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Aspects of life history of *Ischnura graellsii* Rambur, 1842 (Zygoptera: Coenagrionidae) in Northeast Algeria

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Abstract	Temperate Odonates exhibit various seasonal adaptive behaviors, and exploring patterns of seasonal regulation, aid in a better understanding of the general biology and evolution of Odonates. In this study, we investigate the life history of a North African damselfly, <i>Ishnura graellsii</i> , in a northeast Algerian population. We combined field and laboratory investigations to assess the embryonic development, larval growth and micro-habitat choice of exuviae during the emergence season of two cohorts (spring vs autumn cohort). The embryonic development was direct and synchronous, with 95.53% of all eggs hatching after nine days of egg laying and a hatching period ranging from 9 to 14 days. The overall hatching success was 75.35%, and the main causes of hatching failure were infertility and unhatchability. The larvae took 75 to 93 days to develop into adults after undergoing I0 to 13 subsequent molts. The emergence season started in April and lasted until the beginning of October with at least three cohorts according to its asynchronous larval population structure. The sex-ratio was slightly, but not significantly male biased. The autumn generation (cohort) had a significantly smaller body size, presumably due to the short growing season and/or higher growth rate. Males and females did not differ in vertical stratification except in autumn cohort, and emergence support choice was random by the species.					
Keywords	s Ishnura graellsii; seasonal regulation; exuviae; embryonic development; vertical stratification; Algeria.					
	Aspects du cycle biologique de <i>Ischnura graellsii</i> Rambur, 1842 (Zygoptera: Coenagrionidae) dans le Nord-Est de l'Algérie					
Résumé	Les Odonates des zones tempérées présentent divers comportements adaptatifs saisonniers, et l'exploration des modèles de régulation saisonnière aide à mieux comprendre la biologie générale et l'évolution des odonates. Dans cette étude, nous étudions le cycle biologique d'une demoiselle d'Afrique du Nord, <i>Ishnura graellsii</i> , dans une population du Nord-Est Algérien. Nous avons combiné des recherches sur le terrain et en laboratoire pour évaluer le développement embry-onnaire, la croissance larvaire et le choix du micro-habitat des exuvies pendant la saison d'émergence de deux cohortes (cohorte de printemps vs automne). Le développement embryonnaire était direct et synchrone, avec 95,53 % de tous les œufs éclos après neuf jours de ponte et une période d'éclosion allant de 9 à 14 jours. Le succès global de l'éclosion était de 75,35 % et les principales causes d'échec de l'éclosion étaient l'infertilité et l'impossibilité d'éclosion. Les larves ont pris 75 à 93 jours pour se développer en adultes après avoir subi 10 à 13 mues successives. La saison d'émergence a commencé en avril et a duré jusqu'au début octobre avec au moins trois cohortes selon sa structure de population larvaire asynchrone. Le sex-ratio était légèrement mais pas significativement biaisé vers les mâles. La génération d'automne (cohorte) avait une taille corporelle significativement plus petite, probablement en raison de la courte saison de croissance et/ou du taux de croissance plus élevé. Les mâles et les femelles ne différaient pas dans la stratification verticale, sauf dans la cohorte d'automne, et le choix du support d'émergence était aléatoire selon l'espèce.					
Mots-clés	Ishnura graellsii ; régulation saisonnière; exuvies ; développement embryonnaire ; stratification verticale ;Algérie.					

Introduction

In temperate regions, seasonality induces many stressors that shape the life history of the species and determine seasonal regulation and population structure of species in which the development is constrained by low winter temperatures in the north and by summer drought in the south (STOKS *et al.*, 2008). Odonate voltinism has been shown to be driven by two main factors, namely temperature and photoperiod (CORBET, 1999; CORBET *et al.*, 2006), and latitude is correlated with both factors. In general, the higher the latitude, the longer the life cycle.

In temperate Odonates, there are three patterns of seasonal regulation (CORBET, 2003). First spring species with synchronized development and short emergence season. Second, summer species with non-synchronized development and long emergence season. The third pattern is obligatorily one-generation summer species (SAWCHYN & GILLOTT, 1974; SNIEGULA & JOHANSSON, 2010; KHELIFA et al., 2015), but (PAULSON & JENNER, 1971; ZEBSA et al., 2014a) remind that there is an intermediate life history at low latitudes.

