

# Florida State University Libraries

---

Electronic Theses, Treatises and Dissertations

The Graduate School

---

2021

## Ecological and Evolutionary Dynamics of Inbreeding and Outbreeding in a Spermcasting Invertebrate

Kevin Olsen

FLORIDA STATE UNIVERSITY  
COLLEGE OF ARTS AND SCIENCES

ECOLOGICAL AND EVOLUTIONARY DYNAMICS OF INBREEDING AND  
OUTBREEDING IN A SPERMCASTING INVERTEBRATE

By

KEVIN C. OLSEN

A Dissertation submitted to the  
Department of Biological Science  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

2021

Kevin C. Olsen defended this dissertation on June 30, 2021.

The members of the supervisory committee were:

Don R. Levitan  
Professor Directing Dissertation

Peter Beerli  
University Representative

Scott C. Burgess  
Committee Member

Joseph Travis  
Committee Member

Alice A. Winn  
Committee Member

The Graduate School has verified and approved the above-named committee members, and certifies that the dissertation has been approved in accordance with university requirements.

For my mother Eileen, sister Sara, and my brother Bill whose love and support shaped me as a person.

## ACKNOWLEDGMENTS

This work would not have been possible without the guidance, support, and encouragement of my major advisor Don Levitan. I would like to thank all the members of my committee, Peter Beerli, Scott Burgess, Don Levitan, Joseph Travis, and Alice Winn for challenging me to become a better naturalist. I especially thank Alice Winn for her willingness to share her incredible knowledge of plant mating systems with marine scientists. I thank Isabelle Basden, Rachael Best, Scott Burgess, Ashley Dawdy, Alex Hooks, Katie Kaiser, Don Levitan, Jose Moscoso, Kate Mcgrath Hill, Jackson Powell, Bobbie Renfro, and Brendan Scherer for assistance in the field, as well as Giuliana Antunez, Cesar Estien, Yueling Hao, and Ellen Kosman for assistance in the laboratory. I am grateful for assistance from Gretchen Lambert and Lauren Stefaniak in identifying ascidian species. I would like to thank the PADI Foundation, the Department of Biological Science at Florida State University, and the Florida State University Coastal and Marine Laboratory for funding that supported my dissertation research.

## TABLE OF CONTENTS

List of Tables .....	vii
List of Figures .....	viii
Abstract .....	x
1. BIPARENTAL INBREEDING MIRRORS RATES OF SELF-FERTILIZATION .....	1
1.1 Introduction .....	1
1.2 Methods .....	5
1.2.1 Overview .....	5
1.2.2 Study species .....	6
1.2.3 Sampling .....	6
1.2.4 DNA extraction, microsatellite design and amplification .....	7
1.2.5 Population genetics .....	8
1.2.6 Assignment tests .....	9
1.2.7 Relatedness .....	9
1.2.8 Inbreeding .....	11
1.2.9 Statistical analyses .....	12
1.3 Results .....	13
1.4 Discussion .....	15
1.4.1 Gene flow, demographic openness, and relatedness .....	16
1.4.2 Biparental inbreeding and differentiating mating systems .....	18
2. INBREEDING TOLERANCE, SIRE SIZE, AND PROXIMITY GOVERN REPRODUCTIVE SUCCESS .....	28
2.1 Introduction .....	28
2.2 Methods .....	31
2.2.1 Overview .....	31
2.2.2 Sampling .....	32
2.2.3 Relatedness .....	33
2.2.4 Spatial patterns of relatedness .....	33

2.2.5 Siring success .....	34
2.2.6 Statistical analyses.....	35
2.3 Results .....	36
2.4 Discussion .....	38
APPENDICES .....	48
A. SUPPLEMENTARY INFORMATION FOR CHAPTER 1 .....	48
B. SUPPLEMENTARY INFORMATION FOR CHAPTER 2.....	50
References.....	53
Biographical Sketch .....	62

## LIST OF TABLES

1.1. Mating system estimates across sites. Sample size of maternal colonies with number of offspring in parentheses, total inbreeding measured with the single locus estimate of selfing ( $S_s$ ), the number of offspring where selfing was excluded by the presence of non-maternal alleles with the proportion in parentheses, and an estimate of the fraction of total inbreeding due to mating among relatives ( $1-r_s$ ). Biparental inbreeding was distinguished from self-fertilization with either the presence of non-maternal alleles or $1-r_s$ .....	26
2.1. Results of multiple logistic regression with mixed effects. Effects of relatedness, relative size, relative distance, and their interactions on the number of offspring sired. Maternal colony included as a nested random effect within site. Significant p-values in bold .....	46
A.1. Location, date, and depth of sites sampled .....	48
A.2. Primer sequences, repeat motif, allelic polymorphism ( $n_a$ ), and $F_{IS}$ of the 10 microsatellite loci designed for this study. All loci were amplified with an M13 tag on the forward primer and two loci (LV809PT and LV2565PT) had an additional ‘Pig-Tail’ on the end of the reverse sequence .....	49
B.1. Results of model fitting with all possible combinations of relatedness, relative size, and relative distance as predictors in multiple logistic regression with mixed effects. Maternal colony included as a nested random effect within site. ....	50
B.2. Results of model comparisons using likelihood ratio testing. Model numbers are as in Table B.1. Significant p-values in bold.....	51



## LIST OF FIGURES

1.1. Pairwise genetic differentiation among <i>L. verrilli</i> colonies was positively associated with the distance between sites (n = 12 sites, 66 comparisons).....	22
1.2. Estimates of pairwise relatedness between individuals with known coancestry. Open circles represent independent pairwise estimates of relatedness ( $r'$ ) with the seven metrics available in the program COANCESTRY (in order: TRIOML, WANG, LYNCHLI, LYNCHRD, RITLAND, QUELLERGT, DYADML). Open squares represent the mean of independent estimates ( $R$ ). Grey horizontal lines represent the expected value of relatedness for each category. Estimators were used on the same pairings of individuals to identify the metric best suited for distinguishing patterns of coancestry in this dataset (Mother-Offspring n = 21, Full Siblings n = 9, Half Siblings n = 5, Unrelated n = 21).....	23
1.3. Distributions of bootstrapped relatedness ( $R$ ) between maternal colonies and their offspring in outbreeding ( $S_s < 0.2$ ) and inbreeding ( $S_s > 0.2$ ) sites. Sites categorized as outbreeding or inbreeding with the single locus estimate of selfing ( $S_s$ ). Solid line represents mother-offspring pairs in outbreeding sites and dotted line represents mother-offspring pairs in inbreeding sites. Vertical lines are the means of bootstrapped estimates. ....	24
1.4. The prevalence of natal philopatry ( <b>A</b> ) and the relatedness among <i>L. verrilli</i> colonies ( <b>B</b> ) varied substantially across sites. Estimates of philopatry and relatedness were greater on artificial sites compared with natural sites and were not significantly associated with population census size. Open symbols represent sites on artificial substrates; closed symbols represent sites on natural substrates. Dashed lines represent the predicted relatedness of first cousins and half siblings following calibration with observed patterns of coancestry.....	25
1.5. Total inbreeding was positively associated with the prevalence of natal philopatry ( <b>A</b> ) and the degree of relatedness among <i>L. verrilli</i> colonies ( <b>B</b> ) but not the population census size ( <b>C</b> ). Open symbols represent sites on artificial substrates; closed symbols represent sites on natural substrates. Regression lines represent linear best fit. Dashed lines represent the predicted relatedness of first cousins and half siblings following calibration with observed patterns of coancestry. ....	27
2.1. Spatial correlogram of relatedness estimates. A global correlogram across 10 sites indicated that relatedness among <i>L. verrilli</i> colonies was elevated at small spatial scales ( $\leq 0.2$ m) and declined sharply with increasing distance. Vertical bars represent the 95% confidence interval of relatedness estimates in each distance class. The global correlogram was partitioned into three subsections for visualizing patterns of relatedness within sites, among proximate sites, and among distant sites.....	43
2.2. Spatial correlogram of relatedness estimates. Local correlograms within the six sites used to evaluate siring success indicated that relatedness among colonies in close proximity ( $< 2.0$ m) varied considerably among sites. Dashed lines represent the predicted relatedness of first cousins and half siblings calibrated with observed patterns of coancestry.....	44

2.3. Proportion of offspring sired for each maternal colony (n = 18 maternal colonies and 102 offspring) in relation to **(A)** relatedness between dam and potential sires, **(B)** relative size of potential sires, and **(C)** relative distance of potential sires. Sires assigned with genetic exclusion or 95% probability (n = 22 offspring with 13 assigned sires). Dashed lines represent the predicted relatedness of first cousins and full siblings calibrated with observed patterns of coancestry.....45

2.4. Probability of siring success associated with **(A)** relative size and **(B)** relative distance. Predictions based on the results of multiple logistic regression with mixed effects (Table 2.1)...47

B.1 Proportion of offspring sired for each maternal colony (n = 18 maternal colonies and 102 offspring) in relation to relatedness between dam and potential sires. In **(A)** sires assigned with genetic exclusion or 95% probability (n = 22 offspring with 13 assigned sires), **(B)** sires assigned with genetic exclusion or 95% probability and unexcluded sires least related to dams (n = 50 offspring with 28 assigned sires), **(C)** sires assigned with genetic exclusion or 95% probability and unexcluded sires most related to dams (n = 50 offspring with 28 assigned sires). Dashed lines represent the predicted relatedness of first cousins and full siblings calibrated with observed patterns of coancestry. ....52

## ABSTRACT

The ecological and evolutionary factors influencing whether hermaphrodites inbreed or outbreed via self-fertilization or outcrossing has long been a theoretical and empirical focus. Recent theory predicts that the conditions favoring the evolution of self-fertilization from outcrossing should also promote tolerance of biparental inbreeding in species with separate sexes or hermaphrodites with mechanisms of self-incompatibility. Yet, there is little evidence of inbreeding tolerance, especially when individuals have the option to outbreed. We quantified biparental inbreeding in a sessile marine invertebrate (*Lissoclinum verrilli*) in which individuals have the potential to cast sperm over large distances to avoid inbreeding. Data from progeny arrays and paternity assignments indicate that unlike many animals, individuals tolerated mating with close kin when natal philopatry placed them nearby. Variation in biparental inbreeding and outbreeding across sites spanned nearly the complete range of possible values and resembled rates of self-fertilization and outcrossing reported in self-compatible taxa. Rather than shared ancestry, colony size and proximity determined paternal success within sites, emphasizing the importance of sperm availability and competition to reproductive success in free spawning invertebrates. Positive associations between philopatry, the relatedness among conspecifics, and biparental inbreeding across sites indicate that either individuals could not discriminate kin from non-relatives or that costs associated with avoiding inbreeding and the genetic benefits of inbreeding counterbalanced the negative effects of inbreeding depression. Our results highlight overlap in the dynamics of uni- and biparental mating systems and suggest that natal philopatry and inbreeding tolerance contribute meaningfully to the substantial variation in inbreeding and outbreeding within species of spermcasting marine invertebrates.

## CHAPTER 1

### BIPARENTAL INBREEDING MIRRORS RATES OF SELF-FERTILIZATION

#### 1.1 Introduction

The amount of coancestry shared between mates is a critical force shaping the structure of genetic variation within and among populations (Charlesworth 2003). The evolutionary causes of inbreeding and outbreeding manifested through self-fertilization or outcrossing has long been a focus in seed plants and more recently hermaphroditic animals (Darwin 1877; Jarne and Auld 2006; Cutter 2019). In general, self-fertilization engenders an inherent genetic transmission advantage over outcrossing and can be adaptive when mates are limiting (Fisher 1941; Kalisz and Vogler 2003), whereas outcrossing tends to be favored if inbred offspring suffer from reduced fitness due to inbreeding depression (Charlesworth and Charlesworth 1987). The dynamic between self-fertilization and outcrossing is paralleled in theory by inbreeding preference and avoidance in interacting individuals (Kokko and Ots 2006; Puurtinen 2011; Duthie and Reid 2016), but few studies have addressed whether the organismal traits and ecological conditions that promote selfing also lead to mating among kin.

The extent to which individuals inbreed or outbreed is a product of traits influencing the likelihood to avoid, tolerate, or prefer inbreeding and past demographic processes that establish the current pool of available mates (Shields 1982; Szulkin et al. 2013). Increased rates of self-fertilization at the periphery of a species range in seed plants (Pannell 2015), and mating among relatives in isolated populations of birds, insects, and mammals highlight the influence of dispersal on mating systems (Keller and Waller 2002). However, self-incompatibility (SI) systems and kin discrimination during mate choice are commonly employed to avoid the costs of inbreeding depression (Barrett 1988; Pusey and Wolf 1996). These recognition systems can

uncouple mating systems from processes that isolate individuals and place relatives together in close proximity (Pannell 2015; Leedale et al. 2020). Even when SI or inbreeding avoidance are absent, strong selection against inbred progeny can disrupt the association between the relatedness of nearby conspecifics and the effective strength of inbreeding (Lande et al. 1994; Winn et al. 2011). Examining the interaction between dispersal, the relatedness among individuals, and the degree of biparental inbreeding is key to understanding the ecological and evolutionary causes of mating among kin.

Theory and empirical examples connecting dispersal with the evolution of self-fertilization emphasize the relationship between SI systems, isolation, mate availability, and the wide variation in selfing rates within and across hermaphroditic species (Goodwillie et al. 2005; Jarne and Auld 2006; Auld and de Casas 2013; Whitehead et al. 2018). In comparison, only recent theory addresses how relatedness among mates, the ability to discriminate kin from non-relatives, and conspecific density combine to influence the evolution of inbreeding and outbreeding given obligate biparental reproduction (Kokko and Ots 2006; Puurtinen 2011; Duthie and Reid 2016). Moreover, empirical methods used to quantify selfing and biparental inbreeding are often distinct, making it difficult to identify similarities or differences in the causes of inbreeding via selfing and mating among relatives. Rates of self-fertilization in hermaphroditic plants and animals are typically measured with the frequencies of genotypes in parents and their offspring (progeny arrays), whereas the strength of biparental inbreeding in animals with separate sexes is commonly measured using pedigrees or genetic estimates of homozygosity (Ritland 2002; Szulkin et al. 2013). Quantifying biparental inbreeding with progeny arrays places both reproductive modes on the same scale, and would permit comparisons of the magnitude of selfing and biparental inbreeding across taxa. Furthermore,

evaluating the factors contributing to biparental inbreeding in species likely to mate with relatives may help identify general conditions driving mating system variation.

A survey of inbreeding coefficients ( $F_{IS}$ ) suggests that the magnitude of inbreeding in sessile and sedentary marine invertebrates is on par with terrestrial seed plants, and that variation in inbreeding and outbreeding across species can be explained by differences in reproductive and dispersal traits (Olsen et al. 2020). Because many marine species have a free-swimming planktonic life stage lasting weeks or months, populations of marine organisms were historically viewed as demographically open, indicating that associations among relatives and opportunities for inbreeding should be rare (Knowlton and Jackson 1993; Caley et al. 1996). However, variation in pelagic larval duration is becoming better recognized (Shanks 2009), and evidence is building that in some populations of invertebrates and reef fish individuals do not disperse away from relatives but remain near their natal patches (Wood and Gardner 2007; Hogan et al. 2012; Burgess et al. 2014). Evidence of natal philopatry and the collective dispersal of siblings in cohesive larval packets (Burgess et al. 2016; Eldon et al. 2016), along with positive estimates of  $F_{IS}$ , suggest that inbreeding may play a larger role in the mating systems of marine invertebrates than commonly recognized (Olsen et al. 2021). Inbreeding coefficients also vary considerably within species of marine invertebrates, especially those with brief planktonic stages and reproductive traits that limit control over mate choice once gametes are spawned (Addison and Hart 2005; Olsen et al. 2020). Variation in  $F_{IS}$  within gonochoristic (dioecious) marine invertebrates suggests that traits affecting the tendency to prefer, avoid, or tolerate inbreeding, and ecological factors such as mate density and habitat isolation contribute meaningfully to the strength of biparental inbreeding in these species just as they do rates of self-fertilization in self-compatible plants and animals.

Spermcasting marine invertebrates typically have a short larval stage lasting only minutes or days, and similar to wind pollination, rely on oceanic currents as an external vector to transport male gametes to conspecifics (Bishop and Pemberton 2006). In general these species are sessile, and larval movement, mixing, and recruitment are responsible for establishing the pool of nearby potential mates. Because time spent in the plankton is limited, dispersal in these species can be comparatively philopatric, leading to spatial associations among relatives and the potential for inbreeding (Yund and O'Neil 2000; Calderon et al. 2007; Blanquer et al. 2009). Yet even though individuals are often near kin, inbreeding coefficients can be surprisingly low in these species (Hellberg 1994; 1996; Blanquer et al. 2009), indicating that either individuals avoid inbreeding via recognition systems and the planktonic transport of sperm, or that lethal inbreeding depression removes most inbred offspring.

