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SIZE AT MATURATION, SPAWNING VARIABILITY, AND FECUNDITY IN THE QUEEN CONCH, *ALIGER GIGAS*[§]

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ABSTRACT: The queen conch (*Aliger gigas*) resource is one of the most important in the Caribbean. While aspects of queen conch reproduction have been studied, e.g., size-at-maturity, spawning season, and density-based Allee effects, there is little information on other important aspects. From 210 lipped queen conch collected off southwest Puerto Rico, histological examination of gonads showed that 50% maturation occurred at 9 mm lip thickness. Experimental caged queen conch held on a natural spawning ground were monitored across the spawning season to evaluate fecundity and its variability across individuals and between nominal density treatments (2,000 vs 143 conch/ha). Near daily monitoring identified all egg masses to specific females, and all egg masses were retained to calculate the number of eggs. Conch in the low-density treatment produced more and larger egg masses over a longer spawning season than those in the high-density treatment. Within each density treatment, individual fecundity varied by a factor of 6. The maximum fecundity estimated was 22 million, the maximum number of egg masses spawned was 25, and the largest single egg mass contained 1.48 million eggs. Variability in fecundity was largely driven by length of the individual spawning season, but this may in turn have been dependent on the degree of maturation of females at the start of the spawning season. These results emphasize the importance of allowing queen conch to mature and further grow in lip thickness to ensure sufficient spawning to sustain reproductive capacity. This experimental approach could be used to assess variations in fecundity based on size (length, biomass) and age (lip thickness).

KEY WORDS: 50% maturation, Egg production, Lip thickness effects, Copulation

INTRODUCTION

The queen conch, *Aliger gigas* (Maxwell et al. 2020), is one of the most important marine resources in the Caribbean region, which supports industrial and artisanal fisheries that supply local consumption and export markets, and it is a cultural icon in coastal communities (Prada et al. 2017). Following severe stock declines into the 1990s, queen conch became listed in 1992 under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Yet, despite its importance and dedicated efforts for its culture (see review in Stoner 2019) relatively little is known of its reproduction. Following copulation, queen conch females can store sperm for at least 6 weeks (D'Asaro 1965) and can spawn multiple egg masses during the spawning season, generally depositing them on clean coarse sand. A single egg mass can contain over 400,000 eggs (Robertson 1959, Buckland 1989), and conch held in a natural enclosure spawned an average of 9.4 egg masses/female over a single spawning season (Davis et al. 1984).

The importance of understanding queen conch reproduction for its fishery management is primarily driven by two issues. First is the manner and intensity of fishing in relation to the queen conch need of copulation for fertilization, and therefore maintaining its density is important (Appeldoorn 1995, Appeldoorn et al. 2011). Reproductive activity declines as density declines, with a pronounced Allee effect (Stoner

and Ray–Culp 2000, Stoner et al. 2012a). Second is the relationship between queen conch size and effective fecundity. Size of adults becomes fixed shortly after they produce the flared shell lip (Appeldoorn 1988). As adults age, their shell thickens and the internal volume and tissue biomass declines (Stoner et al. 2012b) suggesting that fecundity, or at least the size of individual egg masses, may decline with age. In long-lived, iteroparous species, such as queen conch, life history theory predicts that reproductive effort should increase with age as long as growth (in size or reproductive efficiency) increases with age (Charlesworth and Leon 1976). For this to hold for queen conch, any decline in size must be offset by an increase in reproductive efficiency. At present it is unknown whether reproductive efficiency in queen conch increases with age, but if it does management strategies should target the preservation of older individuals. To determine this, a first step might be to ascertain what aspects of reproduction (e.g., size or number of egg masses, frequency of spawning, individual length of the spawning season, density or size of eggs within egg masses) are subject to variation and therefore potentially altered to enhance reproductive efficiency. Furthermore, significant declines in adult size have been documented (Tewfik et al. 2019), and assuming fecundity is proportional to biomass, these smaller sizes will result in a permanent reduction in egg production.

[§]This article is based on a presentation given in November 2019 at the 72nd annual Gulf and Caribbean Fisheries Institute conference in Punta Cana, Dominican Republic.

The purpose of this paper is to determine the relationship between size, maturation and the individual variability in female productivity of queen conch, *Aliger gigas*, in La Parguera, in the southwest of Puerto Rico. Size was measured as the lip thickness of the flared shell lip of adult conch, and reproductive characteristics examined include length of the spawning season, spawning (deposition) rate, number of eggs per mass, number of eggs/g of egg mass, and total fecundity.

MATERIALS AND METHODS

Size at Maturation

To estimate size at maturation, 210 individuals with flared lips ranging from 4–25 mm in thickness were collected during the spawning season on the insular shelf off La Parguera (17 m depth), in the south west of Puerto Rico, generally in the area studied by Appeldoorn (1987). Following Appeldoorn (1988), all individuals were measured for shell length (maximum distance from the apex of the spire to the tip of the siphonal groove), lip thickness (measured 35 mm in from the lip margin), both to the nearest 1 mm, and for whole live weight and whole tissue weight (i.e., without the shell), both to the nearest 1 g. Gonad samples were removed, preserved in Davidson's solution for 24 h, and prepared for histological examination by dehydrating in 95% ethanol and embedding in paraffin. Sections of 6–10 μm thickness were cut and mounted on albuminized slides and stained with hematoxylin and eosin according to Harris' regressive method (Howard and Smith 1983). Stages of gonad development for males and females followed that of Egan (1985), with individuals in Stages II and higher (i.e., Developing, Ripe, Spent) being considered mature. This is equivalent to Stages 3 and higher used by Delgado et al. (2004) and Bowman et al. (2018).

