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Regional variation in multiple paternity in the brown smooth-hound shark *Mustelus henlei* from the northeastern Pacific

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Abstract

Multiple paternity (MP) in the brown smooth-hound shark (*Mustelus henlei*) was assessed in 15 litters (15 mothers and 97 embryos) collected in the northern Gulf of California of which 86.7% were sired by more than one male (*i.e.*, from 2 to 4 sires). When taken together with results from previous studies, this record indicates that there is regional variation in MP in *M. henlei* in the northeastern Pacific. This pattern is associated with variations in the reproductive traits of each population (*e.g.*, female size and litter size). In the Gulf of California, the results of a generalized linear model (GLZ) indicated that the litters of larger females had a higher probability of MP compared to those of smaller females.

KEYWORDS
multiple paternity, paternity analysis, polyandry, reproductive behaviour, shark, sireship skew

1 | INTRODUCTION

The management of commercially exploited elasmobranch species is frequently conducted under the assumption that the mortality and natality rates of a given stock are in equilibrium, but this is not the case for most fish stock. Moreover, many elasmobranch species are commercially important yet vulnerable to overfishing because of life-history characteristics and environmental degradation (Davidson *et al*., 2016). To address these issues, research efforts aimed at understanding life-history characteristics and the reproductive behaviour of the species and stocks in question are needed (Rowe & Hutchings, 2003). In particular, assessments of elasmobranch mating systems are required for the development of successful management and conservation measures (Pratt & Carrier, 2001).

In almost all studies that have assessed mating systems in elasmobranch species, polyandry (*i.e.*, females mate with multiple partners during the reproductive season) has been identified (review in Lamarca *et al*., 2020; Lyons *et al*., 2021). In polyandrous populations, multiple matings can result in a high number of reproductive partners [multiple paternity (MP)]. Most MP studies in sharks focus on a single or very few populations of a given species because of logistical constraints that arise from the large geographical distributions of most elasmobranch species. Thus, spatial characterizations of the reproductive biology of many elasmobranch species have been overlooked. In some cases, regional variation in MP has been associated with mate encounter rates, which could be related to the size of the population (Chabot & Haggin, 2014); nonetheless, in other cases variation in MP is not clearly associated with anything (Boomer *et al*., 2013). Moreover, little attention has been paid to other aspects of reproductive biology, such as the synchrony between the time window for insemination (the period between parturition and the next ovulation) and the encounter rate (Boomer *et al*., 2013; Veríssimo *et al*., 2011), which could cause MP to vary by population.

The brown smooth-hound shark, *Mustelus henlei* (Gill, 1863) is a small placental viviparous species that is distributed from California to the Gulf of California in Mexico and from Ecuador to Peru (Compagno, 1984). The reproductive cycle of *M. henlei* is annual with a gestation period of *c*. 10–11 months (Ebert, 2003; Pérez-Jiménez & Sosa-Nishizaki, 2008; Silva-Santos, 2012). The ovarian cycle and gestation are concurrent in this species (Pérez-Jiménez & Sosa-Nishizaki, 2008), and thus females may mate again shortly after parturition.

