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NUMBER AND DURATION OF THE JUVENILE INSTARS OF THE NEOTROPICAL GRASSHOPPER *Cornops aquaticum* (ACRIDIDAE: LEPTYSMINAE) IN ARGENTINA

Soledad Capello and M. Celeste Franceschini

SUMMARY

Cornops aquaticum is a semiaquatic grasshopper that lives in close association with Pontederiaceae, especially the *Eichhornia* genus. In its native distribution range, information is only available about life history traits of populations from Brazil and Uruguay. The aim of this work was to assess the number of juvenile instars and duration of nymphal development of *C. aquaticum* in summer and spring nymphs from Argentinean populations. Wild newly emerged nymphs were individually reared in Corrientes and Santa Fe cities under local climate, without direct sun exposure. In both sites, nymphs showed males with five instars and females with five or six instars. Females with six instars were more frequent in summer nymphs. The highest mortality occurred in the first two instars. Nymphal development was longer in nymphs from the summer peak. Lon-

ger nymphal development in females was determined by females with six juvenile instars. Our results, as those from other South American locations about the life cycle of *C. aquaticum*, show that when the instar number and nymphal development are the life history traits to be studied, rearing should be carried out at several sites within its distribution, and in the periods when newly hatched nymphs have peaks of abundance in wild populations. Different juvenile instars in native populations respect to quarantine populations of *C. aquaticum* show the importance to consider life history traits throughout the native range and the effect of environmental factors as useful information for its possible use as a biological control agent of *Eichhornia crassipes* in non-native areas.

Introduction

Cornops aquaticum Bruner (1906) is a semi-aquatic grasshopper that lives in close association with Pontederiaceae floating meadows, especially of the genus *Eichhornia* (Center *et al.*, 2002). Nymphs and adults of this grasshopper feed on the leaves of these macrophytes, and eggs are laid in an ootheca within the leaf petiole (Zolesi, 1956). Currently, several aspects of the life history of *C. aquaticum* are intensely studied for its possible release in non-native ecosystems as a biological control agent of the water hyacinth *Eichhornia crassipes* (Lhano, 2002; Adis *et al.*, 2004; Franceschini *et al.*, 2005; Capello *et*

al., 2007; Franceschini, 2008; Capello, 2010).

C. aquaticum seems to have originated in the Amazonian floodplain (Amedegnato, 1977) and currently is widely distributed in Latin America, from Southeastern Mexico to central Argentina and Uruguay (Roberts and Carbonell, 1979; Lhano, 2006; Adis *et al.*, 2007). Climatic variables such as insolation, temperature and precipitation have been shown to vary greatly over its area of distribution (Adis *et al.*, 2004).

Recent studies carried out in natural populations of *C. aquaticum* from South America and quarantine populations reared in South Africa have confirmed that this species shows impor-

tant variations in its life history traits, mainly with respect to the number of juvenile instars and duration of nymphal development (Zolesi, 1956; Silveira Guido and Perkins, 1975; Medeiros, 1984; Hill and Oberholzer, 2000; Lhano, 2002; Vieira and Santos, 2003; Adis and Junk, 2003; Adis *et al.*, 2004; Brede *et al.*, 2007; Capello *et al.*, 2007). Several scenarios have been proposed to explain the causes of these variations (Adis *et al.*, 2004). However, the most plausible explanation seems to be that the variation is a result of the phenotypic plasticity in response to different climatic factors of the respective environments where populations live (Brede *et al.*, 2007).

In this context, temperature and insolation have been mentioned as key factors determining the number of juvenile instars and duration of nymphal development (Lhano, 2002; Adis *et al.*, 2004; Capello *et al.*, 2007).

