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Variation in polyandry, reproductive output, and within-brood genetic diversity in a marine snail population across seasons and years

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ABSTRACT: The frequency of polyandry has important implications for effective population size, genetic variation, and reproductive output. Compared to terrestrial organisms with complex social behaviors, the patterns and consequences of polyandry in marine populations are relatively less clear. Here we quantified polyandry in the Florida crown conch *Melongena corona* in the field under natural settings. We assessed the extent to which additional mates increase genetic diversity within broods, how polyandry relates to female reproductive output, and how consistent patterns are across their 5 mo reproductive season in 2 separate years. We found large variation in polyandry (2 to 19 sires per brood) and reproductive output among females. However, the number of sires per brood was unrelated to reproductive output. The number of sires increased genetic diversity within broods regardless of year or time of season. The number of sires per brood and reproductive output did not vary over the season or among years. Overall, our results show natural variation in polyandry upon which selection could act, but increased polyandry did not lead to females producing more hatchlings, and neither polyandry nor reproductive output increased over time when females could accumulate and store sperm. Any benefits of polyandry in terms of genetic diversity are expected to occur after hatching, if at all, rather than inside the egg capsule. Variation in polyandry could arise because males control mating and polyandry is less costly for females than trying to prevent superfluous matings.

KEY WORDS: Polyandry · Multiple mating · Seasonal patterns · *Melongena corona* · Reproductive output · Genetic diversity

1. INTRODUCTION

Multiple mating by females (i.e. polyandry) has important implications for sexual selection and the maintenance of genetic and phenotypic variability in populations (Karl 2008, Lotterhos 2011, Kvarnemo & Simmons 2013, Taylor et al. 2014). Benefits of polyandry include an increase in female fitness through an increase in reproductive output by increasing the quantity of sperm received, increasing male-provided resources if males provide nutrition or care to females or offspring, or increasing the diversity and quality of offspring produced (Arnqvist & Nilsson 2000, Jennions & Petrie 2000). While polyandry is ubiquitous in the animal kingdom (Taylor et al. 2014), much of our understanding of the cost and benefits of this common mating system comes from terrestrial species. Polyandrous females in the ocean face different challenges such as dilution of sperm when sperm are released into the water column (Levitan & Petersen 1995, Johnson & Yund 2007, McLeod & Marshall 2009), or mobility or encounter rates (Le Cam et al. 2014). In particular, species that copulate and encapsulate many offspring together (e.g. in egg cases) are subject to post-copulatory processes that potentially alter the costs and benefits of polyandry through con-

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flict between parents and offspring, and between offspring from different males (Sprenger et al. 2008, Kamel et al. 2010, Avise et al. 2011, Kamel & Grosberg 2012, Collin & Ochoa 2015, Kamel & Williams 2017, Ylitalo et al. 2019). Therefore, studying variability within marine populations in terms of the number of sires per female brood, and in the relationships between the number of sires, genetic diversity, and fecundity, is therefore a key research priority to contextualize the importance of polyandry as a mating system in marine taxa.

Being able to control conditions while manipulating mating in laboratory settings is seen as a strength to isolate causal mechanisms, but it can also can bias the conclusions on the amount, costs, and benefits of polyandry (Arnqvist & Nilsson 2000, Simmons et al. 2007, Gowaty 2013). For instance, in dumpling squid *Euprymna tasmanica*, higher levels of paternity were found in wild-caught individuals compared to those reared in the lab (Squires et al. 2014). Therefore, conclusions about the strength of sexual selection may not be transferable from the laboratory to the wild. Such differences can occur when laboratory conditions remove important sources of selection that would otherwise alter mating rates, such as increased mating displays by males when predation pressure is removed in laboratory settings (Godin 1995, Candolin 1997). Other laboratory-specific patterns can arise such as when males that would not be given the opportunity to mate in the wild are given that opportunity in lab settings (e.g. smaller males in *Drosophila nigrospiracula*, Markow 1988). Laboratory populations can have fewer sires represented in a brood with greater paternity skew compared to their wild counterparts (Oneal & Knowles 2015). Mismatches between laboratory and field studies also occur when environmental factors like thermal conditions, density of conspecifics, and strength of social interactions are excluded from laboratory studies (Gowaty 2013).

Inferences about the consequences of polyandry likely depend on the degree of polyandry, necessitating the quantification of natural variation in the number of sires in a brood in natural settings (Johnson & Yund 2007, Kupfernagel et al. 2010). For instance, the pattern of last mated male siring the most offspring when 2 males are present can be weakened (Laturney et al. 2018) or completely broken down (Zeh & Zeh 1994) when a third mate is added. Ronkainen et al. (2010) found a cost to mating in terms of number of eggs produced in the polyandrous water strider *Aquarius paludum* only after females mated with 4 males and not when mated to 1 or 2 males. Limiting the number of mates in experimental studies below what is found in wild populations can artificially reduce the strength of postcopulatory sexual selection, limiting our understanding of the role of sexual selection in the evolution of polyandry (Simmons & Beveridge 2010).

The degree and consequences of polyandry can vary over time as population density, resources, or environmental conditions change. For instance, Simmons et al. (2007) and Demont et al. (2011) both found that more sires were represented in the sperm stores of nonsocial insects later in the breeding season, compared to earlier, as females accumulated and stored sperm from more mates. Similarly, the number of sires in a brood can change over time if population density or male encounters vary through the reproductive season (Xue et al. 2016). In large-coiled whelk *Solenosteira macrospira*, the number of sires within a brood increased throughout the season, resulting in greater rate of cannibalism and greater offspring size at hatching (Kamel et al. 2010). Environmental conditions can also play a role in mediating the level of polyandry. Torres-Vila et al. (2005) found that time of season affected what food was available for female larvae of the European grapevine moth *Lobesia otrana*, which affected the number of mates a female had. An increase in rainfall can result in lower mating frequencies (El-Niweiri & Moritz 2011) while an increase in temperature can increase the reproductive output of polyandrous females (Grazer & Martin 2012). Each of these examples shows that an assessment of the degree of polyandry in the field cannot be restricted to a single sampling time point, as the strength of sexual selection (Simmons & Beveridge 2010), sperm competition (Simmons 2005), and offspring competition (Kamel et al. 2010) will vary as the number of sires varies.

In this study, we investigated the temporal patterns of polyandry and reproductive output in a marine gastropod, the Florida crown conch *Melongena corona*. Along the northern Gulf coast of Florida, USA, this species lays several egg strings throughout its 5 mo reproductive season, providing the opportunity for temporal environmental changes through time to impact mating behavior, sperm storage, and reproductive output. We quantified the degree of polyandry and multiple components of reproductive output early (April) and late (July) in the reproductive season in 2 years (2018 and 2020). From egg capsules laid in the field by known dams, we genotyped offspring at 10 microsatellite loci to estimate paternity within broods. We also quantified the relationship between the number of sires within a brood, genetic diversity, and reproductive output across the reproductive season in both years.