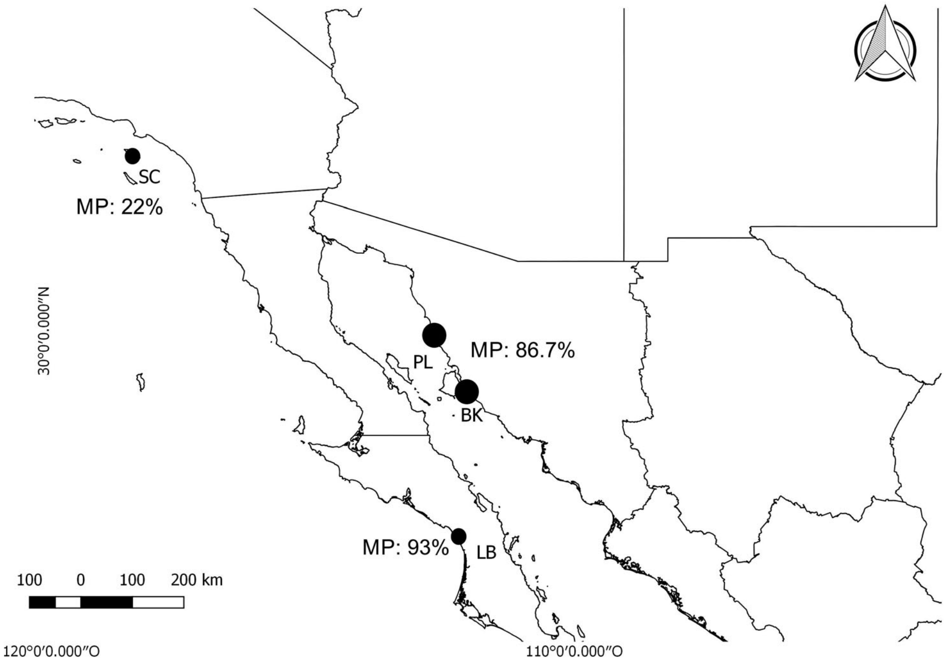

FIGURE 1 Locations where *Mustelus henlei* mothers and their litters were collected: Bahía de Kino (BK) and Puerto Libertad (PL). The Santa Catalina Island (SC; Chabot & Haggin, 2014) and Las Barrancas (LB; Byrne & Avise, 2012) locations were included from previous studies as spatial references. MP: multiple paternity

Research has indicated contrasting frequencies of MP between distant populations of *M. henlei*. MP has been found to be high in Las Barrancas, Baja California Sur, and low in Santa Catalina Island, California (93% and 22% respectively; Byrne & Avise, 2012; Chabot & Haggin, 2014). This pattern has been attributed to clear population differences, both demographically and genetically (Chabot *et al*., 2015; Sandoval-Castillo & Beheregaray, 2015), which is reflected in differences in densities and fertility (Chabot & Haggin, 2014).

Previous studies have evaluated the relationship between female reproductive traits and MP levels in elasmobranchs. These studies suggested that if a relationship exists, then polyandry will likely increase female fecundity, guaranteeing the fertilization of most eggs (Boomer *et al*., 2013; Chapman *et al*., 2004; Farrell *et al*., 2014; Nosal *et al*., 2013; Portnoy *et al*., 2007; Veríssimo *et al*., 2011). Nonetheless, this has not yet been verified. Moreover, it should only take a single male to fertilize every egg. In the *M. henlei* population of the Gulf of California, a positive relationship between maternal size and fertility has been established (*e.g*., litter size; Pérez-Jiménez & Sosa-Nishizaki, 2008), yet whether relationships among these variables and the MP frequency are present in this species remains unknown.

Over the past three decades, *M. henlei* has been one of the most frequently caught elasmobranch species in the Gulf of California (Saldaña-Ruiz *et al*., 2017), indicating that this species is capable of withstanding intense fishing pressure. Therefore, the objective of this study was to assess MP in *M. henlei* in the northeastern Pacific by incorporating a new record of MP in a population located at an intermediate position within the distribution range of the species: the northern Gulf of California. In addition, to explore the direct benefits of polyandry on female fertility, an interpopulation analysis was conducted to evaluate the relationships between the probability of MP and female size and fertility.

2 | MATERIALS AND METHODS

2.1 | Sample collection and DNA extraction

Muscle tissue samples from 23 gravid females and 113 embryos were collected from landings of the artisanal fisheries of Bahia de Kino (28° 49′ 22″ N, 111° 56′ 27″ W) and Puerto Libertad (29° 54′ 15″ N, 112° 40′ 59″ W) in the northeastern region of the Gulf of California, Mexico (Figure 1). All samples were preserved in saturated saline solution with 20% dimethyl sulfoxide for subsequent analyses. Genomic DNA was extracted using a standard proteinase K digestion, followed by organic extraction and subsequent ethanol precipitation (Aljanabi & Martinez, 1997).

