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Differences in prevalence of multiple paternity in the spiny spider crab *Maja brachydactyla* in two localities that differ in female fecundity, fishing intensity, and management measures

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Many marine species are under high fishing pressure, which can cause alterations in their mating systems and the structure of their populations. The spiny spider crab *Maja brachydactyla* is a commercial decapod on the east Atlantic coast. In Galicia, the most exploited region in Spain, the landing of ovigerous females is prohibited, favouring exploitation focused on males. The removal of males, especially the largest ones, may lead to sperm limitation and a reduction in the average size over generations. In these cases, polyandry can work as a mechanism to protect females against sperm limitation and to increase genetic diversity and effective population size. This study analyses the multiple paternity in *M. brachydactyla* in two localities that differ in female fecundity, fishing intensity, and management measures. Our results showed multiple paternity in this species for the first time, with a moderate bias between sires. However, the frequency of multiple paternity was almost seven times higher in the intensely exploited Golfo Ártabro (NW Spain; 69%) than in Carna (W Ireland; 10%), where exploitation levels and female fecundity are lower.

Keywords: Brachyura, *Maja brachydactyla*, microsatellite markers, multiple paternity, size- and sex-selective fishing, sperm limitation, spider crab

Introduction

For an adequate management of commercial species, a detailed understanding of their mating systems and biological traits, such as size and age distributions and the intensity of sexual selection, is essential (Emlen and Oring, 1977). In marine species, the selective mortality caused by fishing produces disturbances in these mating systems (Hankin *et al.*, 1997; Gosselin *et al.*, 2005; Sato *et al.*, 2010; Robertson and Butler, 2013; Rains *et al.*, 2016; Pardo *et al.*, 2017). When management measures are aimed at protecting particular sizes or a specific sex, these disturbances are accentuated and an alteration of the structure of the population and a reduction of reproductive potential may occur. This is the case of several crustacean fisheries regulated by minimum landing sizes and prohibitions on the landing of ovigerous females. In these fisheries, the largest males, with a high commercial value, are the main target (Orensanz *et al.*, 1998), resulting in changes in the sex ratio and in the relative male/female size (Kennelly, 1992; Pillans *et al.*, 2005; MacDiarmid and Sainte-Marie, 2006; Fenberg and Roy, 2008; Pardo *et al.*, 2015; Alborés *et al.*, 2019). In female-biased populations, males have more mating opportunities. In such situations, sperm reserves may not recover between copulations or mating seasons (Kendall and Wolcott, 1999; Sato *et al.*, 2005; Sainte-Marie, 2007; Pardo *et al.*, 2015) or males may invest small amounts of sperm across many mates (Rondeau and Sainte-Marie, 2001; Pardo *et al.*, 2015). In addition, the removal of the largest males will force many females to copulate with

smaller males that may be unable to fill their seminal receptacles and whose sperm plugs may be too small to be effective in preventing further inseminations. All of these situations may cause sperm limitation, promote promiscuity of females, and the consequent multiple paternity of their clutches (Kendall *et al.*, 2002; Gosselin *et al.*, 2003, 2005; Hines *et al.*, 2003; Hill *et al.*, 2017). It has been suggested that overexploited sex- and size-selectively fished populations use multiple paternity as a compensation mechanism to maintain genetic diversity (Morán and García-Vázquez, 1998; Jennions and Petrie, 2000) and effective population size (Sugg and Chesser, 1994; Martínez *et al.*, 2000; Pearse and Anderson, 2009), as well as to reduce inbreeding (Stockley *et al.*, 1993; Yasui, 1998). Moreover, it has been reported that in populations where there is selective extraction of males, the remaining ones invest less time in mate guarding and they may be smaller than the female they protect (Wilber, 1989; Rondeau and Sainte-Marie, 2001; Pardo *et al.*, 2016), increasing the probability of polyandry and the risk of injury or death of females (Sainte-Marie and Hazel, 1992; Sainte-Marie *et al.*, 2008).

Maja brachydactyla Balss, 1922, is a brachyuran crab of the family Majidae (De Grave *et al.*, 2009; Ng and Richer de Forges, 2015) that occurs in the northeastern Atlantic, from the south of the North Sea to Senegal, including Madeira, the Azores, the Canary Islands, and Cape Verde (Monod, 1966; Kergariou, 1984; Neumann, 1998; Udekem d'Acoz, 1999; Sotelo *et al.*, 2008). Females of eubrachyuran crabs have a pair of seminal receptacles that allow them to store sperm from different copulations and use it for fertilization of successive broods without the need for new matings (Hartnoll, 1968; Diesel, 1991; González-Gurriarán *et al.*, 1998; McLay and López Greco, 2011). The number of broods per annual cycle and the breeding season of *M. brachydactyla* varies with latitude due to differences in water temperature (Kergariou, 1984). Females lay up to three broods annually in Galicia (NW Spain) (González-Gurriarán *et al.*, 1998), up to two in France (Kergariou, 1984) and one in Ireland (Rodhouse, 1984; Fahy, 2001).

Maja brachydactyla is a species of great commercial value fished in several countries, such as Spain, United Kingdom, Ireland, France, Portugal, and Morocco (Kergariou, 1984; Le Foll, 1993; González-Gurriarán *et al.*, 1998). Fishing of this species is conducted using multiple gears, although tangle and gill nets are most common in the south of the distribution area and traps in the north (Kelly *et al.*, 2003). The present study is focused on two populations of spiny spider crabs: one in the Golfo Ártabro (43°21′49″ N 8°21′54″ W, Galicia, Spain) and the other in Carna (53°19′13″ N 9°50′36″ W, Co. Galway, Ireland). The *M. brachydactyla* fisheries in these two regions differ in their characteristics, such as landings (Table 1) and management measures. Galicia is the principal fishing region of this species in Spain, with around 330 t landed annually (Xunta de Galicia, 2010–2019). In the Ría de Arousa (Galicia), it has been suggested that more than 90% of newly mature specimens are caught each year (Freire *et al.*, 2002). In Galicia, there is a fishing closure from June to November, coinciding with the period of terminal moult to adulthood and reproductive migration (González-Gurriarán *et al.*, 1993, 1998; Bernárdez *et al.* 2005; Corgos *et al.*, 2006), and the landing of ovigerous females is not permitted during the whole annual cycle. In addition to this, there is a minimum legal landing size of 120 mm of carapace length (CL) for both sexes (DOG N°133, 2019). In contrast, in Ireland, the only regions with an established spider crab fishery to date are Brandon Bay, Tralee Bay, and some regions of the southeast coast (Tully *et al.*, 2006; Fahy and Carroll, 2009; Tully, 2017). Some targeted fishing for large spider crabs occurs periodically in other regions, but populations are not heavily exploited and the species has expanded its range in Ireland in recent decades. In Carna, the spiny spider crab are only caught in small amounts as by-catch in fisheries targeting *Homarus gammarus* and *Cancer pagurus*. On the Irish coast, the only restriction on spider crab fishing is a minimum landing size of 130 mm of CL for males and 125 mm for females (Kelly *et al.*, 2003; Fahy and Carroll, 2009). 2003

Table 1. Landings of *Maja brachydactyla* (in tonnes) for Galicia and County Galway from 2004 to 2016.