Besides these stressors, the habitat selection is also an important factor in determining the life history of a species in which the optimal habitat used by species is usually followed by higher survival and reproductive success. Odonates carry out several molts during the aquatic larval stage in order to reach the final instar and choose the site where they leave the water, and at this stage, they spend a substantial time in an immobile state, vulnerable and unable to escape any predation attempt (CORBET, 1999). Therefore, site selection plays a significant role to minimize mortality risks at emergence (CORBET, 1999; PURSE & THOMPSON, 2003; KHELIFA *et al.*, 2013). Starting from this point, it is reasonable to think that larvae choose emergence height based on the height of the support, type of support (ZEBSA *et al.*, 2014b; HADJOUDJ *et al.*, 2014).

Ischnura graellsii is ranked as least concern, according to the red list IUCN and is probably the commonest nonterritorial damselfly in Europe and North Africa. Coenagrionidae is a good biological model and has been the subject of several studies on larval development (THOMPSON, 1987; GRIBBIN & THOMPSON, 1990), demography (PARR & PALMER, 1971; PARR & PARR, 1972; VAN NOORDWIJK, 1978; HINNEKINT, 1987), behavior and sperm competition (CORDERO, 1990, 1992). To our knowledge, in all populations of *I. graellsii*, three female color morphs are present in which have polychromatic females, one phenotype resembling the male and two or more less dissimilar phenotypes (CORDERO, 1989).

The present study investigates some aspects of the life cycle of *lschnura graellsii* (Coenagrionidae) in northeast Algeria that widely common in streams, rivers, and ponds. This species has not been studied before its southern distribution range.

Combining field and laboratory studies, we aim to explore the embryonic development time, larval growth of the species in a northeastern Algerian natural population. In addition, we investigate the seasonal pattern of body size during the emergence season, vertical stratification, based on exuviae in two generations in spring and autumn.

Material and methods

Study site

The study site is an artificial pond, 3 km northwest from El Fedjoudj province, Guelma, Algeria, near the national road number 80 (36°31' 54.30" N, 7°22'48.08" E). The Odonata community that lived in this fish farming pond consisted of *Anax imperator, Anax parthenope, Crocothemis erythraea, Erythromma lindenii, Platycnemis subdilatata, Ischnura graellsii, Orthetrum cancellatum, Sympetrum fonscolombii, Trithemis annulata* and *Trithemis arteriosa*. Bank emergent vegetation was heterogeneous, i.e. dense in Southern parts of the pond and sparse in the Northern parts, and it consisted mainly of *Typha angustifolia, Scirpus lacustris, Cyperus longus* and *Paspallum distichum.*

Clutch size and embryonic development

In order to get eggs from natural oviposition, twenty-three females were captured and measured after copulation and induced artificially to lay their eggs in plastic vials containing a wet paper towel. After oviposition, the females were released on the site and the plastic vials were taken to the laboratory, the number of eggs was counted to determine the clutch size, and the length and width of 32 eggs from two clutches were measured to the nearest 0.01 mm using image analysis software (Digimizer). Eleven clutches were placed in separate vials with ancient water $(20 \times 10 \times 5 \text{ cm})$ under natural light conditions. Air temperature in the laboratory was taken three times a day (at 9:00 am, 12:00 am and 5:00 pm) with an electronic thermometer to the nearest 0.1°C.Water was replaced three times each week before hatching and after hatching to avoid algae growth. After hatching, larvae were counted and returned to their original site. Forty-two of the larvae hatched in mid-July from a single female were maintained, and reared individually in separate plastic boxes filled with water to prevent cannibalism, with brine shrimps (Artemia sp.) as food. Time to full development was recorded.

Larval population structure

From November 2015 to October 2016, we monthly collected larvae with a rectangular hand net $(40 \times 25 \text{ cm}, 0.5 \text{ mm mesh})$. A 10 m-stretch of bank vegetation, where most larvae occur was sampled by intensively searching. We followed SEIDENBUSCH (2010) to identify the species and sex. In the laboratory, larvae were counted and measured (body length, head width, and length of wing sheath) to the nearest 0.01 mm using a digital calliper and a dissecting microscope. LUTZ's (1968) nomination of instars was followed (F-0: final, F-1: penultimate, F-2: antepenultimate, etc.). After measurements, about 80% of larvae were returned to their original site and the rest was conserved in 70% ethanol for further investigations.

Spring vs. autumn (Body size, habitat selection) of exuviae

Since the bivoltinism and partial bivoltinism were recorded in some Odonates studies in that region (ZEBSA et al., 2014b; MAHDJOUB et al., 2015), we expected that *l. graellsii* could have more than two generations. Therefore, surveys were conducted every three days to determine the onset



Figure I

Frequency distribution of Ischnura graellsii (Rambur, 1842) (Odonata: Coenagrionidae) hatching dates under laboratory conditions. Distribution des fréquences des dates d'éclosion de Ischnura graellsii (Rambur, 1842) (Odonata : Coenagrionidae) dans les conditions de laboratoire.

of the emergence season, then we switched to the daily visits when the emergence started (mid-March to the beginning of April) and (September to the beginning of October). During the study, we noted the support height (SH), height of the exuviae fixation (He) (distance from the water surface to the tip of exuviae abdomen).

