of Tampa Bay, FL (Fig. 1), using standard commercial plastic stone crab traps, as described in Bert et al. (2016). To ensure that the size distribution of the females captured in the traps was consistent through time, each trap was cleaned of fouling organisms and debris during alternate sampling trips. External trap surfaces and spaces between trap slats were cleaned by scraping with hand axes; internal surfaces were similarly cleaned of large fouling organisms.

For each female, the following data were recorded: CW to the nearest 0.1 mm; spawning condition; and on females with missing claws, molting condition, as estimated by absence of or presence and stage of claw or leg buds (Savage & Sullivan 1978; Table 1). During almost all months between February 1989 and December 2004, subsets of at least 15 females per month were examined for gonad condition by dissecting the females and observing ovarian color, which was recorded in more detail than in Gerhart and Bert (2008) in that early and late developmental stages were separated (Table 1). The sampling objective for collecting females for dissection was to examine the same proportions of females, partitioned into 10-mm-CW size classes (Table 2), as were present in the total sample. To accomplish that objective, the sample sizes of size classes with few females were increased during 2005 ($n = 192$) and 2006 ($n = 18$).

With few exceptions, at each station during each sampling event bottom temperature (°C) and salinity (psu) were recorded with thermometer and refractometer (bottom water, retrieved using a Niskin bottle) or YSI water-monitoring instrument package. When data were not taken, the geographically and temporally most proximal readings were used to interpolate temperature or salinity values for the station with the missing data.

Statistical

Statistical analyses followed Sokal and Rohlf (1995) unless otherwise stated. Correlations and regressions were performed using the Microsoft Excel Trendline option. For most analyses, frequency-gravid or frequency-ovigerous, as appropriate, were calculated for 10-mm-CW size classes (Table 2). The size classes used were the same as those used by numerous researchers who previously studied aspects of stone crab reproduction (summarized in Bert et al. 1978, 1986, Wilber 1989, Gerhart & Bert 2008) enabling direct comparisons between the results of this study and results from the previous studies.

Data Considerations

Because genotypic differences in reproduction related to hybridization between *Menippe mercenaria* and *Menippe adina* were expected to be minimal or absent and because the stone crab fishery is managed as a single taxonomic group in Florida, previous studies in this series (Gerhart & Bert 2008, Bert et al. 2016) analyzed the data without genotype as a factor. All genotypes were combined into a single data set for this study, as well.

Sampling in 1988 covered only part of the year and in 2005 and 2006 was conducted only to complete sample sizes. Therefore, those data are omitted from analyses exploring variation in mean frequency-gravid or mean frequency-ovigerous with month or year as a factor.

TABLE 1.

Characteristics for identifying gonad stage, stage of eggs in an egg mass (ovigerous stage), and immediacy of molting in female stone crabs (genus *Menippe*).

Developmental stage	Attribute		
	Gonad condition	Spawning condition	Molting condition–bud stage*
0	–	–	Intermolt—no bud
1	Dormant–white ovaries	Non-ovigerous–no eggs	Early premolt–very small bud, small bump in center of declawing suture zone; claw segments undiscernible
2	Actively developing†–pale orange ovaries	Ovigerous–orange eggs	Premolt–bud approximately 2–5 mm in length; bulge covers most of suture zone; claw segments barely discernible
3	Mature†–bright orange ovaries, individual eggs discernable	Ovigerous–red-brown eggs	Imminent premolt–bud approximately 5–10 mm in length, bulging from suture zone; all claw segments identifiable, propodus pronounced
4	–	Ovigerous–brown eggs, zoeae's eyes evident	–

* Applicable only to females missing claws or legs.

† Actively developing and mature are collectively termed, ''developing.''

–, Not applicable

TABLE 2.

Size-class frequencies of female stone crabs (genus *Menippe*) sampled for reproductive activity.

	Gravid				Ovigerous				
Size class (size range*)	Total number dissected	Freq. total dissected	Number gravid	Freq. Gravid†	Total number examined	Freq. total examined	Number ovig.	Freq. ovig.†	Freq. gravid/ freq. ovig.
20 (20.0–29.9)	38	0.004	1	0.026	935	0.031	1	0.001	
30 (30.0–39.9)	56	0.006	5	0.089	1,230	0.041	12	0.010	10.6 (20+30)
40 (40.0–49.9)	297	0.030	29	0.098	1,371	0.046	58	0.042	2.3
50 (50.0–29.9)	549	0.055	135	0.246	1,606	0.054	166	0.103	2.4
60 (60.0–69.9)	870	0.086	412	0.474	2,280	0.077	458	0.201	2.4
70 (70.0–79.9)	1,805	0.179	1,058	0.586	4,829	0.162	1,176	0.244	2.4
80 (80.0–89.9)	3,176	0.315	1,978	0.623	8,655	0.291	2,314	0.267	2.3
90 (90.0–99.9)	2,544	0.253	1,647	0.647	6,819	0.229	1,867	0.274	2.4
100 (100.0–109.9)	674	0.067	424	0.629	1,857	0.062	431	0.232	2.7
110 (≥110)‡	58	0.006	34	0.586	199	0.007	40	0.201	2.9
Total	10,067		5,723		29,781		6,523		

Freq., frequency; ovig., ovigerous.

* CW (mm).

† Calculated within size class.

‡ For some size-class-specific analyses, females in this size class were combined with those in size-class 100, which was then designated as size-class 100+.

The data were examined for biases in sample size among years and months (heterogeneity in sample sizes among size classes was obvious) using the Goodness-of-fit *G*-test with expected proportions of equal sample size among those factors, applied to each size class. When the *G*-test was significant, size-class-specific regressions of sample size on year or month were also calculated to search for biases in sample size through time or among temperatures.

Reproduction

The data set for gravid females (''total-dissected data set'') and the data set for ovigerous females (''total-examined data set'') were respectively analyzed for overall differences in frequency-gravid or frequency-ovigerous among size classes, years, or months (other factors combined) using the $R \times C$ *G*-test for independence to detect significant differences and Simultaneous Test Procedure for Frequencies to locate significant differences (collectively, the ''$R \times C$ test''). When the $R \times C$ test was significant, regressions were calculated to search for patterns in the variation of frequency-gravid or frequency-ovigerous and to estimate the proportion of differentiation attributable to the targeted factor. The coefficient of determination (r^2) was calculated for each regression and the regression coefficient (slope of the line, b) was tested for significant differences from $b = 0$. When linear regression r^2 values were low, higher-order polynomial or exponential regressions were calculated to find the best-fit regression of the lowest order using the program from udel.edu/~mcdonald/statpolyreg.xls and by statistically comparing the r^2 values for regressions with increasing power levels using the equation

$$F = \mathrm{df}_j\left(r_j^2 - r_i^2\right)\Big/\left(1 - r_j^2\right),$$

where ${r_i}^2$ and ${r_j}^2$ are the coefficients of determination for, respectively, the lower-power equation and higher-power equation. The F value is distributed with j degrees of freedom in the numerator and $n - j - 1$ degrees of freedom in the denominator, where j is the power of the higher-order polynomial. For all regression analyses, frequencies were transformed into percentages to mitigate for the very small r^2 and b values generated when frequencies were regressed on large numbers such as years. Gerhart and Bert (2008) similarly compensated for the same problem. When the data suggested further analyses, additional *ad hoc* statistical tests (described in Results) were used.

A similar statistical approach was used to analyze interannual and within-year variation in frequency-gravid or frequency-ovigerous for each size class and to test the various stages of ovarian development and clutch maturation for differences in frequencies among size classes and among months: the $R \times C$ test was applied to evaluate variation among years or months for each size class, followed by regression analysis to discern patterns in the temporal variation when G was significant. The influences of the factors molting and temperature on variation in frequency-gravid and frequency-ovigerous in each size class through the year, and of temperature on the frequency of molting females (''frequency-molting'') in size classes were also analyzed similarly. Only gravid or ovigerous females were included, as appropriate, in analyses involving stages.

For all analyses, some size classes were combined or results were summarized when numbers of females in the size classes were small. When females in size classes were combined, the combined size class is represented as, for example, size-class-20+30. When an average of values for multiple size classes was used, the value is represented as, for example, size-class 20&30 when two values were averaged or as, for example, size-class 70–100+ when multiple size classes were averaged.

Correlations were calculated to test for relationships between frequency-gravid and frequency-ovigerous among size classes and, overall and for all pairs of size classes, through years or months. Similarly, correlations were used to search for relationships between frequency-gravid stages and frequency-ovigerous stages and relationships between frequency-gravid or frequency-ovigerous and frequency-molting through months and across temperatures.

Size-class-specific frequency-gravid and frequency-ovigerous were also examined for relationships with extreme annual, or periodically high or low, temperatures and El Niño or La Niña periods by searching for coincidence of unusually high or low frequency-gravid or frequency-ovigerous values and extreme temperatures or temperature periods. Extreme temperature periods were defined as three or more sequential months during which temperature exceeded or was less than the overall mean ± SD for each of those months. El Niño periods were identified using the sea surface temperature record published in the National Centers for Environmental Prediction, National Oceanographic and Atmospheric Administration website http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml and the categorized list of El Nino and La Niña events provided in http://ggweather.com/enso/oni.htm.

To compensate for unequal numbers of samplings among months, annual means and overall monthly means for frequency-gravid, frequency-ovigerous, frequency-molting, and temperature were calculated using mean monthly averages throughout the analyses.

No tests of the relationship between salinity and frequency-gravid or frequency-ovigerous were conducted because salinity was typically high and well within the tolerance range of *Menippe mercenaria* (Bert et al. 1986, Brown et al. 1992, Brown & Bert 1993) (mean psu, SD, range: RSK, 31.5, 2.4, 22–37; BNP and PSK, 33.1, 2.3, 20–39; AMI, 24.0, 1.8, 21–39; $n = 2{,}151$ measurements). At all stations, low-salinity events were rare and transient.