2. MATERIALS AND METHODS

2.1. Study species

The Florida crown conch *Melogena corona* (Gastropoda, Melongenidae) is an intertidal, gonochoristic gastropod that lives along the coasts of Florida and eastern Alabama (Hathaway & Woodburn 1961, Woodbury 1986, Bowling 1994). Females lay egg strings that consist of several circular egg capsules (~5–25 egg capsules averaging 43 mm in perimeter) that are laid within the warmer months of March–August at our field site (Fig. 1A,B). Each egg capsule encapsulates multiple offspring (~50–200 individuals; Fig. 1C) that emerge as crawl-away larvae after developing for ~16–35 d (Fig. 1D; Hooks & Burgess 2021). The study population inhabited oyster and seagrass habitat west of the Florida State University Coastal and Marine Laboratory (29° 54′ 57.87″ N, 84° 30′ 38.66″ W).

2.2. Sampling

During the reproductive season, females can be seen laying egg strings at low tides (Fig. 1A). We placed temporary cages around individual females while they were laying, which did not disturb them and ensured that the maternal identity and the age of all egg capsules were known prior to sampling the egg strings for subsequent analyses. In 2018, 11 dams were caged in the field while laying egg strings. Five females were caged early in the reproductive season (April) and 6 were caged late in the reproductive sea-

Fig. 1. (A) Female crown conch laying an egg string in the field. (B) Egg string with multiple egg capsules laid in the field. (C) Single egg capsule packaged with offspring (small yellow dots inside capsule) early (~5 d) in development. (D) Newly emerged hatchling

son (July). After 24 h, well after females finished laying, both the females and their egg capsules were collected and brought to the lab. Adult snails were frozen for later genotyping. Egg capsules were individually removed from their egg strings (Fig. 1C) using sterile scissors, then photographed. To estimate egg capsule size, the perimeter of the egg capsules was measured from photographs using Image Insight Pro (v. 9.1). Egg capsules were then individually placed in 100 ml plastic containers with 500 µm openings to allow water flow. Each mesh cage was marked with the identity of the dam and a unique egg capsule number to allow the tracking of individual egg capsules throughout the experiment. Egg capsules in mesh containers were maintained in 189 l tank filled with artificial seawater (Instant Ocean) that was maintained with a carbon filter and mesh bag filter. Seawater was maintained at 28°C and 29 ppt. To prevent protist outbreaks, egg capsules with larvae close to hatching (indicated by a fully formed foot visible through the egg capsule) were transferred into individual 50 ml conical tubes and placed in an environmentally controlled chamber at the same temperature and salinity as in the recirculating tank. The seawater inside the tubes was replaced 6 d wk^{-1}, and the presence of newly emerged hatchlings was checked every day. Once the first hatchling emerged from an egg capsule (Fig. 1D), the egg capsule was cut open and all hatchlings were counted and preserved in 95% EtOH for later DNA extraction.

In 2020, 45 females were caged in the field while laying egg strings: 17 were caged early in the reproductive season (April) and 28 were caged late in the reproductive season (July). Females and their egg strings were handled the same as in 2018, except that the egg capsules in mesh containers were placed in the field rather than in the laboratory, so that offspring could develop under natural field conditions. Containers were zip-tied to 0.5 inch (~1.27 cm) diameter PVC pipes, each approximately 1 m long, and fixed horizontally at 10 cm above the seagrass in a similar area to where egg capsules were laid. Egg capsules were transferred to new plastic containers every week. Egg capsules inside of the containers were checked for hatchlings every 2 d, and hatchlings were handled the same as in 2018.

2.3. DNA analysis

Offspring from 11 dams from 2018 (5 in April and 6 in July) and 13 dams from 2020 (6 in April and 7 in July) were sub-sampled for DNA paternity analysis. Dams and their offspring were genotyped at 10 microsatellite loci (Tables A1–A3 in the Appendix) to estimate the offspring paternity within a brood. From each female, 16 offspring from each of 3 egg capsules were sampled (total of 48 offspring per female, except for 1 dam that had 64 offspring genotyped). Overall, a total of 1152 offspring from 24 dams across 2 separate years were analyzed.

To obtain DNA from offspring, the entire hatchling was placed in a solution of 150 µl of Chelex 100 Resin (Bio-Rad) and 3 µl of 25 mg ml^{-1} concentrated Proteinase K (IBI Scientific). To obtain DNA from dams, a small piece of tissue dissected from the foot was used. To extract DNA, the sample was placed in a thermocycler at 55°C for 60 min, followed by 95°C for 15 min.

DNA extractions were amplified for 10 microsatellite loci in 2 multiplex reactions (Table A1). The PCR was done on a 10 µl volume made up of 5 µl Qiagen Multiplex PCR master mix, 2 µl of 0.05 mg ml^{-1} BSA (New England Biolabs), 1.2 µl of primer stock mix, and 0.8 µl of sterile deionized water. Forward primers were tailed with Tail A–D universal primers described by Blacket et al. (2012) and tagged with fluorescent dyes (NED, VIC, FAM, or PET [Applied Biosystems]; Table A2).

The primer stock mix for the first multiplex included a 1:2:1:1.5:1:1 ratio of Mcor3010, McoA4, Mcor652, Mco2, Mco10, and Mcor4853 primers, respectively, each at a 20 µM concentration. The primer stock mix for the second multiplex included 1:1:1:1.5 ratio of Mcor4007, Mco12, Mco3, and Mcor 6632 primers, respectively, each at a 20 µM concentration. An equal molar mix of forward and universal tagged primers was added at equal concentration to the reverse primer so that the resulting mix would be a 1:1:2 ratio of forward:universal:reverse primers. Thermal cycling conditions for PCRs were: 95°C for 15 min; followed by 32 cycles of 94°C for 30 s, 57°C for 90 s, and 72°C for 90 s; followed by a final extension at 72°C for 10 mins.

Fragment analysis used 1 µl of PCR product, 0.02 µl of Liz-500 size standard, and 10 µl of Hi-Di formamide (Applied Biosciences) and was done using a 3730 Genetic Analyzer at the Biology Core Facility at Florida State University. Fragment lengths were scored manually using Geneious v. 9.1.8 (Biomatters). For consistent allele calling, the same individual scored all samples and repeated the scoring of 48 individuals to ensure quality scoring. Fragment analyses did not produce reliable genotype calls (missing at 5 or more loci) for 56 offspring, so those individuals were removed from the data sets, resulting in 1096 offspring being analyzed.

2.4. Paternity reconstruction

Sires were inferred based on full- and half-sibling groups within each dam, which were estimated using Colony v2.0.6.6 (Jones & Wang 2010). Paternal reconstruction using dam—offspring progeny arrays was used where the likelihood of all paternal alleles is evaluated from the population allele frequency and Mendelian rules of inheritance (Jones et al. 2010). Input parameters for Colony were polygamous for both sexes, dioecious, no inbreeding, diploid, one long run of full-likelihood model, high likelihood precision, and updating of allele frequencies. Importantly, Colony takes account of locus-specific genotyping errors, such as null alleles and mutations, to provide robust multilocus relationship estimates in the presence of genotyping error (Wang 2004). We set the error rates for all markers at 0.002, but the estimated error rate was often 0 and the highest was 0.025.

