

Factors Modulating the Female Reproductive Performance of the Fiddler Crab *Leptuca uruguayensis* with Short Reproductive Season

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Abstract. This study aimed to evaluate the factors modulating the female reproductive performance of the fiddler crab *Leptuca uruguayensis* (Nobili, 1901) during the short reproductive season of a temperate population. We proposed two modulating factors: the age of females (young and old) and the periods of the reproductive season (beginning, middle, and end); we then evaluated the fecundity, reproductive output, egg volume, and biochemical composition of eggs. The fecundity of *L. uruguayensis* was affected by the size of females, a variable related to their age. Although young females showed lower fecundity, the reproductive output was not affected by the age or by the periods of the reproductive season, suggesting a constant reproductive effort, proportional to female size. The egg volume decreased, and carotenoid content increased at the end of the season for both female ages, probably as a consequence of variations in food availability and changes in the breeding strategies during the season. However, the content of protein and lipids in the egg clutches decreased at the end of the season only in old females spawning for the second time in the season. The main differences in the reproductive parameters were recorded between the beginning and the end of the reproductive season, probably because in these periods females exclusively use one of the breeding strategies. Finally, we determined that both factors, that is, fe-

male age and the periods of the short reproductive season, can modulate the reproductive performance of *L. uruguayensis* in temperate estuaries.

Introduction

Biotic and abiotic factors modulating reproduction have ecological and evolutionary implications for animal species. Knowledge of the effects of these factors on reproduction may allow predicting the ideal conditions for successful production of offspring (Giménez and Anger, 2001; Marshall and Keough, 2004; Birkeland and Dayton, 2005). Biotic factors influencing the female reproductive performance include the physiological condition (Wu *et al.*, 2010; Tropea and López Greco, 2015), the age (Wouters *et al.*, 1999; Jones *et al.*, 2014), and the nutritional status of the females (Ruffino *et al.*, 2014; Stahlschmidt and Adamo, 2015; Domitflia *et al.*, 2016), whereas abiotic factors include temperature, salinity, and photoperiod (Steele and Steele, 1991; Menassol *et al.*, 2012; Thatje *et al.*, 2019).

The physiological condition and nutritional status of females determine their ability to achieve gamete production, mating, and maternal care, all of which in turn influence the quality and success of the offspring (Prasad *et al.*, 2011; Horváthová *et al.*, 2012; Hayes *et al.*, 2013). Therefore, females in good physiological and nutritional conditions can achieve higher egg quality (size and biochemical composition), higher number of eggs (fecundity), higher number of clutches, and higher survival of newly hatched individuals (Hernández Herrera *et al.*, 2001; Wootton and Fletcher,

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Abbreviation: CW, carapace width.

2009; Ruffino *et al.*, 2014; Stahlschmidt and Adamo, 2015; Guzmán *et al.*, 2020). The age of the female has also been shown to affect the offspring in different ways. For instance, young mature females of different taxa have shown lower fecundity and lower-quality offspring than old mature females (Massot *et al.*, 2011; Chang *et al.*, 2017), probably because of the trade-off between reproduction and growth (Jones *et al.*, 2014; Cohen *et al.*, 2020). Some studies have suggested that the improvement of reproductive traits with the age of the female could be related to the increase in body size, which allows the production of more offspring (DelGiudice *et al.*, 2007; Tsujimoto *et al.*, 2016). In decapod crustaceans, some studies suggest that senescent females have higher-quality offspring (Verísimo *et al.*, 2011; Marciano *et al.*, 2018), whereas others have found that the age of the female does not affect fecundity or egg size (Arcos *et al.*, 2003; Graham *et al.*, 2012).

One of the most important abiotic factors that affect several reproductive traits of populations is temperature (Clarke *et al.*, 1991; Yampolsky and Scheiner, 1996; Cardoso and Defeo, 2003; Bywater *et al.*, 2010). In tropical climates, the small annual thermal amplitude allows continuous reproductive activities, and successive broods tend to be homogeneous throughout the year (Bauer, 1989). In contrast, in temperate climates, the reproductive seasons are usually restricted to the warmer months (Boa and García, 2015; Grassi *et al.*, 2018). In decapods inhabiting temperate climates, the quality and quantity of the progeny can vary between the beginning and the end of the same reproductive season (Guzmán *et al.*, 2016; Naderi *et al.*, 2018), even in short reproductive seasons extending for three or four months (Vázquez *et al.*, 2013).

