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HISTÓRIA NATURAL E BIOLOGIA POPULACIONAL EM *Euryades corethrus* (Boisduval, 1836) (Lepidoptera: Papilionidae:Troidini), UMA ESPÉCIE BRASILEIRA AMEAÇADA DE EXTINÇÃO.

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Orientador: Prof. Dr. Nicolás Oliveira Mega

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Aprovado em __________de__________de 2016

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"What is the point of the frustrating,

dirty and tedious job of ecology?

It's because of the jazz. The jazz is when

you figure something out, when you

discover one small part of how life works

on this planet"

Robert Denno, entomologista

(1945 – 2008)

Agradecimentos

Durante minha trajetória como biólogo fui levado a acreditar, primeiro de uma maneira ingênua, de manual, depois de uma maneira um pouco mais crítica e pessoal, que a ciência é feita de trocas entre campos de conhecimentos e entre pessoas. Apesar da tendência que temos (eu tive) em inicialmente ver somente o lado bonito e enlevante disso, esse tipo de interação pode ser também muito difícil de se lidar. Para o bem e para o mal, lidar com pessoas envolve política, concessão, *mea culpa*, fatos e suas interpretações variadas. Bichos sociais são complexos. Não saio desse período de formação como uma pessoa pessimista, mas saio muito mais cético em relação a como é feita essa busca da realidade. Acredito que isso tenha sido o mais importante que internalizei.

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RESUMO

Euryades corethrus (Boisduval, 1836) é uma borboleta da tribo Troidini que tem distribuição meridional na América do Sul. É uma espécie que usa como hospedeira plantas do gênero *Aristolochia.*A borboleta é categorizada como Vulnerável (VU) no Sul do Brasil, sendo que as maiores ameaças que sofre estão relacionadas à perda e fragmentação de seu habitat, que gera diminuição na oferta de sua planta hospedeira. Os principais motivos de conversão dessas áreas de campo são o uso na agricultura, principalmente monoculturas de eucalipto e soja, e a pecuária intensiva com pastos cultivados. Durante um ano nós conduzimos um estudo de captura-marcação-liberação-recaptura (CMLR) em uma área de campo nativo presente na Estação Experimental Agronômica da UFRGS, localizada no município de Eldorado do Sul, Rio Grande do Sul, com o intuito de investigar a dinâmica populacional, história natural e características ligadas ao uso de habitat da espécie. Durante esse período capturamos 955 indivíduos, sendo 367 fêmeas e 588 machos, dos quais 7.6% das fêmeas e 14.12% dos machos foram recapturados pelo menos uma vez. A maior abundância de indivíduos foi observada uma semana antes do Equinócio de Primavera. O comprimento de asa não diferiu entre machos e fêmeas, mas o tamanho de asas em geral variou durante o ano, com as maiores medias de comprimento sendo observadas em Dezembro. A estrutura etária variou em uma sucessão de picos de indivíduos jovens seguidas por um aumento no envelhecimento da população. Nenhum adulto foi observado de Junho a Agosto de 2014, e de Abril a Maio de 2015, o que sugere diapausa nesses períodos. Indivíduos geralmente preferiam áreas abertas e as fêmeas foram significativamente mais capturadas próximas a manchas da planta hospedeira. Os resultados encontrados sugerem que os esforços de conservação da espécie deveriam ser voltados para o aumento da conectividade da matriz ambiental, com a promoção da criação de gado de

uma maneira extensiva, com o uso sustentável de pastagens naturais e rotatividade, mas com atenção à época em que isso é feito, e para o aumento de extensão de habitat, com a implementação de Unidades de Conservação para as formações de campo.

CAPÍTULO 1

Introdução Geral

O Brasil é um país megadiverso cuja diversidade é altamente desconhecida e bastante ameaçada (Lewinsohn & Prado 2005). A conservação dos Biomas brasileiros é voltada, principalmente, para a preservação e conservação das suas formações florestais, ambientes com maior apelo carismático. Apesar de ser cada vez mais necessária, pouca atenção é direcionada para ambientes e formações que possam parecer menos atraentes para o público. Este é o caso dos chamados Campos, formações encontradas nos Biomas Mata Atlântica e Pampa e que apresentam fisionomias diversas, com presença de espécies vegetais gramíneas e arbustivas e alta diversidade de fauna associada. Pouco conhecimento a respeito de seu estado ecológico chega ao público em geral, e as políticas de conservação direcionadas às formações em questão vão de pouca a nenhuma (Overbeck et al. 2015).

O principal impacto aos Campos é relacionado ao seu mau uso, a conversão extensiva e a fragmentação da paisagem natural. A expansão das fronteiras agrícolas para suprir a necessidade dos chamados biocombustíveis no Brasil e o mercado de celulose no Uruguai promoveram a conversão de áreas que eram originalmente de gramíneas em campos de monocultura de soja e de eucalipto (Vélez-Martin et al. 2015). Por outro lado, o uso de campos naturais para a pecuária, especialmente quando esta é feita em uma escala pequena, causa um impacto menor, quando em comparação com sistemas intensivos de criação de gado de corte que usam pastos semeados (Borba e Trindade 2009), proporcionando uma alternativa econômica mais sustentável para a região de Argentina, Brasil e Uruguai. Contudo, os métodos de criação de gado na região permanecem arcaicos e muito intensos para que seja possível a manutenção da

produtividade e a preservação do ecossistema de forma conjunta. Atualmente, a agricultura e a pecuária suprimiram as áreas de campos naturais da Savana Uruguaia para 51% da sua cobertura original no território brasileiro (Crawshaw et al. 2007) e para 70% no território uruguaio (Gautreau, 2010).

Invertebrados terrestres, dependendo do tipo de reação que apresentam às mudanças no ambiente, são categorizados em diferentes grupos. Os *detectores* são espécies nativas que são sensíveis a alterações ambientais e que reagem a elas com declínios populacionais; *exploradores* são organismos que, por outro lado, tiram vantagem na mudança e respondem com um aumento em abundância, e os *acumuladores*, espécies que tendem a acumular químicos presentes no ambiente e que podem ser usados como uma medida indireta da presença desses químicos (Gerlach et al. 2013). De qualquer modo, se mostra necessário um conhecimento prévio sobre o estado da fauna local para que seja possível a aplicação de alguma métrica para investigar modificações posteriores causadas por um eventual impacto. Entre a fauna, borboletas são tidos como bons indicadores (grupo dos *detectores*) do estado geral de conservação de uma área estudada, principalmente porque, dentre os insetos, são provavelmente o grupo mais conhecido, taxonomica e ecologicamente (Thomas 2005, Bonebrake et al. 2010).

O gênero *Euryades* Felder & Felder, 1864 (Papilionidae: Troidini) é um grupo de borboletas Neotropicais que tem distribuição concentrada no sul na América do Sul. O grupo é encontrado na Argentina, Brasil, Paraguai e Uruguai e apresenta duas espécies: *Euryades duponchelii* (Lucas, 1839) e *Euryades corethrus* (Boisduval, 1836). Os adultos de ambas espécies ovipositam somente em plantas do gênero *Aristolochia*, e os compostos tóxicos secundários que são absorvidos dessas plantas acabam tornando larvas e adultos impalatáveis (Tyler 1994).

Euryades corethrus é uma borboleta que apresenta um acentuado dicromatismo sexual: machos são pretos com manchas amarelas e vermelhas, enquanto fêmeas apresentam uma coloração amarela translúcida, bem como alguns *redspots* nas asas posteriores (Fig 1). Além das sinapomorfias de Papilionidae, que incluem o osmetério, a morfologia da veia A2 da asa posterior, e escleritos cervicais fundidos localizados posteriormente à cabeça do imago; a espécie também apresenta uma estrutura notável chamada *sphragis:* um tampão genital com duas pequenas alas, que é transferido pelo macho para a fêmea durante o acasalamento e que impede fisicamente que fêmeas previamente copuladas tenham novos encontros sexuais. *Euryades corethrus* pode ser encontrada nos meses de Fevereiro, Abril, Maio, Setembro, Outubro e Dezembro, e é propensa a ser visualizada em áreas abertas de campo e campos de altitude, exibindo seu comportamento de voo mais intenso durante as horas mais quentes do dia, quando está procurando por parceiros ou plantas para ovipositar (Link et al. 1977). Ela parece preferir áreas de arbustos e de campo próximas a bordas de florestas para executar esses comportamentos. Devido à sua oviposição característica, principalmente em *Aristolochia sessilifolia*, e devido à absoluta especialização das larvas à fonte de alimentação a qual foram inicialmente expostas, o fator mais importante de ameaça que parece afetar *E. corethrus* é a progressiva diminuição de áreas de campo nativo onde a planta hospedeira pode ser encontrada.

