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**Spatial and temporal variability of Biological and
Fisheries indicators of a small-scale crustacean
fishery in southern Chile.**

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I. GENERAL INTRODUCTION

Stock assessments models required long-term data associate to abundance and life-history parameters for the estimation of maximum sustainable yield (MSY)-which allow the calculation of Biological Reference Points (Collie and Gislason, 2001; Morgan *et al.*, 2014). Unfortunately, only 10% of the global fisheries had enough data to perform this conventional stock assessment (FAO, 2017; Fitzgerald *et al.*, 2018). Most of the fisheries, classified as small-scale, are often limited in data hence their management is very poor compared to their importance for local economies. It had been studied that small-scale fishery not only provide with employment but also are the main protein intake of coastal communities (FAO, 2004). The same global tendency is seen in Chile, where at least 70% of the species landed in the country do not have estimates of MSY, stock abundance, life history and fishery parameters. In southern Chile, in northern Patagonia, fjords, channels and numerous islands characterize the inland sea, here many coastal families rely almost exclusively in small-scale fisheries. Since the colonization (that took place during the second half of the 19th century) local economies depend on the exploitation of natural resources (Molinet *et al.*, 2014). Particularly the fishing sector was developed in the 80s with the so-called southern hake boom and “fiebre del loco” (intensive extraction of the gastropod *Concholepas concholepas*). Since then, the region has sustained a mainly demersal artisanal fishery (Figure 1) associated with the extraction of southern hake (*Merluccius australis*) and golden conger eel (*Genypterus blacodes*) and a benthic resource fishery associated with red sea urchin (*Loxechinus albus*), crustaceans (*Metacarcinus edwardsii* and *Lithodes santolla*) and seaweed (*Gigartina skottbergii*).

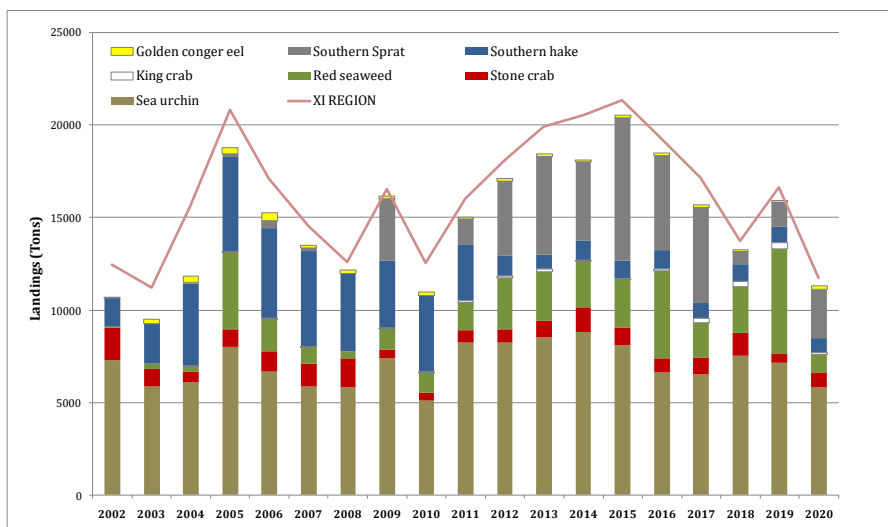


Figure 1. Main species contribution to Aysen regional small-scale fisheries landings (tons). Pink line represents total regional landings. Data obtained from National Fisheries Service, SERNAPESCA.

Marble crab, *Metacarcinus edwardsii* (Bell, 1835), is one of the important species, that contribute some years up to 30% of the total national landings and 66% of fishers have permission to extract this resource. This exclusively artisanal fishery is monitored annually under a scheme that allows the registration of biological and fishing information, mainly from the landing ports and secondarily, from fishing grounds (Olguín and Mora, 2021). This monitoring strategy have had limitations, especially south of 45°, where the remoteness of fishing grounds (8 to 48 hours of sailing from the main landing port) combined with rough weather conditions, made data collection difficult and expensive. Product of scarce and limited data, this fishery has a low level of regulation since only the entry of fishermen is limited, but there are no restrictions on the fishing effort (e.g., number of traps per boat) or the total catch. So far, management measurements include the permanent ban of ovigerous females and a Minimum Legal Size of extraction (MLS) which was set empirically for all national territory in 120 mm carapace width (CW) for both sexes in 1990. In 2011, this MLS was reviewed considering estimations of size at maturity performed for both sexes in individuals collected in Los Lagos Region (40°13'–44 °38' S) were after deliberation, was diminished to 110 mm CW.

The estimation of parameters that could reflect the health status of a fishery had been a challenged since they must be sensitive enough to be affected by the activity and thus could be used as a proxy of harvest intensity. In the case of decapods, reproductive potential usually quantified in terms of fecundity, size at maturity, proportion of females in each size class had been used as indicator of population status. The common strategy of females' decapods to brood their eggs prior to hatching presents an opportunity to estimate fecundity potential, and, in the longer term, forecast recruitment, a key parameter adopted in models of fisheries dynamics (Maunder and Thorson, 2019). In other words, the detection of a decrease in fecundity may be an early sign of recruitment overfishing (Orensanz *et al.*, 1998) that could alert and trigger fisheries management actions. In crabs, sperm depletion is one of the known causes of reducing female fecundity since harvesting target largest males with greater sperm reserves leaving under-sized males with a lower sperm supply to fertilize mature females (Sato *et al.*, 2005; Sato and Goshima, 2007; Pardo *et al.*, 2015; Di Salvatore *et al.*, 2019). In decapods males store sperm and eubrachyuran females, storage them after mating, in very specialized organs called vasa deferentia and seminal receptacles, respectively (Sainte-Marie, 2007). The relative weight of these organs, with respect to their body weight, has been used

as an indicator of sperm transference capacity, which can be adopted as a proxy of sperm depletion associated with fishing effort (Pardo *et al.*, 2015) and therefore could be complementary to studies associated to female fecundity. Additionally, temporal variation of these indicators can give insights of reproductive cycle, since a decay of sperm reserves in males with the concomitant increase in the weight of seminal receptacles; can be associated to the mating season (Pardo *et al.*, 2015). The understanding of the reproductive cycle of a species and its spatial variation is essential for a proper regulation that assures population reproductive success. Moreover, variability of the catch per unit effort, which used often as an estimation of natural abundance, can be more comprehensively understood when reproductive cycle is known. In this context, it is recommended that reproductive potential could be estimated regularly and ideally at the beginning of the exploitation of a fishery (Militelli *et al.*, 2020). Size at maturity also can be used as a reference point to establish MLS (Bianchini *et al.*, 1998), a common regulation that if not set with scientific bases can lead to growth-overfishing and recruitment-overfishing (Ungfors, 2007, Pardo *et al.*, 2009). This indicator could also help to estimate the percentage of mature specimens in the catch which also can show trends of the status of a fishery. In a fishery where larger individuals are target, size is also an important indicator that could lead to conclusions associated with harvest pressure. *M. edwardsii* display sexual dimorphism where males could be as much as 13 mm larger in carapace width than females after reaching morphometrical sexual maturity (Pardo *et al.*, 2017). In species that mates immediately after molt, as *M. edwardsii*, studies had found that females preferentially mate with larger males since they need a pre- and post-copulatory embrace to assure survival (Pardo *et al.*, 2016). Also, a positive correlation had been found between sperm production and size, *i.e.*, bigger males can provide with more ejaculated sperm, and they can copulate with bigger females also, which will lead to a great female fecundity (Sato and Goshima 2007, Pardo *et al.*, 2015).

In this context, estimating reproductive indicators to be used as a reference point to follow a fishery could give insides of its health status and could give the scientific bases to promote certain management strategies. Reproductive indicators can also increment knowledge about reproductive cycle of the species, which can help to understand its variability and promote management measurements, for example seasonal closure during the mating period. In species with a wide distribution as *M. edwardsii*, it is important to develop local derived data to implement management measures since reproductive and fisheries indicators can vary associated to habitat characteristics and fishing intensity. So far monitoring included only catch per unit effort, which in vast territory as Aysen, have had limitations. Most of the data

obtained is gathered from landings which do not allow knowing soak time of the traps neither the presence of escape windows, both variables that can affect catch. In this context, the gathering of information directly from the fishing grounds could allow to have a more precise catch per unit effort which combined with reproductive indicators would help to have a more comprehensively understanding of the fisheries variability and hence, fishery status.

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II. OBJECTIVE AND SCIENTIFIC QUESTIONS

The main objectives of the thesis were to estimate fisheries, biological and reproductive indicators associated to the small-scale fishery of *Metacarcinus edwardsii* in main fishing grounds in northern Patagonia, Chile.

For this purpose, the thesis was focused on the following questions:

1. What is the spatial and temporal variability of reproductive indicators of *M. edwardsii*?
This question was addressed comparing reproductive indicators along a latitudinal gradient (between 36° and 45°S) in three fishing grounds that represent different fishing intensity. These indicators were (1) Size at the onset of maturity, which was estimated associated to physiological and morphometrical sexual maturity of females and males separately; (2) Sperm transference capacity, which was evaluated through Vaso-Somatic and Receptacle-Somatic indices and (3) Fecundity potential, in terms of number of eggs per ovigerous female. Results are discussed associated with the spatial and temporal variability and health status of the fishery.
2. What is the spatial and temporal variability of fisheries and biological indicators in main fishing grounds south of 45°S? Fisheries indicators included catch per unit effort (over and below MLS) and weight and size relationships. Biological indicators were based on size and sexual ratio. The results are discussed associated with recommendations for improving a monitoring program that could allow a stock assessment of a data-limited fishery in the channels of northern Patagonia.

III. MANUSCRIPTS IN PREPARATION

Chapter 1- Manuscript I

Effects of latitudinal gradient on reproductive indicators of a small-scale crab fishery in the South-East Pacific. Regional Studies in Marine Science (posiblerevista)

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ABSTRACT

Reproductive potential are key parameters in the life traits of exploited marine populations since they can change due to local environmental conditions and also due to different degrees of fishing intensity. The common strategy of most decapods to brood their eggs prior to hatching presents an opportunity to estimate fecundity potential. The capacity of decapods' males and females (eubrachyuran) to storage sperm in specialized organs also can be used as reproductive indicator associated to sperm transference capacity. In this context the present study estimated these reproductive indicators on a crustacean small-scale fishery (*Metacarcinus edwardsii*) along a latitudinal gradient (between 36° and 45°S) in three zones along the Chilean coast that represents comparatively different fishing intensities. These indicators were (1) Size at the onset of maturity, which was estimated associated to physiological and morphometrical sexual maturity of females and males separately; (2) Sperm transference capacity, which was evaluated through Vaso-Somatic and Receptacle-Somatic indices and (3) Fecundity potential, in terms of number of eggs per ovigerous female. Results indicated that differences in size at maturity along the latitudinal gradient where minimal, and where in the range estimated by other studies. Reproductive cycle also showed no differences with latitude. Sperm transference capacity showed no clear latitudinal pattern, in fact presented no differences between low and high fishing intensity zones. The exception was the gonadal size at maturity in males in the southernmost area that where in the lowest range estimated, which in conjunction with low females' fecundity could be suggesting that the fishery is affecting its reproductive condition or could be the result to local environmental adaptations since this area is in the southern range of this species distribution.

Key words: decapods, fecundity, sperm transference capacity, size at maturity, small-scale fishery

1. INTRODUCTION

Reproductive potential of an exploited marine population is a key parameter to understand population resilience associated to perturbations such as fishing. In crustaceans, changes in reproductive potential could be quantified in terms of fecundity and size at maturity since both are fundamental life-history parameters that are sensitive to changes and therefore, can be affected by the fishery (Poos *et al.* 2011; Lappalainen *et al.*, 2016; Militelli *et al.* 2020). Both can be used as reference points to be monitored and establish local regulations. Especially in species widely distributed, these parameters can vary spatially and temporally depending upon habitat characteristics (*e.g.* water temperature, food availability, depth) and fishing pressure (Smith & Saint-Marie 2004; Lappalainen *et al.* 2016; Ondes *et al.* 2017).

The common strategy of decapods to brood their eggs prior to hatching presents an opportunity to estimate fecundity potential, and, in the longer term, forecast recruitment, a key parameter adopted in models of fisheries dynamics (Maunder and Thorson, 2019). In other words, the detection of a decrease in fecundity may be an early sign of recruitment overfishing (Orensanz *et al.*, 1998) that could alert and trigger fisheries management actions. In crabs, sperm depletion is one of the known causes of female fecundity reduction since harvesting target larger males with greater sperm reserves leaving for reproduction undersized males with a lower sperm supply (Sato *et al.*, 2005; Sato *et al.*, 2007; Pardo *et al.*, 2015; Di Salvatore *et al.*, 2019). In decapods males store sperm and eubrachyuran females, storage them after mating, in specialized organs. The relative weight of these organs, with respect to their body weight, has been used as an indicator of sperm transference capacity, which can be expressed as Vaso-Somatic and Receptacle-Somatic Indices in males and females, respectively (Saint-Marie, 2007; Pardo *et al.*, 2015). The estimation of both indices can be adopted as a proxy of sperm depletion associated with fishing effort (Pardo *et al.*, 2015) and therefore could be complementary to studies associated to female fecundity. On the other hand, size at maturity has been proved to be sensitive to overexploitation, such that when sufficient energy is stored to invest in maturation, maturity can be attained at smaller sizes (Gangl and Pereira 2003). The continual removal of larger individuals from a fishery could have long-term implications associated to size at sexual maturation. This has in fact been demonstrated in an invasive lobster species in the United Kingdom, where high-effort removal of larger individuals resulted in a decrease in size at sexual maturation for the remaining population (Atherley *et al.*, 2021). On the other hand, the estimation of size at maturity also can be used as a reference point to establish minimum legal size (Bianchini *et al.*, 1998), a common

regulation that if not set with scientific bases can lead to growth-overfishing and recruitment-overfishing (Ungfors 2007, Pardo *et al.*, 2009).

Metacarcinus edwardsii (Bell, 1835) is a eubrachyuran crustacean distributed in the South-East Pacific from southern Ecuador down to the Strait of Magellan in southern Chile (Retamal, 1981). In Chile, catches are typically concentrated in the southern zone from 36°6' to 45°3'S, and in the last 10 years, total landings have averaged 4300 tons (Chilean national fishery statistics, SERNAPESCA). This species is only harvested by artisanal fishers, and therefore many coastal families depend on the resource for subsistence. Processing infrastructure is present at a range of scales from small scale family plants to more industrial units, depending on the harvesting volume. In 2017, 46 plants were processing this resource, although 10 of these processed 86% of the landings (provided by Sernapesca data base). A total of 11943 fishing licenses had been delivery (of a total of 17988) and up today the system does not allow new entries. Despite the socio-economic importance of this fishery for coastal communities, the availability of data to evaluate exploitation intensity is limited. In this context, *M. edwardsii* fishery is managed through Minimum Legal Size of extraction (MLS) and the permanent banned of females carrying eggs. MLS had been set at 120 mm carapace width for both sexes throughout most of the country, except between 40 and 44° S where MLS is 110 mm, according to size estimation at maturity in this region (Subpesca, 2011). Harvesting is permitted throughout the year with no seasonal closure. Because of the wide range of distribution of this species, it could be expected that reproductive parameters could varied spatially.