The combination between the general status of females and the environmental conditions generates different scenarios that demand appropriate strategies for females to maximize reproductive success (Reznik, 1985; Barnes and Partridge, 2003). *Leptuca uruguayensis* (Nobili, 1901) is a fiddler crab species inhabiting estuarine areas, from Rio de Janeiro state, Brazil (tropical estuaries), to Buenos Aires province, Argentina (temperate estuaries). To the north of its geographical distribution, this crab reproduces throughout the year (Costa *et al.*, 2006; Hirose *et al.*, 2013), whereas to the south, its reproductive season is short, occurring in the warmer months (November to February) (Colpo and López Greco, 2017). In recent years, the habitat of *L. uruguayensis* has expanded toward higher latitudes (Truchet *et al.*, 2019), which makes relevant the study of the factors that modulate its reproduction. In temperate estuaries, the life expectancy of *L. uruguayensis* is around two years (Spivak *et al.*, 1991), and adult females experience two reproductive seasons during their lives. In their first reproductive season, adult females are small and young and have only one clutch in the whole season, whereas in their second reproductive season, females are larger and older and can produce a maximum of two clutches in the season (Marciano *et al.*, 2021). Young and

old females of *L. uruguayensis* spawn during the short reproductive season, which is divided into the beginning (November), middle (December and January), and end (February). At the beginning of the reproductive season, most females spawn using reserves previously stored during the pre-reproductive period (capital breeding strategy) (Marciano *et al.*, 2021). In the middle of the season, some females use capital breeding strategy, and other females use resources acquired during the season by feeding to mature their ovaries and then spawn (income breeding strategy) (Marciano *et al.*, 2021). Therefore, in the middle of the reproductive season, it is possible to find females spawning using both breeding strategies at the same time. Finally, at the end of the reproductive season, the predominant strategy is income breeding (Marciano *et al.*, 2021).

The aim of this study was to evaluate whether the reproductive performance of *L. uruguayensis* females in a temperate population is modulated mainly by the age of females (young and old) or by the periods of the reproductive season (beginning, middle, and end). The reproductive performance of females was assessed by estimating fecundity, reproductive output, egg volume, and biochemical composition of eggs (contents of carotenoids, total proteins, and total lipids), considering that total proteins are the main component of the mature oocytes of *L. uruguayensis* (Bert *et al.*, 2016; Colpo and López Greco, 2018). We expected that the age of females modulates fecundity and reproductive output due to the strong relationship of these traits with female size (Fox and Czesak, 2000). Also, we predicted that the periods of the reproductive season will affect the volume and biochemical composition of eggs, especially between the beginning and the end of the season, because the different breeding strategies used by females in these periods might impact offspring quality.

Materials and Methods

Study area and reproductive biology

The fieldwork was performed in the locality of General Lavalle, Samborombón Bay, Río de la Plata estuary, Buenos Aires province, Argentina. In this study area, *Leptuca uruguayensis* (Nobili, 1901) inhabits a muddy sand area of about 3500 m² in the intertidal zone of the Saladero canal (36° 25'0.3" S–56°57'11" W; Fig. 1). The tides are semi-lunar and semi-diurnal, with amplitude of about 1 m in spring tides (Acha *et al.*, 2008). Crabs were collected during low tides in the high intertidal zone.