The proportion of mature individuals was calculated at 2 mm intervals of lip thickness for analysis. Two models were used to estimate the proportion mature versus lip thickness. One was the logistic model as recommended by Boman et al. (2018), with the upper asymptote fixed at 1 (i.e., 100% mature):

$$\text{(Equation 1): } PM = 1/(1+(1/a - 1)^{-b \cdot LT})$$

where PM = proportion mature, LT = lip thickness, and a and b are the estimated model parameters. However, inspection of the data suggests that the logistic model might give unrealistic projections, especially at thin lip thicknesses where histological studies have shown no evidence of concomitant gonadal development (Egan 1985, Buckland 1989). Alternatively, because the logistic model is symmetric, forcing the model to go through the origin would cause substantial distortions at higher lip thicknesses. This may be because the starting point is lip formation, and not actual age, which can be variable at the time of lip formation. Consequently, a second model was used that does not assume an inflection point, which was a decreasing exponential:

$$\text{(Equation 2): } PM = a(1 - \exp(-b(LT - c)))$$

where a , b , and c are the estimated model parameters. Both models were fit via nonlinear regression using the nonlinear

least squares (nls) regression procedure in R (RStudio Team 2018). The resulting models were used to calculate the lip thickness at 50% maturation.

Spawning and Fecundity

Queen conch with flared shell lips were held in 2 large adjacent enclosures located in 18 m (60 ft) of water off La Parguera. The specific area was a sand–algal plain located off the forereef of San Cristobal, an emergent coral reef platform. The area has a clean, coarse sand bottom interspersed with patches of rubble and benthic algae. Prior observations over several spawning seasons have shown the area to be a focus of spawning activity. The enclosures were bounded by walls made of 2.5 mm square, vinyl–coated wire mesh fencing. The fencing was cut into short lengths (2–3 m) to facilitate handling. Fence panels were folded longitudinally to form a double wall about 40 cm high and 35 cm wide at the base. The base was buried into the sediment and the panel was held in place by hooks made of re–enforcing rod driven into the sediment until the hook pressed down on the top of the fence. This low profile and double wall arraignment provided a strong framework with little vertical structure exposed to currents and wave surge. The double wall structure also prevented direct contact among conch between the adjacent enclosures.

One enclosure was 200 m^2 (~14 x 14 m) while the second enclosure had boundaries twice as long (~28 x 28 m) and enclosed 770 m^2 , sharing one side with the smaller enclosure. There was no discernable difference in the benthic habitat characteristics between the 2 enclosures. These 2 enclosures constituted the nominally high–density and low–density treatments, respectively.

All conch used in the experimental enclosures were captured from the field from the same broad area as those for the maturity study, about 3.2 km from the enclosures. All conch were brought to the laboratory for processing, where they were maintained in a 5 m diameter tank with natural bottom consisting of clean coarse carbonate sand with a slight growth of algae and benthic diatoms. All individuals were tagged (Appeldoorn 1987) and then measured as above for shell length, shell lip thickness, and live weight. Each individual was turned on its side and, when extending the foot to right itself, observed for the presence of a verge or egg groove to determine sex. Processing time was variable but limited to a few days.

Other than with respect to sex, individuals were allocated to treatment haphazardly. Forty individuals were added to the high–density treatment, with an equal number of males and females, yielding a final density of 1 conch/5 m^2 , or 2,000/ha. This approximated the density of conch in the breeding enclosure reported by Davis et al. (1984). In the low–density treatment, 10 females and one male were added, yielding a final density of 1 conch/70 m^2 , or 143/ha. While the two treatments were meant to compare the largest difference in density possible, the functional design of the enclosures was driven by 2 constraints. The first was that the area and number of conch had to be small enough to survey in one dive (~60 min). The second constraint was that a sample size of 10 females was con-

sidered the lowest number that could be used and still have a representative number. As such, this design was not meant to replicate actual spawning conditions, either with respect to densities in natural spawning areas or in allowing the degree of movement that conch may undergo during the spawning season (e.g., Stoner and Sandt 1992).

The enclosures were stocked on 14 May 1991 and spawning activity was monitored as close to every day as possible over the 5-month season, with gaps driven by weather and mechanical problems. During surveys, all individuals were accounted for, and all instances of egg deposition (spawning) were recorded to individual. Because it takes 24–36 h to produce an egg mass (Randall 1964, D'Asaro 1965) it is thought that nearly all egg masses produced were recorded. All instances of copulation were recorded to individual; however, it is assumed that copulation is a relatively short process such that only a small proportion of daily copulations were observed during the survey period. Surveys always took place at the approximate same time each morning. Bottom water temperature was measured to the nearest 0.1 °C with a mercury thermometer. After terminating the experiment (23 October), all individuals were reweighed and measured as above; they were then sacrificed and weighed to obtain measures of shell weight and tissue weight.