2.5. Genetic diversity

Genetic diversity within a brood was estimated as 2 components: allelic richness and expected heterozygosity. Allelic richness, corrected for sample size, was calculated using the 'PopGenReport' package (v. 3.0.7; Adamack & Gruber 2014) in R. Expected heterozygosity, which expresses the probability that 2 randomly drawn alleles from a sample are different based on Hardy-Weinberg assumptions (Nei 1978), was calculated using the 'poppr' package (v. 2.9.3; Kamvar et al. 2014) in R (v. 4.1.1).

2.6. Statistical analyses

All statistical analyses were performed in R v. 3.6.3 (R Core Team 2018). All data and R code are publicly available at https://zenodo.org/records/10904782. Residual diagnostics were assessed using the package 'DHARMa' (v. 0.4.6; Hartig 2022). Significance was assessed at $\alpha = 0.05$. Time of season (Early = April versus Late = July) and year (2018 versus 2020) were included as fixed effects, and all non-significant 2- and 3-way interactions were removed from the final model (but main effects were always kept). Binomial and negative binomial generalized linear models, and hurdle models, were fit using the package 'glmmTMB' (Brooks et al. 2017), and model terms were assessed for significance using χ^2 log-likelihood ratio tests. For Gaussian linear models, model terms were assessed for significance using *F*-tests.

A negative binomial generalized linear model was used to estimate the effect of time of season (April versus July) and year on the number of sires at hatching per brood. A Gaussian linear model was used to estimate the relationship between the number of sires, time of season, and year on allelic richness and expected heterozygosity. Curvilinearity was first assessed by comparing a second- and first-order polynomial using *F*-tests. A Gaussian linear model was used to estimate the relationship between the number of sires on estimated total reproductive output within a brood. Total reproductive output was estimated by multiplying the average number of offspring within an egg capsule per dam in a subset of egg capsules (50% of egg capsules a dam produced) by the number of egg capsules produced.

A negative binomial generalized linear model was used to estimate the effect of time of season on the number of egg capsules per dam. A Gaussian linear model was used to estimate the relationship between the number of sires on the number of hatchlings per egg capsule. A binomial generalized linear model was used to estimate the effect of the time within reproductive season and egg capsule size on egg capsule viability. In 2020, egg capsules were considered viable if they produced hatchlings and not viable if all offspring within the egg capsule failed to develop to the hatchling stage. Due to complete mortality of offspring within some egg capsules in 2020 (which did not occur in the lab in 2018), estimates of the total number of hatchlings produced in a brood (i.e. an egg string) were analyzed separately for each year. For the 2018 data, the effect of timing within the reproductive season on total number of hatchlings was estimated with a negative binomial generalized linear model. For the 2020 data, the effects of timing within the reproductive season on total number of hatchlings was analyzed using a hurdle model. The 2-part hurdle model included a binomial model with a logit link function and a zero-truncated negative binomial model with a log link function ('truncated_nbinom2') ('ziformula=~time of season').

3. RESULTS

3.1. Paternity reconstruction and number of sires

All known dams were correctly inferred from the genotype data, which was checked by setting the threshold number of loci showing a mismatch (Mendelian incompatibility where alleles in the offspring

do not occur in the known dam) to 0 and checked that no known dam–offspring relationship were rejected. Offspring were assigned to full sib groups, indicating different sires.

On average, there were 9.13 (95% CI: 7.64–10.90) sires per egg string per dam. One dam had only 2 sires per egg string, while another had 19 sires per egg string (Figs. 2 & 3). The time of season (χ^2 = 2.27, df = 1, p = 0.131) or year (χ^2 = 2.28, df = 1, p = 0.131) had no detectable effects on the number of sires within a brood (Fig. 3b). From a total of 24 females and 1096 offspring, the reconstructed paternal genotypes indicated that there was a minimum of 118 unique sires in 2018 and 81 unique sires in 2020. The estimated number of sires for these 24 females is still likely only a fraction of the number of males in the population, given that the population is relatively large and many hundreds of individuals can be seen in the field during the reproductive season (approximately 1 m^{-2}).

We also found evidence that males sired offspring from multiple dams (Fig. 2). In 2018, 3 reconstructed paternal genotypes were present in 2 maternal broods (1 paternal genotype from 1 maternal pair, and 2 paternal genotypes from a different maternal pair). In 2020, 7 reconstructed paternal genotypes were present in 2 maternal broods (1 paternal genotype from 1 maternal pair, 2 paternal genotypes from another maternal pair, and 4 paternal genotypes from yet another maternal pair).

3.2. Genetic diversity within broods

There was no evidence for curvilinearity in the relationship between the number of sires and allelic richness ($F_{1,12}$ = 1.30, p = 0.326) or expected heterozygosity ($F_{1,12}$ = 1.12, p = 0.392). There was also no evidence for any 2- or 3-way interactions between the number of sires, time of season, and year on allelic richness ($F_{1,16}$ = 1.97, p = 0.147) or expected heterozygosity ($F_{1,16}$ = 2.14, p = 0.123). Overall, a higher number of sires was related to greater allelic richness ($F_{1,20}$ = 6.93, p = 0.016; Fig. 4a) and expected heterozygosity ($F_{1,20}$ = 7.90, p = 0.011; Fig. 4b) within broods.

3.3. Reproductive output

Dams laid between 6 and 21 egg capsules on an egg string and produced 355 to 2190 estimated total number of hatchlings within an egg string. However, the number of sires per brood did not explain the estimated total number of hatchlings within a brood ($F_{1,20}$ = 3.21, p = 0.088; Fig. 3b). The 95% confidence interval for the effect of the number of sires on the total number of hatchlings was −93.78 to 7.11, indicating that the maximum effect size supported by the data is 7.11 additional hatchlings per sire. In other words, if each additional sire increases female reproductive output by less than ~7 offspring (approx-

