Journal of Experimental Marine Biology and Ecology 506 (2018) 91–99

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Prevalence of polygyny in a critically endangered marine turtle population

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ARTICLE INFO

Keywords:
Hawksbill
Conservation genetics
Mating systems
Multiple paternity
Microsatellite
Global warming

ABSTRACT

Genetic analyses of nuclear DNA (e.g., microsatellites) are a primary tool for investigating mating systems in reptiles, particularly marine turtles. Whereas studies over the past two decades have demonstrated that polyandry (i.e., females mating with multiple males) is common in marine turtles, polygyny (i.e., males mating with multiple females) has rarely been reported. In this study we investigated the mating structure of Critically Endangered hawksbill turtles (*Eretmochelys imbricata*) at Bahía de Jiquilisco in El Salvador, one of the largest rookeries in the eastern Pacific Ocean. We collected genetic samples from 34 nesting females and hatchlings from 41 clutches during the 2015 nesting season, including one nest from each of 27 females and two nests from seven additional females. Using six highly polymorphic microsatellite loci, we reconstructed the paternal genotypes for 22 known male turtles and discovered that seven (31.8%) sired nests from multiple females, which represents the highest polygyny level reported to date for marine turtles and suggests that this is a common mating structure for this population. We also detected multiple paternity in four (11.8%) clutches from the 34 females analyzed, confirming polyandrous mating strategies are also employed. The high level of polygyny we documented suggests there may be a limited number of sexually mature males at Bahía de Jiquilisco; a scenario supported by multiple lines of empirical evidence. Our findings highlight key management uncertainties, including whether polygynous mating strategies can compensate for potential ongoing feminization and the low number of adult males found for this and possibly other marine turtle populations.

1. Introduction

Mating strategies are life history features under selective pressure to maximize reproductive success (Uller and Olsson, 2008). These strategies vary within and among species according to numerous biotic and abiotic influences (Bollmer et al., 1999; Pearse and Avise, 2001). Marine turtles are reptiles that present particular challenges for mating system research as they are wide-ranging and spend the majority of their lives at sea. Although direct observations of mating can lend insights into mating behavior, direct approaches provide limited information on fertilization levels (versus strictly mating) and paternity.

During the past two decades, nuclear (n) DNA markers (e.g., microsatellites) have emerged as a primary tool to understand mating systems in marine turtles (Fitzsimmons, 1996; Hoekert et al., 2002; Zbinden et al., 2007; Jensen et al., 2013; Phillips et al., 2013). These studies have demonstrated that polyandry, or females mating with

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https://doi.org/10.1016/j.jembe.2018.06.004
Received 23 August 2017; Received in revised form 30 May 2018; Accepted 7 June 2018

Table 1
Available genetic studies of hawksbill mating structure. MP = Multiple Paternity.

Author	Ocean region	Location	Nesting females	Clutches analyzed	Hatchlings per clutch	Males Identified	# loci used	MP levels	Polygyny levels
Joseph and Shaw, 2011	South China Sea	Gulisaan, Malaysia	10	12	14–40	12	5	20.0%	0%
Phillips et al., 2013	West Indian Ocean	Cousin Island, Seychelles	43	85	20	47	32	9.3%	0%
Phillips et al., 2014a, 2014b	West Indian Ocean	Cousin Island, Seychelles	128	249	3–20	91	32	9.3%	0%
González-Garza et al., 2015	Caribbean Sea	Yucatan Peninsula, Mexico (various rookeries)	41	50 (2–16)	25	45	12	6.0%	0%
Natoli et al., 2017	Arabian/Persian Gulf	United Arab Emirates (Various rookeries)	53[a]	68 (5–40)	1–5	74–80	33	0–67%	0–15%
Gaos et al. this study	East Pacific Ocean	Bahia de Jiquilisco, El Salvador	34	41	15–20	22	6	14.7%	31.8%

[a] Maternal genotypes were not used in study.

multiple males (i.e., as evidenced via multiple paternity of clutches), is a common mating strategy employed by the taxon (Theissinger et al., 2009; Stewart and Dutton, 2011; Wright et al., 2012; Lasala et al., 2018). This strategy may confer selective advantages, such as fertility assurance, heightened offspring viability, and increased genetic diversity (Chapman et al., 2009; Phillips et al., 2017). In contrast, polygyny, or males mating with multiple females, has rarely been reported for marine turtles (but see Crim et al., 2002; Stewart and Dutton, 2014; Natoli et al., 2017), despite the existence of > 30 studies that have assayed > 1000 maternal families (see Tedeschi et al., 2015; Lee et al., 2018).

Hawksbill turtles (*Eretmochelys imbricata*) are a highly threatened marine turtle species that inhabit tropical and subtropical regions of the world's oceans. Previous genetic research of hawksbill mating systems in different ocean regions (Table 1) has found varying levels of polyandry and differences in mating strategies, highlighting variation among hawksbill populations. Hawksbills within the regional management unit (RMU, sensu Wallace et al., 2011) of the eastern Pacific Ocean are considered some of the world's most endangered and least resilient populations (Fuentes et al., 2013), with < 700 reproductively active females estimated to remain in the region (Gaos et al., 2017a). One of the two primary rookeries in this RMU is located at Bahía de Jiquilisco, El Salvador, where a research and nest conservation program has operated since 2008 (Liles et al., 2011, 2015). Recent genetic research has indicated that hawksbills nesting at this site represent a distinct management unit (MU sensu Moritz, 1994) that is strongly differentiated from other rookeries in the region, including a nearby rookery located only 115 km to the south (F_{ST} = 0.3706, P < .001; Gaos et al., 2016, 2018). Nesting beach monitoring efforts at Bahía de Jiquilisco have identified < 200 individual females to date (Gaos et al., 2017a), highlighting the need for acute conservation attention.

Despite the depleted status of hawksbills at Bahía de Jiquilisco and across the eastern Pacific, genetic research on hawksbill mating strategies and male population status in the region is lacking. Understanding male marine turtle populations and their mating strategies in particular, is likely to be increasingly important with the projected rise in global temperatures and the potential for feminization of many marine turtle populations (Hawkes et al., 2009; Eckert et al., 2012; Pike, 2014; Jensen et al., 2018). In an effort to fill current data gaps, we used nDNA microsatellite markers to evaluate mating systems in hawksbills nesting at Bahía de Jiquilisco. More specifically, we sought to assess levels of polygamy for both males and females, gain insights into the number of breeding males during the 2015 nesting season, and estimate a single-season breeding sex ratio (i.e., proportion of males and females that successfully mate; Stewart and Dutton, 2014) for the population.

2. Materials and methods

2.1. Study site and sample collection

This study used tissue samples collected in 2015 from nesting female hawksbills and their offspring (i.e., hatchlings) within the Bahía de Jiquilisco Biosphere Reserve (6°33′ N, 80°02′ E), a large mangrove-lined estuary in eastern El Salvador (Fig. 1). Bahía de Jiquilisco represents one of the two largest hawksbill rookeries in the eastern Pacific Ocean, hosting an average of 168.5 (± 46.7) nests per season and accounting for 24.4% of annual nesting by the species in the region (Gaos et al., 2017a). Because nest poaching is virtually 100% without protection efforts (Liles et al., 2014), the ongoing conservation activities include relocating most nests to a protective hatchery (Liles et al., 2015).