The length of the hind femur is the most constant and precise morphometric trait to distinguish each juvenile instar of *C. aquaticum*, because it is subject to little measuring bias and it is easy to measure when working with a high number of individuals (Franceschini *et al.*, 2005). In Argentina, the reproduction of this grasshopper takes place during spring and summer, when abundance peaks of instar I nymphs occur in populations living on *E. azurea* (France-

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NÚMERO Y DURACIÓN DE LOS ESTADIOS NINFALES DE LA TUCURA NEOTROPICAL *Cornops aquaticum* (ACRIDIDAE: LEPTYSMINAE) EN ARGENTINA

Soledad Capello y M. Celeste Franceschini

RESUMEN

Cornops aquaticum es un acridio semiacuático que vive en estrecha asociación con Pontederiaceas, especialmente del género *Eichhornia*. En su área de distribución nativa, la información existente respecto al ciclo de vida esta referida a poblaciones de Brasil y Uruguay. En este trabajo se determinó el número de estadios ninfales y la duración del desarrollo ninfal de *C. aquaticum* en Argentina, en ninfas originadas en verano y primavera. Las crías se realizaron en dos localidades: Corrientes y Santa Fe, utilizando ninfas recién eclosionadas en campo, criadas individualmente en recintos cerrados bajo influencia del clima local y sin exposición solar directa. En ambas localidades, los machos presentaron cinco estadios ninfales; las hembras cinco o seis. Las hembras con seis estadios fueron más frecuentes en las ninfas de verano. La mayor mortalidad de

ninfas se produjo en los dos primeros estadios. La mayor duración del desarrollo ninfal en las hembras estuvo determinada por aquellas de seis estadios. Estos resultados, y los de otros sitios de Sudamérica sobre el ciclo de vida de *C. aquaticum*, muestran que al analizar el número de estadios y el desarrollo ninfal de un acridio conviene realizar crías en distintos lugares y en periodos cuando las ninfas de estadio I presentan mayor abundancia en las poblaciones naturales. Las diferencias en el ciclo de vida de *C. aquaticum* en ninfas nativas y en cuarentena muestran la importancia de considerar las particularidades del desarrollo ninfal en el área de distribución nativa y los factores climáticos implicados, como información valiosa ante su posible utilización en el control biológico de *E. crassipes* en áreas no nativas.

NÚMERO E DURAÇÃO DOS ESTÁGIOS NINFAIS DO GAFANHOTO NEOTROPICAL *Cornops aquaticum* (ACRIDIDAE: LEPTYSMINAE) NA ARGENTINA

Soledad Capello e M. Celeste Franceschini

RESUMO

Cornops aquaticum é um acrídeo semiaquático que vive em estreita associação com Pontederiaceas, especialmente do gênero *Eichhornia*. Nos seus limites de distribuição nativa, a informação existente relativa ao ciclo de vida esta referida a populações de Brasil e Uruguai. Neste trabalho se determinou o número de estágios ninfais e a duração do desenvolvimento ninfal de *C. aquaticum* na Argentina, em ninfas originadas no verão e primavera. As crías se realizaram em duas localidades: Corrientes e Santa Fe, utilizando ninfas recém-ecloídas em campo, criadas individualmente em recintos fechados sob a influência do clima local e sem exposição solar direta. Em ambas as localidades, os machos apresentaram cinco estágios ninfais; as fêmeas cinco ou seis. Fêmeas com seis estágios foram mais frequentes nas ninfas de verão. A maior mortalidade

de ninfas se produziu nos dois primeiros estágios. A maior duração do desenvolvimento ninfal nas fêmeas esteve determinada por aquelas de seis estágios. Estes resultados, e os de outros lugares da América do sul sobre o ciclo de vida de *C. aquaticum*, mostram que ao analisar o número de estágios e o desenvolvimento ninfal de um acrídeo convém realizar crías em distintos lugares e em periodos quando as ninfas de estágios I apresentam maior abundancia nas populações naturais. As diferenças no ciclo de vida de *C. aquaticum* em ninfas nativas e em quarentena mostram a importância de considerar as particularidades do desenvolvimento ninfal nos limites de distribuição nativa e os fatores climáticos implicados, como informação valiosa diante de sua possível utilização no controle biológico de *E. crassipes* em áreas não nativas.

schini *et al.*, 2007) and *E. crassipes* floating meadows (Capello *et al.*, 2004; Franceschini *et al.*, 2008).

In the native range of distribution of *C. aquaticum*, information is only available about life history traits of populations from Brazil and Uruguay. Thus, the aim of this work was to assess the number of juvenile instars and duration of nymphal development of *C. aquaticum* in summer and spring nymphs from Argentinean populations.