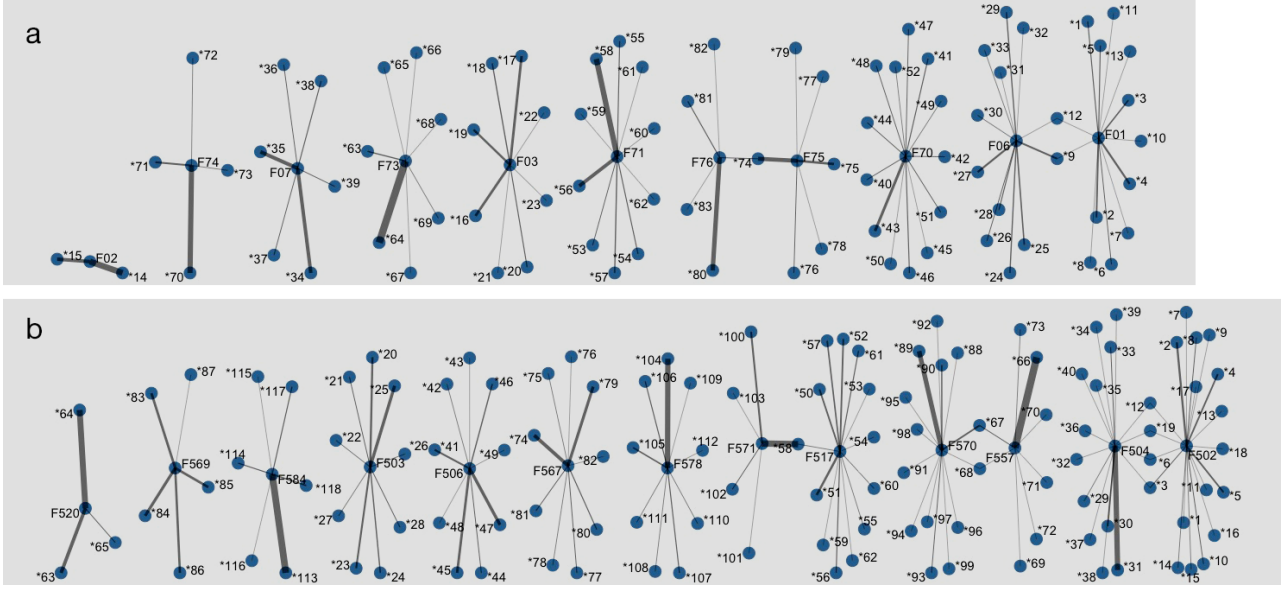

Fig. 2. Network visualization of paternity reconstruction in the crown conch from known maternal–offspring arrays estimated in (a) 2018 and (b) 2020 created with Colony. Dots (nodes) in the center marked with 'F' denote known females, and numbered dots with a '*' denote reconstructed paternal identity. Lines (edges) connect sire identity to dam identity. The width of the line indicates the relative number of full-sib offspring assigned to a given sire within each maternal brood

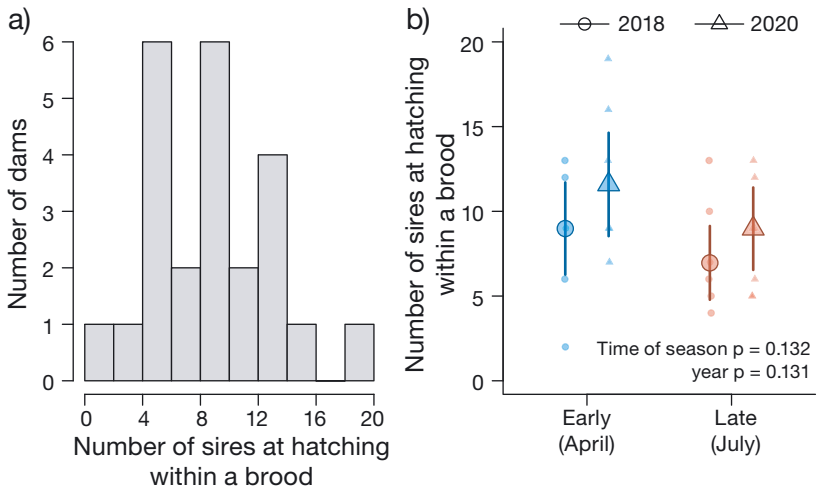

Fig. 3. Minimum number of sires detected (a) within a brood and (b) across the reproductive season in 2018 and 2020. Larger symbols represent average number of sires within a brood while smaller symbols represent individual dams. Lines represent 95% confidence intervals; p-values denote the additive effects of each factor from a chi-squared test

imately 1–2.5% of the brood), we would not have detected it with the current sample size.

The number of egg capsules per egg string did not differ between the time of season they were laid (χ^2 = 0.15, df = 1, p = 0.702, Fig. 5a) nor between years (χ^2 = 0.22, df = 1, p = 0.640). The mean number of hatchlings per egg capsule per dam differed between time of season ($F_{1,52}$ = 6.54, p = 0.014; Fig. 5b) but not between years ($F_{1,52}$ = 0.20, p = 0.655). On average, there were 72.27 (95% CI: 61.09–83.46) hatchlings per egg capsule early in the reproductive season and 90.67 (95% CI: 81.53–99.80) hatchlings per capsule late in the reproductive season.

Egg capsule viability in the field did not depend on egg capsule size (χ^2 = 1.29, df = 1, p = 0.255) but did depend on the time in the season in which they were laid (χ^2 = 25.69, df = 1, p < 0.001; Fig. 5c). The odds of egg capsule survival late in the season were 18% (95% CI: 9–38%) of that early in the reproductive season. The total number of hatchlings per egg string did not differ between time of season in 2018 (χ^2 < 0.001, p = 0.995) nor in 2020 (χ^2 = 0.004, df = 1, p = 0.950; Fig. 5d).

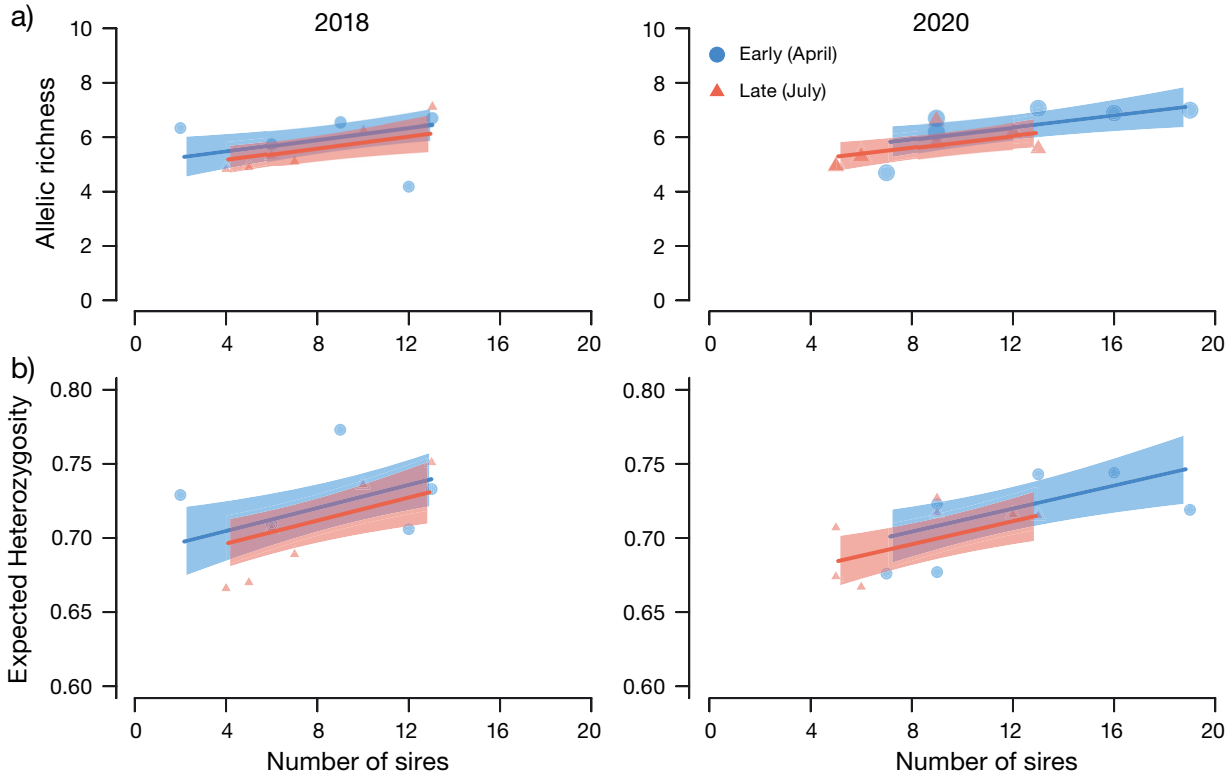

Fig. 4. Interactive effects time of season and year on number of sires for (a) allelic richness and (b) expected heterozygosity. Circles and triangles represent individual dams. Lines represent the relationship between number of sires and the tested factor, and the shading around each line represents 95% confidence intervals

