



High fishing intensity reduces females' sperm reserve and brood fecundity in a eubrachyuran crab subject to sex- and size-biased harvest

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Size-selective male fisheries may result in sperm limitation whereby the number of sperm is insufficient to fertilize all oocytes produced by females. In eubrachyuran crabs, females have seminal receptacles for sperm storage which may reduce the risk of sperm limitation over their lifetime. In this study on the commercially exploited eubrachyuran *Metacarcinus edwardsii*, we evaluate the sperm limitation hypothesis by measuring female reproductive success in five Chilean populations subjected to low or high fishing intensity. The quantity and viability of sperm stored by females was measured in each season and population, and related to resulting brood fecundity. Females' sperm reserve was larger when fishing intensity was low than when it was high—paralleling previously demonstrated differences in males' sperm reserve—and especially in the season before oviposition. Sperm viability was in general high (92%) and independent of fishing intensity. Mean brood fecundity adjusted to constant female size was about 60% greater under low compared with high fishing intensity. Thus, in *M. edwardsii*, population reproductive output could be depressed by male-biased fishing in spite of female sperm storage capability.

Keywords: artisanal fishery, crab, fecundity, management, sperm load, sperm viability.

Introduction

Commercial fisheries for marine species are typically size selective. With few exceptions, these fisheries target the largest individuals due to minimum legal size (MLS) management rules as well as market demand (Miller, 1976; Tsikliras and Polymeros, 2014). This artificial selection caused by fishing can have a direct effect on reproduction and recruitment by altering life history traits, mating systems and the sexual selection process (Willson, 2002; Allendorf and Hard, 2009). Moreover, many crab and lobster fisheries are also subject to management rules that bias landings towards males or exclude females, which may exacerbate the negative consequences on reproduction and recruitment through a

cascade of effects involving changes in the operational sex ratio, the intensity of male competition and mating behaviour including sperm allocation (Jivoff, 2003; MacDiarmid and Sainte-Marie, 2006; Sainte-Marie, 2007; Sato *et al.*, 2010; Pardo *et al.*, 2015).

Most crab fisheries are managed by male-biased or male-only harvest strategies (Orensanz *et al.*, 1998). The rationale for this type of management is to preserve high levels of egg/progeny production, assuming or knowing that males are polygynous and that, among the Eubrachyura, females can store sperm over long periods of time. Under these assumptions, only a relatively small number of males is necessary to fertilize enough oocytes to ensure

population renewal. Thus, females are presumed to acquire and store enough viable sperm to allay sperm limitation (Sainte-Marie et al., 2002; Hines et al., 2003).

However, eubranchyuran females may receive less sperm when the abundance of large (dominant) males is reduced and the operational sex ratio becomes strongly biased towards females by selective fishing. This may occur for a variety of non-exclusive reasons depending on the mating system. On one hand, males may partially or completely exhaust their sperm reserve through single or multiple matings, and then transfer less or no sperm to subsequent females (Kendall et al., 2001; Carver et al., 2005; Pardo et al., 2015). In highly promiscuous species, males may also allocate sperm strategically, reducing the amount passed to individual females with increasing number of perceived mating opportunities and declining risk of sperm competition (Rondeau and Sainte-Marie, 2001). On the other hand, when males are relatively fewer, females might be unable to find a mate (Hankin et al., 1997) or promiscuous females may have fewer mates (Rondeau and Sainte-Marie, 2001).

When multiparous eubranchyuran females do not refresh their sperm reserve prior to spawning, they must rely on stored sperm (if any) from previous mating seasons to fertilize a new brood, as demonstrated in *Romaleon polyodon* and *Metacarcinus magister* (Hankin et al., 1989; Fischer and Thatje, 2008). Although seminal receptacles seem to be a suitable environment for maintaining sperm alive (Anilkumar et al., 1996), some sperm mortality may be expected especially after spawning or a relatively long-storage time. Multiparous female eubranchyurans that do not re-mate prior to spawning can be less fecund than females that do (Paul and Paul, 1982; Sainte-Marie and Carrière, 1995; but see Nagao and Munehara, 2007), probably because of low residual sperm reserves and/or decreased sperm viability. Direct measures of sperm viability in female crabs have been infrequent (but see Wolcott et al., 2005), even though this would provide insight into the quality of stored sperm.

A decrease in the quantity or quality of females' sperm reserve due to fishing of large males could result in sperm limitation and a reduction of female individual and population reproductive output (Kendall et al., 2001; Sainte-Marie et al., 2002). However, determining how many viable sperm are needed to fertilize a female's reproductive potential, and demonstrating empirically the reality of sperm limitation, are big challenges. Some studies were able to demonstrate a reduction of female per capita fecundity due to sperm limitation in the laboratory (e.g. Rondeau and Sainte-Marie, 2001) and others measured natural temporal and spatial variability of females' sperm reserve and estimated theoretically what fecundity losses might be incurred at the population level (e.g. Ogburn et al., 2014); however, there are very few demonstrations of sperm limitation and associated fecundity costs in wild crab populations.

Eubranchyuran crabs in the family Cancridae support intense fisheries along several coasts around the world. In Chile, four cancrinid species are harvested, but *M. edwardsii* is the largest in body size and is the most important to artisanal fisheries, with mean annual landings of 5000 tons (Servicio Nacional de Pesca, 2011–2014). The harvest of *M. edwardsii* is concentrated in southern Chile (40–48°S), but fishing effort and removals vary considerably across localities within this region (Olguin and Parraga, 2011). At present, *M. edwardsii* is fished year-round and is managed by means of a MLS of 110 mm carapace width (CW) and a ban on landing females when they are brooding embryos, usually for a period of 4–5 months in late austral fall and winter (from

April to August). Thus, males represent 60–80% of landed crabs between May and October (Olguin et al., 2006).