The number of embryos and the total length (L_T) of all organisms were recorded. The dorsal fin distance (DFD; from the origin of the first dorsal fin to the origin of the second dorsal fin) was also measured after the head of each specimen was removed (Supporting Information Table S1).

2.2 | Ethical statement

Specimen collection complied with Mexico government laws, guidelines and policies as approved by Comisión Nacional de Acuacultura y Pesca.

2.3 | Amplification and genotyping

Four microsatellite loci (*Mh*1, *Mh*13, *Mh*15 and *Mh*25) developed for *M. henlei* (Chabot, 2012) were standardized and amplified. The amplifications were fluorescently labelled (6-FAM, VIC, NED or PET) with

M13 universal primer (De Arruda *et al*., 2010). Each PCR reaction consisted of 1× PCR-buffer, 2 mM of $MgCl_2$, 0.2 mM of dNTP mix, 0.5 μM of each forward and reverse primer, ½ U of Taq DNApol (NEB, Ipswich, MA, USA), 0.5 μM of fluorescently labelled forward primer and 10 ng of template DNA in a total volume of 10 μl. The thermocycling profile for each PCR was carried out in two phases. The first phase consisted of 5 min at 94°C, followed by 25 cycles of 94°C for 30 s, the annealing temperature of each pair of primers (Supporting Information Table S2) for 30 s, and 72°C for 1 min. Before the second phase, a universal M13 primer with a fluorophore was added to each reaction. The second phase of amplification consisted of 20 cycles at 94°C for 30 s, 53°C for 30 s, 72°C for 1 min and a final extension of 72°C for 30 min. Products were electrophoresed on an ABI 3100 capillary sequencer (Applied Biosystems Inc., Foster City, CA, USA), and the genotypes were scored by eye using Gene Marker v. 2.7.0 (Softgenetics, State College, PA, USA). Null alleles and allelic dropout were assessed using Microchecker v. 2.2.3 (Van Oosterhout *et al*., 2004).

2.4 | Data analysis

For each litter, the authors verified that embryos shared at least one allele per locus with their respective mothers. Genepop v. 4.1 (Rousset, 2008) was used to calculate observed heterozygosity (*Ho*), expected heterozygosity (*He*), conformance to the expectations of Hardy–Weinberg equilibrium (HWE) for each microsatellite locus (using only data from mothers). In addition, the authors evaluated the number of alleles per locus (*A*) in all data sets. To calculate the probability of detecting MP, the authors used PrDM (Neff & Pitcher, 2002), which ran different scenarios with varied litter sizes, number of males, and litter paternity percentages. The PrDM analyses were run 10 times for each scenario, and the averages were obtained. To evaluate the number of sires in each litter and potential male reproductive skew, the authors used GERUD v. 2.0 (Jones, 2005) and COLONY v. 2.0 (Wang, 2004). GERUD generates paternal genotypes and the minimum number of sires per litter, whereas COLONY uses a maximum likelihood algorithm to assign pups in full or half sibling groups based on parent–offspring relationships. COLONY analyses were run several times with different parameters to identify the best run (consistent results and high probabilities of inclusion and exclusion). The best run corresponded to the population allele frequencies with the following settings: "update allele frequency" unselected, "long run" selected, allelic dropout set to 0 and an error rate of 0.01. Both programmes were run twice to evaluate the consistency of the analyses. Comparing paternity results may be considered to be a test of congruence, as GERUD calculates the minimum number of sires per litter (Jones, 2005), whereas COLONY overestimates the putative number of sires per litter when data are limited (Sefc & Koblmüller, 2009). In all programmes used to evaluate MP, the authors used the population allele frequencies from unrelated *M. henlei* adults from the Upper Gulf of California from this study (23) and those from specimens (12) that had been previously genotyped by Chabot *et al*. (2015). Similar to the methods of Chabot and Haggin (2014), alleles were recorded in embryos but not for the population allele frequencies were set to 0.01. The rest of the frequencies were adjusted accordingly.