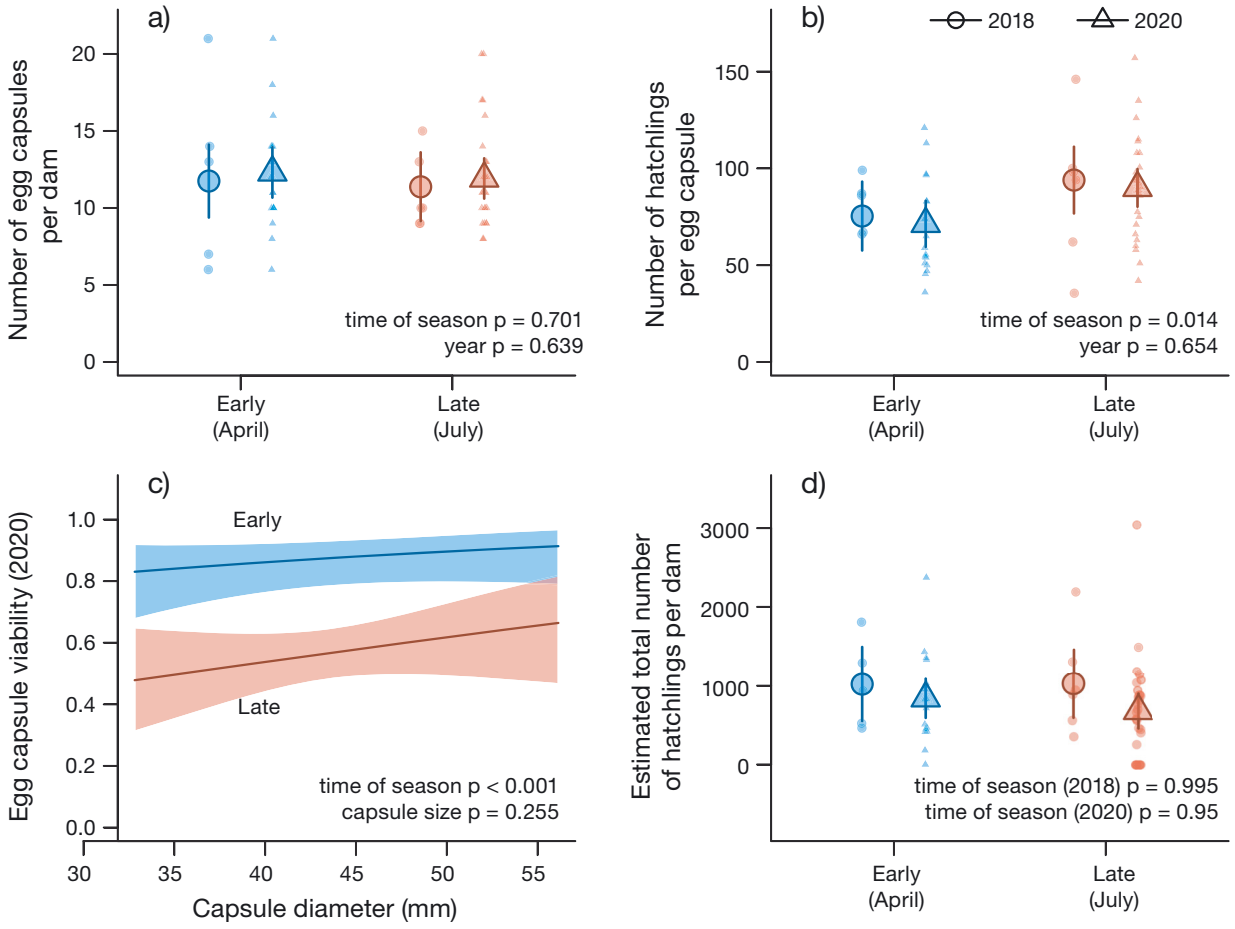

Fig. 5. Effects of the timing of reproduction (early vs. late in the reproductive season) on the (a) average number of egg capsules per dam in an egg string, (b) average number of hatchlings per egg capsule per dam, (c) relationship between egg capsule perimeter (mm) and egg capsule survival early and late in the season in the field (2020), and (d) the total number of hatchlings per dam. Larger symbols represent averages while smaller symbols represent individual dams. Lines in panels a,b,d and shaded areas in panel c represent 95% confidence intervals

4. DISCUSSION

We genotyped 1096 offspring from 24 dams across 2 months in 2 years and uncovered large variation among females in the number of males that sired their offspring (2 to 19 sires), as well as large variation among females in the number of offspring they produced (355 to 2190 within a single egg string). However, females with more sires did not produce more hatchlings, indicating there is no viability selection on polyandry in this population. Females with more sires did, however, produce more genetically diverse broods, as would be expected in a large population with random mating. The number of hatchlings per egg capsule increased from early to late in the reproductive season, but because egg capsule viability decreased over that time, total reproductive output was similar throughout the reproductive season in both 2018 and 2020. Overall, our results indicate that if there is selection on polyandry, it would have to occur via the effects of genetic diversity on offspring survival after hatching, but it does not occur via the effects of polyandry on the number of hatchlings, regardless of time of season. A likely explanation is that polyandry in this system occurs through the convenience of avoiding male harassment (Panova et al. 2010, Johannesson et al. 2016, Boulton et al. 2018).

A lack of direct benefits to polyandry has been reported in many species of both invertebrates (e.g. Bretman et al. 2004, Portnoy et al. 2007) and vertebrates (e.g. Pai & Yan 2002, Zajdel et al. 2019) and often leads to the hypothesis that there must instead be potential genetic benefits to polyandry, if there are benefits at all. This then, leads to another, often un-

tested assumption, that additional mates increase genetic diversity, which may not be true in small populations, female-biased populations, or populations with large variance in male reproductive success (Karl 2008, Lotterhos 2011). However, we confirmed that genetic diversity in terms of allelic richness and expected heterozygosity increased with number of sires. While effects of season on polyandry have been tested in some wild populations (crickets: Simmons et al. 2007; yellow dung fly: Demont et al. 2011), our results provide evidence for the consistency of seasonal and yearly effects on polyandry, especially in marine populations. Genetic diversity within a brood increasing with number of sires could be beneficial in terms of preventing complete reproductive failure from, for example, severe infections (Tarpy 2003) or unpredictably poor environmental conditions that reduce offspring survival after hatching (Sakaluk et al. 2002).

