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Phenology of aquatic insects in a protected wetland (Natura 2000 network) in northwestern Spain

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ABSTRACT

Phenology of aquatic insects in a protected wetland (Natura 2000 network) in northwestern Spain

The aim of this study was to gather new data about the life cycle phenologies of several species of aquatic insects in the "Gándaras de Budiño" (Galicia, NW Spain) protected wetland, included in the Natura 2000 network. During an annual cycle (2004-2005), three shallow lakes and four streams were sampled monthly using a semi-quantitative sampling method. The body lengths of the larvae and nymphs of thirteen species were measured, and their life cycles were analysed. All species had univoltine or semivoltine cycles. Additionally, a possible correlation between larval and nymphal lengths and water temperature was examined. We found a significant correlation for two species: the water beetle (*Noterus laevis*) and the dragonfly (*Boyeria Irene*). It is important to highlight the presence in the studied area of a species included in the Habitats Directive: the damselfly (*Coenagrion mercuriale*).

Key words: Life cycle, aquatic insects, larvae, wetland, Natura 2000.

RESUMEN

Fenología de insectos acuáticos en un humedal protegido (Red Natura 2000) del noroeste de España

El objetivo principal de este estudio fue aportar nuevos datos al conocimiento sobre los ciclos de vida de varias especies de insectos acuáticos en un humedal protegido, las Gándaras de Budiño (Galicia, NO España), incluido en la Red Natura 2000. Se muestrearon tres lagunas y cuatro arroyos mensualmente durante un año (2004-2005) mediante un método semicuantitativo. Se midieron las larvas y ninfas de trece especies, y se analizaron sus ciclos de vida. Todas las especies presentaron ciclos univoltinos o semivoltinos. También se examinó la posible correlación entre la longitud de las larvas y ninfas, y la temperatura del agua. Se encontró una correlación significativa para dos especies, el coleóptero acuático Noterus laevis*, y la libélula (*Boyeria irene*). Es importante destacar la presencia de una especie incluida en la Directiva Hábitats, el caballito del diablo (*Coenagrion mercuriale*).*

Palabras clave: *Ciclos de vida, insectos acuáticos, larvas, humedal, Natura 2000.*

INTRODUCTION

Wetlands are habitats with high biodiversity (Mitsch & Gosselink, 2000; Gopal *et al.*, 2001). They have recognised social and economic uses (Chapman *et al.*, 2001). These sites are one of the ecosystems most at risk of being seriously or irreversibly disturbed not only because of their extreme fragility but also because of the human pressure to which they are subjected. The proximity of wetlands to urban areas and the drainage and extension of agricultural lands into these areas negatively impact the shallow water levels found in wetlands. During the twenty-first century, wetland losses reached 40-90 % in a number of north-western European countries (Hull, 1997). In Spain, it was estimated that more than 60 % of wetlands had disappeared by the end of the last century (Casado & Montes, 1995). Due to this great loss of habitat, many wetland species are endangered. It is well known that wetland management and conservation is closely linked to an understanding of the biodiversity and ecology of the biota they host (Gee *et al.*, 1997; Gaston *et al.*, 2005; Céréghino *et al.*, 2008), and there are an increasing number of studies focusing on this type of habitat (Nicolet *et al.*, 2004; Della Bella *et al.*, 2005; García-Criado *et al.*, 2005; Hinden *et al.*, 2005; Trigal *et al.*, 2006; Oertli *et al.,* 2005, 2008; Garrido & Munilla, 2008; Pérez-Bilbao & Garrido, 2008).

Aquatic insects constitute an important part of the animal life within wetlands (Oertli, 1993), and these insects are tightly integrated into the structure and function of their habitats (Cayrou & Céréghino, 2005). The temporal variability of habitats is a key factor that influences the biodiversity of insect communities (Hanquet *et al.*, 2004). Information on the life cycles of aquatic species is of fundamental importance (Cayrou & Céréghino, 2005). Knowledge of these life cycles is fairly limited, but in the last decade, interest has increased considerably (Vega & Durant, 2000; Derka *et al.*, 2004; Cayrou & Céréghino, 2003; Corbet *et al.*, 2006; López-Rodríguez & Tierno de Figueroa, 2006; Braccia *et al.*, 2007; Fernández, 2007; Navarro-Martínez *et al.*, 2007). The possible application of this knowledge in the management and conservation of aquatic ecosystems has been discussed (Cayrou & Céréghino, 2005).