The indeterminate growth of fiddler crabs allows the relation of their age with their size (Yamaguchi, 2002). Therefore, knowing that adult females of *L. uruguayensis* can experience 2 reproductive seasons during their lives, we considered that females sized between 7 and 12 mm of carapace width (CW) are young adult females that are having their first reproductive season and those larger than 12 mm of CW are



Figure 1. Geographical distribution of *Leptuca uruguayensis*. The northern limit of the *L. uruguayensis* geographical distribution is Rio de Janeiro state, Brazil, where females reproduce continuously during the year (Costa *et al.*, 2006), whereas the southern limit is Buenos Aires province, Argentina, where most females reproduce once a year during the warmest months (November to February) and only some large females can reproduce twice in the same reproductive season (Marciano *et al.*, 2021). The study area was the General Lavalle locality, in Samborombón Bay.

old females that are in their second reproductive season (Spivak *et al.*, 1991; Marciano *et al.*, 2021). The moment of hatching determines the size, period, and energy management strategy for reproduction of young females. Females that hatch early (November) have time to grow and mature before the beginning of their first reproductive season as adults. These females spawn once at the beginning or in the middle of the reproductive season, using accumulated reserves (capital breeding strategy). Females that hatch late (February) have less time to grow and mature before the beginning of the reproductive season (November); thus, they spawn either in the middle or at the end of the reproductive season. To reproduce, these females use the food resources available in the

environment at the moment (income breeding strategy). In this first reproductive season, young females have only one clutch in the whole season, because they are also allocating energy to somatic growth. After this season, young females (both of early and late hatching) grow during the non-reproductive period (March to October) and reach the second reproductive season of their lives as old adult females. These old females allocate less energy to growth and can thus produce a maximum of two clutches in the season. They initiate the reproductive season with sufficient accumulated reserves to spawn by using the capital breeding strategy at the beginning or in the middle of the reproductive season. Some old females can have a second spawn in this season, about 60 days later, using the income breeding strategy.

Sampling and laboratory processing

Leptuca uruguayensis females were sampled at the beginning (November), middle (December and January), and end (February) of the 2018–2019 and 2019–2020 reproductive seasons. Crabs were sampled two or three times in each period of the reproductive seasons to ensure an appropriate number of replicates.

On each sampling day, a trained person collected ovigerous females for one hour. Because females of *L. uruguayensis* remain in the burrow during the egg incubation period (Ribeiro *et al.*, 2016; Colpo and López Greco, 2017), it was necessary to dig 40 cm deep to sample them. Ovigerous females were taken to the laboratory, where they were examined under a stereoscopic microscope to determine the stage of embryonic development. Only females with eggs at an initial stage of development were used. The initial stage of development was characterized by eggs in the early blastula stage, identified by the uniform distribution of yolk, small embryo size, and no visible eye pigments (Litulo, 2005; Guzmán *et al.*, 2016). Females were sacrificed by cold anesthesia for 10 minutes. The female maximum CW was measured with a calliper. The egg clutch was removed from the pleopods with fine forceps, dried with absorbent paper, and weighed (egg clutch wet weight). Females were dried with absorbent paper and weighed (female wet weight). The accuracy of the balance was 0.1 mg. The ovigerous females were randomly distributed to determine the reproductive traits evaluated.

Fecundity

The fecundity in decapod crustaceans is usually estimated by counting the number of eggs carried by ovigerous females in the pleopods and is called realized fecundity (Steachey and Somers, 1995; Ramirez Llodra, 2002; Militelli *et al.*, 2019). In this study, the realized fecundity of each female was estimated by counting eggs from five clutch subsamples of about 3 mg (~200 eggs) and extrapolating the average number of eggs to the wet weight of the total clutch.

Reproductive output

The reproductive output is a widely used estimator of reproductive effort in decapod crustaceans because it relates the size of the female with the size of the egg clutch, indicating the amount of resources that individuals use for reproduction (Bell, 1980; Hines, 1991; Tsujimoto *et al.*, 2016). In this study, the egg clutches and females were dried at 45 °C for 3 days until they reached a constant weight (egg clutch dry weight and female dry weight). This information was used for the determination of the reproductive output of *L. uruguayensis* (%reproductive output = (egg clutch dry weight/female dry weight) × 100) (Clarke *et al.*, 1991).