The number of sires found in the Florida crown conch was high compared to previous estimates in copulating animals. For instance, in other gastropods that encapsulate their young, a brood tends to range from 1 to 7 sires (e.g. *Rapana venosa:* Xue et al. 2014, 2016; *Littorina obtusata*: Paterson et al. 2001; *Busycon carica*: Walker et al. 2007). Very few gastropod species exceed the number of sires within in a brood reported here with the exception of the ovoviviparous gastropod *L. saxatilis*, that can range from 15 to 23 sires within a brood (Panova et al. 2010). Other invertebrates that encapsulate their young in egg strands or egg masses, such as several species of squid (Buresch et al. 2001, Shaw & Sauer 2004) or octopuses (Voight & Feldheim 2009), rarely exceed 5 sires within a brood. Many polyandrous copulating invertebrates, such as crustaceans (e.g. Yue et al. 2010, Jorquera et al. 2016), spiders (Matzke et al. 2022), and some insects (e.g, Arbuthnott et al. 2015, Browne & Gwynne 2022), rarely exceed 9 sires within a brood. In polyandrous copulating vertebrates, such as reptiles (e.g. Zbinden et al. 2007, Noble et al. 2013) and mammals (e.g. Firman & Simmons 2008, Connell & Russell 2010), broods rarely exceed 6 sires, while broods in bony fish (e.g. Liu & Avise 2011, Dekker et al. 2020) rarely exceed 9 sires. In copulating species, with the exception of *L. saxatilis*, only social insects have been found to have a higher degree of polyandry within a brood than what we found in the Florida crown conch (e.g. Rheindt et al. 2004).

Broods having many sires suggest that females likely mate multiple times before laying each egg string, then mate again before laying the next egg string, or that they accumulate and store sperm from multiple matings to different males and lay consecutive egg strings. Many sires within a brood could also indicate that males are not able to effectively displace a previous male's sperm as seen in other invertebrate species (e.g. Tuni et al. 2020). In terms of accumulating and storing sperm from multiple males, female crown conch can store sperm for up to 4 mo (A. P. Hooks pers. obs.). If females accumulate sperm from multiple males over the season, we expected to see the number of sires increase over the season. Instead, we found no evidence that sperm storage resulted in more sires within a brood later in the reproductive season, or that seasonal environmental changes affect the number of sires and genetic diversity within a brood. This is in contrast with other studies where environmental factors affect the level of polyandry (Torres-Vila et al. 2005) or sperm storage, leading to an increase in sires later in the reproductive season (Simmons et al. 2007, Demont et al. 2011). Our results suggest that conducting experiments in the crown conch at one point in time within a lab setting should not alter inferences about polyandry in this population, but such findings are likely species or population dependent.

Finally, variation in polyandry could be explained by variation in female resistance to male harassment and superfluous matings (e.g. McLean & Stuart-Fox 2010, Johannesson et al. 2016). Cases of convenience polyandry have been documented in taxa when mating is costly via female harm during mate refusal (Crudgington & Siva-Jothy 2000), attraction of predators during the act of refusal (Han & Jablonski 2010), or if the act of rejecting a mate takes too much time away from foraging (Rowe 1992). We predict in female crown conch that the cost of avoiding multiple mating outweighs the benefits of multiple mating past a few males. Refusing mates may come as an energetic cost when males pile onto females, each trying to mate, when large males continuously flip females for easier access to mating, or when females clamp down on their foot to deter mating access for males (A. P. Hooks pers. obs.). However, future studies estimating convenience polyandry explicitly, as well as selection on males for traits like guarding or mate finding, will be needed to test this expectation.

Data accessibility. Data and R code can be found at https://zenodo.org/records/10904782

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LITERATURE CITED

Adamack AT, Gruber B (2014) PopGenReport: simplifying basic population genetic analyses in R. Methods Ecol Evol 5:384–387

Arbuthnott D, Crespi BJ, Schwander T (2015) Female stick insects mate multiply to find compatible mates. Am Nat 186:519–530

Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. Anim Behav 60:145–164

Avise JC, Tatarenkov A, Liu JX (2011) Multiple mating and clutch size in invertebrate brooders versus pregnant vertebrates. Proc Natl Acad Sci USA 108:11512–11517

Blacket MJ, Robin C, Good RT, Lee SF, Miller AD (2012) Universal primers for fluorescent labelling of PCR fragments — an efficient and cost-effective approach to genotyping by fluorescence. Mol Ecol Resour 12:456–463

Boulton RA, Zuk M, Shuker DM (2018) An inconvenient truth: the unconsidered benefits of convenience polyandry. Trends Ecol Evol 33:904–915

Bowling C (1994) Habitat and size of the Florida crown conch (*Melongena corona* Gmelin): why big snails hang out at bars. J Exp Mar Biol Ecol 175:181–195

Bretman A, Wedell N, Tregenza T (2004) Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. Proc R Soc B 271:159–164

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A and others (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378–400

Browne JH, Gwynne DT (2022) Paternity sharing in insects with female competition for nuptial gifts. Ecol Evol 12: e9463

Buresch KM, Hanlon RT, Maxwell MR, Ring S (2001) Microsatellite DNA markers indicate a high frequency of multiple paternity within individual field-collected egg capsules of the squid *Loligo pealeii*. Mar Ecol Prog Ser 210: 161–165

Candolin U (1997) Predation risk affects courtship and attractiveness of competing threespine stickleback males. Behav Ecol Sociobiol 41:81–87

Collin R, Ochoa I (2015) Access to multiple mates increases fecundity but does not affect per-offspring maternal investment in a marine gastropod. Invertebr Biol 134: 271–281

Connell SD, Russell BD (2010) The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. Proc R Soc B 277:1409–1415

Crudgington HS, Siva-Jothy MT (2000) Genital damage, kicking and early death. Nature 407:855–856

Dekker ML, Hagmayer A, Leon-Kloosterziel KM, Furness AI, Pollux BJA (2020) High degree of multiple paternity and reproductive skew in the highly fecund live-bearing fish *Poecilia gillii* (Family Poeciliidae). Front Ecol Evol 8: 579105

Demont M, Buser CC, Martin OY, Bussière LF (2011) Natural levels of polyandry: differential sperm storage and temporal changes in sperm competition intensity in wild yellow dung flies. Funct Ecol 25:1079–1090

El-Niweiri MAA, Moritz RFA (2011) Mating in the rain? Climatic variance for polyandry in the honeybee (*Apis mellifera jemenitica*). Popul Ecol 53:421–427

Firman RC, Simmons LW (2008) The frequency of multiple paternity predicts variation in testes size among island populations of house mice. J Evol Biol 21:1524–1533

Godin JGJ (1995) Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). Oecologia 103:224–229

Gowaty PA (2013) Adaptively flexible polyandry. Anim Behav 86:877–884

Grazer VM, Martin OY (2012) Elevated temperature changes female costs and benefits of reproduction. Evol Ecol 26: 625–637

Han CS, Jablonski PG (2010) Male water striders attract predators to intimidate females into copulation. Nat Commun 1:52

Hartig F (2022) 'DHARMa': residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.6. https://CRAN.R-project.org/package=DHARMa

Hathaway RR, Woodburn KD (1961) Studies on the crown conch *Melongena corona* Gmelin. Bull Mar Sci Gulf Caribb 11:45–65

Hayes KA, Karl SA (2004) Characterization of microsatellite markers from the gastropod genus *Melongena*. Mol Ecol Notes 4:755–757