We applied Inconel flipper tags (National Band & Tag, Newport, KY, USA) and Passive Integrated Transponder (PIT; Avid, Norco, CA, USA) tags to all female turtles encountered to confirm and track identity throughout the nesting season. Using sterile techniques, we took a skin

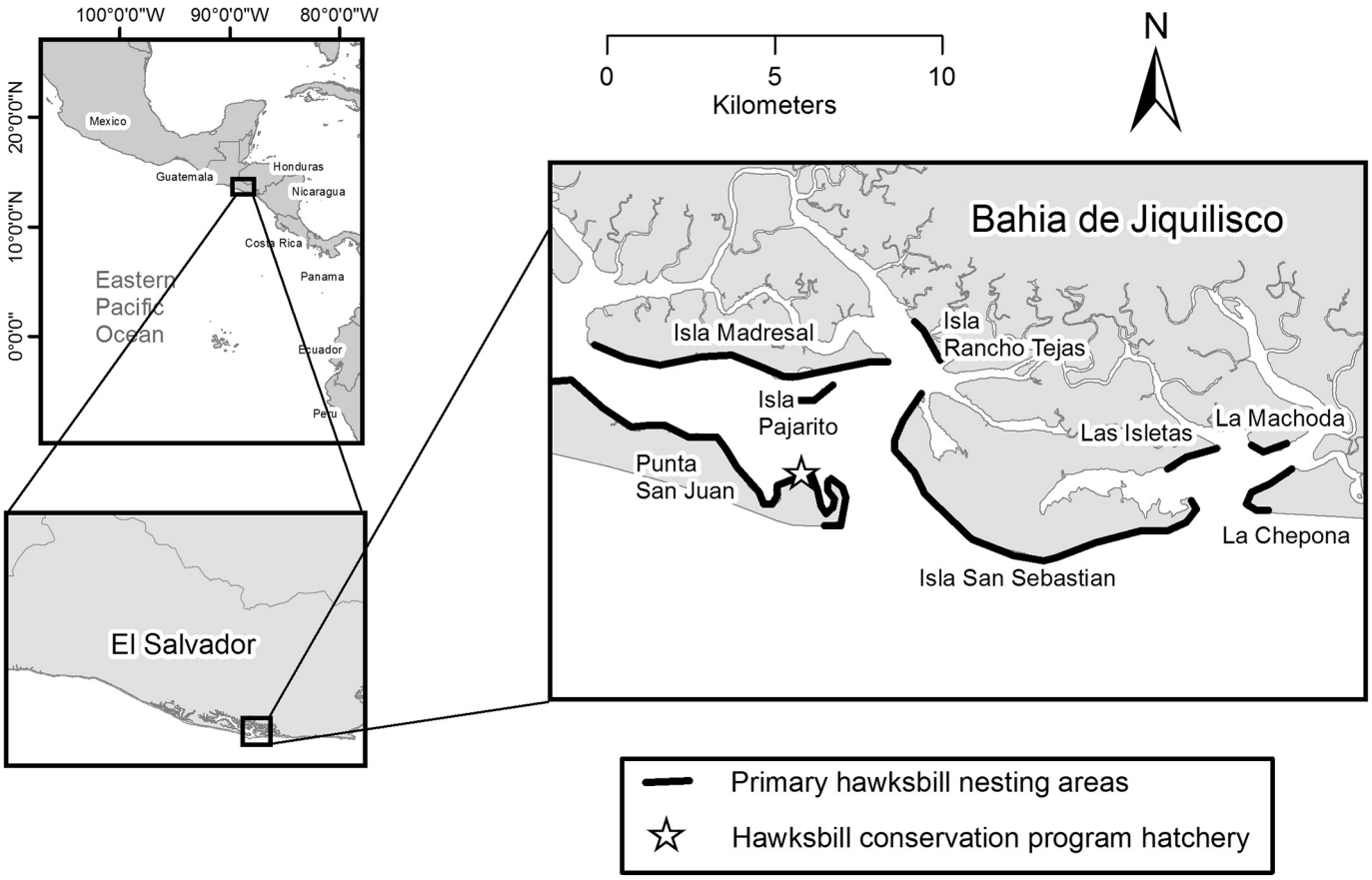

Fig. 1. Map depicting Bahía de Jiquilisco in El Salvador, including the primary nesting beaches and the location of the hawksbill conservation program hatchery.

sample (~0.5 cm^2) from the shoulder area of each female using a razor blade. Nests were moved to the hatchery and data were recorded on the corresponding mother. After hatchlings emerged from the nests, we took one small sample from the trailing edge of the front right flipper using a 2 mm biopsy punch (Miltex Inc., York, PA). Hemostatic agents (styptic pencil: ammonium sulfate 56%) were then applied to the flipper to prevent bleeding (Dutton and Stewart, 2013). Tissue was stored in vials containing saturated NaCl solution and was placed in a refrigerator within one week of the sampling date. When possible, we sampled 20 live hatchlings from each clutch deposited by known females in Bahía de Jiquilisco between May and September 2015. We sampled 41 nests deposited by 34 known mothers (69.4% of all nesting females in 2015) to evaluate paternity, including single nests from 27 females and two successive nests from seven females.

2.2. Laboratory processing

We extracted total genomic DNA from the tissue samples using an NaCl extraction method (modified from Miller et al., 1988). We used six loci that we determined as having high levels of polymorphism for hawksbill samples from the Pacific Ocean. The six primers [Cc5H7, Cc7G11 (Shamblin et al., 2007), CcP7D4, CcP7E5 (Shamblin et al., 2009), Eim11 (Miro-Herrans et al., 2008), and ERIM28 (Shamblin et al., 2013)] were labeled with fluorescent dyes (FAM or HEX) and used to amplify DNA in a polymerase chain reaction (PCR) (Innis et al., 1990). Amplifications were carried out on an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) according to the following schedule: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and lastly one cycle of 60 °C for 5 min.

Amplification of PCR product was checked using 2% agarose gels with ethidium bromide staining (Maniatis et al., 1982) and diluted using milli-Q H_2O based on band brightness (no band = no dilution, light band = 1:20 dilution, medium band = 1:40 dilution, bright band = 1:60 dilution), then analyzed in two multiplex sets on an ABI Prism 3730 Genetic Analyzer using ROX500 fluorescent size standard (PE Applied Biosystems, Foster City, CA, USA). We scored alleles using GeneMapper 5.0 (Applied Biosystems, Foster City, CA, USA) and subsequently manually verified the accuracy of allele assignments by the program. We used negative controls in all extractions and PCR reactions to account for potential contamination, and replicated 1 to 8 hatchlings per female to ensure genotype agreement. We calculated the genotyping error rate derived from replicates presenting conflicting alleles, as well as all original offspring and replicate samples that did not display a maternal allele (Bonin et al., 2004; Stewart and Dutton, 2011). Any samples that failed to amplify at three or more loci were excluded from further analyses.

2.3. Data analysis

We calculated allele frequencies, heterozygosity, and tested for deviations from Hardy-Weinberg equilibrium and linkage disequilibrium for all 34 nesting females using GENEPOP v.4.2 (Raymond and Rousset, 1995) and the package StrataG (Archer et al., 2016) in the R programming language (R Core Team, 2015). We calculated the probability that two unrelated individuals shared a genotype at a single locus (q) and across all loci (Q) (Hanotte et al., 1991), as well as the probability of detecting multiple paternity at a single locus (d) and across all loci (D) (Westneat et al., 1987). However, because we detected linkage disequilibrium between two pairs of loci (see Results), indicating these loci are not completely independent, we calculated a single d and q value for these loci (Table 2).

We identified the maternal alleles in each female's genotype and

Table 2
Number of alleles, expected (He) and observed (Ho) heterozygosity, Hardy-Weinberg *P*-value, probability of detecting multiple paternity (d), probability of individuals sharing a genotype (q), and error rate for the six loci used to examine 34 nesting females. Asterisks indicate a single d and q value was calculated for loci due to the detection of limited linkage disequilibrium, indicating a lack of independence among these loci. The probability of detecting multiple paternity and the probability of sharing a genotype across all loci, represented by D and Q, respectively, are provided below the table.