To determine the total number of eggs spawned per egg mass, each egg mass observed being spawned was marked without disturbing the depositing female. The following day, the egg mass was collected and brought to the laboratory where it was first blotted dry and then wet weighed, including attached sand particles. A single subsample was taken, similarly weighed and the number of eggs counted. The resulting eggs/g was then multiplied by the total weight of the egg mass to get total estimated number of eggs. Furthermore, the percentages of infertile or moribund egg capsules were noted. Lastly, the egg masses were retained in aquaria to monitor hatching success. These data were then used to calculate the overall fecundity of each female over the reproductive season.

Comparisons of means (among groups or individuals) were conducted using either a Student's *t*-test or analysis of variance (ANOVA) models. All tests assumed normality; any suspected variations were verified by calculating skewness and kurtosis and testing for significance following Zar (1999). All tests assumed homogeneity of variance (confirmed with Levine's test), except for comparison of mean total fecundity/female between the low- and high-density treatments, when a Student's *t*-test for unequal variances was used. Trends were analyzed using linear least squares regression. Comparison of trends (rates of egg production among females; slopes) was done using analysis of covariance comparing slopes (Zar 1999) among treatments with tissue biomass as the covariate. The relationship between lip-thickness and total fecundity used log-transformed data, assuming an allometric relationship between linear and biomass parameters (Gould 1966). For all analyses, the significance level was $p \leq 0.05$ unless stated otherwise.

RESULTS

Size at Maturation

The proportion mature within the 2 mm size categories ranged from 0.22 to 1, gradually increasing with increasing lip thickness. Number of individuals within each size category ranged from 3 to 41, with the smaller numbers associated with the thinner size categories. Only lip thickness categories above 23 mm showed 100% maturation. Both models resulted in statistically significant regressions (Table 1, Figure 1), yield-

TABLE 1. Parameters and standard errors (se) for 2 models relating the proportion of mature queen conch to the thickness of the shell lip.

Model	Parameter	Estimate	se
Logistic	a	0.13228	0.07533
	b	0.23316	0.0634
	Lip thickness at 50% maturation (mm)		8.07
Decreasing Exponential	a	0.99795	0.11335
	b	0.1567	0.07379
	c	3.29222	1.37303
	Lip thickness at 50% maturation (mm)		7.73

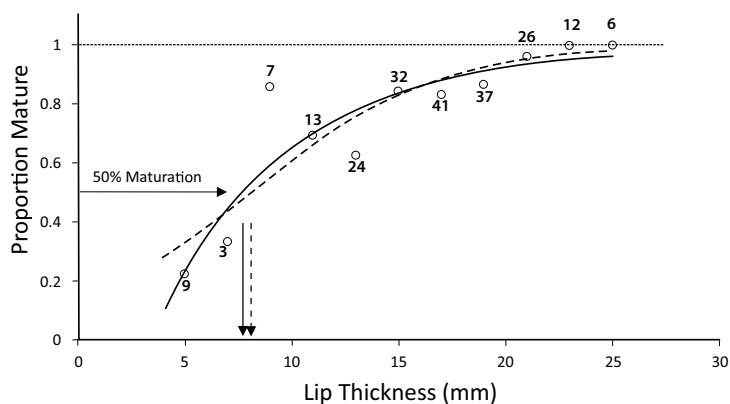


FIGURE 1. Proportion mature of queen conch versus lip thickness within 2-mm size classes. Numbers give the number of conch in each size class. Predicted relationships are given for the logistic (dashed line) and decreasing exponential (solid line) models. Vertical arrows indicate the predicted lip-thickness at 50% maturation for each model, respectively. Dotted horizontal line indicates 100% maturation.

ing estimates of lip thickness at 50% maturation of 8.1 mm (logistic) and 7.7 mm (decreasing exponential).

Spawning and Fecundity

Females were observed spawning in both treatments; however, important differences were observed (Table 2). In the low-density treatment, all females were observed to spawn, while in the high-density treatment, only 12 of the 20 females were observed to spawn. There was no difference in mean initial lip thickness between spawning females in the 2 treatments, but mean lip thickness growth of spawning females in the high-density treatment (6.8 mm) was greater (unequal variance Student's $t = 2.813$, $p = 0.012$) than in

TABLE 2. Comparison of mean (\pm sd) queen conch reproductive output per female in nominally low-density ($1/70\text{ m}^2$) and high-density ($1/5\text{ m}^2$) treatments. Means in the high-density treatment include only those females that spawned at least once. *Values excluding female 4F5, which only produced 2 egg masses 47 days apart (see Table 3).