This study provides new data on the life cycle phenologies of several species of aquatic insects in the little-studied area of "Gándaras de Budiño" (NW Spain), which is included in the Natura 2000 network.

MATERIAL AND METHODS

Study area

The "Gándaras de Budiño" wetland is located in the province of Pontevedra (NW Spain) (Fig. 1). This wetland is included in the Galician Natura

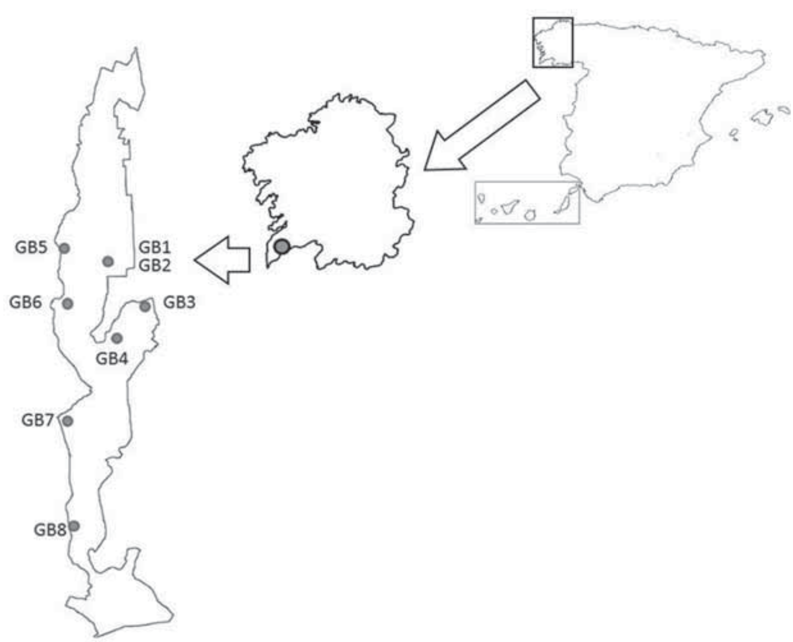

Figure 1. Location of the sampling sites in the studied area. The line delimits the SAC, and the dots represent the sampling sites. *Localización de los puntos de muestreo en el área de estudio. La línea delimita el LIC y los puntos representan las estaciones de muestreo.*

2000 network as a Special Area of Conservation (SAC) (site ES1140011) in the Atlantic biogeographical region (DOCE L387 of 29/12/2004). The climate of the area is characterised by an average annual rainfall of 1715 mm and an average annual temperature of 14 °C. This wetland area includes several permanent ponds, marshes and streams that extend to cover a total area of 834 Ha at an average altitude of 24 m. The water in this wetland comes from the water table and the Louro River, which is one of the tributaries of the Miño River, the main watercourse in the Galician Hydrological Network.

The "Gándaras de Budiño" wetland is a marshy area with an important riparian forest. It is home to species such as *Alnus glutinosa* (L.) Gaertner, *Quercus robur* L., *Fraxinus excelsior* L., *Castanea sativa* Miller and *Salix atrocinerea* Brot. Aquatic and peat bog vegetation are also widely represented (*Typha latifolia* L., *Juncus* sp. L. or *Erica cinerea* L.). While it is a place of great interest with regard to vertebrate fauna and flora, there are also important invertebrate communities, including many endemic water beetle species (Pérez-Bilbao & Garrido, 2008). In the last few years, this area has undergone considerable transformation due to the building of an industrial estate, a road and a railway, which have fragmented the area and seriously damaged the environment.