Metacarcinus edwardsii exhibits sexual size dimorphism and size-assortative mating. Males develop relatively larger chelae and are about 13 mm larger in CW than females upon reaching morphological sexual maturity (Pardo et al., 2009). This species is polygamous and females copulate exclusively in soft-shell condition, during a molting/mating season that extends from October to January (Pardo et al., 2016). Copulation is preceded by a male pre-copulatory embrace and followed by a post-copulatory embrace lasting for 50–150 h depending on sex ratio (Pardo et al., 2016). In the field and laboratory, sexually paired males are always larger than females (Pardo, unpublished data). Sperm transferred by males are stored in a pair of seminal receptacles where they are retained through female molt (Pardo et al., 2013). *Metacarcinus edwardsii* is a univoltine species, with females spawning typically during late fall and early winter, from April to June (Pardo et al., 2013).

This study aims to assess the effects of the current fishery management strategy (male-biased and minimum size) for Chilean *M. edwardsii* on three female reproductive features—sperm load, sperm viability and brood fecundity—in order to detect possible sperm limitation. We compare these features across localities with *M. edwardsii* populations subjected to different fishing intensities. A previous study demonstrated that operational sex ratio and sperm reserve in males varied across these localities in a pattern consistent with different levels of fishing intensity, with sex ratio more biased to females and males having a smaller sperm reserve in localities with more intense fishing (Pardo et al., 2015), so parallel variability in the quality and quantity of females' sperm reserve and brood fecundity was expected.

Material and methods

Localities

Five localities in southern Chile (Figure 1), spread over 400 km of coastline, were selected for study. These localities differ in fishing intensity, based on an index derived from the ratio of local landings to local density of legal-size crabs determined in diver surveys (Pardo et al., 2015). Crab fishing in southern Chile is completely artisanal; fishermen primarily use baited traps, and activities are concentrated close to landing ports (Olguin and Parraga, 2011), such that landings reflect local removals and fishing intensity (Pardo et al., 2015). Mean annual landings were <0.25 t in Los Molinos and Calbuco which were categorized as having a low fishing intensity, and around 400 t in Ancud, 500 t in Quellón and 900 t in Dalcahue, which were categorized as having a high fishing intensity.

Mature females were collected in discrete seasons from 2012 to 2013 (Table 1) and transported to Laboratorio Costero Calfuco (Universidad Austral de Chile), measured for CW (to the nearest 0.01 mm), and dissected to extract the seminal receptacles and/or remove the brood. Non-ovigerous females were used for estimations of sperm load and sperm viability by season, and for pre-spawn sperm viability, whereas ovigerous females were used for estimations of post-spawn sperm viability and per capita fecundity.

Estimation of sperm load

The sperm load, expressed as number of sperm per seminal receptacle, was estimated from the right seminal receptacle of females.

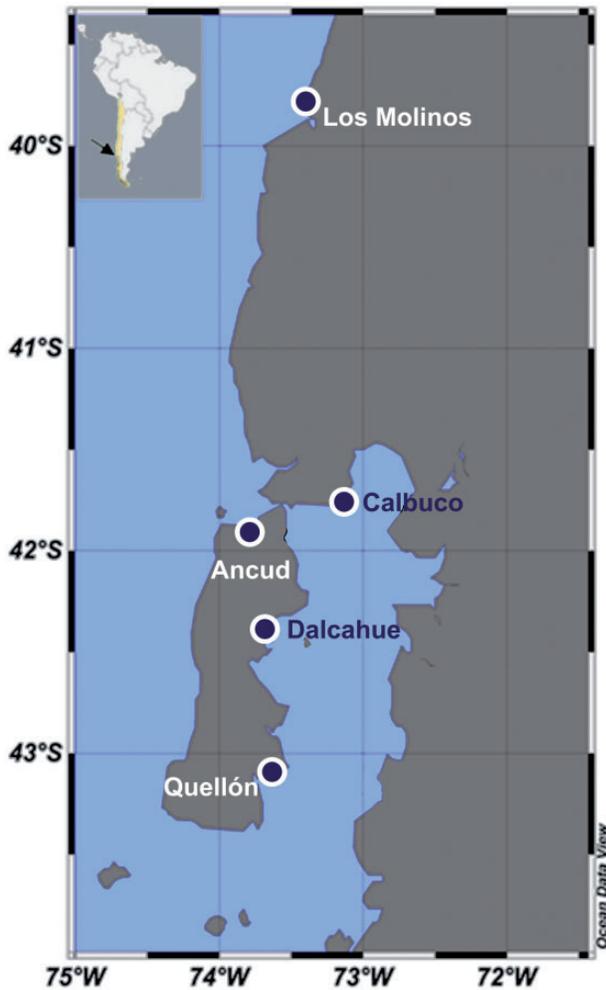


Figure 1. Sampling localities in southern Chile.

The receptacle was extracted from the female, then the receptacle wall was slit with forceps and the content was placed in a pre-weighed Eppendorf tube (1.7 ml) with 0.5 ml of artificial sea water (ASW; Premium Sera[®] Marin Reef Salt) at 30 psu. The translucent receptacle wall was checked for remaining content under a binocular in all cases.

To estimate the number of sperm, 0.1 ml of formalin (5%) was added to the Eppendorf tube and the content was homogenized with a Dounce homogenizer for at least 20 min prior to sperm count. Sperm are generally free in the receptacle of *M. edwardsii* because spermatophores are ruptured soon after transfer (Pardo *et al.*, 2013). Sperm counts were performed in five haphazardly selected grids of a Petroff–Hausser counting chamber (volume: 0.02 mm³) under a light microscope at 1000 \times magnification. Three replicates of each seminal receptacle homogenate were taken and the resulting 15 sperm counts were averaged.