Year	Landings in Galicia	Landings in Co. Galway
2004	214.90	16.27
2005	352.52	11.60
2006	382.31	13.23
2007	244.33	9.86
2008	290.43	63.80
2009	342.60	70.64
2010	259.59	51.15
2011	276.41	13.00
2012	334.01	2.22
2013	213.88	0.62
2014	241.00	46.09
2015	291.96	2.03
2016	272.40	6.95

Source: Marine Institute Ireland; Xunta de Galicia (2010–2019).

The aim of this work is to analyse the paternity of the broods of the spider crab *M. brachydactyla* in two populations with different female fecundity, fishing intensity, and management strategies.

Material and methods

Study specimens

For paternity analysis, 23 ovigerous females ranging in CL from 122 to 180 mm were captured. Thirteen of these were caught by divers in the Golfo Ártabro, Spain, with a permit emitted by the Consellería do Medio Rural e do Mar of the Xunta de Galicia (regional government, resolution of 18 February 2014) and 10 were caught by local fishermen in Carna, Ireland. The 13 Spanish ovigerous females were captured in January to February, when they were carrying the first brood of the annual cycle. The ten Irish ovigerous females were captured in June, when they were carrying their only brood of the year.

In addition to this, another 118 adult specimens were collected for microsatellite characterization. Sixty of these spider crabs were bought from local fishermen in Galicia, 44 females from the Ría de Arousa (42°35′00″ N 8°53′02″ W) and 4 females and 12 males from the Golfo Ártabro. The remaining 58 spider crabs, 29 females and 29 males, were imported from Rosslare (52°15′26″ N 6°20′28″ W, Co. Wexford, Ireland) by Airmar SL (Spain).

Sample collection

The females from Arousa and Rosslare were dissected and gonads were sampled. All these specimens were cold-anaesthetized at −20°C for 10 min before being sacrificed. From the remaining adult individuals, including the ovigerous females, autotomy of a pereiopod was induced by performing a puncture in one of its

joints in order to collect a muscle sample. Ovigerous females were kept in captivity until their first brood reached the developmental stage B described by González-Gurriarán *et al.* (1993), because eggs from that brood stage are the most suitable for DNA extraction (Rodríguez-Pena *et al.*, 2017). For brood sampling, the eight egg masses adhered to the pleopods were numbered from one to eight, and eggs were collected in a zigzag pattern from pleopods 1, 4, 5, and 8 (Figure 1). This sampling method allowed us to assess if there was an orderly distribution of the eggs fertilized by different males in the abdominal cavity of the female. If a pattern existed, sampling eggs from different areas of the brood would be more appropriate for detection of multiple paternity than completely random sampling.

The egg samples of the females from Golfo Ártabro were preserved in absolute ethanol until DNA extraction. On the other hand, the samples collected in Carna (Ireland) were frozen and lyophilized to facilitate their transport.

DNA extraction

DNA was extracted from the muscle or gonad of the 141 adult individuals, including the 23 ovigerous females. In addition to this, pooled and individual eggs were processed. For six of the females from the Golfo Ártabro, DNA was extracted from a pool of five eggs from each pleopod sample, resulting in a total of four pools per female (20 eggs per female). Moreover, the DNA from 8 to 12 individual eggs (2–3 eggs from each pleopod) was extracted for all the ovigerous females.

DNA extraction was conducted using the NZY Tissue gDNA Isolation kit (NZYTech, Portugal), following the protocol indicated by the manufacturer for DNA extraction from animal tissues. After adding the lysis buffer and proteinase K, the samples were homogenized and incubated at 55°C overnight.

Microsatellite development and analysis

Sotelo *et al.* (2007) described specific microsatellites for *M. brachydactyla*. These microsatellite markers were tested but there were problems in amplifying them correctly. In order to develop new specific markers, a microsatellite-enriched genomic library from a gonad sample was constructed at AllGenetics & Biology SL (A Coruña, Spain). The library was prepared using Nextera XT DNA Library Preparation kit (Illumina), following the manufacturer's instructions, and was enriched with the microsatellite motifs AC, AG, ACG, and ATCT. Once enriched, the library was sequenced in the Illumina MiSeq PE300 platform (Macrogen Inc., Seoul, Korea). A total of 4 035 302 reads were obtained and processed using Geneious 11.1.2 (Kearse *et al.*, 2012) and in-house developed scripts. For primer design, sequences containing microsatellite loci were selected. Primer pairs were designed using Primer3 (Untergasser *et al.*, 2012) for PCR amplification of 500 microsatellite loci, with primers hybridizing at the flanking regions of tandem repeats.

After preliminary studies, nine microsatellite loci (GenBank accession numbers: MT211631 to MT211639) were selected for having amplified in 73 individuals from the Galician sampling location and 68 from the Irish location. PCRs were performed in a final reaction volume of 12.5 µl, containing 1 µl of DNA (10 ng/µl), 6.25 µl of the Type-it Microsatellite PCR Kit (Qiagen), 4 µl of PCR-grade water and 1.25 µl of the primer mix. The optimal PCR protocol consisted in an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 57°C for 90 s, 72°C for 30 s; 8 cycles of 95°C for 30 s, 53°C for 90 s, 72°C for 30 s; and a final extension step at 68°C for 30 min. All PCR batches included a negative control to check for potential cross-contamination. Oligonucleotide tails were attached to the 5′ ends of the primers to allow for fluorescent labelling. The oligonucleotide tails used were the universal sequences M13 (5'-GGAAACA GCTATGACCAT-3'') and CAG (5'-CAGTCGGGCGTCATC-3''). The two oligonucleotides were labelled with the HEX dye and the FAM dye, respectively. The software Geneious 11.1.2 was used for the analysis of fluorescent profiles and to identify the allele peaks. For each locus (except AG_Mbr_215) and study area, number of alleles (N_a), observed (H_o), and expected (H_e) heterozygosity, deviations from Hardy–Weinberg equilibrium (HWE) and inbreeding coefficient (FIS) were estimated using GenAlEx 6.5 (Peakall and Smouse, 2006). Null allele frequency was estimated using FreeNA (Chapuis and Estoup, 2007).