Sampling

During an annual cycle, three shallow lakes and four streams (eight points in total) were sampled monthly (from February 2004 to February 2005) using a semi-quantitative sampling method (Table 1). The fauna were collected with a round entomological water net (500 μm mesh, 30 cm diameter and 60 cm deep), and sweepings were done along a stretch of approximately 5 meters for periods of 1 minute. Invertebrates were preserved in 99 % ethanol in the field and then identified in the laboratory. After identification, organisms were stored in 70 % ethanol in hermetically sealed tubes. Odonata adults were observed flying, but they were not captured. The water temperature (°C) was measured at each sampling point where the fauna were sampled.

Data analysis

Thirteen species (seven Odonata, two Heteroptera and four Coleoptera) were selected for this study. Other species collected in the samples were not used because the number of individuals was too low to analyse. The total body length of all collected larvae and nymphs was measured with the ocular micrometer of a binocular microscope (Olympus BX51 model). All data from the different sampling points were used together to analyse the life cycles of the studied species. Box plots were constructed to represent these life cycles. The correlation between the water temperature and the total body length of the larvae was calculated. Both analyses were performed using Statistica 7.0 for Windows (Statsoft, 2004). The data were previously tested for a normal distribution using the Kolmogorov-Smirnov test.

Table 1. Sampling sites in the studied area, their respective codes, the type of ecosystem and their coordinates. *Estaciones de muestreo en el área de estudio, su respectivo código, tipo de ecosistema y coordenadas.*

Sampling sites name	Code	Ecosystem	Coordinates	
Laguna de Budiño (Observatorios)	GB1	Pond	N 42°06′46.6″ W	008°37′42.37″
Laguna de Budiño (Canal periférico)	GB2	Pond	N 42°06′56.63″ W	008°37′44.49″
Viza	GB3	Pond	N 42°06′16.38″ W	008°37′22.95″
Orbenlle	GB4	Pond	N 42°06′02.6″ W	008°37′50.68″
Folón	GB5	Stream	N 42°07′06.75″ W	008°39′11.31″
Penedo	GB6	Stream	N 42°06′22.74″ W	008°38′54.52″
San Simón	GB7	Stream	N 42°05′54.72″ W	008°38′43.4″
Delque	GB8	Stream	N 42°04′06.91″ W	008°38′18.72″

RESULTS AND DISCUSSION

A total of 2917 adults, nymphs and larvae (996 Odonata, 675 Hemiptera and 1246 Coleoptera) were studied (Table 2). Of the Odonata, three species belonged to the family Coenagrionidae (*Coenagrion mercuriale* (Charpentier, 1840), *Coenagrion puella* (L., 1758) and *Pyrrhosoma nymphula* (Sulzer, 1776)), one to Calopterygidae (*Calopteryx virgo* (L., 1758)), one to Aeshnidae (*Boyeria irene* (Fonscolombe, 1838)), one to Gomphidae (*Onychogomphus uncatus* (Charpentier, 1840)) and one to Cordulegastridae (*Cordulegaster boltonii* (Donovan, 1807)). The two species of Heteroptera belonged to the families Gerridae (*Aquarius najas* (De Geer, 1773)) and Notonectidae (*Anisops sardeus* Herrich-Schaeffer, 1849). Most Coleoptera (three species) belonged to the family Elmidae (*Dupophilus brevis* (Mulsant & Rey, 1872), *Limnius perrisi carinatus* (Pérez-Arcas, 1865) and *Limnius volckmari* (Panzer, 1793)), and the other species belonged to the family Noteridae (*Noterus laevis* (Sturm, 1834)).

We should highlight the presence of the damselfly *C. mercuriale*, which is included in Annex II of the Habitats Directive. This species is considered of "special interest" in the National Catalogue of Threatened Species (MARM, 2008). *C. mercuriale* and *O. uncatus* are also included in the Red Book of Invertebrates of Spain as "vulnerable" (Verdú & Galante, 2006). However, *C. mercuriale* is widespread in Galician coastal areas, and it is not rare on the regional scale (Azpilicueta *et al.*, 2007).