Devido à falta de conhecimento e descrição detalhada das características da espécie na natureza e sua biologia populacional, o objetivo do presente estudo é descrever as métricas e dinâmicas da população e alguns aspectos da história natural de *Euryades corethrus*, passando pelo seu comportamento de voo, comportamento reprodutivo e preferências alimentares.

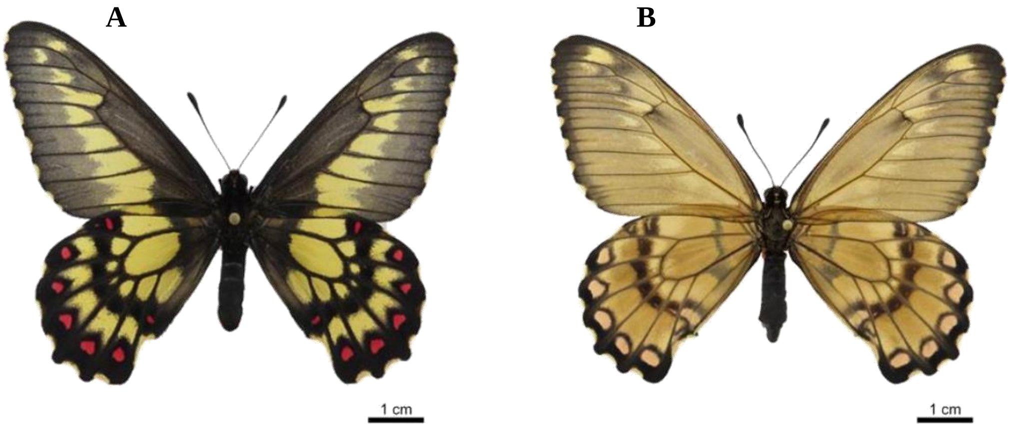

Figura 1. Espécimes de *Euryades corethrus* (Boisduval,1836) em vista dorsal: A, macho; B, fêmea.

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CAPÍTULO 2

Population Biology and Natural History of the grassland butterfly *Euryades corethrus* (Papilionidae: Troidini), an Endangered Brazilian species

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Population biology and natural history of the grassland butterfly *Euryades corethrus* (Papilionidae: Troidini), an endangered Brazilian species

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Abstract

Euryades corethrus is a swallowtail butterfly with a southern South American distribution. It only oviposits in plants of the *Aristolochia* genera. The butterfly is categorized Vulnerable (VU), the main threats it suffers are habitat loss and fragmentation, and decrease in supply of host-plants, due to extensive conversion of the *Campos* for monocultures and intensive cattle raising. We conduct a yearlong mark-release-recapture (MRR) study in a South-Brazilian area of native *Campos* aiming at population dynamics, natural history, and life traits related to the use of habitat. We captured 955 specimens (367 females and 588 males); 7.6% of females and 14.12% of males were recaptured. The most individuals were observed a week prior the spring equinox. Male and female wings sizes did not differ, general wing size showed variation between months, the biggest length in December. Age structure varied in a succession of peaks of young individuals outbreaks followed by increased aging. No adults were observed from June to August 2014, and from April to May 2015, suggesting diapause. Individuals generally preferred meadows, and female were prone to be found next to *Aristolochia* patches.

Conservation efforts should aim at landscape connectivity, by promoting the rearing of cattle in an extensive way, with a rotating, season-wise use of small suitable areas of natural pasture, and at extension of overall suitable are, by implementation of Conservation Units of *Campos* formations.

Keywords

Aristolochia, *Campos*, conservation, mark and recapture, Pampa biome, phenology

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Introduction

Brazil is a megadiverse country that presents heavily threatened species and that has a still widely unknown diversity (Lewinsohn and Prado 2005). The matter of conservation regarding Brazilian Biomes is mainly linked to the care and maintenance of its forests: very little attention is directed to non-forestall environments, despite the high biodiversity, noteworthy endemism rates and ecosystem services they display (Overbeck et al. 2015). Such is the case of the so called *Campos*, the grassland formations found in both Atlantic Forest and Pampa Biomes. These grasslands bear a high diversity of vegetal species (Boldrini 2009) and associated fauna, and are largely forgotten when it comes to public awareness of their condition and political efforts directed to their conservation (Overbeck et al. 2007, 2015).

Because their high suitability for agricultural enterprises (Lambin et al. 2013), the leading impact related to the *Campos* conservation is linked to their misuse, especially the one that leads to fragmentation and extensive conversion. The expansion of biofuel markets in Brazil and the cellulose pulp trade in Uruguay have promoted the conversion of original native grasslands to soybean and *Eucalyptus* monocultures (Vélez-Martin et al. 2015). On the other hand, cattle raising on natural grasslands, especially when performed at a small scale, is expected to cause lower impact when compared to intensive livestock systems using sown pastures (Borba and Trindade 2009), providing a more sustainable economical alternative for the Uruguayan Savanna. However, cattle raising methods in the region remain archaic and too intense to manage productivity and ecosystem preservation in concert. Nowadays, agriculture and livestock have suppressed the natural grassland areas of the Uruguayan Savanna to 51% of its original coverage in the Brazilian territory (Crawshaw et al. 2007) and up to 70% in the Uruguayan territory (Gautreau 2010).

Among the fauna, invertebrates are thought to be good indicators of the overall conservation state of a studied area. Depending on what is their reaction to environmental change, they are categorized into different groups, namely, *detectors* (native species that are sensitive to environmental alteration whose population decrease when it happens), *exploiters* (organisms that take advantage on the change and increase in abundance), and *accumulators* (species that tend to accumulate chemicals of the ambient and can be used as an indirect mean of measure) (Gerlach et al. 2013). Anyhow, it is necessary a previous knowledge of the local fauna species status in order to make possible a metric of further modifications caused by impact.

The butterfly genus *Euryades* Felder & Felder, 1864 (Papilionidae: Troidini) is a Neotropical group of insects that have a southern South American distribution, found in areas of Atlantic Forest, Pampa and Chaco biomes from Argentina, Brazil, Paraguay and Uruguay. One of those species, *Euryades corethrus* (Boisduval, 1836), oviposits solely on plants from the genus *Aristolochia*, and the toxic secondary compounds absorbed from the host-plants end up making both larvae and adults unpalatable (Tyler et al. 1994). The butterfly presents an accentuated sexual dichromatism: males are black with yellow and red stains, while females present a translucent yellow coloration, as well as few red spots in the hindwings (Fig 1). The species also presents a noteworthy structure called *sphragis*: a mating plug that is transferred by the male and carried by the female that physically inhibits mated females from further copulation. *Euryades corethrus* can be found during the months of February, April, May, September, October and December (Link et al. 1977), and is prone to be found in open areas of grasslands and altitude fields, exhibiting its most intense flight behavior during the hottest hours of the day, when it`s looking for either mating partners or plants to oviposit (Link et al. 1977). It seems to prefer shrubland and grassland areas close to forest edges to perform such behaviors.

