The influence of the post-autotomy regeneration of a sexual trait and mating tactics in a fiddler crab

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Abstract

Male fiddler crabs own an enlarged claw which is a weapon and an ornament. The enlargement of this claw begins from the juvenile stage and continues throughout life. Males may voluntarily lose (i.e. autotomise) this claw. After several moults males may regenerate a new claw, called leptochelous, which acquires a similar length but a lower muscle mass area than the original one, called brachychelous. In some species, regenerated claws develop permanently as leptochelous, the population having two discrete claw morphologies. Other species present morphological variations with leptochelous and brachychelous being two ends of a continuum. In the species *Leptuca uruguayensis*, we studied the morphological variation of this enlarged claw, whether it may be caused by its regeneration at different male sizes, and its consequences on mating success. We found that claws could not be discriminated as discrete morphs, suggesting a morphological continuum from brachychelous to leptochelous. Regenerated claws in the laboratory were initially small and proportional to body size, while a field experiment confirmed that claw size is recovered after several moults. Morphological variation may be caused by energetic limitations where males of different sizes must differently trade-off between restitution of claw length (ornament function) or claw muscle area (weapon function). Fiddler crabs use two mating tactics with different levels of female choice. However, regardless of the mating tactic, leptochelous males were at a disadvantage at high densities, while not at low densities, suggesting that the consequences of autotomy and regeneration on mating success may depend on the social context.

Key words: multifunction trait, claw allometry, *Leptuca uruguayensis,* sexual selection

Exaggerated sexual traits often have more than one function, such as ornaments and weapons, but it is not always clear how sexual selection operates on each one (Bildstein 1983; Berglund et al. 1996). Sexual selection often favours positive allometry in secondary sexual traits, which means a disproportionate increase in trait size relative to that in body size (Hartnoll 1978). This effect may occur in pure ornaments, because positive allometry amplifies small differences in body size, which influences mating success (Wallace 1987; Green 1992; O'Brien et al. 2018). However, it does not always occur in pure weapons (van Lieshout and Elgar 2009; McCullough and O'Brien 2022) because strong allometry may cause the weapon to lose efficiency (Levinton and Allen 2005). In species that grow throughout life, the design of multifunction traits may be even more complex, if the relative importance of each function changes throughout life, and if each function is favoured by a different allometric pattern (Bonduriansky and Day 2003; Pélabon et al. 2013). Therefore, sexual selection may end up favouring all functions together, one function over the other and/or the development of compensatory mechanisms due to favouring one function over another (Dennenmoser and Christy 2013).

Fiddler crabs comprise a group of more than 100 species (Shih et al. 2016), which offer an interesting opportunity to address these topics for several reasons. First, fiddler crabs have indeterminate growth (Crane 1975), so they continue to grow after sexual maturity, resulting in body size being correlated with age (Yamaguchi 2002). Second, male fiddler crabs develop an enlarged claw that generally represents more than 30% of total body weight (Crane 1975). Males gradually develop this enlarged claw from juveniles and through successive molts (Nath et al. 2023). Initially, juvenile males, like females, own two symmetric small feeding claws (Yamaguchi and Henmi 2001, 2008). Then the juvenile male loses one of these small claws, which regenerates again as a feeding claw, while the remaining one begins to increase in size through successive moults (Morgan 1923). Consequently, the enlarged claw switches

to function as a weapon and as an ornament (Crane 1975; Ahmed 1978). However, the claw may suffer partial damage or its complete autotomy (i.e. the detachment from the body) due to male-male combats or to escape predators (Bildstein et al. 1989; McLain et al. 2003; Mace III and Curran 2011; Martin 2019; Darnell et al. 2020). Males can regenerate a new claw, which after several moults may acquire a similar length but with a less robust structure than original ones observed in males of the same size (Yamaguchi 1973; Backwell et al. 2000; Reaney et al. 2008). Consequently, within the population some males own robust original claws, called brachychelous, while other males own slighter regenerated claws, called leptochelous (Crane 1975; Backwell et al. 2000). For similar claw lengths (see Fig. 1), leptochelous have relatively longer fingers and a shorter manus in comparison to brachychelous (Backwell et al. 2000; Yamaguchi 2001; Rosenberg 2002). The manus is the segment (Fig. S1) containing the muscle mass that provides the claw closing force. Thus, a relatively shorter manus in leptochelous makes them a worse weapon than brachychelous (Backwell et al. 2000; Reaney et al. 2008; Lailvaux et al. 2009; McLain et al. 2010; Muramatsu and Koga 2016).

For some species, such as *Austruca annulipes*, brachychelous and leptochelous appear to occur as discrete morphologies in which the regenerated claws develop permanently as leptochelous, and with no possibility of reverting to brachychelous (Backwell et al. 2000). In other species, such as *Leptuca pugilator* (McLain and Pratt 2011) or *L. uruguayensis* (pers. obs), major claws occur rather as a continuous morphological variation from brachychelous to leptochelous (see Fig. 1) making it difficult to distinguish some regenerated claws from original ones. Therefore, in these species, brachychelous and leptochelous are likely two ends along the same continuum (McLain and Pratt 2011), caused by size-dependent trade-offs between investment in claw length or in claw robustness. Size-dependent trade-offs may originate for two main reasons. First, available energy and physiological constraints may

prevent claw regeneration from fully restoring both functions. Second, while the energy available to regeneration is likely proportional to body size, the structure to be regenerated is not proportional: large males must regenerate relatively larger claws and throughout a fewer number of moults than small males (Rosenberg 2002). Therefore, as the size of the male increases, so does the gap between the energy available and that required to regenerate a fully functional claw after autotomy. Therefore, leptochelous claws seem to be the result of the regeneration primarily focused to recover claw length at the cost of shortening the length of the manus (Backwell et al. 2000; Yamaguchi 2001). Consequently, a degree of mild to more accentuated leptochelous forms may result from claw autotomy at different body sizes (McLain and Pratt 2011), with more extreme leptochelous cases expected to occur the larger the male is at the time of autotomy.

In some fiddler crab species, in addition to male size females evaluate the characteristics of the male's burrows before mating with them (Backwell and Passmore 1996; Latruffe et al. 1999). Thus, if leptochelous males have a less efficient armament to acquire and defend high-quality burrows (Reaney et al. 2008) they may potentially have less mating success than brachychelous males of the same size. In addition, some species use two different mating tactics, underground and surface mating (deRivera and Vehrencamp 2001). Underground mating involves a complex courtship display in which males wave the enlarged claw to attract females. Mating occurs inside the male's burrow where the pair stay together for 2–3 days after which the female always lays a clutch of eggs (Yamaguchi 1998; Reaney et al. 2012). Then the male leaves the burrow to the female to incubate her eggs, and must dig a new, find or steal another's one burrow (Christy 1982). In surface mating, males do not use the waving courtship, but approach neighbouring females to mate on the surface. In these cases, females do not always lay eggs, but if they do, incubation occurs in her own burrow (Nakasone and Murai 1998; Reaney et al. 2012). Therefore, in species that use both mating

tactics, it may be expected brachychelous males to mate more often underground because their claws are fully functional as an ornament to attract females and as a weapon to acquire and defend high-quality burrows outcompeting other males. Conversely, leptochelous males may be expected to mate more often on the surface, because their burrows are not assessed and given to the females for egg incubation, and female choice is much lower than for underground matings (Nakasone and Murai 1998; Ribeiro et al. 2010). However, up to date there is no clear evidence that males with leptochelous are at a disadvantage in attracting females for underground mating (Backwell et al. 2000; Reaney et al. 2008; Clark and Backwell 2016). Additionally, there is no available information about the role of claw morphology in surface matings (Clark and Backwell 2016).

In the present work, we inspected the morphological variation of males' major claws in the species *Leptuca uruguayensis* (Nobili 1901) (formerly *Uca uruguayensis*, see Shih et al. 2016) and its relationship with mating tactics. The study had three goals. First, we used allometric analysis to unravel whether brachychelous and leptochelous morphologies can be associated to discrete categories or are rather two extremes along a continuum. Secondly, we conducted autotomy experiments to evaluate whether claw regeneration may account for morphological variation depending on the male size at autotomy. Finally, we evaluated the relationship between claw morphology and the outcome of different mating tactics.

Materials and Methods

Study site

The study was conducted between 2002 and 2005. We collected male fiddler crabs from two different sites. One site was in the Mar Chiquita coastal lagoon (Argentina, 37º45'S and 57º25'W), a 46 km^2 body of brackish water, and the other site was near the mouth of the San Clemente tidal creek (eastern Bahía Samborombón, Argentina, 36°22′S, 56°45′W). Both sites

are affected by semidiurnal low-amplitude tides (mean amplitude < 1 m) (Fasano et al. 1982; Dragani et al. 2014), with fiddler crabs occurring in tidal flats above the mean low tide line, adjacent to the edge of cordgrass (*Sporobolus densiflorus*) marshes (Bogazzi et al. 2001). Crabs dig burrows that they use as a refuge during high tide, as a shelter against predators, and as mating and moulting sites (Crane 1975; de la Iglesia et al. 1994; Ribeiro et al. 2010). Fiddler crabs are active on the surface from September to April, remaining inside their closed burrows for the rest of the time (Spivak et al. 1991). There are no differences in the size frequency distribution at both populations (Bogazzi et al. 2001). Crab density, quantified by digging sediment, is generally low in Mar Chiquita, ranging between 10 to 40 crabs $\cdot$ m^{-2} (Bogazzi et al. 2001). In contrast, in San Clemente, crabs form patches of variable densities, some with densities comparable to the Mar Chiquita population and others reaching densities of up to 140 crabs $\cdot$ m^{-2} (Ribeiro et al. 2005).