RESULTS

The 567 sampling trips were conducted an average of 14.5 days apart (SD, 4.3). Altogether, 29,871 females were examined for ovigerous stage (monthly mean, 112; SD, 45; range, 13–317); 6,538 were carrying egg masses. A subset of 10,067 females were dissected to observe ovarian stage; of those, 5,731 had developing ovaries. For the collection of data for the total-dissected data set, the goal of dissecting 15 females/month was achieved 91% of the time between January 1989 and December 2004; and for all except a few months, sample sizes were much larger (monthly mean, 95; SD, 165; range, 8–288). Preliminary analyses indicated that the unevenness among months in sample numbers did not bias the results. In both data sets, all data were not recorded for all females, but data omissions were rare.

Data and Analytical Considerations

The size-class frequency distributions of females in the total-dissected and total-examined data sets were highly similar; and similarly departed from the assumption of equal sample sizes among size classes (*G* values: total dissected, 9,321; total examined, 16,990; $P << 0.0001$ for both; Table 2). Therefore, most analyses were performed separately for each size class.

The proportions of females in each data set varied significantly among years (range: total-dissected, 335–1,099; total-examined, 926–1,846, months combined) and months (range: total-dissected, 520–1,270; total-examined, 1,965–3,105; years combined) ($P << 0.0001$ for both). But no *b* values for the regressions of sample size through years or through months were significant, indicating no directional change in sample sizes. From 2006 through 2010, the number of females in size-classes 20 and 30 markedly declined, resulting in the near absence of females in those size classes in samples from those years.

In figures depicting annual variation, only the last two digits of the year are shown; in figures depicting monthly variation, month 1 is January, month 12 is December; intervening months are numbered accordingly.

Reproduction

Variation among Size Classes

Third-order polynomial equations explained virtually all variation among size classes in frequency of females in either reproductive state (gravid: $y = -0.45x^3 + 4.72x^2 - 1.37x + 1.15$, $r^2 = 0.98$, $P = 0.007$; ovigerous: $y = -0.24x^3 + 3.16x^2 - 7.01x + 4.05$, $r^2 = 0.99$, $P = 0.98$) (Fig. 2). Size-classes 80 and 90 had the highest proportions of females in both reproductive states, whereas very little reproductive activity occurred in females of size-classes 20 through 40. Only one size-class-20 female (26.4-mm CW; collected September 1996) had developing ovaries and only one (27.4-mm CW; collected September 2005) was ovigerous. Gravid size-class-30 females ($n = 5$) were collected in June 1995 and 1996, September 1995 and 2001, and November 1997. Ovigerous females in that size class ($n = 11$) were collected during 1989–2010 (four during 1997), from May through October (four during September).

Size-class-specific frequencies of gravid females and ovigerous females were highly correlated ($r = 0.99$, $P < 0.01$; size-classes 20 and 30, and 100 and 110, combined), but the frequency-gravid:frequency-ovigerous ratio for females in combined size-classes 20+30 and 100+110 were significantly higher than the ratios for females in size-classes 40 through 90 [Table 2; $P < 0.001$ for both, *t*-test comparing a single value with the sample mean (size-classes 20+30 and 100+110, tested separately, versus the mean for size-classes 40 through 90)]. The ratio for size-class 20+30 was more than four times higher than the mean for size-classes 40 through 90.

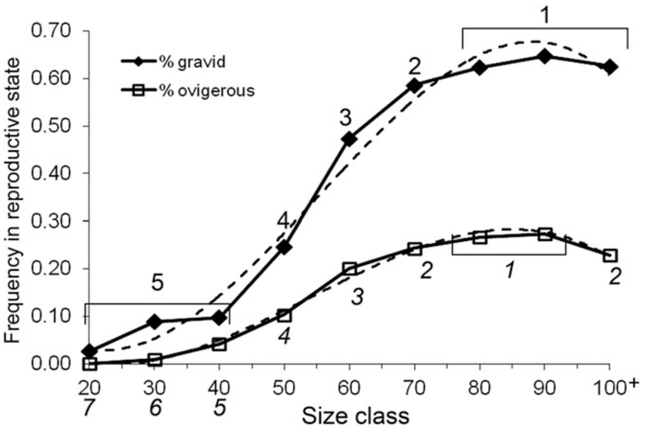

Figure 2. Frequency distributions of reproductively active female stone crabs (genus *Menippe*) grouped into size classes (defined in Table 2). Numbers and brackets, statistically significant groupings of size-class frequencies for gravid (above plot) or ovigerous (below plot, italicized) females; some statistical groups consist of only one value. Dashed lines, polynomial regression lines fitted to the data sets (equations and r^2 values in Results).

Approximately 20% of all gravid females were in stage 2 and 80% were in stage 3. Of all ovigerous females, 65% were in stage 2, 12% in stage 3, and 23% in stage 4. Generally, frequencies of stage-2 gravid and stage-2 ovigerous females were significantly high in size-classes 20+30 and 40 and high in size-classes 90 and 100+ (Fig. 3).

Variation among Years

Overall, the frequencies of gravid females and ovigerous females varied significantly among years (respectively, $G = 117$ and $G = 149$; both, $P < 0.001$) (Fig. 4). Years 1997, 1998, and 2000 constituted the group with the lowest frequencies of gravid females; but 1997 and 1998 had the highest frequencies of ovigerous females. Years 1989 and 1991–1996 composed the group with the highest frequencies of gravid females. The slope of the regression for frequency-gravid on year (–0.49) was significant ($P < 0.05$), but accounted for little of the interannual variation (r^2, 0.17); in part because the frequencies cycled significantly from greater than to less than the overall mean ($P < 0.005$; Runs Test for Dichotomized Data). Frequency-ovigerous varied significantly among years, but without a clear pattern. Consequently, overall frequency-gravid and frequency-ovigerous were not correlated through years ($r = 0.24$).

Patterns of variation among years in frequency-gravid were highly correlated for females in size-classes 80 through 100+; whereas annual patterns of variation in frequency-ovigerous were highly correlated between females in size-classes 70 through 90 (Table 3). Frequencies of females in either reproductive state did not differ significantly among years for size classes less than 70 but did differ significantly for size classes greater than or equal to 70 (gravid, 70 through 90 and ovigerous, 70 through 100+, $P < 0.001$; gravid, 100+, $P < 0.025$). Pairwise comparisons between size classes in the values of annual means relative to (i.e., greater than or less than) their respective overall means revealed multiple patterns of variation between size classes in frequency-ovigerous. (1) The patterns of variation through years for females in size-classes 30&40, 50, and 60 were not similar to each other (Fig. 5A) or to the pattern for any other size class; but the patterns for females in size-classes 70 and 80 were highly similar, as were the patterns for females in size-classes 90 and 100+. The annual mean values for size-classes 70 and 80 were similarly greater than or less than their respective overall mean values 19 of the 22 years of the study

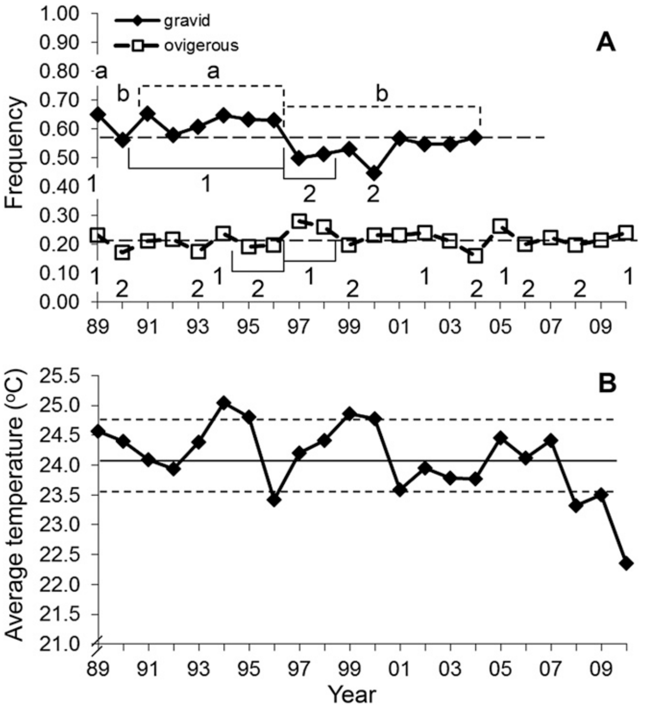

Figure 4. Variation among years in (A) frequency of reproductively active female stone crabs (genus *Menippe*). Numbers and solid-line brackets below frequency graphs, groupings of statistically similar frequencies, as described in Figure 3. Long-dashed lines, means. Gravid only: letters and dashed-line brackets above frequency graph, sequences of frequencies greater than (a) or less than (b) the overall mean. (B) Mean annual water temperature in the study area. Solid straight line, overall grand mean; dashed lines, overall mean ± SD.

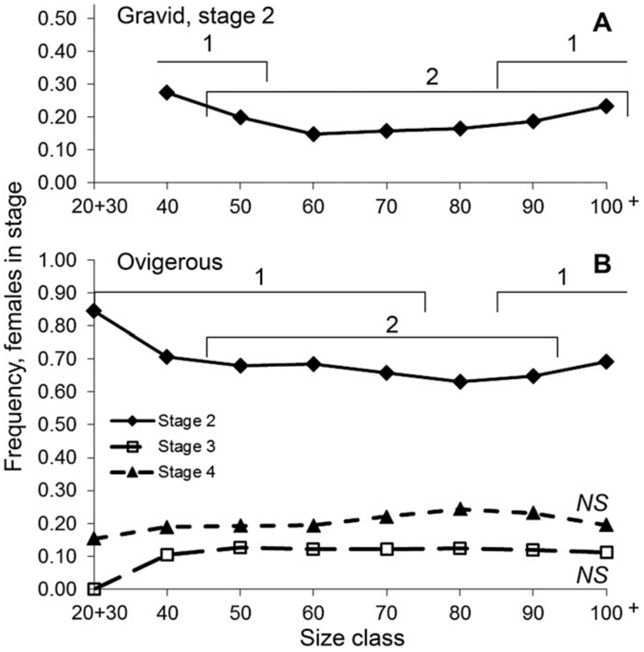

Figure 3. Size-class-specific frequencies of female stone crabs (genus *Menippe*) in gravid stage 2 (A) and in all ovigerous stages (B). Numbers and brackets above plots show statistically homogeneous groupings; only the groups that include the highest (1) and lowest (2) values are shown. Size classes are defined in Table 2. All gravid females in size-class 20+30 were in stage 3. NS, not significant.

TABLE 3.