Percentage of viable sperms

Sperm viability may be assessed by a fluorescence-based assay that distinguishes live cells from dead cells (green and red, respectively). The LIVE/DEAD[®] Sperm Viability Kit, with the dual fluorescent stains SYBR-14 and propidium iodide, was used to stain crab sperm as in Wolcott *et al.* (2005). The content of the left

seminal receptacle was extracted and homogenized as for sperm load estimation. From the homogenate, an aliquot of 10 μ l was diluted in 60 μ l ASW and buffered by 30 μ l of HEPES saline solution in an Eppendorf tube. Then, 1.5 μ l of SYBR-14 dye, previously buffered with 50 μ l of HEPES, was added to the tube which was then incubated in darkness for 10 min at room temperature. Finally, 0.5 μ l of propidium iodide was added to the tube, whose content was homogenized gently, and the tube was incubated in darkness for an additional 10 min at room temperature. To avoid the photo-degradation of fluorescent stains, the Eppendorf tube was covered with aluminium foil. To estimate the percentage of live sperm by receptacle, 25 μ l of the incubated sample were placed onto a slide and observed under a fluorescence microscope at 400 \times , at a wavelength of 500 nm for SYBR-14 which stains live sperm bright green and of 600 nm for propidium iodide which stains dead sperm red. Sperm counts (live/dead) were performed on photographs of randomly chosen microscope fields. Sampling from the homogenate was replicated five times for each receptacle to obtain a mean percentage of sperm viability. Sperm samples killed by incubation at 70 $^{\circ}$ C for 5 min were used as controls.

Estimation of female fecundity

Fecundity was determined in winter 2013 for ovigerous females carrying early stage eggs (i.e. without ocular spots), assumed to be all fertilized, to minimize bias due to embryo losses during development (Brante *et al.*, 2004). The fecundity of these females can be related to the condition of the sperm reserve (number and viability) of non-ovigerous mature females measured before the spawning season. In the laboratory, eggs were carefully separated from the female's pleopods and rinsed with distilled water to remove salt. Three subsamples of 500 eggs were randomly taken from each brood, and these and the remaining brood were oven-dried at 75 $^{\circ}$ C for 48 h, and then weighed on an analytical balance (\pm 0.01 mg). Fecundity was estimated by extrapolating the quotient of 500 eggs over mean dry weight of subsamples to total brood dry weight.

Statistical analysis

Full factorial ANCOVA was performed to test for differences in sperm load among localities and seasons. The CW of females was used as the covariate and data were ln-transformed to linearize relationships between sperm load and female CW. The full model showed significant interactions among localities, season and CW, therefore the analyses were performed by season separately. For each seasonal analysis, data were checked for normality and homogeneity of variance (Cochran test). After all factorial analyses, pair-wise comparisons between localities were performed by specific contrast.

To evaluate the effect of high and low fishing intensity on sperm load of females, data from localities in each category of fishing intensity were pooled. Linear regression was used to describe the relationship between sperm load and female CW by combination of fishing intensity and season. ANCOVA by season, using female CW as the covariate, tested for differences in sperm load between categories of fishing intensity. In this case, ln-transformation was not used as it did not clearly improve the linear relationship between sperm load and female CW.

Sperm viability was not correlated with female CW in any season and data showed heterogeneous variance even after transformation, therefore a Kruskal–Wallis test was used to detect

Table 1. Number and carapace width (CW) of mature females sampled by Chilean locality (fishing intensity, low or high, in parentheses) and season for determination of sperm load, sperm viability and fecundity.

Reproductive trait Sampling locality (fishing intensity)	Number of females per sampling season					Female CW (mm)	
	Winter 2012	Spring 2012	Summer 2012–2013	Fall 2013	Winter 2013	Range	Mean
Sperm load							
Los Molinos (low)	32	15	30	22	–	90–160	118
Calbuco (low)	17	7	19	21	–	86–152	106
Ancud (high)	26	26	28	30	–	83–132	112
Dalcahue (high)	30	17	29	26	–	97–149	109
Quellón (high)	16	24	30	30	–	96–135	112
Sperm viability							
Los Molinos (low)	–	14	14	11	13	82–144	107
Calbuco (low)	–	15	15	14	14	77–134	108
Ancud (high)	–	14	15	18	14	89–160	111
Dalcahue (high)	–	18	14	21	20	89–121	106
Quellón (high)	–	14	15	16	13	93–153	109
Fecundity							
Los Molinos (low)	–	–	–	–	15	99–121	108
Calbuco (low)	–	–	–	–	17	92–148	119
Ancud (high)	–	–	–	–	19	99–148	108
Dalcahue (high)	–	–	–	–	27	99–144	112
Quellón (high)	–	–	–	–	10	105–133	117

Seasons are winter 2012 (18–28 August), spring 2012 (26 September to 9 November), summer 2013 (27 January to 28 February), fall 2013 (2 April to 9 May) and winter 2013 (29 May to 12 July).

differences among localities for each season. In addition, a Kruskal–Wallis test was performed to compare sperm viability among localities at two times of the sperm usage cycle: (i) soon after mating, based on females with a visible sperm plug (a reliable indicator of recent copulation: Oh and Hankin, 2004) collected mainly in summer and fall 2013; and (ii) soon after spawning, based on females with early-stage eggs (as for fecundity analyses) collected in winter 2012 and 2013.

Finally, fecundity was analysed by one-way ANCOVA with locality as the factor and CW as the covariate. Ln-transformation was necessary to linearize relationships between fecundity and female CW. After analysis, the pair-wise comparisons between localities were performed by specific contrast. All analyses were done using R or STATISTICA software.

Results

When comparing the effect of localities on sperm load by season, female CW was a significant covariate in all cases. Moreover, the slopes of the relationship between sperm load and female CW were significant and did not statistically differ among localities, except in summer (interaction Locality \times CW: Table 2). The sperm load, adjusted to constant female CW by season, was highest or equal to highest in Calbuco (low fishing intensity) and was significantly different there from localities with high fishing intensity in summer and fall. In general, the adjusted sperm load was similar around the year in all other localities except in the fall, when Los Molinos, the other locality with low fishing intensity, matched the value in Calbuco (Figure 2).