Material and Methods

Wild newly emerged nymphs were collected and individually reared in a screened outdoor

enclosure under influence of the local climate, without direct sun exposure. Rearings were carried out in Corrientes City, in the northeast of Argentina (27°28'14"S; 58°50'24"W), and in Santa Fe City, 500km further south (31°40'14"S; 60°34'44"W).

Experiments started in February 2004 (Corrientes N= 29; Santa Fe N= 29) and November 2004 (Corrientes N= 28; Santa Fe N= 26), in order to obtain nymphs of *C. aquaticum* populations from the summer and spring peaks, respectively (Capello *et al.*, 2004; Franceschini *et al.*, 2007, 2008). Observations were made every two days, recording the date of each moult

in order to calculate the duration of each nymphal instar and the nymphal development. Experiments were started with wild newly emerged nymphs to include a high number of individuals in the data analysis; thus, the duration of first-instar nymphs was determined from nymphs that hatched from ovipositions contained in *Eichhornia* plants in the summer of 2006.

Study sites

The climate of the study area is classified as subtropical; summers are warm and prolonged, while winters are shorter and mild (Bruniard, 1996), with a frequency of frost days of 0.25

days/year in Corrientes and 1 day/year in Santa Fe (Bruniard, 1981). In both sites, the mean temperature during rearing varied from 13.9°C to 27.3°C. Monthly mean of maximum temperature varied from 30.3 to 35.2°C in summer and from 27.3 to 32.8°C in spring. Monthly mean of minimum temperature varied from 16.8 to 22.2°C in summer and from 17.2 to 20.7°C in spring (Figure 1). High levels of insolation were recorded during summer 2004 (241 to 351h of sun/month); during spring, insolation varied from 228 to 304h of sun/month. Total hours of sun during 2004 were 2732 in Corrientes and 2679 in Santa Fe.

Statistical analysis

The frequency of occurrence of the different juvenile instars was assessed by means of a contingency table. The duration of nymphal development was assessed using ANOVA with post hoc Tukey tests, after having \log_{10} transformed this variable to normalize the distribution and stabilize the variance. Values of $p < 0.05$ were considered significant. Statistical analyses were performed using InfoStat (2002).

Results

In the summer nymphs of Corrientes, 72.2% of individuals were males and 27.8% females; males required only five instars to complete nymphal development, whereas females required six. In the spring nymphs, 52.9% of individuals were females and 47.1% males; all males developed with five instars, whereas females developed with five or six instars (66.67% and 33.33 %, respectively).

In the summer nymphs of Santa Fe, 31.25% of individuals were males and 68.75% females; males had five instars, while females went through five or six instars (54.54% and 45.45%, respectively). In the spring nymphs, 50% of individuals were males and 50% females; all males had five instars, whereas females had five or six instars (60% and 40%, respectively).

Frequency of females that developed with six instars was not significantly different between sites ($X^2 = 0.002$; $df = 1$; $p = 0.96$). In the summer nymphs, females with six instars were significantly more frequent than females with five instars. Conversely, in the spring nymphs, females with five instars were significantly more frequent than females with six instars ($X^2 = 4.690$; $df = 1$; $p = 0.03$).

The survival rate of nymphs in Corrientes and Santa Fe was higher in the summer (62.1 and 55.17%, respectively) than in the spring nymphs (60.7 and 38.46%, respectively). The high-

est mortality of nymphs occurred mainly in the first two instars (40-50%).

Development of nymphs from the summer peak took place more slowly, from February to the end of June (summer to beginning of winter), whereas development of nymphs from the spring peak occurred from November to the end of December (spring to beginning of summer). The duration of each instar varied from 6 to 19 days in the instars I to IV, whereas in the instars V to VI varied from 9.6 to 48 days. In the summer nymphs, the mean of nymphal development reached 52 days in males and 81 days in females. In the spring nymphs, the mean of nymphal development reached 49.6 days in males and 51 days in females (Tables I and II). Nymphs from the spring peaks had significantly shorter development times than nymphs from the summer peak (ANOVA $p < 0.0001$). Nymphal development was significantly longer in females than in males (ANOVA $p < 0.0001$). Comparing females that developed with five and those that had six instars, nymphal development was significantly longer in females with six juvenile instars (ANOVA $p < 0.0014$). No significant differences in nymphal development were obtained

comparing males and females that developed with five juvenile instars (ANOVA $p = 0.332$).