Hooks AP, Burgess SC (2021) Behavioral variability of hatchlings modifies dispersal potential in crown conch (*Melongena corona*): Why do larvae crawl away but sometimes swim? Biol Bull (Woods Hole) 241:92–104

Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. Biol Rev Camb Philos Soc 75:21–64

Johannesson K, Saltin SH, Charrier G, Ring A-K, Kvarnemo C, André C, Panova M (2016) Non-random paternity of offspring in a highly promiscuous marine snail suggests postcopulatory sexual selection. Behav Ecol Sociobiol 70: 1357–1366

Johnson SL, Yund PO (2007) Variation in multiple paternity in natural populations of a free-spawning marine invertebrate. Mol Ecol 16:3253–3262

Jones AG, Small CM, Paczolt KA, Ratterman NL (2010) A practical guide to methods of parentage analysis. Mol Ecol Resour 10:6–30

Jones OR, Wang J (2010) COLONY: A program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Jorquera E, Kenchington E, Ruzzante DE (2016) High prevalence of multiple paternity in the deep-sea shrimp *Acanthephyra pelagica*. Mar Biol 163:89

Kamel SJ, Grosberg RK (2012) Exclusive male care despite extreme female promiscuity and low paternity in a marine snail. Ecol Lett 15:1167–1173

Kamel SJ, Williams PD (2017) Resource exploitation and relatedness: implications for offspring size variation within broods. Oikos 126:1219–1226

Kamel SJ, Oyarzun FX, Grosberg RK (2010) Reproductive biology, family conflict, and size of offspring in marine invertebrates. Integr Comp Biol 50:619–629

Kamvar ZN, Tabima JF, Grünwald NJ (2014) *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281

Karl SA (2008) The effect of multiple paternity on the

genetically effective size of a population. Mol Ecol 17: 3973–3977

Kupfernagel S, Rusterholz HP, Baur B (2010) Variation in multiple paternity and sperm utilization patterns in natural populations of a simultaneous hermaphrodite land snail. Biol J Linn Soc 99:350–361

Kvarnemo C, Simmons LW (2013) Polyandry as a mediator of sexual selection before and after mating. Philos Trans R Soc Lond B Biol Sci 368:20120042

Laturney M, van Eijk R, Billeter JC (2018) Last male sperm precedence is modulated by female remating rate in *Drosophila melanogaster*. Evol Lett 2:180–189

Le Cam S, Riquet F, Pechenik JA, Viard F (2014) Paternity and gregariousness in the sex-changing sessile marine gastropod *Crepidula convexa*: comparison with other protandrous *Crepidula* species. J Hered 105:397–406

Levitan DR, Petersen C (1995) Sperm limitation in the sea. Trends Ecol Evol 10:228–231

Liu JX, Avise JC (2011) High degree of multiple paternity in the viviparous shiner perch, *Cymatogaster aggregata*, a fish with long-term female sperm storage. Mar Biol 158: 893–901

Lotterhos KE (2011) The context-dependent effect of multiple paternity on effective population size. Evolution 65: 1693–1706

Markow TA (1988) Reproductive behavior of *Drosophila melanogaster* and *D. nigrospiracula* in the field and in the laboratory. J Comp Psychol 102:169–173

Matzke M, Toft S, Bechsgaard J, Pold Vilstrup A and others (2022) Sperm competition intensity affects sperm precedence patterns in a polyandrous gift-giving spider. Mol Ecol 31:2435–2452

McLean CA, Stuart-Fox D (2010) Do female Lake Eyre dragon lizards adjust courtship rejection behaviour under higher predation risk? Behaviour 147:1803–1818

McLeod L, Marshall DJ (2009) Do genetic diversity effects drive the benefits associated with multiple mating? A test in a marine invertebrate. PLOS ONE 4:e6347

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Noble DWA, Keogh JS, Whiting MJ (2013) Multiple mating in a lizard increases fecundity but provides no evidence for genetic benefits. Behav Ecol 24:1128–1137

Oneal E, Knowles LL (2015) Paternity analyses in wild-caught and laboratory-reared Caribbean cricket females reveal the influence of mating environment on post-copulatory sexual selection. J Evol Biol 28:2300–2307

Pai A, Yan G (2002) Polyandry produces sexy sons at the cost of daughters in red flour beetles. Proc R Soc B 269:361–368

Panova M, Boström J, Hofving T, Areskoug T and others (2010) Extreme female promiscuity in a non-social invertebrate species. PLOS ONE 5:e9640

Paterson IG, Partridge V, Buckland-Nicks J (2001) Multiple paternity in *Littorina obtusata* (Gastropoda, Littorinidae) revealed by microsatellite analyses. Biol Bull (Woods Hole) 200:261–267

Portnoy DS, Piercy AN, Musick JA, Burgess GH, Graves JE (2007) Genetic polyandry and sexual conflict in the sandbar shark, *Carcharhinus plumbeus*, in the western North Atlantic and Gulf of Mexico. Mol Ecol 16:187–197

R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact test and ecumenicism. J Hered 86:248–249

Rheindt FE, Gadau J, Strehl CP, Hölldobler B (2004) Extremely high mating frequency in the Florida harvester ant (*Pogonomyrmex badius*). Behav Ecol Sociobiol 56:472–481

Roche Applied Science (2006) GS guide to amplicon sequencing. Manual No. USM-0022.B-December 2006. Roche Diagnostics, Mannheim

Ronkainen K, Kaitala A, Kivelä SM (2010) Polyandry, multiple mating, and female fitness in a water strider *Aquarius paludum*. Behav Ecol Sociobiol 64:657–664

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106

Rowe L (1992) Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Anim Behav 44:189–202

Sadek MM (2001) Polyandry in field-collected *Spodoptera littoralis* moths and laboratory assessment of the effects of male mating history. Entomol Exp Appl 98:165–172

Sakaluk SK, Schaus JM, Eggert AK, Snedden WA, Brady PL (2002) Polyandry and fitness of offspring reared under varying nutritional stress in decorated crickets. Evolution 56:1999–2007

Shaw PW, Sauer WHH (2004) Multiple paternity and complex fertilisation dynamics in the squid *Loligo vulgaris reynaudii*. Mar Ecol Prog Ser 270:173–179

Simmons LW (2005) The evolution of polyandry: sperm competition, sperm selection, and offspring viability. Annu Rev Ecol Evol Syst 36:125–146

Simmons LW, Beveridge M (2010) The strength of postcopulatory sexual selection within natural populations of field crickets. Behav Ecol 21:1179–1185

Simmons LW, Beveridge M, Kennington WJ (2007) Polyandry in the wild: temporal changes in female mating frequency and sperm competition intensity in natural populations of the tettigoniid *Requena verticalis*. Mol Ecol 16:4613–4623

Sprenger D, Anthes N, Michiels NK (2008) Multiple mating affects offspring size in the opisthobranch *Chelidonura sandrana*. Mar Biol 153:891–897

Squires ZE, Wong BBM, Norman MD, Stuart-Fox D (2014) Multiple paternity but no evidence of biased sperm use in female dumpling squid *Euprymna tasmanica*. Mar Ecol Prog Ser 511:93–103