Egg volume

Egg volume is usually used as an indicator of the quality of the clutch, because small eggs could indicate not only a smaller quantity of yolk but also a smaller size of the embryo (Kuris, 1990; Moran and McAlister, 2009). To estimate the egg volume of *L. uruguayensis*, 10 eggs per clutch were randomly selected to be photographed under an Axioplan microscope equipped with a Zeiss Axiocam ERc 5 camera (Oberkochen, Germany). The images obtained were analyzed with the Zeiss Zen 2.3 Lite software. Egg volume (mm^3) was calculated according to the formula $1/6\pi D^3$ (Jones and Simons, 1983; Peiró *et al.*, 2014), where D represents the mean of the maximum and minimum diameter of the egg.

Biochemical composition of eggs

In decapod crustaceans, the biochemical composition of the eggs depends on the nutrients that females transfer for embryo development (Koopman *et al.*, 2015). Consequently, this is a measure of the nutritional status and physiological condition of the female (Vargas Ceballos *et al.*, 2018). In this study, the egg contents of carotenoids, total proteins, and total lipids were determined. For this, the egg clutches were taken from the pleopods, dried with absorbent paper, weighed, and kept in a freezer at -80 °C until biochemical analysis.

For carotenoid content determination, egg clutch samples of about 15 mg were homogenized with 1.5 mL of acetone and then left for 72 hours in the refrigerator at 4 °C. Carotenoid content was determined spectrophotometrically at 475 nm, according to the methods described by Renström *et al.* (1981) and Torrissen and Naevdal (1984), with modifications. Values are expressed as microgram carotenoids per milligram eggs.

For total protein determination, egg clutch samples of about 20 mg were homogenized in 4 : 1 volume (μL) : weight (mg) with 50 mmol L^{-1} Tris-HCl buffer (pH 7.5) and then centrifuged at 10,000 g for 30 min in a refrigerated centrifuge (4 °C). Supernatants were diluted 1 : 20 (v/v) with distilled water. Total protein was determined by the Coomassie blue dye method, in a spectrophotometer at 595 nm (Bradford, 1976). Bovine serum albumin was used as standard. Values are expressed as milligram total proteins per gram eggs.

For total lipid determination, egg clutch samples of about 50 mg were homogenized in 20 : 1 volume (μL) : weight (mg) of a mixture of chloroform and methanol (2 : 1 v/v), then mixed and centrifuged with 0.9% NaCl to separate the lipid fraction. Lipids were determined by the sulfophosphovanillin method, according to Folch *et al.* (1957) and modified by Frings and Dunn (1970), and measured with a spectrophotometer at 530 nm. Extra-virgin olive oil diluted with absolute ethanol was used to build the standard curve. Values are expressed as milligram lipids per gram eggs.

Statistical analysis

To evaluate how the factors studied modulate the reproductive performance of *L. uruguayensis* females, we performed statistical analysis where the age of females was a fixed factor, with two levels (young and old), and the periods of the reproductive season were considered a fixed factor, with three levels (beginning, middle, and end). Fecundity was analyzed by multiple linear regressions that related the CW with the number of eggs of young and old females in each period of the reproductive season. Afterward, the fecundity equations were compared by two-way ANCOVA, using CW as a covariable. The variables CW, reproductive output, egg volume, and biochemical composition of eggs (contents of carotenoids, total proteins, and total lipids) were analyzed by two-way ANOVA. The values are expressed as mean \pm standard deviation (SD). The number of ovigerous females used to estimate each reproductive trait was different, because of the technique used for the determination of each trait.

Graphical and analytical methods such as Levene's test and Shapiro Wilks were used to verify the assumptions of homoscedasticity and normality. When necessary, the variance structure was modeled (Zuur *et al.*, 2010). The alpha level of statistical significance was set at 0.05. Tukey's test was used to make multiple comparisons. Statistical analyses were performed using R 4.0.3 software (RStudio Team, 2015).

Results

A total of 252 ovigerous females were sampled: 134 at the beginning of the reproductive seasons, 92 in the middle, and 26 at the end. The smallest ovigerous female had a CW of 7.5 mm, whereas the largest had a CW of 14.5 mm. The comparison of CW showed significant interaction between the age of the females and the periods of the reproductive season (Table 1). Young females were 11.1% larger, on average, at the beginning of the reproductive season than in the middle and at the end. However, old females that were, on average, 20% larger than young females showed similar sizes in all periods of the reproductive season (Table 1; Fig. 2).