Despite sessile reproductive stages, spermcasting marine invertebrates have the capacity to mate over large spatial scales and for dispersing sperm to contribute to gene flow among populations (Grosberg 1991; Yund 1995; Yund et al. 2007; Johnson and Yund 2009). This is in part because the spermatozoa of these species remain viable for hours or days (Johnson and Yund 2004) and because internal fertilization is efficient due to the ability to collect, filter, and concentrate sperm from the water column (Pemberton et al. 2003). Even though sperm can be transported over hundreds of meters (Yund et al. 2007), proximity among conspecifics and sperm competition strongly influence patterns of mating in these species (Grosberg 1987; Yund and McCartney 1994; Pemberton et al. 2003). However, a somatic-gametic recognition system comparable to the SI system of some terrestrial plants has been described in spermcasting species that can prevent self-sperm and the sperm of relatives from fertilizing retained eggs (Bishop 1996). This control over paternity may allow sperm recipients to select less related mates and

avoid inbreeding (Bishop et al. 1996; Olsen et al. 2021), but like SI in plants, the presence and effectiveness of this recognition system varies among and within species (Grosberg and Hart 2000). Thus, the way in which sperm availability and competition interact with the relatedness of proximate conspecifics to influence fertilization, and how this relationship may vary in populations with different demographic histories is largely unknown.

The potential to avoid inbreeding via recognition systems and by projecting planktonic sperm makes spermcasting invertebrates an interesting group to assess associations between demographic openness, the degree of relatedness among individuals, mate availability, and biparental inbreeding. Specifically we address two main questions with the ascidian *Lissoclinum verrilli*: 1) *What is the extent of variation in the degree of relatedness among individuals in comparatively closed and open populations?* and 2) *Is the magnitude of biparental inbreeding associated with the relatedness of nearby conspecifics or are mating systems uncoupled from larval movement and mixing by inbreeding avoidance, long dispersing sperm, and/or early onset inbreeding depression?*

## **1.2 Methods**

### *1.2.1 Overview*

We evaluated genetic variation among *L. verrilli* colonies and their offspring to make inferences about natal philopatry, relatedness, and inbreeding in sites with different degrees of spatial isolation. We quantified inbreeding and distinguished self-fertilization from mating among relatives with progeny arrays, which are often used to estimate selfing rates in seed plants and hermaphroditic animals, but have rarely been employed to measure biparental inbreeding in animals. Associations between natal philopatry, relatedness, and the frequency of biparental inbreeding across sites were used to evaluate whether individuals avoided, tolerated, or preferred



mating with relatives. If inbreeding avoidance was widespread, we expected to find low estimates of inbreeding across all sites. Similarly, if inbreeding preference was prevalent, we expected to find consistently high estimates of inbreeding. If individuals tolerated inbreeding, we expected to find positive linear relationships between the frequency of biparental inbreeding, natal philopatry, and relatedness among conspecifics.

### 1.2.2 *Study species*

*Lissoclinum verrilli* is a colonial ascidian in the family Didemnidae with a documented range that extends throughout the Caribbean and into the Gulf of Mexico (Van Name 1945; Rocha et al. 2012). This species reproduces sexually on a lunar cycle via spermcasting, in which sperm is released into the sea and then captured for internal fertilization and embryonic development. Mature tadpole larvae develop from a maternal energy source and are released after approximately 12 days. In the laboratory, released larvae settle and metamorphose within minutes (*pers. observation*). The zooids of *L. verrilli* (the physiological units of ascidian colonies) are hermaphroditic, but a somatic-gametic recognition system that blocks self-sperm has been described in a species of the same family (Bishop 1996; Bishop et al. 1996), and our current findings suggests that self-fertilization is absent or rare in this species.

### 1.2.3 *Sampling*

We sampled *L. verrilli* colonies April - October in the years 2016 - 2019 from twelve sites in the northeastern Gulf of Mexico. Sampling sites (~10 - 22 m depth) consisted of natural limestone hard-bottom reefs (“Allegedly” and “Sargassum”), artificial concrete substrates (“Two Dogs Reefballs”, “Carrabelle Three Mile Culverts”, “K Tower”, “O Tower”), and a man-made inlet rock jetty (“St. Andrews State Park”) (Table A.1). We selected these sites because they harbored the study species in varying densities and differed in the degree of isolation from other

substrates with *L. verrilli*. Natural reefs in this region consist of a patchwork of limestone outcroppings separated by small spits of sand, and sampling sites on these reefs were in close proximity to other outcroppings inhabited by *L. verrilli* (<10 m). In comparison, artificial substrates were surrounded by large expanses of sand without hard substrate close by. These artificial substrates were sampled because their remote locations were known and permitted an assessment of mating systems under geographic isolation. At each site transect tape was extended along the longest length and width of substrate and the position and size of each *L. verrilli* colony was recorded. Sampled sites ranged in size from 5 × 3 m to 19 × 7 m. Effort was made to locate every *L. verrilli* within the sampling area and each physically distinct colony was sampled by excising a tissue sample (~5 × 5 cm).

Tissue samples of *L. verrilli* were transported to Florida State University, fixed in 95% ethanol, and maintained at -20°C. Subsequently, samples were dissected to isolate adult zooids and brooded offspring for DNA extraction. Tissue samples from seven of the twelve sites contained internally developing offspring. The developmental stage of offspring varied within tissue samples and ranged from newly formed embryos to fully developed larvae nearing release. Approximately fifteen larvae at the most advanced developmental stage were dissected from three maternal colonies from each of the seven sites.

#### *1.2.4 DNA extraction, microsatellite design and amplification*

*L. verrilli* adult zooids were digested in a solution of CTAB and proteinase K in a 65°C water bath, and DNA was extracted and purified with a magnetic-bead protocol (SprintPrep DNA purification kit, Agencourt Bioscience Corporation Beverly, MA). The DNA of larval offspring was extracted and purified using the protocol of the QIAamp DNA Micro Kit (Qiagen, Germantown, MD). DNA concentrations were quantified with a NanoDrop ND-1000

spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and with a Qubit broad-range assay (Thermo Fisher Scientific).

A genomic DNA library enriched for microsatellite loci was developed for *L. verrilli* by the Evolutionary Genetics Core Facility at Cornell University. Ninety potential contigs were screened from this library and 10 loci were selected based upon repeat motif, product size, and degree of polymorphism (Table A.2). Loci were amplified with the Qiagen Type-it Microsatellite PCR Kit under the following cycling parameters: 95°C for 4 minutes, 30 cycles of 95°C for 30 seconds, 57°C for 40 seconds, 72°C for 45 seconds, 9 cycles of 95°C for 30 seconds, 53°C for 40 seconds, 72°C for 40 seconds, and a final extension of 72°C for 10 minutes. Fragment analysis was performed by the DNA Sequencing Facility at Florida State University with GeneScan 500 ROX standard (Thermo Fischer Scientific) and alleles were called in the program Geneious 9.1.8 (Biomatters, New Zealand).

#### *1.2.5 Population genetics*

Allelic count, linkage disequilibrium ( $\bar{r}d$ ), and the number of unique multi-locus genotypes detected by the 10 microsatellite loci were assessed in the R statistical program (version 4.0.3) in the package ‘poppr’ (Kamvar et al. 2021). To evaluate loci for the presence of non-amplifying variants (null alleles), heterozygosity at each locus was compared to Hardy-Weinberg Equilibrium in the R package ‘hierfstat’ (Goudet et al. 2020). The spatial extent of gene flow was evaluated by quantifying genetic differentiation among colonies between each site with Nei’s  $F_{ST}$  (Nei 1987), and the distance between sites was calculated with their G.P.S. coordinates and the haversine great-circle distance formula. Because some sites were sampled in different years and temporal variation could confound spatial patterns, genetic differentiation over time in closely situated sites was also assessed. Specifically, pairwise genetic differentiation among four sites

within the reef system “Allegedly” was evaluated over three consecutive years to test if genetic differences increased over the sampling period.

Occasionally, identical multi-locus genotypes were detected in physically distinct colonies that were in close proximity (<1 m). These putative clonemates were likely formed by a regressing colony that split into multiple smaller colonies rather than asexual propagation (*pers. laboratory-based observation*), and were treated as a single genetic individual.

#### *1.2.6 Assignment tests*

Assignment tests were used to estimate the strength of philopatric recruitment within sites with the program GENECLASS 2 (Piry et al. 2004). This program uses the multi-locus genotypes of individuals to probabilistically assign or exclude reference populations as possible sites of origin. The most probable site-of-origin was assigned to each *L. verrilli* colony with the likelihood criteria of Rannala and Mountain (1997), and the proportion of individuals reassigned to their sampling location was estimated for each site. This approach is comparable to those with probabilistic cutoffs (Berry et al. 2004) and was used here because we were interested in associations among philopatry, isolation, and inbreeding across all sites, rather than dispersal patterns within any single site. Offspring genotypes were included in assignment tests to assess the capacity of the analysis to correctly identify sites of origin.

#### *1.2.7 Relatedness*

The degree of relatedness among individuals was estimated in the program COANCESTRY (Wang 2011). This program implements seven estimates of relatedness that differ in the methodology used to infer identity by descent from allelic states (Weir et al. 2006). To select and calibrate the metric used in this study, estimates of relatedness were compared to theoretical expectations in pairings of individuals where recent coancestry was known. The seven

relatedness estimators were assessed across four categories of pairings with either the same or differing degrees of coancestry (mother-offspring, full siblings, half siblings, and unrelated). Because pairwise estimates are interdependent (*e.g.* the relatedness of Mother A to offspring B is not independent of the relatedness between Mother A and offspring C), means of pairwise estimates were compared. For example, with  $M$  maternal colonies let  $m(i)$  be maternal colony  $i$  and  $o(i, j)$  be the  $j$ th offspring of maternal colony  $i$ . Let  $n(i)$  be the number of offspring of maternal colony  $i$  so that there are  $n(i)$  of  $o(i, j)$  and  $M$  such families. For each  $m(i)$ , the relatedness with each  $o(i, j)$  was estimated and the mean of these pairwise values was calculated ( $r'$ ). The mean of  $r'$  across all maternal colonies ( $R$ ) was compared among estimators. A similar approach with focal individuals was used to estimate relatedness within full sibling, half sibling, and unrelated pairings.

Identifying mother-offspring pairings was straightforward as offspring were dissected from maternal colonies. Full and half siblings were identified with parentage analyses that assigned either the same or a different siring colony by genetic exclusion or 95% assignment probability in the program CERVUS (Marshall et al. 1998). To evaluate the capacity of the estimators to distinguish relatives from non-relatives, individuals that were unlikely to share recent common ancestry were also incorporated. Larval offspring dissected from maternal colonies that originated from geographically separated sites (O Tower and Sargassum-2, distance = 27 km) were used to assess an “unrelated” category. Individuals in this category do not share the same dam and are unlikely to share the same sire or have recent common ancestry prior to the parental generation. The metric that best distinguished patterns of coancestry across categories was used for analyses incorporating relatedness. Additional unreported analyses evaluating associations between relatedness, inbreeding, and census size with the remaining estimators had

comparable results. Variance in site-level relatedness was estimated by resampling individual genotypes with replacement within the program.

While the categories used to assess metrics of relatedness reflect phylogenetically recent shared ancestry, background inbreeding and outbreeding can contribute to variation in the relatedness among individuals and may bias estimates above or below theoretical expectations (Weir et al. 2006). To explore this, the degree of relatedness between maternal colonies and their offspring was compared in outbreeding and inbreeding sites (see below for how site-level outbreeding/inbreeding was quantified). A resampling with replacement approach was used to populate  $M$  families with the same distribution of offspring number as the actual data set (outbreeding sites  $n = 9$  maternal colonies with 52 offspring; inbreeding sites  $n = 12$  maternal colonies with 66 offspring) and  $r'$  and  $R$  were calculated. Resampling with replacement and averaging were repeated 1,000 times to create a bootstrapped distribution of  $R$  in outbreeding and inbreeding sites. The mean of bootstrapped  $R$  in outbreeding sites was used to adjust the threshold values of relatedness expected between first cousins and half-siblings.

### *1.2.8 Inbreeding*

The magnitude of inbreeding within sites was estimated with progeny arrays in the program MLTR (Ritland 2002). This program compares allelic frequencies in parental and offspring generations to quantify the total strength of inbreeding due to both self-fertilization and biparental inbreeding and distinguishes their relative contributions to the mating system. For the seven sites where offspring were found, total inbreeding was measured with the single locus estimate of selfing ( $S_s$ ) and biparental inbreeding was distinguished from self-fertilization by genetic exclusion (*e.g.* the offspring genotype contains an allele that is not present in the maternal genotype) and by evaluating the correlation of selfing rates among loci ( $r_s$ ). Inbreeding

due to self-fertilization increases the correlation of selfing rates among loci compared with biparental inbreeding, and  $1 - r_s$  is an estimate of the fraction of total inbreeding due to mating among relatives (Ritland 2002). Two loci (LV493 and LV607) had greater than expected homozygosity (Table A.2) and showed signs of frequent null alleles in progeny arrays. Consequently, these loci were not included when estimating inbreeding. Variance in mating system parameters was estimated in the program by resampling individuals within families with replacement 100 times.

### 1.2.9 Statistical analyses

Analyses in this study focused on evaluating the spatial extent of gene flow, and associations between natal philopatry, relatedness, census size, and inbreeding. Importantly, estimates of philopatry and relatedness both use allelic frequencies to make inferences about the source of immigrants and the amount of coancestry shared among individuals. Thus, there are methodological as well as biological reasons why these estimates may be correlated, and we used caution in attempting to delineate one from the other. Rather, both metrics were incorporated in this study, and were analyzed independently, to demonstrate the robustness of associations between genetic similarity among *L. verrilli* colonies and the magnitude of inbreeding across sites.

Genetic differentiation among *L. verrilli* colonies in space and over time was assessed by testing the correlation between pairwise genetic differentiation measured with the linear approximation  $F_{ST}/1-F_{ST}$  and either the logarithm of the distance between sites or the number of years between samples with a Mantel test in the R package ‘ade4’ (Dray et al. 2020). The proportion of individuals identified as philopatric and the degree of relatedness among colonies on artificial and natural reefs were compared with census size as a covariate in the R package

‘car’ (Fox et al. 2020). The association between the magnitude of total inbreeding and the relatedness among *L. verrilli* colonies was evaluated with an errors-in-variables (EIV) regression in the R package ‘eivtools’ (Lockwood 2018). EIV regression accounts for error in the measurement of an independent variable and was used here because relatedness among individuals varied within sites. Linear regression was used to evaluate relationships between inbreeding and the proportion of philopatric colonies and inbreeding and population census size. The assumptions of inferential tests were confirmed prior to reporting results.

### 1.3 Results

In total, 144 *L. verrilli* colonies were sampled across 12 sites. After removing clonemates, the dataset consisted of 138 colonies with 137 unique multi-locus genotypes, as one of the repeated genotypes was not spatially consistent with colony regression. Linkage disequilibrium in the adjusted dataset was low ( $\bar{r}d < 0.01$ ), suggesting that the 10 microsatellite loci segregated independently and were appropriate for population genetic analyses. Genetic differentiation among colonies was positively correlated with the distance between sites (Mantel Test; correlation = 0.663;  $p = 0.01$ ; Fig. 1.1). Moreover, genetic differentiation among colonies sampled in consecutive years within the same reef system was low ( $F_{ST}$  0.008 – 0.048), and the magnitude of differentiation was not associated with the number of years between sampling (Mantel Test; correlation = 0.236;  $p = 0.43$ ).

Estimates of philopatry and the degree of relatedness among *L. verrilli* colonies were used to assess patterns of genetic mixing across sites. Offspring genotypes were included in population assignments and estimates of relatedness to test the accuracy of these analyses in identifying sites of origin and distinguishing among categories of known coancestry. Assignment tests correctly identified the site of origin in 80% of offspring (95/118) collected from 7 sites.



Given the small size of sampling areas ( $\sim 10 \times 10$  m) and the lack of genetic differentiation between some sites separated by short distances, we found this resolution to be sufficient for evaluating associations between philopatry, habitat isolation, and inbreeding in *L. verrilli*.