TABLE 1 Genetic diversity of four microsatellite loci of *Mustelus henlei*

Marker	A	He	Ho	*P*-value
*Mh*25	9	0.736	0.696	0.494
*Mh*15	3	0.124	0.130	1
*Mh*13	4	0.405	0.522	0.425
*Mh*1	12	0.812	0.826	0.386

Note: The number of alleles (*A*), expected heterozygosity (*He*), observed heterozygosity (*Ho*) and significance values for the Hardy–Weinberg equilibrium test are shown only for mothers (*P*-value).

It was not possible to measure L_T in some females. In these cases, L_T was estimated with Equation (1) using data from 30 females ($R^2 = 0.894$, $F_0 = 236.63$, $F_{1,28} = 4.196$, $N = 30$, $P < 0.0001$; Supporting Information Table S1):

$$LT = 2.358(DFD) + 12.81. \tag{1}$$

The relationships between female L_T and litter size, and litter size and MP were determined *via* linear regression analysis. To assess the relationships between female L_T and MP, a generalized linear model (GLZ) was constructed in STATISTICA v. 7.0 (StatSoft, Tulsa, OK, USA). Female size was used as an explanatory variable, and the number of sires was used as the response variable. An ordinal multinomial distribution and a logit-type ligation function were chosen. The parameters of the linear equations, in addition to female size, were used to calculate the occurrence probability of MP in a litter given the size of the mother (1, 2, 3 or 4 sires). In this case, only females with more than two embryos were included in the GLZ. To obtain the occurrence probability of MP (probability function) for each event (number of sires), the linear values were transformed with the inverse of the logit-type ligation function.

3 | RESULTS

3.1 | Genetic diversity

Null alleles were detected in two of the four microsatellite loci (*Mh*25 and *Mh*1); nonetheless, these were not in disequilibrium according to the HWE results. The microsatellite loci showed genetic variability that ranged from 3 to 12 alleles per locus and *Ho* values that ranged from 0.130 to 0.826 (Table 1).

3.2 | Paternity analysis

The number of embryos per litter was recorded and ranged from 1 to 14. From 29 pregnant females, only 23 were genotyped because

TABLE 2 Probability of detecting multiple paternity (MP) under equal and skewed scenarios using four microsatellite loci and a variable number of sires and litter sizes

		Litter size									
Males	**PF**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**20**
2	(0.5/0.5)	0	0.437	0.696	0.832	0.999	0.938	0.959	0.971	0.979	0.993
	(0.667/0.333)	0	0.388	0.626	0.760	0.839	0.887	0.918	0.940	0.955	0.991
3	(0.333/0.333/0.333)	–	0.586	0.838	0.933	0.971	0.986	0.993	0.996	0.998	1
	(0.57/0.285/0.145)	–	0.503	0.749	0.864	0.922	0.953	0.970	0.981	0.987	0.999
4	(0.25/0.25/0.25/0.25)	–	–	0.892	0.962	0.990	0.995	0.998	0.999	0.999	1
	(0.52/0.27/0.14/0.07)	–	–	0.798	0.901	0.947	0.971	0.983	0.999	0.994	1
5	(0.20/0.20/0.20/0.20/0.20)	–	–	–	0.976	0.992	0.998	0.999	1	1	1
	(0.50/0.26/0.13/0.07/0.04)	–	–	–	0.914	0.956	0.977	0.987	0.992	0.996	1

Note: PF: proportion of fertilization by each male.

5 females had only one embryo and 1 female could not be genotyped because of poor quality DNA (Supporting Information Table S1). Similarly, it was not possible to genotype one embryo from one of the largest litters. From the 23 females and litters genotyped, all embryos shared at least one allele with their respective mothers. The simulation results showed that the probability of detecting MP using four microsatellite loci increased with both litter size and the number of sires. According to the results of the PrDM analyses, the probability of detecting MP was moderate to high in litters with four or five embryos (0.696–0.914), both with and without skewed fertilization (Table 2). The remaining analyses were conducted with females with litters containing at least three embryos ($N = 15$).