Fecundity

Fecundity, which was estimated for 109 females, ranged from 1447 to 13,172 eggs. The fecundity equations for

Table 1

Results of two-way ANOVAs testing the effects of the age of females (young and old) and the periods of the reproductive season (beginning, middle, and end) on female size and reproductive traits (reproductive output, egg volume, and contents of carotenoids, total proteins, and total lipids in the egg clutches) of the fiddler crab *Leptuca uruguayensis*

Reproductive traits	Factors	df	Mean square	F	P
Female size (carapace width, mm)	Age	1	300	405	<0.001
	Periods	2	5.82	7.9	<0.001
	Age × periods	2	7.7	10.38	<0.001
	Error	246	0.74		
Reproductive output (%)	Age	1	0.004	1.05	0.31
	Periods	2	0.0001	0.04	0.96
	Age × periods	2	0.002	0.53	0.59
	Error	152	0.003		
Egg volume (mm ³)	Age	1	6.74×10^{-6}	2.01	0.15
	Periods	2	5.92×10^{-5}	18.28	<0.001
	Age × periods	2	1.09×10^{-6}	0.34	0.71
	Error	130	3.23×10^{-6}		
Carotenoid content ($\mu\text{g mg}^{-1}$ eggs)	Age	1	0.0005	0.18	0.67
	Periods	2	0.01	3.20	0.04
	Age × periods	2	0.0006	0.21	0.81
	Error	55	0.003		
Total protein content (mg g ⁻¹ eggs)	Age	1	113	0.29	0.59
	Periods	2	417	1.08	0.35
	Age × periods	2	1691	4.37	0.01
	Error	60	387		
Total lipid content (mg g ⁻¹ eggs)	Age	1	45.8	0.37	0.55
	Periods	2	1159	9.39	<0.001
	Age × periods	1	0.32	0.003	0.96
	Error	38	123		

young and old females in each period of the reproductive season showed a positive relationship between female size (CW) and number of eggs (multiple linear regression, $P < 0.05$). The ANCOVA that compared these equations indicated that fecundity was affected by the covariable (CW) (ANCOVA result $df = 1$; $F = 174.30$; $P < 0.001$). Additionally, the ANCOVA showed interaction between the age of the females and the periods of the reproductive season (ANCOVA result: $df = 2$; $F = 2.45$; $P = 0.044$). Consequently, Tukey's test was performed to compare the fecundity equations for all the levels of the factors age and periods (Table 2). The fecundity of young females was significantly higher at the beginning than in the middle and at the end of the reproductive season, whereas the fecundity of old females did not vary along time (Table 2); and they showed, on average, 38% more eggs (between 5078 and 13,172 eggs) than young females (between 1447 and 9893 eggs).

Reproductive output

The reproductive output was estimated for 158 ovigerous females. The egg clutch represented $22\% \pm 6\%$ of the body weight, a value that did not differ significantly between young and old females or between the periods of the reproductive season (Table 1).

Egg volume

Egg volume was determined in 136 ovigerous females. It ranged between 0.008 and 0.017 mm³ and did not differ significantly between young and old females, but it was affected by the periods of the reproductive season (Table 1). At the end of the reproductive season, the eggs were, on average, 24% smaller than those at the beginning and in the middle of the reproductive season (Table 1; Fig. 3A).

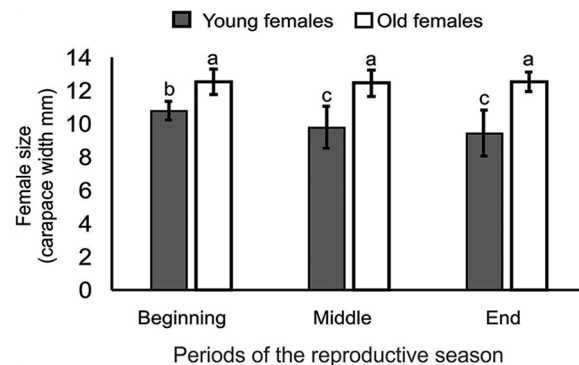


Figure 2. Female size. *Leptuca uruguayensis* carapace width comparing young (gray) and old (white) females in the periods of the reproductive season (mean \pm SD). Different lowercase letters indicate significant variation ($P < 0.05$).