In this context, the present study aims to evaluate reproductive indicators of *M. edwardsii* along a latitudinal gradient (between 36° and 45°S) in three zones that represents different fishing intensity. These indicators were (1) Size at the onset of maturity, which was estimated associated to physiological and morphometrical sexual maturity of females and males separately; (2) Sperm transference capacity, which was evaluated through Vaso-Somatic and Receptacle-Somatic indices and (3) Fecundity potential, in terms of number of eggs per ovigerous female.

2. MATERIALS AND METHODS

2.1. Study areas

Three sampling areas in southern Chile were chosen to compare reproductive indicators of *Metacarcinus edwardsii* (Figure 1): Tomé (36°37'3.2" S; 72°57.35'W), Los Molinos (39° 51' 16.7" S; 73°23' 40.3"W) and Aguirre (45° 14' 24.37" S; 73° 40' 18" W). In these three localities, harvesting of crabs is only permitted using artisanal baited traps. According to annual statistics obtained over the last 10 years (2010-2020; Sernapesca, 2021), average landings have been 1.5± 1.1 tons in Tomé, 37± 31tons in Los Molinos, and 334 ± 289tons in Puerto Aguirre and Puerto Chacabuco (the main landing port near Aguirre). Because crab fishery operates close to landing ports in the three studied localities, landing statistics are considered to represent a reasonable proxy for the local fishing pressure. In this context, harvest intensity ranged from low in Tomé, high in the south (Aguirre) and intermediate fishing intensity in Los Molinos.

2.2. Field sampling

Individuals of *M. edwardsii* were collected directly from fishing vessels at the different localities. The fishing gear used by fishers, called nasa traps, is designed in the form of a truncated pyramid made of iron, enclosed with fishing nets. Although not all size classes enter the creels, enough individuals were collected with sizes between 33–154 mm for females and 44–175 mm for males (Table 1), ranges that are considered appropriate to include both immature and mature individuals (Olguín *et al.* 2006, Pardo *et al.* 2009). Individuals were collected over an annual cycle to detect seasonal changes associated with reproductive condition. Ovigerous females of different sizes were collected in June (2018) in Aguirre (n=51) and in March (2012) in Los Molinos (n=30) to determine fecundity (number of eggs per females) and reproductive yield (percentage of ovigerous biomass related to body biomass). All specimens were frozen on deck (-8°C) and remained frozen until analysis at the laboratory. Unfortunately, in Tome, fecundity could not be estimated since not enough ovigerous females were collected.

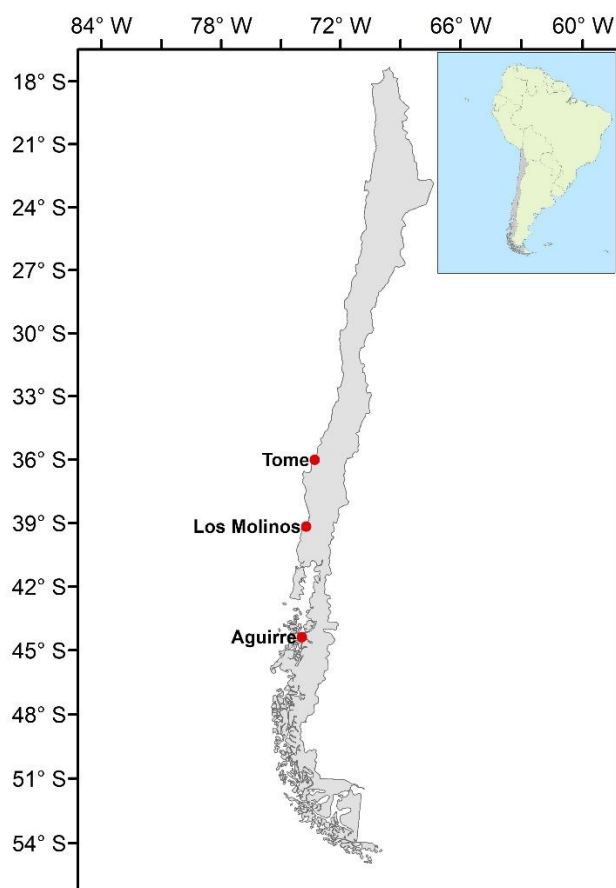


Figure 1. Sampling sites at the three localities in southern Chile: Tomé, Los Molinos and Aguirre.

Table 1. Sampling dates, carapace width (mm) of *M. edwardsii* for estimation of morphometric and gonadal maturity, and the number of dissected individuals for the estimation of reproductive indices in each studied area.

Area	Sampling date (month/year)	Size range for morphometric maturity (females/males)	Size range for gonadal maturity (females/males)	Number of individuals collected for reproductive indices (females/males)
Tomé	12/2015; 01/04/06/2012	78-138/86-164	78-138/86-164	72/43
Los Molinos	07-08-09-12/2011; 01-04-06/2012	71-148/44-167	71-148/66-176	40/433
Aguirre	04/2018; 06-08/2018; 11-12/2018; 03/2019	33-154/56-168	71-147/56-170	196/189

2.3. Laboratory methods

Crabs were allowed to defrost at room temperature in the laboratory before further processing. Before dissection, individuals were examined to determine sex, and morphometric measurements were made using a Vernier calliper (± 0.1 mm). Measurements to determine morphometric maturity included (1) a primary sexual character; carapace width

(CW, independent variable) representing the maximum width of the carapace measured from perpendicular to the antero-posterior midline of the carapace. And (2) secondary sexual character (dependent variable); in males this included height of the biggest cheliped, and in females, width of the fifth abdominal segment at its broadest part (Ungfors, 2007). To determine gonadal maturity, both males and females were dissected to determine development state according to the method of Pardo *et al.* (2009) which assigns to each individual a maturity category based on the macroscopically analysis of the vasa deferentia in the case of males, and development of ovaries in females.

Sperm transference capacity was evaluated through Vaso and Receptacle Somatic Indices. They were estimated through the dissection of both right and left vasa deferentia and seminal receptacles, in males and females, respectively. The dissected organs (vasa deferentia and seminal receptacles) and remaining body were dried in an oven for 72 hours at 70°C prior to determination of dry weight. Reproductive organs were weighted using an analytical balance (± 0.001 mg), and body parts using a standard balance (± 0.1 g). Vaso-Somatic Index (VSI) and Receptacle-Somatic Index (RSI) were calculated summing the dry weight of both right and left organs, which were expressed as a percentage of dry body weight without chelaes (methodology that had been improved from Sainte-Marie, 2007).

Female fecundity was estimated through the quantification of early-stage eggs (*i.e.* without ocular spots) of ovigerous females. In the laboratory, three subsamples of 500 - 600 eggs were randomly taken from each brood; the subsamples and the remaining brood were added separately with ammonium formate (desiccant) previously dried in the oven for 72 hours at 70°C. After removal from the oven, each subsample and remaining brood were brought to room temperature in a desiccator before being weighed using an analytical balance (± 0.001 mg). Fecundity was then estimated by extrapolating from the number of eggs in a known dry weight of subsample, to total number of eggs for the overall brood dry weight (ovigerous mass). Reproductive output was expressed as percentage of the weight of ovigerous mass (dry) to body dry weight. Weight of each egg was estimated relating the ovigerous mass with the total number of eggs.

Gonadosomatic Index (GSI) which reflects the degree of ovary development was estimated through an annual cycle in Tomé and Aguirre. To estimate this index, ovaries were removed and dried in the oven along with the remaining body for 72 hours at 70°C. GSI was then estimated by expressing the dry weight of the ovaries as a percentage of dry weight of the whole body.

2.4. Data analysis

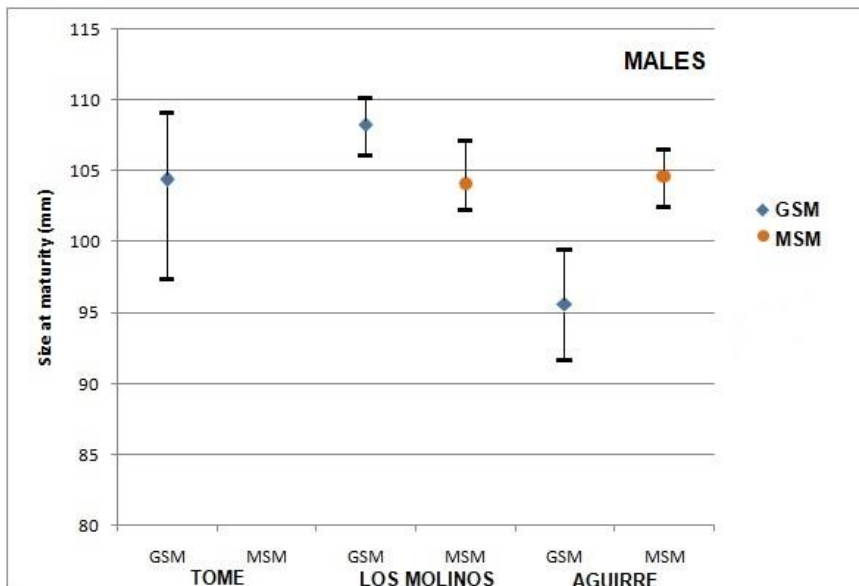
Estimation of size at gonadal (GSM) and morphometric maturity (MSM) for females and males were performed in R studio statistical software (version 2021.09.2) using the “SizeMat” package (Torrejon-Magallanes, 2020). The program classified individuals into two groups (juveniles = 0 and adult = 1) using principal component analysis with two allometric variables (x: independent variable, y: dependent variable) in log base. In the case of GSM estimation, both females and males were classified as mature when gonadal development attained stage 3 (Pardo *et al.* 2009). Maturity ogive estimation used the logit approach, and 50% maturity (L50) is estimated as the CW at which a randomly chosen specimen has a 50% chance of being mature (Somerton, 1980; Roa *et al.*, 1999). For MSM, the program estimates the relation between CW and the secondary sexual character (chela height in the case of males and fifth abdominal segment for females), at some point the program detected an inflection (breakpoint) where the increment in the size of the sexual character is greater than the increment in size of CW. The slopes before and after the breakpoint are estimated and compared statistically. The estimation of the breakpoint is found by iteration. This breakpoint is considered as the size at which MSM takes place. To test for MSM differences between areas, the overlap between confidence intervals was considered; therefore, no significant difference was established when intervals overlap. Statistical significances between two maturity curves associated to GSM estimations were calculated following Olson *et al.* (2018); maturity curves were estimated separately for each area and combined areas using a log-likelihood ratio test.

Data was tested for normality distribution using Kolmogorov-Smirnov test and Levene’s test was used for analyses of variance homogeneity. When data did not fit normality and homogeneity of variance was not established, non-parametric ANOVA (aovp) with permutation tests (lmPerm package, Torchiano, 2016) were used to search for differences. Posthoc tests used the rcompanion package to perform pairwise comparisons using permutations (Mangiafico, 2019). ANOVA test (aov) was used when data was normally distributed, and Scheffe test was used for posthoc analyses. To test for correlations between variables, Spearman rank (non-parametric) or Pearson correlation (parametric) coefficients were used depending on the variables were normally distributed or not. All the analyses were performed in Rstudio (2021.09.1+372 "Ghost Orchid" Release) for Windows.

3. RESULTS

3.1. Size at Gonadal (GSM) and Morphometric Maturity (MSM)

Data for estimation of CW at gonadal maturity of *M. edwardsii* were collected from 580 males and 363 females. Although low numbers of individuals were collected in some areas, maturity curves fit the data well and gonadal size at sexual maturity (GSM) could be estimated with statistical confidence (Table 2, Figure 2 and Supplementary Figures a-f). Size at gonadal maturity of females did not show a clear latitudinal pattern. Females in Los Molinos showed the lowest GSM (97.6 mm CW), with GSM being similar between Aguirre (100.5 mm CW) and Tome (101.7 mm CW), though still statistically different ($p < 0.001$). GSM estimates in males showed no differences between the northern areas (Tome and Los Molinos). Only GSM estimated in the southern area (95.6 mm CW in Aguirre) presented significant difference ($p < 0.001$) with Los Molinos (108.3 mm CW). Except for Aguirre, females showed lower GSM than males when comparing the same areas.



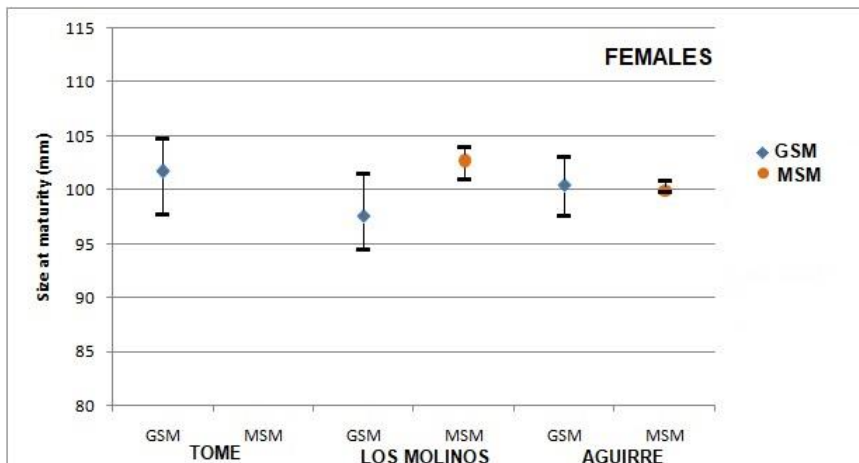


Figure 2. Size estimated for Gonadal and Morphometric Maturity for males and females of *M. edwardsii* at the studied areas, bars represent confidence intervals.

Table 2. Size estimated for Gonadal Maturity for males and females of *M. edwardsii* at the studied areas. N represents, number of individuals considered in the analysis.

Area	N	Size at maturity (mm)	Confidence interval	R ²	p-value
MALES					
Tomé	43	104.4	97.1-109.4	0.75	<0.001**
Los Molinos	322	108.3	106.1-110.1	0.78	<0.001**
Aguirre	215	95.5	91.7-99.4	0.74	<0.001**
FEMALES					
Tomé	71	101.7	97.7 - 104.8	0.64	<0.001**
Los Molinos	47	97.6	94.5 - 101.5	0.76	<0.001**
Aguirre	245	100.5	97.6 - 103.0	0.68	<0.001**

Data for estimations of morphometric size at maturity (MSM) were collected from 341 males and 429 females. In Tome, although the program estimated a breakpoint for MSM in males and females, the slopes before and after the breakpoint did not show significant difference (Table 3). Confidence intervals between MSMs overlap in Los Molinos and Aguirre for both males and females showing no significance differences. In males MSM varied only 1 mm between Los Molinos (104 mm CW) and Aguirre (105 mm CW). In females varied between 100 mm CW in Aguirre and 103 mm CW in Los Molinos. When comparing the same areas, females showed lower MSM than males.