Population variation in claw morphology

We conducted a population screening of the morphology of male's enlarged claws. To do this, 259 male fiddler crabs were randomly collected from the Mar Chiquita population. The collection was done by digging sediment to include all individuals, not only those active on the surface. Several measurements were made on each claw (Fig. S1), which resulted in highly correlated variables (average correlation = 0.94; see Supporting Information). Based on this correlation and considering previous studies (Backwell et al. 2000; Yamaguchi 2001; McLain and Pratt 2011), propodus length and manus length (Fig. S1) were used to describe claw morphological variation. Carapace width was measured as a proxy of body size and handedness was also registered.

Classification of claws

Based on the general robustness and the development of tubercles, each claw was visually classified into 1 of 5 different groups of claw variants or morphs (see Fig. 1), corresponding one group to newly regenerated claws and 4 groups to different variants of full-size claws. A newly regenerated claw was one whose size was suspiciously small, generally close to the size of carapace width. Newly regenerated claws were often opaque in colour. Brachychelous claws were those robust claws and with significant development of tubercles and teeth. At the other extreme, leptochelous claws were those slight claws with absence of tubercles or teeth. Intermediate cases were those that could not be clearly classified but were closer to either brachychelous or leptochelous (see Fig. 1). Although this classification may be reasonable, it is still arbitrary. The arbitrary classification into several claw morphs may segregate the morphological variables potentially creating artefacts in their relationships (Tönnies et al. 2022). To address this, finite mixture models were fit (McLachlan et al. 2019) to seek discontinuities in the morphological variables which can constitute different claw morphs (Prates et al. 2013; Palaoro et al. 2022). These models parameterize the distribution of a determined morphological variable as a mixture of one to k-number of skew-normal distributions. Then, bias-corrected Akaike Information Criterion (AICc) is used to identify the most parsimonious number of groups, based on the log-likelihood values and the number of parameters involved. This approach has been used to detect polymorphism in several species (Rowland and Emlen 2009; Buzatto et al. 2014; Palaoro et al. 2022). However, the method is univariate and thus may be appropriate when polymorphism is associated with abrupt discontinuities in the scaling of the trait along body size. For example, some dung beetles have three types of males: small males with no horns, medium males with short horns, and large males with long horns (see Rowland and Emlen 2009). Thus, in dung beetles the univariate inspection of horn length is enough to detect different morphs. In our study, we

seek for bivariate discontinuities, for example, claw length should be evaluated in relation to male size. To address this, finite mixture models were applied to the residuals obtained by fitting three different linear regression: (1) propodus length in relation to carapace width, (2) manus length in relation to propodus length, and (3) the ratio manus/propodus in relation to carapace width. Then allometric differences were evaluated between the differentiated groups. Variables were log-transformed so the resulting slopes in the fitted relationships estimated the allometric exponents (see Gayon 2000).

Brachychely index

We developed an index that measures quantitatively how brachychelous a claw is. The index gives the likelihood of a determined claw being brachychelous based on the propodus length, manus length and carapace width. The index computes the joint probability that a claw has both a propodus and manus length larger than their expected mean. The brachychely index was computed as the following joint probability:

$BI = p_p \cdot p_m$

where BI is the brachychely index, and p_p and p_m the distribution functions of both propodus and manus, respectively. To do so, the relationship between log propodus length and log carapace width was used to obtain, for each claw, the expected mean and the sd for the propodus length given the carapace width of the male. Similarly, the relationship between log manus length and log propodus length was used to obtain the expected mean and the sd for the manus length given the propodus length of the male. The "pnorm" distribution function from "Stats" R core package (R Core Team 2020) was used to compute each p_p and p_m probabilities. The brachychely index was modeled following the beta distribution (Ferrari and

Cribari-Neto 2004), as it ranges between 0 and 1, and it is asymmetric since it is the product of two probabilities, a claw with average propodus and manus lengths yields an index value of 0.25 (p_p x p_m = 0.5 x 0.5). The index is not an absolute measure of brachychely, but rather the relative status of an individual claw within the whole population, which is useful for comparison.

Claw morphs, male size, and handedness

Beta regression, with loglog link function (Ferrari and Cribari-Neto 2004) was fit to evaluate whether brachychely index varied between the 5 claw classes. This allows us to determine whether the 5 arbitrarily defined classes corresponded to claw groupings with a different degree of brachychely. For each claw class, 95% bootstrap confidence limits were computed for the mean brachychely index. Brachychelous claws were expected to show index values greater than 0.25, and leptochelous claws to show index values less than 0.25.

Handedness in the species *L. uruguayensis* is evenly distributed between left and right-handed males (Spivak et al. 1991). However, the relationship between handedness and claw morphology is unknown. In addition, if a differential trade-off between the regeneration of claw length and manus length increases with male size at autotomy, then it can be expected brachychely index to vary with body size. A beta regression, with logit link function was used to evaluate whether brachychely index varied both with carapace width and handedness of males. In this analysis we excluded data from the newly regenerated claws.

Laboratory experiment

A laboratory experiment was conducted in which male fiddler crabs were induced to autotomise their major claw, and presumably, to regenerate it after moulting. The purpose of this experiment was to determine if the morphological and allometric pattern after claw regeneration varied between large and small males, and between males that were brachychelous or leptochelous before autotomy. From the San Clemente population, we collected 40 juvenile males (carapace width 7.07 to 9.15 mm) and 80 adult males (carapace width 10.30 to 13.99 mm). Adult males were collected such that 40 were visually identified as brachychelous and 40 as leptochelous. These crabs were individually placed in 250 ml recipients, filled with seawater adjusted with fresh water to a salinity of 23 PSU, fed with surficial mud sediment extracted from the field site, and subjected to a daily 12 light:12 dark cycle regime. The water and the feeding mud sediment were renewed daily. The individuals were kept under these conditions to acclimatise for 10 days, after which 20 juveniles, 20 brachychelous adults, and 20 leptochelous adults were induced to autotomise the major claw pressing hard with forceps on the merus. The remaining 60 individuals were kept intact as control. All individuals were cared for and fed as before. The experiment ended for each individual when it moulted, died, or after 9 months if none of these events occurred. Carapace width and claw morphometric variables of all individuals were measured at the beginning and end of the experiment.

A generalised linear model (GLM) with binomial distribution and logit link function compared the proportion of crabs that moulted, between male types and autotomy treatments. A GLM with negative binomial distribution and log link function compared the number of days to moult, between male types and autotomy treatments. The change in body size after moult, computed as the difference between the start and the end of the experiment, was

compared between male types and autotomy treatments using a linear model with normal distribution. Linear models with normal distribution fitting (1) propodus length in relation to carapace width, (2) manus length in relation to propodus length, and (3) the ratio manus/propodus in relation to carapace width were used to evaluate allometric changes in claw morphology between male types and autotomy treatments. Variables were log transformed.

We computed the brachychely index of each claw, at the beginning and at the end of the experiment. The index was computed as before but obtained the expected mean to compute the joint probabilities from male samples of the San Clemente population ($N = 209$ males, obtained by digging sediment). A beta regression, with logit link function, compared the brachychely index after one moult between male types and autotomy treatments. For each moulted individual, the change in the brachychely index was computed as the difference between the index value after and before the moult, and a linear model compared the change in the index between male types and autotomy treatments. Bootstrap confidence limits (95%) were computed for the mean difference in the brachychely index.

Field experiment

We ran a field experiment in a mudflat area of the Mar Chiquita coastal lagoon in which 12 male fiddler crabs were induced to autotomise their major claw, and presumably, to regenerate it after moulting. To do this, we searched for burrows with small-sized males (carapace width range: 5.60—6.68 mm) without neighbours around (50 cm radium). Males were captured without digging, but by filling water into the opening of their burrows until individuals emerged. This procedure kept the structure of burrows intact and the surface sediment as little disturbed as possible. Males were induced to autotomise and returned to

their burrows. To keep autotomised males individualised and to prevent other individuals to come inside we set circular enclosures (20 cm radium) delimited by a plastic mesh that was 20 cm above the surface and buried 30 cm deep. Enclosures were placed so that the male was in the centre. Enclosures were checked periodically to detect the presence of other individuals than the target male. The experiment lasted from October 2003 to March 2004, after which all males were captured and measured. We do not have a control for this experiment. However, even if we had it, we could not be aware if a control individual autotomised and regenerated the claw during the experiment without noticing it, since our monitoring was not continuous. Despite this, individuals in the field experiment might have moulted more than once, so we predicted that their claws should not look like newly regenerated claws, but rather like an intermediate morphological type between brachychelous and leptochelous claws. We computed the brachychely index of regenerated claws and the 95% bootstrap confidence limits for the mean. We expected field regenerated claws to show an index greater than newly regenerated claws but not greater than 0.25 which is the value for an average claw.