The seven marker-based estimates of relatedness were similar in their ability to discriminate among patterns of observed coancestry (Fig. 1.2). On average, the metric described by Wang (2002) best distinguished mother-offspring, full sibling, and half-sibling pairings from individuals unlikely to share recent coancestry, and was used in analyses evaluating associations between relatedness, census size, and inbreeding. Across all metrics, estimates of relatedness were biased above theoretical expectations in mother-offspring, full sibling, and half-sibling pairings. To evaluate the contribution of inbreeding to this positive bias, bootstrapped estimates of relatedness in mother-offspring pairings were compared in inbreeding and outbreeding sites (Fig. 1.3). Bootstrapped distributions of relatedness between mothers and their offspring were significantly greater in inbreeding sites compared with outbreeding sites (KS test;  $D = 1$ ;  $p < 0.001$ ). Furthermore, the mean of bootstrapped estimates in outbreeding sites (0.547) was only slightly biased above the expectation ( $R = 0.5$ ), and this deviation was used to conservatively adjust the predicted relatedness between first cousins and half-siblings in this dataset.

Philopatry and the degree of relatedness among *L. verrilli* colonies varied considerably across sites (Fig. 1.4). Genetic estimates of philopatric recruitment and relatedness were positively but not significantly correlated (Pearson's correlation;  $t = 1.886$ ;  $DF = 10$ ;  $p = 0.089$ ). Nonetheless, the proportion of locally recruiting colonies was greater on artificial substrates compared with natural sites (ANCOVA;  $DF = 1$ ;  $F = 16.574$ ;  $p = 0.003$ ), and was not associated with census size (ANCOVA;  $DF = 1$ ;  $F = 3.097$ ;  $p = 0.112$ ). Similarly, relatedness among colonies was higher on artificial substrates compared with natural sites (ANCOVA;  $DF = 1$ ;  $F =$

7.386;  $p = 0.024$ ), and was also not associated with census size (ANCOVA;  $DF = 1$ ;  $F = 0.963$ ;  $p = 0.352$ ).

In total, 118 offspring dissected from 21 maternal colonies were used in progeny arrays to quantify differences in inbreeding across 7 sites (Table 1.1). Mating systems were highly variable, and rates of total inbreeding ranged from 0.0 to 0.8 (Fig. 1.5). Moreover, the magnitude of inbreeding was positively associated with the proportion of philopatric colonies (linear regression;  $F(1, 5) = 9.305$ ; slope = 1.360;  $p = 0.028$ ) and the degree of relatedness among colonies (EIV regression; slope = 0.949;  $p = 0.034$ ), but was not associated with population census size (linear regression;  $F(1, 5) = 3.859$ ; slope = 0.018;  $p = 0.107$ ). The majority of offspring (100/118) harbored non-maternal alleles in their multi-locus genotypes indicating that they were not the product of self-fertilization (Table 1.1). The remaining 18 offspring could have been produced by either self-fertilization or biparental inbreeding, but estimates of the fraction of total inbreeding due to mating among close relatives were high in these sites ( $1-r_s$  0.8 – 1.0; Table 1.1). Furthermore, analyses with these 18 offspring removed still found an association between total inbreeding and the proportion of philopatric colonies (linear regression;  $F(1, 5) = 17.56$ ; slope = 1.102;  $p = 0.009$ ), and total inbreeding and relatedness among *L. verrilli* colonies (EIV regression; slope = 0.835;  $p = 0.021$ ), but no significant relationship between inbreeding and census size (linear regression;  $F(1, 5) = 0.616$ ; slope = 0.007;  $p = 0.468$ ).

#### **1.4 Discussion**

Despite the potential to avoid inbreeding by casting planktonic sperm, mating among relatives was common in *L. verrilli*. Moreover, variation in biparental inbreeding and outbreeding could be explained by the prevalence of natal philopatry and the degree of relatedness among individuals in a location. Inbreeding increased linearly with the relatedness of

nearby conspecifics, suggesting that *L. verrilli* colonies mate randomly with respect to shared ancestry, and that open or closed population demographics maintain or drive deviations from general panmixia in this species. These patterns draw attention to parallels in the dynamics of selfing and biparental inbreeding, and highlight mechanisms contributing to the substantial variation in inbreeding and outbreeding within species of spermcasting marine invertebrates.

#### 1.4.1 Gene flow, demographic openness, and relatedness

Genetic differentiation among *L. verrilli* colonies was strong over relatively small spatial scales, and sites separated by 18 km or more were consistently distinct (Fig. 1.1). Populations of spermcasting marine invertebrates with indirect development can be homogenized by gene flow via free-swimming larvae and/or the planktonic transfer of sperm (Grosberg 1991; Yund 1995). Yet, the spatial extent of migration is generally restricted in members of this group because the cumulative time spent in the plankton is shorter than for other forms of development where larvae must feed for weeks prior to settlement (Selkoe and Toonen 2011). While species with reduced larval duration tend to exhibit increased genetic subdivision, many exceptions point to the importance of larval behavior, habitat specificity, adult rafting, and water movement in the spatial extent of gene flow in marine invertebrates (Severance and Karl 2006; Ayre et al. 2009; Dupont et al. 2009).

Differences in the magnitude of  $F_{ST}$  across studies should be interpreted carefully (Hellberg 2009), but the distance over which populations of *L. verrilli* began to differentiate was surprisingly small even relative to other species with the same reproductive and developmental modes (Duran et al. 2004; Dupont et al. 2009; Ledoux et al. 2010). Temporal differences in sampling are an unlikely explanation for the genetic differentiation detected among sites, as *L. verrilli* colonies often persist for several years (*pers. observation*) and  $F_{ST}$  between closely

situated sites sampled over multiple years was low. Instead, isolation by distance suggests there are broad restrictions to dispersal in this species, either due to traits that limit sperm and larval longevity or because the patchiness of hard substrate and physical oceanography in the sampling region restrict the movement of gametic and larval propagules.

Given the limitations to migration detected in *L. verrilli*, it could be expected that populations of this species would primarily be closed at small spatial scales. However, demographic openness at the scale of meters varied markedly in different sites (Fig. 1.4A). Some sampling areas were comparatively open with most colonies likely originating from outside the collection site. Other sites were almost completely closed with up to 94% of colonies reassigned to the  $\sim 10 \times 10$  m substrate from which they were collected. Analyses evaluating the degree of relatedness among *L. verrilli* colonies also support stark differences in patterns of genetic mixing among sites (Fig. 1.4B). Estimates of relatedness ranged from values indicating that colonies shared little or no recent ancestry to those suggesting that individuals were, on average, first cousins or half-siblings even after conservative calibration. Closed dynamics and shared ancestry within populations have been reported in reef fish and invertebrates with planktonic larvae that develop for weeks (Hogan et al. 2012; Burgess et al. 2014), signifying that philopatry over small spatial scales in *L. verrilli* can partially be attributed to larval duration lasting only minutes. However, variability in the degree of openness across sites was striking, and suggests that factors other than larval longevity contribute meaningfully to dispersal within and among populations of this species.

The proportion of colonies identified as philopatric and the degree of relatedness among them were greater on artificial sites compared with natural reefs (Fig. 1.4). This pattern suggests that artificial substrates had some combination of characteristics that made their inhabitants more

susceptible to demographic isolation. Artificial and natural substrates did not differ significantly in area or in the density or size of *L. verrilli* colonies (*unreported analyses*). Instead, artificial substrates tended to be located within large expanses of sand, presumably increasing the distance between these sites and exogenous sources of sperm and larval propagules. These spatial patterns are apparent, as artificial reefs are often purposefully deployed in locations that lack natural physical structure to supplement fisheries limited by hard substrate (Strelcheck et al. 2005). The demographic isolation detected on artificial reefs points to the importance of considering population connectivity and the nearest stepping-stone when deploying these substrates. This being said, genetic signatures of philopatry and relatedness among colonies on natural and artificial reefs overlapped, and other than location, there is no fundamental reason why demographic openness or patterns of mating and inbreeding should be different in the two habitat types. Rather, artificial reefs located on the periphery of a source population's dispersal kernel likely reflect the demographics of naturally isolated reefs, and were incorporated in this study because their remote locations were known, and they offered the opportunity to assess the interaction between geographic isolation and mating systems.

#### *1.4.2 Biparental inbreeding and differentiating mating systems*

Instead of avoiding inbreeding, *L. verrilli* tolerated mating with close kin when natal philopatry placed relatives nearby (Fig. 1.5). This pattern is markedly different from those reported for most animals where individuals avoid the costs of inbreeding depression through kin recognition and the preferential use of sperm from unrelated conspecifics (Pusey and Wolf 1996; Bretman et al. 2004; Szulkin et al. 2013; Leedale et al. 2020). Yet, avoiding inbreeding can also be costly, particularly when mating opportunities are missed completely (Nichols et al. 2014;

Reynolds et al. 2014), and our results suggest that proximity rather than shared ancestry between individuals was critical to reproductive success in *L. verrilli*.

Inbreeding tolerance is predicted to evolve as an effective mating strategy when losses in fitness caused by inbreeding depression are counterbalanced by costs associated with avoiding relatives (Kokko and Ots 2006; Duthie and Reid 2016). Given that spermcasting marine invertebrates can reproduce with conspecifics separated by hundreds of meters (Yund et al. 2007; Johnson and Yund 2009), and that a somatic-gametic incompatibility system has been described in a related species (Bishop 1996; Bishop et al. 1996), we thought that *L. verrilli* colonies might avoid inbreeding by preferentially using the sperm of non-relatives. This notion was further supported in that laboratory-based experiments often find a limited capacity for marine invertebrates to self-fertilize (Heyward and Babcock 1986; Levitan et al. 2004; Johnson 2010; Fogarty et al. 2012) and biparentally inbreed (Palmer and Edmands 2000; Burgess et al. 2019). Instead, inbreeding occurred frequently both in geographically isolated sites and on a natural outcrop where unrelated sperm was likely available but presumably at lower concentrations than that of kin (“Allegedly-4” Table 1.1). In this particular site, the average relatedness among individuals was lower than expected given the strength of biparental inbreeding. Multiple families within this site could explain why biparental inbreeding was elevated when mean relatedness among all individuals was low. Nonetheless, the positive association between biparental inbreeding and relatedness across sites suggests that individuals either lacked the ability to discriminate kin from non-relatives and/or that potential reductions in fitness imposed by avoiding inbreeding were equal to or greater than those from inbreeding depression.

The need for reproductive assurance when unrelated sperm is less available may be an important element in the mating system of *L. verrilli*. Like self-compatibility in seed plants and

aquatic snails, individual fitness in this species may be increased by traits that permit mating with a relative over not mating at all (Kalisz and Vogler 2003; Escobar et al. 2011), so long as inbreeding depression does not eliminate this advantage. The magnitude of inbreeding depression over the lifespan of *L. verrilli* is unknown, but selection against inbred progeny was not strong enough to disassociate the strength of inbreeding and subsequent larval development from spatial genetic structuring. Thus, depending on the severity of inbreeding depression manifested after larvae are released, as well as the cost of avoiding relatives, inbreeding tolerance might or might not be adaptive in *L. verrilli*. On one hand, missed mating opportunities while waiting for unrelated sperm and the benefits of maintaining locally adapted allelic combinations might favor inbreeding tolerance. On the other, strong inbreeding depression manifested after larvae are released could make mating among kin deleterious. The former scenario mirrors the evolution of predominant self-fertilization from outcrossing (Goodwillie et al. 2005) and suggests that traits associated with somatic-gametic recognition and sperm and larval longevity may respond to selection to increase biparental inbreeding (Olsen et al. 2021). The latter indicates that despite occurring frequently, mating among relatives is not adaptive in this species, and like unintentional selfing in wind pollinated gymnosperms or biparental inbreeding in higher animals, cannot facilitate the evolution of stable inbreeding mating systems (Lande et al. 1994; Keller and Waller 2002; Winn et al. 2011).

Regardless of whether biparental inbreeding is adaptive in *L. verrilli*, the frequency and magnitude with which it occurs suggests that mating among kin is an integral component of this species' mating system. We did not find a negative relationship between census size and natal philopatry, the relatedness among colonies, or the magnitude of biparental inbreeding across sites. In fact, the two sites with the greatest census sizes exhibited the strongest inbreeding. This

pattern is opposite what would be expected if inbreeding were an exception that only occurred in small ephemeral populations. Moreover, increased relatedness between mothers and their offspring in inbreeding compared with outbreeding sites suggests that differences in these mating systems are persistent and may be correlated with the evolution of other traits (Fig. 1.3).

Further research evaluating the fitness consequences of biparental inbreeding in species where mating among relatives is common is needed to better understand the degree of overlap in the dynamics of uni- and biparental mating systems. We found that biparental inbreeding in a spermcasting invertebrate varied to a similar extent as rates of self-fertilization in seed plants and hermaphroditic animals. While selfing is known to be adaptive under certain conditions and is associated with the evolution of reproductive and dispersal traits (Cutter 2019), our understanding of the adaptive value of biparental inbreeding is far less complete. Given that inbreeding is common in marine invertebrates and that variation in inbreeding and outbreeding is associated with an incredible diversity of reproductive and developmental traits in this group (Olsen et al. 2020), marine invertebrates offer substantial opportunity to address the adaptive value of biparental inbreeding and expand the general study of mating systems.



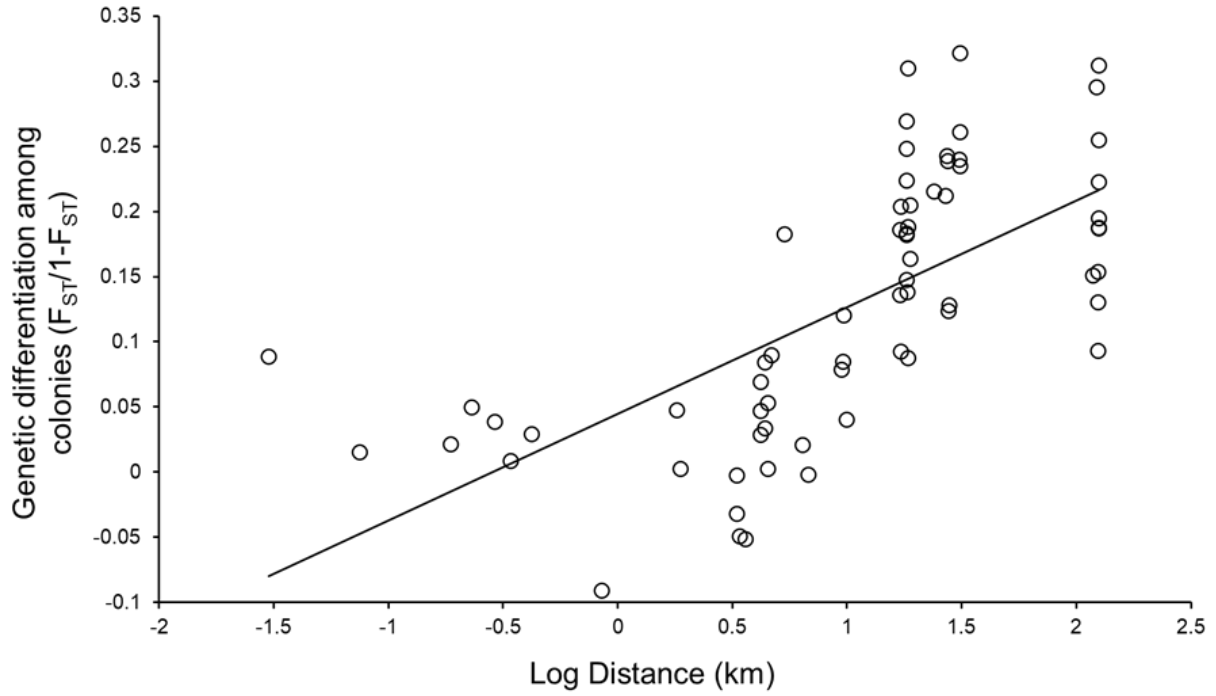


Figure 1.1. Pairwise genetic differentiation among *L. verrilli* colonies was positively associated with the distance between sites (n = 12 sites, 66 comparisons).