Table 3. Size estimated for Morphometric Maturity for males and females of *M. edwardsii* in the studied areas. N represents, number of individuals considered in the analysis.

Area	N	Size at maturity (mm)	Confidence interval	R ²	p-value
MALES					
Tome	43	108.2	107.1 - 111.6	1	0.06
Los Molinos	105	104.2	102.3 - 107.1	0.94	<0.001**
Aguirre	193	104.7	102.5 - 106.5	0.96	<0.001**
FEMALES					
Tome	73	113.3	112.4 - 115.2	0.97	0.9
Los Molinos	64	102.7	101.0-104.0	1	0.046*
Aguirre	292	100.0	99.8 - 100.8	0.99	0.001**

In all the areas, when comparing GSM and MSM they do not present significant differences within the same area and sex. The exception was males in Aguirre, where GSM (95.6 mm CW) was smaller than MSM (104.7 mm CW).

3.2. Reproductive indexes

Vaso-Somatic and Receptacle-Somatic Indices (VSI and RSI, respectively) were estimated in the three studied areas over an annual cycle to examine different reproductive conditions. Since during some seasons fewer individuals could be collected (especially in Tome, see Table 4), the annual cycle was reduced to a warm period (spring + summer) and a cold period (autumn + winter). Both indices were calculated only for mature individuals, with size selected according to GSM estimations (for each area and considering sex differences). Size selection considered all individuals exceeding the confidence interval lower limit associated to GSM estimation. In this context, indices were analyzed to explore differences between periods and areas and considered potential interactions between both factors. Results indicated that VSI was significantly different between areas (aovp, $p < 0.001$) and an interaction between season and areas were found (aovp, $p < 0.001$). The only area where VSI presented seasonal variability was Los Molinos, where VSI in the cold period was higher (average $0.21 \pm SE 0.01\%$, aovp, $p < 0.001$) than in the warm one (average $0.14 \pm SE 0.005\%$). Estimated VSI in Los Molinos was higher than in Aguirre (average $0.13 \pm SE 0.004\%$) and Tome (average $0.12 \pm SE 0.01\%$) (aovp, $p < 0.05$). For Los Molinos and Aguirre, there were sufficient data to perform a more detailed analysis considering seasons not grouped as cold and warm. In Aguirre, no significant differences were found between seasons (aovp, $p > 0.1$). In contrast, in Los Molinos, statistical differences between seasons were estimated (aovp, $p < 0.001$); being higher in autumn (average $0.24 \pm SE 0.02\%$) than in the other seasons ($0.14 \pm SE$

0.004%). Other seasons showed statistical differences (pairwise comparison, $p < 0.05$), being higher in summer (average $0.15 \pm \text{SE } 0.007\%$) compared to spring (average $0.12 \pm \text{SE } 0.008\%$).

Table 4. VSI and RSI indices (average and number of individuals dissected) for males and females estimated in each season and area

Period	Season	RSI		VSI	
		Average (%)	n	Average (%)	n
AGUIRRE		0.08	175	0.13	169
COLD	Autumn	0.07	28	0.12	36
	Winter	0.08	71	0.14	32
WARM	Spring	0.07	38	0.14	56
	Summer	0.07	38	0.12	45
MOLINOS		0.16	51	0.17	429
COLD	Autumn	0.17	20	0.24	92
	Winter	0.13	14	0.19	90
WARM	Spring	0.17	3	0.12	98
	Summer	0.17	14	0.15	149
TOME		0.08	59	0.12	37
COLD	Autumn	-	-	0.16	2
	Winter	0.08	33	0.11	18
WARM	Spring	0.08	11	0.11	8
	Summer	0.07	15	0.14	9

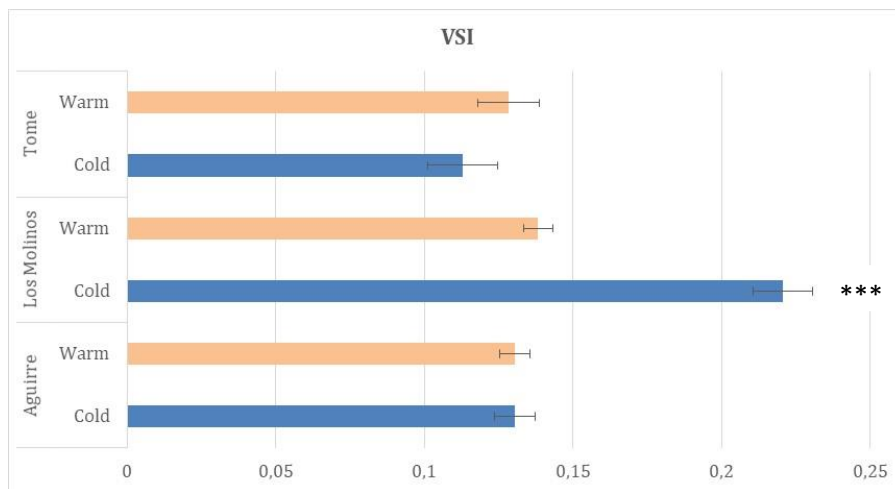


Figure 2. Vaso-Somatic Index in three areas during cold and warm periods. Data is presented as averages with standard errors. Asterisk represents significant different between periods in the same area.

Receptacle-Somatic Index (RSI) varied significantly between areas (aovp, $p < 0.001$) but not between cold and warm periods (Figure 3) and no interactions were found between both factors (aovp, $p > 0.1$). Within the same area no significant differences were found between cold and warm periods (aovp, $p > 0.1$). In Aguirre where enough data allowed seasonal comparison, no seasonal variations in RSI was detected (pairwise permutation, $p > 0.1$). Los

Molinos showed the highest RSI (average $0.16 \pm \text{SE } 0.009\%$) and was significantly different to Aguirre and Tomé (aovp, $p < 0.001$, Figure 3).

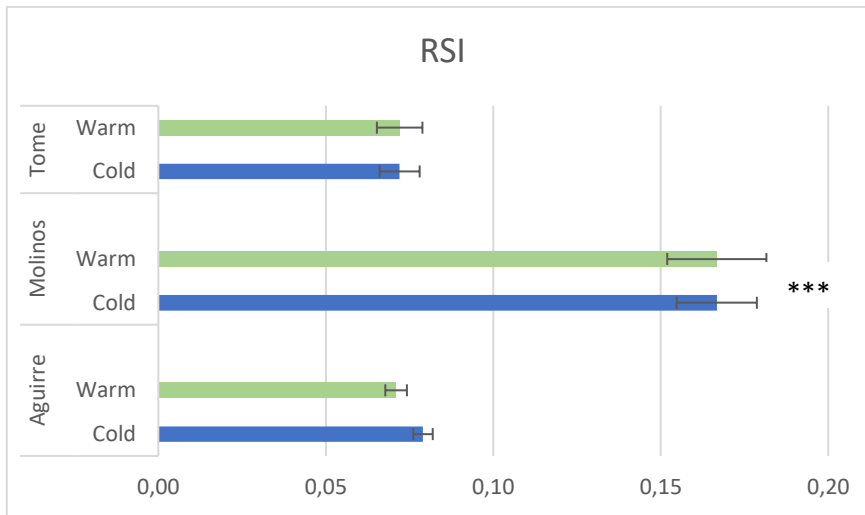


Figure 3. Receptacle-Somatic Index in three areas during cold and warm periods. Data is presented as averages with standard errors. Asterisk represents significant different between Los Molinos and the other area.

3.3. Fecundity

Expressed as the numbers of eggs carried by females, was estimated in Los Molinos ($n=30$) and Aguirre ($n=50$). This parameter was associated significantly with area (aovp, $p < 0.001$) and CW (aovp, $p < 0.001$); for the same CW class interval, females in Los Molinos carried 1.7-3 times more eggs than in Aguirre (aovp, $p < 0.001$, Figure 4a). In Los Molinos, fecundity was estimated to be 987,207 eggs per female ($\pm \text{SD } 285,143$), with CW of these females varying between 84.5 and 121.4 mm (average 104.8 ± 8.7 mm). Fecundity in this area was positively correlated with CW (Pearson correlation test; $p < 0.001$). In contrast, in Aguirre, no correlation was found between fecundity and CW ($p < 0.05$; Spearman correlation test; $p > 0.1$) being average fecundity estimated in 493,280 ($\pm \text{SD } 200,617$) eggs per female. CW of these females ranged from 97.9 to 140.5 mm (average $116.6 \pm \text{SD } 9.2$ mm). Regarding total weight of eggs per female (ovigerous mass), a positive relationship was observed with body weight and CW, in both Los Molinos (Pearson correlation test; $p < 0.001$) and Aguirre (Spearman correlation test; $p < 0.001$). An additional finding indicated that body weight of females carrying eggs in Los Molinos were heavier (average $44 \pm \text{SE } 2$ g) than in Aguirre (average $39 \pm \text{SE } 1$ g) (aovp, $p < 0.001$). This result led to have smaller reproductive output (which represents the relationship between ovigerous mass and body weight), in Los Molinos (average $35 \pm \text{SE}$

0.9%), compared to Aguirre (average $41 \pm \text{SE } 0.8\%$), with significant differences between them (aovp, $p < 0.01$). Low fecundity in Aguirre, was explored preliminary analyzing the weight of each egg, the results indicated that eggs in Aguirre (average 0.04 mg) could be as 3 times heavier than in Los Molinos (average 0.015 mg) being statistically different (aovp, $p < 0.001$). Higher frequency of ovigerous females ($>45\%$) were found between similar size range in Aguirre (110- and 120-mm CW) and Los Molinos (100- and 110- mm CW).

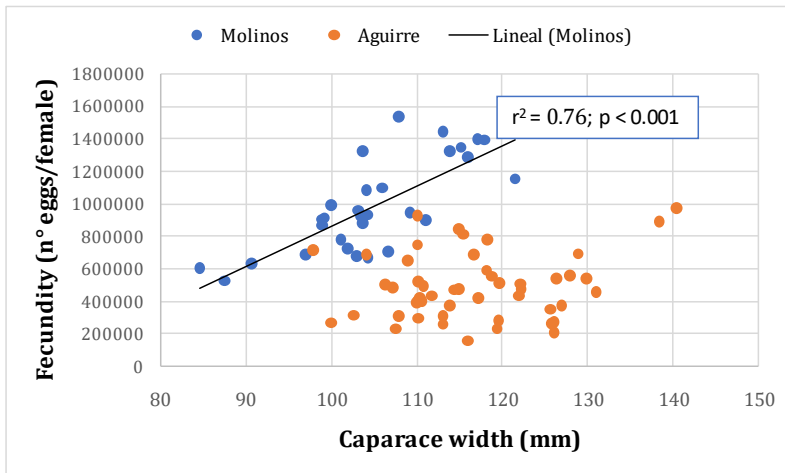


Figure 4. Fecundity (number of eggs per female) related to CW (mm) in Aguirre (orange dots) and Los Molinos (blue dots).

Gonadosomatic index (GSI) indicates the state of development of female gonads in relation to body weight, this index was evaluated over an annual cycle in Aguirre and Tome (autumn season missing). No significant differences in GSI were detected between areas (aovp; $p > 0.05$) but differences were observed between seasons (aovp; $p < 0.001$); GSI showed maximum gonadal development during summer (average $16 \pm \text{SE } 2\%$) and autumn in Aguirre (average $16 \pm \text{SE } 1\%$) and in summer in Tome (average $20 \pm \text{SE } 2\%$). In spring and winter, IGS was low in both areas, varying between 1 and 4% (Figure 5).

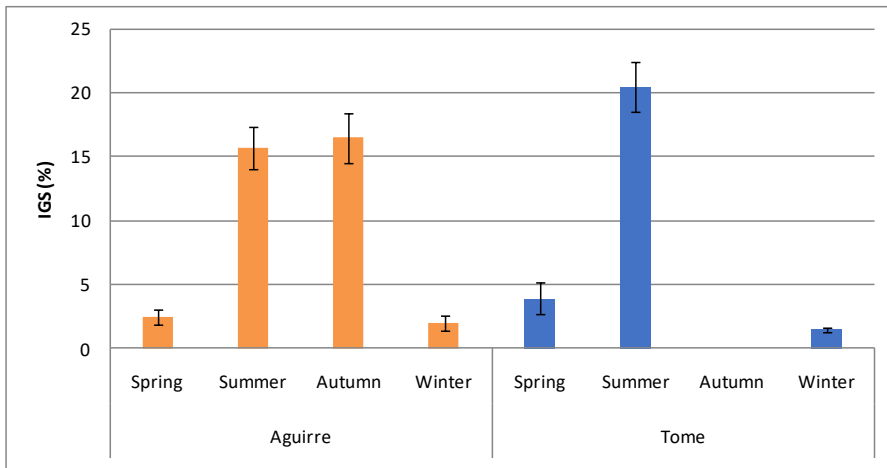


Figure 5. Gonadosomatic index (%) estimated over an annual cycle in Tome and Aguirre. No data was collected in autumn in Tome. Data is presented as averages with standard errors.

4. DISCUSSION

Reproductive potential of *M. edwardsii* was evaluated associated to sperm transference capacity, fecundity, and size at maturity in three different fishing areas, which represent different exploitation intensity, along a coastal latitudinal gradient separated as far as 1000 km (from 36° to 45°S). Since these reproductive parameters have been proved to be sensitive to environmental changes and exploitation intensity (Smith & Saint-Marie 2004; Poos *et al.*, 2011; Lappalainen *et al.*, 2016; Ondes *et al.*, 2017; Militelli *et al.*, 2020; Atherley *et al.*, 2021), a latitudinal pattern was expected. Despite this, most reproductive parameters did not respond to the north-south gradient of exploitation intensity described earlier in the methodology. Size at maturity was estimated examining gonadal development (physiological capacity to produce gametes) and morphometric measurements (morphological capacity to mate). GSM presented latitudinal differences but only for males, since it was significantly lower in Aguirre (96 mm CW) than in the northern areas; largest differences (of 13 mm CW) were found between this area and Los Molinos. No latitudinal differences were found in MSM for both sexes varying between 100- and 105-mm CW. Estimated GSM was similar to MSM for females and males in Los Molinos and only in females in Aguirre. Only for males in Aguirre, MSM was larger than GSM (~ 9 mm). No comparison could be done in Tome, since MSM could not be validated statistically (probably due to low number of individuals collected). It is expected that reproductive behaviour should begin after secondary sexual characters are developed specially in males in order to successfully mate. This because mating occurred during female moulting and to protect them during this vulnerable period, males need to have their chelipeds fully developed (Smith, 1992; Jivoff, 1998; Pardo *et al.*, 2018). In our findings, this difference (between gonadal and morphometric maturity) was not found, except for males in Aguirre, meaning that gonadal and morphometric development were almost synchronize. Studies done in Chiloe Island (41°S) in the same species (Pardo *et al.*, 2009), reported differences only for males where MSM was 17 mm larger than GSM (101 mm), being similar in females (GSM=101 mm; MSM=106 mm). Whether these differences should be larger or more similar in terms of deducing the fishery health status is a matter for discussion. For management, it could be more important to compare spatial differences and monitor temporal changes of both size at maturity since it could expect that over-exploited populations mature earlier to assure offspring (Pollock 1995). In this context, an earlier gonadal maturation in Aguirre compared to the other areas could be associated to high fishing pressure. Moreover, in an area classified as being of high intensity (41°S, Pardo *et al.*, 2017) a

similar size at maturity as in Aguirre had been reported (102 mm CW, Pardo *et al.*, 2009). Nevertheless, since growth models have not been developed for this species, is not possible to know the correspondence age associated to size at maturity, in other words, we have estimations related to size at maturity but the age at which this size is achieved might vary. This is especially feasible along the coast of Chile where a gradient in water temperature is clear (Camus, 2001), and probably other factors as quality and size of the food are different and hence, are affecting somatic growth (Bakke *et al.*, 2018). In this context, a smaller size at maturity estimated in Aguirre could be the result of slow growth due to low temperatures in southern Chile, which might lead to a similar age at maturity between individuals of different areas. For the moment, since these are the first estimates of size at maturity in Aguirre, they can serve as a baseline to monitor this fishery. Indeed, since this parameter had been detected to be sensitive to fishing intensity, the European Union (2010) recommends its estimations every three years. MSM estimates also serves to compare some management measures, such as the minimum legal landing size (Ungsfor 2007; Pardo *et al.*, 2009; Waiho *et al.*, 2017) since this measure pretend to ensure reproductive success, allowing individuals to reproduce at least once before being caught.