Pairwise correlations between size classes for temporal variation in annual frequencies (months combined) of gravid female (above diagonal) or ovigerous female (below diagonal) stone crabs (genus *Menippe*).

Size class	50	60	70	80	90	100+
50	–	0.07	0.25	0.28	0.22	0.45
60	0.11	–	0.13	0.22	0.11	0.10
70	0.13	0.43	–	0.60	0.47	0.48
80	0.01	0.22	**0.81**	–	**0.70**	**0.72**
90	0.32	0.33	**0.63**	**0.69**	–	**0.72**
100+	0.31	0.20	0.32	0.14	0.42	–

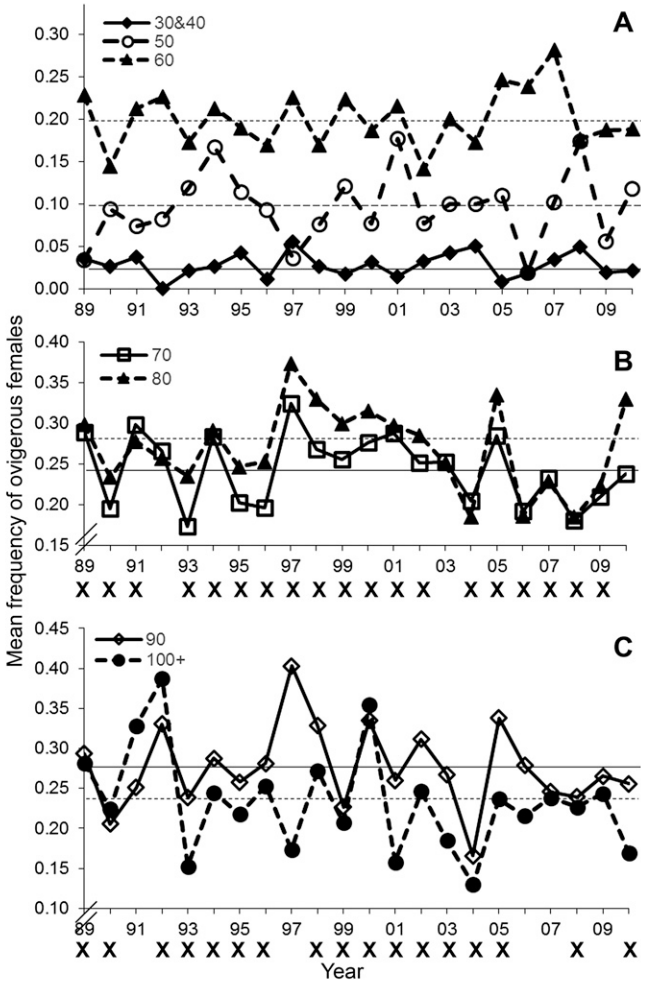

Figure 5. Interannual variation in size-class-specific frequency of ovigerous female stone crabs (genus *Menippe*). Size classes are defined in Table 2. Horizontal lines, means; form of line (solid, long dashed, short dashed) matches form of associated line connecting annual values. (A) Size-classes 30&40, 50, and 60. (B) Size-classes 70 and 80. (C) Size-classes 90 and 100+. X in B and C denotes years when annual values for frequency-ovigerous in both size classes were similarly above or below their respective annual means. Groupings of significantly high or low years not shown because they followed no broad patterns.

(Fig. 5B), a proportion that departed significantly from expected frequencies of 50% similarly directed, 50% opposed in direction (Goodness-of-fit *G*-test, extrinsic hypothesis; expected frequency ratio, 1:1; $P < 0.01$). The positions of the annual means for size-classes 90 and 100+ were similarly greater than or less than their overall means 17 years of the study ($P = 0.05$) (Fig. 5C). When the annual means for size-classes 70 or 80 were paired with the annual means for size-classes 90 or 100+, the paired values of their annual means relative to their respective overall means did not differ significantly from random. (2) Females in size-classes 90 and 100+ exhibited a unique pattern in frequency-ovigerous. The direction of a size-class-90 frequency-ovigerous value relative to the size-class-90 overall mean opposed the direction of the size-class-100+ frequency-ovigerous value, advanced by one year, relative to its overall mean significantly frequently (16/21 pairs of years; Goodness-of-fit *G*-test, extrinsic hypothesis $P = 0.05$) (Table 4). (3) Finally, the annual mean

TABLE 4.

Pattern of values for annual mean frequencies of ovigerous large female stone crabs (genus *Menippe*) relative to size-class-specific overall mean frequencies.

		Year																					
Size class	**Mean freq.ovig.**	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
90	0.28	A	B	B	A	B	A	B	a	A	A	B	A	B	A	b	B	a	a	B	B	B	B
100+	0.23	a	b	a	A	B	a	b	a	b	a	b	a	b	a	b	B	a	b	a	b	a	b

freq., frequency; ovig., ovigerous. a or A, yearly mean higher than overall mean; b or B, yearly mean lower than overall mean. Capital letters, values differed significantly from respective overall mean. Gray shades differ to distinguish sequential-year pairs when positions of mean frequency-ovigerous relative to their respective means opposed each other (e.g., 1989 size-class-90 mean was above overall size-class-90 mean, whereas 1990 size-class-100+ mean was below overall size-class-100+ mean).

frequency-ovigerous values for size-class-100+ females alternated between greater than and less than the overall size-class-100+ mean in sequential years significantly frequently (Runs Test for Dichotomized Data, 19 changes from greater than overall mean to less than overall mean, or vice versa, in sequential years in a total of 21 possible sequential-year pairs, $P < 0.005$; Table 4).

Through the years, frequency-gravid generally decreased in females of many size classes; but the decrease was much less for females in size-classes 30 through 70 (mean b value –0.01) than for females in size-classes 80 through 100+ (mean b value –0.91). Regressions revealed that the frequencies of ovigerous females in size-classes 70 through 100+ gradually decreased through years (proportional decrease: size-classes 70 through 90, 10% to 12%; size-class 100+, 26%), whereas the frequencies of ovigerous females in size-classes 50 and 60 increased, respectively 5% and 29% (Fig. 6). In large females, frequency-gravid and frequency-ovigerous were more highly influenced by the factor "year" than were females in other size classes (Table 5). The r^2 values of the regression lines for frequency-gravid in females in size-classes 80 through 100+ and for frequency-ovigerous in females in size-class 100+ were much greater than they were for females in smaller size classes.

Variation among Months

Frequencies of females in all size classes except 20+30 varied significantly among months in each reproductive state (ranges; G values, 22.4–2,114; $P < 0.025$, size-class-40 gravid females, to $P \ll 0.001$, nearly all other size classes, both reproductive states). Overall, October was the singular month with the lowest frequencies of gravid females (all size classes, nearly all years), followed by November and December. January was always in the statistical group with the lowest frequencies of ovigerous females (all size classes, all years) followed by November and December (all size classes, all years but one). August was the month most frequently in the group with the highest frequency-gravid; high frequencies of females in most size classes were gravid during that month (Fig. 7) almost every year. August and September were the months most frequently in the group with the highest frequency-ovigerous (Fig. 8), for the same reasons.

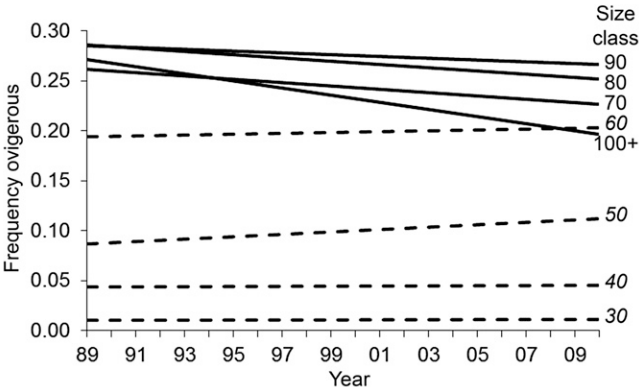

Figure 6. Regression lines for size-class-specific frequencies of gravid or ovigerous female stone crabs (genus *Menippe*) through years (Figure 5). Dashed lines show regressions with positive slopes; solid lines show regressions with negative slopes. Size classes are defined in Table 2. Regression equations are provided in Table 6.

TABLE 5.

Adjusted regression equations (frequency × 100) and coefficients of determination (r^2) for size-class-specific graphs of frequency of reproductively active females on year (Fig. 6).

Size class	Ovarian development: Equation	Ovarian development: r^2	Spawning: Equation	Spawning: r^2
30	FO = –0.18Y + 9.86	0.002	FO = 0.004Y + 1.03	>0.000
40	FO = 0.12Y + 7.01	0.007	FO = 0.008Y + 4.37	>0.000
50	FO = –0.44Y +28.99	0.076	FO = 0.12Y + 8.52	0.032
60	FO = 0.17Y + 44.64	0.011	FO = 0.04Y + 19.33	0.006
70	FO = –0.26Y + 60.20	0.026	FO = –0.17Y + 26.29	0.061
80	FO = –0.97Y + 71.00	0.388	FO = –0.16Y + 28.71	0.040
90	FO = –0.70Y + 71.19	0.237	FO = –0.09Y + 28.57	0.012
100+	FO = –1.05Y + 72.19	0.223	FO = –0.36Y + 27.46	0.130

FO, frequency ovigerous; Y, year.

Variations in frequency-gravid and frequency-ovigerous in size-class-20+30 and size-class-40 females were similar to each other and fundamentally different from those of larger females (Table 6). No size-class-30 females were reproductively active during the initial months of the year and very few were active through the remainder of the year (Figs. 7A and 8A). Size-class-40 females followed a similar pattern, but with higher frequencies of reproductively active females (Figs. 7B and 8B). For both size classes and in each reproductive state, frequencies of reproductively active females were highest during September. Females in size-class 50 exhibited reproductive patterns similar to those of females in size-class 40 (Figs. 7B and 8B), except that both frequency-gravid and frequency-ovigerous were distinctively higher and peak spawning month was August, when frequency-gravid was essentially the same as frequency-gravid in all larger size classes (Fig. 7B, C). The spring spawning pulse evident in all larger size classes (Fig. 8C) was also becoming apparent in size-class-50 females (Fig. 8B), and contributed to the correlation of size-class 50 with larger size classes (Table 6).