Treatment	Females	Males	Spawning Females	N masses	Interval	Total eggs	eggs/mass	Egg/g
Low Density	10	1	10	13.60 \pm 7.81	6.40 \pm 1.57	10,061,389 \pm 7,348,522	677,726 \pm 169,432	12,332 \pm 1,576
Low Density	9*	1	9*	14.890 \pm 7.06	6.40 \pm 1.57	11,084,509 \pm 6,998,190	705,623 \pm 153,428	12,129 \pm 1,5276
High Density	20	20	12	5.83 \pm 4.67	8.08 \pm 3.25	3,097,878 \pm 2,371,570	532,313 \pm 131,767	15,437 \pm 3,188

the low-density treatment (3.8 mm). Similarly, in the high-density treatment there was no difference in mean initial lip thickness between those females that spawned and those that did not (Table S1), but mean lip growth among the spawning females was 70% greater compared to non-spawners (Student's $t = 1.858$, $p = 0.080$). Excluding female 4F5, which only produced 2 smaller egg masses 47 days apart, spawning females in the low-density treatment had on average a higher number of egg masses/female (13.6; Student's $t = 2.762$, $p = 0.015$), higher number of eggs/mass (677,000; Student's $t = 2.180$, $p = 0.044$), and a higher overall fecundity/female (10 million; unequal variance Student's $t = 3.274$, $p = 0.008$). While the interval between egg mass deposition was shorter in the low-density treatment, it was not statistically significant. In contrast, egg masses in the high-density treatment had significantly more eggs/g of egg mass (Student's $t = -2.868$, $p = 0.012$).

There was substantial variability in the cumulative egg production among spawning females within treatment with respect to individual length of the spawning season, number of egg masses, and total fecundity (Figure 2, Table 3). The largest single egg mass (1,649,336 eggs) was produced 19 September by female 4F9, while the highest fecundity (22 million) was registered for female 4F1. These 2 females produced 41% of all eggs spawned in the low-density treatment. Overall fecundity

is driven largely by the length of the spawning season (Figure 2A). Once a female starts producing egg masses on a regular basis, its rate of egg production appears to be fairly constant. However, differences in the rate of egg production among females were observed (ANCOVA, $F_{5,106} = 198.8$, $p < 0.001$) among the top 6 producing females; 4 produced eggs at a similar sustained rate (mean = 176,198 eggs/day), while female 4F9 was more productive, averaging 214,686 eggs/day, and 4F8 was less productive, averaging only 107,353 eggs/day.

In the low-density treatment there was a positive relationship ($r^2 = 0.5031$, $F_{1,8} = 8.10$, $p = 0.022$) across females between the number of egg masses spawned/female and the mean number of eggs/mass, but a negative correlation ($r^2 = 0.6995$, $F_{1,7} = 16.30$, $p = 0.011$) between the number of egg masses spawned and the mean interval between egg masses (Figure 3). In the high-density treatment there was also a negative relationship between number of egg masses spawned/female and mean interval between eggs/mass ($r^2 = 0.5777$, $F_{1,8} = 10.95$, $p = 0.011$). However, the trend between the number of egg masses spawned/female and the mean number of eggs/mass was negative, but this was not statistically significant.

Within the low-density treatment, there was a significant difference among females in the estimated number of eggs/g of egg mass (ANOVA; $F_{9,126} = 3.224$, $p = 0.001$). Furthermore, the mean number of eggs/g produced by a female (E/g) was

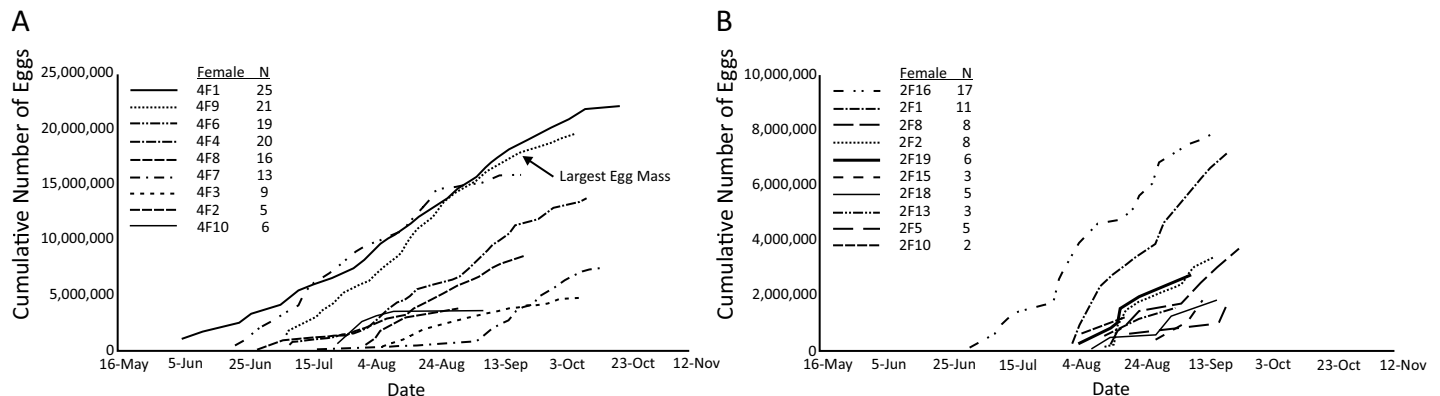


FIGURE 2. Cumulative egg production of individual female queen conch over the course of the spawning season. A. Low-density treatment ($1/70\text{ m}^2$). Not plotted is female 4F5, which only produced 2 egg masses 47 days apart. B. High-density treatment ($1/5\text{ m}^2$). Females producing only one egg mass (2F6, 2F11) are not plotted. Note change in vertical axis relative to low-density treatment. N = number of egg masses spawned.