Mating tactics, patch density, and claw morphology

To evaluate the relationship between claw morphs and the outcome of different mating tactics we used stored samples of males from the population and stored samples of males from mating pairs obtained in a previous study conducted at the San Clemente population (Ribeiro et al. 2010). Population samples encompassed males from two contrasting patch densities (High-density: mean density of 70—90 crabs m^2; Low-density: mean density of 10—30 crabs m^2). In the species *L. uruguayensis,* both mating tactics occur irrespectively of patch densities, however, underground matings are more frequent at high densities while surface

matings are at low densities (Ribeiro et al. 2010). Mating pair samples included males that mated underground at high densities (UH; $N = 73$), or at low densities (UL; $N = 32$), and males that mated on the surface at high densities (SH; $N = 51$), or at low densities (SL; $N = 66$). We measured carapace width and claw morphological variables of all these males.

Linear models with normal distribution were used to evaluate differences in claw morphology between population densities by fitting (1) propodus length in relation to carapace width, (2) manus length in relation to propodus length, and (3) the ratio manus/propodus in relation to carapace width to evaluate Variables were log transformed.

We evaluated whether males mating with different tactics at different densities differed in claw morphology. These comparisons cannot be done straightforwardly mainly because female choice for large males is stronger in underground matings than in surface matings (Ribeiro et al. 2010). Then, if the brachychely grade of regenerated claws depends on male size at autotomy and on population density (De Grande et al. 2021), this may lead to differences in the brachychely grade between mating tactics, simply because the size frequency distribution of mated males differs between mating tactics.

To explore this possibility, a randomization numerical method (Manly 2006) computed the expected brachychely index for each mating tactic at each density based on male size and compared it to that observed in mating pairs. For each mating tactic and density the procedure applied the following steps: (1) take a random sample with replacement of mating males (n size of the sample being the number of matings of the corresponding tactic sample) and compute the brachychely index for each male, (2) take a random sample with replacement of males from the population of the corresponding density (n size equal to step 1) such that each male matched the carapace width of each male obtained in step 1, and then compute the brachychely index for each male, (3) calculate the mean for the difference between the

brachychely indices obtained in step 1 and step 2. The procedure was repeated 100,000 times, obtaining the distribution for the mean difference between the expected and observed brachychely index. To serve as a test of hypothesis, bootstrap confidence limits for the mean difference were obtained by extracting the 2,5 and 97,5 % percentiles of the generated distribution. Confidence limits excluding 0 indicated significant differences in the brachychely index between mating males and those expected from the population males that cannot be explained solely by female choice of male size.

Statistical analysis and software

All analyses were conducted in the R environment (R Core Team 2020) (R Core Team, 2020). Linear models were fit using the "Stats" core package, and GLMs were fit using the "lme4" package (Bates et al. 2015). The statistical significance of each effect was assessed with ANOVA or analysis of the deviance using the "car" package (Fox and Weisberg 2019). If covariates did not interact with fixed factors, differences were assessed through estimated marginal means using the "emmeans" function from the homonym package (Lenth 2022). When the interactions were significant, the differences between slopes were assessed using the "emtrends" function from the "emmeans" package (Lenth 2022). Bootstrap confidence limits were obtained using the "boot.ci" function of the "boot" R package (Canty and Ripley 2022).

Results

Population variation in claw morphology

Of the 259 collected male claws, 43 were classified as newly regenerated and 216 as full-size claws; these later being 108 brachychelous, 56 inter-brachychelous, 37 inter-leptochelous, and 15 leptochelous. The proportion of left-side ($n = 127$) and right-side claws ($n = 132$) did not differ from evenness (49:51; Exact Binomial Test: $P = 0.8038$).

Finite mixture models identified 2 groups of claws when evaluating the propodus length in relation to carapace width (Table 1). One group (group A: 217 claws) included all brachychelous, all inter-brachychelous, 36 of 37 inter-leptochelous, 12 of 15 leptochelous, and 5 of 43 newly regenerated claws. The second group (group B: 42 claws) included 38 of 43 newly regenerated claws, 3 of 15 leptochelous, and 1 of 37 inter-leptochelous. Thus, the group A contained the 98.15% of the claws which were a priori classified as full-size claws, and the group B contained the 88.37% of the claws which were a priori classified as newly regenerated claws. Finite mixture models (Table 1) did not identify different groups of claws when evaluating the manus length or the ratio manus/propodus (Table S1). Therefore, finite mixture models only support morphological differentiation in the length of newly regenerated claws and full-size claws. Consequently, allometric analysis compared the 3 morphometric variables between two groups defined by the a priori classification, one corresponding to the whole group of full-size claws ($n = 216$) and the other to the group of newly regenerated claws ($n = 43$).

The linear model for the log of propodus length in relation to the log of carapace width (Fig. 2A) showed a lower slope in newly regenerated claws than for the full-size claws (ANOVA interaction term: $F_{1, 255} = 40.77$, $SSR = 0.2937$, $P < 0.0001$; Table S1). The linear model for the log of manus length in relation to the log of propodus length (Fig. 2B) showed

that full-size claws have allometric slopes that are further from 1 than the newly regenerated ones (ANOVA interaction term: $F_{1, 255} = 5.08$, $SSR = 0.0073$, $P = 0.0251$; Table S1). The linear model for the log of ratio manus/propodus in relation to the log of carapace width (Fig. 2C) showed flatter slopes in newly regenerated claws than for the full-size claws (ANOVA interaction term: $F_{1, 255} = 19.53$, $SSR = 02891$, $P < 0.0001$; Table S1).

The brachychely index (Fig. 3) was different between the claw classes (Beta regression: deviance change $\chi^2 = 727.7$, $df = 4$, $P < 0.0001$). As expected, brachychelous claws showed index values greater than 0.25 and leptochelous claws showed index values less than 0.25. The brachychely index did not vary with carapace width (Beta regression: deviance change $\chi^2 = 1.43$, $df = 1$, $P = 0.2323$) nor with male handedness (Beta regression: deviance change $\chi^2 = 1.24$, $df = 1$, $P = 0.2646$).

Laboratory experiment

During the laboratory experiment, 8 of the 120 individuals died (Supporting Information, Table S2). The proportion of crabs that moulted varied separately between treatments (Deviance change: $\chi^2 = 33.38$, $df = 1$, $P < 0.0001$), and between male types (Deviance change: $\chi^2 = 8.11$, $df = 2$, $P = 0.0173$), but without an interaction effect between treatments and male types (Deviance change: $\chi^2 = 4.27$, $df = 2$, $P = 0.1183$). Post-comparisons found that the proportion of crabs that moulted (Supporting Information, Fig. S2A) was higher for autotomised individuals than for control ones, and for juveniles than for brachychelous adults, but not between leptochelous adults and both juveniles and brachychelous adults. The difference in carapace width after moulting varied between treatments (ANOVA: $F_{1, 69} = 7.05$, $SSR = 0.1432$, $P = 0.0098$), and between male types (ANOVA: $F_{2, 69} = 36.14$, $SSR = 1.4677$, $P < 0.0001$), but without an interaction effect between them (ANOVA: $F_{2, 69} = 2.28$,

$SSR = 0.0926$, $P < 0.1098$), where only juveniles increased their size, but adults did not grow or reduced their size, especially the autotomised individuals (Supporting Information, Fig S2B). The time that crabs took to moult varied between autotomy treatments (Deviance change: $\chi^2 = 15.26$, $df = 1$, $P < 0.0001$) and male types (Deviance change: $\chi^2 = 12.85$, $df = 2$, $P = 0.0016$), but without an interaction effect between them (Deviance change: $\chi^2 = 1.53$, $df = 2$, $P = 0.4655$). Post-comparisons found that autotomised males moulted sooner than control males, and juveniles moulted sooner than both brachychelous and leptochelous adult males (Supporting Information, Fig. S2C, D).

The linear model for the log of propodus length in relation to the log of carapace width did not find different slopes between male types and autotomy treatments (Tables S3, S4). However, after moult males with autotomised claws regenerated a shorter claw in comparison to control males (Fig. S3A). The linear model for the log of manus length in relation to the log of propodus length, found different slopes between autotomy treatments (Table S3), where autotomised crabs showed slopes around 1 and control crabs showed slopes below 1 (Fig. S3B, Table S4). The linear model for the log of the ratio manus/propodus in relation to the log of carapace width showed negative slopes for control males and flat slopes for autotomised males (Fig. S3C, Table S3).

The brachychely index after one moult depended on treatment and on male type (Deviance change for the interaction: $\chi^2 = 18.51$, $df = 2$, $P < 0.0001$; Fig. S4). As expected, the change in the brachychely index (Fig. 4) was higher for autotomised males than for control males (ANOVA: $F_{1, 68} = 9.67$, $SSR = 0.1622$, $P < 0.0027$). However, the change in the brachychely index was also higher for brachychelous males than for leptochelous and juveniles (ANOVA: $F_{2, 68} = 16.24$, $SSR = 0.5451$, $P < 0.0001$), and without an interaction between treatment and male type (ANOVA: $F_{2, 68} = 2.19$, $SSR = 0.0736$, $P < 0.1196$).