Discussion

All males of *C. aquaticum* reared in Argentina under the influence of the local climate show five juvenile instars, whereas females develop with five or six juvenile instars. Nymphs of this species having between five and six instars is a finding in agreement with the results obtained in other sites of South America (Zolessi, 1954; Medeiros, 1984; Adis and Junk, 2003; Vieira and Santos, 2003). In non-native areas, quarantine populations of this grasshopper

show six or seven juvenile instars (Hill and Oberholzer, 2000).

High frequency of *C. aquaticum* nymphs with six instars in Argentina is coincident with high values of insolation recorded during summer 2004. This result agrees with those found by other authors (Lhano, 2002; Adis and Junk, 2003; Vieira and Santos, 2003; Capello *et al.*, 2007). In laboratory rearing, a high frequency of females and males with six instars has been observed in *C. aquaticum* nymphs reared in constant (24h) light at 27°C (Capello *et al.*, 2007). In Manaus, where the insolation per year is lower and the monthly temperature is high-

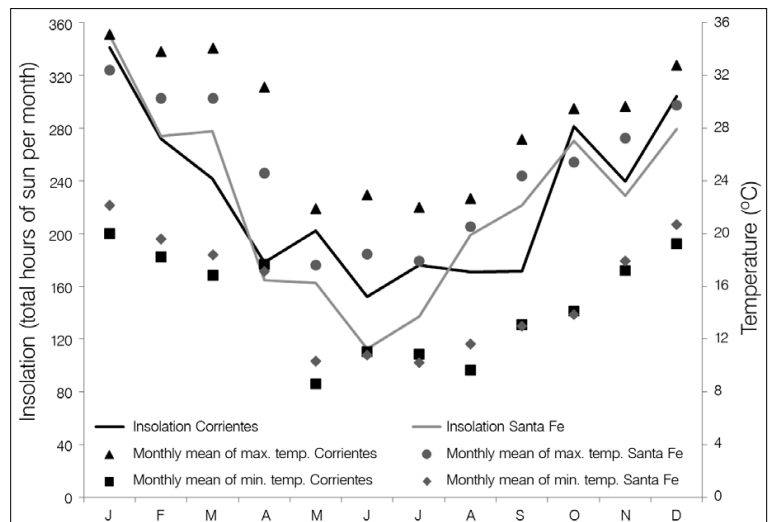


Figure 1. Insolation (total hours of sun per month) and temperatures during the rearing of *Cornops aquaticum* nymphs in Corrientes and Santa Fe, Argentina. Autumn is from Mar 21 to Jun 21 and spring is from Sept 21 to Dec 21.

TABLE I
DURATION OF THE JUVENILE INSTARS (FROM STAGE I TO VI) AND NYMPHAL DEVELOPMENT OF *Cornops aquaticum* NYMPHS REARED FROM THE SUMMER PEAK, IN CORRIENTES AND SANTA FE *

	Males	I	II	III	IV	V	Nymphal development	
		Corrientes	\bar{x}	6.00	11.67 ±1.92	10.54 ±2.29	10.07 ±1.32	14.69 ±3.35
	Range	-	9-15	7-15	7-13	10-22	41-57	
Santa Fe	\bar{x}	6.00	7.60 ±0.55	10.20 ±1.67	16.60 ±2.28	16.60 ±2.19	51 ±3.16	
	Range	-	7-8	8-12	7-19	14-20	48-56	
	Females	I	II	III	IV	V	VI	Nymphal development
		Corrientes	\bar{x}	6.00	9.80 ±2.38	13.00 ±3.39	10.40 ±4.03	17.60 ±17.00
	Range	-	7-11	9-18	4-15	9-48	13-42	62-132
Santa Fe	\bar{x}	6.00	9.91 ±1.04	11.27 ±2.00	11.55 ±1.81	17.45 ±5.84	22.71 ±5.15	70.64 ±10.24
	Range	-	9-12	8-15	9-14	12-33	16-30	55-82

Values are average ± standard deviation. Range: lowest and highest values recorded in the nymphal instars and nymphal development.