TABLE 3. Reproductive output throughout the spawning season of individual queen conch females from low-density (1/70 m²) and high-density (1/5 m²) treatments. Spawning interval = days. Lip = shell lip thickness. Weights are from the end of the spawning season. ● = eclosion occurred before egg number could be estimated.

Female	Egg masses	Eggs/mass		Spawning Interval		Total Fecundity	Eggs/g		Length (cm)	Lip (mm)		Live Wt (g)	Tissue Wt (g)
		Mean	sd	Mean	sd		Mean	sd		Start	End		
Low-Density Treatment													
4F1	25	881,231	276,482	5.75	2.54	22,030,779	10,029	2,608	24.6		17	2,627	430
4F2	5	756,225	212,334	7.25	6.85	3,781,127	14,227	1,546	24.1	14	18	2,405	424
4F3	9	532,224	219,529	7.63	3.07	4,790,014	11,937	1,761	24.8	10	16	2,145	412
4F4	20	700,670	230,265	5.00	3.62	14,013,409	13,790	2,911	25.0	16	19	2,405	442
4F5	2	426,654	71,298	47.00		853,307	14,154	6,040	24.6	5	11	2,106	468
4F6	19	833,375	335,451	5.00	3.25	15,834,127	10,613	2,967	23.3	17	19	2,279	396
4F7	13	580,418	279,300	7.58	8.60	7,545,436	13,092	2,765	24.4	16	19	2,426	534
4F8	16	535,775	262,042	5.60	4.42	8,572,394	10,303	2,335	24.5	11	17	2,833	542
4F9	21	933,945	389,722	4.60	2.76	19,612,842	12,393	4,850	27.9	14	16	3,153	670
4F10	6	596,742	417,052	9.20	10.57	3,580,455	12,777	4,158	27.0	11	14	2,913	676
Mean ±	13.60±	677,726±		6.40±		10,061,389±	12,332±		25.0±	12.7±	16.5±	2,529±	499±
sd	7.81	169,432		1.57		7,348,522	1,576		1.4	3.9	2.4	345	104
High-Density Treatment													
2F1	11	651,099	206,419	4.80	2.10	7,162,094	11,351	1,823	24.5	14	17	2,477	504
2F2	8	431,705	224,291	5.00	3.96	3,453,638	19,313	2,993	26.4	15	20	2,802	452
2F5	5	398,159	227,744	10.75	9.39	1,592,636	17,142	4,135	25.3	3	16	2,083	390
2F6	1	795,675				795,675	19,671		26.3	5	15	2,535	432
2F8	8	464,378	184,673	5.71	3.64	3,715,025	14,010	3,770	27.5	17	21	2,828	464
2F10	2	634,966	35,102	14.00		1,269,933	10,257	315	25.0	14	19	2,406	366
2F11	1	●				●	●		26.5	8	17	2,558	448
2F13	3	534,755	121,040	11.50	0.71	1,604,266	15,664	2,547	23.3	11	18	1,865	364
2F15	3	642,144	206,962	7.50	3.54	1,926,431	17,280	8,029	24.7	13	25	2,720	438
2F16	17	464,810	324,297	4.75	2.67	7,901,776	13,698	4,619	23.8	12	18	2,432	398
2F18	5	371,328	266,444	9.75	4.92	1,856,640	13,105	3,127	23.5	11	15	2,019	342
2F19	6	466,424	218,253	7	6.16	2,798,545	18,318	4,607	26.0	15	20	2,461	472
Mean ±	14.89±	705,623±		6.40±		11,084,509±	12,129±		25.2±	11.6±	18.4±	2,432±	423±
sd	7.06	153,428		1.57		6,998,190	1,527		1.3	4.1	2.9	305	50

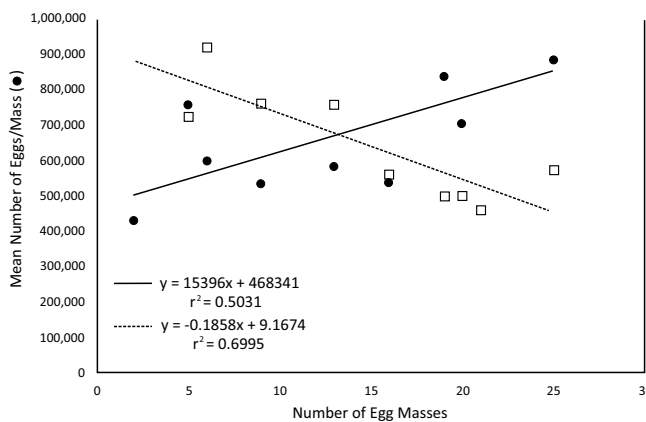


FIGURE 3. Relationship between the number of egg masses spawned per female and (1) the mean number of eggs/egg mass (left axis, solid circles, solid line) and (2) the mean number of days between egg masses (right axis, open squares, dashed line) for queen conch females in the low-density treatment (1/70 m²). Female 4F5 is excluded from the latter relationship; time between the 2 egg masses was 47 days.