Regressions between sperm load and female CW (Figure 3) by fishing intensity and season were positive and significant in all but one case (high fishing intensity in spring), and the determination coefficients (R^2) were greater with low compared with high fishing intensity (supplementary materials). In the full ANCOVA model testing for the effects of fishing intensity (pooled data) on

sperm load, the covariate female CW was significant ($p < 0.001$) but the Season \times CW interaction was also significant ($p = 0.006$), therefore ANCOVA was performed separately by season. In winter and spring, no interaction between fishing intensity and the covariate CW was detected (winter, $F = 0.21$, $p = 0.64$; spring, $F = 2.68$, $p = 0.10$) and adjusted sperm load did not differ with fishing intensity (winter, $F = 0.14$, $p = 0.70$; spring, $F = 1.13$, $p = 0.29$). In summer, an interaction between fishing intensity and CW was detected ($F = 13.46$, $p < 0.001$) due to the slope of the regression of sperm load on CW being steeper in areas with low fishing intensity than in areas with high fishing intensity (Figure 3), resulting in larger females (> 110 mm CW) having relatively smaller sperm loads in the latter compared with the former areas (Figure 3). In fall, the slopes were parallel between high and low fishing intensity areas (Fishing intensity \times CW: $F = 2.40$; $p = 0.13$), with the latter showing greater values of sperm load across the size range of females (contrast high—low fishing intensity: $t = 6.7$, $p < 0.01$; Figure 3). The relative difference in sperm reserve between females in high compared with low fishing intensity areas in fall was about 151% at 100 mm CW and 78% at 120 mm CW. Overall, these observations indicate that females acquired more sperm through the spring–summer mating period and had a larger sperm reserve at the outset of the spawning season in fall in areas with low fishing intensity than in areas with high fishing intensity.

Considering all mature females, viability of stored sperm was independent of female CW ($R^2 = 0.06$; $n = 288$) and was high overall (average $91.7 \pm \text{SE } 8.7\%$). Considering localities and seasons, the viability of stored sperm differed among localities only in summer (Kruskal–Wallis; $H_{4,73} = 22.5$, $p = 0.002$) and winter ($H_{4,74} = 17.5$, $p = 0.002$). This heterogeneity was due to sperm viability being relatively low in Calbuco (low fishing intensity) in summer and relatively high in Dalcahue (high fishing intensity) in winter (Table 3), so fishing intensity was seemingly not in

Table 2. ANCOVAs by season and year to detect differences in female sperm load among five localities from southern Chile.

Season and year				
Source of variation	Df	MS	F	P
Winter 2012				
Locality	4	0.61	1.32	0.268
CW	1	8.16	17.54	0.000
Locality×CW	4	0.48	1.04	0.389
Error	111	0.47		
Spring 2012				
Locality	4	4.76	8.77	0.001
CW	1	12.13	22.35	0.000
Locality×CW	4	1.19	2.20	0.080
Error	79	0.58		
Summer 2013				
Locality	4	5.17	18.60	0.000
CW	1	16.27	58.60	0.000
Locality×CW	4	0.45	1.60	0.020
Error	126	0.28		
Fall 2013				
Locality	4	3.50	11.80	0.000
CW	1	25.30	85.60	0.000
Locality×CW	4	0.29	1.00	0.426
Error	119	0.30		

The covariate is female carapace width (CW).

cause. Sperm viability in recently mated females (with sperm plug present) sampled in spring 2012 and summer 2013 was similar and very high (average 92–94%) across all localities (Figure 4). Sperm viability was comparatively less in recently spawned females (with early-stage eggs) from winters 2012 and 2013, varying from 75% to 87% on average across localities (Figure 4); the smallest value was observed in Calbuco (low fishing intensity) but there was no overall difference (Kruskal–Wallis; $H_{4,61} = 6.14, p = 0.18$).

Fecundity was strongly related to female CW (ANCOVA, covariate: $F = 54.7, p < 0.001$) and this relationship was similar among localities (ANCOVA, interaction Locality × CW, $df = 4, F = 2.2, p = 0.07$). Contrast analysis showed that fecundity adjusted to constant female size was similarly high in Calbuco and Los Molinos, lowest in Ancud and Dalcahue, and intermediate in Quellón (Supplementary materials; Figure 5). When localities were pooled by fishing intensity, adjusted fecundity was significantly greater (about 62% more) in low than in high fishing intensity areas (contrast high—low fishing intensity; $t = 12.4, p < 0.001$). Mean fecundity adjusted to 112 mm CW considering all females analysed was 893 113 eggs, and the maximum value was recorded in Calbuco where a 139-mm CW female carried 3 304 400 eggs.

The relationship between the number of viable stored sperm (i.e. sperm load × sperm viability) per seminal receptacle available prior to spawning (measured on non-ovigerous females in fall 2013) and CW-adjusted fecundity realized in winter 2013 (females carrying early-stage eggs) by locality is shown in Figure 5. There was a positive relationship (correlation) between