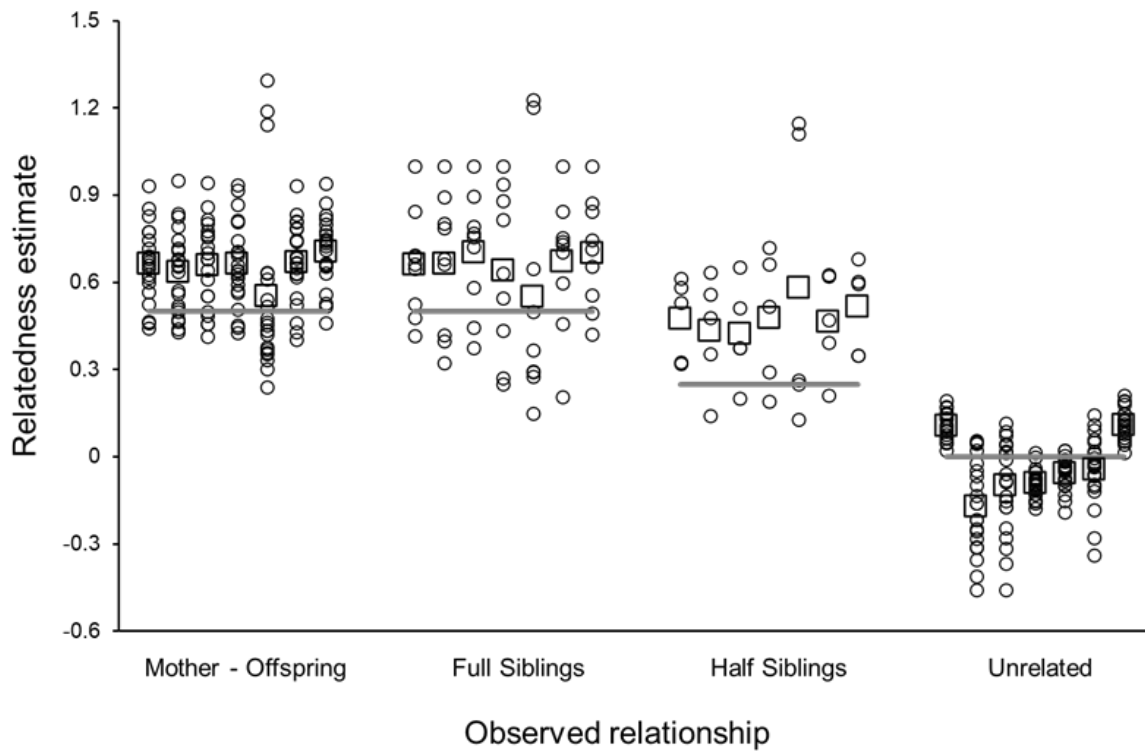


Figure 1.2. Estimates of pairwise relatedness between individuals with known coancestry. Open circles represent independent pairwise estimates of relatedness ( $r'$ ) with the seven metrics available in the program COANCESTRY (in order: TRIOML, WANG, LYNCHLI, LYNCHRD, RITLAND, QUELLERGT, DYADML). Open squares represent the mean of independent estimates ( $\bar{R}$ ). Grey horizontal lines represent the expected value of relatedness for each category. Estimators were used on the same pairings of individuals to identify the metric best suited for distinguishing patterns of coancestry in this dataset (Mother-Offspring  $n = 21$ , Full Siblings  $n = 9$ , Half Siblings  $n = 5$ , Unrelated  $n = 21$ ).

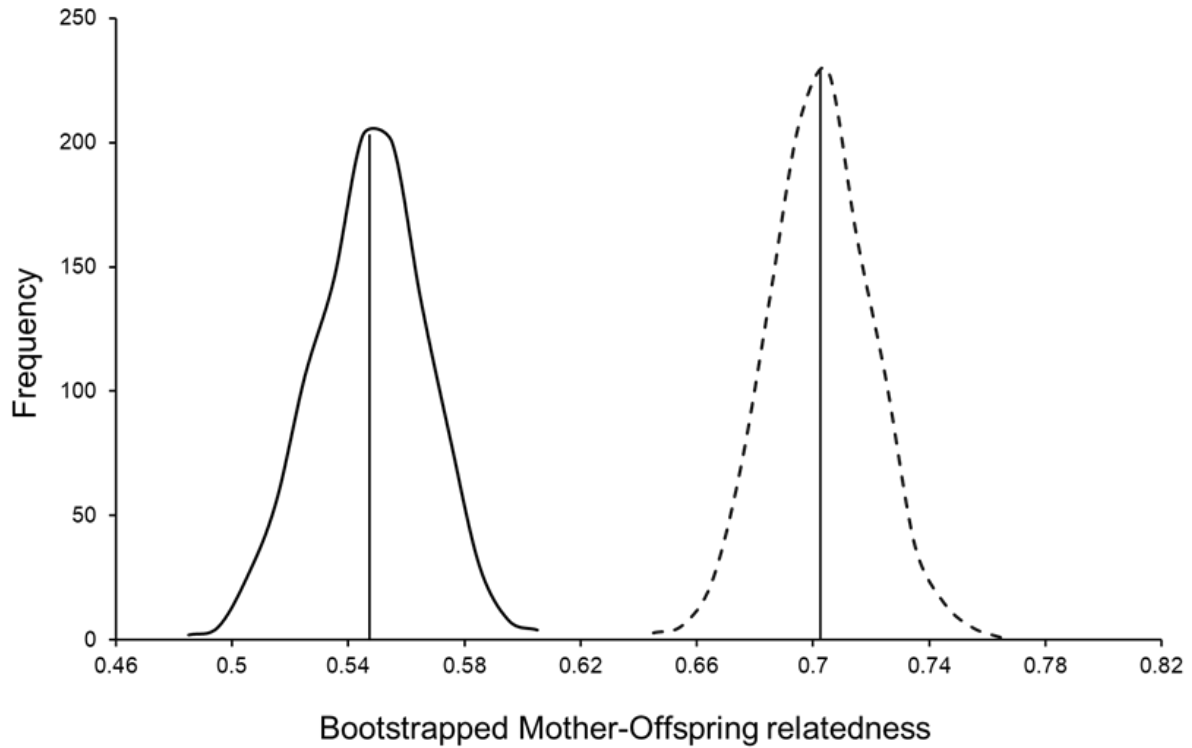


Figure 1.3. Distributions of bootstrapped relatedness ( $R$ ) between maternal colonies and their offspring in outbreeding ( $S_s < 0.2$ ) and inbreeding ( $S_s > 0.2$ ) sites. Sites categorized as outbreeding or inbreeding with the single locus estimate of selfing ( $S_s$ ). Solid line represents mother-offspring pairs in outbreeding sites and dotted line represents mother-offspring pairs in inbreeding sites. Vertical lines are the means of bootstrapped estimates.

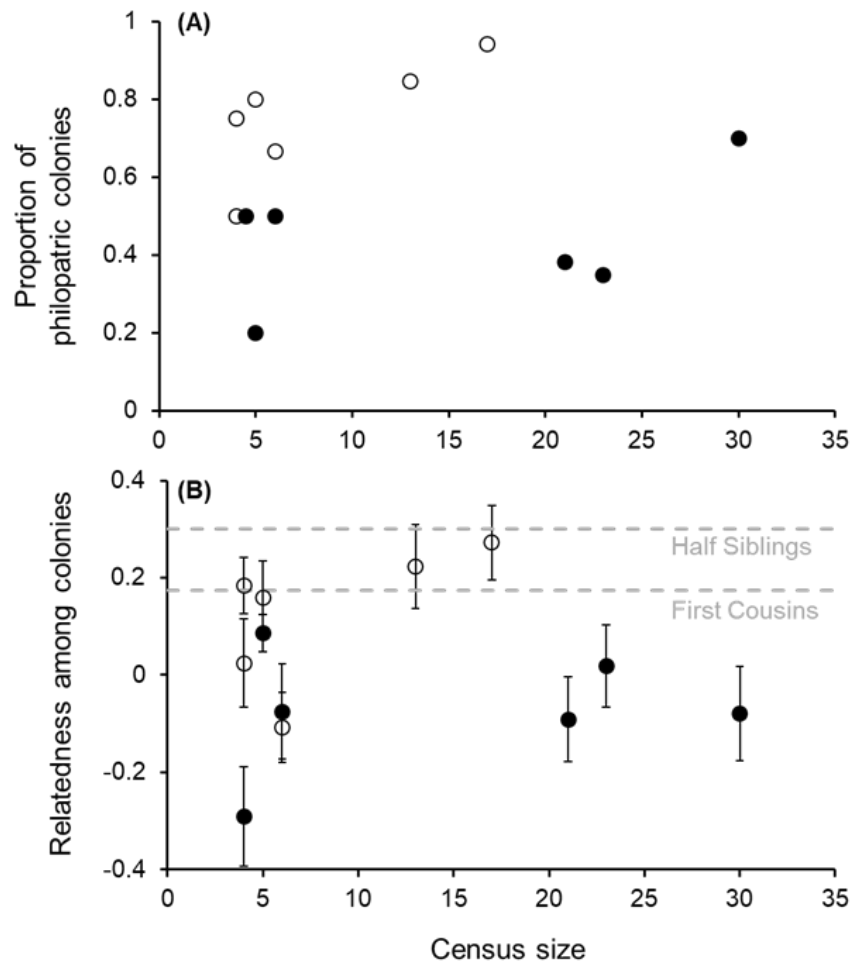


Figure 1.4. The prevalence of natal philopatry **(A)** and the relatedness among *L. verrilli* colonies **(B)** varied substantially across sites. Estimates of philopatry and relatedness were greater on artificial sites compared with natural sites and were not significantly associated with population census size. Open symbols represent sites on artificial substrates; closed symbols represent sites on natural substrates. Dashed lines represent the predicted relatedness of first cousins and half siblings following calibration with observed patterns of coancestry.

Table 1.1. Mating system estimates across sites. Sample size of maternal colonies with number of offspring in parentheses, total inbreeding measured with the single locus estimate of selfing ( $S_s$ ), the number of offspring where selfing was excluded by the presence of non-maternal alleles with the proportion in parentheses, and an estimate of the fraction of total inbreeding due to mating among relatives ( $1-r_s$ ). Biparental inbreeding was distinguished from self-fertilization with either the presence of non-maternal alleles or  $1-r_s$ .

<b>Site</b>	<b>Sample Size</b>	<b>Total Inbreeding (<math>S_s</math>)</b>	<b>Selfing Excluded</b>	<b><math>1-r_s</math></b>
Allegedly-4	3 (15)	$0.455 \pm 0.085$	9/15 (0.6)	0.8
Carrabelle Three Mile Culverts	3 (15)	$-0.011 \pm 0.066$	15/15 (1.0)	1.0
K Tower-West Wall	2 (15)	$0.291 \pm 0.086$	13/15 (0.9)	1.0
O Tower	4 (21)	$0.742 \pm 0.063$	12/21 (0.6)	1.0
Sargassum-1	3 (16)	$0.083 \pm 0.089$	15/16 (0.9)	0.6
Sargassum-2	3 (21)	$0.027 \pm 0.068$	21/21 (1.0)	0.6
Two Dog Reefballs	3 (15)	$0.233 \pm 0.086$	15/15 (1.0)	1.0

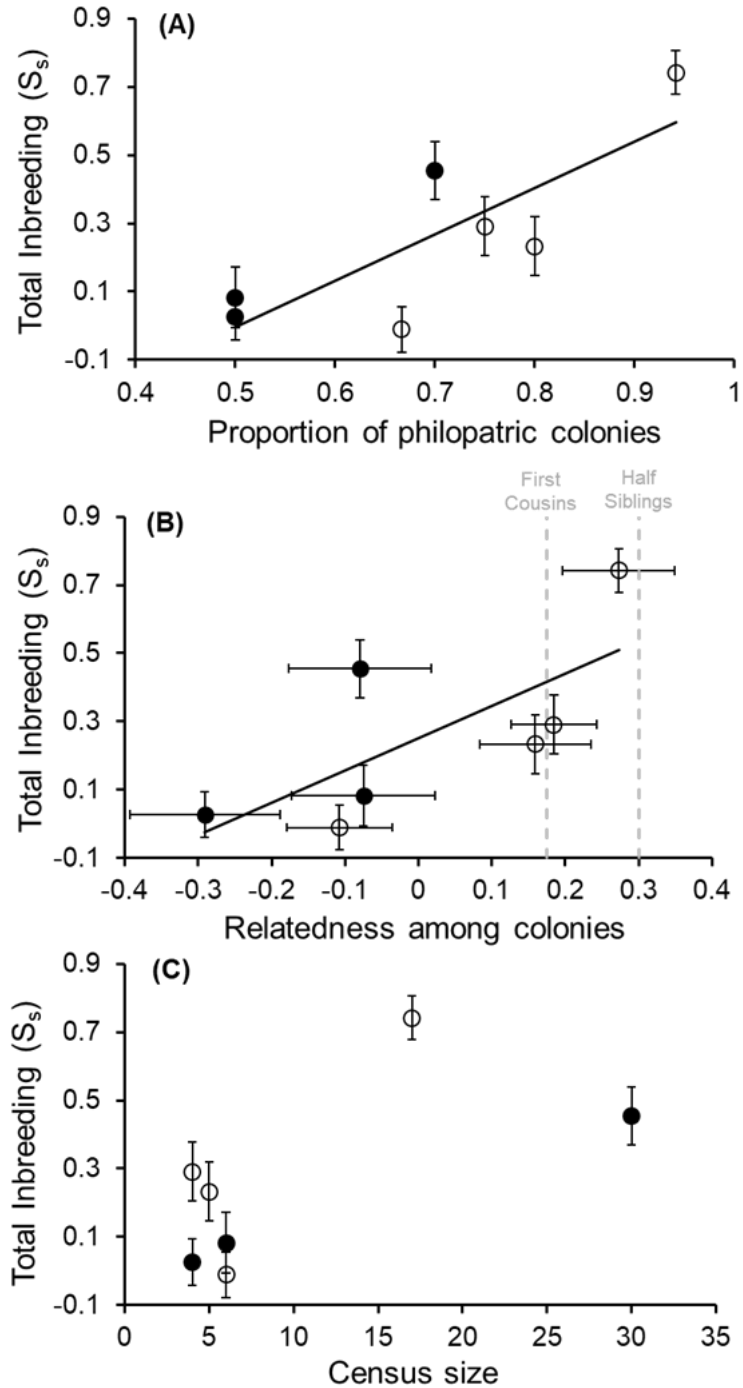


Figure 1.5. Total inbreeding was positively associated with the prevalence of natal philopatry (A) and the degree of relatedness among *L. verrilli* colonies (B) but not the population census size (C). Open symbols represent sites on artificial substrates; closed symbols represent sites on natural substrates. Regression lines represent linear best fit. Dashed lines represent the predicted relatedness of first cousins and half siblings following calibration with observed patterns of coancestry.

## CHAPTER 2

### INBREEDING TOLERANCE, SIRE SIZE, AND PROXIMITY GOVERN REPRODUCTIVE SUCCESS

#### 2.1 Introduction

The amount of common ancestry shared among mates is an important element in the evolutionary trajectory of populations and species (Charlesworth 2003). Inbreeding and outbreeding are relative phenomena reflecting a continuum of shared ancestry among mates spanning from self-fertilization to interspecific hybridization (Thornhill 1993). Both inbreeding and outbreeding have costs and benefits to fitness that can stabilize or propel mating systems toward one end of this spectrum or the other (Lande and Schemske 1985; Goodwillie et al. 2005; Ellison and Burton 2008). Examining the interplay between inbreeding and outbreeding, and how this dynamic interacts with mate availability and competition, is critical to understanding the general forces shaping mating systems (Thomson and Barrett 1981; Zavada and Taylor 1986; Arnold 1994).

To an individual, limits on the magnitude of inbreeding and outbreeding are set by dispersal and demographic processes that establish the pool of available mates (Shields 1982). Within these bounds, individuals inbreed or outbreed due to traits influencing the likelihood to avoid, tolerate, or prefer mating with relatives (Szulkin et al. 2013). While floral traits in angiosperms have long been recognized as adaptations to promote or block self-fertilization in environments where conspecific density and the severity of competition for mates differ (Barrett 1988), corresponding traits that govern inbreeding and outbreeding in species with obligate biparental reproduction have received less attention (Szulkin et al. 2013; Olsen et al. 2021). Moreover, self-incompatibility (SI) systems are not only a means of inbreeding avoidance, but facilitate the ability to choose among mates and bolster the significance of sexual selection (Thomson and

Barrett 1981; Zavada and Taylor 1986). Addressing how inbreeding avoidance, tolerance, and preference interrelate with competition for mates is key to determining why inbreeding and outbreeding vary considerably among populations and species (Jarne and Auld 2006; Whitehead et al. 2018; Olsen et al. 2020).

Theory suggests that the conditions favoring the evolution of high selfing rates should commonly promote inbreeding preference and tolerance in species with biparental reproduction (Kokko and Ots 2006; Puurtinen 2011; Duthie and Reid 2016). Yet, inbreeding has historically been viewed as comparatively rare in many taxonomic groups, such as animals, where avoidance of kin during mating has been well documented (Pusey and Wulf 1996; Leedale et al. 2020). However, recent meta-analyses indicate that inbreeding avoidance in animals may be less widespread than often expected (de Boer et al. 2021), and that distributions of the inbreeding coefficient ( $F_{IS}$ ) are similar in seed plants and some animals (Olsen et al. 2020).