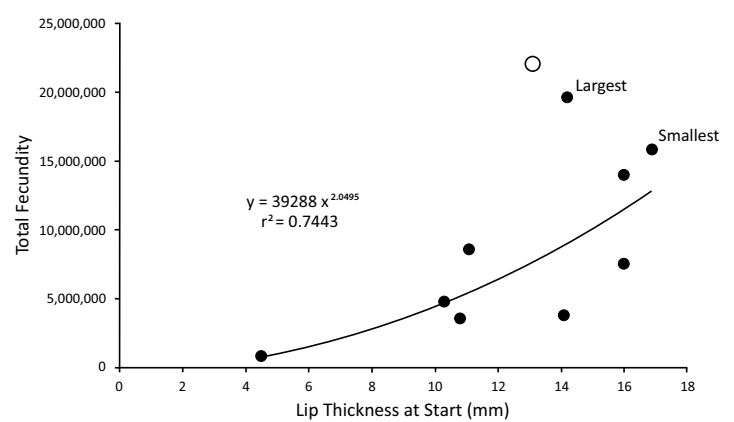


FIGURE 4. Relationship between shell lip thickness at the start of the spawning season and the total fecundity (eggs spawned) over the spawning season for queen conch in the low-density treatment (1/70 m²). Fitted line is a quadratic equation. Largest and Smallest refer to the tissue weights at the end of the season. Not included is female 4F1 (open circle), for which initial lip thickness was not available; position is estimated from the lip thickness at the end of the experiment minus the mean lip growth rate within the treatment.

negatively associated with the number of egg masses spawned per female (N_{EM}): $E/g = 14,069 - 127.8 * N_{EM}$ ($r^2 = 0.40$, $F_{1,8} = 5.348$, $p = 0.049$). Additionally, there was a significant posi-

tive nonlinear relationship ($F_{1,7} = 2.037$, $p = 0.003$) between shell lip–thickness at the start of the experiment and total fecundity (Figure 4). Unfortunately, the initial lip thickness

measure for the most fecund female was missing. The residuals from the regression were not related to the tissue weight.

In the high-density treatment, 43 copulations were observed, involving 12 of the 20 males. The most copulations observed for an individual male was 11. The number of copulations per male (N_{cop}) was negatively correlated with the date when first copulation was observed (Date): $N_{\text{cop}} = 2806 - 0.0838 * \text{Date}$ ($r^2 = 0.68$, $F_{1,10} = 21.53$, $p < 0.001$) indicating that, as with females, males with a longer reproductive season had more copulations overall. However, this trend was largely driven by 4 males observed to start copulating in early June. The other 8 males (13 copulations total) were not observed to copulate until late July or August. The number of females a male copulated with increased with the number of copulations per male observed; roughly 70% of an individual male's copulations were with different females. The number of egg masses produced per female was positively correlated ($r^2 = 0.585$; $F_{1,17} = 23.96$, $p < 0.001$) with the number of copulations (Figure 5). Notably, 3 females observed to undergo copulation never produced any egg masses. The lone male in the low-density treatment had a total of 14 observed copulations among only

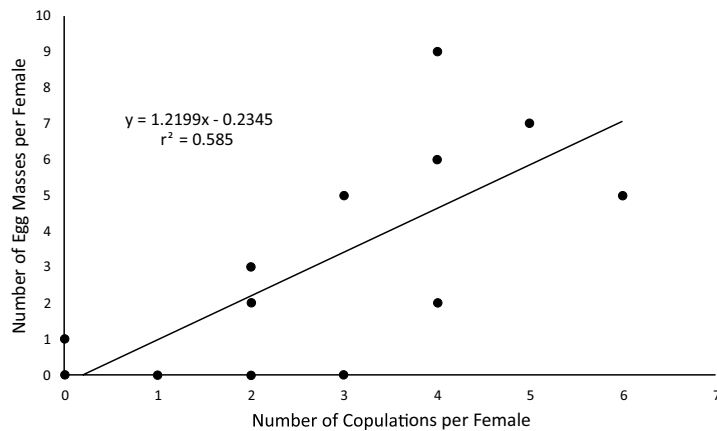


FIGURE 5. Relationship between the number of copulations observed per female queen conch and the number of egg masses produced per female for all females in the high-density treatment (1/5 m²).

6 of the 10 females, with all but one of these females having been observed copulating twice.

DISCUSSION

Estimates of lip thickness at 50% maturation in queen conch were similar for both models at 8 mm. However, this probably represents an underestimation due to the effect of variability introduced by low numbers of observations in the small size classes, which disproportionally affect the degree of curvature and, hence, the point of 50% maturation. In particular, the value for the 9 mm size class, with only 7 individuals, seems high relative to the overall trend. This would tend to raise the lower end of the regression lines, leading to a lower lip-thickness estimate of 50% maturation. Eliminating this point increases the estimate about 1 mm in both models. Thus, a more conservative estimate would be to assume 50%

maturation at 9 mm lip thickness. This value is consistent with the range of values reported by Boman et al. (2018), who also obtained a mean lip thickness at 50% maturation of 9 mm applying the logistic model using data across 7 sites.