In the laboratory, the sex was determined and the body length and the head width were measured to the nearest 0.01 mm using a digital caliper, and broken or fragmented exuviae were not measured and, therefore, were not included in statistical analyses related to body size.

Statistical analyses

Statistical analyses were carried out by R 3.3.2 (R Development Core Team, 2017). Non-parametric tests were applied when residuals were not normally distributed. Chi-square tests were used to reveal sex ratio deviations from equilibrium at emergence for both cohorts.

Mann-Whitney U-tests were used to detect potential significant differences in exuviae body length, head width, height of exuviae fixation, support height, and ratio *He/Hs* between males and females in spring and autumn cohort and between both cohorts. Spearman correlation was used between body lengths, head width and emergence date to show if individual body size increased or decreased over the emergence season in both cohorts.

To test if body size had an effect on individual microhabitat choice, Spearman correlations were also used between exuviae body length, head width, and the three following parameters: height of exuviae fixation, support height, ratio He/Hs. Values are mean \pm SD.

Results

Clutch size, embryonic and larval development

Only females those induced to lay their eggs in the morning were considered, because they could produce a full clutch size. The mean artificial clutch size of *l. graellsii* was 180.43 \pm 43.86 eggs (N = 23) with a maximum of 251 eggs. The eggs length was 0.80 \pm 0.03mm (0.72-0.88 mm, N = 32) and width was 0.14 \pm 0.01mm (0.12–0.16 mm, N = 32). In the laboratory, air temperature varied within and between days showing a mean of 27.6 \pm 1.04°C. The time between the oviposition and the first hatching of all eggs was ranged between 9-14 days with mean 10.72 \pm 1.51 days, it was well



Figure 2

Temporal pattern of larval growth and number of molts of females (A) and males (B) of *lschnura graellsii*. Schéma temporel de la croissance larvaire et du nombre de mues des femelles (A) et des mâles (B) de lschnura graellsii.



Figure 3

Frequency distribution of *lschnura graellsii* (Rambur, 1842) larval instars collected monthly in 2015/2016.

> Distribution des fréquences des stades larvaires de Ischnura graellsii (Rambur, 1842) collectés mensuellement en 2015/2016.

synchronous with 95.53% of hatching within 9-11 days, while the other 4.45% of eggs hatching took up to 14 days (Figure 1). A total of 982 eggs were collected (11 clutches) and only 740 eggs hatched, resulting in an overall hatching success of 75.35%. The mean hatching success between different clutches was 72.17% \pm 26.27.

Thirty five of the larvae developed successfully into adults after 75 to 93 days (mean \pm SD:81.05 \pm 3.77 days) passing through 10 to 13 subsequent molts (Figure 2).

Larval population structure

A total of 84 larvae of *I. graellsii* were collected in one year of study. Figure 3 presents the frequency of distribution of larval instars based on monthly larvae collecting during one year. F-0 larvae first appeared in February and continued until July.A small proportion of individuals entered the winter in the final instar larva, but most of them presented a substantial variation in size (F-4 to F-1).

During the period prior to emergence (March, April), two cohorts could be identified: one consisted mainly of F-0 to F-1 larval instar and the other consisted of smaller individuals from F-3 to F-5. From September to January, the last instar larva was not appearing.

Spring vs. autumn (Sex ratio, Body size, habitat selection) of exuviae

The emergence seasonstarted in April and lasted until the beginning of October. The sex ratio was slightly, but not significantly male biased. The overall sex ratio at emergence was similar in the spring generation and slightly but not significantly male biased in the autumn generation (x^2 -test: P > 0.68) with 52.7%.

Females exuviae $(13.16 \pm 1.00 \text{ cm}; 11.68 \pm 0.81 \text{ cm})$ were longer than males $(13.05 \pm 1.33 \text{ cm}; 11.03 \pm 0.90 \text{ cm})$ for both spring and autumn generations, but significantly only in the autumn cohort (U = 476.5, P = 0.63; U = 218.5, P = 0.00) respectively. There was a significant difference in body length between both generations of males and females (Table 1).

Head width of females was greater but significantly only in the autumn cohort (U = 492.5, P = 0.78; U = 256.0, P = 0.03). There was a significant difference in head width between both generations of males and females and was greater in the spring cohort than the autumn cohort (Table I).