The maximum number of paternal alleles by locus was seven (*Mh*1), which belonged to the largest litter (13 embryos). According to the paternal allele count, from the 15 litters analysed, 13 litters (86.7%) presented at least three alleles in at least one locus. Moreover, six litters (26.1%) presented at least three alleles in at least two loci (Table 3). GERUD revealed that at least 13 litters (86.7%) were sired by multiple males, whereas the COLONY results indicated that 14 litters (93.3%) were sired by multiple males. The minimum and maximum numbers of sires per litter calculated with COLONY were one and seven, respectively, whereas GERUD calculated a minimum of one and a maximum of four sires per litter (Table 3). The average number of sires estimated by COLONY was greater than that estimated by GERUD (average of 4.2 and 2.1, respectively). Similarly, COLONY estimated a greater male reproductive skew (85.7%; 12 of 14 litters with MP) than GERUD (69.2%; 9 of 13 litters with MP). Given the different results obtained with both approaches when calculating MP, the authors decided to consider the conservative approach of GERUD instead of that of COLONY because the latter overestimated the number of sires by obtaining over-split sibships. Over-split sibships occur when there is a low probability of exclusion (group of siblings is exclusive without the possibility that other groups of full siblings may form) despite there being a high probability of inclusion in the results (group of individuals composed of full siblings), which results in an overestimated number of sires.

3.3 | Relationship between MP, litter size, and maternal size

There was a significant relationship between maternal size and litter size ($R^2 = 0.676$, $F_0 = 56.47$, $F_{1,27} = 4.210$, $P < 0.0001$; Figure 2). There was also a significant relationship between litter size and MP using GERUD ($R^2 = 0.68$, $F_0 = 27.66$, $F_{1,13} = 4.667$, $P = 0.0002$; Figure 3). The GLZ with four scenarios (*i.e.*, 1, 2, 3 and 4 sires) showed a significant relationship between maternal L_T and the number of sires (Table 4). The model indicated a high probability of females with L_Ts between *c.* 62 and 79 cm having litters with two sires, whereas females between *c.* 90 and 95 cm L_T had a high probability of having litters with four sires (Figure 4).

4 | DISCUSSION

The MP frequency reported in the present study for *M. henlei* in the Gulf of California (86.7%) is higher than those reported for species of the same genus, such as *Mustelus antarticus* (Günther, 1870) (31%; Boomer *et al.*, 2013), *Mustelus mustelus* (Linnaeus, 1758) (47–67%; Marino *et al.*, 2015; Rossouw *et al.*, 2016), *Mustelus punctulatus* (Risso, 1827) (54%; Marino *et al.*, 2015), and *Mustelus californicus* (genetic monogamy; Tárula-Márin & Saavedra-Sotelo, 2021). Nevertheless, the authors consider that the percentage of MP obtained in the northern Gulf of California in this study should be viewed with caution as the sample size was only 15 females (eight litters of two pups were removed).

The MP analysis carried out in this study allowed the authors to assess the local reproductive behaviour of *M. henlei* in the Upper Gulf of California. It also allowed them to compare the results of this study with previous MP records for the species in the northeastern Pacific (Figure 1). In Santa Catalina Island, an MP value of 40% was reported for a single reproductive season and a value of 22.2% was reported over 3 years (Chabot & Haggin, 2014), whereas in Las Barrancas, which is located on the Pacific side of Baja California Sur, the MP value was 93% for a single reproductive season (Byrne & Avise, 2012). In the Upper Gulf of California, the results of this study

TABLE 3 Summary of sampled *Mustelus henlei* individuals with regard to female size (L_T: total length), litter size, estimated number of sires and the maximum number of paternal alleles