Table 2

Fecundity equations relating the carapace width (CW) with number of eggs (F) of young and old females of *Leptuca uruguayensis*, in each period of the reproductive season (beginning, middle, and end)

Age	Period	n	Fecundity equation	R ²	Tukey's result
Young	Beginning	16	F = 1303.3 CW - 7787.9	0.80	b
	Middle	22	F = 1048.9 CW - 5671.9	0.80	c
	End	5	F = 1558 CW - 10,499	0.88	c
Old	Beginning	38	F = 1303.5 CW - 7863.3	0.56	a
	Middle	22	F = 1115.4 CW - 5973.4	0.48	a
	End	6	F = 1838 CW - 14,936	0.66	a

Different lowercase letters indicate a significant variation in fecundity equations compared by Tukey's test, after ANCOVA interaction result ($P < 0.05$).

Biochemical composition of eggs

The carotenoid content was determined for 61 egg clutches. The carotenoid content in egg clutches did not differ significantly between young and old females but varied significantly between the periods of the reproductive season (Table 1), because the eggs produced at the end of the reproductive season showed, on average, 19% more carotenoids than the eggs produced at the beginning.

The total protein content was determined for 66 egg clutches. The two-way ANOVA showed significant interaction between age and period (Table 1). The egg clutches of young females showed similar protein content (71.2 ± 17.5 mg total protein

g^{-1} eggs) throughout the reproductive season. By contrast, the eggs produced by old females at the end of the reproductive season showed, on average, 28% less total protein content than the eggs produced in the other periods (Fig. 3C).

The total lipid content was determined for 43 egg clutches. The mean lipid content in clutches did not differ significantly between young and old females during the beginning and the middle of the reproductive season (54.1 ± 11.9 mg total lipids g^{-1} eggs; Table 1; Fig. 3D). At the end of the reproductive season, the eggs produced by old females presented, on average, 32% less total lipid content than the eggs produced at the beginning and middle of the reproductive season (Fig. 3D). In this period, the young females recorded were few and had

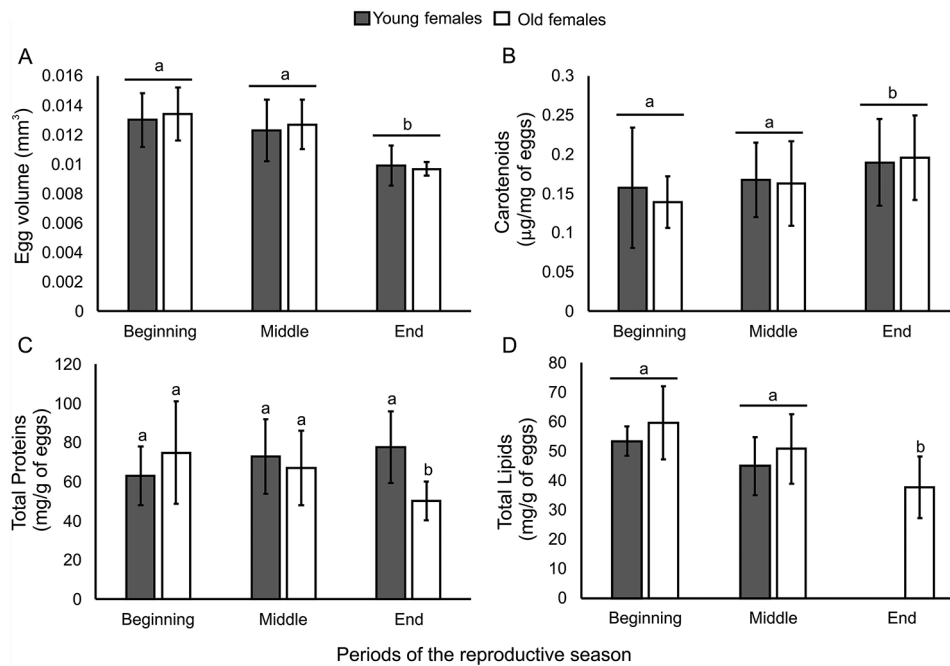


Figure 3. Traits of eggs of *Leptuca uruguayensis*. (A) Egg volume, (B) content of carotenoids, (C) content of total proteins, and (D) content of total lipids in eggs of young females (gray) and old females (white) in the different periods of the reproductive season (mean \pm SD). Different lowercase letters indicate significant variation ($P < 0.05$).