There was not a seasonal pattern in body length for the spring cohort (r = -0.02, P = 0.84, N = 64), but there was a seasonal decline in body size for the autumn cohort (r = -

Table I

Differences in exuviae body length, head width, height of exuviae fixation, support height and ratio He/Hs between males and females in spring and autumn cohorts and between both cohorts.

Différences de la longueur du corps des exuvies, de la largeur de la tête, la hauteur de la fixation de l'exuvie, la hauteur du support et le rapport He/Hs entre les mâles et les femelles des cohortes de printemps et d'automne et entre les deux cohortes.

		Body size (mm)	Head width (mm)	Heightof exuviae (cm)	Support height (cm)	Ratio HS\He (cm)
Spring						
Male		13.05 ± 1.33	3.34 ± 0.21	5.73 ± 6.03	56.18 ± 44.94	0.18 ± 0.20
Female		13.16 ± 1.00	3.35 ± 0.22	4.78 ± 5.48	61.61 ± 46.93	0.17 ± 0.30
Autumn						
Male		11.03 ± 0.90	3.05 ± 0.2	7.54 ± 4.46	68.94 ± 58.90	0.19 ± 0.17
Female		11.68 ± 0.81	3.18 ± 0.2	4.64 ± 3.75	62.76 ± 55.83	0.15 ± 0.17
			Spring vs	Autumn		
Male	U-test	U = 85.50	141.0	U = 317.0	U = 389.5	U = 398.5
	P value	P = 0.00	P = 0.00	P = 0.03	P = 0.28	P = 0.34
Female	U-test	U = 95.50	U = 238.5	U = 370.5	U = 394.5	U = 356.5
	P value	P = 0.00	P = 0.00	P = 0.47	P = 0.73	P = 0.35

Table 2

Effect of body size on individual microhabitat choice. Effet de la taille du corps sur le choix individuel du microhabitat.

		Support Height	Height of exuviae	Ratio He/Hs
Body length	P value	0.44	0.85	0.22
	N	119	119	119
Head width	P value	0.47	0.56	0.75
	N	119	119	119

0.44, P = 0.00, N = 55) (Figure 4). Contrarily to body size, head width was significantly increased in the autumn cohort over the emergence season (r = 0.34, P = 0.00, N = 55), but no seasonal pattern for the first cohort (r = -0.5, P = 0.67, N = 64) (Figure 5).

Ischnura graellsii used Typha angustifolia to emerge at a mean height (5.42 \pm 6.03 cm) in the spring cohort and (6.24 \pm 4.18 cm) in the autumn cohort with a maximum of 26 cm above the water surface. Males chose higher heights than females during emergence in both cohorts, but significantly only for the second cohort (U = 439.5, P = 0.33; U = 223.5, P = 0.01) respectively, and there was a significant difference in height of the exuviae fixation in males between cohorts but not in females (Table 1).

There were not significant differences in support height between male and female in both cohorts (U = 467.5, P = 0.55; U = 365.5, P = 0.84) respectively, and between cohorts. The ratio *He/Hs* did not differ either between males and females in spring or autumn cohort, nor between the two cohorts (Table 1).

Table 2 shows that there is no significant correlation between exuviae size (body length and head width) and mean height above water, neither between exuviae size and support height.



Figure 4

Seasonal pattern in *lschnura graellsii* body lengths for the spring cohort and the autumn cohort.

Évolution saisonnière de la longueur des corps de Ischnura graellsii pour la cohorte de printemps et la cohorte d'automne.

Discussion

Using the data from the combination of embryonic development and larval growth, we showed that *I. graellsii* is a multivoltine summer species in Northeast Algeria.

Clutch size of *I. graellsii* (180.43 ± 43.86 eggs) with a maximum of 251 eggs was slightly smaller than that reported by CORDERO (1992) and of its three European Coenagrionidae, namely Ischnura elegans (COOPER et al., 1996) Pyrrhosoma nymphula (BENNETT & MILL, 1995), Coenagrion puella (BANKS & THOMPSON, 1987), reasons for this may be differences in sample size and/or differences in age between females of *I. graellsii* in the present study and the other European studies (FINCKE, 1986; BANKS & THOMPSON, 1987; CORDERO, 1990b). In addition, the duration of egg hatching reveals that the species has direct embryonic development. This duration was synchronized; lasting forleast 9 days from the first to the last hatching under laboratory air temperature of 27.6 ± 1.04°C, whereas longer duration was observed in *I. graellsii* in Spain (SANCHEZ-GUILLEN, 2013). Moreover, the reared larvae in the present study had a faster development rate (3 months) than those reared in Spain (6 months) (CORDERO & MILLER, 1992), which reflects a greater voltinism, hence the achievement of more generations



Figure 5

Seasonal pattern in *Ischnura graellsii* head widths for the spring cohort and the autumn cohort.