Locus	# alleles	He	Ho	HW	d	q	Error rate
CcP7D4t	11	0.77	0.75	0.18	0.42	0.089	0.001
Cc5H7t	14	0.90	0.88	0.11	0.23	0.020	0.005
ERIM28t[a]	13	0.69	0.69	0.68	0.14	0.008	0.012
EiM11t	4	0.42	0.44	0.72	0.78	0.065	0.000
CcP7E5t[a]	12	0.88	0.91	0.26	0.14	0.008	0.004
Cc7G11t[a]	8	0.81	0.76	0.39	0.14	0.008	0.006

D = 0.989, Q = 1.03×10^{-6}.

[a] A single q and d calculation was calculated for these loci due to detection of limited LD.

used the results from that analysis to determine the allelic contribution to hatchlings. We then deduced paternal genotypes by assessing the remaining alleles. We determined clutches were multiply sired if we could confirm three or more paternal alleles at two or more loci (Stewart and Dutton, 2011). We evaluated the number of mating males by comparing the resulting paternal genotypes. We then compared male genotypes and considered males to be the same individual if their genotypes matched across available loci.

We used the program GERUD1.0 (Jones, 2001) to confirm our parentage analysis. There are often ambiguities when reconstructing male genotypes from clutches exhibiting multiple paternity due to multiple allelic combinations that are not fully resolvable. Due to these ambiguities, for nests exhibiting multiple paternity, we based our findings of male genotypes on GERUD1.0 probability scores (Jones, 2001). Probability scores were based on the segregation of paternal alleles and its deviation from Mendelian expectations (Jones, 2001), as well as the frequencies of genotypes in the population, which we calculated from our known females (Crim et al., 2002). We calculated the number of males and polygyny rates for single paternity clutches, as well as single and multiple paternity clutches combined to account for uncertainty in genotypes for the latter, providing an estimated range of males that successfully bred with females. We calculated breeding sex ratios of females to males for single paternity, and both single and multiple paternity clutches combined, by dividing the number of females by the number of males under each scenario.

3. Results

We successfully genotyped 34 nesting females, including 30 females at all six loci and four females at five loci. We found 4–14 alleles for each locus, with EiM11t and Cc5H7t representing the least and most polymorphic loci, respectively (Table 2). Expected and observed heterozygosity ranged from 0.42 to 0.90 and 0.44 to 0.91, respectively, and no locus showed significant deviation from Hardy-Weinberg equilibrium ($P > .05$). We found limited evidence of linkage disequilibrium ($P < .05$) at two pairs of loci, including Cc7G11t and ERIM28t ($P < .001$) and Cc7G11t and CcP7E5t ($P = .024$). However, given the family sizes used, the variability of our markers, and because we estimated multiple paternity manually, we believe our results are robust. The probability of detecting multiple paternity at a single locus (d) ranged from 0.14 to 0.78 and rose to 0.989 when calculated across all loci (D) (Table 2). The probability of any two individuals sharing a genotype at a single locus (q) ranged from 0.008 to 0.089 and was 1.03×10^{-6} across all loci (Q).

Female clutch size ranged from 97 to 239 eggs (mean = 173.4 ± 33.8) (Table 3). We successfully genotyped between 15 and 20 hatchlings (mean = 19.2 ± 1.4) for single clutches and 35 to 40 hatchlings (mean = 39.1 ± 1.9) for nest pairs. In total, we successfully genotyped 793 (96.9%) of 818 hatchlings. We replicated a total of 143 samples (18.0%; mean = four hatchlings per female) and had a genotyping error of 55 allele calls out of 11,907 (0.005 error).

3.1. Paternity

We reconstructed the paternal genotypes for 22 known males when only looking at nests exhibiting single paternity, and an additional eight potential males when including nests exhibiting multiple paternity (Table 4). When looking solely at single paternity clutches, seven (31.8%) of the 22 males were sires of multiple nests (i.e., polygyny), including six males that sired clutches of two separate females and one male that sired clutches from three separate females. When looking at both single and multiple paternity clutches combined, eight (26.6%) of the 30 males were sires of multiple nests, including seven males that sired clutches of two separate females and one male that sired clutches from three separate females. One of the fathers of single paternity clutches also contributed portions of offspring to a clutch that exhibited multiple paternity (Table 4). We calculated a breeding sex ratio in favor of females of 1.41/1 for single paternity clutches and of 1.13/1 for single and multiple clutches combined.

We found evidence of polyandry (i.e., multiple paternity) in nests of four (11.8%) of the 34 females analyzed (Table 3). Three (75.0%) of the four clutches in which we detected multiple paternity were sired by two males and one (25.0%) was sired by three. Of the seven females for which we collected successive clutches, in all cases both clutches were sired by the same male.

4. Discussion

This study provides the first insights into hawksbill mating systems in the eastern Pacific Ocean. We found polygynous mating strategies are common at Bahía de Jiquilisco and detected the highest levels of polygyny (approximately 32% for known males) reported to date for a marine turtle population (Crim et al., 2002; Stewart and Dutton, 2014; Natoli et al., 2017). We attribute the prevalence of this mating strategy to a limited number of males in the reproductive population (Phillips et al., 2013, 2014a, 2014b). We also found evidence of multiple paternity (i.e., polyandry), albeit limited, indicating polyandry is also employed by female hawksbills at this site.

4.1. Polyandry

Multiple paternity (i.e., polyandry) is now a well-established mating strategy in marine turtles and reptiles in general, but can vary greatly among populations (Uller and Olsson, 2008). While the high levels of polygyny encountered in our study may signify that females have difficulty encountering and mating with multiple males (Phillips et al., 2013, Phillips et al., 2014b), we did detect multiple paternity in four (11.8%) of the 34 females analyzed. This level of multiple paternity is low for marine turtles in general (Tedeschi et al., 2015; Lee et al., 2018), but within the range of values reported for hawksbills in other ocean regions (Table 1), suggesting females at Bahía de Jiquilisco are able to effectively employ this mating strategy despite the low male population size. Successive clutches from single females were all sired by the same male, showing parentage patterns indicative of within-season sperm storage (Sakaoka et al., 2013; Phillips et al., 2013).

Although various theories have been proposed for the prevalence of multiple paternity in marine turtles (e.g., Lee and Hays, 2004; Theissinger et al., 2009; Phillips et al., 2013), including selective advantages facilitated through fertility assurance, sperm competition, heightened offspring fitness, and increased genetic diversity (Moore and Ball, 2002; Theissinger et al., 2009), studies actually demonstrating a benefit from polyandry remain scant (Chapman et al., 2009; Phillips

Table 3
Information on the females and the 41 clutches analyzed in this study, including clutches per female, clutch size, hatchlings successfully genotyped, hatchlings replicated, female genotypes, and number of inferred fathers (hatchlings assigned to each father and unassigned for multiple paternity clutches shown in parentheses). Successfully genotyped hatchlings include those for which we could confirm four or more loci. Dash indicates data unavailable.