Tarpy DR (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proc R Soc B 270:99–103

Taylor ML, Price TAR, Wedell N (2014) Polyandry in nature: a global analysis. Trends Ecol Evol 29:376–383

Torres-Vila LM, Rodríguez-Molina MC, McMinn M, Rodríguez-Molina A (2005) Larval food source promotes cyclic seasonal variation in polyandry in the moth *Lobesia botrana*. Behav Ecol 16:114–122

Tuni C, Schneider J, Uhl G, Herberstein ME (2020) Sperm competition when transfer is dangerous. Philos Trans R Soc Lond B Biol Sci 375:20200073

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Voight JR, Feldheim KA (2009) Microsatellite inheritance and multiple paternity in the deep-sea octopus *Graneledone boreopacifica* (Mollusca: Cephalopoda). Invertebr Biol 128:26–30

Walker DE, Power AJ, Sweeney-Reeves M, Avise JC (2007) Multiple paternity and female sperm usage along egg-case strings of the knobbed whelk, *Busycon carica* (Mollusca; Melongenidae). Mar Biol 151:53–61

Wang J (2004) Estimating pairwise relatedness from dominant genetic markers. Mol Ecol 13:3169–3178

Woodbury BD (1986) The role of growth, predation, and habitat selection in the population distribution of the crown conch, *Melongena corona* Gmelin. J Exp Mar Biol Ecol 97:1–12

Xue D, Zhang T, Liu JX (2014) Microsatellite evidence for high frequency of multiple paternity in the marine gastropod *Rapana venosa*. PLOS ONE 9:e86508

Xue DX, Zhang T, Liu JX (2016) Influences of population density on polyandry and patterns of sperm usage in the marine gastropod *Rapana venosa*. Sci Rep 6:23461

Ylitalo H, Oliver TA, Fernandez-Silva I, Wood JB, Toonen RJ (2019) A behavioral and genetic study of multiple paternity in a polygamous marine invertebrate, *Octopus oliveri*. PeerJ 7:e6927

Yue GH, Le Li J, Wang CM, Xia JH, Wang GL, Feng JB (2010) High prevalence of multiple paternity in the invasive crayfish species, *Procambarus clarkii*. Int J Biol Sci 6:107–115

Zajdel J, Lance SL, Rainwater TR, Wilkinson PM, Hale MD, Parrott BB (2019) Mating dynamics and multiple paternity in a long-lived vertebrate. Ecol Evol 9: 10109–10121

Zbinden JA, Largiadèr CR, Leippert F, Margaritoulis D, Arlettaz R (2007) High frequency of multiple paternity in the largest rookery of Mediterranean loggerhead sea turtles. Mol Ecol 16:3703–3711

Zeh JA, Zeh DW (1994) Last-male sperm precedence breaks down when females mate with three males. Proc R Soc B 257:287–292

Appendix. Detailed information of the 10 microsatellites used in this study

Table A1. Characterization of 10 microsatellite markers for *Melongena corona*. Stars (*) are microsatellites developed by Hayes & Karl (2004). Plus signs (+) indicate microsatellites developed in this study. Underlined bases consist of the universal fluorescent tails described in Table A2

Locus	Primer sequences (5′ - 3′)	Repeat motif	Allele size range (bp)	Multiplex number
Mcor3010+	F: GCC TTG CCA GCC CGC ATT ATC CAT GCA TCT TCT GGG R: CAT CTG TCG TGC GGT AAA GAG	$(ACAT)_{25}$	129–197	1
McoA4*	F: CAG GAC CAG GCT ACC GTG TGC TTA GAT TGG AGG TGT TGG R: CGT CGG GAC AGA TTG TGA TAC	$(GAAAA)_4$	264–333	1
Mcor652+	F: GCC TCC CTC GCG CCA TTG CAG AGT TAT GTT GCC TCG R: CGT TCT ACT GTA CTG TGC TGT G	$(ACTCT)_{22}$	170–245	1
Mco2*	F: GCC TCC CTC GCG CCA CGA CAG GTG GCG TTA GGT T R: GTT GGA TTT ATT TGT CTG GTT CG	$(GAA)_{38}$	424–466	1
Mco10*	F: CGG AGA GCC GAG AGG TGC GTG CAT GTT ACT TCC CAC A R: GAT TCC GTT GCA ACT TTT CGT	$(CAAA)_{16}$	299–339	1
Mcor4853+	F: CGG AGA GCC GAG AGG TGA TCA GTA TGC CAC AGA CCT CG R: TAT CAC ATG TGC TTG GTG GTC	$(ACT)_{24}$	191–242	1
Mcor4007+	F: CGG AGA GCC GAG AGG TGT GTA AGT ACA GGC TGC TGG R: AAC TGC GAA GTG AGT CCA ATC	$(AAC)_{13}$	216–249	2
Mco12*	F: CAG GAC CAG GCT ACC GTG AGG ATT AAT GGG AAA TCA TTG CT R: GAG CTT GAA GTA CAC GCT TGA	$(GC)_5(AC)_{13}$	202–242	2
Mco3*	F: GCC TTG CCA GCC CGC TCT GAA AGA ATT TTC GCT TCT TA R: CCT GGT CAA TAA TCT TCA CAA AA	$(GTTT)_{19}$	187–255	2
Mcor6632+	F: GCC TCC CTC GCG CCA ACG GCT TTG TTT GTA AGT GAC R: ATT GAC AGG AGG GAC ACT TTC	$(AAAG)_{12}$	190–250	2

Table A2. Characterization of the universal fluorescent tails that attached to the forward primers

Universal fluorescent tail	Primer sequence (5′ – 3′)	Reference
Tail A (FAM)	GCC TCC CTC GCG CCA	Roche Applied Science (2006)
Tail B (VIC)	GCC TTG CCA GCC CGC	Roche Applied Science (2006)
Tail C (NED)	CAG GAC CAG GCT ACC GTG	Blacket et al. (2012)
Tail D (PET)	CGG AGA GCC GAG AGG TG	Blacket et al. (2012)

Table A3. Summary statistics of microsatellite markers for *Melongena corona* for 46 randomly sampled adults. Na: number of alleles; $H_{o:}$ observed heterozygosity; H_e: expected heterozygosity; p-values for Hardy-Weinberg equilibrium test. No linkage was detected between any loci ($p > 0.10$) using Genepop v4.7.5 (Raymond & Rousset 1995, Rousset 2008). No null alleles were detected for any loci using Microchecker v. 2.2.3 (Van Oosterhout et al. 2004)

Locus	Na	H_o	H_e	F_{IS}	p
Mcor3010	14	0.913	0.899	−0.016	0.1552
McoA4	16	0.870	0.903	0.038	0.4621
Mcor652	17	0.826	0.931	0.114	0.0044
Mco2	14	0.804	0.889	0.121	0.0177
Mco10	9	0.804	0.745	−0.081	0.3532
Mcor4853	15	0.891	0.910	0.021	0.9472
Mcor4007	9	0.761	0.804	0.054	0.0475
Mco12	8	0.652	0.751	0.133	0.2243
Mco3	15	0.848	0.909	0.069	0.5516
Mcor6632	11	0.804	0.823	0.023	0.5320

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