Sperm transference capacity was evaluated spatially and temporally through VSI and RSI in males and females, respectively. Results indicate that these indicators did not respond to the latitudinal gradient associated to fishing intensity. In fact, higher VSI and RSI were found in Los Molinos and in other sampled areas (Tome and Aguirre), both indices were lower and similar. Since this resource is not a target species in Tome (represents only 3% of crustaceans landings with an average of 1.5 tons in the last 10 years) as in Aguirre (where, excluding, king crab, marble crab represents 100% of crustaceans landings with an average of 334 tons in the last 10 years), our results apparently do not agreed with other studies that had found an inverse correlation between VSI and fishing intensity (Saint-Marie, 2007; Pardo *et al.*, 2015,). Due that no data is available to compare our results (this is the first time that this indicator is estimated in Aguirre, and in Tome, previous studies used a different methodology for the estimations) we used a different approach to be able to compare our results; Pardo *et al.* (2015) correlated *Vasa Deferentia* weight (VDw) with CW in areas with different fishing intensity during the mating season (spring-summer) and then, compared their slopes. Their results showed that in areas with high fishing intensity slopes were steepness compared to low fishing intensity. Our estimations indicated an increment of 1 mg in VDw per mm CW in the three studied areas, showing no differences between their slopes (Ismeans test; $p>0.1$). This increment was between values obtained in the study done by Pardo *et al.* (2015) where it

varies from 0.4 to 2 mg VDw per mm CW in high and low fishing intensity areas, respectively. So, even when in Aguirre and Tome, estimated VSI was lower compared to Los Molinos, no latitudinal differences found in the estimated slopes is indicating that the fishery is not affecting sperm reserves, this is especially true in Aguirre, where landings are 1-2 order of magnitude higher than in the other areas.

Reproductive cycle can be deduced from seasonal variability of VSI and RSI since after mating, VSI should decrease with a concomitant increase in RSI. Despite this, only VSI in Los Molinos reflected this pattern showing lower values in winter (late winter) and spring, followed by a recovered of sperm reserves in summer and to be fully recovered in Autumn (where it presented the highest value). Lack of temporal variability of the VSI in Tome and Aguirre could be associated with sampling dates. In both areas, sampling was conducted in late spring when mating could have already occurred and at the time of sampling, sperm reserves may have not been recovered. In Aguirre, where females carrying eggs were considered in the estimations of RSI, no seasonal changes were observed; suggesting that females do not deplete sperm reserves after mating. In Los Molinos, RSI and VSI were higher than in other areas. Fecundity estimations agreed with other indicators, being higher in Los Molinos compared to Aguirre (unfortunately no ovigerous females could be gathered in Tome). Low fecundity in Aguirre ($493,280 \pm \text{SD } 200,617$) could be comparable with populations in areas of high fishing intensity (Pardo *et al.*, 2017) but other considerations should be considered. Relationship between fecundity and weight of the ovigerous mass in Aguirre indicated that eggs were around 3 times heavier than in Los Molinos. Sampling dates in both areas were different, in Los Molinos it was in March and in Aguirre, in June, so heavier eggs could be the result of different degree of embryo development (Crisp 1959) or could be hypothesized that female in Aguirre invest more energy in the quality of their eggs that in the quantity, which coincides with lower VSI and fecundity registered in Aguirre compared to Los Molinos. This pattern where females in high latitudes invest in larger and hence better quality of the eggs at the cost of reducing fecundity had been registered in other species of Brachyuran along Chile (Brante *et al.*, 2003, 2004; Fischer *et al.*, 2009). So, it could be associated with local adaptation.

No latitudinal differences in IGS seasonal variability were found between Tome and Aguirre, observed seasonality coincides with previously described reproductive cycle for this species (Pardo *et al.* 2017); high IGS in summer and autumn and low during spring and winter are indicative of a reproductive cycle where, molt and mate occurred during spring, followed by gonadal development in summer and autumn, being winter, the season were females carrying embryos are found (low IGS, Figure 6).

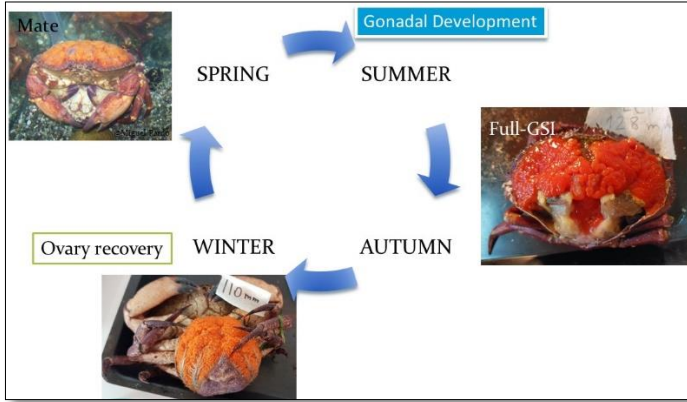


Figure 6. Reproductive cycle estimated for *M. edwardsii* in the three studied areas.

The present study is the first to analyze reproductive parameters of *M. edwardsii* along a latitudinal gradient in Chile, although in some areas, low number of individuals could be collected for analysis, preliminary results indicated no spatial variability. Low values of GSM and VSI in males in Aguirre in conjunction with low RSI and females' fecundity need further studies to confront if it is a reproductive strategy to adapt to local environmental conditions. Since *M. edwardsii* is a species that is distributed from Guayaquil (Ecuador) to the Strait of Magellan (Nation 1975), it is reasonable to think that adaptative strategies had been developed in Aguirre (southernmost distribution) in order to optimize its reproductive potential.

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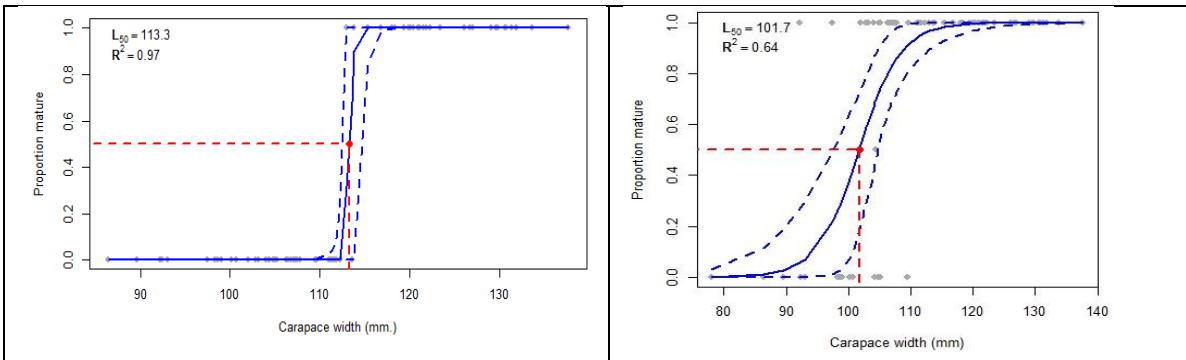
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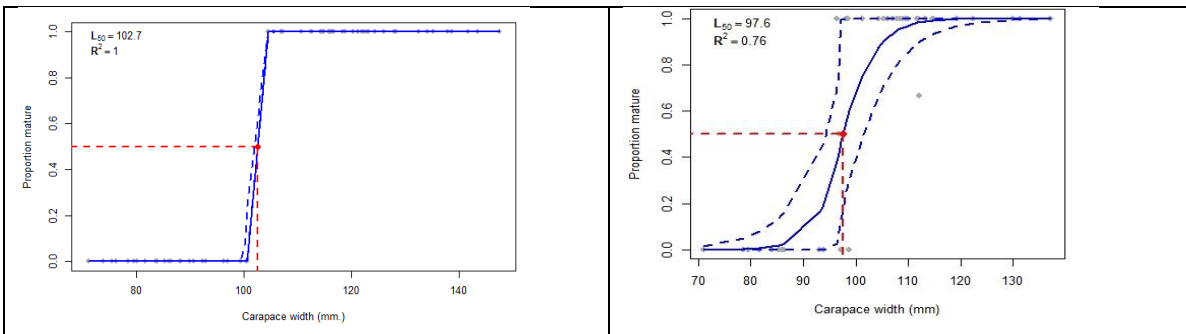
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6. SUPPLEMENTARY MATERIAL

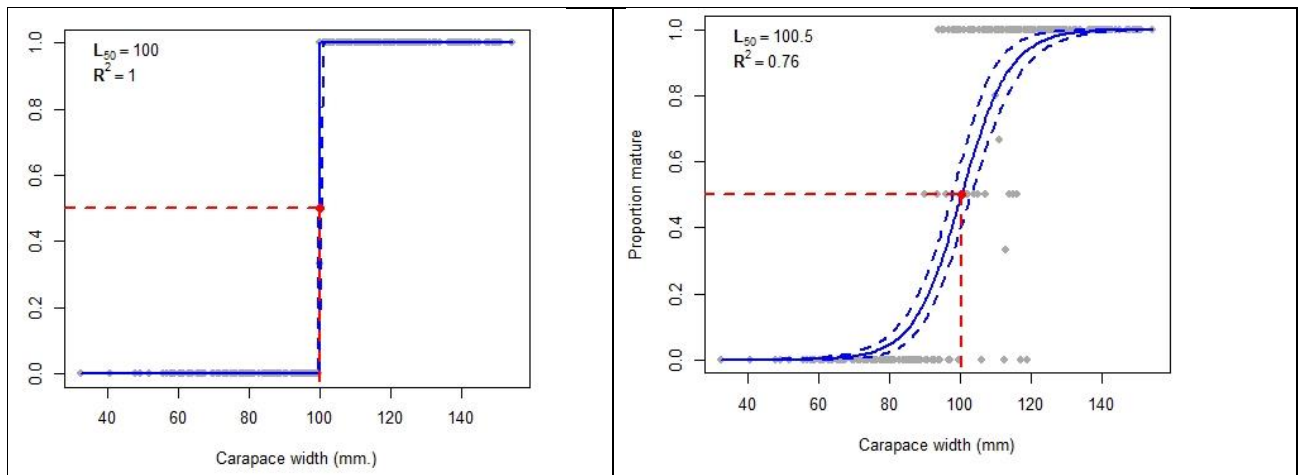
a) Females; morphometric (left) and gonadal (right) size at maturity: Tome



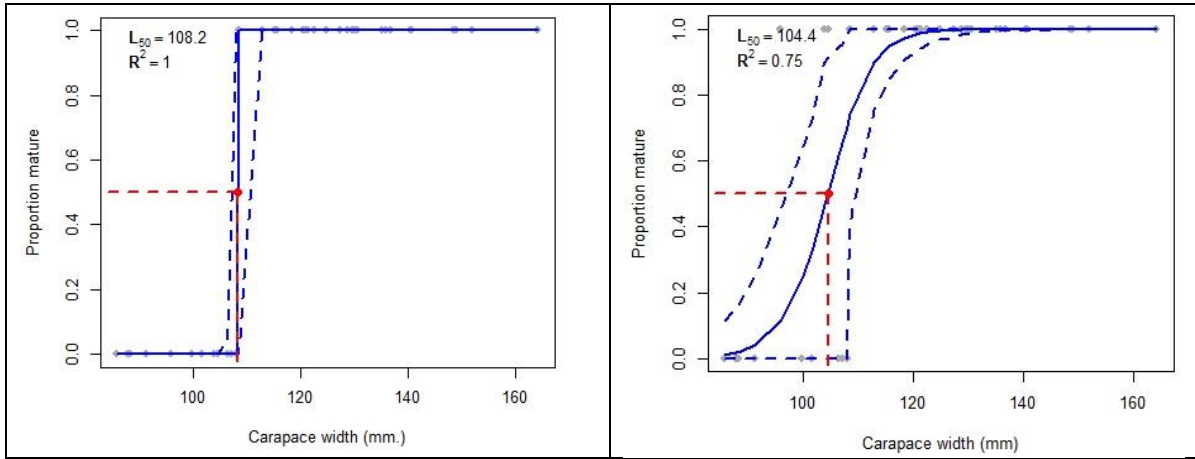
b) Females; morphometric (left) and gonadal (right) size at maturity: Los Molinos



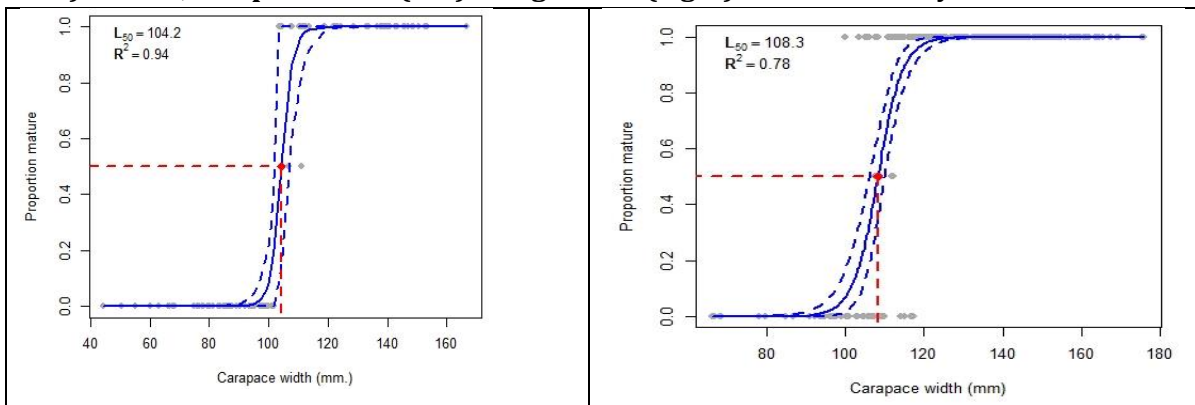
c) Females; morphometric (left) and gonadal (right) size at maturity: Aguirre