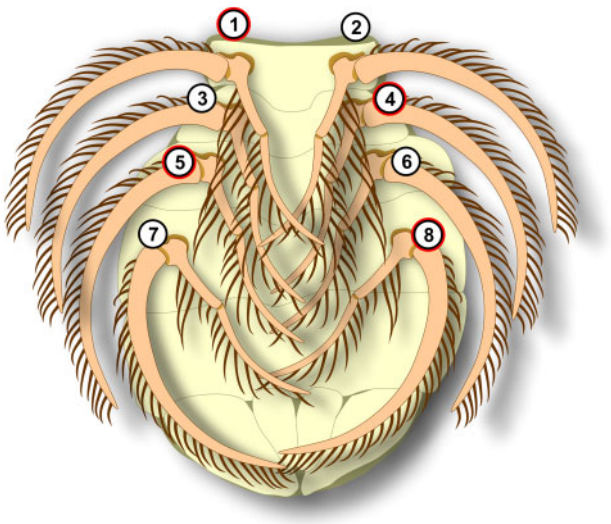

Figure 1. Abdomen of a spider crab female with numbered pleopods for sample collection in zigzag. The egg samples for paternity analysis were collected from pleopods 1, 4, 5, and 8.

Considerations for the paternity analysis

First, the PrDM software (Neff and Pitcher, 2002) was used to determine the probability of detecting multiple paternity in a brood. This programme is based on a Monte Carlo simulation, so the probability of detecting multiple paternity may vary slightly in each run. To minimize this effect, ten iterations were performed for each case and the average was computed. Simulations were performed varying the number of loci, the prevalence of multiple paternity, the percentage of contribution of the different sires, and the number of eggs analysed. In addition to this, the software GERUD 2.0 (Jones, 2005) was used to calculate the exclusion probabilities for different loci combinations. The method described by Veliz *et al.* (2017) for species with high fecundity was used to evaluate the statistical power at population level. In this case, the following parameters were varied: the number of females analysed, the number of eggs analysed per female, the prevalence of multiple paternity, and the contribution of male 1 (the one that fertilizes most of the brood) and male 2 (other males that participate in the fertilization of the brood). This method takes into account the fecundity of the study species. Verísimo *et al.*

Table 2. Characterization of the nine selected microsatellite loci for *Maja brachydactyla*.

Locus name	Accession no.	Primer sequence 5′–3′	Dye	Repeat motif	Allele size (bp)	NW Spain							SW Ireland						
						N	N_a	H_o	H_e	PHWE	FIS	Null	*N*	N_a	H_o	H_e	PHWE	FIS	Null
AG_Mbr_050	MT211631	CTTCCCTTC AGATAGCCCGC	HEX	AC	218–230	64	6	0.516	0.591	0.852	0.128	0.24866	5	0.485	0.516	0.013	0.060	0.116
		GCTTGTTAAC AACCCGCCTG																
AG_Mbr_177	MT211632	CGGAGCGTG ACTACCTATCC	6-FAM	AC	109–119	65	3	0.877	0.505	0.000*	−0.736	0.13068	2	0.838	0.497	0.000*	−0.686	0.000
		GACTGAAGA GGCCGCTTAGG																
AG_Mbr_204	MT211633	TCCCTCCA CTACCCTTCCTT	HEX	ATC	129–183	48	2	0.979	0.500	0.000*	−0.959	0.34750	2	1000	0.500	0.000*	−1	0000.265
		CAAACACCA CGACAACCACC																
AG_Mbr_235	MT211634	ATCGAGCTG CGGGTTAAGAG	HEX	AC	107–121	53	4	0.415	0.553	0.000*	0.249	0.44057	5	0.456	0.610	0.000*	0.252	0.322
		GGTACCACG GGACCACTGTA																
AG_Mbr_317	MT211635	ATGCACAC TTCTTCCTCCGC	HEX	GT	97–125	67	15	0.716	0.857	0.000*	0.164	0.17967	11	0.761	0.818	0.321	0.069	0.070
		CCTGCCGAT ATCAGCTGACT																
AG_Mbr_325	MT211636	ACACTGTTGG ATTCCTGCCT	6-FAM	TG	94–102	43	4	0.209	0.584	0.000*	0.642	0.61736	4	0.139	0.610	0.000*	0.772	0.679
		CTCTGCCTACA CAGGAGGGT																
AG_Mbr_415	MT211637	AACACTCCCAT AACGCCACT	6-FAM	GT	102–116	52	4	0.981	0.519	0.000*	−0.891	0.29254	4	0.889	0.515	0.000*	−0.725	0.230
		CGGCCGAAC AGTGAACAGTT																
AG_Mbr_438	MT211638	GCGTATGCTC CCAACTTTACC	HEX	TTG	259–292	59	11	0.864	0.846	0.216	−0.021	0.23662	12	0.871	0.827	0.015	−0.053	0.131
		TCGCTTGCTC TTTGCCTACT																
AG_Mbr_215	MT211639	GTGTAGCTCA GGCCCTGTAA	HEX	CA	220–244	9	7	–	–	–	–	–	9	6	–	–	–	–	–
		AAAGTCCTC CTTGACGCTGG																

Accession no., GeneBank accession number; *N*, sample size; N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; PHWE, Hardy–Weinberg equilibrium *p*-values; FIS, inbreeding coefficient; Null, null allele frequency. *Significant departure from HWE after the sequential Bonferroni correction ($p < 0.00625$). H_o, H_e, PHWE, FIS, and null allele frequency were not estimated for AG_Mbr_215 due to the small sample size ($N = 9$).

(2011) determined that the fecundity of female spider crabs ranged from 125 081 to 530 309 eggs, with an average of 300 192 ± 92 912 eggs. For simulations used here, the approximate value of 300 000 eggs was used.

Paternity analysis

Three loci (AG_Mbr_050, AG_Mbr_317, AG_Mbr_438) were selected for the paternity analysis. In a first phase, the egg pools of the six females were analysed to determine if multiple paternity existed in *M. brachydactyla*. In a second phase, the individual eggs of the 23 broods were genotyped. The 23 corresponding mothers were also genotyped from muscle samples. The PCR protocol was the same as that described for the amplification of the nine microsatellite loci in the subsection "Microsatellite development and analysis".