Some species were captured only in stagnant water (*N. laevis*, *A. sardeus*, *P. nymphula, C. puella* and *C. mercuriale*) or only in running water (*D. brevis*, *L. perrisi carinatus, A. najas*, *B. irene*, *O. uncatus*, *C. boltonii* and *C. virgo*), but one species, *L. volckmari*, was collected in both types of ecosystem, although this species is usually found in running waters (Garrido, 1990).

The last nymphal instars and the highest nymph abundance were observed in the summer for *A. najas*, in the spring for *N. laevis*, and in the autumn-winter for *P. nymphula* and *C. puella*. The last larval instars of *O. uncatus* and *C. boltonii* appeared in the spring, autumn and winter, and those of *L. perrisi*, *L. volckmari*, *B. irene*, *C. mercuriale* and *C. virgo* appeared in the spring, summer and winter. Larvae of *D. brevis* were observed in the autumn and winter, and nymphs of *A. sardeus* were found in the winter. Larvae of *L. volckmari*, *D. brevis*, *B. irene*, *C. boltonii* and *O. uncatus* were captured through-

Table 2. List of the thirteen species collected at the sampling sites and the state in which they were captured. A: adults, L: larvae, N: nymphs. *Listado de las trece especies recogidas con los puntos de muestreo y estadío en los que se capturaron. A: adultos, L: larvas, N: ninfas.*

Species	Month												
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Calopteryx virgo (Linnaeus 1758)	L	L	L	L	L	L			L	L	L		L
*Pyrrhosoma nymphula (*Sulzer 1776)	L							L	L	L	L	L	
Coenagrion mercuriale (Charpentier 1840)	L			L		L	L		L	L	L		
Coenagrion puella (Linnaeus 1758)		L	L	L	L			L	L		L	L	L
Boyeria irene (Fonscolombe 1838)	L	L	L	L	L	L	L	L	L	L	L	L	L
Onychogomphus uncatus (Charpentier 1840)	L	L	L	L	L	L	L	L	L	L	L	L	L
Cordulegaster boltonii (Donovan 1807)	L	L	L	L	L	L	L	L	L	L	L	L	L
Aquarius najas (De Geer 1773)	A	A	A	A	A+N	A+N	A+N	A	A	A		A	A
Anisops sardeus Herrich-Schaeffer 1849	A	A			N	N	N	A+N	N	A	N	A	
Noterus laevis Sturm 1834	L	A	A+L	A+L	A+L	A+L	L						
Dupophilus brevis Mulsant & Rey 1872	A+L	A+L	A+L	A+L	A+L	L	L	L	L	A+L	L	A+L	A+L
Limnius perrisi carinatus (Pérez-Arcas 1865)	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A		A+L	L	A+L	A+L
Limnius volckmari (Panzer 1793)	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A+L	A+L