small clutches; thus, the mass of eggs required for the determination of lipids was insufficient, and the total lipid content in egg clutches of young females at the end of the reproductive season could not be determined.

Discussion

The reproductive performance of *Leptuca uruguayensis* was affected by female age and by periods of the reproductive season. Female age mainly affected fecundity, because young females showed lower fecundity than old females. Reproductive output was not affected either by female age or by period of the reproductive season. However, egg volume and carotenoid content were mainly modulated by period of the reproductive season, and nutritional content of the eggs (total protein and lipids) was influenced by both factors.

The positive relationship between female size and fecundity has been well documented in many decapod species (Fox and Czesak, 2000; Yosho, 2000; Muiño, 2002; García Guerrero and Hendrickx, 2004; Birkeland and Dayton, 2005; Marshall and Keough, 2007; Bert *et al.*, 2016; Tropea *et al.*, 2019 among others). Larger females have more space to develop the gonad in the cephalothorax and greater capacity to carry eggs in the abdomen (Hines, 1982, 1988). Therefore, fecundity variations between *L. uruguayensis* females of different ages were expected. The greater fecundity of young females at the beginning of the reproductive season was related to the larger size of these females in this period (10–12 mm of CW). The decrease in the fecundity of young females was related not to the periods but to the size of the females, which also decreased throughout the reproductive season. Young females smaller than 10 mm of CW only spawn from the middle of the reproductive season (Marciano *et al.*, 2021). The inclusion of these smaller females in the reproductive population decreases the fecundity of *L. uruguayensis* in the middle and at the end of the season, as a consequence of their sizes. A similar trend has been previously found for the shrimp *Palaemon macrodactylus* Rathbun, 1902 and the crab *Pyrhila pisum* (De Haan, 1841 [in De Haan, 1833–1850]), in which large females predominate at the beginning of the reproductive season and smaller females are incorporated toward the end, resulting in lower fecundity values (Vázquez *et al.*, 2013; Kobayashi and Vazquez Archdale, 2017).

Measuring the cost of reproduction is an interesting but complex issue (Reznik, 1985). Therefore, an alternative is to determine reproductive output (Clarke, 1987), because, in brachyuran crabs, almost the total content of the ovary is extruded in the spawn, making the egg clutch weight : female body weight ratio a valid approximation of reproductive effort (Hartnoll, 2006). The reproductive output of brachyuran crabs can vary between 10% and 22%, on average (Hines, 1982, 1991). Thus, the value here recorded for *L. uruguayensis* (22%) indicates that females of this fiddler crab make a high investment in reproduction. Although *L. uruguayensis* fecun-

dity was modulated by size, the reproductive output was similar for young and old females, indicating that the effort made in reproduction was proportional to the female age and/or size, regardless of the number of eggs in the clutch. Moreover, reproductive output did not vary between periods of the season. These results suggest that the resources allocated by *L. uruguayensis* females to reproduction can be similar in every spawn of their lives. However, reproductive output is not a measure that includes the full complexity of energy investment in reproduction, because it does not consider variables such as egg volume and egg nutritional composition (Callow, 1979; Hartnoll, 2006; Tsujimoto *et al.*, 2016).