The patterns of variation in frequency-gravid and frequency-ovigerous displayed by females in size-classes 60 through 100+ were nearly identical (Figs. 7C and 8C) and, thus, highly correlated (Table 6). Frequency-gravid was highest from February through August, dramatically decreased during September, was lowest in October and November, and consistently increased in December through February. Frequency-ovigerous was high in April through September, peaked July and August and secondarily during May, prior to a small—but in some size classes significant—decrease during June. Frequency-ovigerous dramatically declined in all size classes from September to October, was near zero November through February, and dramatically increased from March to April. The greatest differences among size-classes 60 through 100+ were the lower frequencies of size-class-60 females during the months of peak ovarian development or spawning. Although seasonal patterns differed somewhat, frequency-gravid and frequency-ovigerous were correlated across months ($r = 0.58$, $P = 0.05$), principally due to the precipitous decline in frequencies of reproductively active females of all size classes October through December.

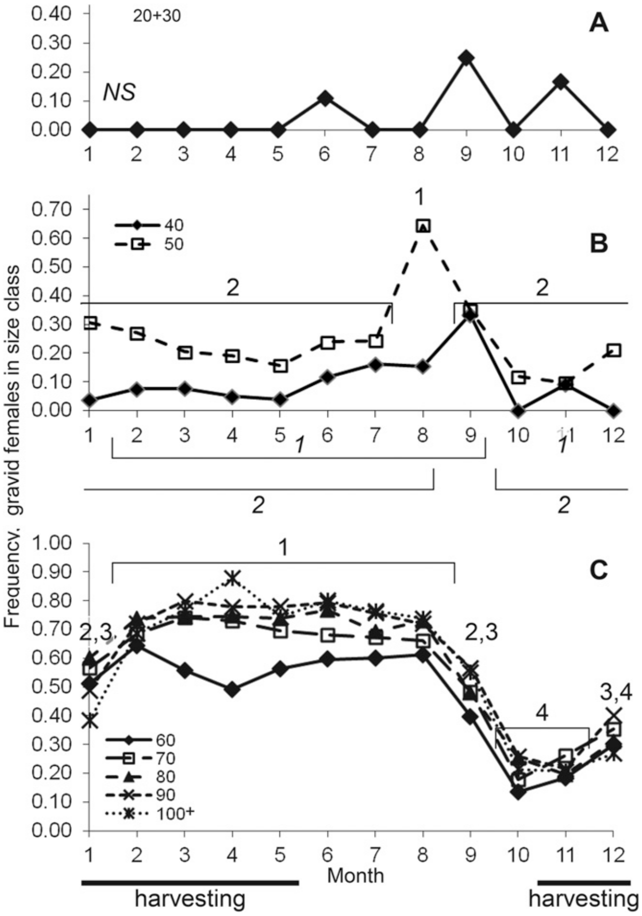

Figure 7. Variation among months in size-class-specific frequency of gravid female stone crabs (genus *Menippe*). Numbers and brackets above or below graphs show statistically similar size-class groupings: group 1 includes highest value; highest number denotes group with lowest value. Size classes are defined in Table 2. Lines beneath graphs, stone crab harvesting season in Florida. (A) Combined size-classes 20 and 30. NS, no significant differences. (B) Size-classes 40 (statistically similar groupings shown below *X* axis) and 50 (statistically similar groupings shown above plot for size-class 50). Only groups that include the highest or lowest frequency are shown. (C) Size classes 60 through 100+. All groupings are shown; groupings 1 and 4 were the same for all size classes. Months with two grouping numbers, groupings varied among size classes.

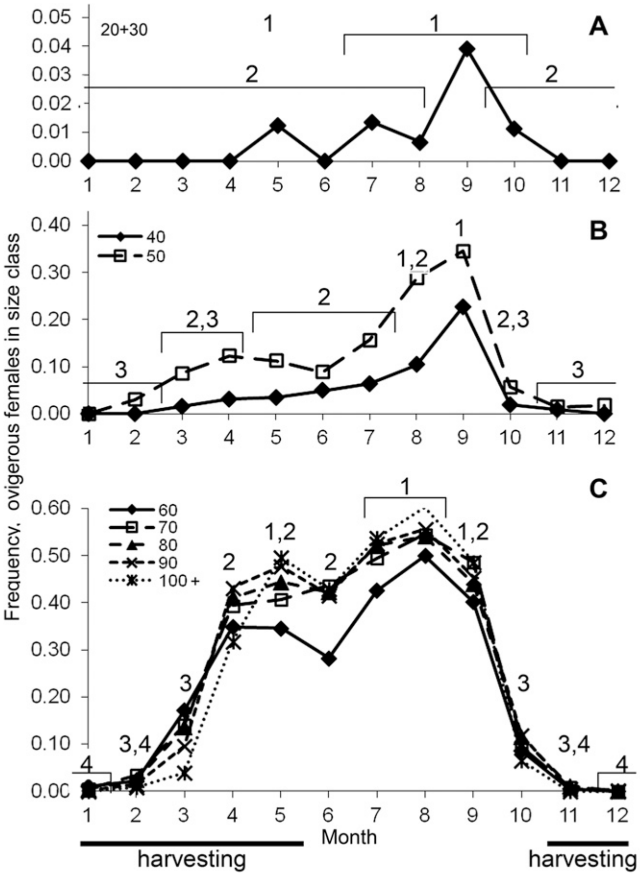

Figure 8. Variation among months in size-class-specific frequency of ovigerous female stone crabs (genus *Menippe*). Numbers and brackets are as in Figure 7. Size classes are defined in Table 2. Lines beneath graphs, stone crab harvesting season in Florida. (A) Combined size-class 20 and 30. Note difference in *Y* axis scale from B and C. (B) Size-classes 40 and 50. Two highest and single lowest groupings are shown. (C) Size classes 60 through 100+. All groupings are shown; months with two grouping numbers, groupings varied among size classes.

The frequencies of females in the two stages of ovarian development and in ovigerous stages 2 and 4 changed significantly through the year (all *G*-tests, $P < 0.001$). Frequencies of females with stage-2 ovaries steadily and significantly increased ($y = 1x + 11$, r^2, 0.72; $P < 0.05$, size-class greater than or equal to 40 combined; all gravid females in size-class 20+30 were in stage 3) and were highest from September through December (Fig. 9A). Frequency of ovigerous stage 2 decreased linearly through the year ($y = -5x + 96$, $P < 0.01$); and frequency of ovigerous stage 4 concomitantly increased ($y = 4x - 4$, $P < 0.01$) (Fig. 9B); high proportions of the changes in those frequencies were explained by the variable "month" (respectively, $r^2 = 0.90$ and 0.87). The frequencies of ovigerous stage 3 were low and nearly constant through most of the year. Frequency-gravid, stage 2 was negatively correlated with frequency-ovigerous, stage 2 and positively correlated with frequency-ovigerous, stage 4 (both $r = 0.86$, $P < 0.01$).

Influential Factors

Molting

To increase sample sizes, size-classes 30 through 50 were combined. No correlations between frequency-gravid and frequency-molting were significant for females in size-class 30–50 or 60. Most molting and ovarian development occurred during summer in those females; and in size-class-30–50 females, frequency-gravid followed a similar pattern of variation as frequency-molting from April to October, with a 1-mo delay in frequency-gravid (Fig. 10A). In contrast, frequency-gravid was highly and inversely correlated with frequency-molting in females of size-classes 70 through 100+; and the relationship was highly similar among those size classes. Therefore, those size classes were combined for final analysis ($r = -0.80$, $P < 0.001$) (Fig. 10B).

Uniquely, frequency-ovigerous and frequency-molting were positively correlated January through September in size-class-60 females ($r = 0.70$, $P < 0.05$) (Fig. 10C). Among other size classes, the highest r value for the correlations between frequency-ovigerous and frequency-molting was 0.63.

TABLE 6.

Pairwise correlations between size classes for temporal variation in monthly frequencies (years combined) of gravid female (above diagonal) or ovigerous female (below diagonal) stone crabs (genus *Menippe*).

Size class	20+30	40	50	60	70	80	90	100+
20+30	–	**0.72**	0.02	0.31	0.30	0.35	0.27	0.20
40	**0.88**	–	0.47	0.22	0.17	0.14	0.23	0.27
50	**0.76**	**0.93**	–	0.49	0.34	0.38	0.33	0.31
60	0.51	0.69	**0.85**	–	**0.95**	**0.96**	**0.92**	**0.86**
70	0.51	0.69	**0.82**	**0.98**	–	**0.98**	**0.97**	**0.94**
80	0.48	0.64	**0.78**	**0.98**	**1.00**	–	**0.97**	**0.94**
90	0.49	0.64	**0.78**	**0.98**	**0.99**	**1.00**	–	**0.98**
100+	0.52	0.68	**0.80**	**0.96**	**0.98**	**0.98**	**0.98**	–

Underlined, $P < 0.05$; bold print $P < 0.01$.

As molting approached (as estimated by appendage bud stage), both ovarian development and spawning ceased. Frequency-gravid and frequency-ovigerous decreased significantly when appendage buds were evident (Fig. 11). When appendage bud stage was advanced, very few females had developing ovaries and none were carrying egg masses.

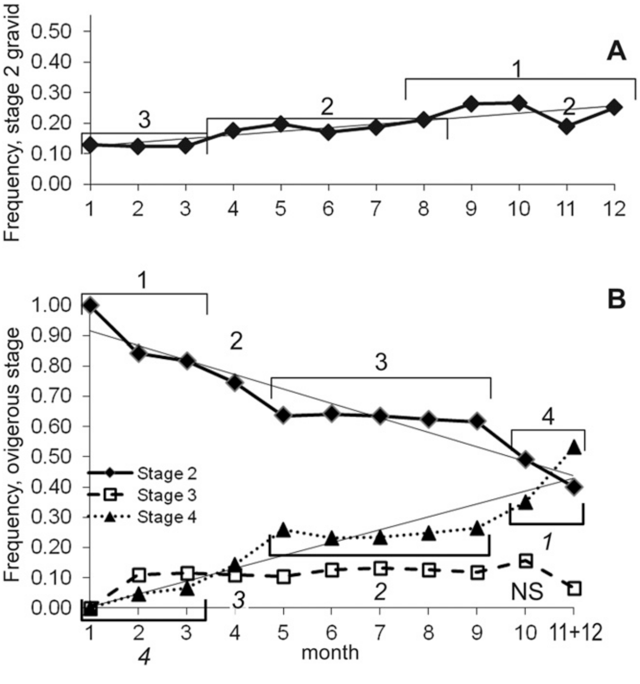

Figure 9. Variation among months in stages of reproductive activity in female stone crabs (genus *Menippe*). Bars and numbers show statistically similar groupings, as in Figure 7, except that all groupings are shown. Straight lines, regressions. Size classes are defined in Table 2. (A) Stage 2 gravid (stage-3 frequencies are the reciprocals). (B) Ovigerous stages. Data taken during November and December were combined to increase sample size. Stage 2 statistically similar groupings above plot; Stage 4 statistically similar groupings below plot, in italics. NS, not significant, stage-3 frequencies did not differ significantly.