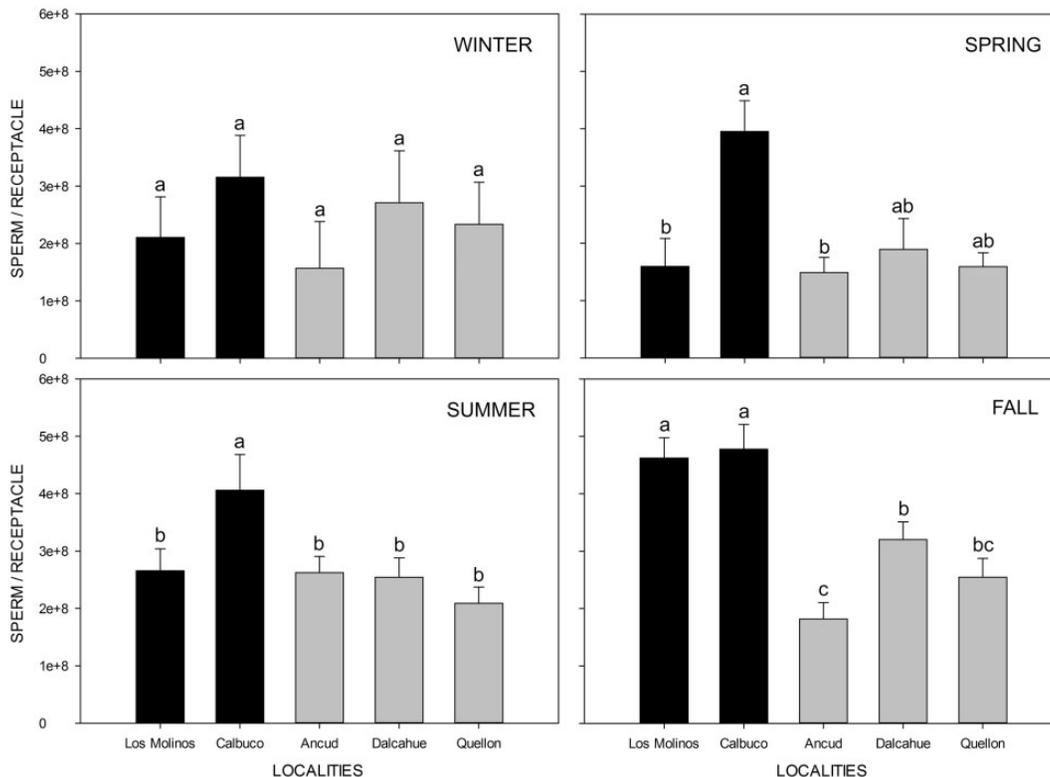


Figure 2. Seasonal variation in sperm load (mean number of sperm by seminal receptacle) for five localities with contrasting fishing intensities (black bars = low intensity, grey bars = high intensity). Standard errors are shown and significant differences ($p < 0.05$) are represented by different letters.

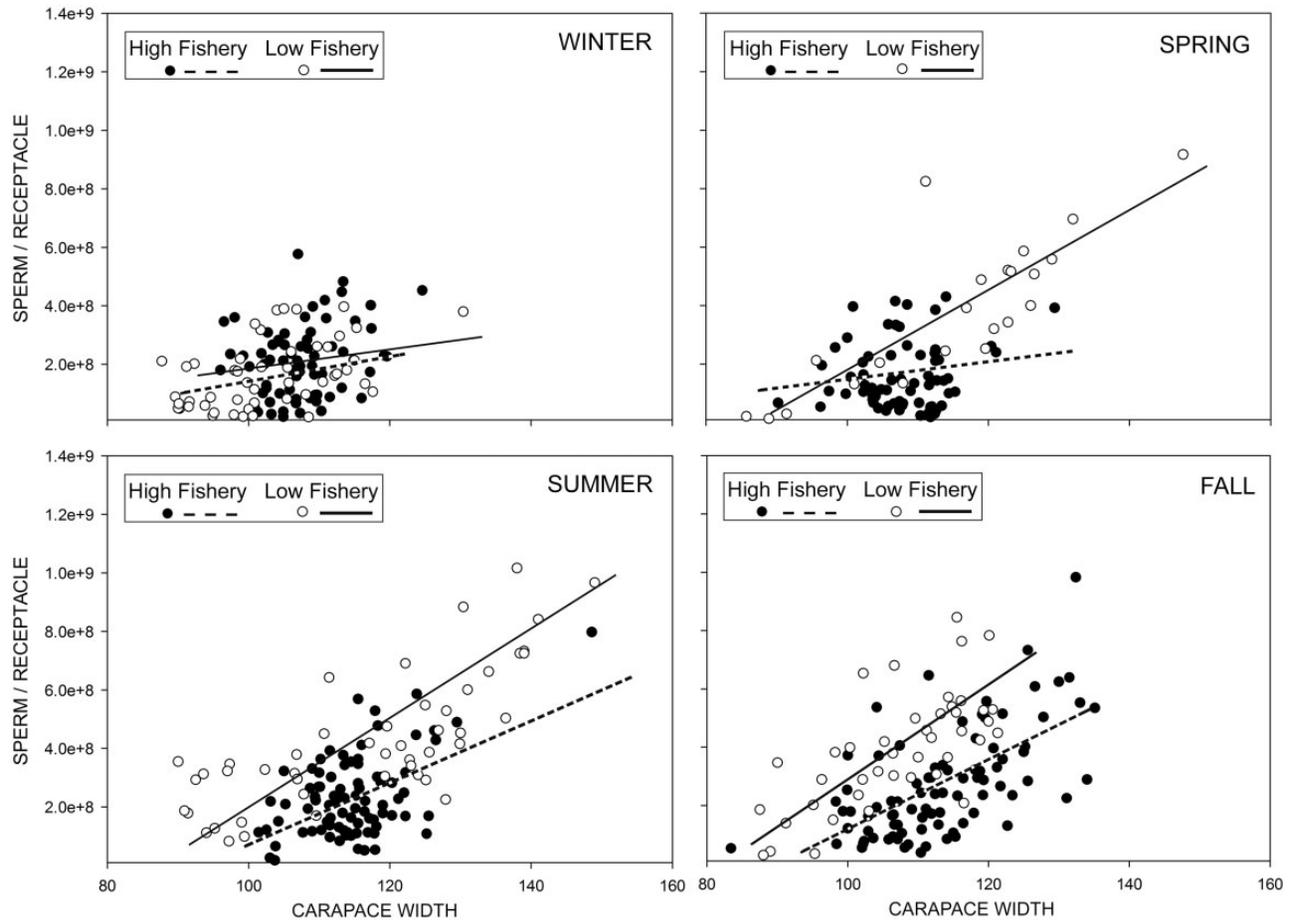


Figure 3. Relationship between female CW and sperm load (number of sperm per seminal receptacle) by season. Full black circles represent females from high fishing intensity localities and open circles represent females from low fishing intensity localities. Lines represent significant linear regression fits (see supplementary materials); the relationship was not significant for high fishing intensity localities in spring.

Table 3. Mean percentage \pm standard error of sperm viability by season for five Chilean localities with contrasting fishing intensity (FI, low or high).

Locality	FI	Winter 2012	Spring 2012	Summer 2013	Fall 2013
Los Molinos	Low	83.7 \pm 1.9	96.1 \pm 1.9	94.4 \pm 1.9	92.2 \pm 2.1
Calbuco	Low	83.0 \pm 1.9	90.5 \pm 1.8	93.6 \pm 1.8	89.9 \pm 1.9
Ancud	High	83.5 \pm 1.9	89.9 \pm 1.9	89.9 \pm 1.8	92.1 \pm 1.6
Dalcahue	High	92.4 \pm 1.6	91.9 \pm 1.6	97.0 \pm 1.9	93.6 \pm 1.5
Quellon	High	83.5 \pm 1.6	95.5 \pm 1.9	98.2 \pm 1.8	90.1 \pm 1.7

the two variables for the localities of Ancud, Quellón, Calbuco and Los Molinos, with localities with a high fishing intensity clustered to the bottom left of the graph and localities with low fishing intensity grouped in the upper right of the graph (Figure 5). Female per capita fecundity increased by a factor of about 1.5 and viable sperm number by a factor of about 2.4 from high to low fishing localities. Dalcahue was somewhat of an outlier to this relationship (Figure 5), its female fecundity being less than expected for the number of viable sperm (or vice versa).