Field experiment

Individuals grew on average 3.82 mm ($n = 12$, $SD = 0.29$), achieving a carapace width between 8.97 to 10.60 mm. Individuals from the field experiment (allometric exponents in Table S5) showed brachychely index below the mean value of 0.25 (Fig. 3; mean = 0.0651, 95% bootstrap confidence limit: 0.0325, 0.1152), but higher than that observed in newly regenerated claws (Fig. 3; mean = 0.0010, 95% bootstrap confidence limit: 0.0001, 0.0025).

Mating tactics, patch density, and claw morphology

There were no differences between patch densities in the propodus length of males from the San Clemente population (Table S6). However, males from low densities showed relatively larger manus and a greater ratio of manus/propodus than males at high densities (Table S6). The brachychely index (Fig. 5) indicated that at high densities mated males, regardless of the tactic used, were more brachychelous than the expected based on male size (Table S7). In contrast, males mating at low densities were as brachychelous as that expected based on male size (Table S7).

Discussion

In this work we analysed the population morphological variation in the major claw of the fiddler crab *Leptuca uruguayensis* and, together with data from laboratory and field experiments, evaluated the possible consequences of autotomy and regeneration on claw morphology and the mating tactic used.

Previous works have shown that after autotomy males regenerate a new claw with a different shape than the original one (Backwell *et al.* 2000; Yamaguchi 2001; McLain and Pratt 2011). However, the pattern of morphological variation of regenerated claws seems to vary among species. While in *L. pugilator* males regenerate their claws along a morphological gradient (McLain and Pratt 2011), in *Autruca annulipes* regenerated claws have a discreetly differentiated morphology (Backwell *et al.* 2000). In our studied species, *L. uruguayensis*, newly regenerated claws could be differentiated from full-size claws; however finite mixture models could not discriminate brachychelous and leptochelous within the group of full-size claws. This supports a pattern of morphological continuum from brachychelous to leptochelous like that of *L. pugilator*.

The comparison of allometric slopes between newly regenerated claws and full-size claws suggest that the first regenerated claw, the one that is developed in the first moult after autotomy, is more isometric than allometric. In fact, the confidence limit for the slope for the propodus length of the regenerated claws included the 1 (Table S1), which means that it is proportional to body size (i.e. isometry). These patterns of initial isometry in regenerated claws appeared clearer in laboratory experiments (Table S4) suggesting that the recovery of the previous allometric size is constrained and must be developed along subsequent moults). This is supported by the field experiment in which males have been able to moult several times and whose regenerated claws were clearly allometric, showing a brachychely index higher than that observed in newly regenerated claws, but lower than those claws visually classified as brachychelous.

In some vertebrate species it has been found that the ability to regenerate may depend on ontogeny (Seifert and Voss 2013). These may be caused by constraints in physiological pathways as well as energetic limitations (Maginnis 2006; Yakushiji et al. 2009). The results showing the initial reduced size of regenerated claws suggest that crabs have a limited energy

budget for claw regeneration, which adds evidence that major claws are costly to regenerate (Allen and Levinton 2007). In fact, the change in the body size of crabs from the laboratory experiment may account for age-dependent energy restrictions, as autotomised juveniles grew less than control ones, but autotomised adults even reduced their size (see Supporting Information, Fig S2B). Furthermore, we observed that in control males brachychely index decreased between the end and the beginning of the experiment, especially for brachychelous adult males (CBA males; see Fig. 4). This may be a consequence of food limitation during the laboratory conditions but suggests that in natural conditions depending on how well fed the male is, its claw could improve or worsen in the next moult, even when it is an original one (McLain and Pratt 2010). In relation to this, the comparison of claw morphology of the San Clemente population found that manus length was shorter for high densities than for low densities, suggesting that leptochely may increase with density. In fact, the brachychely index was below the whole population mean at high densities, and above the whole population mean at low densities (Fig. 5). These differences between densities may be due to (1) energetic differences between sites and to (2) different rates of autotomy and regeneration. First, although crab density is positively related to organic matter content of sediment (Ribeiro et al. 2005), energy expenditure may also increase with density due to social interaction, as an increased frequency of fights to defend burrows or the access to a food patch (di Virgilio and Ribeiro 2013). Then males at different densities could have a different energy budget to invest in claw development (McLain and Pratt 2010). Second, previous works have shown that predation by shorebirds species which exploit male crabs' vulnerability during courtship is higher at high densities (Iribarne and Martínez 1999; Ribeiro et al. 2003). Therefore, a higher proportion of leptochelous claws at high densities are likely also the outcome of more autotomy and regeneration due to higher risk of predation in comparison to low densities.

The morphological variations of major claws due to regeneration may be interpreted by the interaction of both physiological and functional mechanisms (Maginnis 2006). Phylogenetic contrasts have shown that the enlarged claw is isometric to the body size across species (Swanson et al. 2013; Levinton and Weissburg 2021), meaning that there is a target claw size that all males of a given species will tend to reach as they grow. This may be schematized as a programmed trajectory of claw size as the male grows (see Fig. 6). Therefore, we propose that morphological variations may represent the deviation from the original trajectory because of autotomy. Given that the first claw size regenerated is rather proportional to body size, the autotomy at different body sizes may imply tracing new trajectories with different grades of deviation from the original one, and with different energetic trade-offs. Then, the larger the male size at autotomy the larger the deviation from the original trajectory. Consequently, new highly deviated trajectories may require larger increments per moult in claw size but given energy budget available seems to be limited at each moult event, this should be at the cost of relatively lower increments in manus length.

The major claw of fiddler crabs is multifunctional (Dennenmoser and Christy 2013). The armament power of claws may allow males to obtain and defend high quality burrows (Backwell et al. 2000), and thus indirectly increasing males' mating success. However, the direct female choice has been found to be linked to claw length rather than to claw stoutness (Oliveira and Custódio 1998; McLain and Pratt 2007; Levinton and Weissburg 2021). Therefore, given the energetic constraints; crabs may be able to regenerate or (1) a long claw with a small manus area, or (2) a short claw with a large manus area. A functional explanation may propose that males of different sizes would adjust energy allocation differently by prioritising claw characteristics that maximise both current and future mating success (Perrin and Sibly 1993). It has been found that male fiddler crabs increase investment in courtship effort with age, because they have higher current mating probabilities and low

residual (or future) reproductive opportunities (Hayes et al. 2013). Therefore, small males with low current but high residual (or future) mating probabilities would benefit from regenerating a claw that initially restores weapon function as much as possible, and with the chances of improving it in successive molts. In contrast, large males may probably obtain low mating opportunities by regenerating a relatively long and weak claw, but no mating opportunities by regenerating a short but armed claw. Hence, the morphological continuum may reflect variations in how individual of different sizes and energetic conditions solve the problem of making the best of a bad job.

It is likely that the same mechanisms responsible for the morphological variation of male claws are present in all fiddler crab species but operate differently resulting in different claw patterns. Discrete claw patterns may be rather a special case of continuous claw patterns in which claw proportions are greatly altered after regeneration (*A. annulipes*, Backwell et al. 2000; *A. mjoebergi*, Reaney et al. 2008), so that successive moults, albeit with improvements, never manage to resemble what they originally were. Therefore, the way in which each species balances between the different claw functions may give the pattern of claw morphological variation after regeneration.

The third goal of this work was to evaluate how variation in claw morphology may be reflected in the outcome of different mating tactics. Since underground mating is associated with a stronger level of female choice than surface matings (Ribeiro et al. 2010) we expected more brachychelous males in underground matings and more leptochelous males in surface matings. In contrast, we found that the brachychely index of matings was higher than the one expected by chance for both mating tactics at high densities but not at low densities. In some species it has been shown that females select males based on the thermal and structural properties of their burrows, which is a crucial characteristic for egg incubation (Christy 1982; Reaney and Backwell 2007). It is likely that brachychelous males outcompete other males in

accessing and defending the best burrow sites for breeding (Backwell et al. 2000). However, why are brachychelous males also more likely to mate on the surface than leptochelous males if female choice is lower than for underground mating? (Ribeiro et al. 2010). One possibility may be due to an indirect effect of the procedural of the tactic. In surface mating, the male temporarily moves away from his burrow, leaving it exposed to being occupied by an intruder male. This situation can occur more frequently at high densities; thus, the male must be sufficiently armed to fight to recover his burrow. Therefore, a higher frequency of brachychelous males in surface matings may not be due to female choice as in underground matings, but rather because brachychelous males are more daring than leptochelous males to move away from their burrows for surface mating. Finally, variation in claw morphology appears not to play a major role in mating tactics under low density conditions. This may be due to several reasons. First, the frequency of brachychelous males is greater at low densities, so the probability to find a brachychelous male to mate without choosing it is greater than at high densities. Secondly, at low densities the competition for burrow sites may be more relaxed than at higher densities. Thus, more males in comparison to high densities are likely to own an appropriate burrow for egg incubation regardless of their claw morphology. Studies in different fiddler crab species found that males with regenerated claws were able to attract females and mate with frequencies that do not differ from the general population (Backwell et al. 2000; Lailvaux et al. 2009; Clark and Backwell 2016). In contrast, our results combining data from different densities suggest that the role of claw morphology on the outcome of mating tactics is highly dependent on the social context.