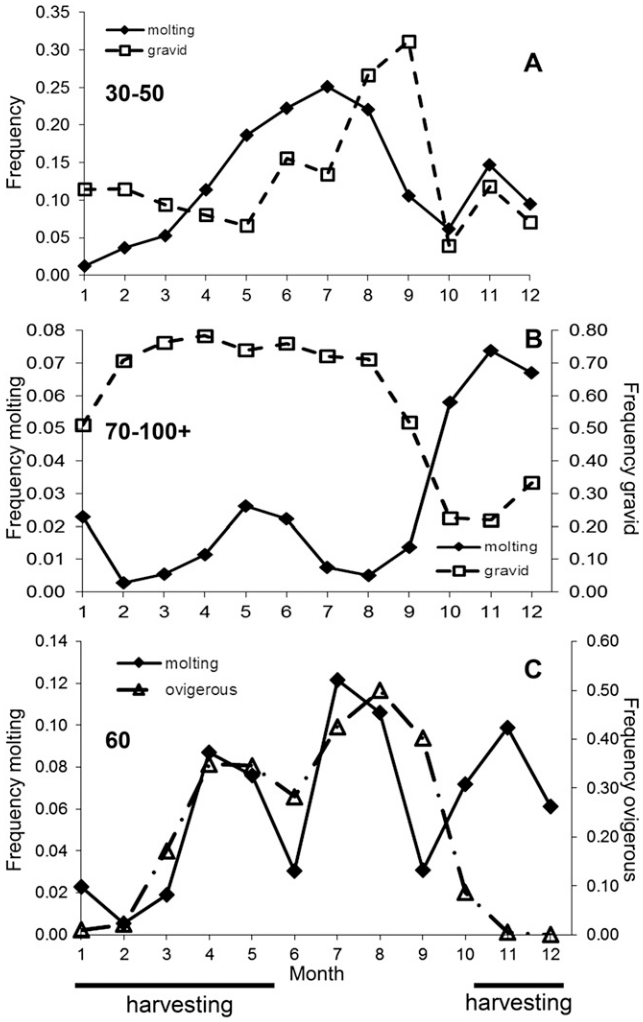

Figure 10. Relationship between molting and ovarian development (A and B) or spawning (C) in female stone crabs (genus *Menippe*). Note differences in *Y* axis scales. Size classes (bold numbers) combined from those defined in Table 2. Lines below graphs, stone crab harvesting season in Florida. Size-class 60 is not included in A or B because no relationship between molting and ovarian development could be ascertained.

Water Temperature

Minimum temperature was 11°C for all stations; maxima were 33°C for AMI, 34°C for RSK and BNP, and 37°C for PSK. Annual mean temperatures for 1994, 1995, 1999, and 2000 were high; and for 1996 and 2008 were low (Fig. 4B); but the most notable temperature event was a prolonged period of unusually cold weather January through April 2010, which included 2 wk of record-breaking cold in January (Wang et al. 2010, Lirman et al. 2011, Colella et al. 2012) and contributed to an exceptionally low mean annual temperature. Nevertheless, neither annual frequency-ovigerous nor frequency-gravid were dependent on variation in annual temperature. In the same manner, size-class-specific monthly variation in frequency-gravid or frequency-ovigerous was not related in any observable way to prolonged periods of high or low temperatures, extreme temperature values, El Niño, or La Niña.

Temperature changed asymmetrically through the year. Average temperature rose slowly February through June, was

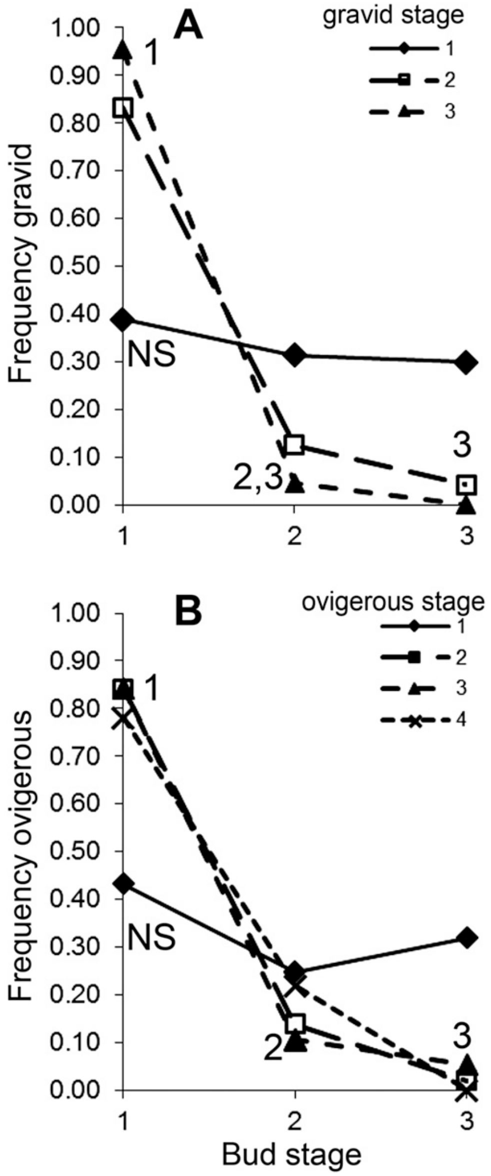

Figure 11. Relationship between temporal proximity of molting and reproduction in female stone crabs (genus *Menippe*). Claw and leg bud development was used as a proxy signaling that molting would soon occur (see Methods). Stages of reproductive states and claw buds defined in Table 1. Numbers above and beside plots show statistical groupings, as described for Figure 8C. NS, no significant differences in frequencies among bud stages. (A) Effects of molting on ovarian development. (B) Effects of molting on spawning. Frequencies of females in ovigerous stages 2 and 3 were the same.

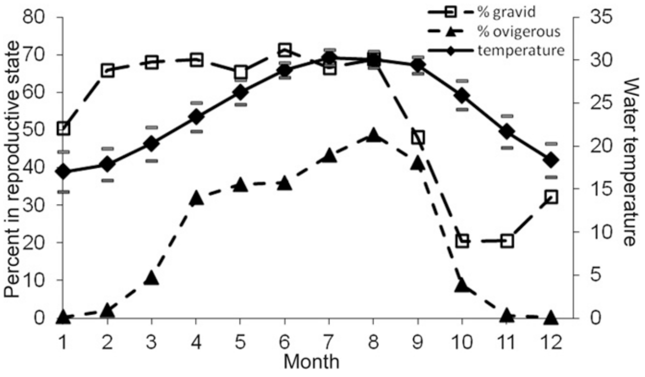

Figure 12. Relationship between average monthly temperature (bars, SD) and average monthly percentage of female stone crabs (genus *Menippe*) in each reproductive state.

highest during August (30°C; SD, 1), fell rapidly September through December, and was minimal during January (17°C; SD, 3) (Fig. 12). The slope of a regression line fitted to the months of ascending temperature was 2.8 ($r^2 = 1.00$); whereas the slope for a regression line for the months of descending temperature was –3.7 ($r^2 = 1.00$). This asymmetry, the asymmetry in the shapes of the curves for average monthly frequency-gravid and frequency-ovigerous (Fig. 12), and a strong dependency of mean monthly frequency-ovigerous on mean monthly temperature ($y = 0.04x - 0.65$, $r^2 = 0.80$; $P < 0.01$) indicated that females may respond in different ways to a specific temperature, depending on when during the reproductive cycle the temperature occurred. Therefore, temperature values and associated frequencies of gravid and ovigerous females during the months of rising temperatures were separated from the corresponding values during the months of falling temperature (Fig. 13) for most analyses of the relationships between reproductive state and temperature. For these analyses, size-classes 20 and 30 were omitted, size-classes 40 and 50 were combined to increase sample size, and size-classes 70 through 100 were combined because monthly variation in frequencies of females in both reproductive states were virtually identical (Figs. 7C and 8C).