Genotypes were established using Geneious 11.1.2. The number of broods with multiple paternity, the number of sires of each brood, and the most likely paternal genotypes were determined using the allele frequencies of each study area and the software GERUD 2.0. Taking into consideration the most probable parents, the egg genotypes were checked to determine how the alleles of the different sires were distributed throughout the brood.

Statistical analysis

Possible differences in the prevalence of multiple paternity between the two study localities and the effect of maternal size were

examined using a multiple logistic regression analysis conducted with the statistical program R 3.6.3 (R Core Team, 2020).

Results

Microsatellite characterization

Preliminary microsatellite analysis showed that the number of alleles varied between 2 and 15 across the nine loci, being quite similar in both regions (Table 2). Moreover, the loci deviating significantly from the HWE were the same in both areas, except for AG_Mbr_317, which was only significant in NW Spain. The FIS values suggest that there is no inbreeding in either population.

Paternity analysis

For the nine loci, the exclusion probability was calculated by indicating that the genotype of one progenitor was known (Table 3). Three loci with a high individual exclusion probability were selected and their combined exclusion probability was compared with the combined exclusion probability of the nine loci. The exclusion probability for the three selected loci (AG_Mbr_317, AG_Mbr_050, and AG_Mbr_438) was >0.9 for the NW Spain and the SW Ireland sites (0.9427 and 0.9166, respectively). Although by including the nine loci the exclusion probability increases slightly, this does not compensate for the large increase in costs that would result from tripling the number of loci analysed. For this reason, only three loci were amplified for the paternity analysis.

In the initial phase of egg pool analysis, the existence of multiple paternity in *M. brachydactyla* was confirmed. More than three non-maternal alleles were detected in four of the six broods examined. For the individual eggs, the results obtained with GERUD 2.0 indicated that at least 10 of the 23 broods analysed had been fertilized by more than one male (Table 4). In the broods with multiple paternity, the minimum number of sires was always two, except in one brood where alleles of three males were detected. The contribution of each male to the fertilization of the broods was calculated over the total number of eggs that could be unequivocally assigned to one father. In the case of broods fertilized by two males, the contribution of the main male ranged from 50 to 87.5% (Figure 2). For the brood with three sires, GERUD 2.0 offered four solutions with the same likelihood, three with equal contribution of the males (33.33% each one) and one with a contribution of 40% for males 1 and 2 and a contribution of 20% for male 3. However, these percentages are an approximation since only a small proportion of the whole brood was analysed. Once the eggs from different pleopods were assigned to each father, it was found that, in at least six of the ten broods, the eggs fertilized by different males were partially or totally mixed in the female abdomen. In the remaining four broods, the possible existence of a pleopod pattern in the distribution of the paternal alleles could not been rejected.

Table 3. Exclusion probability (EP) when the genotype of one progenitor is known.

Locus	EP NW Spain	EP SW Ireland
AG_Mbr_415	0.2121	0.2105
AG_Mbr_204	0.1874	0.1875
AG_Mbr_317	0.7094	0.6519
AG_Mbr_050	0.3500	0.2931
AG_Mbr_177	0.1968	0.1868
AG_Mbr_235	0.2565	0.3327
AG_Mbr_325	0.3237	0.3245
AG_Mbr_438	0.6967	0.6612
AG_Mbr_215	0.4454	0.5067
AG_Mbr_317 AG_Mbr_050 AG_Mbr_438	0.9427	0.9166
All loci	0.9918	0.9903

EPs were calculated for each individual locus, for a combination of three loci (AG_Mbr_317, AG_Mbr_050, AG_Mbr_438) and for the combination of the nine loci, for both study areas.

The calculation of the probability of detecting multiple paternity in a brood made with the PrDM software indicated that this probability was considerably higher when analysing three loci (AG_Mbr_438, AG_Mbr_317, AG_Mbr_050) than when analysing two (AG_Mbr_317, AG_Mbr_050), but the increment was moderate using five loci (AG_Mbr_325, AG_Mbr_438, AG_Mbr_317, AG_Mbr_050, AG_Mbr_215) instead of three. Table 5 shows only cases in which two males were considered, since in similar conditions, as the number of sires increase, the probability of detecting multiple paternity also increases. For equal contributions from two males, three loci are sufficient to obtain a PrDM around 0.9 analysing 8 eggs and higher than 0.95 analysing 12 eggs. Nevertheless, for a skewed contribution, the probability of detecting multiple paternity decreases considerably.

To estimate the statistical power at the population level, the method described by Veliz *et al.* (2017) was used, which takes into account the fecundity of the species and the number of females analysed. For the simulations, it was assumed that 50% of the broods in the population were fertilized by more than one male and that the male contribution was biased (95:5), taking into account data reported in other crustacean species (Veliz *et al.*, 2017). For 10 females, the statistical power was 0.8416 analysing 8 eggs and 0.9266 analysing 12. By increasing the number of females to 20, the statistical power also increased, being 0.9749 for 8 eggs and 0.9946 for 12.

Influence of maternal size and population on the prevalence of multiple paternity

Nine of the ten broods fertilized by more than one male came from the Golfo Ártabro (NW Spain) while only one came from Carna (W Ireland). To determine if this apparent difference between populations was significant and to check if multiple paternity depends on maternal size, a multiple logistic regression model was used. In this model, the paternity of the broods of *M. brachydactyla* was studied as the independent variable with two levels (0: simple paternity, 1: multiple paternity). In addition, the independent variables "population" (with two levels; 0: Carna, 1: Golfo Ártabro) and "maternal size" (continuous) were considered. The model proposed was the following: $\log(p/(1-p)) = A + BX + CZ + \text{Error}$, where p is the probability of multiple paternity in the Golfo Ártabro, X is the maternal size variable, Z is the variable indicating population ($Z = 1$ for the Golfo Ártabro and $Z = 0$ for Carna) and the coefficients of the linear model are A, B, and C. Once the model was adjusted with the available data, the estimates for the parameters were obtained: −9.75 for A, 0.054 for B, and 2.71 for C. The contrasts of significance for each parameter showed that C was significant (p-value = 0.031), that is, the population of the female influences the probability of multiple paternity, but A and B were not significant (p-value = 0.070

Table 4. Paternity results for the analysis of individual eggs from 23 broods.