Regeneration of body parts after autotomy has been observed in several invertebrate and vertebrate taxa (Maginnis 2006; Fleming et al. 2007; Higham et al. 2013; Dunoyer et al. 2021). The study of regeneration has been focused on understanding its evolutive significance (Reichman 1984; Elchaninov et al. 2021) as well as for practical reasons for

example when the autotomy is the product of the exploitation of a resource of economic importance (e.g. Walus et al. 2023). However, except for studies in fiddler crabs (Backwell et al. 2000; Reaney et al. 2008; Lailvaux et al. 2009), less attention has been given to reproductive consequences of autotomy and regeneration of sexually selected traits (Uetz et al. 1996; Galeotti et al. 2008; Yasuda et al. 2014; Joseph et al. 2018). Our results suggest that autotomy introduces a disruption in the ontogenetic program of resource allocation driving the development of the trait. Therefore, we believe that the study of autotomy and the outcome of regenerated sexually selected structures can contribute to understanding the underlying mechanisms by which individuals balance investment in sexual traits (Arévalo and Heeb 2005) by differential weighing the costs and benefits of different functions throughout ontogeny.

Acknowledgements

References

Ahmed M, 1978. Development of asymmetry in the fiddler crab *Uca cumulanta* Crane, 1943 (Decapoda Brachyura). *Crustaceana* **34**: 294–300.

Allen BJ, Levinton JS, 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler crab. *Funct Ecol* **21**: 154–161.

Arévalo JE, Heeb P, 2005. Ontogeny of sexual dimorphism in the Long-tailed Manakin *Chiroxiphia linearis*: long maturation of display trait morphology. *Ibis* **147**: 697–705.

Backwell PRY, Christy JH, Telford SR, Jennions MD, Passmore NI, 2000. Dishonest signalling in a fiddler crab. *Proc R Soc Lond B* **267**: 719–724.

Backwell PRY, Passmore NI, 1996. Time constraints and multiple choice criteria in the sampling behaviour and mate choice of the fiddler crab, *Uca annulipes*. *Behav Ecol Sociobiol* **38**: 407–416.

Bates D, Mächler M, Bolker B, Walker S, 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw* **67**: 1–48.

Berglund A, Bisazza A, Pilastro A, 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol J Linn Soc.* **58**: 385–399.

Bildstein KL, 1983. Why white-tailed deer flag their tails. *Am Nat* **121**: 709–715.

Bildstein KL, McDowell SG, Brisbin IL, 1989. Consequences of sexual dimorphism in sand fiddler crabs, *Uca pugilator*: differential vulnerability to avian predation. *Anim Behav* **37**: 133–139.

Bogazzi E, Iribarne OO, Guerrero R, Spivak E, 2001. Wind pattern may explain the southern limit of distribution of a southwestern Atlantic fiddler crab. *J Shellfish Res* **20**: 353–360.

Bondurlansky R, Day T, 2003. The evolution of static allometry in sexually selected traits. *Evolution* **57**: 2450–2458.

Buzatto BA, Tomkins JL, Simmons LW, Machado G, 2014. Correlated evolution of sexual dimorphism and male dimorphism in a clade of neotropical harvestmen. *Evolution* **68**: 1671–1686.

Canty A, Ripley BD, 2022. boot: Bootstrap R (S-Plus) Functions. R package, version 1.3-28.1.

Christy JH, 1982. Burrow structure and use in the sand fiddler crab, *Uca pugilator* (Bosc). *Anim Behav* **30**: 687–694.

Clark HL, Backwell PRY, 2016. Male mating success in a fiddler crab: a lesson in sample sizes. *J Ethol* **34**: 119–126.

Crane J, 1975. *Fiddler crabs of the world: Ocypodidae: genus* Uca. Princeton: Princeton University Press.

Darnell MZ, Backwell PRY, Munguia P, 2020. Frequency and latency of autotomy of a sexually selected fiddler crab appendage. *J Exp Mar Biol Ecol* **523**.

De Grande FR, Fogo BR, Costa TM, 2021. Losers can win: thermoregulatory advantages of regenerated claws of fiddler crab males for establishment in warmer microhabitats. *J Therm Biol* **99**: 102952.

Dennenmoser S, Christy JH, 2013. The design of a beautiful weapon: compensation for opposing sexual selection on a trait with two functions. *Evolution* **67**: 1181–1188.

deRivera CE, Vehrencamp SL, 2001. Male versus female mate searching in fiddler crabs: a comparative analysis. *Behav Ecol* **12**: 182–191.

di Virgilio A, Ribeiro PD, 2013. Spatial and temporal patterns in the feeding behavior of a fiddler crab. *Mar Biol* **160**: 1001–1013

Dragani WC, Codignotto JO, Romero SI, Molina S, Alonso G, et al, 2014. Evolución geomorfológica de Punta Rasa, Pcia. de Buenos Aires, Argentina. *Rev Mus Argent Cienc Nat* **16**: 107–113.

Dunoyer LA, Seifert AW, Van Cleve J, 2021. Evolutionary bedfellows: Reconstructing the ancestral state of autotomy and regeneration. *J Exp Zool B* **336**: 94–115.

Elchaninov A, Sukhikh G, Fatkhudinov T, 2021. Evolution of regeneration in animals: a tangled story. *Front Ecol Evol* **9**: 621686.

Fasano JL, Hernández MA, Isla FI, Schnack EJ, 1982. Aspectos evolutivos y ambientales de la Laguna Mar Chiquita (Provincia de Buenos Aires, Argentina). *Oceanol Acta* **SP**: 285–298.

Ferrari S, Cribari-Neto F, 2004. Beta regression for modelling rates and proportions. *J Appl Stat* **31**: 799–815.

Fleming PA, Muller D, Bateman PW, 2007. Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biol Rev* **82**: 481–510.

Fox J, Weisberg S, 2019. *An R companion to applied regression*. Thousand Oaks, CA: Sage.

Galeotti P, Rubolini D, Pupin F, Sacchi R, Fasola M, 2008. Sperm removal and ejaculate size correlate with chelae asymmetry in a freshwater crayfish species. *Behav Ecol Sociobiol* **62**: 1739–1745.

Gayon J, 2000. History of the concept of allometry. *Am Zool* **40**: 748–758.

Green AJ, 1992. Positive allometry is likely with mate choice, competitive display and other functions. *Anim Behav* **43**: 170–172.

Hartnoll RG, 1978. The determination of relative growth in crustacea. *Crustaceana* **34**: 281–293.

Hayes CL, Booksmythe I, Jennions MD, Backwell PRY, 2013. Does male reproductive effort increase with age? Courtship in fiddler crabs. *Biol Lett* **9**: 20121078.

Higham TE, Russell AP, Zani PA, 2013. Integrative biology of tail autotomy in lizards. *Physiol Biochem Zool* **86**: 603–610.

de la Iglesia HO, Rodríguez EM, Dezi RE, 1994. Burrow plugging in the crab *Uca uruguayensis* and its synchronization with photoperiod and tides. *Physiol Behav* **55**: 913–919.

Iribarne OO, Martínez MM, 1999. Predation on the southwestern Atlantic fiddler crab (*Uca uruguayensis*) by migratory shorebirds (*Pluvialis dominica, P. squatarola, Arenaria interpres*, and *Numenius phaeopus*). *Estuaries* **22**: 47–54.

Joseph PN, Emberts Z, Sasson DA, Miller CW, 2018. Males that drop a sexually selected weapon grow larger testes. *Evolution* **72**: 113–122.

Lailvaux SP, Reaney LT, Backwell PRY, 2009. Dishonest signalling of fighting ability and multiple performance traits in the fiddler crab *Uca mjoebergi*. *Funct Ecol* **23**: 359–366.

Latruffe C, McGregor PK, Oliveira RF, 1999. Visual signalling and sexual selection in male fiddler crabs *Uca tangeri*. *Mar Ecol Prog Ser* **189**: 233–240.

Lenth RV, 2022. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package, version 1.7.4-1.

Levinton JS, Allen BJ, 2005. The paradox of the weakening combatant: trade-off between closing force and gripping speed in a sexually selected combat structure. *Funct Ecol* **19**: 159–165.

Levinton JS, Weissburg M, 2021. Length of a sexually selected ornament-armament in fiddler crabs (Decapoda: Brachyura: Ocypodidae): One way, over deep time and space. *J Crustac. Biol* **41**: ruab066.

van Lieshout E, Elgar MA, 2009. Armament under direct sexual selection does not exhibit positive allometry in an earwig. *Behav Ecol* **20**: 258–264.

Mace III MM, Curran MC, 2011. Differences in the use of cheliped autotomy by the mud fiddler crab *Uca pugnax* (Smith, 1870) (Decapoda, Ocypodidae) when escaping predation by the blue crab *Callinectes sapidus* (Rathbun, 1896). *Crustaceana* **84**: 1281–1293.

Maginnis TL, 2006. The costs of autotomy and regeneration in animals: a review and framework for future research. *Behav Ecol* **17**: 857–872.

Manly BFJ, 2006. *Randomization, bootstrap and Monte Carlo methods in biology*. Boca Raton: Chapman and Hall/CRC.

Martin BE, 2019. Autotomy and running performance of fiddler crabs (Decapoda: Brachyura: Ocypodidae). *J Crustac Biol* **39**: 613–616.

McCullough EL, O'Brien DM, 2022. Variation in allometry along the weapon-signal continuum. *Evol Ecol* **36**.

McLachlan GJ, Lee SX, Rathnayake SI, 2019. Finite mixture models. *Annu Rev Stat Appl* **6**: 355–378.