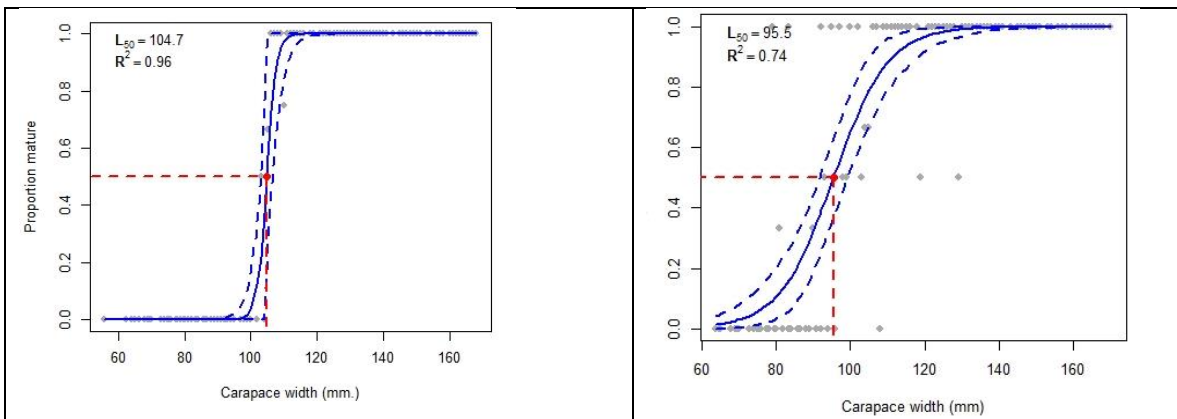
d) Males; morphometric (left) and gonadal (right) size at maturity: Tome



e) Males; morphometric (left) and gonadal (right) size at maturity: Los Molinos



f) Males; morphometric (left) and gonadal (right) size at maturity: Aguirre



Chapter 2- Manuscript II

Biological and fisheries indicators for the small-scale marble crab fishery in Northern Patagonia: recommendations for improving a monitoring program and stock assessment of a data-limited fishery

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ABSTRACT

Marble crab, *Metacarcinus edwardsii*, is a specie distributed along the coast of Chile, but main landings are concentrated in the south. Despite its socio-economic importance for local communities the fishery had remained data limited, and monitoring had yet not allowed to detect spatial and temporal variability that could indicate its health status. This exclusively artisanal fishery is monitored annually under a scheme that allows the registration of biological and fishing information, mainly from the landing ports and secondarily, from fishing grounds. This monitoring strategy have had limitations, especially south of 45°, since due to remoteness of fishing grounds combined with rough weather conditions, logistic and cost of data collection is difficult and expensive. In this context, data limitation had prevented the development of stock assessments models and so far, monitoring had not allowed to detect spatial and temporal trends associated to biological and fisheries indicators. In the present study we collected data in fishing grounds on board of artisanal vessels south of 45° during an annual cycle. Data collected were used to construct indicators based on catch per unit effort (over and lower minimum legal size), size, sex-ratio and relationship between weight and size. Results indicated that seasonal variability influenced biological, and fisheries indicators and some patterns could be concluded between fishing zones. Detected variability was in concordance with reproductive cycle being registered for this species. In this context, a monitoring design should select one or two seasons and zones that should be monitored along a time series to derive statistically significant patterns. The collection of information on board of artisanal vessels allowed the recording of some variables, otherwise no notice. Traps soak time was one variable that was probed to influence estimated indicators hence should be considered not to misinterpret catch and other indicators.

KEYWORDS: *M. edwardsii*, monitoring, data-limited fishery, North Patagonia, small-scale fishery

1. INTRODUCTION

Chilean fisheries are regulated by a new legislative framework since 2013 (General Law of Fisheries and Aquaculture, LGPA), which establishes the Maximum Sustainable Yield (MSY) as the main management objective (Tsikliras and Froese, 2018). This requires the implementation of stock assessments models, based on long term abundance data and life-history parameters which allow the estimation of Biological Reference Points (PBRs) (Collie and Gislason, 2001; Morgan *et al.*, 2014). In Chile, there are currently 44 species declared as fisheries, 27 of which have PBRs (SUBPESCA, 2021), a low percentage, considering that *ca.* 150 species of fish, crustaceans, mollusks, and other resources are reported in the national landing statistics. This implies that at least 70% of the species landed in the country do not have estimates of MSY, stock abundance, life history and fishery parameters. Most of these data-limited fisheries are identified in the category of Small-Scale Fisheries (SSF) (Béné, 2006). In Aysén region (43°38'-49°16'S), only SSF are allowed in the so-called "inland sea" since the marine ecosystem is restricted to narrow channels and fjords. Strong tides, strong seasonal changes in radiation, freshwater discharge and precipitation determine different oceanographic conditions creating numerous habitats that harbour a rich biodiversity (Försterra *et al.*, 2016). In these habitats, several fishing grounds have been developed over the years, maintaining a demersal, pelagic and benthic fisheries that are the main sustained for local economies. In these environments, temporal and spatial variation in harvest patterns could be expected not only in response to local fishing activities but also related to species distributions and behaviors. The detection of this variability through a monitoring strategy is crucial to identify seasons and/or fishing grounds that could be more vulnerable to fishing pressure.

Marble crab, *Metacarcinus edwardsii* (Bell, 1835), is one of the important species that is harvested in Aysén; in fact, 83% of local fishermen have fishing licenses to harvest it and at a national level some years the region had contributed up to 30% of the landings. This exclusively artisanal fishery is monitored annually under a scheme that allows the registration of biological and fishing information, mainly from the landing ports and secondarily from carrier vessels which transport commercial catch between fishing grounds and landing ports (Olguín and Mora, 2021). This monitoring strategy has limitations, especially south of 45°, where the fishery is concentrated, due to remoteness of fishing grounds (8 to 48 hours of sailing from the main landing port) and rough weather conditions, logistics are difficult and cost of data

collection is higher compared to northern areas. In this context, the data limitation has made it difficult to establish the state of the fishery, while current monitoring of the fishery has not made it possible to detect spatial and temporal changes in biological and fishery indicators. As a result, the regulation of this fishery is restricted only to limiting the entry of fishermen and boats, and the fishing gear. However, there are no regulations on fishing effort or total catch. Additionally, there is a prohibition of landing ovigerous females, and a minimum size of extraction for both sexes, separated in 110 mm carapace width for Los Lagos Region (40°13'-44°38' S) and 120 mm carapace width, for the rest of the country, Aysén Region included.

The main objective of this study was to estimate the temporal and spatial trends of the biological and fishery indicators of *Metacarcinus edwardsii* in its southernmost fishing grounds, the Aysén region. The results will allow making recommendations for the monitoring program.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in Chilean Western Patagonia, in an area located between 45°10'-45°30'S and 73°50'-73°20'W (Figure 1). The marine ecosystem in this region is influenced by a main channel, called Moraleda, that separates the archipelago to the west from the continental coast to the east. Fjords, channels, and numerous islands characterize the area, water exchange from the open ocean, glacier coverage, light regime and freshwater discharge varied latitudinal presenting a strong seasonal cycle that determine hydrographic conditions and hence productivity patterns (Aracena *et al.*, 2011; Montero *et al.*, 2011). Near the southern extreme of the Moraleda channel, there is a shallow sill (60 m deep), called the “Meninea constriction”, which partially isolate the southern part of the Moraleda channel and Aysén fjord from the open ocean, forming a deep basin. The sill constrains the circulation forming two basins (North and South); being the southern basin, warmer, less saline, and more oxygenated than the northern one (Silva *et al.*, 1995; Silva *et al.*, 2000). The Northern basin in its eastern part is influenced by water masses from Moraleda channel (Estuarine waters in the surface, modified subantarctic waters as an intermediate water mass, and at deep, equatorial subsurface waters) and Aysén fjord. Two water masses are present during the year at the mouth of this fjord, varying their depth according to the season. In summer, salty estuarine water dominates over 40 m, and then gets deeper in winter (around 100 m).

Below this water mass, modified subantarctic water mass dominate at different depth depending on the season (Guzman, 2004).

The study area was selected since it is the main regional fishing grounds associated to the extraction of crustaceans, i.e., marble crab (*Metacarcinus edwardsii*) and king crab (*Lithodes santolla*). Fishers live with their families very close to the fishing grounds (10 min to couple of hours) and the extraction is all year-round except for a summer break (usually between the middle of December till the beginning of March) being this cease related to commercial agreement with the buyers. In this context, ten fishing grounds were sampled trying to cover all seasons through an annual cycle (Figure 1). Five fishing grounds were localized in the northern basin above the Meninea constriction and the other five, in the southern basin. Zones were grouped considering oceanographic conditions associated to the Meninea constriction and the influence of the Aysén fjord, in this sense the northern basin was divided in west and east zones, and the southern basin, remained as a south zone (see polygons in Figure 1).

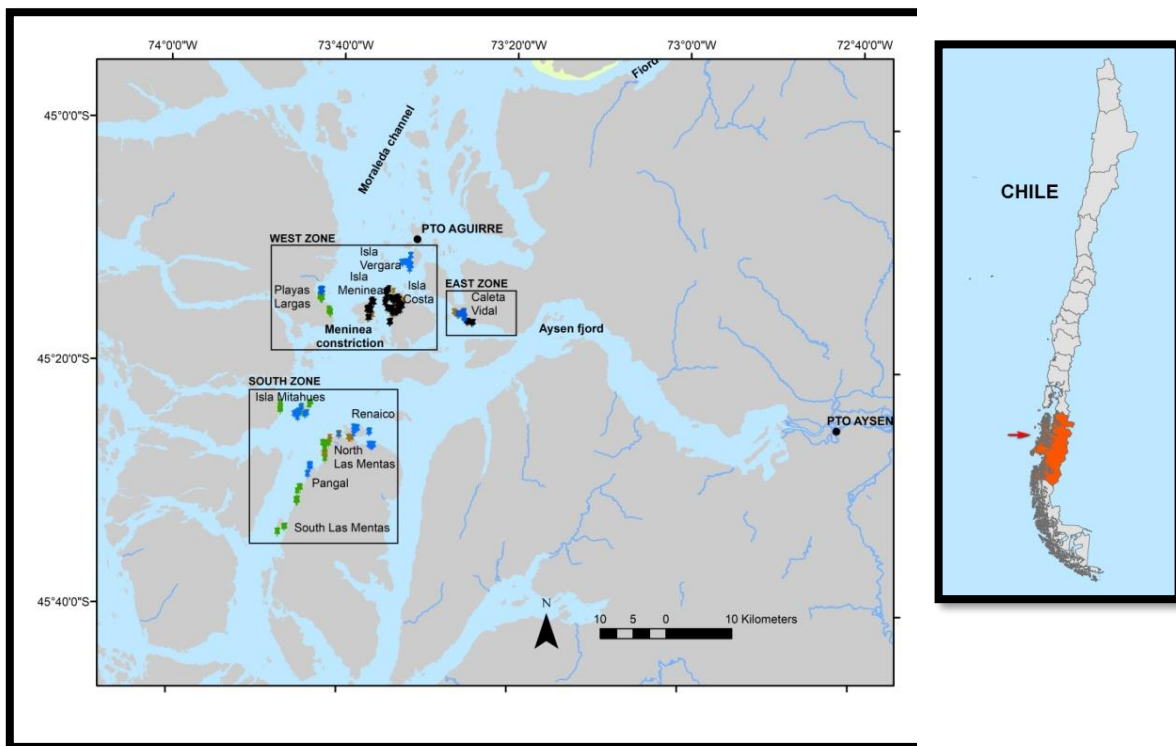


Figure1. Study area, red squares showed the main marble crab fishing grounds, south of Puerto Aguirre cove (45°10'S-45°30'W). Colors indicate the location of the fishing traps during autumn (brown), winter (black), spring (green) and summer (blue).

2.2. Sampling

In the study area, the fishery was monitored in different zones in four seasonal surveys (Table 1). Unfortunately, due to bad weather conditions, the southern zone could not be sampled during the winter season. Sampling was planned daily according to fishers' activity since the assessment was made onboard of fishing boats using their traps as the sample unit. Traps are the only fishing gear legally approved for crustaceans' extractions. Traps used by the fishers were conical and composed of 3 metal rings, one in the bottom, other in the middle, and a third upper ring to which a plastic entrance tunnel is attached; the diameter of the entrance varied between 25 and 35 cm (Figure 2). Most traps (84%) had escape windows with a diameter equivalent to 8 cm. Traps were individually set on the seabed at a distance greater than 50 meters between them, therefore, it is assumed that there is no overlap between the effective fishing areas (Aedo and Arancibia, 2003). The number of traps that each boat picked up per day varied between 10 and 37, which depends mainly on the size of the boat. The soak time registered for the traps varied between 1 and 31 days. Under good weather conditions and sufficient bait availability, fishermen go daily through all the traps (up to 26 in open boats with manual hauling and up to 37 in partly open boats equipped with davits), in these cases, the soak time was equal to 1 day. However, in bad weather conditions or limited bait availability, the soak time was longer. All the traps (99%) used during the study period had the same bait, which consists in a mesh of 3 to 4 kg of Chilean mussels (*Mytilus chilensis*). For each trap, the catch was recorded in number of individuals separated by retained (Carapace Width, CW, ≥ 120 mm) and released (CW < 120 mm) (D. Ex. No. 9 of 1990 establishes minimum legal size of extraction, MLS, of 120 mm CW). This procedure allowed the estimation of Catch Per Unit Effort (CPUE) in number of individuals. During each fishing trip, 3 or 4 traps were randomly selected to collect enough individuals that allowed biological sampling (Table 2). These traps were taken to the coast where individuals were measured, weighed, and sexed. For the estimation of size of individuals, CW was measured in their widest part of the carapace using a Vernier caliper with a precision of 0.1 mm, and body wet weight was (Total Weight, TW) registered using an electronic balance with a precision of 1 g.



Figure 1. Photographs showed boats and fishing gear commonly used by fishers to catch marble crabs in Aysén region.

Table1. Seasonal sampling in the different fishing zones, Aysén Region.