A survey of published inbreeding coefficients suggests that inbreeding in sessile and sedentary marine invertebrates occurs frequently, and similarly to land plants, variation in the degree of inbreeding across species can be explained by reproductive and dispersal modes (Olsen et al. 2020). Because of sessile or sedentary adult stages, larval movement and mixing are primarily responsible for establishing the pool of nearby potential mates in marine invertebrates (Johnson and Woollacott 2010). Thus, the amount of time spent in the plankton, oceanographic conditions, and traits influencing the likelihood that individuals remain near or disperse away from relatives are important elements in the interplay between inbreeding and outbreeding (Knowlton and Jackson 1993; Addison and Hart 2005; Burgess et al. 2016; Eldon et al. 2016; Chapter 1). This notion is reflected in that species with comparatively brief developmental stages display substantial variation in  $F_{IS}$  (Addison and Hart 2005; Olsen et al. 2020), making this an



ideal group for examining the dynamic between inbreeding and outbreeding within the context of mate availability and competition.

Due to the fluid media of the ocean, the rapid dilution of gametes is central to marine mating systems (Strathmann 1990; Levitan and Peterson 1995), with substantial consequences for the relationships between fertilization success, conspecific density and proximity (Grosberg 1987; Levitan et al. 1992), and competition for mates (Levitan 1996; Hughes et al. 2009). The concentration of conspecific sperm in the vicinity of females and their eggs is particularly important to sexual selection and mate competition in the marine environment (Pemberton et al. 2003; Levitan 2018). As a result, factors that affect sperm concentration, such as distance from the source, or the amount of sperm released, have important implications for fertilization success (Levitan 1991; Levitan et al. 1992). In modular invertebrates, sperm production and the potential to sire offspring is correlated with colony size, this is partly because as a colony grows so too does the size and number of anatomical units producing sperm (McCartney 1997; Yund et al. 1997; Coffroth and Lasker 1998). Because of dilution and sperm competition, paternal success in spawning invertebrates is well understood to be proximity dependent (Grosberg 1987; Warner et al. 2016), and positively associated with sperm production (McCartney 1997; Yund 1998).

Even though the significance of sperm concentration and competition in marine mating systems is well recognized, our understanding of how these factors may be altered by the relatedness of conspecifics is far less complete (Olsen et al. 2021). Inbreeding avoidance or preference manifested via spawning synchrony, behavioral mate choice, and/or gametic recognition systems have the potential to disrupt the documented associations between mating success, proximity, and sperm production (Grosberg 1988; Palmer and Edmands 2000; Bishop et al. 1996). For example, a somatic-gametic recognition system comparable to the SI system of

angiosperms has been described in some free spawning invertebrates that can prevent self-sperm and the sperm of relatives from fertilizing retained eggs (Bishop 1996; Bishop et al. 1996). When kin are nearby, reduced compatibility among relatives and sperm concentration may result in an optimal distance between conspecifics for fertilization success (Grosberg 1991). However, the way in which SI systems and inbreeding avoidance or preference interact with sperm availability and competition to influence fertilization is largely unknown. Accordingly, we addressed three main questions associated with relatedness, inbreeding, and sperm availability with the colonial ascidian *Lissoclinum verrilli*: 1) *Are individuals near relatives so that the dynamic between inbreeding and outbreeding may be an important element of the mating system?* 2) *Does the amount of coancestry shared among individuals influence patterns of mating via inbreeding avoidance or preference?* and 3) *How does relatedness between conspecifics interact with sperm availability and competition to affect paternal success?*

## **2.2 Methods**

### *2.2.1 Overview*

We estimated the amount of coancestry shared among *L. verrilli* colonies at varying spatial scales to address if relatedness between proximate conspecifics had the potential to influence mate choice. We characterized the relatedness between dams and conspecifics, as well as the size and proximity of nearby colonies to evaluate how these factors influenced the number of offspring sired. Patterns of siring success associated with the relatedness between dams and sires, and the size and proximity of sires, were used to make inferences about the relative contributions of inbreeding avoidance, tolerance, or preference and sperm availability and competition to the mating system of *L. verrilli*. We expected to find a negative relationship between siring success and the relatedness of conspecifics if inbreeding avoidance was prevalent, a positive association

given inbreeding preference, or no relationship if *L. verrilli* tolerated inbreeding. Because of sperm competition, we anticipated that distance among colonies would be negatively associated with paternal success, whereas colony size would have a positive association.

### 2.2.2 Sampling

Components of the dataset analyzed in this study were also used to evaluate variation in the frequency of biparental inbreeding across populations of *L. verrilli* (Chapter 1). Here, we address how factors within sampling sites such as colony size, proximity, and relatedness interact to influence patterns of mating. Thus, methods used in sampling and genotyping are the same as described in Chapter 1. Briefly, *L. verrilli* colonies inhabiting twelve sites in the northeastern Gulf of Mexico were sampled from April to October 2016 – 2019. At each site transect tape was extended along the longest length and perpendicular width of hard substrate and the position and size of each *L. verrilli* colony was recorded. Effort was made to locate every *L. verrilli* within the sampling area and each physically distinct colony was sampled by excising a tissue sample (~5 × 5 cm).

Occasionally, identical multi-locus genotypes were detected in physically distinct colonies that were in close proximity (<1 m). These putative clonemates were treated as a single genetic individual and their total size was estimated by summing the individual sizes of clonemates. At two of the twelve sites, *L. verrilli* were sampled and their sizes were measured, but strong currents prevented us from mapping the spatial coordinates of colonies. Consequently, these colonies were not included in analyses evaluating spatial patterns of relatedness or siring success. Unreported analyses incorporating these individuals did not qualitatively change our results associated with the influence of relatedness or colony size on siring success.

Tissue samples of *L. verrilli* were transported to Florida State University, fixed in 95% ethanol, and maintained at -20°C. Samples were then dissected to isolate adult zooids (the physiological units of ascidian colonies) and brooded offspring for DNA extraction. Tissue samples from six of the ten sites contained internally developing offspring. The developmental stage of offspring varied within tissue samples, ranging from newly formed embryos to fully developed larvae nearing release. Approximately fifteen offspring at the most advanced developmental stage were dissected from three maternal colonies from each of the six sites. DNA extractions from *L. verrilli* colonies and offspring were used to amplify ten microsatellite loci for genetic marker-based assessments of relatedness and paternity.

### 2.2.3 Relatedness

The multi-locus genotypes of 138 *L. verrilli* colonies and 118 offspring were used to generate a matrix of pairwise relatedness estimates with the program COANCESTRY (Wang 2011). In this dataset, the estimator described by Wang (2002) best distinguished mother-offspring, full sibling, and half-sibling pairings from individuals unlikely to share recent coancestry (Chapter 1). Furthermore, estimates of relatedness between mothers and their offspring in outbreeding sites were used to conservatively calibrate the expected relatedness between first cousins, half-siblings, and full siblings from  $R = 0.125$ ,  $R = 0.25$ , and  $R = 0.5$  to  $R = 0.175$ ,  $R = 0.30$ , and  $R = 0.55$ , respectively (Chapter 1). Twelve colonies and 16 offspring that were not mapped spatially were removed from the matrix prior to assessing spatial patterns of relatedness and siring success.

### 2.2.4 Spatial patterns of relatedness

We evaluated how relatedness among *L. verrilli* colonies varied at differing spatial scales with correlograms in the R package EcoGenetics (Roser et al. 2017). We used a global

correlogram across ten sites to quantify mean relatedness among colonies in nine distance classes (0 – 0.2, 0.2 – 1.2, 1.2 – 5.0, 5.0 – 40.0, 40.0 – 70.0, 70.0 – 150.0, 150.0 – 1000.0, 1000.0 – 8000.0, > 8000.0 m). These groupings were chosen to evaluate the association between relatedness and proximity at scales representing estimates within sampling sites, among proximate sampling sites, and among distant sites. Three approaches were used to evaluate the global correlogram: 1) a Mantel Test was used to test the correlation between relatedness and distance, 2) permutation tests were used to determine if relatedness within distance classes were different from those drawn at random from all classes, and 3) the 95% confidence intervals of relatedness estimates were calculated for each distance class to determine if they overlapped with zero (*i.e.* the value expected in individuals without shared ancestry).

Local correlograms were also constructed to visualize spatial patterns of relatedness among *L. verrilli* colonies within the six sites used to evaluate siring success. Because there were few pairwise estimates of relatedness within some sites, we tailored distance classes in each site to evenly distribute the number of relatedness estimates among classes and did not use inferential statistics or estimate 95% confidence intervals.

### 2.2.5 Siring success

Siring success was assessed by assigning paternity to offspring with either genetic exclusion (*e.g.* when an offspring's genotype could be explained by the combination of its dam and only a single sire) or likelihood-based paternity assignments in the program CERVUS (Marshall et al. 1998). When an offspring's genotype could be explained by more than a single paternal colony, sires were assigned when they had at least 95% assignment probability. Two loci (LV493 and LV607) showed frequent null alleles in comparisons between maternal and offspring genotypes and were not included in paternity assignments. To test if our ability to

detect paternity varied in different sites due to either assignment biases or inbreeding avoidance/preference, we assessed the association between the fraction of offspring with an assigned sire and the mean relatedness among colonies in each site with errors-in-variables (EIV) regression in the R package ‘eivtools’ (Lockwood 2018). EIV regression accounts for error in the measurement of an independent variable and was used here because relatedness among individuals varied within sites.

### 2.2.6 Statistical analyses

The association between siring success, the relatedness between dams and potential sires, relative size, and relative distance was evaluated with multiple logistic regression in a mixed effects model with the R package ‘lme4’ (Bates et al. 2015). For each dam (*i.e.* colony with a tissue sample harboring offspring), the number of offspring sired by other colonies inhabiting the same site and the characteristics of those colonies were evaluated. Specifically, a data frame consisting of site, focal dam, potential sire, the relatedness between dam and potential sire, the distance between dam and potential sire, the size of potential sire, the number of offspring sired, and the number of offspring not sired was assessed. The sizes and distances of potential sires relative to other colonies inhabiting the same site were used to standardize predictors and resolve rescaling warnings. Maternal colony was included as a nested random effect within site [*e.g.* (1 | Site / Maternal colony)] so that random intercepts varied among sites and among maternal colonies within sites. Multicollinearity among predictors was assessed with variance inflation factors in the R package ‘car’ (Fox et al. 2020). Inferences were made using a combination of hypothesis testing and model fitting with the Wald Z test statistic in the R package ‘LmerTest’ (Kuznetsova et al. 2017) and likelihood ratio testing in the R package ‘lmerTest’ (Zeileis and

Hothorn 2002). Given a significant interaction, prediction lines were simulated from model results in the R package ‘visreg’ (Breheny and Burchett 2017).

### 2.3 Results

The association between relatedness and geographic distance was evaluated in 126 *L. verrilli* colonies sampled across 10 sites. Across sites, mean relatedness among colonies declined with increasing distance (Mantel Test correlation = -0.289,  $p = 0.01$ ; Fig. 2.1). Moreover, colonies in close proximity ( $\leq 0.2$  m) were more related than expected by chance (permutation test;  $p = 0.01$ ), and the 95% C.I. of this distance class did not overlap with zero. In larger distance classes, the 95% C.I. overlapped with zero and/or mean relatedness was not significantly different from random. Local correlograms were also constructed for the six sites with brooded offspring to visualize how relatedness between closely situated conspecifics varied in different sites (Fig. 2.2). Estimates of relatedness between proximate colonies ( $< 2.0$  m) indicated that individuals were first cousins or half siblings in some sites and comparatively unrelated in others.

In total, 102 offspring dissected from 18 maternal colonies were used to evaluate how relatedness between dams and potential sires, relative size, and relative distance influenced siring success in 6 sites (Fig. 2.3). Paternity was assigned to a single siring colony either by genetic exclusion or with 95% probability in 22% (22/102) of offspring. A single sire was assigned to at least one offspring in 11 of the 18 maternal broods and in all 6 sites. Moreover, the proportion of offspring with an assigned sire was not associated with the mean relatedness among colonies across sites (EIV regression; slope = -0.209,  $p = 0.277$ ). For those offspring where a single paternal colony was not identified, 28 (27% of all offspring) could have been sired by two or

more colonies within the site, whereas in 52 offspring (51% of all offspring) all nearby sampled colonies were excluded as possible sires.

The relatedness between dams and sires assigned with exclusion or 95% probability varied from estimates suggesting they shared little or no coancestry to those indicating that mates were first cousins, full siblings, or parents and offspring (Fig. 2.3A). We did not find a significant effect of relatedness on siring success when evaluated either as the only predictor (GLMM; slope = -0.335, S.E. = 0.827,  $z = -0.405$ ,  $p = 0.686$ ), or when included with relative size and distance (Table 2.1). Multicollinearity among predictors was low ( $VIF < 2$ ), suggesting that multiple regression was appropriate for evaluating the relationship between siring success, relatedness, relative size, and relative distance. Siring success was best explained when relatedness, relative size, relative distance, and their interaction terms were included as predictors (Table B.1). However, the log likelihood of the full model was not significantly greater than when only relative size and relative distance were considered (Table B.2). Nonetheless, the interaction between relative size and distance was significant both when relatedness was included in the model (Table 2.1) and when it was removed (GLMM; slope = 9.783, S.E. = 3.355,  $z = 2.916$ ,  $p = 0.004$ ). The probability of siring offspring was predicted to increase with relative size and decrease with the relative distance between colonies (Fig. 2.4).

We also conducted analyses to address if either inbreeding avoidance or preference were evident when incorporating both exclusively assigned sires and possible sires that were not excluded in paternity assignments (Fig. B.1). For the 28 offspring that could have been sired by two or more colonies, we selected the least related candidates to test if there was evidence of inbreeding avoidance (Fig. B.1B). When we biased siring success with the least related candidates there was no association between the number of offspring sired and relatedness



(GLMM; slope = 0.146; S.E. = 0.464;  $z = 0.314$ ;  $p = 0.753$ ). However, when we biased siring success with the most related candidates, we found a positive association between the number of offspring sired and relatedness (GLMM; slope = 2.373; S.E. = 0.633;  $z = 3.750$ ;  $p < 0.001$ ; Fig. B.1C).

## 2.4 Discussion

Predominately, siring success was not associated with the degree of relatedness between dams and paternal colonies, suggesting that there were no barriers to consanguineous mating in *L. verrilli* when kin were nearby. Rather than shared ancestry, colony size and proximity determined paternal success, even when the relatedness of conspecifics was on the order of first cousins, full siblings, or parents and offspring. These patterns indicate that neither inbreeding avoidance or preference are important elements in the mating system in *L. verrilli*, and reinforces the notion that sperm availability and competition are critical to reproductive success in free spawning invertebrates.

Across sites, mean relatedness among *L. verrilli* colonies was elevated at small spatial scales and declined with increasing geographic distance (Fig. 2.1A). Relatedness and phenotypic similarity among proximate conspecifics have also been reported in other invertebrates with the same developmental mode (Yund and O'Neil 2000; Calderon et al. 2007; Blanquer et al. 2009). Limited planktonic duration lasting only minutes or hours and/or natal philopatry in these species increases the probability that individuals settle near relatives and can establish opportunities for inbreeding (Knowlton and Jackson 1993; Addison and Hart 2005; Olsen et al. 2020; Chapter 1). Moreover, our results emphasize that within species with comparatively brief larval stages, variation in the degree of relatedness among proximate conspecifics can be substantial (Fig. 2.2). Habitat isolation and patchiness, water movement, and intraspecific differences in larval

settlement behavior likely contribute meaningfully to genetic mixing in this study and others (Johnson and Woollacott 2010; Olsen et al. 2016; Chapter 1). Nonetheless, spatial associations among relatives do not necessarily indicate that inbreeding is prevalent (Hellberg 1994; 1996; Blanquer et al. 2009), and variation in the relatedness among individuals provides the opportunity for evaluating how inbreeding avoidance or preference may alter patterns of mating in *L. verrilli*.

We assigned paternity with either genetic exclusion or 95% probability in 22% of the offspring evaluated. This assignment rate is comparable to other studies evaluating spawning invertebrates in natural settings (see Lasker et al. 2008; Warner et al. 2016). Yet, in a substantial fraction of offspring (51%), all colonies within sampling areas were excluded as potential sires. Because we had no control over which colonies harbored offspring, dams at the periphery of sampling sites, and offspring sired by colonies that were nearby but beyond sampling areas could have contributed to this pattern. Even so, the strong signature of unexplained paternity suggests that at least some of these offspring were sired by colonies over larger distances than those sampled. Considering relatively large colonies sired offspring over distances of 19 m, we suggest that sperm from large colonies located outside of sampling areas could have contributed to the fraction of offspring with unexplained paternity. However, the transfer of sperm over distances greater than  $\sim 10 \times 10$  m is unlikely to be an artifact of inbreeding avoidance or preference, as unexplained paternity was just as common in sites where relatedness was high as those where individuals shared little or no coancestry.