McLain DK, Pratt AE, Berry AS, 2003. Predation by red-jointed fiddler crabs on congeners: interaction between body size and positive allometry of the sexually selected claw. *Behav Ecol* **14**: 741–747.

McLain DK, McBrayer LD, Pratt AE, Moore S, 2010. Performance capacity of fiddler crab males with regenerated versus original claws and success by claw type in territorial contests. *Ethol Ecol Evol* **22**: 37–49.

McLain DK, Pratt AE, 2007. Approach of females to magnified reflections indicates that claw size of waving fiddler crabs correlates with signaling effectiveness. *J Exp Mar Biol Ecol* **343**: 227–238.

McLain DK, Pratt AE, 2010. Food availability in beach and marsh habitats and the size of the fiddler crab claw, a sexually selected weapon and signal. *Oikos* **119**: 508–513.

McLain DK, Pratt AE, 2011. Body and claw size at autotomy affect the morphology of regenerated claws of the sand fiddler crab, *Uca pugilator*. *J Crustac Biol* **31**: 1–8.

Morgan TH, 1923. The development of asymmetry in the fiddler crab. *Am Nat* **57**: 269–273.

Muramatsu D, Koga T, 2016. Fighting with an unreliable weapon: opponent choice and risk avoidance in fiddler crab contests. *Behav Ecol Sociobiol* **70**: 713–724.

Nakasone Y, Murai M, 1998. Mating behavior of *Uca lactea perplexa* (Decapoda: Ocypodidae). *J Crustac Biol* **18**: 70–77.

Nath A, Jose Priya TA, Kappalli S, 2023. On sexual maturity in male fiddler crab, *Gelasimus hesperiae* (Crane, 1975) (Brachyura: Ocypodidae). *Invertebr Zool* **20**: 417–432.

O'Brien DM, Allen CE, Van Kleeck MJ, Hone D, Knell R, et al., 2018. On the evolution of extreme structures: static scaling and the function of sexually selected signals. *Anim Behav* **144**: 95–108.

Oliveira RF, Custódio MR, 1998. Claw size, waving display and female choice in the European fiddler crab, *Uca tangeri*. *Ethol. Ecol. Evol.* **10**: 241–251.

Palaoro AV, García-Hernández S, Buzatto BA, Machado G, 2022. Function predicts the allometry of contest-related traits, but not sexual or male dimorphism in the amazonian tusked harvestman. *Evol Ecol* **36**: 605–630.

Pélabon C, Bolstad GH, Egset CK, Cheverud JM, Pavlicev M, et al., 2013. On the relationship between ontogenetic and static allometry. *Am Nat* **181**: 195–212.

Perrin N, Sibly RM, 1993. Dynamic models of energy allocation and investment. *Annu Rev Ecol Syst* **24**: 379–410.

Prates MO, Lachos VH, Cabral CRB, 2013. Mixsmsn: fitting finite mixture of scale mixture of skew-normal distributions. *J Stat Softw* **54**: 1–20.

R Core Team, 2020. R: a language and environment for statistical computing.

Reaney LT, Milner RNC, Detto T, Backwell PRY, 2008. The effects of claw regeneration on territory ownership and mating success in the fiddler crab *Uca mjoebergi*. *Anim Behav* **75**: 1473–1478.

Reaney LT, Maurer G, Backwell PRY, Linde CC, 2012. Paternity analysis of two male mating tactics in the fiddler crab, *Uca mjoebergi*. *Behav Ecol Sociobiol* **66**: 1017–1024.

Reaney LT, Backwell PRY, 2007. Temporal constraints and female preference for burrow width in the fiddler crab, *Uca mjoebergi*. *Behav Ecol Sociobiol* **61**: 1515–1521.

Reichman OJ, 1984. Evolution of regeneration capabilities. *Am Nat* **123**: 752–763.

Ribeiro PD, Iribarne OO, Jaureguy L, Navarro D, Bogazzi E, 2003. Variable sex-specific mortality due to shorebird predation on a fiddler crab. *Can J Zool* **81**: 1209–1221.

Ribeiro PD, Iribarne OO, Daleo P, 2005. The relative importance of substratum characteristics and recruitment in determining the spatial distribution of the fiddler crab *Uca uruguayensis* Nobili. *J Exp Mar Biol Ecol* **314**: 99–111.

Ribeiro PD, Daleo P, Iribarne OO, 2010. Density affects mating mode and large male mating advantage in a fiddler crab. *Oecologia* **164**: 931–941.

Rosenberg MS, 2002. Fiddler crab claw shape variation: a geometric morphometric analysis across the genus *Uca* (Crustacea: Brachyura: Ocypodidae). *Biol J Linn Soc* **75**: 147–162.

Rowland JM, Emlen DJ, 2009. Two thresholds, three male forms result in facultative male trimorphism in beetles. *Science* **323**: 773–776.

Seifert AW, Voss SR, 2013. Revisiting the relationship between regenerative ability and aging. *BMC Biol* **11**: 2.

Shih HT, Ng PKL, Davie PJF, Schubart CD, Türkay M, et al., 2016. Systematics of the family Ocypodidae Rafinesque, 1815 (Crustacea: Brachyura), based on phylogenetic relationships, with a reorganization of subfamily rankings and a review of the taxonomic status of *Uca* Leach, 1814, sensu lato and its subgenera. *Raffles B Zool* **64**: 139–175.

Spivak ED, Gavio MA, Navarro CE, 1991. Life history and structure of the world's southernmost *Uca* population: *Uca uruguayensis* (Crustacea, Brachyura) in Mar Chiquita lagoon (argentina). *Bull Mar Sci* **48**: 679–688.

Swanson BO, George MN, Anderson SP, Christy JH, 2013. Evolutionary variation in the mechanics of fiddler crab claws. *BMC Evol Biol* **13**: 137.

Tönnies T, Kahl S, Kuss O, 2022. Collider bias in observational studies. Consequences for medical research. Part 30 of a series on evaluation of scientific publications. *Dtsch Arztebl Int* **119**: 107–122.

Uetz GW, McClintock WJ, Miller D, Smith EI, Cook KK, 1996. Limb regeneration and subsequent asymmetry in a male secondary sexual character influences sexual selection in wolf spiders. *Behav Ecol Sociobiol* **38**: 253–257.

Wallace B, 1987. Ritualistic combat and allometry. *Am Nat* **129**: 775–776.

Walus AM, Schneider EVC, Parker EN, Brittain C, McGaw IJ, et al., 2023. A comparison of claw removal methods on the survival and claw regeneration of stone crab (*Menippe mercenaria*). *J Exp Mar Biol Ecol* **563**: 151895.

Yakushiji N, Yokoyama H, Tamura K, 2009. Repatterning in amphibian limb regeneration: A model for study of genetic and epigenetic control of organ regeneration. *Semin Cell Dev Biol* **20**: 565–574.

Yamaguchi T, 1973. Asymmetry and dimorphism of chelipeds in the fiddler crab, *Uca lactea* De Haan. *Zool Mag* **82**: 154–158.

Yamaguchi T, 1998. Evidence of actual copulation in the burrow in the fiddler crab, *Uca lactea* (De Haan, 1835) (Decapoda, Brachyura, Ocypodidae). *Crustaceana* **71**: 565–570.

Yamaguchi T, 2001. Dimorphism of chelipeds in the fiddler crab, *Uca arcuata*. *Crustaceana* **74**: 913–923.

Yamaguchi T, 2002. Survival rate and age estimation of the fiddler crab, *Uca lactea* (De Haan, 1835) (Decapoda, Brachyura, Ocypodidae). *Crustaceana* **75**: 993–1014.

Yamaguchi T, Henmi Y, 2001. Studies on the differentiation of handedness in the fiddler crab, *Uca arcuata*. *Crustaceana* **74**: 735–747.

Yamaguchi T, Henmi Y, 2008. Cheliped differentiation and sex ratio of the fiddler crab *Uca arcuata*. *Crustac Res* **37**: 74–79.

Yasuda CI, Matsuo K, Wada S, 2014. Rapid regeneration of the major cheliped in relation to its function in male-male contests in the hermit crab *Pagurus middendorffii*. *Plankton Benthos Res* **9**: 122–131.

Table 1. Summary of finite mixture models used to detect the most parsimonious k-number of morphologically differentiated groups of claws, where each group is described by a skew normal distribution (parameters: mean, variance, and skewness). The total number of parameters of each model are computed as 3k + p, where k is the number of distributions, and p = k - 1, the number of parameters describing the proportion of males in each distribution. Analysis was applied to the residuals after fitting linear regression to (A) Propodus length in relation to carapace width, (B) Manus length in relation to propodus length, and (C) Ratio manus/propodus in relation to carapace width. Values are the bias-corrected Akaike Information Criterion AICc. The best fit for each morphological variable is indicated with * and corresponds to the one with lower AICc.

k-number of distributions	parameters	Propodus length	Manus length	Ratio manus/ propodus
1	3	-359.46	-808.21 *	-913.23 *
2	7	-436.31 *	-806.90	-904.85
3	11	-428.42	-798.66	-901.36
4	15	-419.72	-799.35	-895.95
5	19	-410.73	-784.23	-886.38

Figure legends

Figure 1. Variability of male claws along the morphological continuum. (A) Brachychelous: robust claws with significant development of tubercles and teeth, (B) Inter-Brachychelous: robust claws with moderate development of tubercles and teeth. (C) Inter-Leptochelous: slight claws with poor development of tubercles and teeth, (D) Leptochelous: slight claws without tubercles and teeth, (E) Newly regenerated claws: small claws with evident signs of recent regeneration. (F) A male fiddler crab *Leptuca uruguayensis* from the San Clemente population. In C, the short line shows the manus length, and the long line shows the propodus length.