Discussion

This is the first study to have assessed mating success for a cancrid crab in terms of the number and viability of sperm stored by females and their resulting brood fecundity. For *M. edwardsii*, this study revealed significant and very substantial variations in females' sperm reserve and brood fecundity within and among fishing localities in southern Chile. Moreover, the number of viable sperm stored by females prior to spawning and their subsequent brood fecundity were greater in localities with low fishing intensity than in localities with high fishing intensity, the differences being greater for sperm number than for per capita fecundity. This pattern was particularly evident when comparing Los Molinos and Calbuco (low fishing intensity) with Ancud and Quellón (high fishing intensity), and it is consistent with observed male demographic and reproductive features that can alter female mating success and potentially lead to reduced fertilization success.

Indeed, Pardo *et al.* (2015) found that the sex ratio of physiologically mature crabs (>101 mm CW) was skewed to males and that, among legal-size crabs (>110 mm CW), males were significantly larger than females in Los Molinos and Calbuco (low fishing intensity) but not in Ancud and Quellón (high fishing

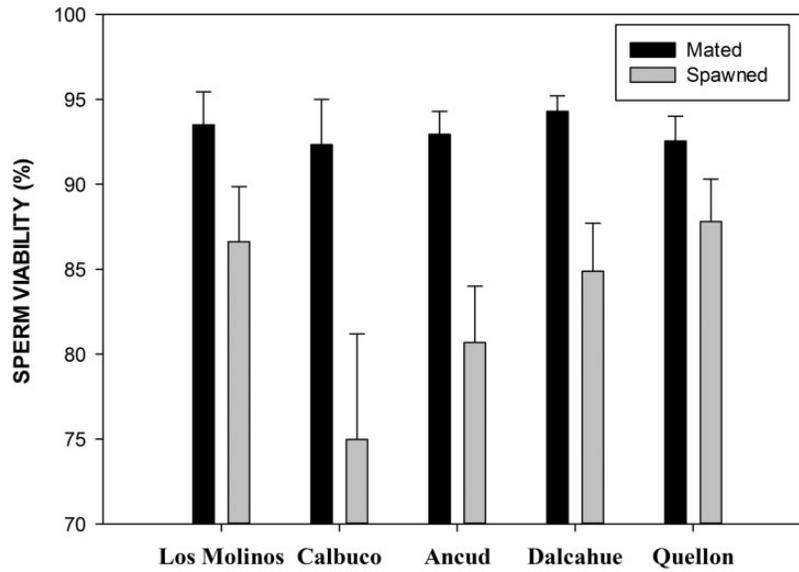


Figure 4. Mean viability of sperm stored by females just after mating (females with a sperm plug) and just after spawning (females carrying eggs without eyespots). Standard errors are shown.

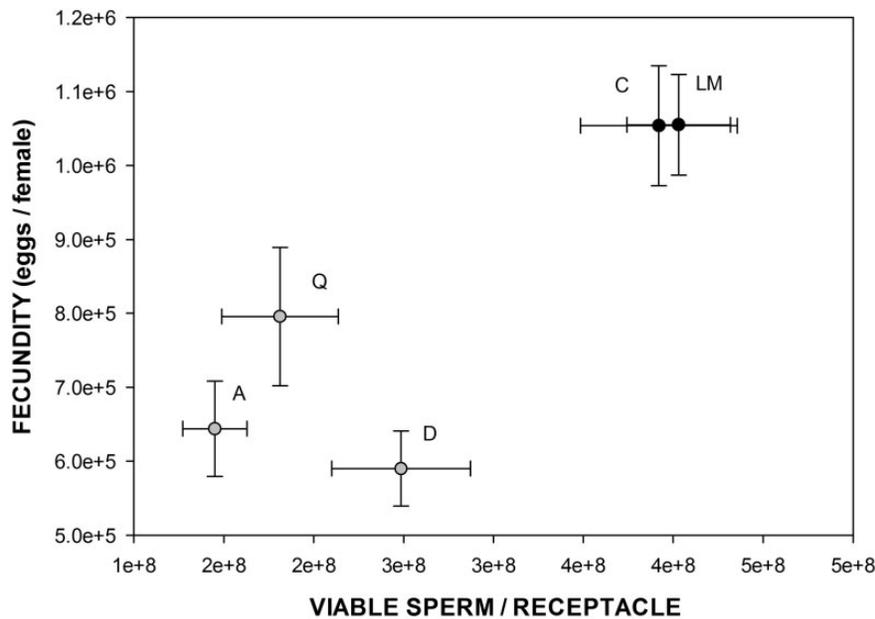


Figure 5. Relationship between female mean viable sperm load (number of viable sperm in storage before spawning) and fecundity measured just after spawning, adjusted to constant female CW of 112 mm by ANCOVA, at five localities with different fishing intensities (low = full black circles; high = grey circles). Standard errors are shown. The number of viable sperm was the product of the number of sperm stored by each female in one receptacle and the mean proportion of viable sperm in each locality during fall. CW of females did not differ significantly (ANOVA, $F_{1,215} = 0.16$; $p = 0.69$) between those used for determinations of fecundity (112.1 mm CW) and sperm load (111.4 mm CW). A, Ancud; Q, Quellón; D, Dalcahue; C, Calbuco; LM, Los Molinos.

intensity). Moreover, physiologically mature males had a smaller sperm reserve (measured by the vaso-somatic index and percent content of spermatozoa) in the two last localities compared with the two first, consistent with partial sperm depletion due to more frequent mating opportunities and insufficient time to recover

(Pardo *et al.*, 2015). The concurrently much smaller sperm reserve of females in Ancud and Quellón thus indicates that females were not able to re-mate (i.e. refresh their sperm reserve) or acquired only a small amount of sperm from their most recent mate, which can happen when opportunities for polygyny are

increased and larger (often dominant or preferred) males pass less sperm due to smaller reserves or strategic reduction of ejaculate size (Rondeau and Sainte-Marie, 2001).