Management regulations in Puerto Rico require either a minimum length of 9 inches (22.9 cm) or a lip thickness of 3/8 inches (9.5 mm). While this minimum lip thickness is similar to the observed value in this study, if the management goal was to allow half the population to become mature and spawn before being harvested, the current regulations would fall substantially short for 2 reasons: (1) the minimum length limit allows large juveniles to be harvested before maturation, and these would be expected to become the most fecund due to their larger size; (2) this minimum lip thickness measure does not account for the fact that females spawn multiple times over the spawning season, while also increasing in lip thickness (an average of 3.8 mm in the low-density treatment) so that a full season of spawning would not be guaranteed.

The enclosures and stocking densities were not designed to mimic natural conditions (other than to be located on a natural spawning ground), and this could have affected observed results. However, the low-density treatment, at 143 conch/ha, was well within the range of densities within spawning sites reported by Stoner and Ray-Culp (2000), Stoner et al. (2012a) and Delgado and Glazer (2020). Indeed, this density was lower than the mean density reported by Stoner and Ray-Culp (2000; 209 conch/ha) and Delgado and Glazer (2020; 610 conch/ha). Although the high-density treatment, at 2,000 conch/ha, is well above average reported values, it still is less than the maxima reported by Stoner and Ray-Culp (2000) and Delgado and Glazer (2020), at 2,293 and 3,133 conch/ha, respectively. In these latter studies, a high proportion of conch was engaged in reproductive activity (spawning, copulating, pairing) at these extreme densities.

Perhaps a more significant factor differing between the enclosures and natural spawning grounds is in the area available for movement. Glazer et al. (2003) and Delgado and Glazer (2007) reported on the movements of adult queen conch using acoustic tagging. Mean home range in the former study was 59,800 m², while that in the latter was 27,705 m², or 2 orders of magnitude greater than that available in the enclosures. Yet, the minimum core area reported by Glazer et al. (2003) was 800 m², which is similar to the 770 m² in the low-density enclosure. Potential limits on the movement of queen conch individuals could have affected behavior by preventing either feeding off the spawning grounds (Stoner and Sandt 1992) or enhancing the encounter rate among males and females. Yet, the habitat layout in La Parguera is quite different than that reported by Stoner and Sandt (1992), where spawning and feeding areas were physically separate. Rather, the habitat distribution within the La Parguera enclosures was patchy, with open sand areas with rubble, the latter supporting the growth of macroalgae, so conch would not have to move much to both spawn and feed, similar to what Glazer and Kidney (2004) reported for their Conch Reef-1 site. Nevertheless, food could become limiting if overgrazing occurred (see below).

Significant differences were found between the 2 treatments, with a higher proportion of females spawning in the low-density treatment, and those females producing, on average, a higher number of egg masses and a higher number of eggs/mass, resulting in higher overall fecundity. The mean number of eggs/mass for both treatments was higher than previously reported (407,000, $n = 1$, Robertson 1959; 415,000, $n = 25$, Buckland 1989). Yet, in the high-density treatment, egg masses contained still 30% more eggs. The mean number of egg masses spawned per female over the season (high-density 5.83; low-density 13.6) differed from the sole previous estimate (9.4, Davis et al. 1984), obtained from queen conch in a natural enclosure. That Davis et al. (1984) reported a higher value than in the high-density treatment, when densities in the 2 studies were the same, suggests that other factors can have a substantial influence on the rate of egg mass deposition. Indeed, during a partial spawning season, albeit during the expected peak, Davis et al. (1984) reported a rate only 70% of that recorded over a full spawning season; however, over a full season incorporating nonpeak months the resulting rate may have been similar with the results observed here. In the low-density treatment, the higher number of egg masses spawned, combined with a greater production of eggs/mass, yielded a much higher mean fecundity, with the mean in the low-density treatment being over 3x that in the high-density treatment. That the number of eggs/g of egg mass was higher in the high-density treatment is interesting, but this could result from either the production of smaller but more numerous eggs or the packing of eggs at a higher density within the egg strand. As such, its significance relative to potential food shortage (or other factors) remains enigmatic.

There are various reasons for these treatment differences given that sex ratio, male density and total density varied, and the effect of incomplete maturation among females could be a factor (see below). Yet, during the reproductive season the effect of density was glaringly apparent with respect to algal cover between the 2 treatments. In the high-density enclosure, all macroalgae were stripped from the sediment and cage mesh, suggesting that queen conch were limited to graze at the rate of algal production. In contrast, the low-density enclosure was characterized by a lush growth of macroalgae in the center, with conch preferring to feed along the fence and the adjacent substratum, leading to a halo of sand near the fence. Whether food supply was actually limiting in the high-density treatment could not be assessed. Stoner and Sandt (1992) found that individual queen conch frequently moved between the spawning ground (coraline sand) and feeding grounds (hard bottom) during the reproductive season, indicating individuals continue to feed during that time. Studies across a wide variety of species (e.g., Vitt and Caldwell 2014, Davis and Cuthbert 2019) have shown that the energy devoted to egg production is largely a function of female body condition and that in high-density populations reproductive effort increases when conditions are good. Interestingly, the start of spawning in the high-density treatment appeared to be later than in the

low-density treatment, yet there was no difference in mean initial lip-thickness. However, spawning females in the high-density treatment put more of an emphasis on lip-growth, so there may have been a trade-off between growth and reproduction between the 2 treatments.