Figure 2. Population variation in claw morphology. (A) propodus length in relation to carapace width, (B) manus lengths in relation to propodus length and (C) ratio manus/propodus in relation to carapace width. Full-size claws are shown in black, and recently regenerated claws in grey.

Figure 3. Mean and 95% confidence limits for the brachychely index computed for each of the claw variants classified based on their visual robustness and development of tubercles (see methods). Brachychelous (Brach), inter-brachychelous (Int-Brach), inter-leptochelous (Int-Lepto), leptochelous (Lepto), and newly regenerated claws (Regen). The Brachychely index for the claws for the field experiment (Field) are also shown. Horizontal red line indicates the value for brachychely index with average propodus and manus length.
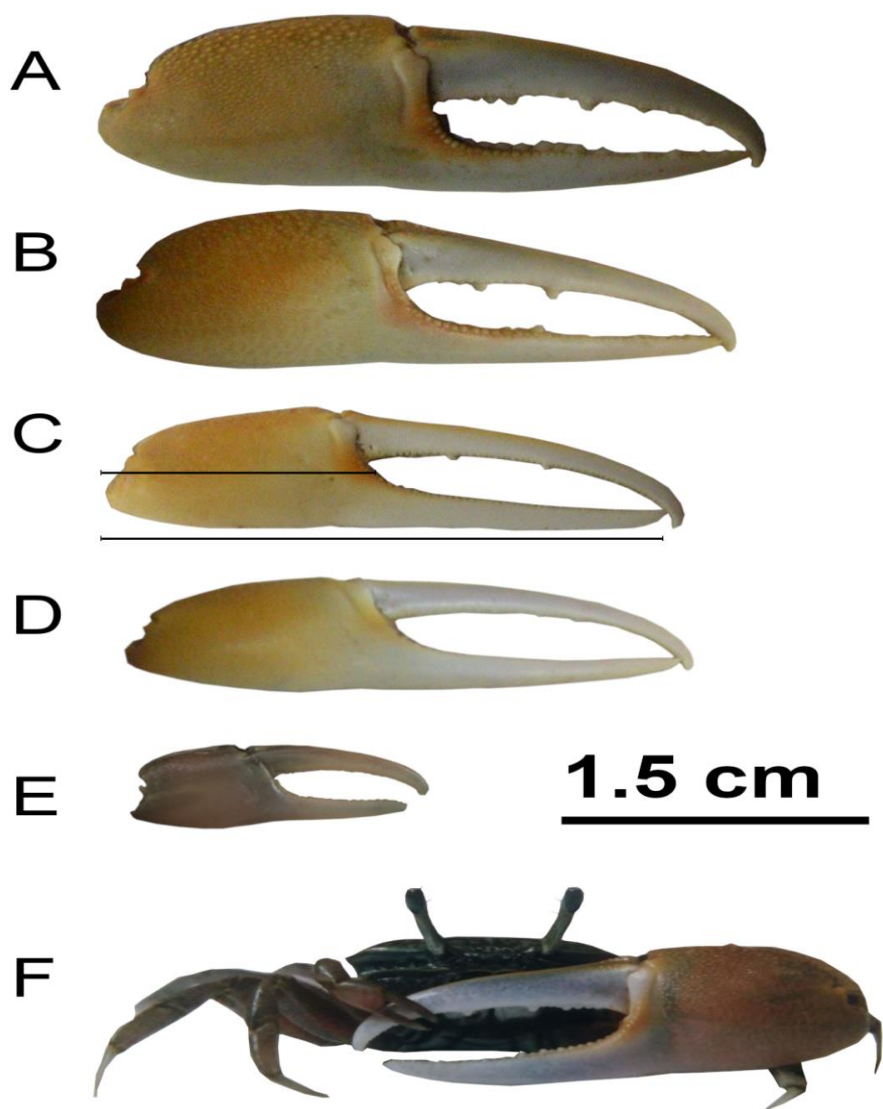
Figure 4. Laboratory experiment. Mean and 95% confidence limits for the change in the brachychely index computed as the value of the index after one moult minus the value at the beginning of the experiment. Autotomized brachychelous adults (ABA), autotomized

leptochelous adults (ALA), autotomized juveniles (AJ), control brachychelous adults (CBA), control leptochelous adults (CLA), and control juveniles (CJ).

Figure 5. Mean and 95% confidence limits for the brachychely index for the population of San Clemente at high (PH) and low-density (PL), for underground matings at high and low density (UH and UL, respectively) and for surface matings at at high and low density (SH and SL, respectively). Expected values for the brachychely index based on the size of mating males are shown for each mating tactic as eUH, eSH, eUL, and eSL. Horizontal red line indicates the value for brachychely index with average propodus and manus length.