SEASON	SAMPLING DATE	FISHING GROUNDS
AUTUMN	24/03 till 03/04- 2018	East: Caleta Vidal West: Isla Costa, Isla Meninea South: Las Mentas, Renaico
WINTER	21- 24/06/2018 13-19/08/2018	East: Caleta Vidal West: Isla Costa, Isla Meninea South: No information
SPRING	26/11 till 06/12 -2018	East: Caleta Vidal West: Isla Vergara, Playas Largas South: Mitahues, West and South Las Mentas
SUMMER	14-23/03/2019	East: Caleta Vidal West: Isla Vergara, Playas Largas South: Mitahues, Pangal, Renaico

2.3. Data Analysis

The seasonal surveys carried out between autumn 2018 and summer 2019, allowed to have 632 independent and validated data associated to Catch Per Unit Effort (CPUE) of *M. edwardsii*. Soak time of this traps varied between 1 day (n = 402), 2 days (n = 126), 3 days (n = 63), 4 days (n = 36), and 31 days (n = 5). Since traps used in the extraction of marble crab have lateral escape windows, soak time could have influenced escape rates and hence CPUE. Accumulated CPUE can increase linearly or asymptotically with soak time or decrease after it has reached its maximum capacity (Fogarty and Addison, 1997). Consequently, the effect of soak time on released and retained CPUE was analyzed separately.

Seasonal and zone effects were analyzed based on 5 indicators: (i) released and retained CPUE, (ii) Proportional Stock Density (PSD), (iii) largest 10% of the sample (Lmax), (iv) sex ratio and (v) length-weight relationship. Total CPUE was not analyzed because it was influenced by both components of the catch in number (*i.e.* released and retained). PSD relates the number of individuals in a specific size class to the total number of individuals collected.

The limit sizes adopted were the MLS, and therefore PSD, estimates the percentage of individuals whose size exceeds these MLS. PSD was calculated regarding sex. This indicator had been proposed by Froesse (2004) describing it as “let them spawn” since is a measured of the percentage of mature specimens in the catch and hence can show trends of the status of a fishery. Size data was also explored considering the average size of the largest 10% of the sample (Lmax) and was selected as a measure that is less affected by environmental effects and recruitment variability (Miethe *et al.*, 2016). Sex ratio was estimated to identify spatial and temporal changes, which could reflect potential differences in the availability of both sexes, associated with reproductive patterns (Sato and Goshima 2006; Sato and Yoseda, 2010; Pardo *et al.*, 2017). Sex ratio was estimated as the ratio of the number of males in each trap to the total number of individuals (females + males), and was estimated separately for the entire size range, as well as for individuals ≥ 120 mm CW. A sexual ratio greater than 0.5 indicate an increased number of males compared to females, on the other hand, if proportion were less than 0.5, number of females exceeded the males. Length-weight relationship was calculated using the formula: $TW = aCW^b$, where TW is body wet weight of crab in gm, CW is carapace width in mm, “ a ” and “ b ” are intercept and slope of equation. Length-weight relationship was transformed to a linear model by taking the natural logarithms of both sides of equation (Patil and Patil, 2012), making the errors additive, and stabilizing the variances about the model. To test the isometric growth hypothesis ($b = 3$) was used a t -test: $t = \hat{b} - b_0 / SE_{\hat{b}}$, where \hat{b} , $SE_{\hat{b}}$ and df (degrees of freedom) are from linear regression and b_0 is the specified value in the H_0 (Maity, 2018). To compare the slopes of the regressions, first the coefficients of the slope were estimated using the nlme package (Pinheiro *et al.*, 2022), then the slopes were compared using least square means (lsmeans in R; Lenth, 2012).

Non-parametric ANOVA with permutations (using function aovp from lmPerm package; Torchiano and Wheeler 2016) were used to search for differences in CPUE (released and retained) and PSD estimated for males since data were not normally distributed (Kolmogorov-Smirnov test, $p < 0.05$) and presented no homogeneity of variance (Levene’s test, $p < 0.05$). Post hoc tests were performed using permutations pairwise comparisons (rcompanion package; Mangiafico, 2019). ANOVA test was used with Tukey test as *post-hoc* analyses for PSD (all data), PSD estimated for females, and sexual ratio since data presented a normal distribution.

All the analyses were performed in Rstudio (2021.09.1+372 "Ghost Orchid" Release) for Windows.

3. RESULTS

3.1. CPUE and soak time

For retained catch, mean, median and interquartile range statistics did not differ between 1 and 2 days of soak time (Figure 3a). After that, retained catch increased when soak time was 3 or 4 days. However, with 31 days of soak time retained catch decreased to values lower than those obtained with shorter soak times. For the released catch, high values were recorded in traps with 1 and 3 days of soak time, while the values were low in those traps with 2 and 4 days of soak time (Figure 3b). Once again, with 31 days of soak time, traps registered the lowest released catches.

The analysis of variance to test the null hypothesis that the means for retained catch for different soak times were the same, provided a value for the test statistic, $F = 15.11$, whose p -value was significant (Table 2, $p < 0.001$). Tukey's multiple comparison test indicated significant differences for retained catch between soak times of 1 and 2 days compared to 3 and 4 days ($p < 0.001$). The catch retained with 31 days of soak time was significantly different from that recorded with 3 and 4 days ($p < 0.05$). When the null hypothesis was relative to released catch, ANOVA also revealed a significant effect of soak time ($F = 19.11$; $p < 0.001$; Table 3). Tukey's multiple comparison test indicated significant differences ($p < 0.001$) for the released catch between soak times of 1 and 2 days, and between 2 and 3 days. For all other possible comparisons, no significant differences were found ($p > 0.05$).

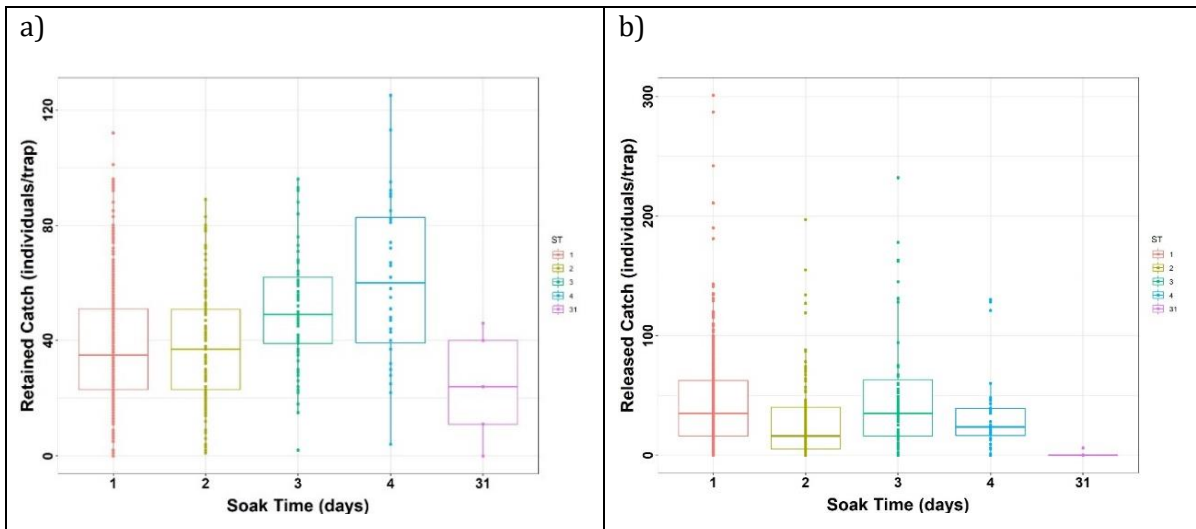


Figure 3. (a) Retained and (b) released catch (individuals/trap) of marble crab as a function of soak time (days).

Table 2. ANOVA results for the hypothesis test of equality means for retained catch between different soak times.

	d.f.	SS	MS	<i>F</i>	<i>p</i> -value
Soak time	4	25928	6482	15.11	< 0.001 ***
Residuals	629	269878	429	–	–

ns is not significant; * significant; ** very significant; *** highly significant

Table 3. ANOVA results for the hypothesis test of equality means for the released catch between different soak times.

	d.f.	SS	MS	<i>F</i>	<i>p</i> -value
Soak time	4	92.4	23.11	19.11	< 0.001 ***
Residuals	629	760.9	1.21	–	–

ns is not significant; * significant; ** very significant; *** highly significant

The effect of soak time on retained and released catch allowed selecting the most appropriate data sets for the analysis of spatial (fishing zones) and temporal (seasons) factors, for the different biological and fishing indicators. The analysis for retained CPUE considered catches with 1 and 2 days of soak time ($n = 528$). In contrast, released CPUE, sexual proportion, Proportional Stock Density and L_{max} , were analyzed only with the catches obtained with 1 day of soak time ($n = 402$). This due to significant differences found for released catch between 1 and 2 days of soak time, which could bias the results for those indicators that considered individuals <120 CW. For the analysis of the length-weight relationship, the information corresponding to all soak times ($n = 632$) was considered, since the relationship between both metric variables is not affected by the rates of entry and escape to the trap.

Table 4 summarizes the spatial and temporal distribution of the number of traps according to soak time.

Table 4. Number of traps analysed depending on soak time.

SEASON/Zone	Soak time (days)				
	1	2	3	4	31
AUTUMN	64	35	-	22	-
West	-	31	-	22	-
East	37	-	-	-	-
South	27	4	-	-	-
WINTER	90	12	40	1	-
West	71	12	40	1	-
East	19	-	-	-	-
South	-	-	-	-	-
SPRING	96	42	-	13	5
West	53	17	-	-	-
East	-	-	-	13	-
South	43	25	-	-	5
SUMMER	152	37	23	-	-
West	15	37	23	-	-
East	29	-	-	-	-
South	108	-	-	-	-
Total	402	126	66	36	5

Table 5. Total number of individuals collected for weight, and sex determination per zone and season collected in traps with one day of soak time.

ZONES	Autumn	Winter	Spring	Summer	Total
East	415	182	-	523	1120
South	554	-	919	1287	2760
West	-	740	613	189	1542
TOTAL	969	922	1532	1999	5422

3.2. Released catch

Due to catch dependence on soak times; when considering only one day of soaked time, released catch data was restrained in a way that no zones had data for all the seasons and only summer had data for all the zones. In this context, zones were analyzed separately searching for seasonal variability and only summer was analyzed to explore for differences between zones.

The only zone that presented seasonality was the west (Figure 3), where winter presented higher released catch (average $77 \pm \text{SE } 4$) compared to the other seasons, *i.e.*, spring and summer (pairwise Permutation Test; $p < 0.01$). When analyzing the summer season, no significant differences in released catch were found between zones (aovp; $p > 0.05$).

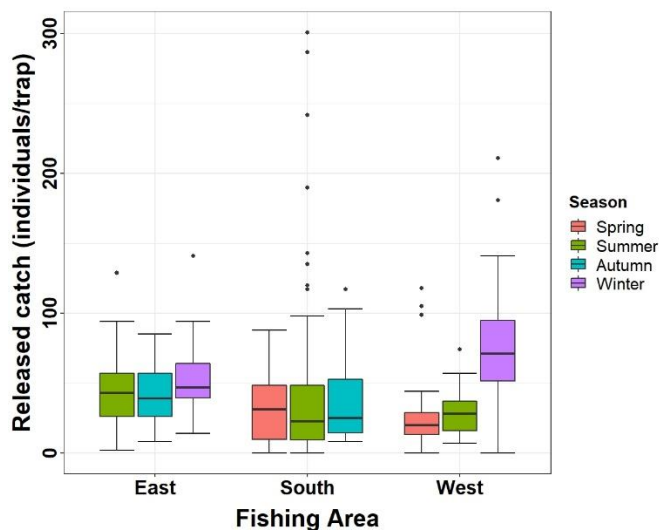


Figure 3. Released catch (n° of individuals/trap) along different seasons in fishing grounds in Aysen region.

Sizes analysis of the released catch showed that from 53 mm CW individuals enter the traps although highest frequencies were found between 104 and 119 mm CW (Figure 4). Seasonal variability in each zone was detected (aovp; $p < 0.05$; pairwise permutation test; $p < 0.05$); in the south zone higher sizes were found in summer and spring (average $105 \pm \text{SE } 0.5$ mm CW) compared to autumn ($103 \pm \text{SE } 1$ mm CW). In the west zone, summer ($105 \pm \text{SE } 1$ mm CW) was significantly higher than in the other seasons (spring and winter, 101 and 103 mm CW, respectively). In the east zone, higher sizes were found in autumn ($112 \pm \text{SE } 0.5$ mm CW) compared to summer and winter ($104 \pm \text{SE } 1$ mm CW). In summer where all zones could be sampled, no differences were found between zones (aovp; $p > 0.1$).

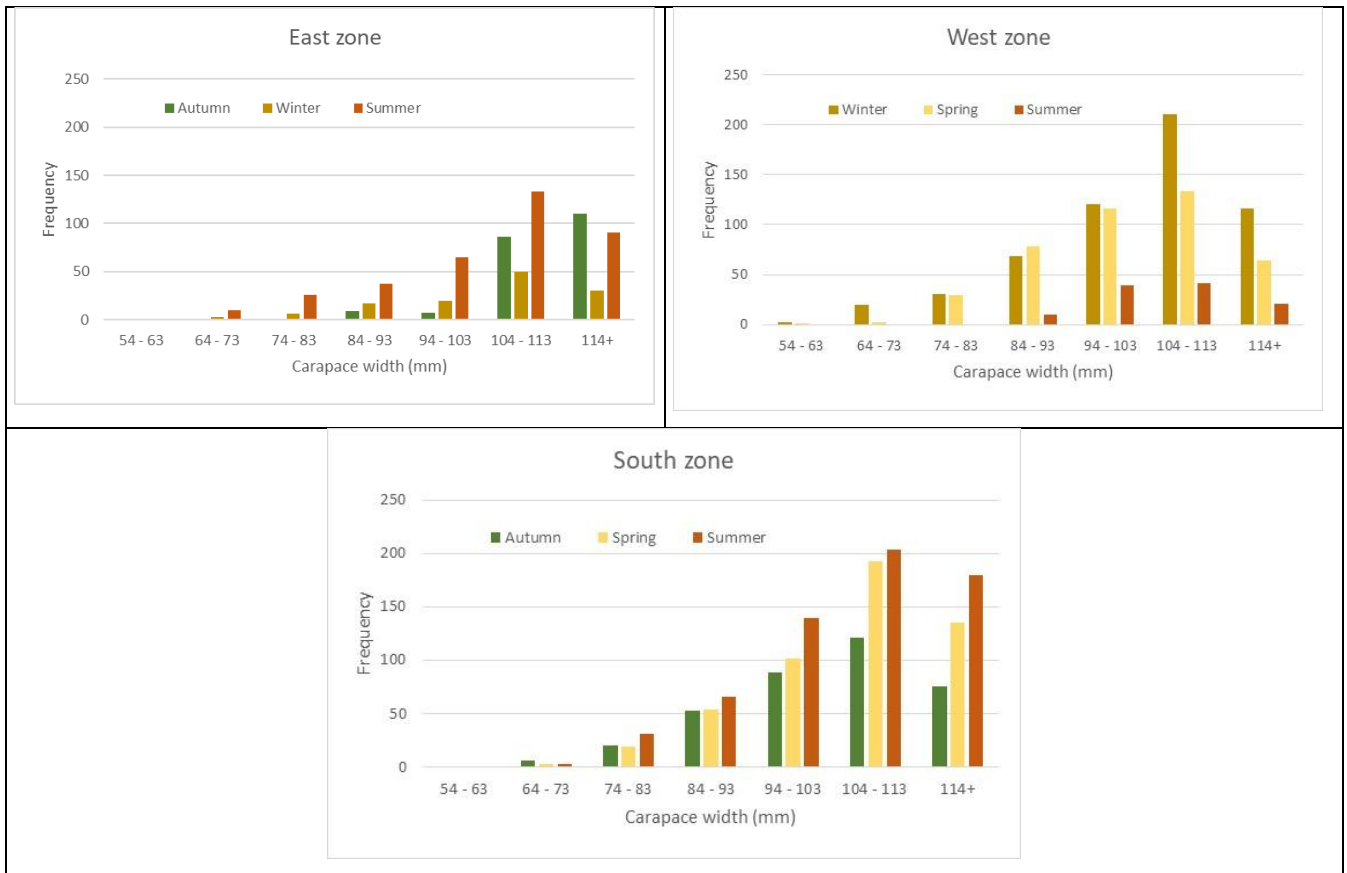


Figure 4. Size frequencies of released catch in the three sampled zones at different seasons.