Évolution saisonnière de la longueur des corps de Ischnura graellsii pour la cohorte de printemps et la cohorte d'automne.

per year. This variability in the duration of embryonic and post-embryonic development between the northeastern Algerian population and the northwestern Spanish population could be the result of environmental differences (latitude-thermal conditions) (CARBONELL & STOKS, 2020).Notable example is the increase in voltinism of I. elegans populations going from semi-voltinism at the cooler, high latitudes (southern Scandinavia) towards trivoltinism and quadri-voltinism in the warmer low-latitudes (southern France) showing a latitudinal cline (CORBET et al., 2006). The larval development consisted of 10 to 13 stages. However, reared larvae of the same species under laboratory conditions in Morocco showed different range of development stages (8 to 12 stages) (BENAZZOUZ et al., 1995). No exclusive hypothesis may explain variation in the number of stages between and within species (CORBET, 2002).

The emergence season started in April and lasted until the beginning of October, showing a typical summer species pattern. Similar temporal pattern of emergence was recorded in Spain (DIJKSTRA & LEWINGTON, 2015). Moreover, sex ratio was slightly but not significantly male biased, similar to the general pattern in Zygopterans (CORBET, 1999). According to the asynchronous larval population structure; emergence season had at least three cohorts. The last instar larvae first appeared in February and continued until July. From September to January, F-0 larval instar was not appearing. A small proportion of individuals entered the winter in the final instar larva, but most of them presented a substantial variation in size (F-4 to F-I). During the period prior to emergence (March, April), two cohorts could be identified: one consisted mainly of F-0 to F-1 larval instar and the other consisted of smaller individuals from F-3 to F-5. The duration of eggs hatching was synchronized, but the larvae showed a non-synchronized development rate and emergence (18 days interval from the first to the last emergence) the difference in the larval development rate may play a role in the asynchrony of larval population structure. Female mature lifetime egg production and longevity, might also determine the larval population structure. One female mated only once may lay sequentially more than a thousand eggs over her lifetime (CORDERO, 1990; 1991), and the first and last ovipositions might take place at two-weeks apart (CORDERO, 1990).

The autumn generation (cohort) had a significantly smaller body size, presumably due to the short growing season and/or higher growth rate. Similar seasonal body size decline was observed in the damselfly *Coenagrion mercuriale* (MAHDJOUB et al., 2015; MELLAL et al., 2018). Head width of the spring cohort was greater than the autumn cohort. This seasonal variation in head width should be related to the number of larval molts (INGRAM & JENNER, 1976), the larvae emerged in the spring perhaps accomplished extra-molts compared to those emerged in autumn.

I. graellsii selected emergence sites with dense vegetation and used *Typha angustifolia* to emerge at a maximum height of 26 cm above the water surface, while the maximum height climbed by the species larva in Spain was 46 cm (CORDERO, 1995). We found a great variation in exuviae height above water within species; some larvae climbed the support just above water (0 cm height), while others chose higher heights. Males climbed higher than females, even though females had significantly longer body length and larger head width, probably due to difference in post-emergence behavior between males and females. Furthermore, as table 2 shows, a larva strata selection was not influenced by the mean support height, suggesting that the support height was not a limiting factor. Further studies are required to reveal other factors influencing vertical stratification of *l. graellsii*. During the field work, exuviae collected were found in sites with low density of other species exuviae. This suggested the possibility of avoidance of disturbance and/or interspecific competition for substrates.

Voltinism was negatively correlated with latitude for most genera of Coenagrionidae (CORBET *et al.*, 2006). *I. graellsii* is bivoltine in northwestern Spain (CORDERO, 1987), trivoltine in southern Spain, and quadrivoltine in Morocco (BENAZZOUZ *et al.*, 1995).

Due to the fast growth rate of larvae observed in the laboratory (ranging from 75 to 93 days) and higher food availability observed at the study site (high densities of alevins and tadpoles), which provides optimal growth conditions, the larvae were able to complete more than two generations per year.

According to the literature, this study takes initiative to fill the gap on the seasonal regulation, embryonic development, and habitat choice of *l. graellsii* at its southern distribution range. Since the species is widespread in the area, we expect that some northwestern populations can have four generations per year, similar to the Morocco population.

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