TABLE II
DURATION OF THE JUVENILE INSTARS (FROM STAGE I TO VI) AND NYMPHAL DEVELOPMENT OF *Cornops aquaticum* NYMPHS REARED FROM THE SPRING PEAK, IN CORRIENTES AND SANTA FE *

	Males	I	II	III	IV	V	Nymphal development
		Corrientes	\bar{x}	6	7.20 ± 0.82	7.00 ± 1.30	7.75 ± 1.04
	Range	-	7-9	4-8	7-10	9-16	36-76
Santa Fe	\bar{x}	6	9.80 ± 2.49	10.00 ± 2.24	10.40 ± 0.89	11.80 ± 1.48	49.60 ± 6.02
	Range	-	8-14	7-13	9-11	10-14	44-59

	Females	I	II	III	IV	V	VI	Nymphal development
		Corrientes	\bar{x}	6	8.75 ± 2.27	7.56 ± 3.28	6.89 ± 3.26	11.67 ± 3.57
	Range	-	4-12	2-14	3-14	7-16	10-16	36-55
Santa Fe	\bar{x}	6	8.20 ± 1.30	10.60 ± 1.52	11.00 ± 2.00	9.60 ± 1.95	14.00 ± 1.41	51 ± 7.91
	Range	-	6-9	9-13	9-14	8-13	13-15	43-60

Values are average ± standard deviation. Range: lowest and highest values recorded in the nymphal instars and nymphal development.

er than Corrientes and Santa Fe, nymphs of *C. aquaticum* reared under the influence of local climate go through five instars during the rainy period (low insolation) and through six instars during the dry period (high insolation) (Adis and Junk, 2003; Vieira and Santos, 2003).

In Curitiba, where the insolation per year is lower than in Corrientes and Santa Fe, *C. aquaticum* nymphs reared under the local photoperiod at 25°C have five instars in males, whereas females have predominately five instars and only 5% of them have six juvenile instars (Medeiros, 1984). In Cuiabá (Pantanal of Mato Grosso), where insolation is lower and temperature is higher than in Corrientes and Santa Fe, males and females reared under the influence of the local climate have five and six instars (Lhano, 2002; Adis *et al.*, 2004). Although temperatures and insolation in Carrasco, Uruguay, are lower than in Corrientes and Santa Fe, the nymphs of *C. aquaticum* develop with six juvenile instars (Zolessi, 1956). In quarantine populations in Pretoria, South Africa, nymphs of this grasshopper

reared under local photoperiod and temperatures from 22 to 30°C go through six or seven juvenile instars (Hill and Oberholzer, 2000) (Figure 2).

Adis *et al.* (2004) pointed that the number of juvenile instars and duration of nymphal development in *C. aquaticum* mirrors the temperature and photoperiod of different climatic conditions in the respective regions, and Capello *et al.* (2007)

demonstrated that both climatic factors have a synergic effect. The number of juvenile instars of this grasshopper has been primarily related to the flood pulse (Adis and Junk, 2003), quantity of food (Hill and Oberholzer, 2000) and rearing conditions (Medeiros, 1984).

The variation in the number of juvenile instars, with additional instars in females, has also been reported in other spe-

cies of *Cornops* (Turk, 1984; Turk and Aquino, 1996) and Leptysminae (Aquino and Turk, 1997; Nunes *et al.*, 1992; Amorim and Adis, 1994, 1995).

Higher mortality of *C. aquaticum* nymphs in the instar I and II is also mentioned by Lhano (2002).

Nymphal development of 81 days found in females from the summer peak in Argentina is a high mean value in comparison with those mentioned for *C. aquaticum* nymphs from native population (Zolessi, 1954; Silveira Guido and Perkins, 1975; Medeiros, 1984; Lhano, 2002; Adis and Junk, 2003; Vieira and Santos, 2003). A similar duration of nymphal development was obtained in males and females reared under constant (24h) dark at 25°C (Lhano, 2002). In quarantine populations in South Africa, nymphal development takes 50 days with temperature between 22 and 30°C (Hill and Oberholzer, 2000). In laboratory rearing, Capello *et al.* (2007) find that the duration of nymphal development in *C. aquaticum* under constant long days with 24h of light is 33 days at 27°C and 75 days at 36°C. The duration of nymphal development is shorter under constant long days with 24h of light in *C. aquaticum* from the Pantanal of Mato Grosso (Lhano, 2002) and *Stenacris fissicauda fissicauda* from Amazonian (Amorim and Adis, 1995).