The potential impact of differences in sex ratio or male density is more difficult to envision but could be important. For example, Allee effects, observed in queen conch, can arise when high male mating success in high densities leads to low mating success at low densities due to trade-offs between a male's ability to cope with competition and its investment in reproduction (Wright et al. 2019). Theory argues that mating (copulation) rate will increase with increasing density, but if females have control over mating (i.e., no male harassment) and partners are chosen at random, mating rates can remain constant across different densities (Sprenger et al. 2011). At present, little is known about the mating systems governing queen conch reproduction, although the proportion of the population engaging in reproduction increases with increasing density up to some plateau (Stoner and Ray-Culp 2000, Stoner et al. 2012, Delgado and Glazer 2020). That there may be tensions between males and females and competition among males is suggested by the observations of a high proportion of copulations occurring during egg deposition (up to 99% in *Strombus pugilis*) when the females remain stationary, of females dragging a copulating male with them when not spawning, and of multiple males attempting to copulate with a single female (Reed 1992, 1995). In the high-density treatment, individual egg mass production was positively correlated with number of copulations, suggesting a potential role of either sexual facilitation (Crews et al. 1986) or sperm limitation (Levitan and Petersen 1995). However, the much greater production observed in the low-density treatment and the high fertilization rates suggest reproductive output was not significantly limited by having only a single male.

Observations on the reproductive output across individual females yielded interesting results. However, interpretation of some of these is limited because of the logistical constraints in the experimental design, differences between the 2 treatments, and the consequences of presumably including immature conch into the enclosures. Due to the question concerning density effects, emphasis here is on the production within the low-density treatment.

Total fecundity estimates across the spawning season are one of the more surprising results. Females were found to produce upwards of 20 million eggs. The factor responsible for most of the variability was individual length of the spawning season, although individual differences in the rate of egg production were found. However, variation in length of the spawning season was probably driven in large part by some females being immature at the start of the experiment, as shown by the relationship between fecundity and shell lip thickness at the start of the experiment. From the relationship between proportion mature and shell lip thickness, it is expected that thinner lipped females were immature at the start of the

experiment but matured during the course of the spawning season. The positive relationship between fecundity and shell lip thickness is also interesting since there was no relation between shell length and fecundity, assuming that larger shell length reflects a larger biomass. This may be an artifact resulting from the fact that the range of shell lengths of females in the experiment was narrow and thus did not incorporate the full biological variability observed in natural populations. In retrospect, this was perhaps fortuitous because this limited variability in the experimental conch and may have allowed the relationship between lip thickness and fecundity to be detected, thus helping to understand the role of potentially immature females when interpreting the results. On the other hand, it precluded being able to assess the effects of biomass in fully mature females.

There was a significant inverse relationship in both treatments between the number of egg masses deposited and the mean interval between egg mass depositions. More interesting was the relationship between the number of egg masses deposited and the mean number of eggs/mass, which tended negative (but not statistically significantly) in the high-density treatment. This result might be expected where resources may be limited, suggesting a trade-off in how egg production is allocated (more smaller masses vs. fewer larger masses). However, the relationship was positive in the low-density treatment, resulting in a more complicated interpretation. Our results suggest that there are substantial differences in the potential individual output among females, which become manifest when resources are not limiting. Alternatively, our results may still reflect the effects of variable degrees of maturation across females. Biomass still increases during initial lip-thickening (Appeldoorn 1988, 1992, Appeldoorn et al. 2018), and thus females with thicker lips may be more productive not only due to achieving maturation earlier, but also that newly maturing females are not as productive as established mature females.

Copulation rate may be another factor affecting egg production, as evidenced by the positive relationship in the number of egg masses spawned per individual female and the

number of observed copulations for each female found in the high-density treatment. This finding could be indicative of either sexual facilitation or sperm limitation, but there was no obvious indication of the latter affecting the females in the low-density treatment where there was only one male. If sexual facilitation occurs, and increased contact with males stimulates gametogenesis and egg production (Crews et al. 1986), this would be still another reason to protect areas with high densities of spawning queen conch adults (Delgado and Glazer 2020).

Lastly, it was observed that the mean number of eggs/g of egg mass decreases with an increase in the number of egg masses spawned. While this is an intriguing result, its interpretation suffers not only from the differing potential mechanisms affecting eggs/g but, again, from the potential effect of variable states of maturation.

Results demonstrate queen conch have a much higher potential reproductive output than previously thought, but this potential can be influenced by various factors, such as the obvious treatment differences of density and sex ratio or male density. State of reproductive development is an important factor affecting the performance of females, and while this potentially explained some of the patterns observed in the study, it also represented the greatest limitation. Not having started the spawning season with fully mature queen conch females potentially masks other important trends. Nevertheless, the study did demonstrate that there is individual variability in factors important for potentially increasing reproductive effort in older queen conch, such as length of spawning season, frequency of spawning, size and number of egg masses, and perhaps density or size of eggs, depending on the factors affecting the eggs/g of egg mass. Although the study was not designed to assess reproductive effort as a function of age or size, it clearly demonstrated these could be assessed using an experimental approach. This experiment would require stocking enclosures with females across a wide range of ages (i.e., including very thick-lipped individuals) or sizes (length, biomass) to determine relevant trends.

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