							Male 1		Male 2		Male 3		
Population	Fem. code	Fem. CL (mm)	N	No. un. eggs	MP	Min. sires	No. eggs	Pl.	No. eggs	Pl.	No. eggs	Pl.	Egg pattern
Golfo Ártabro	Sp1	170.89	10	10	Yes	2	6	1, 4, 5, 8	4	1, 4, 5	–	–	No
Golfo Ártabro	Sp2	129.86	12	12	No	1	12	–	–	–	–	–	–
Golfo Ártabro	Sp3	171.09	11	8	Yes	2	7	1, 4, 5, 8	1	8	–	–	Possible
Golfo Ártabro	Sp4	122.33	10	10	Yes	2	8	1, 4, 5, 8	2	8	–	–	Possible
Golfo Ártabro	Sp5	179.34	12	9	Yes	2	5	1, 4, 8	4	1, 4, 8	–	–	No
Golfo Ártabro	Sp6	124.37	12	12	No	1	12	–	–	–	–	–	–
Golfo Ártabro	Sp7	153.89	6	A: 5; B, C, D: 6	Yes	3	A, B, C, D: 2	A: 1, 8; B: 1; C: 1; D: 1	A, B, C, D: 2	A: 1, 5; B: 4, 5; C: 4; D: 4, 8	A: 1; B, C, D: 2	A: 4; B: 4, 8; C: 5, 8; D: 4, 5	A, B, D: No; C: Possible
Golfo Ártabro	Sp8	153.09	6	6	No	1	6	–	–	–	–	–	–
Golfo Ártabro	Sp9	146.11	8	8	No	1	8	–	–	–	–	–	–
Golfo Ártabro	Sp10	131.66	8	8	Yes	2	6	1, 4, 5, 8	2	1, 5	–	–	No
Golfo Ártabro	Sp11	134.24	8	8	Yes	2	7	1, 4, 5, 8	1	5	–	–	No
Golfo Ártabro	Sp12	152.95	6	6	Yes	2	4	1, 5, 8	2	4, 8	–	–	No
Golfo Ártabro	Sp13	179.76	8	8	Yes	2	4	1, 4, 5	4	4, 5, 8	–	–	No
Carna	Ir1	132.60	7	7	No	1	7	–	–	–	–	–	–
Carna	Ir2	147.80	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir3	145.66	8	6	Yes	2	4	4, 5, 8	2	1, 4	–	–	Possible
Carna	Ir4	153.10	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir5	149.36	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir6	121.92	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir7	145.04	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir8	127.05	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir9	133.37	8	8	No	1	8	–	–	–	–	–	–
Carna	Ir10	125.87	7	7	No	1	7	–	–	–	–	–	–

Fem. code, female code; Fem. CL, female carapace length in mm; *N*, number of analysed eggs per female; No. un. eggs, number of unambiguous eggs (eggs that could be unequivocally assigned to one father); MP, detection of multiple paternity; Min. sires, minimum number of sires; No. eggs, number of eggs assigned to a particular male; Pl., pleopods where eggs assigned to a particular male were detected; Egg pattern, organized distribution of the eggs fertilized by different males throughout the brood. This table shows the solutions with the highest likelihood provided by GERUD 2.0. For female Sp7, there are four solutions with the same likelihood (A, B, C, and D).

and 0.146, respectively), which indicates that the maternal size does not influence the probability of multiple paternity of the broods.

For this reason, a simpler model excluding maternal size variable was formulated, using a Fisher's exact test to assess if prevalence of multiple paternity was independent of site. In this model, the dependent variable continued to be the paternity of the broods, while the only independent variable was the population of origin of the female. This test confirmed that the probability of multiple paternity is significantly higher in the Golfo Ártabro than in Carna (Table 6), multiple paternity being almost seven times more frequent in the Spanish population (0.69) than in the Irish one (0.1).

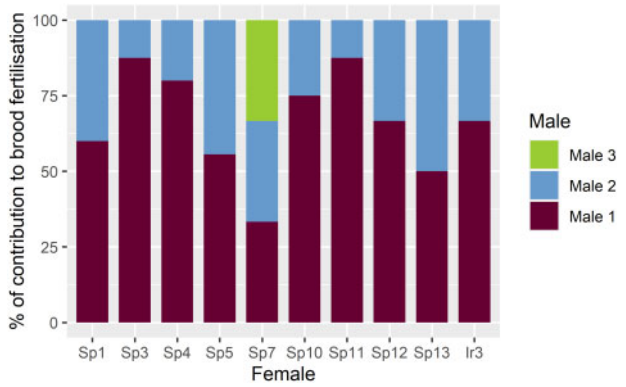

Figure 2. Percentage of contribution of each sire in the broods with multiple paternity. For the female Sp7, GERUD 2.0 offers another solution with the same likelihood, with contributions to brood fertilization of 40:40:20 (see Table 4).

Moreover, a simple logistic regression model was used to check if the size of the females was correlated with their population of origin. The results showed that the correlation between these variables was not significant (p-value = 0.127).

Discussion

In decapods, broods fertilized by more than one male are the norm (Dennenmoser and Thiel, 2015; McLay and Dennenmoser, 2020). Table 7 summarizes the results of several paternity studies conducted in recent years. In general, the prevalence of multiple paternity is higher in species without specialized sperm storage structures, such as carideans and anomurans (in seven of the nine species, the prevalence was higher than 73%). These two taxa, with relatively few exceptions (e.g. Rasch and Bauer, 2016), do not have sperm storage structures, although they may have receptor areas for temporary holding of the spermatophores, which would allow multiple paternity. Within astacids, the species with annulus ventralis also showed high levels of prevalence (87% on average), while in species with thelycum, the percentage of broods fertilized by more than one male was considerably lower (28% on average). Regarding the analysed brachyurans, which had ventral type seminal receptacles, multiple paternity was not reported in 22% of the species and, in those in which it was detected, its prevalence was 37% on average. Nevertheless, the increase in the complexity of the sperm storage organs is usually associated with an increase in fecundity, since sperm storage allows for a greater number of eggs to be fertilized. This implies that, in species with more developed sperm storage organs, the proportion of offspring analysed in paternity studies is usually lower, which reduces the probability of detecting multiple paternity.

Paternity studies in species with dorsal seminal receptacles are scarce. Hill *et al.* (2017), analysed the paternity of one *Callinectes sapidus* clutch, a crab with dorsal seminal receptacles. They

Table 5. Probability of detecting multiple mating (PrDM) in a brood.