We found that *L. verrilli* colonies often engaged in inbreeding when relatives were in close proximity. This pattern contrasts with laboratory-based tests that often find a low but variable capacity for marine invertebrates to self-fertilize or biparentally inbreed (Heyward and Babcock

1986; Carlon 1999; Levitan et al. 2004; Burgess et al. 2019). Given that behavioral (Palmer and Edmands 2000) and gametic (Bishop 1996; Bishop et al. 1996) mechanisms of inbreeding avoidance have been described in some marine invertebrates, we thought that *L. verrilli* might preferentially use unrelated sperm from exogenous sources when kin were nearby. However, the proportion of offspring with a proximate assigned sire was not correlated with the relatedness among colonies across sites. Furthermore, we found little evidence for an association between paternal success and the relatedness among dams and potential sires within sites. Only when we selected the most related candidate sires and purposefully biased paternal success toward inbreeding preference did we find a positive relationship between the number of offspring sired and relatedness (Fig. B.1C). These patterns indicate that *L. verrilli* did not shift to exogenous sources of sperm when relatives were close, nor did they preferentially mate with the least related neighboring conspecific.

Our results support previous *in situ* assessments of paternity and self-fertilization reporting that spermcasting and copulating marine invertebrates often do not discriminate among mates based on relatedness (Grosberg 1991; Brazeau et al. 1998; Carlon and Lippe 2011; Plough et al. 2014; Chapter 1). Discrepancies between laboratory-based and *in situ* assessments of inbreeding in marine invertebrates could be due to population and species-specific differences in the capacity to fertilize related gametes, and/or might suggest that low and variable signatures of inbreeding in the laboratory are representative of more substantial patterns in nature (Olsen et al. 2021). Tests of compatibility among related gametes combined with assessments of the frequency of inbreeding under natural conditions are warranted to distinguish these possibilities.

Irrespective of the relatedness among *L. verrilli*, siring success was greatest for the largest colonies and for those colonies closest to dams (Fig. 2.3B; 2.3C). In offspring where sires were

assigned with confidence, the mean distance between dams and paternal colonies was only 3.8 m ( $\pm 1.6$  S.E.), which is shorter but comparable to those reported in other spermcasting species sampled over similar spatial scales (Lasker et al. 2008; Warner et al. 2016). These patterns suggest that sperm availability and competition are more prominent than inbreeding avoidance or preference in the mating system of *L. verrilli*. Given that large *L. verrilli* colonies persist multiple years (*pers. observation*), and the magnitude of relatedness between some dams and sires ( $R = 0.5$ ), we suggest that mating among parents and their offspring may have contributed to the inbreeding reported here. Moreover, our results emphasize the importance of sperm availability and competition in free spawning invertebrates (Levitan et al. 1992; Yund and McCartney 1994; Levitan and Peterson 1995; Levitan 2018), and support other studies that found associations between paternal success, male size and investment, and proximity (Grosberg 1987; McCartney 1997; Warner et al. 2016).

We found that relatedness among conspecifics did not influence reproductive success in *L. verrilli*. Inbreeding tolerance suggests that larval movement and mixing plays a significant role in structuring mating systems in this species (Chapter 1), and likely contributes to the substantial variation in inbreeding and outbreeding recorded across species of marine invertebrates with similar reproductive and developmental modes (Olsen et al. 2020). However, while the causes of inbreeding and outbreeding in marine invertebrates are becoming better recognized, our understanding of the evolutionary consequences of inbreeding in this group are far behind (Olsen et al. 2021). Taking the adaptive benefits of inbreeding into consideration, manifested via the genetic transmission advantage (Fisher 1941; Kokko and Ots 2006), reproductive assurance (Kalisz and Vogler 2003), and the maintenance of locally adapted genotypes (Shields 1982), and integrating the well documented influences of sperm concentration and competition on rates of

fertilization (Levitan et al. 1992; Levitan 2018), could facilitate studies of how these factors interact to shape marine mating systems and broaden the general study of inbreeding and outbreeding.

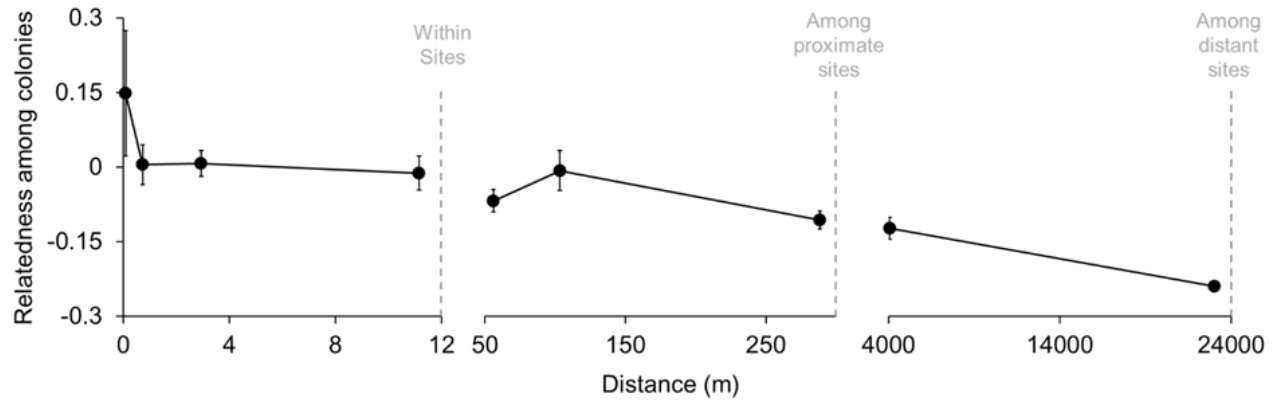


Figure 2.1. Spatial correlogram of relatedness estimates. A global correlogram across 10 sites indicated that relatedness among *L. verrilli* colonies was elevated at small spatial scales ( $\leq 0.2$  m) and declined sharply with increasing distance. Vertical bars represent the 95% confidence interval of relatedness estimates in each distance class. The global correlogram was partitioned into three subsections for visualizing patterns of relatedness within sites, among proximate sites, and among distant sites.

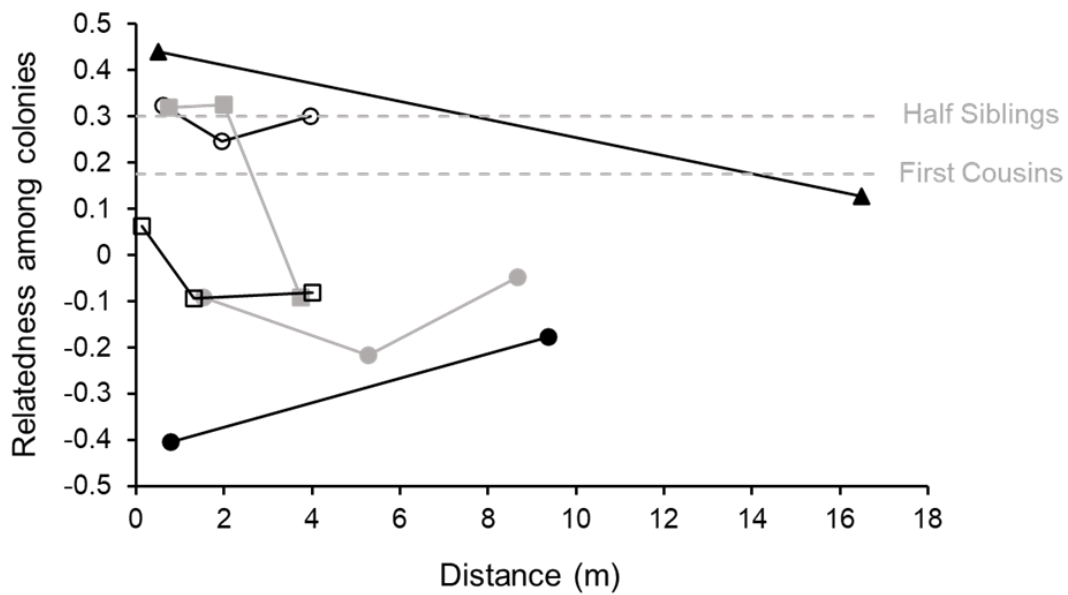


Figure 2.2. Spatial correlogram of relatedness estimates. Local correlograms within the six sites used to evaluate siring success indicated that relatedness among colonies in close proximity (< 2.0 m) varied considerably among sites. Dashed lines represent the predicted relatedness of first cousins and half siblings calibrated with observed patterns of coancestry.

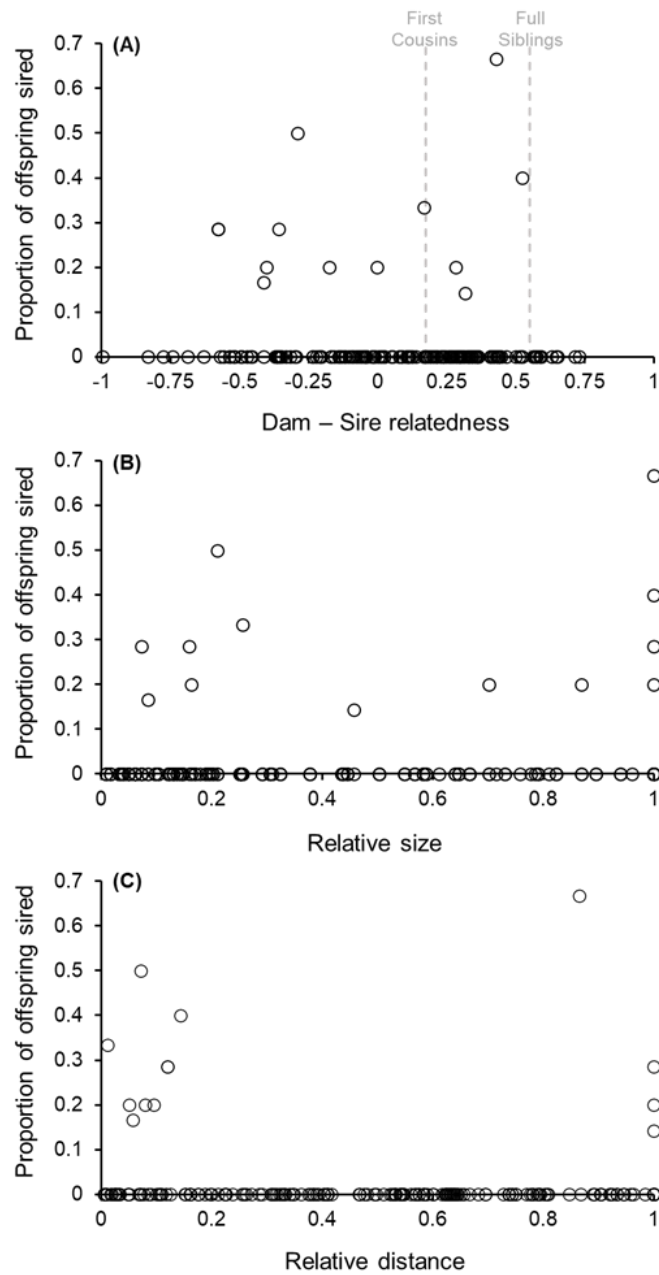


Figure 2.3 Proportion of offspring sired for each maternal colony ( $n = 18$  maternal colonies and 102 offspring) in relation to (A) relatedness between dam and potential sires, (B) relative size of potential sires, and (C) relative distance of potential sires. Sires assigned with genetic exclusion or 95% probability ( $n = 22$  offspring with 13 assigned sires). Dashed lines represent the predicted relatedness of first cousins and full siblings calibrated with observed patterns of coancestry.



Table 2.1 Results of multiple logistic regression with mixed effects. Effects of relatedness, relative size, relative distance, and their interactions on the number of offspring sired. Maternal colony included as a nested random effect within site. Significant p-values in bold.

Source	Estimate	Std. Error	Z - value	p - value
relatedness	-2.6693	1.8715	-1.426	0.154
relative size	0.3283	1.3201	0.249	0.804
relative distance	-9.6848	3.8134	-2.540	<b>0.011</b>
relatedness * relative size	0.0541	3.2891	0.016	0.987
relatedness * relative distance	6.5318	9.3065	0.702	0.483
relative size * relative distance	9.7289	3.9991	2.433	<b>0.015</b>
relatedness * relative size * relative distance	-0.7809	9.9719	-0.078	0.938

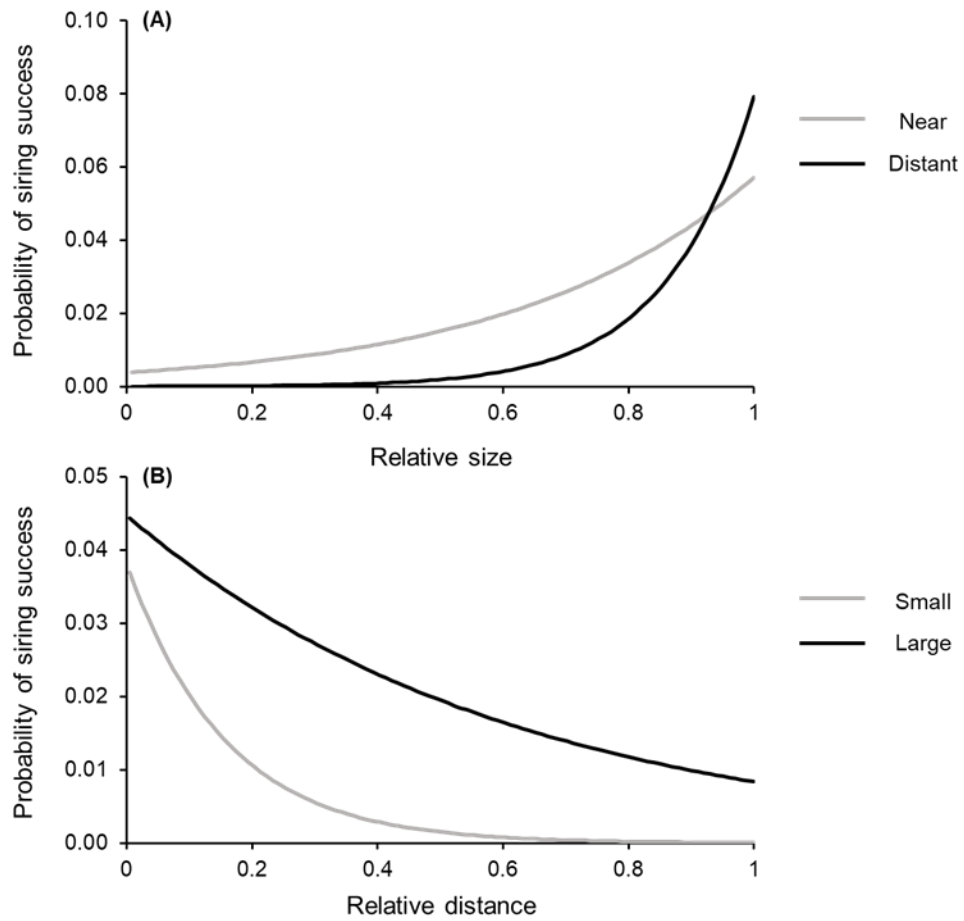


Figure 2.4. Probability of siring success associated with **(A)** relative size and **(B)** relative distance. Predictions based on the results of multiple logistic regression with mixed effects (Table 2.1).

**APPENDIX A**  
**SUPPLEMENTARY INFORMATION FOR CHAPTER 1**

Table A.1. Location, date, and depth of sites sampled.

<b>Site</b>	<b>Sampling Date</b>	<b>Depth (m)</b>	<b>G.P.S. Coordinates</b>
Allegedly-1	August 16 2016	10	29.79394°N, -084.48871°W
Allegedly-2	August 19 2016	11	29.79562°N, -084.48575°W
Allegedly-3	July 7 2017	10	29.79564°N, -084.48870°W
Allegedly-4	October 25 2019	11	29.79368°N, -084.48942°W
Carrabelle Three Mile Culverts	July 11 2018	12	29.75150°N, -084.57482°W
K Tower-East Wall	May 5 2018	18	29.66190°N, -084.37310°W
K Tower-West Wall	May 10 2019	18	29.66190°N, -084.37279°W
O Tower	July 16 2019	21	29.53667°N, -084.61780°W
Saint Andrews Jetty	June 19 2018	11	30.12457°N, -085.73074°W
Sargassum-1	August 8 2018	13	29.76033°N, -084.50911°W
Sargassum-2	June 18 2019	13	29.79394°N, -084.504831°W
Two Dog Reefballs	June 26 2018	11	29.76983°N, -084.53194°W

Table A.2. Primer sequences, repeat motif, allelic polymorphism ( $n_a$ ), and  $F_{IS}$  of the 10 microsatellite loci designed for this study. All loci were amplified with an M13 tag on the forward primer and two loci (LV809PT and LV2565PT) had an additional ‘Pig-Tail’ on the end of the reverse sequence.