Mother ID	Clutches analyzed	Clutch size	Hatchlings gentoyped	Hatchlings replicated	Replication (%)	Female genotype						# of fathers
						CcP7D4t	Cc5H7t	Erim28t	EiM11t	CcP7E5t	Cc7G11t	
166708	1	155	19	2	10.5	342/342	255/259	134/134	197/205	164/164	268/272	1
166635	1	160	16	2	12.5	342/362	211/227	134/138	197/197	160/216	268/284	1
166697	1	133	19	4	21.1	342/346	239/255	134/178	197/197	176/216	280/288	1
	2	152	20	4	20.0							
166712	1	168	20	4	20.0	342/350	239/255	134/186	197/197	172/192	268/272	1
	2	171	20	1	5.0							
166664	1	129	20	1	5.0	342/366	215/255	134/134	197/205	172/192	272/284	1
166669	1	129	20	4	20.0	366/370	215/247	154/182	197/197	160/208	272/300	1
166651	1	97	17	4	23.5	318/322	207/235	166/166	197/217	172/172	272/284	1
	2	196	18	4	22.2							
139198	1	232	20	1	5.0	326/362	239/251	134/158	197/205	164/192	268/268	1
139201	1	190	16	4	25.0	–	215/215	166/234	197/197	160/172	272/284	1
139196	1	221	20	4	20.0	342/362	227/251	134/134	197/205	160/196	268/272	1
166709	1	188	20	4	20.0	334/342	227/243	–	197/217	172/172	280/288	1
166628	1	239	20	4	20.0	342/362	219/223	134/174	197/217	160/204	272/280	1
166681	1	147	19	1	5.3	342/346	247/251	162/186	197/197	172/200	272/300	1
166648	1	154	20	2	10.0	342/366	227/255	134/134	197/217	160/204	284/284	1
157790	1	99	20	4	20.0	342/342	227/243	134/182	197/197	172/204	284/284	1
166698	1	168	18	4	22.2	350/366	239/239	134/178	197/205	192/196	272/272	1
166629	1	161	18	1	5.6	342/366	211/243	134/182	197/197	160/204	284/284	1
166637	1	217	20	4	20.0	342/342	211/255	134/134	197/197	172/204	284/284	1
166641	1	129	20	4	20.0	342/362	219/231	134/134	197/197	176/180	280/284	1
	2	152	20	1	5.0							
166661	1	158	20	4	20.0	350/362	215/239	134/138	197/197	164/176	268/284	1
166654	1	170	20	4	20.0	322/322	235/251	134/166	197/197	160/172	272/284	1
139189	1	182	20	1	5.0	350/350	239/239	134/134	197/197	160/192	272/284	1
166705	1	189	20	4	20.0	342/362	239/243	134/182	197/197	160/208	272/280	1
	2	211	20	4	20.0							
139188	1	214	20	4	20.0	–	215/247	134/198	197/197	160/208	272/280	1
	2	190	20	4	20.0							
166659	1	148	20	4	20.0	322/358	239/251	134/134	205/205	172/192	272/292	2 (16,2,2)
139183	1	189	19	4	21.1	346/350	215/247	134/190	197/197	188/204	268/300	2 (6,8,2)
139187	1	222	15	4	26.7	342/342	247/259	134/134	197/197	180/204	280/284	1
166656	1	195	20	1	5.0	342/350	215/251	138/174	197/217	172/204	284/300	1
166642	1	203	20	4	20.0	342/362	215/247	134/174	197/217	192/208	272/296	1
139195	1	191	20	4	20.0	342/342	215/215	134/234	197/205	204/208	272/296	3 (6,5,5,4)
166677	1	167	20	1	5.0	318/342	215/231	158/234	197/217	172/180	272/300	1
166666	1	178	20	4	20.0	318/342	223/247	134/154	197/221	196/204	280/296	2 (16,3,1)
166685	1	136	19	4	21.1	342/342	215/239	134/138	197/197	172/192	280/280	1
166699	1	177	20	4	20.0	346/350	215/239	–	197/205	172/204	268/268	1
	2	201	20	4	20.0							

et al., 2017; Lee et al., 2018). Regardless, multiple paternity is expected to increase as females encounter more males, i.e., at higher population densities (Weigensberg and Fairbairn, 1994; Jensen et al., 2006, 2013; Lasala et al., 2018). However, our findings suggest that polyandrous mating strategies can manifest even in populations that may have limited numbers of males.

4.2. Polygyny

We found evidence of polygyny for approximately 32% of known male hawksbills that sired nests at Bahía de Jiquilisco during the 2015 nesting season. To our knowledge, these are the highest levels of polygyny reported to date for any marine turtle species (Crim et al., 2002; Stewart and Dutton, 2014; Natoli et al., 2017). Although polygyny has been documented in various mammals, birds and reptiles (Moore et al., 2008; Stiver et al., 2008; Pérez-González et al., 2009; Bro-Jørgensen, 2014), it is a phenomenon that has rarely been detected in marine turtles, despite > 30 genetic studies investigating polygamy in the taxon (Tedeschi et al., 2015; Lee et al., 2018).

Of the previous genetic studies investigating mating strategies in hawksbills (Table 1), only the most recent found evidence of polygyny, at a rate (15%) of approximately half of what we report here (Natoli et al., 2017), although the estimates from this study should be treated tentatively given the relatively small family sizes assayed (1–5 hatchlings per clutch) and the lack of maternal genotypes (Table 1). The absence of polygyny in the remaining studies may be due to larger effective population sizes of source rookeries, which host approximately 600–4500 nests annually (Mortimer and Bresson, 1999; Pilcher, 1999; Allen et al., 2010; Joseph and Shaw, 2011; Phillips et al., 2013, Phillips et al., 2014a, 2014b; González-Garza et al., 2015), compared to roughly only 170 nests for Bahía de Jiquilisco (Gaos et al., 2017a). An understanding of the differences in mating strategies among geographically discrete, conspecific reproductive populations underscores the need for site-specific evaluations, and is important for accurate life-history characterizations and population evaluations, as well as effective management planning.

Although early studies suggested polygyny might reduce genetic diversity due to its negative effects on effective population size (Nunney, 1993; Briton et al., 1994), recent research indicates that may not always be the case for wildlife populations (Engen et al., 2007; Stiver et al., 2008; Martínez-Ruiz and Knell, 2016). These findings suggest that the genetic implications of polygyny remain unclear and likely depend on the interplay of various demographic and evolutionary factors (Engen et al., 2007; Stiver et al., 2008; Wheelwright et al., 1992).

Polygyny is rarely reported in marine turtles but has recently been

Table 4
Information on male hawksbills, including inferred genotypes, nests sired and fertilized female IDs for the 41 clutches analyzed. Genotypes for males M23-M30 were inferred by GERUD1.0 based on likelihood and could not be completely resolved manually. Dash indicates data unavailable.

Paternity status	Male ID	Clutches sired	Male genotype						Mother ID(s)	Father # (MP only)
			CcP7D4t	Cc5H7t	Erim28t	EiM11t	CcP7E5t	Cc7G11t		
Single paternity clutches	M1	1	318/342	215/247	146/182	197/205	172/180	272/284	166712	
	M2	2	318/342	219/255	134/226	197/205	180/192	284/284	166656	
									166651	
	M3	1	322/342	231/267	134/138	205/205	164/196	280/296	166685	
	M4	1	322/362	215/255	134/134	197/217	172/176	280/296	166708	
	M5	1	326/362	235/247	134/182	197/205	172/192	284/284	166635	
	M6	2	342/346	239/255	134/138	197/197	172/196	268/280	139187	
									139196	
	M7	1	342/346	243/247	170/170	197/217	146/184	268/280	166642	
	M8	1	342/358	215/231	134/138	197/205	192/220	276/280	166664	
	M9	1	342/358	239/271	–	197/205	188/204	268/272	166709	
	M10[a]	2	–	243/271	134/186	197/205	188/204	272/272	139188	
			342/358	243/271	–	197/205	188/204	272/272	166699	
	M11	1	342/362	219/251	146/202	217/217	192/192	268/300	139198	
	M12	1	342/362	227/255	134/138	201/205	172/196	268/280	166628	
	M13	2	342/362	243/243	146/158	197/205	192/204	276/280	166648	
									157790	
	M14	3	342/366	227/255	134/134	197/217	172/220	284/284	166637	
									166698	
									166629	
	M15	1	350/362	215/215	138/174	197/197	172/184	268/300	166681	
	M16ϖ	2	350/370	215/223	134/154	197/197	160/192	268/300	166654	
									139183	
	M17	1	–	219/251	146/202	217/221	192/192	268/300	139201	
	M18	2	350/366	211/255	134/134	197/197	172/204	276/284	166661	
									166641	
	M19	1	350/366	211/255	134/134	197/217	172/204	276/284	166677	
	M20	2	358/362	215/239	134/178	197/197	164/192	268/268	166705	
									139189	
	M21	1	358/362	219/239	134/202	197/217	–	268/300	166669	
	M22	1	362/366	215/239	134/134	197/197	160/172	280/288	166697	
Multiple paternity clutches	M23	1	342/346	215/243	134/138	197/205	192/220	276/296	166659	Father 1
	M24	1	346/362	215/215	134/178	205/217	172/220	268/296		Father 2
	M25	1	342/346	239/255	138/234	197/197	172/200	268/280	139195	Father 1
	M26	1	342/346	239/255	138/226	197/197	176/196	268/280		Father 2
	M27	1	342/346	239/255	134/226	197/197	172/196	268/280		Father 3
	M28	1	350/370	223/227	190/198	197/197	192/192	268/268	139183	Father 1
	M16	2								Father 2
	M29	1	350/362	215/239	134/134	197/197	192/204	268/280	166666	Father 1
	M30	1	350/362	239/239	134/134	221/221	192/208	280/300		Father 2