In general, temperature greatly influenced frequency-ovigerous (Table 7), but not frequency-gravid ($r^2 = 0.01$; NS), principally because high frequencies of females were gravid for most of the year regardless of temperature. Nevertheless, strong seasonal temperature-specific shifts in frequencies of females in both reproductive states occurred in all size classes (all P values < 0.0001) (Fig. 13). Frequencies of females in both reproductive states plunged from highest values to zero or near-zero as mean temperature fell to 25°C, which typically occurred quickly during October. Spawning essentially ceased when temperature fell to 20°C in late fall or early winter and remained at or near zero until temperature increased to 18°C in early spring, when females in all size classes began to spawn at low frequencies. In contrast, in all size classes and almost immediately after the reproductive cessation at 25°C, ovarian development commenced again and continued throughout the cold temperatures of winter. During the rising temperatures of spring and early summer, changes in frequency-gravid and frequency-ovigerous differed among size classes. In size-class 40+50, frequencies of both reproductive states remained low until temperature was warmest (≥30°C); then, frequencies attained high levels. In size-classes 60 and 70–100+, frequency-gravid continually increased after the fall 25°C reproductive pause and remained high through winter's low temperatures. Frequency-gravid declined in size-class-60 females when temperature reached 23°C during spring but returned to high levels when temperature reached 30°C during summer; whereas frequency-gravid continued to remain high through the rising temperatures of spring and early summer in females of size-class 70–100+. In females of both size-classes 60 and 70–100+, frequency-ovigerous increased through rising spring

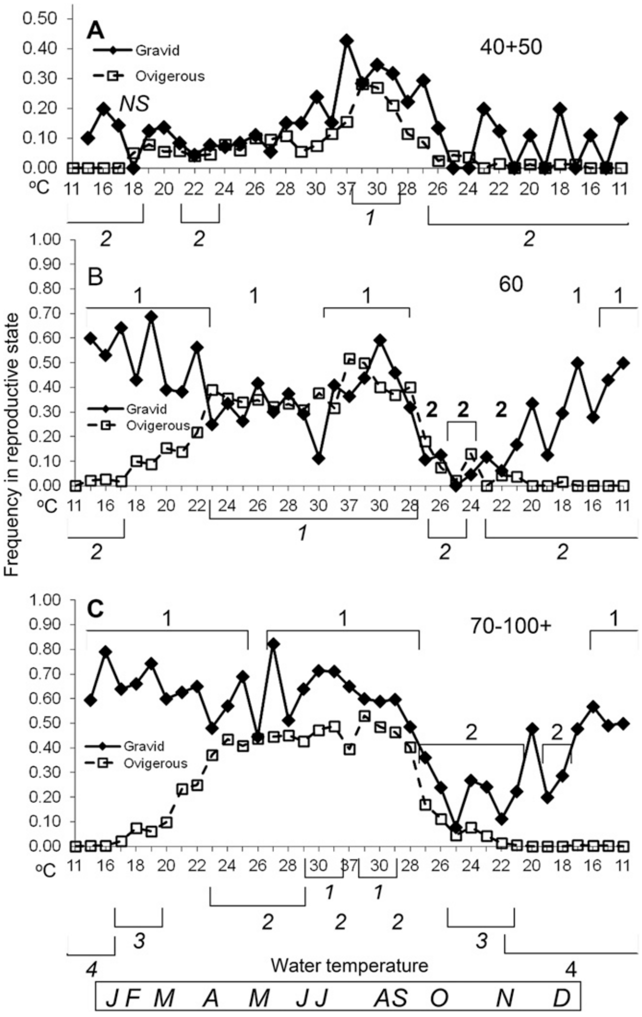

Figure 13. Relationship between size-class-specific variation in frequency of gravid or ovigerous female stone crabs (genus *Menippe*) and seasonal change in water temperature. Bars above and below plots respectively show statistically similar groupings for frequency-gravid and frequency-ovigerous. Plots showing two groupings, only the groupings that include the highest or lowest frequencies are shown. Boxed letters below graphs: abbreviations of months, placed approximately below their mean temperatures. (A) Size-classes 40 and 50 combined; NS, not significant. (B) Size-class 60. (C) Size-classes 70 through 100+, averaged; for frequency-ovigrous, the two highest (1, 2) and two lowest (3, 4) groupings are shown.

TABLE 7.

Relationship between water temperature and frequency of female stone crabs (genus *Menippe*) in designated ovigerous stage (defined in Table 1).

Category	Regression equation	r^2	*P*
All ovigerous	FO = 0.032T – 0.047	0.97	<0.001
Ovigerous stage 2	FO2 = –0.019T – 0.010	0.96	<0.001
Ovigerous stage 3	FO3 = 0.004T + 0.000	0.94	<0.001
Ovigerous stage 4	FO4 = 0.010T – 0.038	0.93	<0.001

r^2, regression coefficient of determination; *P*, probability; FO, frequency ovigerous; T, water temperature.

temperatures to 23°C, plateaued at intermediate to high levels through increasing summer temperatures, and initiated the precipitous fall decline when temperature decreased to 28°C in fall.

Sample sizes for size-class-20 and -30 females were adequate for analysis of the effects of temperature on molting; other size classes were grouped as in the analyses for frequency-gravid and frequency-ovigerous. Females in all size classes molted at all temperatures except when temperature initially decreased to approximately 15°C in early winter (Fig. 14; *P* range, B–D: 0.005–0.001). In size-classes 20, 30, and 40+50, frequency-molting remained very low through winter's low temperatures and began to increase when temperature rose to approximately 18°C in early spring (Fig. 14A, B). Females in size-classes 20 and 30 exhibited little seasonality in molting frequency throughout the rest of the year, whereas frequency-molting in size-class-40+50 females increased through warming spring-summer temperatures, peaked when temperatures were highest, and decreased through cooling fall and early-winter temperatures (linear regression, frequency-molting on temperature: increasing, 17–32°C; r^2, 0.94; $P < 0.001$; decreasing, 33–15°C; r^2, 0.50; $P < 0.05$). The intermittent peaks in frequency-molting of size-class-60 females were reminiscent of both high frequencies of size-class-40+50 females when temperature was warmest and the high frequencies of size-class-70–100+ females when temperature was decreasing during fall (Fig. 14B–D). Size-class-70–100+ females molted in high frequencies principally as temperatures descended from 27°C to 15°C through fall and early winter (Fig. 14D). A small pulse in molting also occurred in those females during spring, initiating at 24°C and peaking at 27°C. Frequency-molting also increased markedly at 24°C in size-class-60 females (Fig. 14C).

Through the annual temperature cycle, frequency molting was positively correlated with both frequency-gravid and frequency-ovigerous in size-class-40+50 females, negatively correlated with the two reproductive measures in size-class-70–100+ females (size-class 40+50; $r = 0.37$, $P < 0.05$ and 0.71, $P < 0.01$; size-class 70–100+; $r = –0.47$, $P < 0.01$ and –0.34, $P < 0.05$), and not correlated with either reproductive measure in size-class-60 females (*r* values: 0.32 and 0.07). But in contrast to the strong inverse correlation between frequency-gravid and frequency-molting in size-class-70–100+ females when values were averaged for each month (Fig. 10), *r* values for correlations between frequency-molting and frequency of reproductively active females never exceeded 0.60 when frequencies were calculated for each temperature.

DISCUSSION

Reproduction

As in other crab species (Norman & Jones 1993), frequencies of ovarian and egg-mass stages change similarly through the year in mature female stone crabs of all size classes. Frequencies of gravid females in the early developmental stage 2 were high from August through December, after the principal spawning season; whereas frequencies were lowest at the beginning of the year, when ovaries were mature for the first spring spawn. As might be expected, frequency of stage-2 eggs was highest during the early months of the year when the first egg masses were spawned and lowest toward the end of the year, when the last

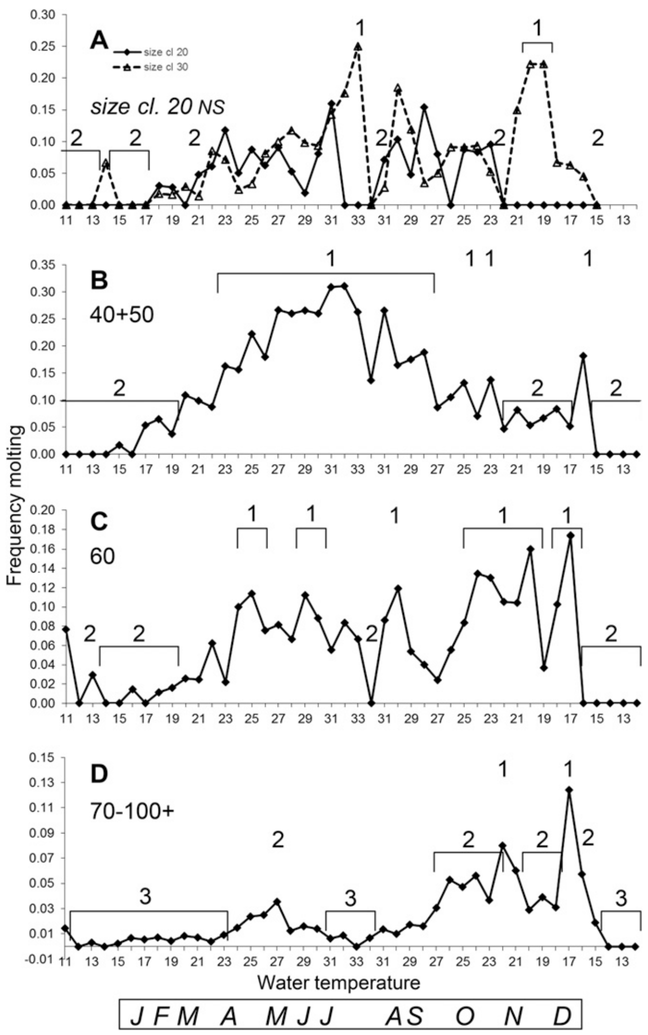

Figure 14. Relationship between size-class-specific variation in frequency of molting female stone crabs (genus *Menippe*) and seasonal change in water temperature. Bars and numbers above plots show statistically similar groupings for frequency-molting. Plots showing two groupings, only the groupings that include the highest or lowest frequencies are shown. Boxed letters below graphs as in Figure 13. (A) Size-classes 20 (NS, not significant) and 30. (B) Size-classes 40 and 50, combined. (C) Size-class 60. (D) Size-classes 70 through 100+, averaged; the two highest and single lowest groupings are shown.

few females were carrying stage-4 eggs at the end of the spawning season.

Assuming that the relative proportions of females in each reproductive stage are representative of the relative durations of stages in females, then the approximate average proportional duration of gravid stages 2 and 3 was 1:4 and of ovigerous stages 2, 3, and 4 was 5:1:2. These proportions suggest that initial ovarian development is rapid and that embryonic development progresses considerably before external changes in egg coloration are detectable.

Variation among Size Classes

As female size increases, changes in the percentages of females carrying eggs are closely associated with changes in the percentages with developing ovaries. Overall, frequencies of both reproductive states follow the same pattern of variation among size classes; frequencies of gravid and ovigerous females are highly correlated across size classes; and the ratio of frequency-gravid to frequency-ovigerous, which could be an estimate of the relative length of time spent in each reproductive state, is constant except for females in the smallest and largest size classes.