3.3. Retained catch

As explained in the previous paragraphs retained catch was analyzed considering one and two days of soak time. In this context, the only zone with data for all seasons was the west and only summer and autumn had data for all the zones. In this context, zones were analyzed separately searching for seasonal variability and only these two seasons (summer and autumn) were analyzed to explore for differences between zones.

All zones presented seasonal variability (Figure 5); in the east zone, retained catch in autumn (average $53 \pm SE 2$) were higher than in winter (average $41 \pm SE 4$) and summer (average $43 \pm SE 4$) (pairwise Permutation Test; $p < 0.05$); in the south zone, autumn and summer presented no differences (average $34 \pm SE 2$ and average $37 \pm SE 2$, respectively) (pairwise Permutation Test; $p > 0.1$); and in both seasons, retained catch was higher than in spring (average $24 \pm SE 2$) (pairwise Permutation Test; $p < 0.001$). In the west zone retained catch in autumn (average $47 \pm SE 4$) and winter (average $50 \pm SE 2$) presented no differences (pairwise Permutation

Test; $p < 0.05$), being higher in both seasons compared to summer (average $38 \pm SE 2$) and spring (average $26 \pm SE 1$) (pairwise Permutation Test; $p < 0.001$). Retained catch in spring in this zone was the lowest of all seasons. In summer no differences were found between zones (aovp test; $p > 0.1$) but in autumn, retained catch was lower in the south (average $34 \pm SE 2$) compared to the east (average $53 \pm SE 2$) and west zones (average $47 \pm SE 4$) (pairwise Permutation Test; $p < 0.001$). Retained catch in these latter zones were similar presenting no significant differences (pairwise Permutation Test; $p > 0.1$).

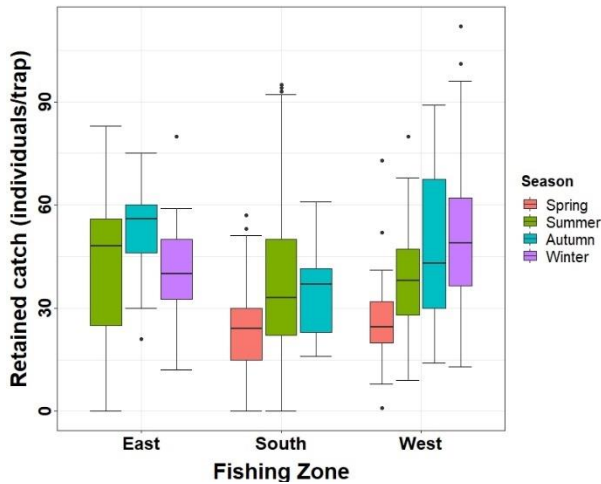


Figure 5. Retained catch in the three zones sampled at different seasons in Aysen region.

Maximum sizes of the retained catch were around 174-193 mm CW, highest frequencies were found between 120 and 130 mm CW (Figure 6). Seasonal variability in each zone was detected (aovp; $p < 0.05$; pairwise permutation test; $p < 0.05$); in the south zone higher sizes were found in spring (average $135 \pm SE 1$ mm CW) compared to summer ($133 \pm SE 1$ mm CW) and autumn ($131 \pm SE 1$ mm CW). In the west and east zone, pattern where the same, winter season (average 134 and 135 mm CW, respectively) was significantly higher than the other seasons (spring, autumn and summer and winter, between 130 and 132 mm CW). Autumn and summer were sampled in all zones, so both were analyzed to search for differences between zones. Results indicated that in autumn no differences were found between zones (aovp; $p > 0.1$) but in summer, the south zone had significantly higher sizes (average 134 mm CW) than the west (average 131 mm CW) and east zones (average 130 mm CW).

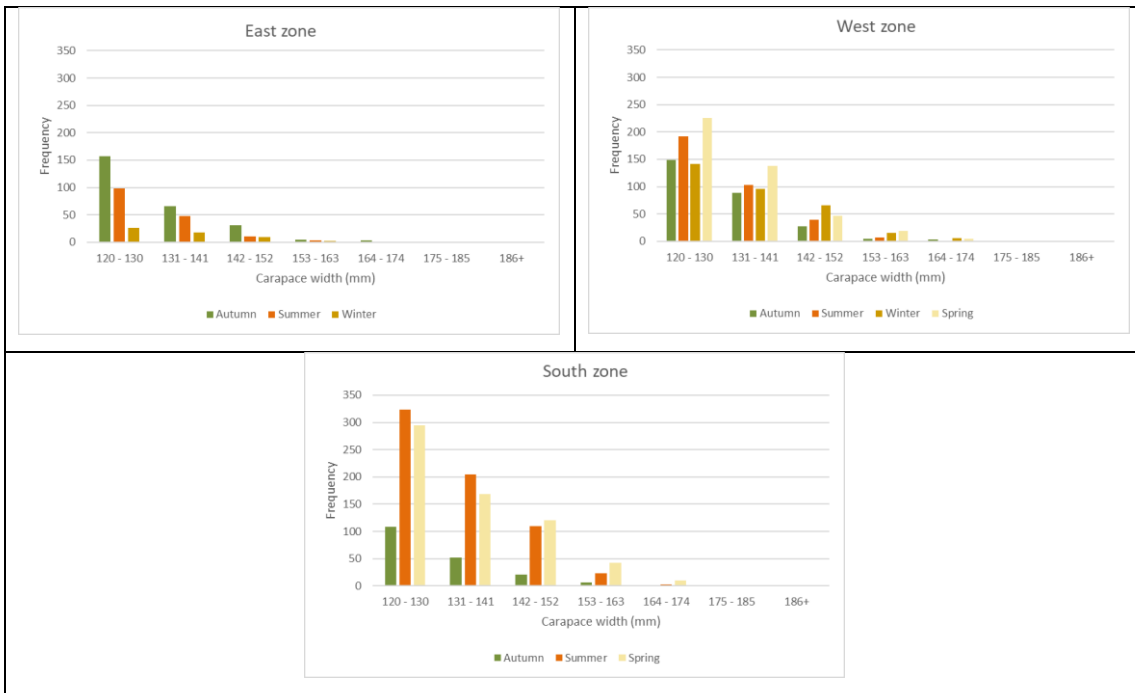


Figure 6. Size frequencies of retained catch in the three sampled zones at different seasons.

3.4. Proportional Stock Density (PSD) and Lmax

PSD estimated the percentage of individuals over the MLS (≥ 120 mm) in the catch and Lmax represented 10% of the biggest individuals in each trap. Both indicators were estimated using one day of soaked time.

Although PSD could not be estimated in all seasons in each of the sampled zones; a tendency could be observed as the seasons of the year progress from a minimum in winter, increasing towards spring and summer till a maximum in autumn (Figure 7). In the east zone, PSD was higher in autumn (average $60 \pm \text{SE } 2$) compared to summer (average $52 \pm \text{SE } 3$; pairwise Permutation Test, $p < 0.05$) and winter (average $44 \pm \text{SE } 3$; pairwise Permutation Test, $p < 0.001$). In the west zone, PSD could not be estimated in autumn, despite this, spring and summer (average $54 \pm \text{SE } 2$ and average $59 \pm \text{SE } 4$), presented a higher PSD compared to winter (average $40 \pm \text{SE } 2$; pairwise Permutation Test, $p < 0.001$). On the contrary, in the South, no significant differences were found between seasons (aov; $p > 0.1$) although winter was not sampled. In summer where all zones were sampled, no significant differences were found (aov; $p > 0.1$).

Lmax presented no differences between seasons in the same zone (aov; $p > 0.1$) and no differences in summer where all zones could be sampled (aov; $p > 0.1$). Lmax varied on average

between 136 and 147 mm CW, having a minimum value of 110 mm CW and a maximum of 170 mm CW.

When exploring for differences between females and males, PSD estimated in males (average $59 \pm \text{SE } 3\%$) were significantly higher than females (average $34 \pm \text{SE } 3\%$) (aov; $p < 0.001$). Lmax also presented significant differences between sexes (aov; $p < 0.001$), being higher in males (average $149 \pm \text{SE } 1$) compared to females ($135 \pm \text{SE } 1$) (Figure 8).

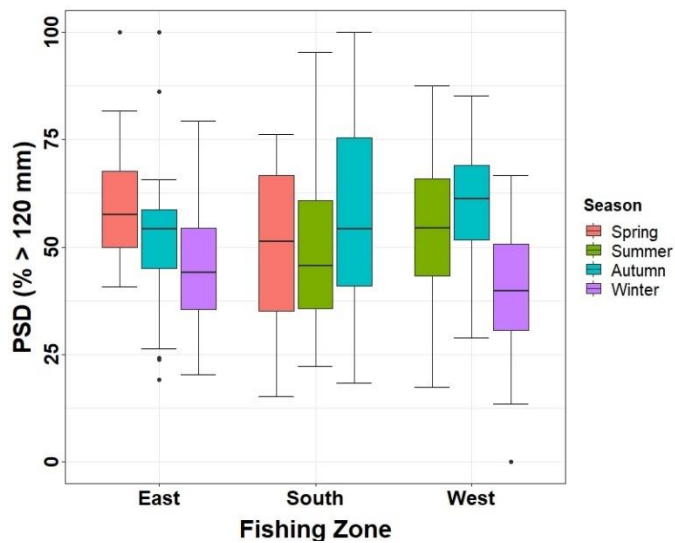


Figure 7. PSD (express as average percentage of individuals over 120 mm with standard error) in the three zones sampled at different seasons in Aysen region.

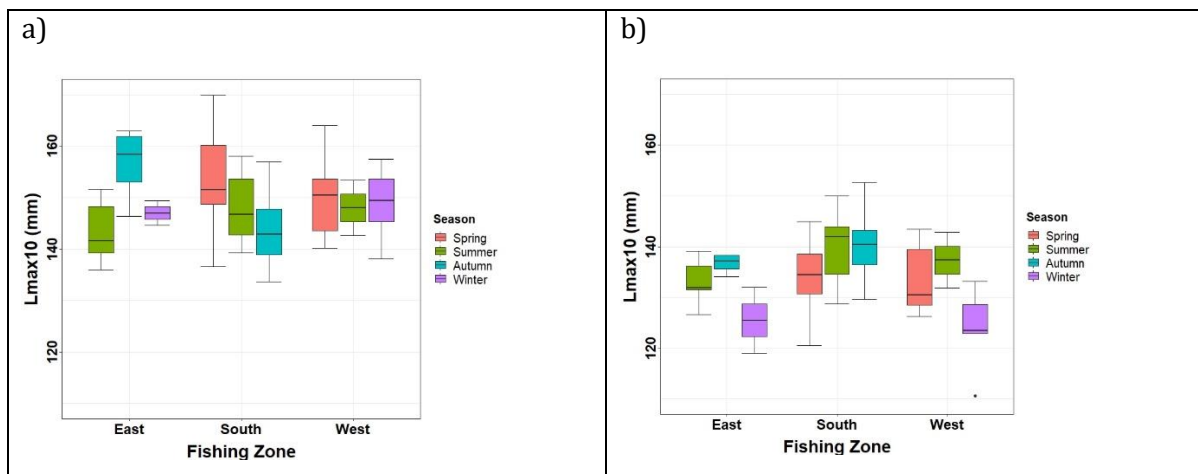


Figure 8. Lmax of (a) males and (b) females in three zones sampled at different seasons in Aysen region

3.5. Sexual ratio

Sexual ratio presented seasonal variability in all zones (Figure 8). In the east zone, winter was significantly different from autumn and summer (pairwise test, $p < 0.05$); a higher proportion of males (average $0.84 \pm \text{SE } 0.03$) were found compared to summer (average $0.48 \pm \text{SE } 0.03$) and autumn (average $0.39 \pm \text{SE } 0.01$). In these latter zones, no significant differences were found between them (pairwise test, $p > 0.05$). Sexual ratio in the west zone in winter present a high proportion of males (average $0.55 \pm \text{SE } 0.06$) but not as high as in the east. This season was significantly different from spring (Tukey test $p < 0.05$) and summer (Tukey test $p < 0.01$) where a higher proportion of females were found in the catch (average $0.39 \pm \text{SE } 0.02$ and average $0.22 \pm \text{SE } 0.05$, respectively). No significant differences were estimated between these two latter seasons (Tukey test $p > 0.01$). Winter season could not be sampled in the South, here, spring (average $0.59 \pm \text{SE } 0.03$) and summer (average $0.50 \pm \text{SE } 0.05$) presented no significant differences (Tukey test, $p > 0.1$), having a greater proportion of males compared to autumn (average $0.32 \pm \text{SE } 0.05$).

In summer, where data could be obtained for all zones, no significant differences were found between the zones (aov; $p > 0.05$).

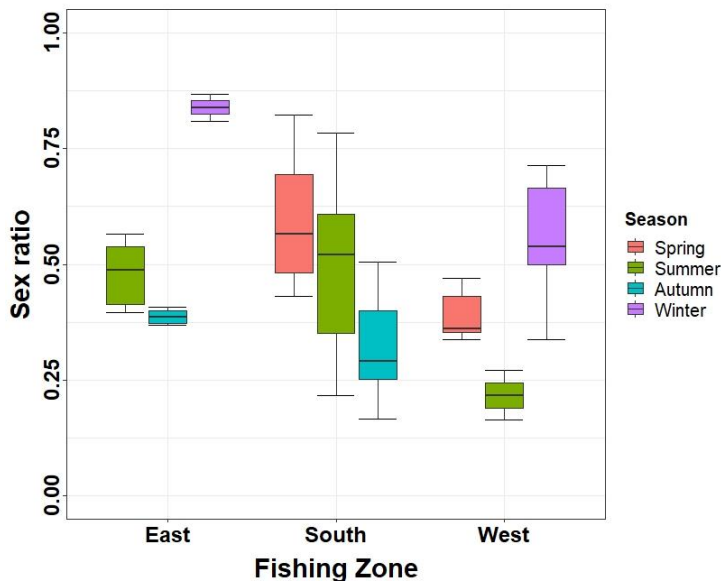


Figure 8. Sexual ratio in the three zones sampled at different seasons in Aysen region

Since sexual ratio could be biased due to *CW*, a new data analyses were incorporated considered the relation with size intervals. Results showed that *CW* intervals (aovp; $p < 0.001$) were significant (aovp; $p < 0.01$) to determine sex ratio. Class intervals showed that from 120 mm, there is a maintained increment of the number of males compared to females (Figure 9). In fact, when sex ratio was analyzed using sizes over 120 mm, seasonal differences in each zone remained the same as when considering all sizes, but a tendency of increasing sex ratio towards males were found in all seasons in each zone, the only exception was autumn in the south zone, where females dominated the catch (average $0.34 \pm \text{SE } 0.08$).