[a] Genotypes for M10 matched at the four loci available ϖMale also contributed to a clutch from a female that exhibited multiple paternity.

documented in two hawksbill populations (Natoli et al., 2017; the present study), raising the possibility that this mating strategy may be influenced by species-level differences in mating behavior. For instance, polygyny has not been reported for green turtles (*Chelonia mydas*) (e.g., Lee and Hays, 2004; Ekanayake et al., 2013; Chassin-Noria et al., 2017) and males of this species are known to vigorously latch onto female turtles and undergo mate guarding for days or even weeks at a time when mating (Cuadrado, 2002; Martínez, 2003; Owens and Blanvillain, 2013). This behavior typically occurs during the early portion of the nesting season, when females are physiologically receptive (Booth and Peters, 1972; Owens and Blanvillain, 2013). Adult hawksbill turtles, particularly those in the eastern Pacific, are typically more sedentary than green turtles and often use foraging grounds in close proximity to their nesting beaches (Witzell, 1983; Bowen et al., 2007; Gaos et al., 2012a, 2012b, 2017b). This movement behavior could facilitate a longer female receptive period, which could provide increased opportunities for individual males to encounter and mate with multiple females.

4.3. Male population status

Our single season breeding sex ratio based on known males at Bahía de Jiquilisco slightly favored females (female/male = 1.41/1). Although a previous three-year study on leatherback turtles (*Dermochelys coriacea*) in the Caribbean found that breeding sex ratios calculated during year one of the study remained consistent during the entire research period (Stewart and Dutton, 2014), expanding the current study to sample females and hatchlings across years will be important to gain a better understanding of mating systems and sex ratios in the Bahía de Jiquilisco hawksbill reproductive population (Phillips et al., 2014a, 2014b). Additional studies will also be important considering that our findings only reflect the minimum number of males that successfully mated (with 69.4% of the nesting females) during the 2015 season and this may not be representative of the total number of males that actual participated in copulation events (i.e., where males mate with females, but do not successfully fertilize any clutches). Additionally, because the 20 hatchlings sampled from each clutch represent approximately only 12% of the average clutch size at Bahía de Jiquilisco (167.8 ± 37.0 eggs year^{-1}; Gaos et al., 2017a), it is possible that some males went undetected. Although the importance of such low-representation males for genetically effective population sizes is arguable, future studies will benefit from larger offspring samples sizes that can better ensure detection of all potential sires for individual clutches.

For rare and highly endangered species, the difficulty in finding members of the opposite sex may lead to reduced mating opportunities

and higher levels of polygyny than would otherwise be expected under normal population conditions (Crim et al., 2002; Frankham et al., 2011; Phillips et al., 2013, Phillips et al., 2014a, 2014b). The high levels of polygyny documented in our study suggest there may be relatively few reproductively mature males in the Bahía de Jiquilisco population (Fitzsimmons, 1996; Crim et al., 2002; Phillips et al., 2013), and this idea is supported by multiple lines of empirical evidence. First, although 70 juvenile and eight adult female turtles were captured during hawksbill in-water monitoring efforts at Bahía de Jiquilisco (between May 2016 and December 2017), not a single adult male turtle was captured during that time (Yañez and Gaos, 2017). Second, the hawksbill nest monitoring program at Bahía de Jiquilisco documented a total of eight clutches during the 2015 season where hatchlings from the same nest emerged with both hawksbill and green turtle morphotypes (i.e., some hatchlings had hawksbill and others had green turtle morphology), and this phenomenon has been documented in other seasons as well (M. Liles, personal communication 21 September 2017). These findings indicate female hawksbills may be mating with male green turtles, supporting the hypothesis of a lack of male hawksbills in the area (Seminoff et al., 2003). Considering that several of the other known hawksbill rookeries in the eastern Pacific are far smaller than Bahía de Jiquilisco (Gaos et al., 2017a), it is possible that polygyny and a lack of males is typical throughout the region. Indeed, juvenile hawksbill and green turtle hybrids have been documented in other parts of the eastern Pacific (Seminoff et al., 2003; Kelez et al., 2016), supporting this assertion.

Furthermore, individual male turtles can mate with different females across successive seasons (e.g., Wright et al., 2012) and may shorten reproductive and migratory cycles to compensate for female-biased sex ratios (Hays et al., 2014), indicating annual male cohorts at nesting grounds are likely not composed of entirely new individuals. Two adult male hawksbills encountered while copulating with females at a neighboring rookery in Nicaragua were tracked via satellite across multiple nesting seasons and never left the area during that time (Gaos and Seminoff, unpub. Data), supporting the possibility that individual males may remain at nesting grounds and mate with multiple females across successive years. If such a scenario applies more generally, the male reproductive population at Bahía de Jiquilisco may be composed of even fewer males than is suggested by our single-year breeding sex ratio estimate. Additionally, if female hawksbills are storing sperm over multiple nesting seasons, as has been suggested for conspecifics in the Indian Ocean (Phillips et al., 2014a, 2014b), we could be further overestimating the number of males.

Lastly, ongoing research into the sex ratios of hawksbill hatchlings emerging from natural and hatchery nests at Bahía de Jiquilisco between 2011 and 2015 suggests a female bias of approximately 95% and 80%, respectively (M. Liles, personal communication 15 June 2017). Ongoing research of foraging juvenile hawksbills in the same area also suggests a female bias of approximately 85% (C. Allan, personal communication, 15 March 2018). Given these estimates, it seems reasonable to suspect that these female-biased sex ratios have manifested in the adult population as well.

4.4. Significance to research and management

Our findings of high levels of polygyny and evidence of a limited number of males at Bahía de Jiquilisco have important research and management implications, particularly in the face of ongoing climate change. The sex of marine turtles is determined by nest incubation temperatures (i.e., temperature-dependent sex determination), with temperatures above the pivotal temperature skewing ratios in favor of females (Mrosovsky and Yntema, 1980; Bollmer et al., 1999). Many studies report highly female-biased hatchling sex ratios in marine turtle populations and temperature increases at nesting beaches due to ongoing climate change have the potential to further drive feminization of populations, or in some cases already have (Hawkes et al., 2009; Eckert et al., 2012; Pike, 2014; Jensen et al., 2018). This is particularly relevant to Bahía de Jiquilisco, where nesting activity is concentrated on low-relief beaches in mangrove estuaries that are especially vulnerable to increasing global temperatures (Gilman et al. 2008; M. Liles, personal communication 15 June 2017).

The polygynous mating strategy we documented here may underlie what appears to be a viable hawksbill nesting population at Bahía de Jiquilisco. Nonetheless, whether this polygynous strategy can continue to compensate for ongoing feminization and the putative low number of adult males in the population remains unclear. Future research on these questions will have major implications for marine turtles worldwide. This study provides valuable baseline genetic data that can be compared to future studies at this and other hawksbill nesting grounds in the region.