Females in size-class 80 and 90 may to be the most important reproductive size classes. The frequencies of ovigerous females in those size classes are significantly higher than the frequencies in other size classes, suggesting that the number of clutches spawned per year is high. The frequency of females with stage-2 eggs is singularly lowest in size-class 80, without compensatory increases in frequency of advanced-stage eggs, suggesting that the eggs produced by those females develop more rapidly than in other size classes. Clutch sizes produced by females in size-class 80 and 90 are high (Ros et al. 1981, Bert et al. 2016). The females in those size classes are abundant in populations (Sullivan 1979, Bert et al. 1986); in this study, they comprised 52% of all females captured. A high proportion of these females is below the minimum legal size for claw harvest (88-mm CW; Gerhart & Bert 2008); any fishery-related negative effects on reproduction (e.g., reduction in number of spawns per year) should be lower than in larger females. Thus the large number of females, high reproductive rate, and sublegal size of most individuals combine to make the reproductive contribution of females in these size classes important.

In size-class-20+30 females, the presence of females in gravid stage 3 illustrates that full ovarian development occurs, but the low frequency of ovigerous females and comparatively very high frequency-gravid:frequency-ovigerous ratio suggests that that the physical ability to spawn has not been fully established or that spawning is inhibited. The high frequency of gravid females in stage 2 (85%) compared with the frequencies of females in larger size classes in gravid stage 2 (65%–71%) suggests that ovarian development is protracted in precociously mature small females. The frequent molting in size-class-20 and -30 females (Gerhart & Bert 2008) might intermittently suspend full ovarian development and inhibit spawning because molting and spawning are antagonistic (Cheung 1969).

Interannual Variation

The multiple different interannual patterns of variation in frequency-gravid and frequency-ovigerous exhibited in female stone crabs are, at least in part, due to the influences of both the internal (molting) and external (temperature) factors investigated in this study. In some cases, these factors differentially influence females in different size classes.

The proportion of females with developing ovaries may fluctuate in multiyear cycles, suggesting the influence of factors that change only every few years. Annual variation in temperature is not among those factors. Annual frequencies of ovigerous females, which vacillated irregularly between values higher or lower than the overall mean, were also not related to interannual temperature variation; nor were they correlated with annual frequency-gravid. These interannual patterns merit further study.

The fluctuations in annual mean frequency-ovigerous that vary similarly for females in size-class 70 and 80 and females in size-class 90 and 100+ but differently between those two groups suggest that internal or external influences affect the frequencies of size-class-70 and -80 ovigerous females differently than they affect those frequencies in size-class-90 and -100+ females. The pattern of alternating above-average and below-average frequencies of ovigerous females in sequential years for females in size-class 90 and 100+ may be indicative of a metabolic stress associated with producing large egg clutches. A large majority of females in size-class 90 that molted in a given year would be in size-class 100+ the following year (Gerhart & Bert 2008). If frequency-ovigerous is an indicator of the magnitude of spawning activity in the female population, then a relatively high level of spawning activity in size-class-90 females in a given year is followed by a relatively low level of spawning activity by most of the same females, which are then in size-class 100+, the following year; and vice versa. These large females are old (Gerhart & Bert 2008); and clutch size is exponentially related to CW (Bert et al. 2016). It may be difficult for these females to spawn in high frequencies for two successive years because developing their disproportionally large egg masses extracts a physiological toll (Ituarte et al. 2004). In addition, size-class-70 females, and most females in size-class 80, are sublegal, whereas most females in size-classes 90 and 100+ have at least one legal-sized claw. Fishery effects may be contributing to the differences between size-class-70 and -80 females versus size-class-90 and -100+ females in interannual patterns of frequency-ovigerous. Thus, the observed interannual differences between females in these pairs of size classes could be due to senescence, fishery effects, both of these factors, a shift in energy allocation to extend lifespan (Bert et al. 2016), or as yet undefined physiological or biological factors.

Intra-annual Reproductive Patterns

The relationship between ovarian development, spawning, and molting in female stone crabs greater than or equal to 70-mm CW is typical of that exhibited by many tropical and temperate crab species in size classes where all females are mature (Heasman et al. 1985, Zimmerman & Felder 1991, Litulo 2004). A prolonged period of ovarian activity ceases when molting is imminent. Soon after molting and mating, which can promote ovarian development (Onyango 2002), the female carapace hardens and ovarian activity initiates again (Nelson 1991, Ituarte et al. 2004). The ovaries develop to an intermediate or advanced state and remain suspended there while environmental conditions (which usually include cooling water temperature) are unfavorable for spawning (Hinsch 1972, Heasman et al. 1985, Armstrong 1988, Nelson 1991, Liu & Li 2000, Hamasaki et al. 2004). As soon as environmental conditions (which often include warming water temperature) are favorable, the ovaries undergo final development in preparation for spawning (Zimmerman & Felder 1991, Ituarte et al. 2006). The ovaries maintain a high level of activity throughout the spawning season, which can permit multiple spawns in rapid succession (Nagao et al. 1996). The onset of less favorable environmental conditions triggers the cessation of spawning (Zimmerman & Felder 1991), arrest of ovarian development (Ituarte et al. 2004), and initiation of molting again.

Several lines of evidence indicate that, unlike the situation in many crab species (Nelson 1991, Ituarte et al. 2004), the ovaries of most female stone crabs are not suspended in some intermediate or advanced developmental state when ecdysis is impending, but are dormant. (1) No females with stage-3 claw or leg buds had developing ovaries; (2) in females greater than or equal to 70-mm CW, the correlation between frequency-gravid and frequency-molting is exponentially negative; and (3) intra-annual changes in frequency-gravid occur parallel to, but opposite of, changes in frequency-molting. The parallel relationship in females greater than or equal to 70-mm CW also indicates that ovarian development reinitiates almost immediately after molting. In contrast, frequency-molting and frequency-gravid are positively related with a 1-mo offset in frequency-gravid during peak reproductive season in size-class-30–50 females. This relationship suggests that initial gonad development begins approximately 1 mo after the maturation molt. The pattern of ovarian development, spawning, and molting exhibited by young stone crabs (<70-mm CW) has also been recorded for other crabs (Zimmerman & Felder 1991, Yoshino et al. 2002): ovarian development, spawning, and molting co-occur during the warm months of the year, as some of these females molt into maturity; and frequency-ovigerous increases principally near the end of the spawning season.

The first spring clutch maybe be the most important clutch of the season. The long ovarian development period during winter and early spring, when the ovaries of most females are in the advanced stage, may be a principal contributing factor to the production of larger, heavier eggs in the spring spawning pulse (Bert et al. 2016). Larvae produced early in the spawning season may take slightly longer to mature into postsettlement juvenile crabs because water temperature is lower (average: April, 23°C; May, 26°C) than optimal (30°C; Brown et al. 1992), but the resultant postsettlement juveniles would have a longer developmental period in warm water, which increases molting frequency, and thereby, growth (Brown et al. 1992). Collectively, these postsettlement juveniles could have a fitness advantage in the form of disproportionately high survival because they are the product of relatively large, heavy eggs (Shakuntala & Reddy 1982, Clarke 1993, Jaeckle 1995) and have a long growth season prior to winter.

The abrupt cessation of ovarian development during October may be facilitated by a gradual decrease in final ovarian development as the spawning season progresses. The ovaries of most mature females are in the advanced developmental stage 3 at the beginning of the spawning season. But as the demand for fully developed eggs proceeds through the spawning season, the proportion of females with ovaries in stage 3 decreases. It seems that depleted ripe eggs are replaced at a consistently slower rate. The small percentages of females gravid during the principal molting period have ovaries in the early developmental stage. A decrease in proportions of females with developed ovaries during molting season also occurs in *Sesarme* sp. (Zimmerman & Felder 1991).

Lastly, this study illustrates the need for data from many years to discern regional long-term reproductive patterns. Past short-term studies (even those conducted in the same latitudinal range–southern Florida) have varied considerably in the reported months of initiation and cessation of the principal spawning season and in the shape of the plot of frequency-ovigerous on month (Fig. 17 in Bert et al. 1986; Wilber 1989). Even greater interannual variation was recorded in this study, but the combined data revealed strongly coherent patterns for both frequency-gravid and frequency-ovigerous for size classes in which virtually all females were mature (≥ size-class 70) and indications of how the maturation of smaller females contributed to the observed overall pattern of variation in frequency-ovigerous among months. The lesser spawning peak in April or

May followed by a decrease in spawning activity seen during June results from some synchrony in gonad maturation during winter and in the initial spawning pulse during spring. The molting of some females in all size classes following this initial spawning pulse contributes to the temporary decrease in spawning during June. The molting into maturity of some juveniles in size-classes 20 through 60 throughout summer and production of multiple spawns at different times among mature females, due to variability in interim time periods between spawning events (Binford 1913, Porter 1960, McConaugha et al. 1980), contribute to the prolonged principal spawning peak from July through September.

The Role of Temperature

Water temperature has long been considered to be a vital factor in regulating reproductive seasons in marine invertebrates (Giese 1959), including crabs (Pillay & Ono 1978, Ituarte et al. 2004). But the generation under selection for temperature regimes is ambiguous. Temperature regulation of reproduction may be underpinned by selection for optimum survival of offspring (Thorson 1950, Giese 1959, Adiyodi 1988, Flores & Negreiros-Fransozo 1988, Nelson 1991). Spawning and hatching are timed so that zoeal broods are released during periods of environmentally benign conditions or when critical ecological factors such as temperature are advantageous. Alternatively or additionally, reproduction occurs during periods of time also advantageous for the spawning population (Emmerson 1994, Cobo 2002) because of the influences that both internal and external factors exert on adults (Sastry 1983, Dayakar & Rao 1992). Warm water and abundant food ease the stress of simultaneous physiological maintenance, production of eggs, preparation for molting, and in some cases, appendage regeneration. Heasman et al. (1985) found that temperatures at the peak of spawning season corresponded to those most conducive to both optimal egg incubation by maternal females and optimal larval development in *Scylla serrata*.

Temperature plays an important role in selection acting on reproduction in female stone crabs, in the form of selection for optimal survival of young life stages. The largest, heaviest eggs are produced during spring when temperatures are less than 27°C (Bert et al. 2016). Both larvae and postsettlement juveniles are most abundant when temperatures for survival are most favorable. Survival of Tampa Bay *Menippe mercenaria* larvae is high in the temperatures of midsummer (25–30°C; Brown et al. 1992) and of *M. mercenaria* postsettlement juveniles in the temperatures of early fall (15–25°C; Brown & Bert 1993). No study of stone crab larval abundance has been conducted in Florida, but based on the range of recorded clutch incubation times (7–18 days) and the spawning season, larvae should be abundant in May or June through September, when average temperature ranges 26–31°C. Postsettlement juvenile stone crabs are abundant at some time between July and November (T. M. Bert & C. Crawford, unpublished data), when average temperature decreases from 30°C to 23°C.