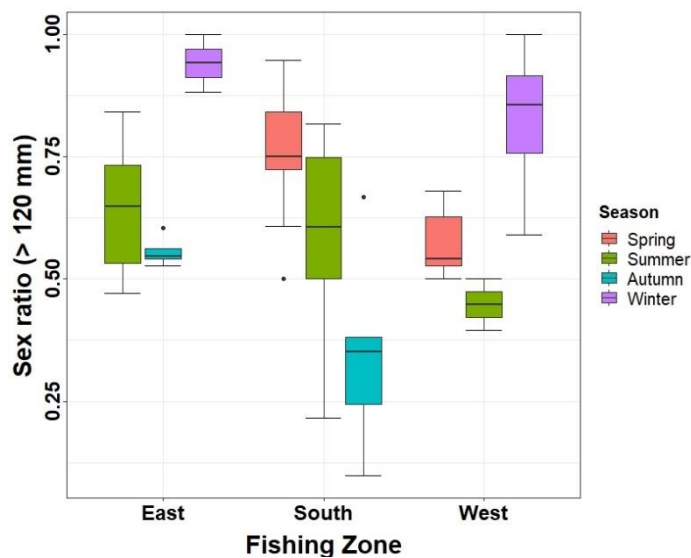


Figure 9. Sex ratio (express as average with standard error) of individuals over 120 mm in the three zones sampled at different seasons in Aysen region.

3.6. Length-weight relationship

A total of 3,289 males and 4,042 females were used for the spatial and temporal analysis of the length-weight relationship. The sample sizes varied between 591 and 2,033 individuals for the spatial analysis (zones), and between 515 and 1,361 individuals for the temporal analysis (seasons). The coefficient of determination R for length-weight relationships was significant in all zones and seasons ($p < 0.05$) (Tables 6 and 7). In terms of growth type, slope b was significantly greater than 3 in males and significantly less than 3 in females, indicating positive and negative allometric growth, respectively. The comparison between the slopes of the regressions determined a significant effect of zone ($p < 0.01$) and the season ($p < 0.001$) only in the case of males, but not so for females (in both factors: $p > 0.05$). The slope b of males collected in the eastern zone was statistically smaller than that estimated for the western

($p < 0.05$) and southern ($p < 0.01$) zones. In addition, the slope b of the length-weight relationship for males collected in spring was statistically higher than that estimated for autumn ($p < 0.001$), winter ($p < 0.001$) and summer ($p < 0.01$).

Table 6. Parameter estimates of the length-weight relationship for males and females of marble crab comparing between fishing zones. N (sample size), a log intercept (SD), b slope (SD) and R coefficient of determination. Lengths are presented for carapace (mm): CW_{\min} minimum observed length, CW_{\max} maximum observed length and mean CW (SD), mean TW in g (SD) and p -value corresponding to the isometric growth hypothesis ($b=3$).

Sex	Zone	N	a	b	R	CW_{\min}	CW_{\max}	Mean CW	Mean TW	p -value
Males	West	1,428	-9.161 (0.072)	3.119 (0.015)	0.968	54	168	122 (18)	362 (165)	<0.001 ***
	East	591	-8.851 (0.096)	3.053 (0.020)	0.975	50	172	120 (17)	337 (145)	0.005 **
	South	1,270	-9.292 (0.075)	3.150 (0.015)	0.970	53	173	125 (19)	402 (183)	<0.001 ***
Females	West	2,033	-8.273 (0.063)	2.931 (0.013)	0.959	64	165	112 (14)	272 (100)	<0.001 ***
	East	616	-8.367 (0.109)	2.949 (0.023)	0.964	65	152	114 (14)	280 (91)	0.014 *
	South	1,393	-8.234 (0.074)	2.932 (0.016)	0.961	62	163	113 (16)	283 (116)	<0.001 ***

ns is not significant; * significant; ** very significant; *** highly significant

Table 7. Parameter estimates of the length-weight relationship for males and females of marble crab comparing between seasons. N (sample size), a log intercept (SD), b slope (SD) and R coefficient of determination. Lengths are presented for carapace (mm): CW_{\min} minimum observed length, CW_{\max} maximum observed length and mean CW (SD), mean TW in g (SD) and p -value corresponding to the isometric growth hypothesis ($b=3$).

Sex	Season	N	a	b	R	CW_{\min}	CW_{\max}	Mean CW	Mean TW	p -value
Males	Autumn	515	-8.976 (0.094)	3.075 (0.019)	0.980	50	172	123 (19)	363 (164)	<0.001 ***
	Winter	733	-8.908 (0.077)	3.065 (0.016)	0.980	63	172	119 (18)	332 (158)	<0.001 ***
	Spring	1,086	-9.466 (0.087)	3.187 (0.018)	0.967	54	173	126 (18)	414 (188)	<0.001 ***
	Summer	955	-9.083 (0.097)	3.105 (0.020)	0.961	64	169	122 (17)	363 (153)	<0.001 ***
Females	Autumn	991	-8.247 (0.091)	2.922 (0.019)	0.960	70	163	117 (14)	299 (98)	<0.001 ***
	Winter	516	-8.352 (0.113)	2.943 (0.024)	0.966	64	149	107 (15)	232 (93)	0.001 **
	Spring	1,174	-8.130 (0.080)	2.901 (0.017)	0.961	62	165	112 (14)	274 (99)	<0.001 ***
	Summer	1,361	-8.376 (0.080)	2.956 (0.017)	0.957	63	156	113 (15)	282 (112)	0.001 **

ns is not significant; * significant; ** very significant; *** highly significant

4. DISCUSSION

Small-scale fisheries are mostly data limited, especially in developing countries since research investment is not imperative due to their low economic impact compared to that of industrial fisheries that contribute significantly to the national Gross Domestic Product (Zeller *et al.*, 2006; Cai *et al.*, 2019). Not considered in this analyze is the socio-economic benefits of this fisheries in coastal communities since in most of them, not only is the primary source of income but due to a strong sense of cultural identity with the activity it is considered a “way of living” (Purcell *et al.*, 2021). Fisheries in southern Chile are not the exception (Castilla and Fernandez, 1998), in Aysen region there are many small coves (fishers’ villages) and small-scale settlements (at a family scale) in which the subsistence primarily relies on artisanal fisheries (Molinet *et al.*, 2014). Despite this, the limited monitoring and therefore, the scarce data had prevented to estimate temporal and spatial trends in fishery and biological indicators that could allow to evaluate the stock status.

In our study we collected data of marble crab in main fishing grounds along Northern Patagonia through an annual cycle. Although data gathered present limitations since in some fishing zones data could not be obtained in all seasons, most estimated indicators presented seasonal variability and some patterns could be identified along the zones. In all the zones, retained CPUE was higher during autumn compared to one or more seasons of the year. On the contrary, in spring retained CPUE was the lowest. PSD also showed seasonal variability in the east and west zones, marked by the winter season, where the lowest PSD was estimated associated to high released catch found during this season. Lack of seasonal variability in the southern zone, could be explained by sampling impossibility in winter. Winter also influenced sex ratio; in this season, in the east and west zones, males dominated the catch. On the contrary, sex ratio in autumn was dominated by females. Seasonal variability of most indicators (CPUE, PSD and sex ratio) could be associated to the reproductive cycle of the studied species, which had been described as beginning with the mating season at the end of spring/beginning of summer followed by females brooding embryos from late austral fall till winter (Pardo *et al.* 2017). Under this reproductive pattern, it is possible that the reduced of retained catch observed in spring could be associated with mating, since it had been registered that males and females reduce their feeding response during this phase (Skinner and Hill, 1987; Kennelly and Watkins, 1994). Same explanation could be associated to the higher proportion of males in winter since in this season most females are brooding embryos

and they are not so easily drawn to fishing gear since it had been demonstrated that their foraging activity is reduced during this stage (Howard, 1982).

When released CPUE was considered, this component of the catch showed seasonality only in the western fishing zone, where the biggest catch occurred in winter. Under the assumption that the catch per trap is proportional to the abundance and availability of juveniles and adults, at least at short soak times (*i.e.* 1 day), an increase in the capture of individuals that could be classified as pre-adults (since was dominated by individuals between sizes of 104 and 119 mm) could be associated with migratory movements. This was not observed in the eastern zone, but it cannot be ruled out in the fishing grounds at the southern zone, since this geographical area was not visited during this season. In crabs, movement patterns in juveniles and adults have been mostly associated with changes in habitat use, to search for refuge sites, and reproductive migration events (Hines *et al.*, 1995; Muñoz *et al.*, 2006; Curtis and McGaw, 2008). Since, higher proportions of pre-adults were found in winter, most probably could be associated to refuge searching or to forage, since during this season food availability could be scarce.

Our results showed that over certain size (110 mm) catch was biased towards males. In winter, sex ratio could get as high as 11 males per female, being on average between 2-3 males per female. Although is not clear the optimal sex ratio, considering the traps as a sample unit that can reflect natural populations, the encountered of big males (L_{max} = average 149 mm) and on the other hand, sexual dimorphism found during the study, where males could be 10 mm larger than females, is concordant to results found in areas of low fishing intensity (Pardo *et al.*, 2017). Since in this species, females' mates after molt, studies had found that they preferentially mate with larger males since they need a pre- and post-copulatory embrace to assure survival (Pardo *et al.*, 2018). Also, a positive correlation had been found between sperm production and size, *i.e.*, bigger males can provide with more ejaculated sperm (Sato and Goshima 2006, Pardo *et al.*, 2015) and they can copulate with bigger females also, which will lead to a great female fecundity (number of eggs per females) (Sato and Goshima, 2006). Sexual dimorphism not only was associated to size but also to the relation between size and weight, negative allometry in females compared to positive in males had been already described for this species (Pool *et al.*, 1998).

These results, although preliminary, allow improving the monitoring of the fishery in Northern Patagonia. Due to remoteness of the fishing grounds and hard weather conditions prevailing in the region, actual monitoring had not allowed to detect temporal and spatial

patterns. In this context, our results indicate that the selection of some monitoring fishing grounds that could be visited over the seasons would be better than having scarce data with great spatial coverage. A good monitoring design to derive statistically significant patterns would be to select one or two seasons and zones that could be monitored along a time series. Moreover, the correspondence of indicators' temporal variability with the reproductive cycle of this species highlights the necessity to monitor in parallel its possible variations. Finally, the monitoring of the fishery on board of artisanal vessels allows the recording of various operational variables that otherwise would not been noticed, as the presence/absence of escape windows and soak time differences of the traps. The effect of escape window on the estimated indicators, could not be established since in our study more than 80% of the traps had them but literature had supporting evidence of a decrease undersized specimens in the catch with this window (Pacheco and Rodriguez, 1996; Aguilar and Pizarro, 2006; Arana *et al*, 2011). This benefits not only the fishermen that pick-up traps by hand decreasing the weight of each trap but also for the species since handling might affect survival. In our study, 56% of the traps had more than 1 day of soak time, and 16% had over 3 days, these allowed us to compare its influence on released and retained catch. The analyses showed that for the released catch, high number of individuals were found in traps with 1 and 3 days of soak time compared to 2 and 4 days. This pattern could be related to the duration of the attraction of the bait, which would encourage the entry of crabs into the trap for at least 4 days. Smaller crabs that enter the trap can later get out through the escape windows, in a cycle of entry and escape. In the case of retained catch, number of individuals increased from day 3 and probably till the bait is consumed, in our studies we skipped from 4 days till 31, so we do not know what the behavior is in between, but at least with 31 days, released and retained catch had the lowest values. Factors affecting the behavior of the specimens in the traps need to be further studied but for data analysis purposes soak time is a factor that should be considered to adequately interpret the data and patterns derived

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IV. GENERAL CONCLUSIONS

The present study is the first to analyze reproductive parameters of *M. edwardsii* in southern Chile (45°S) and to compared them in a latitudinal gradient considering different levels of exploitation. In parallel fisheries and biological indicators were estimated from data collected directly in fishing grounds (at 45°S) through an annual cycle to have a representative spatial coverage. Although due to bad weather conditions, not all zones could be visited in all seasons, estimated indicators were consistent, showing a seasonal variability consistent along the zones. In this context, our results indicate that an improved monitoring could consist in the selection of some fishing grounds that could be visited systematically in one or two seasons of the year to derived statistically significant patterns. The monitoring of the fishery on board of artisanal vessels allows the recording of various operational variables that otherwise could not been noticed, as soaked time of the traps and the presence/absence of escape windows. The combined use of the catch for the construction of the released catch and biological indicators obtained with different soaked times and with/without escape windows, could lead to errors in the data and diagnosis of the fishery.

Since the estimated indicators varied temporally and apparently, this variability is related to reproductive cycle, it is important to have this in consideration in order not to misunderstand the fisheries indicators (mainly catch per unit effort). Biological indicators mainly the size-based and sex ratio analyses showed that the fishery is in a healthy state. On the other hand, reproductive indicators, where not so clear for defining the state of the fishery, since gonadal size at maturity estimated for males in conjunction with low females' fecundity could be suggesting that the fishery is affecting the reproductive condition of the population. These results are not conclusively since when comparing weight of sperm reserves in males with other studies in areas with low fishing intensity results where similar. Reproductive cycle was deduced from reproductive indicators, showed no differences compared to northern areas being as follow: molt and mate occurred during spring, followed by gonadal development in summer and autumn, and hence in winter, females are found carrying embryos. Low values of reproductive indicators in Aguirre need further studies to confront if it is a strategy to optimize its reproductive potential in response to environmental conditions.

V. OTHER PUBLICATIONS DURING THE PERIOD

Madeleine Hamame & Paula Ortiz. 2022. Assessment of Exploitation Intensity of Commercial Species and Associated Benthic Communities, in Chilean Marine Management Areas of North Patagonia. *Front. Mar. Sci.*, 8 <https://doi.org/10.3389/fmars.2021.635756>

Paula Ortiz, Eduardo Quiroga, Paulina Montero, **Madeleine Hamame**, Federico Betti. 2021, Trophic structure of benthic communities in a Chilean fjord (45°S) influenced by salmon aquaculture: Insights from stable isotopic signatures. *Marine Pollution Bulletin* 173(2):113149. DOI: 10.1016/j.marpolbul.2021.113149