Acknowledgements

All hawksbill handling procedures were approved (APF#: 09-05-015L – San Diego State University) by the Institutional Animal Care and Use Committee (IACUC) in accordance with the requirements pertaining to animal subjects protections within the Public Health Service Policy and USDA Animal Welfare Regulations. Tissue import [United States (10-15)US844694/9] and export [El Salvador (0977)] permits to meet requirements established by CITES were acquired from the USFWS and MARN, respectively. We recognize the field support of Neftali Sanchez and Obed Rivera in El Salvador. At the Southwest Fisheries Science Center (La Jolla) we thank Gabriela Serra-Valente, Erin LaCasella, Vicki Pease, and Amy Lanci for sample archiving, optimizing, and processing support, and Robin LeRoux for administrative help. We are grateful to Karl P. Phillips for his helpful review of this manuscript.

Contributions

All authors approve the final article. ARG carried out lab work and drafted the manuscript; RLL, PHD, MJL, KS, ILY, TTJ helped developed the manuscript; ARG conducted the analyses with support from AF and KS; MJL, AH and SC collected field data.

Funding

Analytical support was provided by the Southwest Fisheries Science Center (NMFS-NOAA). Several organizations enabled the operation of the hawksbill conservation project at Bahía de Jiquilisco, during which the samples presented in this paper were collected, including the National Fish and Wildlife Foundation (NFWF), U.S. Fish & Wildlife Service (USFWS), International Seafood Sustainability Foundation (ISSF) and SeeTurtles.org.

References

Allen, Z., Mirmal, J., Alastair, G., Gilles-David, D., Bell, D., 2010. Hawksbill turtle monitoring in Cousin Island Special Reserve, Seychelles: an eight-fold increase in annual nesting numbers. End. Sp. Res. 11, 195–200.

Archer, F.I., Adams, P.E., Schneiders, B.B., 2016. Stratag: an R package for manipulating, summarizing and analysing population genetic data. Mol. Ecol. Resour. 17 (1), 5–11.

Bollmer, J.L., Irwin, M.E., Rieder, J.P., Parker, P.G., 1999. Multiple paternity in Loggerhead Turtle clutches. Copeia 1999, 475–478.

Bonin, A., Bellemain, E., Eidesen, P.B., Pompanon, F., Brochmann, C., Taberlet, P., 2004. How to track and assess genotyping errors in population genetics studies. Mol. Ecol. 13, 3261–3273.

Booth, J., Peters, J.A., 1972. Behavioral studies on the green sea turtle (*Chelonia mydas*) in the sea. Anim. Behav. 20, 808–812.

Bowen, B.W., Grant, W.S., Hillis-Starr, Z., Shaver, D.J., Bjorndal, K.A., Bolten, A.B., Bass, A.L., 2007. Mixed stock analysis reveals the migrations of juvenile hawksbill turtles (*Eretmochelys imbricata*) in the Caribbean Sea. Mol. Ecol. 16, 49–60.

Briton, J., Nurthen, R.K., Briscoe, D.A., Frankham, R., 1994. Modelling problems in conservation genetics using *Drosophila*: consequences of harem. Biol. Conserv. 69, 267–275.

Bro-Jørgensen, J., 2014. Will their armaments be their downfall? Large horn size increases extinction risk in bovids. Anim. Conserv. 17, 80–87.