Figure 6. Schematic representation of the development of claw size in fiddler crabs. The solid black line represents the developmental trajectory of an original claw, as the individual grows through moults. The black dot indicates the target claw size, the claw size that all males are programmed to reach. The solid red line represents the initial state after autotomy from which the individual traces a new development trajectory towards the target claw size. Two examples of new trajectories are indicated with grey dotted lines. After a determined moult, the male grows a determined amount in its carapace size (segment a-b) and a determined amount in its claw size (segment b-c). Autotomising and regeneration at large body size, may imply larger b-c increment per moult than regeneration at small body size or along the original trajectory. The required large increments in claw size per moult may be at the cost of reducing relative increments in other claw features, such as manus length, causing the leptochelous morphology.
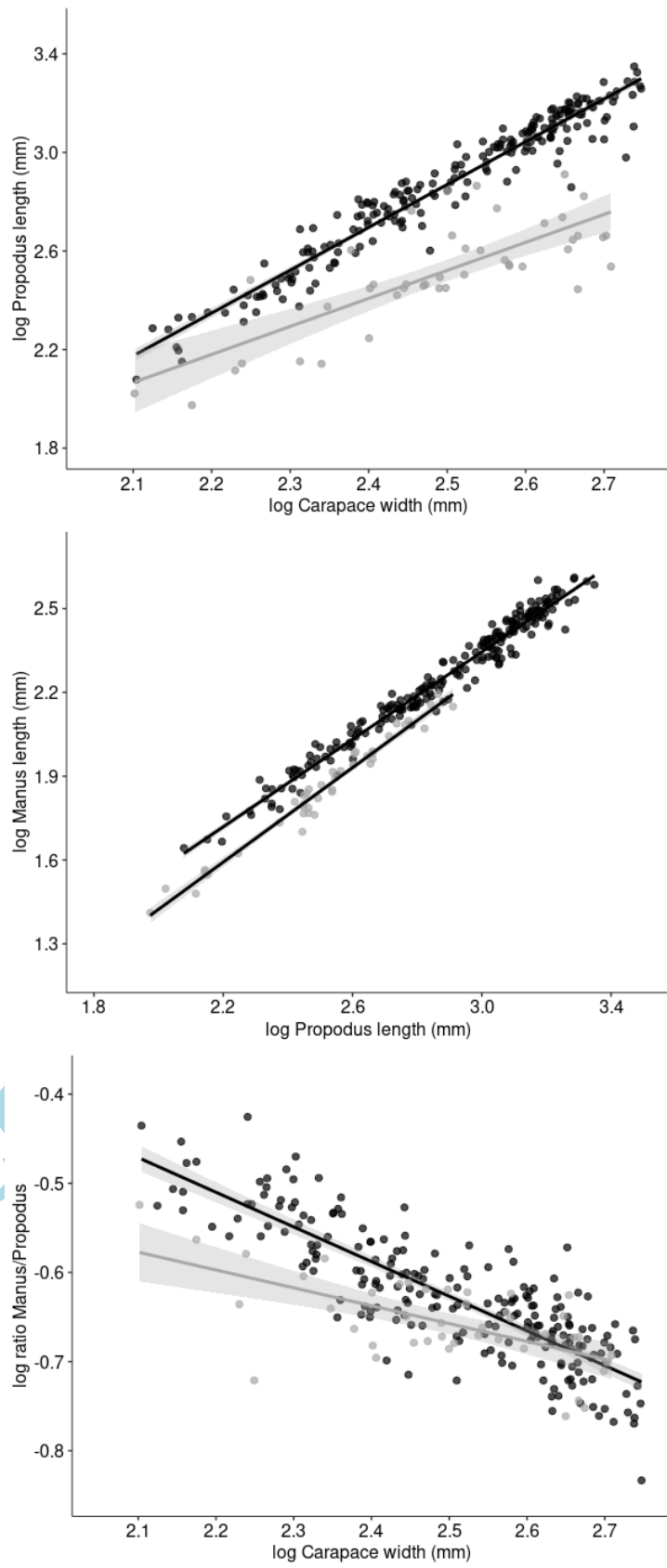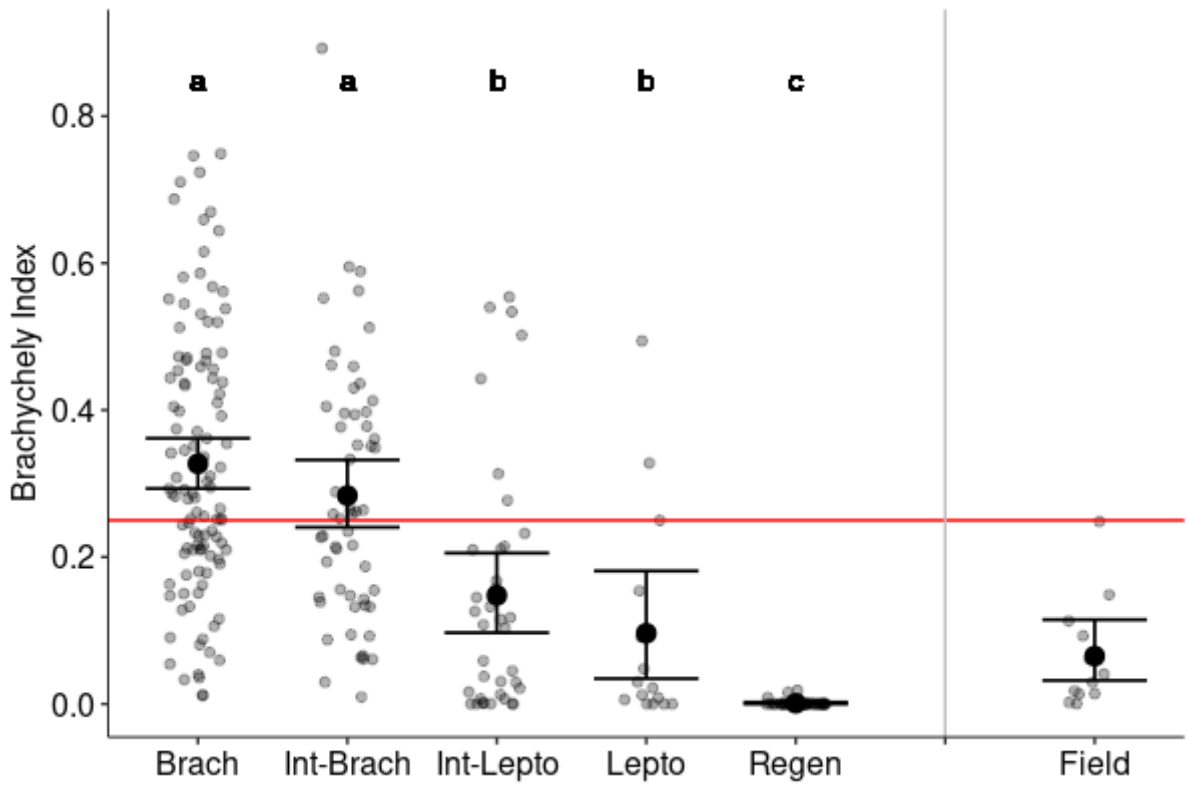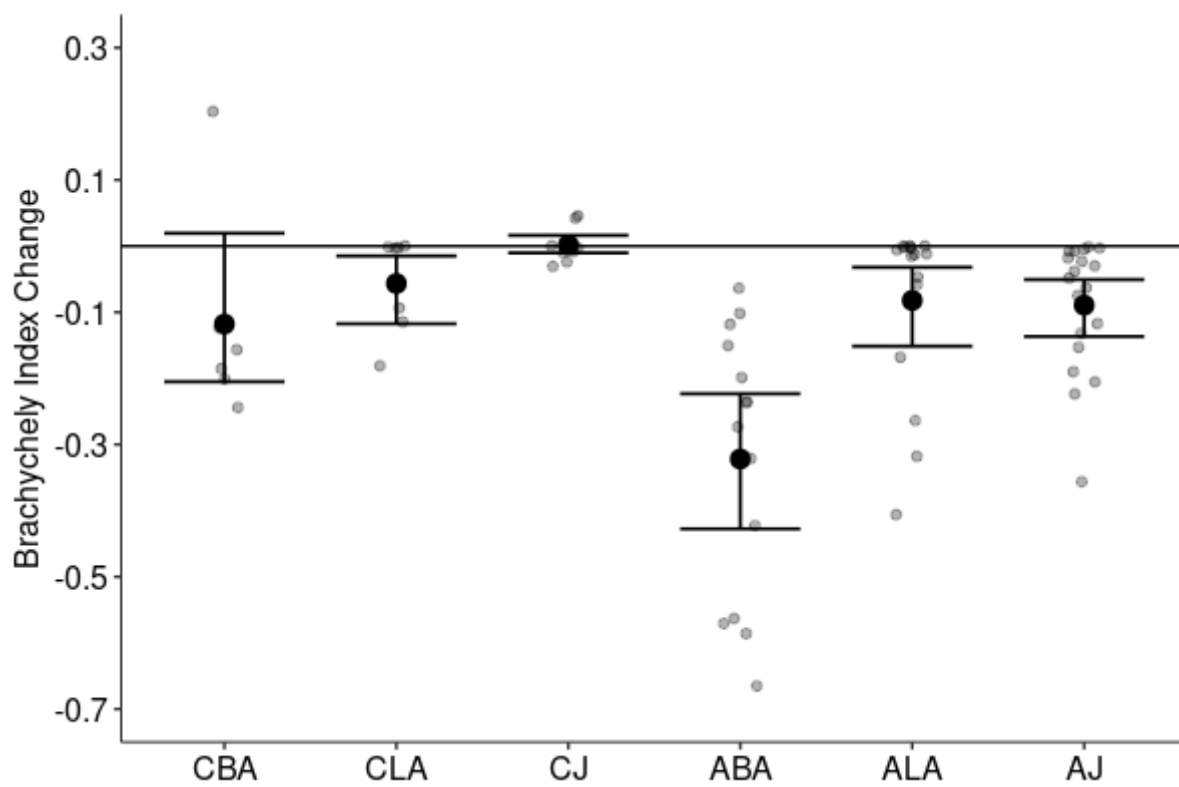
Figure 1

Figure 2

Figure 3

Figure 4
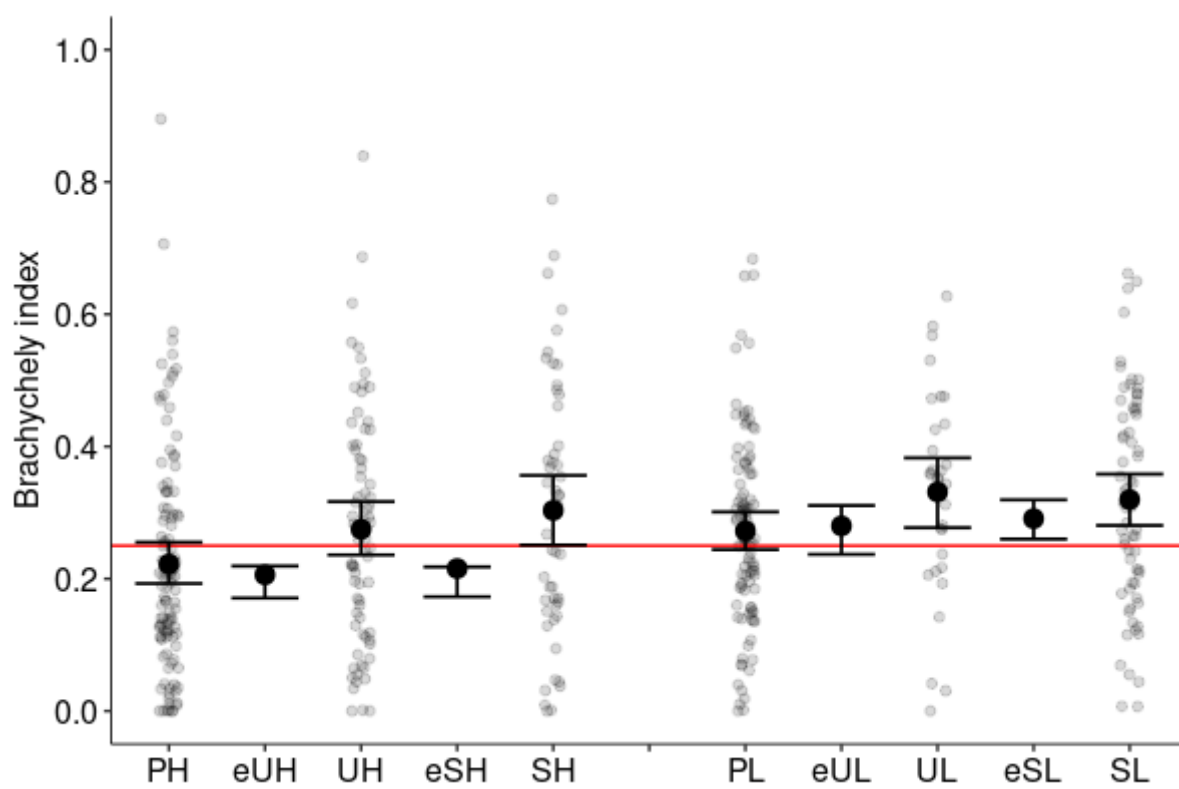
Figure 5

Figure 6