In a recent actualization of the Red List of Threatened Species of Rio Grande do Sul State by the Zoobotanic Foundation of Rio Grande do Sul State (FZB 2013), *E. corethrus* was categorized as Vulnerable (VU), being labeled “B2ab(iii)”, which stands for faunal individuals that are at menace because of factors impacting their area of occupancy. The species’ area of occupancy is either severely fragmented or presents an observable decline in its extent and quality. In the Red List of Threatened Species from Paraná State (Mielke &

Casagrande 2004) the species is labeled as Endangered (EN), being labeled EN B2ab(ii,iii) (Doilibaina et al. 2010), an even worse condition of threat. Natural history records point to characteristic oviposition on five *Aristolochia* species, namely *A. sessilifolia*, *A. brevifolia*, *A. angustifolia*, *A. fimbriata*, and *A. Lingua* (Beccaloni 2008, Klitzke and Brown Jr 2000, Biezanko et al. 1974, Tyler et al. 1994, Núñez-Bustos 2010). Due to the specialization, the most important factor of threatening that seems to affect *E. corethrus* is the progressive shrinkage of native grassland areas where the host-plant can be found.

Given the lack of knowledge on the *E. corethrus* biology, the objectives of our study were to describe the population dynamics and some aspects of the species' natural history that can help to establish parameters for the conservation of *E. corethrus*. For that, we selected a native grassland area preserved from any environmental impact to evaluate oviposition and host-plant use, adult feeding preferences, dispersal, and mating strategies. We expect that population size will vary directly with the day length and temperature, leading to adult local extinction during later autumn, winter, and early spring; if summer conditions get too harsh, temporary adult extinction may also occur. High ecological demands are common to all endangered swallowtail butterflies, thus we expect that *E. corethrus* presence will be strongly correlated with larval host-plants and adult resources abundance.

Material and Methods

Study site

The study area is in an experimental agronomic station, a property of Federal University of Rio Grande do Sul, which is located in the municipality of Eldorado do Sul, state of Rio Grande do Sul, southern Brazil (30°05'27"S, 51°40'18"W). The site upholds approximately 800 ha of native Pampa meadows, which are mainly dedicated to cattle raising and agronomic experimentation. Apart those activities there is a 30 ha area that did not suffer any management in the last 30 years and are suited to conservation studies. We have conducted the study in the least transformed area.

The site is primarily plane, with small hills in its East face, as well as a depression in its middle. The site vegetation is composed by a myriad of grassland species and shrubs. There are also a few water courses that support gallery forests and swamps in the lower part of the terrain (Fig 2).The climate in the region is categorized as Cfa in the Köppen-Geiger classification (Peel 2007), which stands for humid subtropical, tending more to the temperate than to the tropical climate, with mean temperatures of the warmest month surpassing 22°C (Moreno 1961).

Butterfly sampling and parameters analyzed

All field work was done along two 750 meters long trails in 30 ha area. Both trails had numbered signs every 50 m, which were used to identify the point of encounter of individuals. Captures were labeled as the closer to the encounter checkpoint in the trail. The areas were next to one another, separated by a small creek and treated as two areas only as a mean of organization. Both areas were covered in a field day, one by the morning and the other by the afternoon. We started each day of work in a different trail to avoid sampling bias.

We used the mark-release-recapture (MRR) technique to monitor the population of *E. corethrus* over a year, from June 2014 to May 2015. Butterflies were captured with insect nets and given a unique number, which was marked with a waterproof felt-tip permanent-ink pen in the discal cell in one of the hindwings (according Ehrlich & Davidson 1960). Field surveys were conducted 1–3 times a week, except during the winter when field expeditions were done each two weeks. Each field day included three researchers with nets. Butterfly sampling was carried out between 09:00 am and 06:00 pm, and the captures were performed on sunny days, with temperatures between 15 and 35°C and mild wind.

The sampling counted 34 field days scoring approximately 290 net-hours of sampling effort. For each butterfly, the following aspects were recorded: point of capture, numerical code, sex and presence of *sphragis*, age, forewing length (mm), and vegetation formation type (meadows, shrublands, forest edges and swamps) it was interacting with, if any, in the place of capture. The captures were made between consecutive marks on the trail. We also account for the efficiency of butterfly sampling, which was the daily ratio between captured individuals and overall individuals (either captured or visualized).

Population dynamics

We estimated the population size by using the MRR data. To that, we took into account the number of specimens captured and marked each day and the records of recaptured individuals. The MRR data was analyzed separately for each sex through the Jolly-Seber model (Seber 1965) using the CLMR_2010 software pack (Francini 2010).

Sex ratio

Individuals were sexed by inspection of genitalia and wing coloration; the sex ratio was calculated by scoring the observed sexual rate based on field days and distributing it monthly, so to observe whether the proportion varied between sexes and between months. To do so we used a chi-square test.

Age structure

The estimates of age followed Ehrlich & Gilbert (1973), using visually distinguishable wing wear differences to allocate individuals into the following categories, ranging from the youngest to the oldest: *teneral*, *young*, *experienced*, *old*, *very old*. To calculate the age structure we assembled both sexes together and calculated the proportion of each age category by each field day. Differences between sexes were tested using a chi-square test for homogeneity.

Residence time

Residence time was estimated by the difference between the first and the last captures (Brussard et al. 1974), and accounts as an indirect measure of longevity. The Mann-Whitney test was used to verify the residence time differences between sexes.

Wing size

Wing overall size is considered to be directly correlated to body size (Miller 1977; Baguette & Stevens 2013), a measure that goes from wing insertion on the thorax to the terminal portion of vein R4, in the apical area of the wing. Such measure was taken with the use of a digital caliper (accuracy 0.01 mm). It was ascertained if wing size had a normal distribution using the Kolmogorov-Smirnov test and, afterwards, we investigated the character for sexual dimorphism with a t-test and variation between months with an ANOVA followed by a post-hoc Tukey test.

Vegetation type preference

In order to analyze for adult preference amongst the vegetation categories, we scored butterfly captures by every 50-m sector, regarding vegetation type (meadows, shrublands, swamps and forest edges), and applied a chi-square test to data. The vegetation type were of each sector was defined based on the most representative vegetation formation around the numbered signs were individuals were captured. After categorization, each sector vegetation type was double-checked using satellite images on Google Earth Pro (Google Inc., Mountain View, USA).

Vagility

The estimation of vagility was calculated using a geographic information system (GIS). We measured the linear distance from the sector of capture to the sector of recapture of individuals. This measure also includes recaptures that were made on the same day. Vagility was zero when an individual was recaptured in the same sector from a previous encounter. We verified movement differences between sexes using a t-test.

Resource phenology, natural history, and behavior

The phenology of flowers from different plants occurring in the study area was recorded to access information about adult foraging behavior. At each sampling occasion, the plants in bloom were identified and the availability of flower resources mapped along the trail using the 50-m sectors. Blossomed flowers were identified with the help of a botanist using both photos and exsiccates of plants. After plant identification, *ad libitum* observations (Altmann 1974) during field surveys were performed to identify which flowers were visited by *E. corethrus* butterflies. The hypothesis of heterogeneous distribution of flowers along the study area was analyzed using a Kolmogorov-Smirnov test. The hypothesis that the presence of nectar sources increases the likelihood of finding a butterfly was tested by the association of flower records with butterfly captures using the Spearman's rank-order correlation test. The presence of the host-plants (*Aristolochia* species) was mapped in the study site to provide data on their availability over the months and details on their use as host-plants. The hypothesis that clustered distribution of host-plants along a sampling area increases the likelihood of finding a butterfly was tested using Pearson's correlation. *Aristolochia* branches present in the study area were measured (branch length, presence of apical bud, number of leaves) once per season, and inspected for the presence of *E. corethrus* immature forms and signs of herbivory. Eventual immature forms from other Troidini species were also recorded to access resource partitioning information. In addition to the population sampling, adult foraging behavior, sexual behavior, male activity and female oviposition were recorded. The observation method used was the Focal-Animal Sampling (Altmann 1974). Behavioral recordings were performed at each sampling occasion, when butterflies were observed during flight before being net-captured. Once chosen, the focal butterfly was followed for as long as possible and all behaviors were annotated.