The tight positive covariation between the viable sperm reserve and brood fecundity of females across Ancud, Quellón, Calbuco and Los Molinos suggests a causal relationship between the two variables related to fishing intensity (Figure 5). The non-exclusive mechanisms by which female fecundity can be reduced under sperm limitation are resorption of unextruded oocytes and dropping or removal (sloughing) of extruded but unfertilized eggs (Sainte-Marie et al., 2010). Moreover, in crab species in which female molting, mating and spawning occur in sequence, delays in mating can lead to fecundity losses even if the female is eventually well inseminated; these occur because of oocyte over-maturation and lower fertilization rate and/or in some cases because of partial ovary atresia (Sato et al., 2005; Sainte-Marie et al., 2010). Additionally, in crab species in which females mate in the soft-shell (postmolt) condition and male guarding plays the dual role of preventing the female from mating with rival (sexually competing) males and protecting her from predators, as in *C. sapidus* (Jivoff, 1997) and *M. edwardsii* (Pardo et al., 2016), females that molt unguarded or guarded by a small male may be injured/killed and then rapidly eaten or scavenged by predators as observed *in situ* for *Chionoecetes opilio* (Sainte-Marie and Hazel, 1992; Sainte-Marie et al., 2008). Mate and/or sperm limitation must thus be seen as having a continuum of effects on individual and population female fecundity, including reduction of female condition or abundance by injury or death, partial or full ovary atresia/resorption, and partial to complete failure to fertilize all oocytes.

Very few studies have examined the spatial (geographic) or interannual variability of the quantity or quality of females' sperm reserve and the associated brood fecundity in commercially exploited crabs. Those that have, however, found as we did that size-specific brood fecundity was less where exploitation was more intense and sperm reserve was consequently smaller (Hines et al., 2003) or older (Taylor, 1996). Monitoring of primiparous female *C. opilio* over 15 years revealed that females' sperm reserve was positively correlated to the relative abundance of legal-size adult males and that brood size adjusted to female size was positively correlated to females' sperm reserve (Sainte-Marie et al., 2002, 2008; also see Webb et al., 2016). Ogburn et al. (2014) documented spatial and temporal variability of females' sperm reserve for *Callinectes sapidus* in Chesapeake Bay, which was related to operational sex ratio and seemingly the intensity of fishing, and predicted by modelling the resulting changes in female lifetime embryo production. However, a more recent investigation by Rains et al. (2016) did not find a relationship between sex ratio and sperm count in *C. sapidus* from six tributaries of Chesapeake Bay. These authors pointed out that the two Chesapeake Bay studies used different methods to assess sex ratio and infer potential for sperm limitation, and that the temporal periods and spatial scales for sampling—and possibly the fishery context—were different, and concluded by recommending further research into the question of sperm limitation.

Dalcahue female *M. edwardsii* also were less well provisioned with sperm and less fecund at size than females from the low fishing intensity localities (Figure 5). However, Dalcahue stood apart from the other high fishing intensity localities in having females with a relatively larger pre-spawn viable sperm reserve yet lesser size-specific fecundity. The larger sperm reserve might be explained by Dalcahue population features not shared by the other

localities with high fishing intensity: a balanced sex ratio for physiologically mature crabs, and males that were significantly larger than females among the legal-size component of the population (Pardo et al., 2015). The greater sperm load of Dalcahue does not appear to be related to polyandry as most females carried only one ejaculate (Pardo et al., 2016), but it could be related to greater sperm allocation by individual, larger males. Indeed, Pardo et al. (2016) observed for *M. edwardsii* that guarding time increased with male sex ratio skew, and in other polygamous eubranchyuran species sperm allocation and guard time are positively correlated (Rondeau and Sainte-Marie, 2001). Thus, sperm limitation is unlikely to be the root cause of the reduction in female size-specific fecundity at Dalcahue. Among invertebrates, fecundity and more generally reproductive investment can vary for many reasons, such as food availability (Ramirez Llodra, 2002). In particular, Dalcahue exhibited the highest density of legal-size crabs of all localities (Pardo et al., 2015), so it is conceivable that density and/or per capita food availability were a factor in reduced fecundity (DeMartini et al., 2003).

Persistent sperm viability through medium- to long-term storage in the female is also critical to assessing the risk of sperm limitation for eubranchyurans. This is especially true in species in which sequential spawning without re-mating can occur or is the rule, as in *Metacarcinus gracilis* (Orensanz et al., 1995), *Pseudocarcinus gigas* (Gardner and Williams, 2002) or *C. sapidus* (Hines et al., 2003; Darnell et al., 2009). In this study, the loss of sperm viability from time of sperm transfer to next spawning (about 5–6 months) was only $\approx 10\%$ and much less than in *C. sapidus* and *Portunus trituberculatus* in which the losses were closer to 50% (Wolcott et al., 2005; Xuan et al., 2014). In *C. sapidus*, viability of sperm stored by females was seemingly not influenced by male size and mating history (Wolcott et al., 2005). There are no other sperm viability studies on crabs with which to compare, but the higher sperm survival rate in female *M. edwardsii* might be explained by the abundant melanin-like granules secreted by the glandular epithelium of the seminal receptacle (Pardo et al., 2013), also reported for the congener *M. magister* (Jensen et al., 1996). Melanin can act as a bactericide and an anti-oxidative agent, absorbing free radicals produced during metabolism (Subramoniam, 1993). It is important to note, however, that sperm viability assays only measure cellular membrane integrity and do not inform on sperm senescence and competency for fertilization (Holman, 2009). Thus, although quantifying sperm number and viability is an essential step towards assessing the state of females' sperm reserve, it does not allow a definitive statement about their potential for fertilizing a brood. Studies on *M. magister* (Hankin et al., 1997) and *C. opilio* (Carrière, 1995; Taylor, 1996) have demonstrated lesser size-specific fecundity for females relying only on old sperm reserves versus females using sperm reserves refreshed by recent mating, assuredly indicating sperm limitation.