Chapman, J.R., Nakagawa, S., Coltman, D.W., Slate, J., Sheldon, B.C., 2009. A quantitative review of heterozygosity-fitness correlations in animal populations. Mol. Ecol. 18, 2746–2765.
Chassin-Noria, O., Macip-Ríos, R., Dutton, P.H., Oyama, K., 2017. Multiple paternity in the East Pacific green turtle (*Chelonia mydas*) from the Pacific coast of Mexico. J. Exp. Mar. Biol. Ecol. 495, 43–47.
Core Team, R., 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Crim, J.L., Spotila, L.D., Spotila, J.R., O'Connor, M., Reina, R., Williams, C.J., Paladino, F.V., 2002. The leatherback turtle, *Dermochelys coriacea*, exhibits both polyandry and polygyny. Mol. Ecol. 11, 2097–2106.
Cuadrado, M., 2002. Sistemas de apareamiento en reptiles: una revision. Rev. Esp. Herp. 61-69.
Dutton, P.H., Stewart, K., 2013. A method for sampling hatchling sea turtles for the development of a genetic tag. Mar. Turt. Newsl. 138, 3–7.
Eckert, K.L., Wallace, B.P., Frazier, J.G., Eckert, S.A., Pritchard, P.C.H., 2012. Synopsis of the biological data on the leatherback sea turtle (*Dermochelys coriacea*). In: US Department of Interior, Fish and Wildlife Service, Biological Technical Publication BTP-R4015-2012.
Ekanayake, E.M.L., Kapurusinghe, T., Saman, M.M., Rathnakamura, D.S., Samaraweera, P., Ranawana, K.B., Rajakaruna, R.S., 2013. Paternity of green turtle (*Chelonia mydas*) clutches laid in Kosgoda, Sri Lanka. Herpetol. Conserv. Biol. 8, 27–36.
Engen, S., Ringsby, T.H., Saether, B.E., Lande, R., Jensen, H., Lillegard, M., Ellegren, H., 2007. Effective size of fluctuating populations with two sexes and overlapping generations. Evolution 61, 1873–1885.
Fitzsimmons, N.N., 1996. Use of microsatellite loci to investigate multiple paternity in marine turtles. In: Bowen, B.W., Witzell, W.N. (Eds.), Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Technical Memorandum NMFS-SEFSC-396, pp. 69–78.
Frankham, R., Ballou, J.D., Eldridge, M.D., Lacy, R.C., Ralls, K., Dudash, M.R., Fenster, C.B., 2011. Predicting probability of outbreeding depression. Conserv. Biol. 25, 465–475.
Fuentes, M.P.B., Pike, D.A., Dimatteo, A., Wallace, B.P., 2013. Resilience of marine turtle regional management units to climate change. Glob. Chang. Biol. 19, 1399–1406.
Gaos, A.R., Lewison, R.L., Liles, M., Nichols, W.J., Baquero, A., Hasbún, C.R., Vasquez, M., Urteaga, J., Seminoff, J.A., 2012a. Shifting the life-history paradigm: discovery of novel habitat use by hawksbill turtles. Biol. Lett. 8, 54–56.
Gaos, A.R., Lewison, R.L., Liles, M., Nichols, W.J., Baquero, A., Hasbún, C.R., Vasquez, M., Urteaga, J., Seminoff, J.A., 2012b. Spatial ecology of critically endangered hawksbill turtles *Eretmochelys imbricata*: implications for management and conservation. Mar. Ecol. Prog. Ser. 450, 181–194.
Gaos, A.R., Lewison, R.L., Liles, M.J., Gadea, V., Altamirano, E., Henríquez, A.V., Torres, P., Urteaga, J., Vallejo, F., Baquero, F., LeMarie, C., Muñoz, J.P., Chaves, J.A., Hart, C.E., Peña de Niz, A., Chácon, D., Fonseca, L., Otterstrom, S., Yañez, I.L., LaCasella, E.L., Frey, A., Jensen, M., Dutton, P.H., 2016. Hawksbill turtle terra incognita: conservation genetics of eastern Pacific rookeries. Ecol. Evol. 6 (4), 1251–1264.
Gaos, A.R., Liles, M., Gadea, V., Peña de Niz, A., Vallejo, F., Miranda, C., Darquea, J., Henriquez, A., Rivera, A., Chavarria, S., Melero, D., Urteaga, J., Pacheco, C.M., Chacon, D., LeMarie, C., Alfaro-Sigueto, J., Mangel, J., Yañez, I.L., Seminoff, J.A., 2017a. Living on the edge; hawksbill turtle nesting and conservation along the Eastern Pacific Rim. Lat. Am. J. Aquat. Res. 45 (3), 572–584.
Gaos, A.R., Lewison, R.L., Jensen, M.P., Liles, M.J., Henriquez, A., Chavarria, S., Pacheco, C.M., Valle, M., Melero, D., Gadea, V., Altamirano, E., Torres, P., Vallejo, F., Miranda, C., Lemarie, C., Lucero, J., Oceguera, K., Chácon, D., Fonseca, L., Abrego, M., Seminoff, J.A., Flores, E.E., Llamas, I., Donadi, R., Peña, B., Muñoz, J.P., Alarcòn-Ruales, D., Chaves, J.A., Otterstrom, S., Zavala, A., Hart, C.E., Brittain, R., Alfaro-Shigueto, J., Mangel, J., Yañez, I.L., Dutton, P.H., 2017b. Natal foraging philopatry of hawksbill turtles in the eastern Pacific Ocean. R. Soc. Open Sci. 4, 170153.
Gaos, A.R., Lewison, R., Jensen, M., Liles, M., Henriquez, A., Chavarria, S., Pacheco, C., Valle, M., Melero, D., Gadea, V., Altamirano, E., Torres, P., Vallejo, F., Miranda, C., Lemarie, C., Lucero, J., Oceguera, K., Chacon, D., Fonseca, L., Abrego, M., Seminoff, J., Flores, E., Llamas, I., Peña, B., Muñoz, J., Alarcón-Ruales, D., Chaves, J., Otterstrom, S., Zavala, A., Hart, C., Brittain, R., Alfaro-Shigueto, J., Mangel, J.C., Yañez, I., Dutton, P.H., 2018. Rookery contributions, movements and conservation needs of hawksbill turtles at foraging grounds in the eastern Pacific Ocean. Mar. Ecol. Prog. Ser. 586, 203–216.
Gilman, E.L., Ellison, J., Duke, N.C., Field, C., 2008. Threats to mangroves from climate change and adaptation options: a review. Aquat. Bot. 89, 237–250.
González-Garza, B.I., Stow, A., Sánchez-Teyer, L.F., Zapata-Pérez, O., 2015. Genetic variation, multiple paternity, and measures of reproductive success in the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Ecol. Evol. 5 (24), 5758–5769.
Hanotte, O.T., Burke, T., Armour, J.A.L., Jeffreys, A.J., 1991. Hypervariable minisatellite DNA sequences in the Indian pea fowl *Pavo cristatus*. Genomics 9, 587–597.
Hawkes, L.A., Broderick, A.C., Godfrey, M.H., Godley, B.J., 2009. Climate change and marine turtles. Endanger. Species Res. 7, 137–154.
Hays, G.C., Mazaris, A.D., Schofield, G., 2014. Different male vs. female breeding periodicity helps mitigate offspring sex ratio skews in sea turtles. Front. Mar. Sci. 1, 1–49.
Hoekert, W.E.J., Neufeglise, H., Schouten, A.D., Menken, S.B.J., 2002. Multiple paternity and female biased mutation at a microsatellite locus in the Olive Ridley Sea Turtle (*Lepidochelys olivacea*). Heredity 89, 107–113.
Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., 1990. PCR Protocols: A Guide to Methods and Applications. Academic Press, London, United Kingdom, pp. 3–12.
Jensen, M.P., Abreu-Grobois, F.A., Frydenberg, J., Loeschcke, V., 2006. Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. Mol. Ecol. 15, 2567–2575.
Jensen, M.P., Fitzsimmons, N.N., Dutton, P.H., 2013. Molecular genetics of sea turtles. In: Wyneken, J., Lohmann, K.J., Musick, J.A. (Eds.), The Biology of SEA turtles. vol. 3. CRC Press, Boca Raton, FL, pp. 135–162.
Jensen, M.P., Allen, C.D., Eguchi, T., Bell, I.P., Lacasella, E.L., Hilton, W.A., Hof, C.A.M., Dutton, P.H., 2018. Environmental warming and feminization of one of the largest sea turtle populations in the world. Curr. Biol. 28, 154–159.
Jones, A.G., 2001. GERUD1.0: a computer program for the reconstruction of parental genotypes from progeny arrays using multilocus DNA data. Mol. Ecol. Notes 1, 215–218.
Joseph, J., Shaw, P.W., 2011. Multiple paternity in egg clutches of hawksbill turtles (*Eretmochelys imbricata*). Conserv. Genet. 12, 601–605.
Kelez, S., Velez-Suaso, X., Pacheco, A.S., 2016. First record of hybridization between green *Chelonia mydas* and hawksbill *Eretmochelys imbricata* sea turtles in the Southeast Pacific. PeerJ. 1712.
Lasala, J.A., Hughes, C.R., Wyneken, J., 2018. Breeding sex ratio and population size of loggerhead turtles from Southwestern Florida. PLoS One 13 (1).
Lee, P.L.M., Hays, G.C., 2004. Polyandry in a marine turtle: females make the best of a bad job. Proc. Natl. Acad. Sci. U. S. A. 101, 6530–6535.
Lee, P.L.M., Schofield, G., Haughey, R.I., Mazaris, A.D., Hays, G.C., 2018. A review of patterns of multiple paternity across sea turtle rookeries. In: Sheppard, C. (Ed.), Advances in Marine Biology. Academic Press, Cambridge, MA, pp. 1–31.
Liles, M.J., Jandres, M.V., López, W.A., Mariona, G.I., Hasbún, C.R., Seminoff, J.A., 2011. Hawksbill turtles (*Eretmochelys imbricata*) in El Salvador: nesting distribution and mortality at the largest remaining nesting aggregation in the eastern Pacific Ocean. Endanger. Species Res. 14, 23–30.
Liles, M.J., Peterson, M.J., Lincoln, Y.S., Seminoff, J.A., Gaos, A.R., Peterson, T.R., 2014. Connecting international priorities with human wellbeing in low-income regions: lessons from hawksbill turtle conservation in El Salvador. Local Environ. 20 (11), 1383–1404.
Liles, M.J., Peterson, M.J., Seminoff, J.A., Altamirano, E., Henríquez, A.V., Gaos, A.R., Gadea, V., Urteaga, J., Torres, P., Wallace, B.P., Peterson, T.R., 2015. One size does not fit all: importance of adjusting conservation practices for endangered hawksbill turtles to address local nesting habitat needs in the EP Ocean. Biol. Conserv. 184, 405–413.
Maniatis, T., Fritsch, E.F., Sambrook, J., 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
Martínez, M.M., 2003. Repertorio del comportamiento y patrones sociales durante el cortejo y apareamiento de Chelonia agassizii (Professional Thesis). Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México.
Martínez-Ruiz, C., Knell, R.J., 2016. Sexual selection can both increase and decrease extinction probability: reconciling demographic and evolutionary factors. J. Anim. Ecol. 86, 117–127.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16 (3), 1215.
Miro-Herrans, A.T., Velez-Zuazo, X., Acevedo, J.P., Mcmillan, W.O., 2008. Isolation and characterization of novel microsatellites from the critically endangered hawksbill sea turtle (*Eretmochelys imbricata*). Mol. Ecol. Resour. 8 (5), 1098–1101.
Moore, M.K., Ball, R.M., 2002. Multiple paternity in Loggerhead Turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. Mol. Ecol. 11, 281–288.
Moore, J.A., Nelson, N.J., Keall, S.N., Daugherty, C.H., 2008. Implications of social dominance and multiple paternity for the genetic diversity of a captive-bred reptile population (tuatara). Conserv. Genet. 9, 1243–1251.
Moritz, C., 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3, 401–411.
Mortimer, J., Bresson, R., 1999. Temporal distribution and periodicity in hawksbill turtles (*Eretmochelys imbricata*) nesting at Cousin Island, Republic of Seychelles, 1971–1997. Chelonian Conserv. Biol. 3 (2), 318–325.
Mrosovsky, N., Yntema, C.L., 1980. Temperature-dependence of sexual-differentiation in sea turtles—implications for conservation practices. Biol. Conserv. 18 (4), 271–280.
Natoli, A., Phillips, K.P., Richardson, D.S., Jabado, R.W., 2017. Low genetic diversity after a bottleneck in a population of a critically endangered migratory marine turtle species. J. Exp. Mar. Biol. Ecol. 491, 9–18.
Nunney, L., 1993. The influence of mating system and overlapping generations on effective population size. Evolution 47, 1329–1341.
Owens, D.W., Blanvillain, G., 2013. Captive reproduction of sea turtles: an important success story. In: Sato, K. (Ed.), Proceedings of the International Symposium on Reproduction of Marine Life, Birth of New Life!. Investigating the Mysteries of Reproduction. Okinawa Churashima Foundation, Motobu, Japan, pp. 23–40.
Pearse, D.E., Avise, J.C., 2001. Turtle mating systems: behaviour, sperm storage and genetic paternity. J. Hered. 92, 206–211.
Pérez-González, J., Mateos, C., Carranza, J., 2009. Polygyny can increase rather than decrease genetic diversity contributed by males relative to females: evidence from red deer. Mol. Ecol. 18, 1591–1600.
Phillips, K.P., Jorgensen, T.H., Jolliffe, K.G., Jolliffe, S.M., Henwoods, J., Richardson, D.S., 2013. Reconstructing paternal genotypes to infer patterns of sperm storage and sexual selection in the hawksbill turtle. Mol. Ecol. 22, 2301–2312.
Phillips, K.P., Jorgensen, T.H., Jolliffe, K.G., Richardson, D.S., 2014a. Potential interseason sperm storage by a female hawksbill turtles. Mar. Turt. Newsl. 140, 13–14.
Phillips, K.P., Mortimer, J.A., Jolliffe, K.G., Jorgensen, T.H., Richardson, D.S., 2014b. Molecular techniques reveal cryptic life history and demographic processes of a critically endangered marine turtle. J. Exp. Mar. Biol. Ecol. 444, 29–37.
Phillips, K.P., Jorgensen, T.H., Jolliffe, K.G., Richardson, D.S., 2017. Evidence of opposing fitness of parental heterozygosity and relatedness in a critically endangered marine turtle? J. Evol. Biol. 30 (11), 1953–1965.
Pike, D.A., 2014. Forecasting the viability of sea turtle eggs in a warming world. Glob. Chang. Biol. 20, 7–15.
Pilcher, N.J., 1999. The hawksbill turtle, *Eretmochelys imbricata*, in the Arabian Gulf.