			Maximum number of paternal alleles by locus				COLONY		GERUD	
Female ID	L_T **(cm)**	**Litter size**	***Mh25***	***Mh15***	***Mh13***	***Mh1***	**Sires**	**Skew**	**Sires**	**Skew**
MHEPL26	62.3	4	2	1	2	3	3	0.5/0.25/0.25	2	0.5/0.5
MHEPL21	63	5	3	1	2	2	4	0.4/0.2/0.2/0.2	2	0.6/0.4
MHEPL14	65	3	2	1	1	1	1	—	1	—
MHEPL18	66	4	3	1	1	3	3	0.5/0.25/0.25	2	0.5/0.5
MHEPL22	67.3	5	3	2	2	4	4	0.4/0.2/0.2/0.2	2	0.6/0.4
MHEPL28	68	4	2	1	1	3	3	0.5/0.25/0.25	2	0.75/0.25
MHEPL31	68	8	2	1	2	3	5	0.5/0.125/0.125/0.125/0.125	2	0.625/0.375
MHEPL15	69	4	4	1	1	4	4	0.25/0.25/0.25/0.25	2	0.5/0.5
MHEPL27	71.8	4	2	1	1	1	3	0.5/0.25/0.25	1	—
MHEPL25	76.5	8	2	2	2	3	4	0.625/0.125/0.125/0.125	2	0.75/0.25
MHEPL13	77.5	4	2	1	1	3	4	0.25/0.25/0.25/0.25	2	0.75/0.25
MHEPL30	77.7	9	3	1	3	4	6	0.333/0.222/0.111/0.111/0.111/0.111	2	0.56/0.44
MHEBK11	78.4	10	5	1	2	4	6	0.5/0.1/0.1/0.1/0.1/0.1	3	0.5/0.3/0.2
MHEPL12	79	12	3	1	3	5	7	0.25/0.167/0.167/0.167/0.083/0.083/0.083	3	0.42/0.33/0.25
MHEPL20	88	13[a]	2	1	1	7	7	0.231/0.231/0.154/0.153/0.077/0.077/0.077	4	0.462/0.23/0.154/0.154

[a] Original size of the litter is 14, but an embryo was not able to be genotyped.

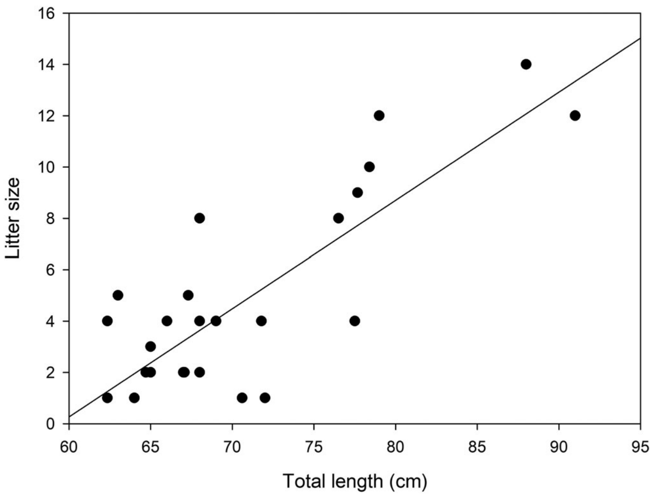

FIGURE 2 Relationship between the total length (L_T) of *Mustelus henlei* females and litter size (LZ). $LZ = 0.422\ (L_T) - 25.05$; $R^2 = 0.676$, $F_0 = 56.47$, $F_{1,27} = 4.210$, $N = 29$, $P < 0.0001$

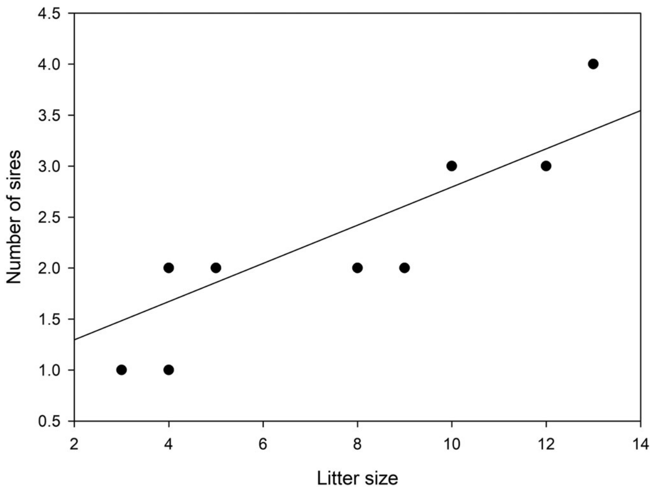

FIGURE 3 Relationship between *Mustelus henlei* litter size (LZ) and the number of sires (SN) determined with GERUD. $SN = 0.187\ (LZ) + 0.921$; $R^2 = 0.68$, $F_0 = 27.66$, $F_{1,13} = 4.667$, $N = 15$, $P = 0.0002$