Results

Population dynamics

We captured and marked a total of 955 individuals (367 females and 588 males). The efficiency of butterfly sampling was 47.34%. The number of daily captures varied from zero to 33 for females (mean ± SD = 12.88 ± 11.45) and from zero to 44 for males (mean ± SD = 22.20 ± 17.87). Males and females were recaptured up to three times. Approximately 7.6% of females (28 individuals) were recaptured at least once. For the males the numbers were slightly larger, with 14.12% of the males recaptured at least once (83 individuals).

The number of butterflies captured was not stable during the year, suggesting a seasonal pattern abundance, though the estimated population size did not differ significantly during some samplings (note error bars in Fig 4). The number of both males and females peaked in early spring and early summer. The number of captures did not correlate with relative humidity ($r_{\text{Pearson}}=-0.361$, p=0.249), month temperature ($r_{\text{Pearson}}=0.460$, p<0.132), and rainfall ($r_{\text{Pearson}}= -0.338$, p=0.282), but was significantly correlated with day length ($r_{\text{Pearson}} = -0.612$; p=0.034).

The maximum number of individuals was observed approximately a week prior spring equinox. There were at least two visible marked peaks in the capture and visualization of butterflies (Fig 4), which were from the beginning of September to the end of October and, after a small gap with an acute descend in encounters, from the beginning of November to the end of December. No adults were observed from June to August of 2014, and from April to May 2015, suggesting the occurrence of overwinter diapause behavior.

Sex ratio

The sex ratio of the marked individuals was slightly male biased (588 males and 367 females were marked, a ratio of 1.6M:1F), however, this sex-ratio differences was not significant among the months of the survey (χ^2=4.759; p=0.691, Fig. 5). Sex ratio was male biased in all months of survey, with the greatest difference observed in September (1.9M: 1F).

Age structure

There was a significant variation in the age structure of the population during the study (χ^2=121.56, p<0.001) (Fig 6). Teneral individuals, which may account as *very* young ones, were found in higher proportions at the beginning and middle of September, late November and from the start of February to the middle of March 2015. Young butterflies scored the most captures until approximately the end of September, when the population seemed to get increasingly old. There was a clear pattern of aging that peaked at the end of October, what coincided with a depletion of the teneral individuals and the dominance of very old individuals in the captures. The same pattern recurred, although lightly peaked, in December 2014, after a mild renewal of the population and, again, past the start of April 2015, when the population appeared to have suffered an intense aging process that culminated with the absence of individuals in the beginning of May 2015.

Wing size

Male and female overall wing size had no significant difference between each other (females, 42.78 ± 2.39 mm; males, 42.60 ± 2.28 mm; *t*= 1.050; p=0.250 - Table 1). The butterflies' wing size showed variation between months. In females it ranged from 36.32 to 53.87 mm, and in males it ranged from 36.75 to 46.46 mm. Both females' and males' largest absolute wing size was found in September 2014. There were significant general differences in wing length throughout the year, both sexes showed their largest wing mean sizes in December. The smaller sizes from September showed increment in size until December and, from there on, a progressive decrease in size.

Vegetation type preference

Butterflies significantly differ regarding the preference for the four types of vegetation (χ^2=819.38, p<0.0001). Forty seven percent of butterfly captures were performed in meadows, 44% in shrublands, 7% swamplands, and 1% in forest edges. No significant differences regarding male and females preferences for any vegetation type were observed (χ^2= 0.910, p= 0.823).

Residence time

Residence time varied from 1 to 23 days, with a significant difference found between the mean residence time of sexes (male median=2.3 days; female median=1.7 days; U= 100645.00, p= 0.0012).

Vagility

Most butterflies were recaptured in a different sector from previous encounters, scoring from 49.2 m up to 1,510 m; five of the recaptures were done in the same sector of previous encounters, scoring 0 m of displacement. The vagility was not statistically different between sexes (t =-1.0031, p=0.317). Males and females averaged very similar traveled distances (males = 388.05 ± 277.53 m; females = 336.46 ± 256.6 m). The maximum male vagility recorded was of 1,510 m and the maximum for a female was 1,177 m. The daily vagility of males varied from 52.6 to 495 m and from 49 to 407 m for females.

Resource phenology, natural history, and behavior

The distribution of flowers along sampling transects was not homogenous (χ^2=819.381, p<0.0001). Near 47% of flowers were found in meadows, 44% in shrubland, 7% in swamp areas and 2% near forest edges. The most abundant nectar resource used by *E. corethrus* was *Senecio riograndensis* Matzenbacher and *Senecio cisplatinus* Cabr. (Asteraceae) flowers, which were available from October to December, followed by *Verbena rigida* Spreng. (Verbenaceae), and *Chromolaena squarrulosa* Hook. & Arn. available year round. *Euryades* *corethrus* adults were eventually sighted feeding on *Baccharis articulata* (Lam.) (Asterace), *Austroeupatorium inulaefolium* Kunth (Asteraceae), *Lantana camara* L. (Verbenaceae), *Richardia grandiflora* (Cham. & Schltdl.) Steud. (Rubiaceae) and *Criscia stricta* (Spreng.) (Asteraceae). The presence of flowers along the study area was strongly correlated with butterfly records (r_{Spearman}=0.581, p=0.003).

Field surveys showed that only one host-plant was present at the study site: *A. sessilifolia*. Fully grown and bigger leafed *A. sessilifolia* plants were only available from late spring to early autumn, being restricted to the open-field and shrubland areas; during winter most of *A. sessilifolia* losses its apical bud and leaves, remaining only low-quality tissues (stalks, stems and damaged leaves with chlorosis). *Aristolochia sessilifolia* had clustered distribution along sampling transect (χ^2=45.401; p<0.001), and the presence of this host-plant along the study site was correlated with records of both sexes (r_{Pearson} = 0.536, p=0.032). When investigated separately, the sexes showed another difference regarding foraging behavior, with only females presenting significant correlation with host-plants distribution (r_{Pearson} = 0.530, p=0.035).

Flight activity started only after the dew deposited on vegetation dried out. In the early hours of activity, both males and females were sighted searching for nectar on flowers. Latter, adults were sighted during dispersal, in a flight moderately slow and less erratic than the one of other swallowtail butterflies found in the same area; if disturbed *E. corethrus* adults may engage in a very fast, erratic and, sometimes, very high flight. Males were frequently sighted during straight flights, probably in search of females.

Very few male-female encounters were observed, and not a single one resulted in mating. When an encounter occurred the individuals engaged in high speed descending spiraling flights, which lasted few seconds, and ended when the male broke off and flew way. During male-male encounters, individuals embraced in a close spiral ascendant flight, reminding a war of attrition. This behavior lasted some seconds, being terminated when one male broke off and flew away. The fugitive male was always chased for a few meters by the other male; the last male returned to his dispersal flight in no time after the pursuit. Females generally flew randomly and at lower speed and closer to the ground when compared to males, searching for suitable host-plants to lay their eggs. The eggs were laid singly on the abaxial face of undamaged *A. sessilifolia* leafs. Females avoided to lay eggs on plants with other oviposition, but in big leafed host-plants , up to five eggs can be found on the same plant. No pupation event was observed, but two events of newly fresh adult emergences from clumps of grass suggested that pupation occurred far from host-plant, using the clumps as shelters. Eggs and larvae of *E. corethrus* were observed in the host-plants from early spring to early autumn.

No courtship was observed. Most of captured females (92.64%) had a *sphragis* attached to her terminalia. The only females that had a free terminalia were the teneral ones, which suggests that mating may occur very early in female adult life. Several mating pairs were found already in copulation (N=14). Some of the females in copulation were in a teneral state, also suggesting that no courtship had occurred. If not disturbed during the copulation, the pair remains motionless on the vegetation; if disturbed the pair either broke apart from each other, or flew away, with the female caring the male attached to her genitalia. The male secretes a large mating plug (*sphragis*) which later hardens around the female terminalia. We speculate that females may mate only once, and that the *sphragis* visually deters other males from attempting to mate with a mated female.

Discussion

Population dynamics

The number of butterflies captured for both sexes were higher in the beginning of September (austral spring) to the beginning of October. This is a period in which diapause termination of many dormant swallowtail butterflies is expected to occur (Tyler et al. 1994), which could explain the outburst of butterflies after a short gap when no butterfly was found.