Chelonian Conserv. Biol. 3 (2), 312–317.
Raymond, M., Rousset, F., 1995. GENEPOP (V.1.2): a population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249.
Sakaoka, K., Sakai, F., Yoshii, M., Okamoto, H., Nagasawa, K., 2013. Estimation of sperm storage duration in captive loggerhead turtles (*Caretta caretta*). J. Exp. Mar. Biol. Ecol. 439, 136–142.
Seminoff, J.A., Karl, S.A., Schwartz, T., Resendiz, A., 2003. Hybridization of a the green turtles (*Chelonia mydas*) and hawksbill turtles (*Eretmochelys imbricata*) in the Pacific Ocean: indication of an absence of gender bias in the directionality of crosses. Bull. Mar. Sci. 73 (3), 643–652.
Shamblin, B.M., Faircloth, B.C., Dodd, M., Wood-Jones, A., Castleberry, S.B., Carroll, J.P., Nairn, C.J., 2007. Tetranucleotide microsatellites from the loggerhead sea turtle (*Caretta caretta*). Mol. Ecol. Resour. 7 (5), 784–787.
Shamblin, B.M., Faircloth, B.C., Dodd, M.G., Bagley, D.A., Ehrhart, L.M., Dutton, P.H., Frey, A.M., Nairn, C.J., 2009. Tetranucleotide markers from the loggerhead sea turtle (*Caretta caretta*) and their cross-amplification in other marine turtle species. Conserv. Genet. 10 (3), 577–580.
Shamblin, B.M., Berry, B.E., Lennon, D.M., Meylan, A.B., Meylan, P.A., Outerbridge, M.E., Nairn, C.J., 2013. Tetranucleotide microsatellite loci from the critically endangered hawksbill turtle (*Eretmochelys imbricata*). Conserv. Genet. Resour. 5 (1), 23–26.
Stewart, K.R., Dutton, P.H., 2011. Paternal genotype reconstruction reveals multiple paternity and sex ratios in a breeding population of leatherback turtles (*Dermochelys coriacea*). Conserv. Genet. 12 (4), 1101–1113.
Stewart, K.R., Dutton, P.H., 2014. Breeding sex ratios in adult leatherback turtles (*Dermochelys coriacea*) may compensate for female-biased hatchling sex ratios. PLoS One 9 (2), e88138.
Stiver, J.R., Apa, A.D., Remington, T.E., Gibson, R.M., 2008. Polygyny and female breeding failure reduce effective population size in the lekking Gunnison sage-grouse. Biol. Conserv. 141, 472–481.
Tedeschi, J.N., Mitchell, N.J., Berry, O., Whiting, S., Meekan, M., Kennington, W.J., 2015. Reconstructed paternal genotypes reveal variable rates of multiple paternity at three rookeries of loggerhead sea turtles (Caretta caretta) in Western Australia. Aust. J. Zool. 62, 454–462.
Theissinger, K., FitzSimmons, N.N., Limpus, C.J., Parmenter, C.J., Phillott, A.D., 2009. Mating system, multiple paternity and effective population size in the endemic flatback turtle (*Natator depressus*) in Australia. Conserv. Genet. 10, 329–346.
Uller, T.A., Olsson, M., 2008. Multiple paternity in reptiles: patterns and processes. Mol. Ecol. 17, 2566–2580.
Wallace, B.P., Dimatteo, A.D., Bolten, A.B., Chaloupka, M.Y., Hutchinson, B.J., Abreu-Grobois, F.A., Mortimer, J.A., Seminoff, J.A., Amorocho, D., Bjorndal, K.A., Bourjea, J., Bowen, B.W., Briseño-Dueñas, R., Casale, P., Choudhury, B.C., Costa, A., Dutton, P.H., Fallabrino, A., Finkbeiner, E.M., Girard, A., Girondot, M., Hamann, M., Hurley, B.J., López-Mendilaharsu, M., Marcovaldi, M.A., Musick, J.A., Nel, R., Pilcher, N.J., Troëng, S., Witherington, B., Mast, R.B., 2011. Global conservation priorities for marine turtles. PLoS One 6, e24510.
Weigensberg, I., Fairbairn, D.J., 1994. Conflicts of interest between the sexes – a study of mating interactions in a semiaquatic bug. Anim. Behav. 48, 893–901.
Westneat, D.F., Fredrick, P.C., Haven Wiley, R., 1987. The use of genetic markers to estimate the frequency of successful alternative reproductive tactics. Behav. Ecol. Sociobiol. 21, 35–45.
Wheelwright, N.T., Schultz, C.B., Hodum, P.J., 1992. Polygyny and male parental care in Savannah sparrows: effects on female fitness. Behav. Ecol. Sociobiol. 31 (4), 279–289.
Witzell, W., 1983. Synopsis of biological data on the hawksbill turtle, Eretmochelys imbricata (Linnaeus, 1766). FAO Fish. Synop. 137, 579.
Wright, L.I., Fuller, W.J., Godley, B.J., Mcgowan, A., Tregenza, T., Broderick, A.C., 2012. Reconstruction of paternal genotypes over multiple breeding seasons reveals male green turtles do not breed annually. Mol. Ecol. 21, 3625–3635.
Yañez, I., Gaos, A.R., 2017. Multi-Year Monitoring of Hawksbills in the Eastern Pacific Ocean. ICAPO (Eastern Pacific Hawksbill Initiative) Programmatic Report to the National Fish and Wildlife Foundation 0104.16.051224.
Zbinden, J.A., Largiader, C.R., Leippert, F., Margaritoulis, D., Arlettaz, R., 2007. High frequency of multiple paternity in the largest rookery of Mediterranean Loggerhead Sea Turtles. Mol. Ecol. 16, 3703–3711.