In stone crabs, spawning is more dependent on temperature than is ovarian development, which is tightly linked to molting. When seasonality is not considered, nearly all variation in frequency-ovigerous can be explained by variation in temperature. When seasonal temperature is considered, the dependency of spawning on temperature lessens; other factors—the most obvious being molting—also influence the timing of spawning. The most striking temperature effects are the strong shifts in frequency-ovigerous at particular temperatures. In mature females of all sizes, both ovarian development and spawning precipitously decrease as temperature falls from 29°C to 25°C and decline to near-zero frequencies at 25°C. This pronounced and abrupt cessation of reproduction indicates that 25°C is a threshold temperature at which the annual ovarian development and spawning cycles are "reset." Some measure of day length or luminescence likely also contributes to the initiation of this reproductive pause because 29°C is not an uncommon temperature during summer. Below 25°C, ovarian development immediately commences again and builds to high frequencies, but spawning is essentially terminated for the winter. The eggs of the few females carrying clutches below this temperature are principally in stage 4.

Other temperatures also initiate shifts in female reproduction (Table 8). In all size classes, frequency-ovigerous essentially decreases to zero as temperature decreases to 20°C; and after an end-of-season pulse at 16–18°C, molting ceases when temperature descends below approximately 15°C. Spawning does not resume until temperature increases from winter lows to 18°C; whereas females molt in low frequencies through winter as long as temperature remains higher than approximately 15°C. When temperature rises to 23°C during spring, frequency-gravid decreases significantly in size-class-60 females due to the entry of newly molted females into that size class, whereas frequency-ovigerous increases significantly in females greater than or equal to 60-mm CW. High levels of spawning are sustained until temperature again decreases from summer highs to 29°C, when the reset process begins again. Thus, multiple aspects of stone crab reproduction seem to undergo phase shifts at specific temperatures. If temperature's influence on reproductive seasonality is as important at the northern distributional limits of *Menippe* as it is in Tampa Bay, then shifts in ovarian developmental and spawning seasons can be expected as climate change proceeds. The biological and ecological outcomes of these shifts are unpredictable.

Fishery Implications

Females in size-classes 100 and 110 have several reproductive characteristics that suggest a reduction in reproductive output. (1) Frequency-ovigerous increases with size class through size-class 90, but decreases in size-class 100+. (2) Mean clutch size increases exponentially as size class increases through size-class 100, but decreases in size-class 110. A reduction in clutch size in the largest size class was also recorded for female *Menippe mercenaria* from Cuban waters (Ros et al. 1981). (3) There is a trade-off between egg size and clutch size in female stone crabs, but mean size of eggs produced by females in size-class 110 does not increase to compensate for the decreased clutch size (Bert et al. 2016). (4) When the frequency of size-class-90 ovigerous females is above the long-term average, the frequency of size-class-100+ ovigerous females (many of which are the same as those in size-class 90 of the previous year) is below average the following year, and vice versa, suggesting that, as a group, these large females may be capable of high levels of spawning only every other year. (5) Through the 22 y of the study, frequency-ovigerous decreased in females of size-classes 70 through 100+, and the rate of decrease in size-class-100+ females

TABLE 8.

Temperature-specific changes in frequencies of reproductive states in female stone crabs (genus *Menippe*).

Temperature, month*	Size class	Condition	Change
18°C, February	All	Spawning	Initiates
23–24°C, April	60	Spawning	Plateaus until temperature reaches summer highs (approximately 30°C)
	70 through 100+		Accelerates to summer high levels
	60 through 100+	Molting	Initiates temporary but significant increase
27°C, late April to May	70 through 100+	Molting	Peak in temporary spring increase
30°C, July	60 through 100+	Molting	Initiation of low-frequency period
30–28°C, July to September	20+30 through 60	Ovarian development, spawning	Attain highest levels
	40, 50	Molting	Attains highest level
29°C, September	All†	Ovarian development, spawning	Initiation of precipitous decline
27°C, September	20+30 through 50	Molting	Initiation of low-frequency period
	60 through 100+		Initiation of high-frequency period
25°C, October	All	Ovarian development, spawning	Simultaneous decrease to zero or near-zero frequency; ovarian development reinitiates immediately
20°C, November	All	Spawning	Ceases, except for occasional spawning by very few females
17–15°C, December	All‡	Molting	Last molting pulse before winter; temporary cessation of molting below these temperatures

* The time period listed is the period during which the specified temperature(s) occur most frequently.

† The temperature interval for sharp decline in ovarian development and spawning for females in size-classes 20+30 through 50 may be 26–27°C.

‡ The temperature interval for the last molting pulse of the season may be 20–19°C for size-class-20+30 females.

was more than double that of smaller females. Characteristics 1–4 support Gerhart and Bert's (2008) suggestion that females in size-classes 100 and 110 are partially senescent; but those females are also heavily impacted by the fishery. Fishery-related physiological stress also could be contributing to the decreases in spawning seen in these females. Declawing negatively affects both ovarian development and spawning in several ways (T. M. Bert, unpublished data). If both claws are legal sized, both can be taken; and both claws are legal sized in females 102-mm CW or larger (regression analysis, total-examined data set). Stone crab traps capture and retain the largest crabs in a local population (Bert 1985). Thus, the largest crabs in a local population would be most affected by fishery operations. Fishers have commercially trapped stone crabs in Tampa Bay for over 30 y (T. M. Bert, personal observation) and the frequency of size-class-100+ females with claws missing is high (approximately 30%, with 22% missing both claws; this study, total-examined data set). In addition to, or rather than, senescence or age-related physiological stress, the decreases observed may be due to the fishery. The lesser decreases in frequency-ovigerous through the years in size-classes 70 through 90 also may be due in part to fishery effects, as well as other factors.

In females, two consequences of intensive fishing for decades are a reduction in size-at-maturity and a smaller minimum maturation size (Dieckmann & Heino 2004). These reductions in maturity size result from selective removal of legal-sized (usually the largest) animals in the local population through harvesting, and they can occur within decades (Conover & Munch 2002). Selection favors individuals that reproduce prior to attaining legal size; i.e., individuals that reproduce at a small size (Dieckmann & Heino 2004). Such selection can reduce the reproductive potential of the population (Kuparinen & Merilä 2007). Fishery-induced reduction in size at maturity has been well documented for fishes, but little work has been done for invertebrates (Fenberg & Roy 2008, Sharp & Hendry 2009).

Selection for reduction in size at maturity may be occurring in stone crabs inhabiting Tampa Bay. Through the years of this study, frequency-ovigerous increased in females of size-classes 50 and 60—the two size classes that include the size range within which females are maturing. Thus, as the proportion of spawning females has generally decreased in large adults, and particularly in those of largest size, the proportion of spawning females in the size range of maturation has increased, particularly in the size class in which maturation is initiating (size class, percentage increase, respectively: 50, 33; 60, 6).

The minimum size at which females spawn also may be decreasing through the years. The smallest ovigerous female recorded from Florida Gulf waters between 1950 and 1980 was 36.9-mm CW (Savage & Sullivan 1978), and the average of the minimum sizes reported in the four studies in which minimum size of ovigerous females was recorded during that period was 46.3-mm CW (McRae 1950, Savage & Sullivan 1978, Sullivan 1979, Bert et al. 1986). During this study, a gravid female of 26.4-mm CW and an ovigerous female of 27.4-mm CW were collected (1996 and 2005, respectively), and the average size of the 10 smallest ovigerous females was 34.8-mm CW. Our sample size was much larger than that of the other studies; but altogether, the collective sample size for the other studies was substantial (n = 7,982). Moreover, those studies ranged from waters off the Cedar Keys in northwestern Florida to the Florida Keys, including Tampa Bay; and the size ranges of females varied greatly among and within studies. Fishery-related reductions in size-at-maturity are most pronounced in species that are several years of age before they mature (Haug & Tjernsland 1986, Bowering & Brodie 1991, Rijnsdorp 1993, Trippel 1995, Rochet 1998, Law 2000, Grift et al. 2003); most female stone crabs mature at ages 2 or 3 (Gerhart & Bert 2008). Similarly documented changes elsewhere in the Florida Gulf would support the idea that prolonged fishing pressure is reducing size-at-maturity and minimum spawning size in female stone crabs.

The stone crab harvesting season overlaps with peaks in the molting-and-mating season in fall, the ovarian development season throughout winter and spring, and both the spawning and molting-and-mating season in spring (see Figs. 7, 8, and 10; Bert 1985). Thus, the season does not protect females during the potentially important first spring spawning pulse, and it overlaps with both the spring and fall peaks in molting and mating. It is illegal to remove the claws of an ovigerous female, but this regulation is difficult to enforce because on-board monitoring of every fishery operation would require great effort and expense; and after harvest, the claws from ovigerous females cannot be differentiated from those of non-ovigerous females. Protection of female stone crabs during the time when the first spring spawn is produced could be achieved by shortening the harvest season by 6 wk in spring. The number and proportion of ovigerous females in traps is high during that time (Bert et al. 1986, Florida Fish and Wildlife Research Institute, unpublished data), and nearly one-half of those females can be ovigerous or molting (Sullivan 1979, Bert et al. 1986). The regulation against harvesting the claws of ovigerous females, coupled with the low market value of claws from recently molted crabs (T. M. Bert, unpublished data), markedly reduce the harvest and its value during this time. Ending the harvest season by March 31 rather than May 15 would be an effective way to separate the harvest season from the initiation of spawning season and the secondary molting season.

ACKNOWLEDGMENTS

We thank A. McMillen-Jackson, K. McCarthy, and R. Hochberg for years of dedicated field work; we also thank numerous other FWRI employees and volunteers who helped with field work through the years. We are grateful to R. Gandy and an anonymous reviewer for reviewing the manuscript. This work was supported by the Department of Commerce, National Oceanic and Atmospheric Administration (PL 88-305 and PL 99-659) and by the State of Florida.

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