Male abundance for late October and late December 2014 estimated the day population numbers to be around 50 and 65 individuals, respectively, but estimates had very large errors. The estimated abundances for the following months were very similar to the butterfly records, showing very low error bars. Females, on the other hand, did not show estimates, when those could be calculated, with that amount of error past the first overflow period of September.

Apart the somewhat gross estimation of high abundance, there is an apparent overall reduction in captures and, assumed, in the population abundance, during the October – November period. The tendency is clearer for females, but is present in males nonetheless. We think that could be explained by the progressive aging of individuals and subsequent slow recruiting of larvae, given that a small but steady increase in young adults abundance is perceived shortly thereafter. Compared to previous studies, we found difference to the months of encounter reported by Link et al. (1977) for *E. corethrus*, especially in the month of May, when we found no individuals.

Wing size

Caporale (2016) indicates that the mean time of *E. corethrus* development from egg to imago is of about 30 days. Although no wing size variation through treatments was found in the former study, the differences we have found *in situ* may be of difficult replication in laboratory conditions. In our study there was a clear pattern of increasing in mean wing length from September to December 2014 and thereafter a progressive shortening in wing length means until June 2015 (Table 1). Wing length, a linear measure representation of wing size, positively correlates with body size in butterflies (Miller 1977), which could be directly interpreted as if the individuals captured in December had bigger bodies. That may be due the increased quality of food those individuals have taken during spring, when they were in larval stage.

Sex ratio

Tyler et al. (1994) indicates that all swallowtail populations tend to show a 1M:1F sex proportion in the wild, while Brown Jr et al. (1995) says the common expected sex ratio for Papilionidae in the field is of 2M:1F. Although the differences found in our study sample had no significance between the months, the results stand between the two aforementioned, showing a sex ratio of 1.6M:1F. A male biased sex ratio may be explained by a myriad of reasons, such as a genetic sex ratio expected to be male biased, differences in the recruitment due to differential larvae or pupae mortality or differences in behavior between sexes that may influence individual's detectability and catchability. Distinct sampling procedures used to assess the spruce budworm sex ratio showed that the estimation may vary widely in response to behavioral, life-history linked, sexual differences (Rhainds and Heard 2015).

In general, swallowtails flight performance is strong and rapid (Tyler et al. 1994), what hinders the capture by the entomological net. Another bias could be due to behavioral differences between males and females. We have observed different patterns of flying between sexes, with females flying lower than males and more constantly amongst shrubs; differences in the sexual distribution of captures seemed to link females to oviposition sites, in a way similarly discussed by Pennekamp et al. (2013). Those are not unexpected findings: a mark-release study of *Erebia epipsodea* with similar data suggested that a 1:1 sex ratio could be assumed and that variations from that could be accepted as result from behavioral variation as well (Brussard and Ehrlich 1970).

Age structure

The sample of captured individuals showed an oscillating age structure. A composition of, mainly, teneral to experienced individuals gave place to a progressively elderly deal of butterflies, and that appeared to happen at least three times, culminating in an acute aging in late May. Thenceforth, we had no captures. September, late October, November 2014 and late February 2015 were periods in which there were present very young butterflies (teneral individuals), which may also indicate a large immature population.

Residence time and vagility

We obtained a low residence time average for both sexes, having a high number of captures. If we compare the maximum residence time *of E. corethrus* with other Neotropical Troidini, it resembles the residence times of *Parides anchises* (30 days) (Freitas and Ramos 2001), *Parides ascanius* (28 days) (Henkenhoff et al. 2013), and *Battus polystictus* (20 days) (Scalco et al. 2015). Other Troidini show a higher residence time, such as *Battus polydamas* (48 days), *Parides proneus* (48 days), *Parides bunichus* (46 days), and *Parides agavus* (56 days) (Brown Jr. et al. 1995). The low values can be due to the previous discussed high vagility or due to low longevity of individuals.

Physical traits are linked to conservation

Environmental features such as the abiotic cues of temperature and photoperiod, as well as factors like competition, food quality and resource availability are known to affect butterflies body size (Mega 2014). Of those, the resources are rather critical in determining adult morphology and life history traits linked to fitness (Boggs and Freeman 2005). Quality and density of host-plant food for the larvae were said to be the best predictors of site quality (Pennekamp et al. 2013). Larvae that fed on spring may have had access to better food sources, mostly because there is a peak in the amount of rainfall, what could induce an nutritional improvement in plants in general (Tourinho and Freitas 2002). Since plant sprouting in spring is very sensitive to photoperiod (total day length) and since most dormant swallowtail pupae will not respond to greater temperature and moisture only, it is likely that increasing photoperiod is a first cue to prepare to break diapause when it starts raining.

A bigger body can influence on the area an individual can cover, or its dispersal distance. An organism's body size is connected to its energetic necessities, which characteristics are both entwined to the amount of quality territory such organisms require (Baguette & Stevens 2013). Tyler et al. (1994) stresses the perceived flight strength of swallowtails that permits they cover a large foraging area and rapidly change places when resources are scarce.

The movement that swallowtail individuals perform is usually big, seeming to cover areas two to five kilometers afar from the site of first encounter (Brown Jr et al. 1995), probably covering up to 10km a day (Tyler et al. 1994). Even though our estimates of distances traveled did not show such superb digits, we think they are an underestimation of the species flight potential due to the low recapture rate we had and due to intrinsic limitations of the experiment design.

Although the study site seems to be fairly large, it may be of the most fragility with respect to its connectivity to other suitable areas. Native grasslands with such a vegetal formation are increasingly less common in the vicinity of the study area. Even though personal observations from other sites point that grass covered areas that suffer periodic management, namely lawn mowing by machine, still present the host-plant resources, we cannot assure such plant populations are enough to maintain a viable population. A feasible conservation plan involves gathering the knowledge relative to the species of interest. The minimum area requirement (MAR) of a species is the quantity of functional habitat that can maintain a viable population. Landscape connectivity and MAR are the two essential, species-specific parameters for the design of conservation networks aiming at preserving biodiversity (Vos et al. 2001).

Previous studies in the *Campos* show that the variation in grassland physiognomy and composition appears to be determined by fire regimens and grazing (Pillar and Quadros, 1997), but Swengel (1996) showed that specialist butterflies had a strong and significant decline in abundance after fire in *Campos*-like grasslands of the central North America, and that this effect persisted for three to five or more years after the disturbance.

The use of grazing as a mean of conservation should not be reckless, either. Grassland management already showed its potential to harm butterflies populations when not done properly. Such is the case of the Pieridae *Colias mymidone* local extinction on a White Carpathians, Czech Republic reserve. Excessive machine mowing as a mean of grazing emulation on the grasslands formations led to a decline of resources heterogeneity in the region and to the subsequent species disappearance (Konvicka 2008). Also, plowing techniques must be used with caution, given the capability to destroy *Aristolochia spp.* underground rhizome.

With respect to butterflies, there is evidence that overgrazing may cause serious harm to the population abundance as well, and that general impact may also strongly depend on the time in which it is applied. Grazing during the hibernating period of *Melitaea cinxia* (Nymphalidae) had a profound impact on the population abundance (Noordwijk 2012). Grazing can lead to incidental omnivory and trampling of individuals that have limited or no dispersal abilities, such as slow moving larvae and immobile pupae.

There are two major agricultural approaches to production in an ecological-wise way. Benayas and Bullock (2012) present them as being the Land Sharing and the Land Separation regimens. The first aims to make production more connected to wildlife and could be summarized as a transformation of a conventional, monoculture paradigm into an agroforestal-like, diversification of crops, while the latter, Land Separation, involves the restoration or creation of refuges amidst agricultural landscapes – what could have a positive impact on connectivity.