No. eggs	No. loci	Loci names	Male contribution	PrDM (NW Spain)	PrDM (SW Ireland)
8	2	AG_Mbr_317, 050	50:50	0.7936	0.7173
	2	AG_Mbr_317, 050	10:90	0.3928	0.3448
	3	AG_Mbr_438, 317, 050	50:50	0.9339	0.8998
	3	AG_Mbr_438, 317, 050	10:90	0.4953	0.4665
	5	AG_Mbr_325, 438, 317, 050, 215	50:50	0.9633	0.9518
	5	AG_Mbr_325, 438, 317, 050, 215	10:90	0.5253	0.5115
12	2	AG_Mbr_317, 050	50:50	0.8678	0.8008
	2	AG_Mbr_317, 050	10:90	0.5232	0.4659
	3	AG_Mbr_438, 317, 050	50:50	0.9730	0.9509
	3	AG_Mbr_438, 317, 050	10:90	0.6451	0.6129
	5	AG_Mbr_325, 438, 317, 050, 215	50:50	0.9884	0.9827
	5	AG_Mbr_325, 438, 317, 050, 215	10:90	0.6740	0.6612

Simulations were performed for 8 and 12 eggs, using two, three, and five loci, and for equal (50:50) and skewed (10:90) contribution of two males. PrDM was calculated for both study populations.

Table 6. Number and percentages of broods with single and multiple paternity in both study populations.

	Multiple paternity		Single paternity		Fisher's exact test p-value
	N	%	***N***	%	0.0065*
Carna (Ireland)	1	10	9	90	
Golfo Ártabro (Spain)	9	69.2	4	30.8	
Total	10	43.5	13	56.5	

N, number of broods. *Significant $p < 0.05$.

Table 7. Paternity studies in decapod crustaceans with different strategies of sperm storage.

Infraorder	Species	Reference	Fecundity (no. eggs per brood)	Seminal receptacle	No. females	No. loci	Multiple paternity (prevalence)	Minimun no. of sires	Contribution of dominant sire* (%)
Caridea	*Acanthephyra pelagica*	Paegelow (2014)	560–3700	No	19	4	100	2–4	ND
	Alpheus angulosus	Mathews (2007)	>200	No	53	5	31	1–2	ND
	Caridina ensifera	Yue and Chang (2010)	16	No	20	4	100	2–11	54
	Palaemonetes pugio	Baragona *et al.* (2000)	95–265	No	10	2	80	ND	ND
	Rhynchocinetes typus	Bailie *et al.* (2014)	1700	No	15	3	73.3	1–4	65
Anomura	*Munida rugosa*	Bailie *et al.* (2011)	32 000	No	25	3	84	1–3	68
	Munida sarsi	Bailie *et al.* (2011)	2 000	No	5	2	100	2–4	50
	Paralithodes camtschaticus	Vulstek *et al.* (2013)	7 900–450 000	No	24	3	0	1	100
	Petrolisthes cinctipes	Toonen (2004)	1 300	No	10	2	80	1–3	ND
Astacidea	*Orconectes obscurus*	Kahrl *et al.* (2014)	250	Yes (annulus ventralis)	3	4	100	2–3	72
	Orconectes placidus	Walker *et al.* (2002)	250	Yes (annulus ventralis)	15	3	60	1–4	83
	Orconectes sanbornii	Kahrl *et al.* (2014)	250	Yes (annulus ventralis)	5	4	100	2–3	68
	Procambarus clarkii	Yue *et al.* (2010)	276	Yes (annulus ventralis)	30	4	96.7	1–4	74
	Homarus americanus	Gosselin *et al.* (2005)	4 800–38 300	Yes (open thelycum)	108	4	13	1–3	70
	Homarus gammarus	Sørdalen (2012)	2 000–40 000	Yes (open thelycum)	73	4	17.8	1–2	ND
	Nephrops norvegicus	Streiff *et al.* (2004)	2 600	Yes (open thelycum)	11	2	54.5	1–3	51
Brachyura	*Cancer pagurus*	McKeown and Shaw (2008)	26 600–2 836 000	Yes (ventral type)	18	3	0	1	100
	Chionoecetes opilio	Sainte-Marie *et al.* (2008)	10 000	Yes (ventral type)	20	1	12.5	1–2	79
	Chionoecetes opilio	Urbani *et al.* (1998)	10 000	Yes (ventral type)	7	2	0	1	100
	Chionoecetes opilio	Roy (2003)	10 000	Yes (ventral type)	79	ND	3.8	ND	ND
	Dissodactylus primitivus	Jossart *et al.* (2014)	200	Yes (ventral type)	18	4	61.1	1–6	86
	Maja brachydactyla	This study	300 000	Yes (ventral type)	23	3	43.5	1–3	66b
	Metacarcinus edwardsii	Pardo *et al.* (2016)	>1 000 000	Yes (ventral type)	31	6	0	1	100
	Metacarcinus edwardsii	Rojas-Hernández *et al.* (2014)	>1 000 000	Yes (ventral type)	5	8	0	1	100
	Metacarcinus magister	Jensen and Bentzen (2012)	1 000 000–2 000 000	Yes (ventral type)	10	3	40	1–3	98[a]
	Scopimera globosa	Koga *et al.* (1993)	4 300	Yes (ventral type)	ND	ND	ND	ND	94
	Uca mjoebergi	Reaney *et al.* (2012)	>3 200	Yes (ventral type)	38	3	56	1–2	98
	Ucides cordatus	Baggio *et al.* (2011)	108 000	Yes (ventral type)	10	6	40	1–2	ND

*For species with multiple paternity, the broods with single paternity were removed from the calculation of contribution of the dominant sire, while for species with single paternity, the 100% of contribution of the male was indicated.

[a]A brood in which the contribution of the dominant sire was 64% was removed from the calculation.

[b]The brood fertilized by three males was removed from the calculation.

ND, no data.

detected multiple paternity in this clutch, but genotyping of the ejaculates stored by many more females suggested a low incidence of polyandry (5.3% of 75 females). This implies that the incidence of multiple paternity may be very low in nature. Due to the lack of more accurate data, *C. sapidus* was excluded from Table 7.

In the cases of species with multiple paternity, male bias in brood fertilization is very evident in taxa with internal organs of sperm storage, while it is not so obvious in groups without these types of organs.

It should be highlighted that in *M. brachydactyla* broods, the contribution of the dominant sire was relatively low (66% even excluding the brood with three sires) compared to that observed in other brachyurans (>79%), which could be explained by the arrangement of the sperm masses and the potential sperm mixing in the seminal receptacles. González-Gurriarán *et al.* (1998) discovered that in *M. brachydactyla*, when there are multiple sperm masses, they are arranged parallel to the major axis of the receptacles, and when these are very full, the masses are not organized in an orderly manner. Moreover, our observations made during dissections of females mated in captivity with various males suggested that, when copulations occur over short periods of time (from hours to weeks between copulations), it is not possible to distinguish defined sperm masses in the seminal receptacles of the female. Furthermore, in *M. brachydactyla*, copulation occurs between hard-shelled individuals, there is no post-copulatory guarding, and the existence of sperm plugs has not been reported (González-Gurriarán *et al.*, 1998). It is a species with a terminal moult in both sexes (Kergariou, 1984; Le Foll, 1993; Sampedro *et al.*, 1999), so sperm stored in the seminal receptacles is not susceptible to be lost during moulting. In addition to this, Corgos *et al.* (2006) suggested that, in *M. brachydactyla*, small males migrate to mating areas earlier than large ones, probably to maximize their mating opportunities by avoiding agonistic interactions with larger males (Sainte-Marie *et al.*, 1997, 1999; Rondeau and Sainte-Marie, 2001; Correa *et al.*, 2003). These small males provide females with small amounts of sperm that may be insufficient to fertilize all their eggs. This set of reproductive traits of *M. brachydactyla* favours polyandry and multiple paternity of broods.