<b>Locus</b>	<b>FWD Sequence</b>	<b>RV Sequence</b>	<b>Motif</b>	<b><math>n_a</math></b>	<b><math>F_{IS}</math></b>
LV646	TTTGTGCATGC TATAACGTTCG	TTGCAGTTTAA GACATTGGCAG	ACT	3	-0.07
LV607	CAGGTGGAAAG GTAGCAAGG	TATTGTCACTT CACGGTTCTCC	AAC	5	0.47
LV2196	ACGATTACCAA GCTGAAGTACG	ATGAAGAGAAA GAGAAGGCTGG	AC	13	-0.02
LV1895	ATTCGCAATTT ATCTGCTTGGC	CTTTCTCAGCG ACACAACAAG	ACT	5	0.13
LV493	GGCATAAACTG TTACACGACTG	AGAGATTTCGA GAAGTGATTGC	ACT	4	0.89
LV2300	CGTGGTCATAT ATCATCCGGTC	GTTTAGCATTC CTGTTTCTCTGC	AAC	3	0.10
LV809PT	TTGCTTGTCAT AACGTCTCTCC	CTGTTCCAAAC TCTACTGAAGC	ACT	3	0.01
LV2565PT	ATCGTTCTGTC ACTGTTTCCTG	ATACTGAAAGA TGGCGGTGTTG	ACT	4	0.06
LV380	TTGCATGTCAC AACGTCTTTAC	TGATTCGTCAC GGATAATTCGG	ACT	6	0.11
LV150	AATCCTTTGAA GACAGCAATGC	TGTTACGAGAA TCTCAATCTCCTG	AAC	4	0.04

## APPENDIX B

### SUPPLEMENTARY INFORMATION FOR CHAPTER 2

Table B.1. Results of model fitting with all possible combinations of relatedness, relative size, and relative distance as predictors in multiple logistic regression with mixed effects. Maternal colony included as a nested random effect within site.

<b>Model</b>	<b>Predictor(s)</b>	<b>Log Likelihood</b>
1.	relatedness	-73.4
2.	relative size	-71.3
3.	relative distance	-71.6
4.	relatedness * relative size	-66.6
5.	relatedness * relative distance	-69.1
6.	relative size * relative distance	-62.0
7.	relatedness * relative size * relative distance	-57.3

Table B.2. Results of model comparisons using likelihood ratio testing. Model numbers are as in Table B.1. Significant p-values in bold.

<b>Model Comparison</b>	<b>DF</b>	<b>Chi- Square</b>	<b>p - value</b>	<b>Best Fit Model</b>
1 & 7	6	32.134	<b>&lt; 0.001</b>	7
2 & 7	6	27.885	<b>&lt; 0.001</b>	7
3 & 7	6	28.454	<b>&lt; 0.001</b>	7
4 & 7	4	18.589	<b>0.001</b>	7
5 & 7	4	23.646	<b>&lt; 0.001</b>	7
6 & 7	4	9.310	0.054	NA

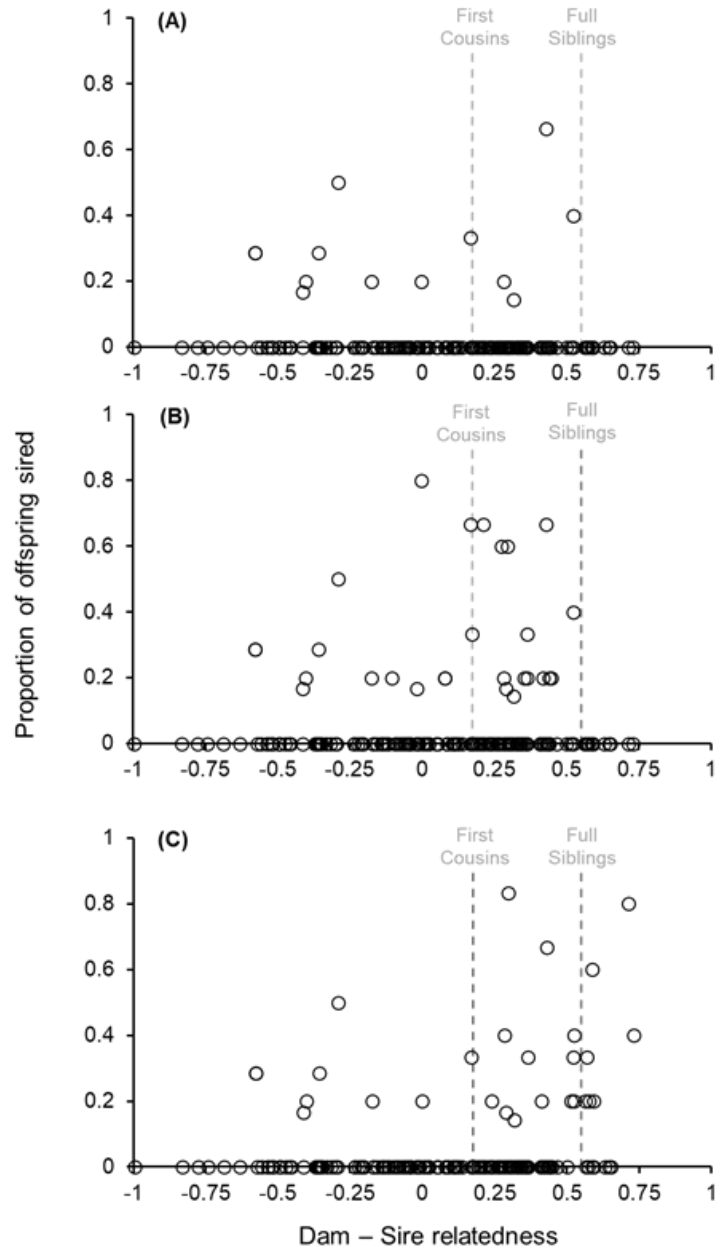


Figure B.1 Proportion of offspring sired for each maternal colony ( $n = 18$  maternal colonies and 102 offspring) in relation to relatedness between dam and potential sires. In **(A)** sires assigned with genetic exclusion or 95% probability ( $n = 22$  offspring with 13 assigned sires), **(B)** sires assigned with genetic exclusion or 95% probability and unexcluded sires least related to dams ( $n = 50$  offspring with 28 assigned sires), **(C)** sires assigned with genetic exclusion or 95% probability and unexcluded sires most related to dams ( $n = 50$  offspring with 28 assigned sires). Dashed lines represent the predicted relatedness of first cousins and full siblings calibrated with observed patterns of coancestry.

## REFERENCES

- Addison, J.A., and M.W. Hart. 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. *Biol. Lett.* 1: 450-453.
- Arnold, S.J. 1994. Bateman's principles and the measurement of sexual selection in plants and animals. *Am. Nat.* 144: S126-S149.
- Auld, J.R., and R.R. de Casas. 2013. The correlated evolution of dispersal and mating-system traits. *Evol. Biol.* 40: 185-193.
- Ayre, D.J., T.E. Minchinton, and C. Perrin. 2009. Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Mol. Ecol.* 18: 1887-1903.
- Barrett, S.C.H. 1988. The evolution, maintenance, and loss of self-incompatibility systems. Pp. 98-124. *in* J. Lovett Doust and L. Lovett Doust, ed. *Plant reproductive ecology, patterns and strategies*. Oxford University Press, New York.
- Bates, D., M. Machler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67: 1-48.
- Berry, O., M.D. Tocher, and S.D. Sarre. 2004. Can assignment tests measure dispersal? *Mol. Ecol.* 13: 551-561.
- Bishop, J.D.D. 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm movements in the female reproductive tract. *Proc. R. Soc. Lond. B* 369-376.
- Bishop, J.D.D., and A.J. Pemberton. 2006. The third way: spermcast mating in sessile marine invertebrates. *Integr. Comp. Biol.* 46: 398-406.
- Bishop, J.D.D., C.S. Jones, and L.R. Noble. 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. II. Investigation of male mating success using RAPD markers. *Proc. R. Soc. Lond. B* 263: 401-407.
- Blanquer, A., M.J. Uriz, and J. Caujape-Castells. 2009. Small-scale spatial genetic structure in *Scopalina lophyropoda*, an encrusting sponge with philopatric larval dispersal and frequent fission and fusion events. *Mar. Ecol. Prog. Ser.* 380: 95-102.
- Brazeau, D.A., D.F. Gleason, and M.E. Morgan. 1998. Self-fertilization in brooding hermaphroditic Caribbean corals: Evidence from molecular markers. *J. Exp. Mar. Biol. Ecol.* 231: 225-238.
- Breheny, P., and W. Burchett. 2017. Visualization of regression models using visreg. *R.J.* 9: 56-71.



- Bretman, A., N. Wedell, and T. Tregenza. 2004. Molecular evidence of post-copulatory inbreeding avoidance in the field cricket *Gryllus bimaculatus*. *Proc. R. Soc. Lond. B.* 271: 159-164.
- Burgess, S.C., K.J. Nickols, C.D. Griesemer, L.A.K. Barnett, A.G. Dedrick, E.V. Satterthwaite, L. Yamane, S.G. Morgan, J.W. White, and L.W. Botsford. 2014. Beyond connectivity: how empirical methods can quantify population persistence to improve marine protected-area design. *Ecol. Appl.* 24: 257-270.
- Burgess, S.C., M.L. Baskett, R.K. Grosberg, S.G. Morgan, and R.R. Strathmann. 2016. When is dispersal for dispersal? *Biol. Rev. Camb. Philos. Soc.* 91: 867-882.
- Burgess, S.C., L. Sander, and M. Bueno. 2019. How relatedness between mates influences reproductive success: an experimental analysis of self-fertilization and biparental inbreeding in a marine bryozoan. *Ecol. Evol.* 9: 11353-11366.
- Calderon, I., N. Ortega, S. Duran, M. Becerro, M. Pascual, and X. Turon. 2007. Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). *Mol. Ecol.* 16: 1799-1810.
- Caley, M.J., M.H. Carr, M.A. Hixon, T.P. Hughes, G.P. Jones, and B.A. Menge. 1996. Recruitment and the local dynamics of open marine populations. *Ann. Rev. Ecol. Syst.* 27: 477-500.
- Carlson, D.B. 1999. The evolution of mating systems in tropical reef corals. *Trends Ecol. Evol.* 14: 491-495.
- Carlson, D.B., and C. Lippe. 2011. Estimation of mating systems in short and tall ecomorphs of the coral *Favia fragum*. 20: 812-828.
- Charlesworth, D. 2003. Effects of inbreeding on the genetic diversity of populations. *Phil. Trans. R. Soc. B.* 358: 1051-1070.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.* 18: 237-268.
- Coffroth, M.A., and H.R. Lasker. 1998. Larval paternity and male reproductive success of a broadcast-spawning gorgonian, *Plexaura kuna*. *Mar. Biol.* 131: 329-337.
- Cutter, A.D. 2019. Reproductive transitions in plants and animals: selfing syndrome, sexual selection and speciation. *New Phytol.* 224: 1080-1094.
- Darwin, C. 1877. The various contrivances by which orchids are fertilized by insects. 2d ed. Reprinted. The University of Chicago Press, Chicago, IL.

- de Boer, R.A., R. Vega-Trejo, A. Kotrschal, and J.L. Fitzpatrick. 2021. Meta-analytic evidence that animals rarely avoid inbreeding. *Nat. Ecol. Evol.* <https://doi.org/10.1038/s41559-021-01453-9>.
- Dray, S., A. Dufour, and J. Thioulouse. 2020. ade4: Analysis of ecological data: exploratory and Euclidian methods in environmental sciences. R package version 1.7-16. Available at <https://cran.r-project.org/web/packages/ade4/index.html>.
- Dupont, L., F. Viard, M.J. Dowell, C. Wood, and J.D.D. Bishop. 2009. Fine- and regional-scale genetic structure of the exotic ascidian *Styela clava* (Tunicata) in southwest England, 50 years after its introduction. *Mol. Ecol.* 18: 442-453.
- Duran, S., M. Pascual, A. Estoup, and X. Turon. 2004. Strong population structure in the marine sponge *Crambe crambe* (Poeciloscerida) as revealed by microsatellite markers. *Mol. Ecol.* 13: 511-522.
- Duthie, A.B., and J.M. Reid. 2016. Evolution of inbreeding avoidance and inbreeding preference through mate choice among interacting relatives. *Am. Nat.* 188: 651-667.
- Eldon, B., F. Riquet, J. Yearsley, D. Jollivet, and T. Broquet. 2016. Current hypotheses to explain genetic chaos under the sea. *Curr. Zool.* 62: 551-566.
- Ellison, C.K., and R.S. Burton. 2008. Interpopulation hybrid breakdown maps to the mitochondrial genome. *Evolution* 62: 631-638.
- Escobar, J.S., J.R. Auld, A.C. Correa, J.M. Alonso, Y.K. Bony, M. Coutellec, J.M. Koene, J. Pointier, P. Jarne, and P. David. 2011. Patterns of mating-system evolution in hermaphroditic animals: correlations among selfing rate, inbreeding depression, and the timing of reproduction. *Evolution* 65: 1233-1253.
- Fisher, R.A. 1941. Average excess and average effect of a gene substitution. *Ann Eugen* 11: 53–63.
- Fogarty, N.D., S.V. Vollmer, and D.R. Levitan. 2012. Weak prezygotic isolating mechanisms in threatened Caribbean *Acropora* corals. *PLoS ONE* 7: e30486.
- Fox, J., S. Weisberg, and B. Price. 2020. car: companion to applied regression. R package version 3.0-10. Available at <https://cran.r-project.org/web/packages/car/index.html>.
- Goodwillie, C., S. Kalisz, and C.G. Eckert. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* 36: 47-79.
- Goudet, J., T. Jombart, Z.N. Kamvar, E. Archer, and O. Hardy. 2020. hierfstat: estimation and tests of hierarchical F-statistics. R package version 0.5-7. Available at <https://cran.r-project.org/web/packages/hierfstat/index.html>.

- Grosberg, R.K. 1987. Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. *Evolution* 41: 372-384.
- Grosberg, R.K. 1988. Life-history variation within a population of the colonial ascidian *Botryllus schlosseri*. I. The genetic and environmental control of seasonal variation. *Evolution* 42: 900-920.
- Grosberg, R.K. 1991. Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. *Evolution* 45: 130-142.
- Grosberg, R.K., and M.W. Hart. 2000. Mate selection and the evolution of highly polymorphic self/nonself recognition genes. *Science* 289: 2111-2114.
- Hellberg, M.E. 1994. Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. *Evolution* 48: 1829-1854.
- Hellberg, M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* 50: 1167-1175.
- Hellberg, M.E. 2009. Gene flow and isolation among populations of marine animals. *Annu. Rev. Ecol. Syst.* 40: 291-310.
- Heyward, A.J., and R.C. Babcock. 1986. Self- and cross-fertilization in scleractinian corals. *Mar. Biol.* 90: 191-195.
- Hogan, J.D., R.J. Thiessen, P.F. Sale, and D.D. Heath. 2012. Local retention, dispersal and fluctuating connectivity among populations of a coral reef fish. *Oecologia* 168: 61-71.
- Hughes, R.N., P.J. Wright, G.R. Carvalho, and W.F. Hutchinson. 2009. Patterns of self compatibility, inbreeding depression, outcrossing, and sex allocation in marine bryozoan suggest the predominant influence of sperm competition. *Biol J Linn Soc* 98: 519-531.
- Jarne, P., and J.R. Auld. 2006. Animals mix it up too: the distribution of self-fertilization among hermaphroditic animals. *Evolution* 60: 1816-1824.
- Johnson, C.H. 2010. Effects of selfing on offspring survival and reproduction in a colonial simultaneous hermaphrodite (*Bugula stolonifera*, Bryozoa). *Biol. Bull.* 219: 27-37.
- Johnson, S.L., and P.O. Yund. 2004. Remarkable longevity of dilute sperm in a free-spawning colonial ascidian. *Biol. Bull.* 206: 144-151.
- Johnson, S.L., and P.O. Yund. 2009. Effects of fertilization distance on male gain curves in a free-spawning marine invertebrate: A combined empirical and theoretical approach. *Evolution* 63: 3114-3123.