A conservatism reasonable aim would be to promote the inevitable economic use of the grasslands of southern South America to the rearing of cattle in an extensive way, without pasture sowing and, if possible, with rotating, season-wise use of the pasture. Furthermore, even in an optimistic scenario of enhanced connectivity due to increase in suitable small areas, a measure aiming the implementation of a greater extent area is urgent, the creation of a Conservation Unit being one of the main directions given in the Red List of Threatened Species of Rio Grande do Sul State, so to protect *E. corethrus.*

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Table 1 Wing size (mm) for females and males of *Euryades corethrus* from July 2014 to June 2015 at UFRGS's Agronomical Experimental Station, Eldorado do Sul, RS, Brazil. Monthly means marked with the same letter did not differ statistically from each other (ANOVA followed by the Tukey's post-hoc test).

Year	Month	Wing size (mean ± standard error)	
		Females (n)	Males (n)
2014	September	41.80 ± 0.27 (83)[a]	41.53 ± 0.16 (141)[a]
	October	42.29 ± 0.23 (53)[a]	41.77 ± 0.19 (94)[a]
	November	43.49 ± 0.34 (31)[ab]	42.67 ± 0.29 (69)[ab]
	December	44.36 ± 0.29 (70)[b]	44.16 ± 0.19 (115)[c]
2015	January	43.09 ± 0.29 (41)[ab]	43.46 ± 0.3 (53)[bc]
	February	42.52 ± 0.38 (40)[a]	42.84 ± 0.28 (62)[bc]
	March	42.32 ± 0.34 (47)[a]	42.52 ± 0.24 (75)[ab]
	April	42.14 ± 0.67 (12)[a]	41.68 ± 0.47 (20)[a]

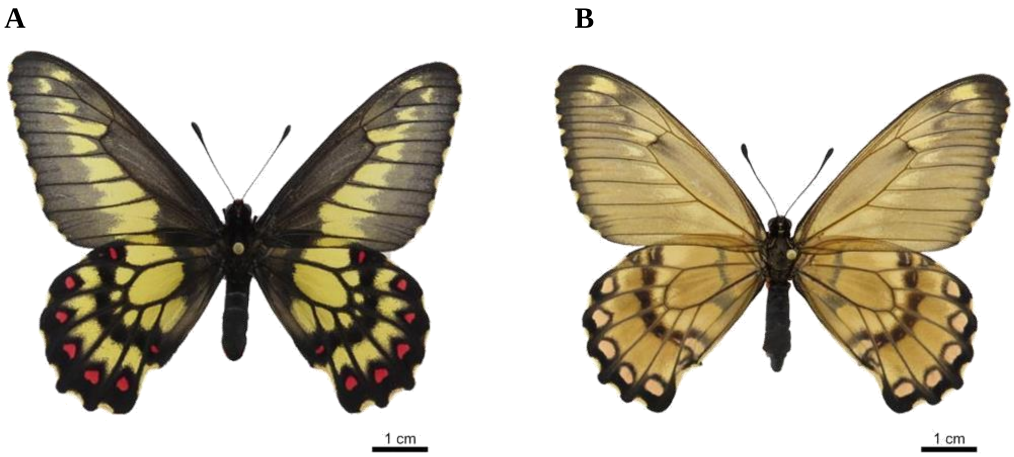

Fig 1 *Euryades corethrus* specimens. A, male; B, female.

Fig 2 Formations present at UFRGS`s EAS Eldorado do Sul, RS, Brazil. The arrows indicate the occurrence of different formations in the landscape of the study site: A, grasslands; B, shrublands; C, swamps; D, forest edges.

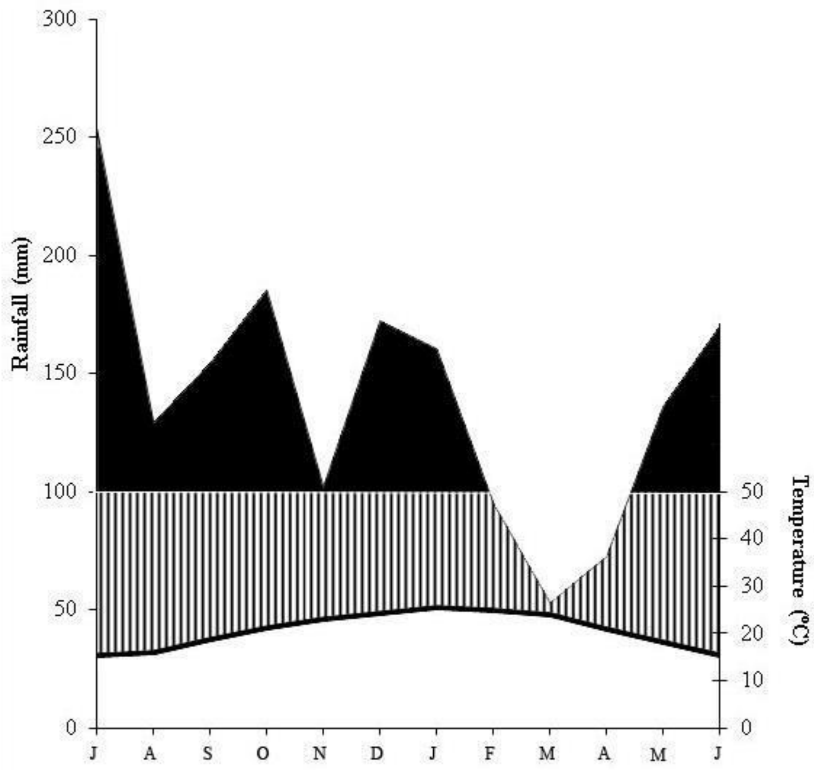

Fig 3 Climatic diagram from July 2014 to June 2015 at Porto Alegre, RS, Brazil (according to Walter 1985). Black, superhumid periods; hatched, humid periods. Polygonal line, temperature