TABLE 4 Generalized linear model (GLZ) parameters (coefficients and standard errors, S.E.) for the count variable (1, 2, 3 or 4 sires)

Effect	Estimates	S.E.	*z*	*P*-value
Intercept 1	20.67210	9.68	4.56	0.032
Intercept 2	26.26919	11.33	5.37	0.020
Intercept 3	28.66846	11.99	5.71	0.016
Slope	−0.33264	0.15	5.16	0.023

Note: The Wald statistic (*z*) and respective *P*-value are shown for each scenario.

indicated an MP value of 86.7%, which is similar to the value reported for Las Barrancas. Given this regional variation, the authors address various possible explanations for these differences.

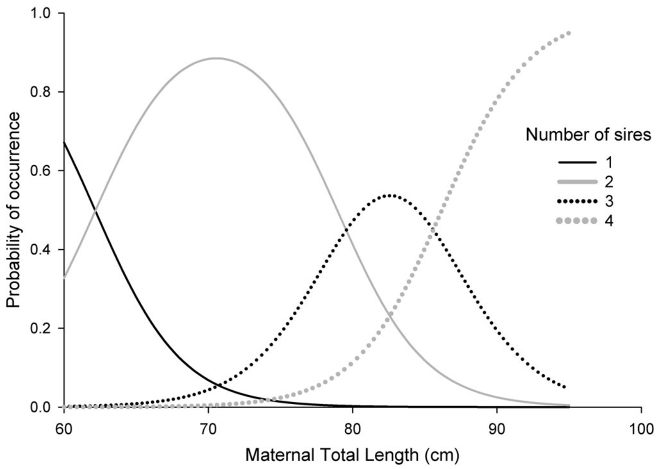

FIGURE 4 Variation in the occurrence probability of multiple paternity (MP) as a function of female size in *Mustelus henlei* from the Gulf of California

The insemination time window (the period between parturition and the next ovulation) and the operational sex ratio can act as predictors of the intensity of MP in a population. If the time window is small and there is strong sexual segregation, the MP frequency is likely to be low (Boomer *et al*., 2013; Daly-Engel *et al*., 2010; Veríssimo *et al*., 2011). The female *M. henlei* population of the western coast of the Baja California Peninsula has an annual reproductive cycle. In addition, the ovarian cycle and gestation are concurrent. After females give birth, they are ready to be newly inseminated (*i.e*., ovulation is present), which is reflected in a short insemination window (Silva-Santos, 2012; Soto-López *et al*., 2018). Nonetheless, because of the high catch volumes of this species in Baja California Sur (Compagno, 1984), it has been hypothesized that the region is a reproductive aggregation zone that allows for multiple matings, and the sex ratio is not significantly different between sexes (1.05 females:1.00 males; Soto-López *et al*., 2018), which could explain the high frequency of MP (93%) that has been reported (Byrne & Avise, 2012).

It has been proposed that the sex ratio changes over time and space along the California coast, with females predominating at times (4.5:1) and males at others (4:1; Ebert, 2003). In addition, it is presumed that in Santa Catalina Island there may be a reduction in the number of males during the breeding season (Chabot & Haggin, 2014), which explains the low frequency of MP (22%). In the northern Gulf of California, the reproductive cycle and ovulation period of *M. henlei* are similar to those that have been observed on the western coast of the Baja California Peninsula (Pérez-Jiménez & Sosa-Nishizaki, 2008), but the sex ratio is significantly female-biased (2.1 females:1 males; Pérez-Jiménez, 2006). Thus, differences in these reproductive features may modulate the frequencies of MP among the three *M. henlei* populations.