In decapod crustaceans, egg size could be influenced by several factors, such as whether the female is multiparous or primiparous (Graham *et al.*, 2012), whether the eggs are summer or winter eggs (Jacobs *et al.*, 2003; Guzmán *et al.*, 2016, 2020), and whether there is an increase or decrease in temperature or latitude (Ituarte *et al.*, 2006; Bas *et al.*, 2007, 2009). This variability could also be due to changes in egg water content (Peiró *et al.*, 2014), maternal exhaustion (Tropea and López Greco, 2015), or food availability (Urzúa *et al.*, 2012). In this research, the egg volume in females of both ages decreased at the end of the reproductive season, probably as a result of more than one factor. Our results suggest that the factors modulating the egg volume of *L. uruguayensis* vary throughout the reproductive season but are not related to the age of females, because this reproductive trait did not change between young and old females during the season.

Regarding the biochemical composition of the eggs, carotenoids provide pigmentation to embryos (Dall, 1995; Long *et al.*, 2017), photoprotection (Britton *et al.*, 2008), and antioxidant defense (Wouters *et al.*, 2001; Wade *et al.*, 2017). The carotenoid content in eggs is directly influenced by the amount of these pigments in the diet of females (Jeckel *et al.*, 1989; Schneider *et al.*, 2016). Because fiddler crabs feed on the sediment (Miller, 1961; Sayão Aguiar *et al.*, 2012), the short-term variations that usually occur in the sediment organic matter contents and in the interstitial communities (Bathmann *et al.*, 1991; Morrisey *et al.*, 1992; Madariaga, 2002; Rodil *et al.*, 2008) can affect the food resources available to *L. uruguayensis*, promoting differences in the carotenoid content of eggs during the reproductive season. Because at the end of the reproductive season *L. uruguayensis* spawned using resources acquired during the season by feeding (income breeding strategy) (Marciano *et al.*, 2021), we suggest that the increase in carotenoid content in the eggs spawned at the end of the reproductive season of young and old females could be explained by variations in the type of food available for *L. uruguayensis* on the sediment and the difference in the mode of using resources destined to egg production.

The nutritional composition of the eggs is a useful indicator of maternal energy investment (Hernández Herrera *et al.*, 2001; Palacios *et al.*, 2001), so a reduction in these yolk

components suggests lower quality of the progeny (Sasaki *et al.*, 1986; Nates and McKenney, 2000; García Guerrero *et al.*, 2003). Although the young females of *L. uruguayensis* here studied were investing energy both in reproduction and in growth, they maintained the protein and lipid supply to egg clutches independently of the breeding strategy used to spawn in each period of the reproductive season. Although we could not estimate the lipid content at the end of the reproductive season, we might suppose that lipid values would not represent an important negative effect on the brood because the proteins are the predominant components recorded in oocytes of *L. uruguayensis* (Colpo and López Greco, 2018; Marciano *et al.*, 2021).

In decapods, successive spawns can produce maternal nutrient depletion; and the biochemical composition of eggs usually changes, affecting the brood condition (Boucard *et al.*, 2004; Yu *et al.*, 2007; Tropea and López Greco, 2015; Marciano *et al.*, 2018). In a study by Marciano *et al.* (2021), the old ovigerous females of *L. uruguayensis* sampled at the end of the reproductive season were spawning for the second time in the season, and their clutches showed, on average, 30% less content of total protein and lipid. After the first spawn of the reproductive season, the content of total proteins in the ovary, as well as the content of glycogen and lipids in the hepatopancreas, decreased (Marciano *et al.*, 2021). This suggests a depletion condition of old females, which would allocate fewer nutrients to eggs, a fact that might in turn result in lower quality of their second clutch in the season. However, we cannot affirm whether this decreased nutritional content in the eggs of old females was a consequence of their senescence or of the change in the breeding strategy (Marciano *et al.*, 2021). Since the egg quality of crustaceans has direct implications on the survival and success of the offspring (Anger, 2001, 2006; Paschke *et al.*, 2004; Urzúa and Anger, 2013), the lower nutritional content of eggs here observed for old females of *L. uruguayensis* at the end of the reproductive season would produce poor-quality larvae.

Finally, it is important to highlight that the most important differences in the reproductive parameters were recorded between the beginning and the end of the reproductive season, probably because, in these periods, the females were exclusively using the capital breeding strategy or the income breeding strategy, respectively. Both female age and the periods of the short reproductive season affected the reproductive performance of *L. uruguayensis* in temperate estuaries.

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