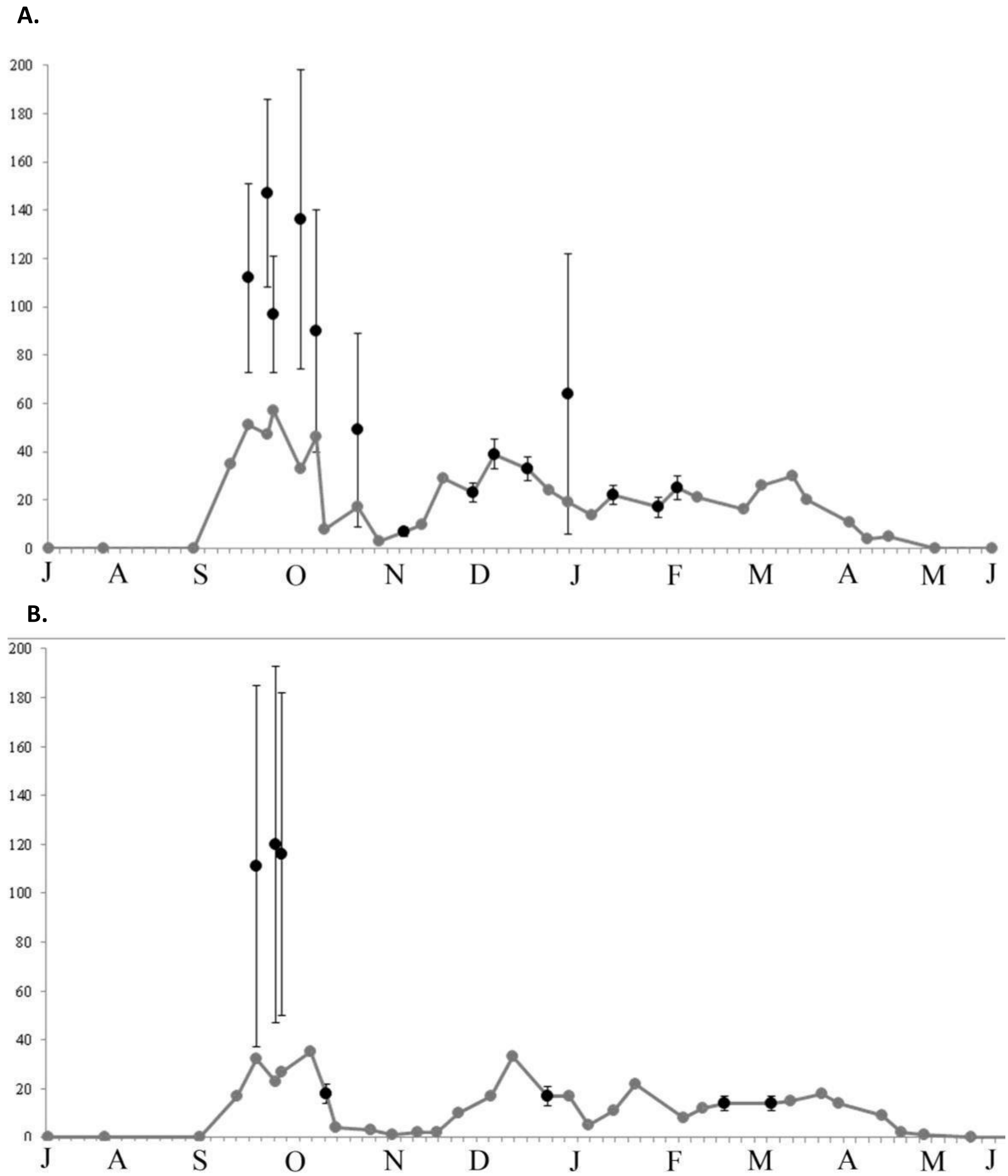

Fig 4 Number of males (A) and females (B) of *Euryades corethrus* from July 2014 to June 2015. Grey circles indicate number of individuals per day; black circles and vertical lines are the estimated number and standard error based on the Jolly-Seber method.

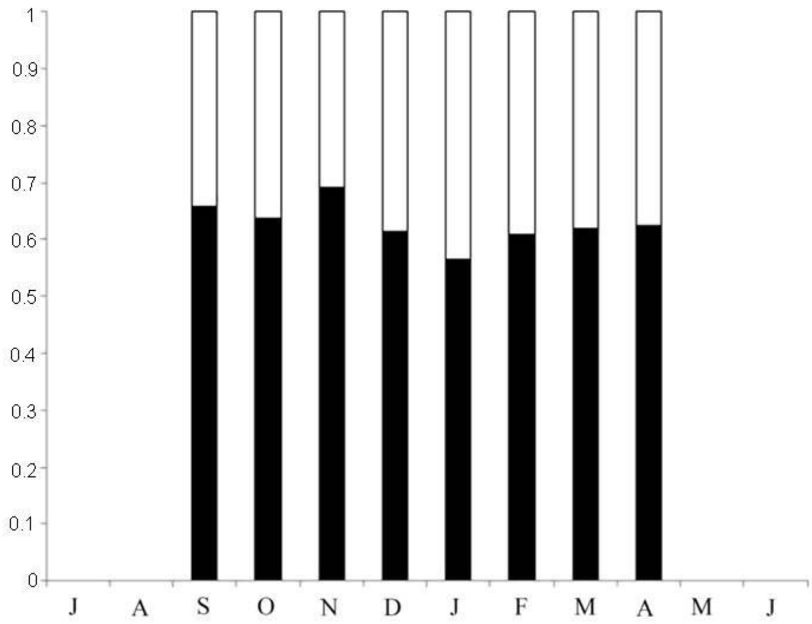

Fig 5 Sex ratio of *Euryades corethrus* from July 2014 to June 2015. Black bars represent the percent of males in each month's capture (means of capture on a daily basis). Blank areas indicate field days with no capture.

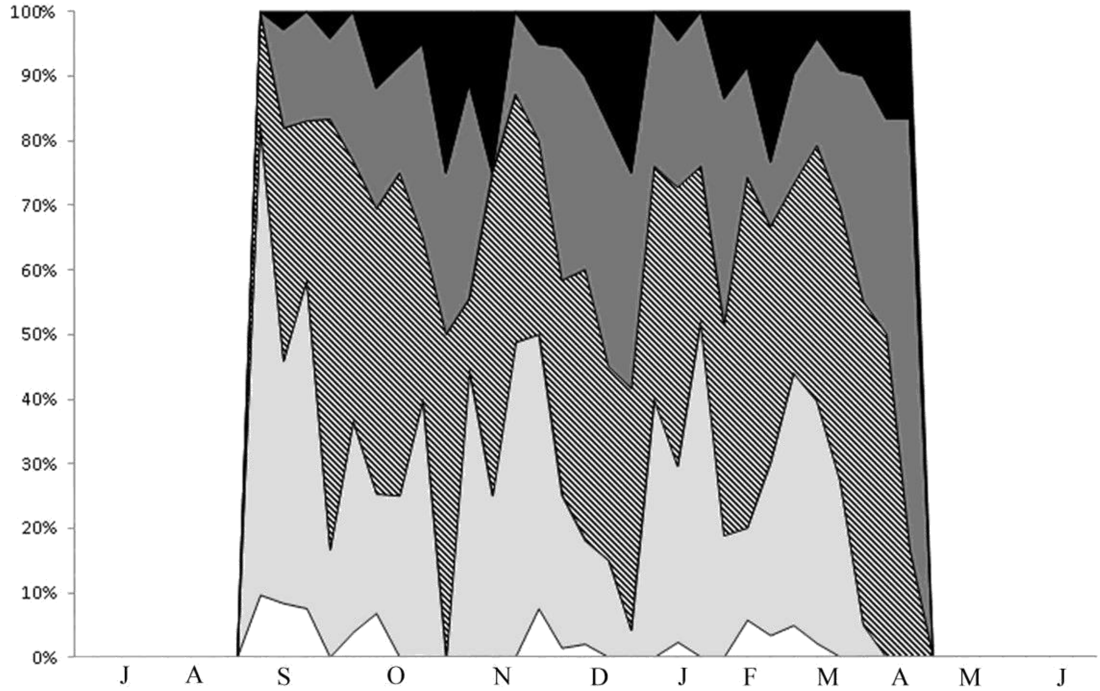

Fig 6 Age structure of *Euryades corethrus* population from July 2014 to June 2015 at . Colors and age categories as follows: White, teneral; bright grey, young; diagonal hatch, intermediate; dark grey, old; black, very old. The blank areas before September 2014 and after April 2015 indicate field days with no capture

CAPÍTULO 3

Considerações finais

Alguns autores indicam que a conversão de formações naturais em campos de cultivo é inevitável. As tecnologias agrícolas cada vez mais facilitam a expansão e a manutenção de plantações onde antes o cultivo era dificultoso, e as demandas industriais e sociais pedem produtos: papel, combustível, ração e, mais por bandeira do que por necessidade, comida. Também entram na conta outros artigos, como o gado de corte que, transformado em *commodity* e requisitado em quantidades condizentes com as que o termo dá a entender, acaba sendo criado em escala imensa.

No sul do Mundo essa realidade não é diferente: o avanço da soja, do eucalipto, e do *Pinus* são patentes, e o impacto é ainda maior quando se leva em conta que uma das formações naturais mais diversas e importantes dessa região não é devidamente protegida. Os Campos, talvez diferente do que o senso comum sugira, apresentam alta diversidade de plantas e animais, além de prestar serviços ecológicos difusos e essenciais; mas acabam sendo esquecidos na conservação no Brasil. Há grande esforço de proteção voltado para formações florestais, mas os campos não parecem ter o mesmo carisma e acabam não recebendo a mesma atenção da sociedade civil e da máquina política.

Euryades corethrus é uma borboleta de distribuição meridional na América do Sul, endêmica do sul do Brasil e que oviposita em plantas do gênero *Aristolochia*, das quais se alimentam as larvas. Justamente por se encontrar no meio dessa disputa de terra, e por não ter nenhuma prioridade em recebê-la, é considerada, no Livro Vermelho do Estado do Rio Grande do Sul, Vulnerável: as causas da sua classificação como tal são a destruição e a falta de conectividade de hábitat e, apesar de ser uma espécie de voo eficiente e longo, isso de nada ajuda na sua proteção se não houver oferta de área e pontes que as liguem.