In some of the female spider crabs where both pooled and individual eggs were analysed, the two methods yielded different results. In one of these females, two sires were detected in the pools but only one in the individual eggs. This may be due to the larger sample size (20 vs. 12 eggs) or due to randomness if the contribution of both males to the fertilization of the brood is skewed. In two other females, multiple paternity was not detected using the pool method but was found analysing individual eggs. This could be because one male with a low contribution to brood fertilization shows a low peak in the electropherogram, which may go unnoticed during genotyping of the eggs of the pools.

The zigzag sampling method did not serve to increase the probability of detecting multiple paternity. Although in four of the ten multiply sired broods a segregated pleopod pattern of the parental alleles could not be excluded, in six of them there was an evident mixing. This lack of organization of the paternal alleles had already been reported in some decapod species with less complex sperm storage organs, such as in *Orconectes placidus* (Walker *et al.*, 2002) or *Homarus americanus* (Gosselin *et al.*, 2005). This could be due to the mixing of different sperms in the seminal receptacles, the mixing of the eggs fertilized by different males in the abdomen of the female prior to their adhesion to the pleopods or, more likely, to a combination of both.

Our observations of specimens in captivity confirmed that, during oviposition, the eggs are free in the female abdomen where they mix with each other before adhering to the pleopods. On the other hand, in species where the oviduct is connected dorsally to the sperm storage organs, as in *C. sapidus* (Johnson, 1980), oocytes must pass through the receptacles to reach the vagina. In this case, it would be expected that the sperm of the first male, which copulated have priority in fertilizing the oocytes, although the entry of the oocytes into the receptacle could also favour multiple paternity (McLay and Becker, 2015). In contrast, in brachyurans such as *Chionoecetes opilio* (Sainte-Marie *et al.*, 2000) and *Inachus phalangium* (Diesel, 1990), which have ventral type seminal receptacles, the oviduct is connected to the sperm storage organs at a point near the vagina, and the masses are stored dorsoventrally when the receptacles are not very full. These characteristics favour the sperm from the last male to copulate before oviposition, which is localized closest to the oviduct and vagina, being used first to fertilize the brood. Furthermore, larger males might deposit more seminal fluid than smaller ones. Since seminal fluid is probably implicated in the formation of the sperm plug and in the stratification of precedent sperm packages in several brachyuran species, larger males may be more successful in fertilization in a sperm competition context (Pardo *et al.*, 2018).

Although *M. brachydactyla* also has ventral type seminal receptacles, the spatial arrangement of the sperm masses described in this species by González-Gurriarán *et al.* (1998) suggests that there is a mixing of the sperm of several males, which would explain the absence of spatial pattern of the paternal alleles within the brood. Koga *et al.* (1993) proposed that, if there is sperm mixing in the seminal receptacles, the male contribution should be equal (50:50), but this would only be true if two males supplied the female with equal quantities of ejaculate with similar spermatozoa concentration and viability. However, large males tend to provide a greater amount of sperm to females (Sato and Goshima, 2007; Butler *et al.*, 2011; Pardo *et al.*, 2018), which could result in broods dominated by eggs fertilized by the larger male.

In the case of Galician spider crabs, first broods of the annual cycle were analysed. This ensured that females carried high amounts of sperm in their receptacles, maximizing the probability of taking samples from a fully fertilized brood. During the breeding season, female spider crabs incubate their broods in shallow waters, while males remain in the mating areas at a depth of 30–100 m (Le Foll, 1993; Hines *et al.*, 1995; Freire and González-Gurriarán, 1998; González-Gurriarán *et al.*, 2002). Although direct observations by fishermen in the Golfo Ártabro indicate that sometimes mating occurs in shallow areas after the return migration, the mating of the spider crab on the Galician coast occurs mainly in deep waters in winter (Corgos, 2004).

In general, both within a brood and at the population level, the simulations performed indicated that the probability of detecting multiple paternity was very high for the number of loci, eggs and females analysed in this study. However, the number of analysed females may be insufficient to establish a relationship between the existence of multipaternity and the maternal size. It would be expected that larger females would be able to store the sperm from more copulations in their receptacles. In addition, as they present a higher fecundity (Verísimo *et al.* 2011), larger females would need to use a greater quantity of the stored sperm,

thereby increasing the probability of finding multiple paternity in broods of multiple-mated females. For this reason, we believe that the absence of a relationship between multiple paternity and maternal size may be an artefact due to insufficient sample size. However, another possibility may be that males adjust the amount of ejaculate delivered to the female according to her size, as has been reported in other crustaceans (MacDiarmid and Butler, 1999; Sato *et al.*, 2006). This would imply that large females may not necessarily store multiple sperm masses more frequently than small ones. Therefore, the analysis of a larger number of females, covering the entire size range of both populations, is necessary to clarify this aspect.

The differences in the prevalence of multiple paternity shown in Table 7 for the same species could be due to the analysis of an insufficient number of broods. Urbani *et al.* (1998) analysed seven broods of *C. opilio* and found single paternity in all of them. In contrast, Roy (2003) and Sainte-Marie *et al.* (2008) analysed the offspring of 79 and 20 females of this species, detecting the existence of multiple paternity in 3.8 and 12.5% of them, respectively. Nevertheless, in these cases the differences in the prevalence of multiple paternity may be also due to the different types of females examined [primiparous in Urbani *et al.* (1998) and Roy (2003); multiparous in Sainte-Marie *et al.* (2008)] and interannual variability in primiparous females [Roy (2003) considered females in 2 years with very different population sex ratios].

In the case of *Metacarcinus edwarsii*, the two paternity studies performed (Rojas-Hernández *et al.*, 2014; Pardo *et al.*, 2016) agreed that all the broods analysed (5 and 31) were fertilized by a single male. However, in this case, Pardo *et al.* (2016) determined that, with the loci and number of eggs analysed in each of the study locations, the PrDM was >0.99. Therefore, they concluded that in the *M. edwarsii* populations analysed there was really no multiple paternity, its prevalence was negligible or the contribution of the additional sires was too low to be detected.