Higher duration of the instars V and VI in comparison with the instars I to IV found in this study is also mentioned to *C. aquaticum* nymphs from the Amazonian floodplain (Adis and Junk, 2003). The duration of nymphal development in summer nymphs from the center of Argentina (Silveira Guido and Perkins, 1975) is similar to nymphal development of the spring nymphs from

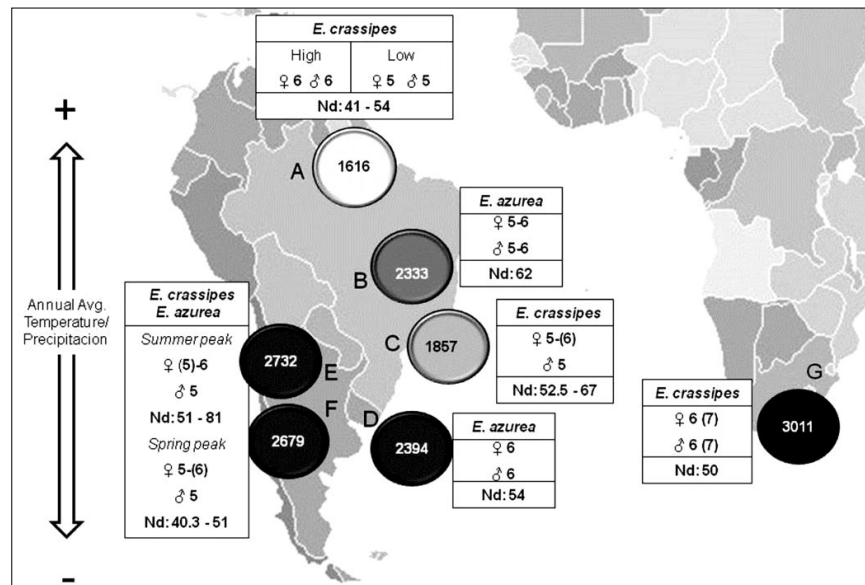


Figure 2. Summary of the variations in the number of juvenile instars and nymphal development of *Cornops aquaticum* in native and quarantine populations. Circles indicate annual average insolation; the darker circles indicate higher number of hours of sun per year. A: Manaus, Brazil (Adis and Junk, 2003; Vieira and Santos, 2003); B: Cuiabá, Brazil (Lhano, 2002; Adis *et al.*, 2004); C: Curitiba, Brazil (Medeiros, 1984); D: Carrasco, Uruguay (Zolessi, 1954); E and F: data from Corrientes and Santa Fe, Argentina; G: Pretoria, South Africa (Hill and Oberholzer, 2000). Values in parenthesis indicate minor frequency of occurrence in number of juvenile instars; Nd indicates number of days to complete nymphal development.

Corrientes and Santa Fe. Longer development in nymphs from the summer peak than those from the spring peak in Argentina may be related to the low temperatures and insolation registered during autumn and at the beginning of winter.

The results show that the longer duration of *C. aquaticum* nymphal development in females than in males is determined by females with six instars. Similar duration of nymphal development in males and females that develop with five instars is also mentioned for *C. aquaticum* in the Central Amazonian region (Adis and Junk, 2003).

Our results, as well as the results obtained in other sites of South America about the life cycle of *C. aquaticum*, show that when the instar number and nymphal development are the life history traits desirable to study in a given species, rearing should be carried out at several geographical sites of its distribution, and in the periods when new hatched nymphs have the peaks of highest abundance in the wild populations. Different juvenile instars in native populations respect to quarantine populations of *C. aquaticum* show the importance to consider life history traits throughout the native range and the effect of environmental factors as useful information for the possible use of this grasshopper as a biological control agent of *Eichhornia crassipes* in non-native areas.

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