Apesar da considerável abundância local e sazonal de *E. corethrus*, sua profunda dependência das plantas hospedeiras se mostra como um fator de fragilidade, e impactos aparentemente pequenos, quando mal colocados, podem levar a grandes desdobramentos negativos.

Mesmo medidas menos impactantes que visem o compartilhamento da terra devem tomar em consideração as dinâmicas populacionais da espécie. Se promovida na época da diapausa da espécie, a pecuária extensiva pode levar ao consumo acidental de pupas. Isso pode causar impactos muito grandes nas populações de adultos e, aliado ao consumo excessivo das gramíneas e ao pisoteio, a extinção local não parece impossível. Ainda, é temerosa, por imaginável, a destruição causada pelo uso de maquinário que destrua não só as folhas de *Aristolochia*, mas também propágulos e pedaços de plantas em situação de resistência.

Uma vez que a recomendação da FZB em relação às direções que devem ser tomadas no que tange a conservação de *E. corethrus* envolvem a busca por populações remanescentes, a conservação direta de seu habitat, o estudo de sua biologia básica e ecologia, e a criação de unidades de conservação que lhe incluam, este trabalho buscou preencher algumas dessas lacunas no conhecimento da espécie.

Para tanto estudamos durante um ano, de Julho de 2014 a Junho de 2015, uma população de *E. corethrus* presente em área remanescente de campo nativo da Estação Experimental Agronômica da UFRGS, localizada no município de Eldorado do Sul, Rio Grande do Sul.

Investigamos aspectos de sua biologia básica e ecologia, como características de alimentação de adultos e larvas, oviposição, voo, seleção sexual e tamanho de asa. Além disso, usamos técnicas de marcação e recaptura para estimar abundância esperada de machos e fêmeas, razão sexual da população, vagilidade, residência, estrutura etária e distribuição na paisagem baseado na presença de plantas hospedeiras e fontes de néctar.

Os animais preferiram, no geral, áreas de campo a vassourais e mato. Também houve significativa diferença entre os sexos na captura em relação a áreas com presença ou não de *Aristolochia sessilifolia*, hospedeira presente na área de estudo: fêmeas foram mais encontradas próximas às áreas com maior densidade da planta, o que só faz eco à já discutida conexão que essa espécie tem com os Campos Sulinos.

Os resultados apresentados aqui reforçam que a conservação de *E. corethrus* deve passar, obrigatoriamente, pela conservação de áreas de campo que aumentem sua área disponível e sua conectividade com outras áreas distantes. Isso não pode ser feito sem investimento em (1) aumento de áreas de conservação de campo, inclusive com presença de pastoreio (leve), mas tomando cuidado para não dizimar a população em diapausa com onivoria acidental e pisoteio e (2) medidas mais profundas, que criem unidade(s) de conservação dedicada(s) às formações de campo, que preserve(m) sua grande diversidade de Flora e Fauna associada, e seus serviços ecológicos e imateriais prestados.

Anexo 1

Fig 1. Paisagem encontrada na área de estudo

Fig 2. Recursos alimentares de adultos

Fig 3. Ramo de *Aristolochia sessilifolia*

Fig 4. Manipulação de indivíduos em campo

Fig 5. A, detalhe da marcação de indivíduos; B, casal em cópula

Fig. 6 Medição de comprimento de asa com paquímetro digital

Anexo 2 – Diretrizes para autores

Authors guidelines for Journal of Insect Conservation

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Editor-in-Chief: John **Dover**

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This result was later contradicted by Becker and Seligman (1996).

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Book

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Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

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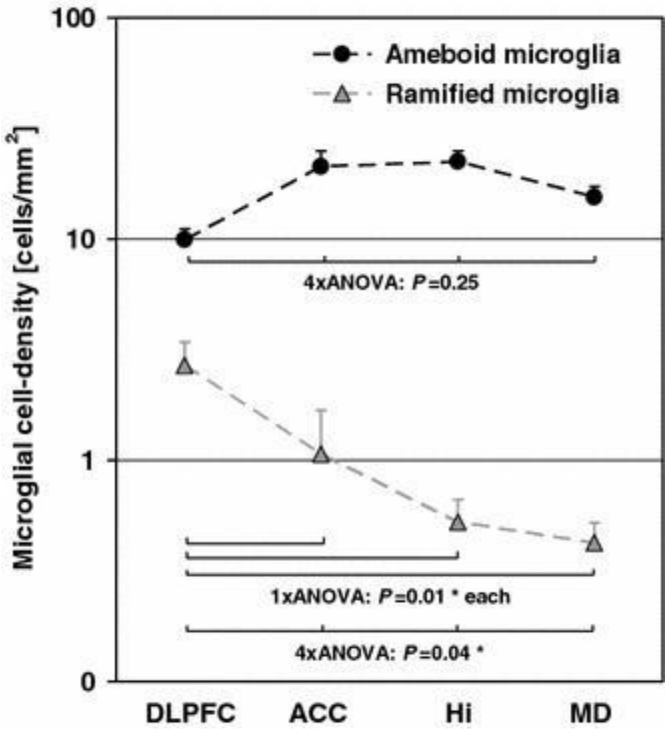

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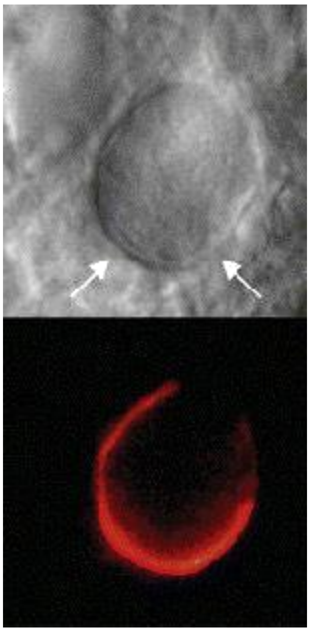

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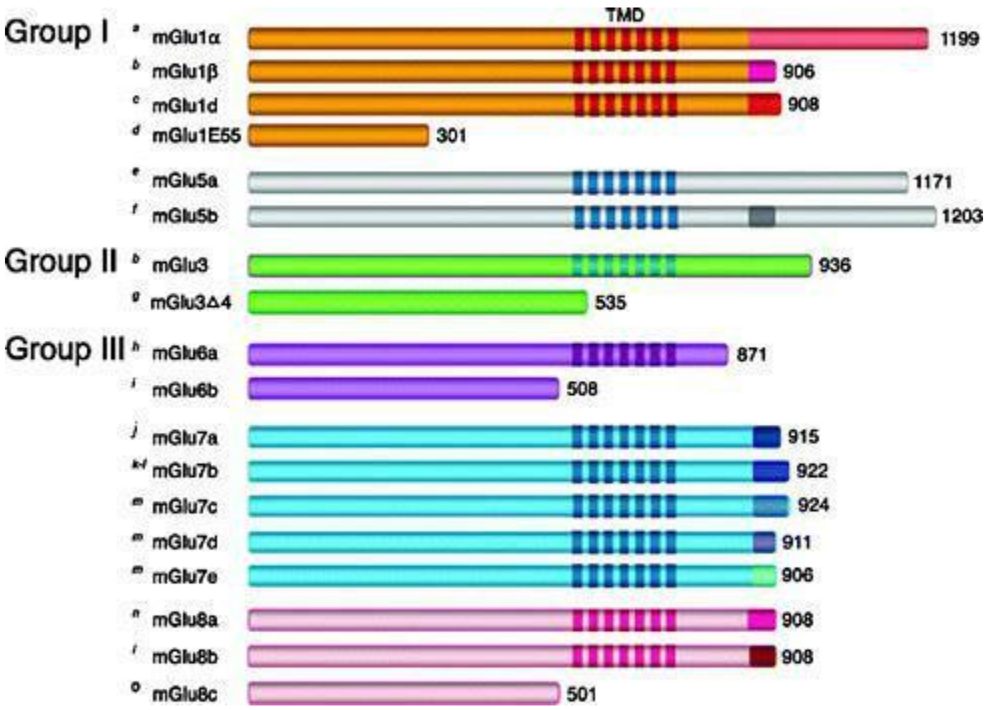

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