- Johnson, C.H., and R.M. Woollacott. 2010. Larval settlement preference maximizes genetic mixing in an inbreeding population of a simultaneous hermaphrodite (*Bugula stolonifera*, Bryozoa). *Mol. Ecol.* 19: 5511-5520.
- Kalisz, S., and D.W. Vogler. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* 84:2928–2942.
- Kamvar, Z.N., J.F. Tabima, S.E. Everhart, J.C. Brooks, and S.A. Krueger-Hadfield. 2021. poppr: Genetic analysis of populations with mixed reproduction. R package version 2.9.1. Available at <https://cran.r-project.org/web/packages/poppr/index.html>.
- Keller, L.F., and D.M. Waller. 2002. Inbreeding effects in wild populations. *Trends. Ecol. Evol.* 17: 230-241.
- Knowlton, N., and J.B.C. Jackson. 1993. Inbreeding and outbreeding in marine invertebrates. Pp. 200-249. in N.W. Thornhill, ed. *The natural history of inbreeding and outbreeding*. Univ. of Chicago Press, Chicago.
- Kokko, H., and I. Ots. 2006. When not to avoid inbreeding. *Evolution* 60: 467-475.
- Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen. 2017. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* 82: 1-26.
- Lande, R., and W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24-40.
- Lande, R., D.W. Schemske, and S.T. Schultz. 1994. High inbreeding depression, selective interference among loci, and the threshold selfing rate for purging recessive lethal mutations. *Evolution* 48: 965-978.
- Lasker, H.R., C. Gutierrez-Rodriguez, K. Bala, A. Hannes, and J.P. Bilewitch. 2008. Male reproductive success during spawning events of the octocoral *Pseudopterogorgia elisabethae*. *Mar. Ecol. Prog. Ser.* 367: 153-161.
- Ledoux, J.B., J. Garrabou, O. Bianchimani, P. Drap, J.P. Feral, and D. Aurelle. 2010. Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. *Mol. Ecol.* 19: 4204-4216.
- Leedale, A.E., M. Simeoni, S.P. Sharp, J.P. Green, J. Slate, R.F. Lachlan, E.J.H. Robinson, and B.J. Hatchwell. 2020. Cost, risk, and avoidance of inbreeding in a cooperatively breeding bird. *Proc. Natl. Acad. Sci. USA* 117: 15724-15730.
- Levitan, D.R. 1991. Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. *Biol. Bull.* 181: 261-268.

- Levitan, D.R. 1996. Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism. *Nature* 382: 153-155.
- Levitan, D.R. 2018. Do sperm really compete and do eggs ever have a choice? Adult distribution and gamete mixing influence sexual selection, sexual conflict, and the evolution of gamete recognition proteins in the sea. *Am. Nat.* 191: 88-105.
- Levitan, D.R., and C. Peterson. 1995. Sperm limitation in the sea. *Trends Ecol. Evol.* 10: 228-231.
- Levitan, D.R., M.A. Sewell, and F.S. Chia. 1992. How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* 73: 248-254.
- Levitan, D.R., H. Fukami, J. Java, D. Kline, T.M. McGovern, K. E. McGhee, C.A. Swanson, and N. Knowlton. 2004. Mechanisms of reproductive isolation among sympatric broadcast-spawning corals of the *Montastrea annularis* species complex. *Evolution* 58: 308-323.
- Lockwood, J.R. 2018. eivtools: Measurement error modeling tools. R package version 0.1-8. Available at <https://CRAN.R-project.org/package=eivtools>.
- Marshall, T.C., J. Slate, L.E.B. Kruuk, and J.M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7: 639-655.
- McCartney, M.A. 1997. Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate. *Evolution* 51: 127-140.
- Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- Nichols, H.J., M.A. Cant, J.I. Hoffman, and J.L. Sanderson. 2014. Evidence of frequent incest in a cooperatively breeding mammal. *Biol. Lett.* 10: 20140898.
- Olsen, K., J.M. Sneed, and V.J. Paul. 2016. Differential larval settlement responses of *Porites astreoides* and *Acropora palmata* in the presence of the green macroalgae *Halimeda opuntia*. *Coral Reefs* 35: 521-525.
- Olsen, K.C., W.H. Ryan, A.A. Winn, E.T. Kosman, J.A. Moscoso, S.A. Krueger-Hadfield, S.C. Burgess, D.B. Carlon, R.K. Grosberg, S. Kalisz, and D.R. Levitan. 2020. Inbreeding shapes the evolution of marine invertebrates. *Evolution* 74: 871-882.
- Olsen, K.C., W.H. Ryan, E.T. Kosman, J.A. Moscoso, D.R. Levitan, and A.A. Winn. 2021. Lessons from the study of plant mating systems for exploring the causes and consequences of inbreeding in marine invertebrates. *Mar. Biol.* 168: 39.
- Palmer, C.A., and S. Edmands. 2000. Mate choice in the face of both inbreeding and outbreeding depression in the intertidal copepod *Tigriopus californicus*. *Mar. Biol.* 136: 693-698.

- Pannell, J.R. 2015. Evolution of mating systems in colonizing plants. *Mol. Ecol.* 24: 2018-2037.
- Pemberton, A.J., R.N. Hughes, P.H. Manriquez, and J.D.D. Bishop. 2003. Efficient utilization of very dilute aquatic sperm: sperm competition may be more likely than sperm limitation when eggs are retained. *Proc. R. Soc. Lond. B* 270: S223-S226.
- Piry, S., A. Alapetite, J.M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. *J. Hered.* 95: 536-539.
- Plough, L.V., A. Moran, and P. Marko. 2014. Density drives polyandry and relatedness influences paternal success in the Pacific gooseneck barnacle, *Pollicipes elegans*. *BMC Evol. Biol.* 14: 81.
- Pusey, A., and M. Wolf. 1996. Inbreeding avoidance in animals. *Trends Ecol. Evol.* 11: 201-206.
- Puurttinen, M. 2011. Mate choice for optimal (k) inbreeding. *Evolution* 65:1501-1555.
- Rannala, B., and J.L. Mountain. 1997. Detecting immigration by using multilocus genotypes. *Proc. Natl. Acad. Sci. USA* 94: 9197-9201.
- Reynolds, S.M., J.A.C. Uy, G.L. Patricelli, S.W. Coleman, M.J. Braun, and G. Borgia. 2014. Tests of the kin selection model of mate choice and inbreeding avoidance in satin bowerbirds. *Behav. Ecol.* 25: 1005-1014.
- Ritland, K. 2002. Extensions of models for the estimation of mating systems using  $n$  independent loci. *Heredity* 88: 221-228.
- Rocha, R.M., T.B. Zanata, and T.R. Moreno. 2012. Keys for the identification of families and genera of Atlantic shallow water ascidians. *Biota. Neotrop.* 12: 269-303.
- Roser, L.G., L.I Ferreyra, B.O. Saidman, and J.C. Vilardi. 2017. EcoGenetics: An R Package for the management and exploratory analysis of spatial data in landscape genetics. *Mol. Ecol. Resour.* 17: e241-e250.
- Selkoe, K.A., and R.J. Toonen. 2011. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Mar. Ecol. Prog. Ser.* 436: 291-305.
- Severance, E.G., and S.A. Karl. 2006. Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. *Mar. Biol.* 150: 57-68.
- Shanks, A.L. 2009. Pelagic larval duration and dispersal distance revisited. *Biol. Bull.* 216: 373-385.
- Shields, W.M. 1982. *Philopatry, inbreeding, and the evolution of sex.* State Univ. of New York Press, Albany, NY.

- Strathmann, R.R. 1990. Why life histories evolve differently in the sea. *Am. Zool.* 30: 197-207.
- Strelcheck, A.J., J.H. Cowan Jr., and A. Shah. 2005. Influences of reef location on artificial-reef fish assemblages in the northcentral Gulf of Mexico. *Bull. Mar. Sci.* 77: 425-440.
- Szulkin, M., K.V. Stopher, J.M. Pemberton, and J.M. Reid. 2013. Inbreeding avoidance, tolerance, or preference in animals? *Trends Ecol. Evol.* 28: 205-211.
- Thomson, J.D., and S.C.H. Barrett. 1981. Selection for outcrossing, sexual selection, and the evolution of dioecy in plants. *Am. Nat.* 118: 443-449.
- Thornhill, N.W. 1993. *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives.* University of Chicago Press, Chicago.
- Van Name, W.G. 1945. The North and South American ascidians. *Bull. Am. Mus. Nat. Hist.* 84: 1-476.
- Wang, J.C. 2002. An estimator for pairwise relatedness using molecular markers. *Genetics* 160: 1203-1215.
- Wang, J.C. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol. Ecol. Resour.* 11: 141-145.
- Warner, P.A., B.L. Willis, and M.J.H. van Oppen. 2016. Sperm dispersal distances estimated by parentage analysis in a brooding scleractinian coral. *Mol. Ecol.* 25: 1398-1415.
- Weir, B.S., A.D. Anderson, and A.B. Hepler. 2006. Genetic relatedness analysis: modern data and new challenges. *Nat. Rev. Genet.* 7: 771-780.
- Whitehead, M.R., R. Lanfear, R.J. Mitchell, and J.D. Karron. 2018. Plant mating systems often vary widely among populations. *Front. Ecol. Evol.* 6: 1-9.
- Winn, A.A., E. Elle, S. Kalisz, P.O. Cheptou, C.G. Eckert, C. Goodwillie, M.O. Johnston, D.A. Moeller, R.H. Ree, R.D. Sargent, and M. Vallejo-Marin. 2011. Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. *Evolution* 65: 3339-3359.
- Wood, A.R., and J.P.A. Gardner. 2007. Small spatial scale population genetic structure in two limpet species endemic to the Kermadec Islands, New Zealand. *Mar. Ecol. Prog. Ser.* 349: 159-170.
- Yund, P.O. 1995. Gene flow via the dispersal of fertilizing sperm in a colonial ascidian (*Botryllus schlosseri*): the effect of male density. *Mar. Biol.* 122: 649-654.
- Yund, P.O. 1998. The effect of sperm competition on male gain curves in a colonial marine invertebrate. *Ecology* 79: 328-339.

- Yund, P.O., and M.A. McCartney. 1994. Male reproductive success in sessile invertebrates: competition for fertilizations. *Ecology* 75: 2151-2167.
- Yund, P.O., and P.G. O'Neil. 2000. Microgeographic genetic differentiation in a colonial ascidian (*Botryllus schlosseri*) population. *Mar. Biol.* 137: 583-588.
- Yund, P.O., Y. Marcum, and J. Stewart-Savage. 1997. Life-history variation in colonial ascidian: broad-sense heritabilities and tradeoffs in allocation to asexual growth and male and female reproduction. *Biol. Bull.* 192: 290-299.
- Yund, P.O., K. Murdock, and S.L. Johnson. 2007. Spatial distribution of ascidian sperm: two-dimensional patterns and short vs. time-integrated assays. *Mar. Ecol. Prog. Ser.* 341: 103-109.
- Zavada, M.S., and T.N. Taylor. 1986. The role of self-incompatibility and sexual selection in the gymnosperm-angiosperm transition: a hypothesis. *Am. Nat.* 128: 538-550.
- Zeileis, A., and T. Hothorn. 2002. Diagnostic checking in regression relationships. *R.J.* 2: 7-10.



## BIOGRAPHICAL SKETCH

Kevin C. Olsen was raised in Daytona Beach, FL and earned his Bachelor of Science degree in Biology from the University of North Florida in 2011. He studied the effects of competition, elevated temperature, and ocean acidification on the recruitment and oxidative stress response of reef-building corals and earned his Master of Science degree in Biology from UNF in 2013. He was employed by the Smithsonian Marine Station in Fort Pierce, FL as a biological research technician until he began his doctoral studies at Florida State University in 2014. He defended his dissertation in 2021.

### Publications:

- Olsen K.C.**, W.H. Ryan, E.T. Kosman, J.A. Moscoso, D.R. Levitan, and A.A. Winn. 2021. Lessons from the study of plant mating systems for exploring the causes and consequences of inbreeding in marine invertebrates. *Marine Biology* 168: 39.
- Olsen K.C.**, W.H. Ryan, A.A. Winn, E.T. Kosman, J.A. Moscoso, S.A. Krueger-Hadfield, S.C. Burgess, D.B. Carlon, R.K. Grosberg, S. Kalisz, and D.R. Levitan. 2020. Inbreeding shapes the evolution of marine invertebrates. *Evolution* 74: 871-882.
- Olsen K.C.**, J.A. Moscoso, and D.R. Levitan. 2019. Somatic mutation is a function of clone size and depth in reef-building corals of the *Orbicella* species complex. *The Biological Bulletin* 236: 1-12.
- Olsen K.**, J.M. Sneed, and V.J. Paul. 2016. Differential larval settlement responses of *Porites astreoides* and *Acropora palmata* in the presence of the green alga *Halimeda opuntia*. *Coral Reefs* 35: 521-525.
- Ross C., **K. Olsen**, M. Henry, and R. Pierce. 2015. Mosquito control pesticides and sea surface temperatures have differential effects on the survival and oxidative stress response of coral larvae. *Ecotoxicology* 24: 540-552.
- Olsen K.**, V.J. Paul, and C. Ross. 2015. Direct effects of elevated temperature, reduced pH, and the presence of macroalgae (*Dictyota spp.*) on larvae of the Caribbean coral *Porites astreoides*. *Bulletin of Marine Science* 91: 255-270.
- Olsen K.**, R. Ritson-Williams, V.J. Paul, and C. Ross. 2014. Combined effects of macroalgal presence and elevated temperature on the early life-history stages of a common Caribbean coral. *Marine Ecology Progress Series* 509: 181-191.
- Olsen K.**, R. Ritson-Williams, J.D. Ochriotor, V.J. Paul, and C. Ross. 2013. Detecting hyperthermal stress in larvae of the hermatypic coral *Porites astreoides*: The suitability of using biomarkers of oxidative stress versus heat shock protein transcriptional expression. *Marine Biology* 160: 2609-2618.

Ross C., R. Ritson-Williams, **K. Olsen**, and V.J. Paul. 2013. Short term and latent post-settlement effects associated with elevated temperature and oxidative stress on larvae from the coral *Porites astreoides*. *Coral Reefs* 32: 71-79.

### **Presentations:**

**Olsen K.C.**, and D.R. Levitan. Biparental inbreeding mirrors rates of self-fertilization in a spermcasting invertebrate. Western Society of Naturalists 2020. Virtual. November 5-8, 2020.

**Olsen K.C.**, and D.R. Levitan. Evolutionary dynamics of inbreeding and outbreeding in a plant-like animal. American Naturalist Annual Meeting 2020. Pacific Grove, CA. January 3-7, 2020.

**Olsen K.C.**, J.A. Moscoso, and D.R. Levitan. Prevalence of somatic mutations is a function of clone size and depth in reef-building corals of the *Orbicella* species complex. 46<sup>th</sup> Annual Benthic Ecology Meeting. Myrtle Beach, SC. April 12-16, 2017.

**Olsen K.**, J.A. Moscoso, and D.R. Levitan. Hidden genetic diversity in reef-building corals? Evidence for limited within-clone variation in corals of the *Orbicella* species complex. 13<sup>th</sup> annual Southeastern Ecology and Evolution Conference. Tallahassee, FL. March 12, 2016.

**Olsen K.**, V.J. Paul, and C. Ross. Global climate change and macroalgal competition have independent impacts on the early recruitment of the reef-building coral *Porites astreoides*. 43<sup>rd</sup> Annual Benthic Ecology Meeting. Jacksonville, FL. March 19-22, 2014.

**Olsen K.**, V.J. Paul, R. Ritson-Williams, and C. Ross. The effects of elevated temperature (global-scale stressor), algal competition (local-scale stressor) and their combined impacts on the early life history stages of the Caribbean hermatypic coral *Porites astreoides*. 42<sup>nd</sup> Annual Benthic Ecology Meeting. Savannah, GA. March 20-24, 2013.

**Olsen K.**, J.D. Ochriotor, R. Ritson-Williams, V.J. Paul, and C. Ross. Utilization of oxidative stress and heat shock protein biomarkers to detect heat-induced sublethal stress in larvae of the reef-building coral *Porites astreoides*. American Society for Cell Biology 2012 Annual Meeting San Francisco, CA. December 15-19, 2012.

### **Awarded Honors, Grants, and Fellowships:**

- Matt Beard Memorial Award. Florida State University Coastal and Marine Laboratory. October 2020
- Jack Winn Gramling Endowed Scholarship. Florida State University Department of Biological Science. October 2017.
- PADI Foundation Grant. “Linking populations and mating systems: roles of spatial scale and degree of relatedness in determining the breeding structure of a sessile ascidian.” April 2016.
- Lerner-Gray Marine Research Grant. American Museum of Natural History. “Contribution of somatic mutation to standing genetic variation in *Orbicella* reef-building corals.” May 2015.

- Link Foundation Fellowship. “Investigating the combined effects of macroalgal competition and elevated temperature on the early life-history stages of a common Caribbean coral.” Smithsonian Institution.
- Eddie M. Skofield Scholarship. University of North Florida Department of Biology. November 2010.