Although overall prevalence of multiple paternity similar to that in other brachyurans has been recorded in *M. brachydactyla* (43.5%), the difference found between the population of the Golfo Ártabro and that of Carna is remarkable. This fact indicates that the paternity of the broods is also influenced by external factors, and not only by the reproductive biology of the species. Below, we discuss a series of hypotheses that aim to explain the differences in the prevalence of multiple paternity between these two populations:

(a) Differences in population density. Carna is a locality at the northern limit of the distribution of the spiny spider crab. This trait and the absence of a permanent fishery of this resource in the area (Tully, 2017) suggest that the population may be less dense than in the Golfo Ártabro. The low density of specimens would limit the availability of sexual partners and, therefore, the level of polygamy. However, some studies have provided data that question the validity of this hypothesis. Kelly *et al.* (2003) conducted a study in Blackshod, Derrinver, and Cleggan (northernmost regions than Carna) to determine if the area was suitable for the establishment of a fishery targeting at *M. brachydactyla*. The results, including a CPUE value of ∼0.76 kg/pot, indicated that the area was appropriate to initiate the exploitation of the spider crab. The same study suggests that the low recapture rate obtained could be due to the existence of a large stock. In addition, Rodhouse (1984) obtained CPUE data of ∼0.4 kg/pot in Kilkieran Bay, near Carna. Bates (1981) had already described the spider crab as a common species on the south and west coast of Ireland, where it was considered by fishermen as a pest due to tangling in large numbers in nets set for crayfish (*Palinurus elephans*). These studies suggest that the population of spider crab in Carna must be dense enough so that there is no shortage of sexual partners.

(b) Differences in female fecundity. As already mentioned, in Galicia females can lay up to three broods in a breeding season while in Ireland they only lay one. Furthermore, fecundity per brood also decreases as latitude increases (Kergariou, 1971, 1984; Verísimo *et al.*, 2011). Since the annual fecundity of Galician females is higher than that of Irish ones, their demand for sperm should also be higher. For this reason, it would be expected that Spanish females will require a greater number of copulations to fill their seminal receptacles and to complete the fertilization of their three broods, while for Irish females one copulation per year may be enough (although another factor to take into consideration would be the quantity of ejaculate passed at each mating). Even though females could carry sperm from previous years in their seminal receptacles, it appears that sperm stored by *M. brachydactyla* females for more than 10 months shows high levels of genetic damage and has low viability (Rodríguez-Pena *et al.*, unpublished data), so multiple paternity would be improbable with only one annual copulation.

(c) To the best of our knowledge, there is no previous works suggesting that females with high fecundity actively search for more copulations. Nevertheless, Paul (1984) and Lovrich and Sainte-Marie (1994) suggested that females of different species of *Chionoecetes* were probably capable of assessing their sperm reserve, because they delayed egg extrusion when insufficiently inseminated, presumably in the 'hope' of encountering another mate.

(d) Different levels of sex- and size-selective exploitation. Differences in the level of polyandry between areas with different fishing intensity have been reported in other decapod species (Gosselin *et al.*, 2005; Pardo *et al.*, 2016).

Galicia is the region of Spain where exploitation of *M. brachydactyla* is most intense, while in Carna there is no stable fishery focused on spider crab. Moreover, in Galicia, *M. brachydactyla* is managed under a 3-S strategy (Season, Sex, and Size Control), while in Ireland the only measure in effect is size control. Management measures prohibiting the landing of ovigerous females result in male-selective fishing, especially of those of larger size with high commercial value (Fahy and Carroll, 2009). In addition, the individuals of extreme sizes are the scarcest in the population, so they are the most susceptible to disappear due to overexploitation. Several studies have provided data on the alteration of the sex ratio and population size structure caused by size- and sex-selective fishing of spider crab. Within lightly exploited populations, males far outnumber and outsize females (Brosnan, 1981; Rodhouse, 1984; Fox, 1985; Kelly *et al.*, 2003). However, in areas with intense male-selective fishing, the proportion of males and females tends to be similar or slightly female-biased, and both sexes tend to be similar in overall size (Fahy, 2001; Corgos *et al.*, 2006; Fahy and Carroll, 2009). This alteration of the population structure can lead to sperm limitation (Hines *et al.*,

2003; Sato and Goshima, 2007; Sato *et al.*, 2010; Pardo *et al.*, 2015), which may promote multiple mating to ensure the fertilization of the broods.

On the other hand, when females mate with multiple small males, even assuming that they obtain a sufficient amount of sperm, the trend over generations may be downwards in the average size of the individuals of the population, as the general ecological and evolutionary literature suggests (Fenberg and Roy, 2008). This will lead to a reduction of the reproductive potential at population level (Sato and Goshima, 2007; Butler *et al.*, 2011; Verísimo *et al.*, 2011). Moreover, our observations under laboratory conditions confirm that small males have difficulties in copulating with females that far outsize them. Therefore, the removal of large males from the population could cause the number of partners compatible with large females to fall.

Taking into account the considerations summarized in point (c), genetic polyandry in the Golfo Ártabro may be functioning as a compensatory mechanism to avoid sperm limitation caused by a shortage of males, especially those of large size.

In the present study, multiple paternity was reported for the first time in *M. brachydactyla*, with an overall prevalence of 43.5% and a moderate bias between sires, unlike in other brachyurans. However, the frequency of multiple paternity was almost seven times higher in the intensely exploited Golfo Ártabro than in Carna, where exploitation levels are low. This difference in the prevalence of multiple paternity could be due to the differences in fertility of *M. brachydactyla* females in these two latitudes or to the different levels of male-selective fishing for this species in these two localities.

To test these hypotheses it would be informative to analyse the paternity in broods from the Magharees region (Ireland). This population is characterized by intense exploitation focused on large males (Rodhouse, 1984; Fahy and Carroll, 2009) and by an annual cycle of one brood. A low prevalence of multiple paternity, similar to that found in Carna, would suggest that the annual fecundity of females is a factor that can influence the level of polygamy in the population. However, a high frequency of multiple paternity would suggest situations of sperm limitation caused by sex- and size-selective fishing. In addition, genetic analysis of ejaculates from the seminal receptacles of *M. brachydactyla* females from Carna would be useful to clarify whether the absence of multiple paternity in this locality is due to the absence of multiple mating or to other factors.

Data availability

The microsatellite data of this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/, and can be accessed with accession numbers MT211631 to MT211639.

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