Female size has been related to MP, and this relationship may depend on whether fecundity is related to maternal size (Boomer *et al*., 2013; Byrne & Avise, 2012; Chapman *et al*., 2004; Farrell *et al.*, 2014; Nosal *et al.*, 2013; Portnoy *et al.*, 2007; Veríssimo *et al.*, 2011). *M. henlei* populations show clear regional differences in female size at maturity, with individuals from the western coast of Baja California tending to be larger (L_{50}: 68 cm, Silva-Santos, 2012; mean size: 87.5 ± 7.5, Byrne & Avise, 2012) than those from the northern Gulf of California ($L_{50} = 58$ cm, Silva-Santos, 2012; mean size: 71.8 ± 7.3, this study). Moreover, female size at maturity in California tends to be the smallest (51–63 cm) in the region (Ebert, 2003). This pattern coincides with the MP frequencies obtained for each population.

Large females with large litters have a greater probability of their litters showing MP than those of small females, although both large and small females may engage in multiple matings. Some studies have attempted to assess the direct benefits that may be potentially enjoyed by females based on increased fecundity derived from polyandry (Lyons *et al.*, 2021; Pratt & Carrier, 2001); nonetheless, no significant differences have been found between the litter sizes of polyandrous and monandrous females of various shark species (Boomer *et al.*, 2013; Farrell *et al.*, 2014; Nosal *et al.*, 2013; Portnoy *et al.*, 2007; Veríssimo *et al.*, 2011). In *Sphyrna tiburo* from the western coast of Florida, large females tended to be polyandrous, although the limited number of polyandrous females in that study impeded any further conclusions on the matter (Chapman *et al.*, 2004). Thus, the observations of this study of large females with litters showing MP are limited, and it cannot be ruled out that small females likely engage in multiple matings, although there is less opportunity to detect MP in their litters given their small sizes (Lyons *et al.*, 2021).

Lyons *et al.* (2021) noted that sperm competition and a lower-than-expected number of sires (based on litter size) can affect male reproductive success and promote sireship skew, whereas female cryptic choice *via* sperm storage can increase or decrease the skew depending on the conditions. *M. henlei* populations from the northeastern Pacific presented sireship skew. In Santa Catalina Island, three of the four litters with MP (75%; Chabot & Haggin, 2014) showed a marked sireship skew (Skew = 0.125 ± 0.138; Lyons *et al.*, 2021), with an average of 1.22 sires per litter. Similarly, in the northern Gulf of California, of the 13 litters with MP, 9 presented a clear sireship skew (70%; Skew = 0.086 ± 0.083; this study), with an average of 2.3 sires per litter. Both skew values fall within the range that has been reported when considering almost all elasmobranch species (0.083–0.380; Lyons *et al.*, 2021). The sireship skew results agree with the sex ratios mentioned above for these populations. On the contrary, no records of a sireship skew exist for the southernmost population (Las Barrancas).

In Mexico, high catch volumes have been reported for *M. henlei* in the Gulf of California (Saldaña-Ruiz *et al.*, 2017), suggesting that this species is resistant to high fishing pressure, which may be associated with its reproductive strategies and mating system. Nonetheless, future studies should focus on assessing the effects of the MP frequency on density or *Ne*.

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Conceptualization: N.C.S.S.; *Data curation*: J.J.R.H.; *Formal analysis*: J.J.R.H.; *Funding acquisition*: N.C.S.S.; *Investigation*: J.J.R.H., N.C.S.S. and J.C.P.J.; *Methodology*: J.J.R.H., N.C.S.S. and J.C.P.J.; *Project administration*: N.C.S.S.; *Resources*: N.C.S.S.; *Supervision*: N.C.S.S. and J.C.P.J.; *Writing – original draft*: J.J.R.H. and N.C.S.S.; *Writing – review and editing*: J.J.R.H., J.C.P.J. and N.C.S.S.

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