Overall, this and a previous study (Pardo et al., 2015) have provided comprehensive and compelling field evidence that fishing biased towards harvesting males and large sizes can trigger a cascade of effects on reproductive traits and success in a population, as conceptualized in MacDiarmid and Sainte-Marie (2006) and Sato (2012), and specified for eubranchyurans in Figure 6. The first line of effects is a reduction of the density and a truncation of the size/age structures of males relative to females, and this is accompanied by a shift in the operational sex ratio in favour of females. In this context, male sexual competition is relaxed and

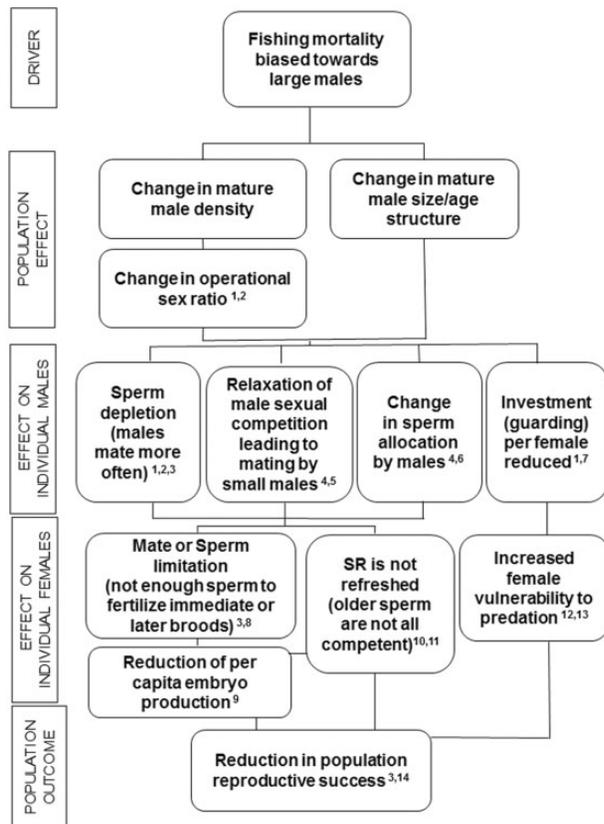


Figure 6. Conceptual model of the cascade of effects triggered by sex and size-biased fishing on brachyuran population traits and reproduction at individual and population levels. The magnitude of effects will depend on mating system and sperm use dynamics in the seminal receptacle. SR, seminal receptacle. (1) Jivoff (2003); (2) Pardo *et al.* (2015); (3) Sainte-Marie *et al.* (2008); (4) Kendall *et al.* (2001); (5) Carver *et al.* (2005); (6) Rondeau and Sainte-Marie (2001); (7) Pardo *et al.* (2016); (8) Hines *et al.* (2003); (9) Ogburn *et al.* (2014); (10) Hankin *et al.* (1997); (11) Taylor (1996); (12) Jivoff (1997); (13) Sainte-Marie and Hazel (1992); (14) this study.

the number of mating opportunities increases for males but decreases for females, especially larger females that may need larger mates. As a result, males may reduce guard time and some females may be guarded ineffectively by smaller or more expedient males, may be mated late or may fail to find a mate, resulting in fecundity losses, injuries or even death. Concurrently, surviving males may become sperm-depleted through multiple mating and pass small or no ejaculates and/or may allocate sperm parsimoniously, resulting in sperm limitation for the surviving females due to insufficient number or viability/competency of acquired or previously stored sperm. The end result is a reduction of population fecundity. This inventory of effects details only the material costs of sex- and size-biased fishing; a reduction of population genetic diversity and selection for slower growth and early maturity are possible outcomes of reduced opportunity for female mate choice and changes in size/age-dependent mortality and reproductive success (Law, 2000; Hutchings and Rowe, 2008; van Wijk *et al.*, 2013).

How this cascade of fishing effects plays out in decapod crustaceans is inevitably a function of the target species' mating system, including the anatomy of female sperm storage areas.

For example, vulnerability to sperm limitation could be expected to vary depending on the nature of sperm storage structures (external versus internal) and variability in *trans*-molt sperm retention capacity (Sainte-Marie, 2007; Sato *et al.*, 2010; McLay and López Greco, 2011). Also species in which females produce multiple broods but mate only once, such as *C. sapidus*, could be less resilient to sex-biased fishing than species with females that are promiscuous or can potentially mate prior to each spawning, such as *C. opilio* and *Metacarcinus* species (Sainte-Marie *et al.*, 2008; Jensen and Bentzen, 2012; Pardo *et al.*, 2013). Thus, fishery management based on size and sex must consider species-specific traits, and especially the dynamics of sperm usage by females. The assumption that few males are needed to secure the full reproductive potential of females is likely true in only some species.

How sperm limitation might impact population recruitment and productivity (Garcia *et al.*, 2012), and eventually the ecosystem (Zhou *et al.*, 2010), is still another matter. This also may depend on the species' ecology. Cancrid crabs are highly fecund, as demonstrated herein, and environmental stochasticity and density-dependent post-settlement processes, including cannibalism, can play important roles in regulating recruitment as demonstrated in *M. magister* (Eggleston and Armstrong, 1995; Higgins *et al.*, 1997). In such species, modest levels of sperm limitation might have little effect on recruitment and productivity, in contrast to species with lesser fecundity and sharper stock-recruitment relationships. Thus, the sustainability of crab fisheries subject to size- and sex-biased harvest requires investigation of the recruitment and productivity impacts of sperm limitation. Depending on the results, alternative or complementary management strategies could be envisaged, for example, the implementation of a maximum legal size to protect individuals with the greatest reproductive potential (Gardner and Williams, 2002), sex-balanced exploitation as in the fishery for *Callinectes trituberculatus* (Xuan *et al.*, 2014, 2016) or reduction of exploitation rates to avoid growth overfishing.

Supplementary data

Supplementary material is available at the ICESJMS online version of the manuscript.

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