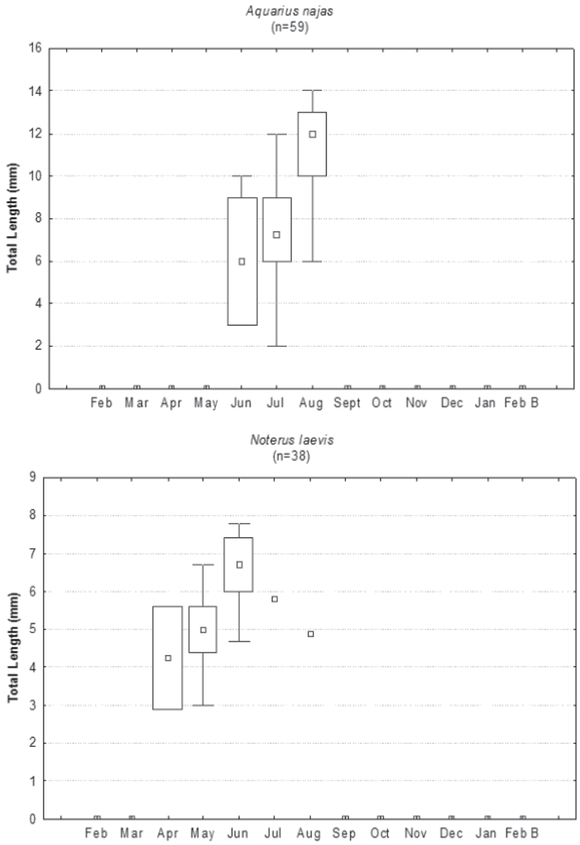

Figure 2. Two examples of box plots representing the univoltine life cycles of the species *Aquarius najas* (up) and *Noterus laevis* (down). The small square represents the median, the box represents the range between the first (25 %) and third (75 %) quartiles, and the bars represent the non-outlier range. *Dos ejemplos de box plots que representan el ciclo univoltino de las especies* Aquarius najas *(superior) y* Noterus laevis *(inferior). El cuadrado pequeño representa la mediana, la caja el rango entre el cuartil uno (25 %) y el tres (75 %), y las barras el rango de valores no atípicos.*

out the year while *C. virgo* and *C. mercuriale* were not collected in some samples.

C. virgo adults were observed in the spring-summer, *P. nymphula* and *C. puella* adults were observed in the spring, and *O. uncatus* and *C. boltonii* adults were observed in the summer. Imagoes of *C. mercuriale* and *B. irene* were not seen during the study. *A. sardeus* adults were captured in the autumn-winter, *D. brevis* adults were captured in the winter-spring, and *N. laevis* adults were captured in the spring-early summer. *A. najas*, *L. perrisi carinatus* and *L. volckmari* adults were collected throughout the year.

The term "voltinism" denotes the number of generations completed within a year (Ferreras-Romero, 1997). In our study, the life cycle patterns of the thirteen studied species could be divided into two main types: (1) univoltine, species that complete one generation per year; in our case, *P. nymphula*, *C. puella*, *A. najas*, *A. sardeus* and *N. laevis* (Fig. 2); and (2) semivoltine, species that complete one generation in two years; in our study, *C. virgo*, *C. mercuriale*, *O. uncatus*, *C. boltonii*, *B. irene, D. brevis, L. perrisi carinatus* and *L. volckmari* (Fig. 3).

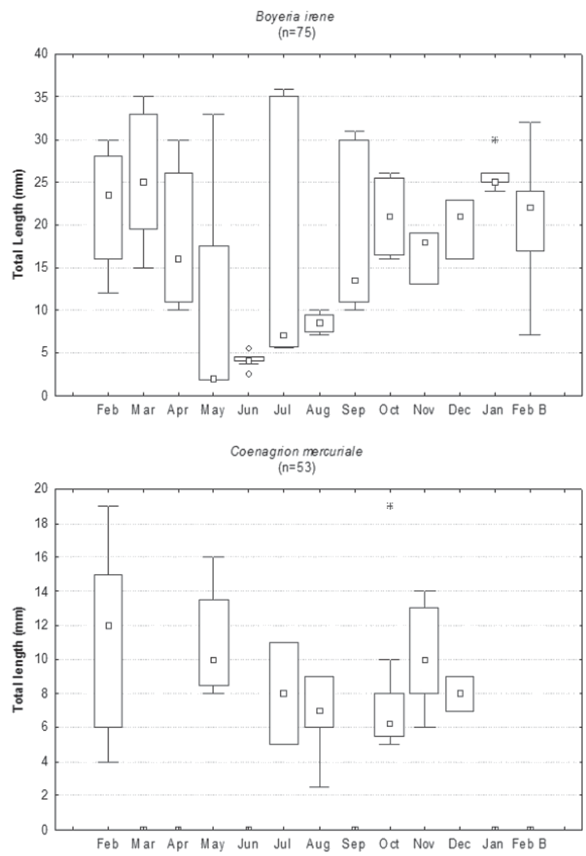

Figure 3. Two examples of box plots representing the semivoltine life cycles of the species *Boyeria irene* (up) and *Coenagrion mercuriale* (down). The small square represents the median, the box represents the range between the first (25 %) and third (75 %) quartiles, the bars represent the non-outlier range, asterisks represent extreme values, and the circles represent the outliers. *Dos ejemplos de box plots que representan el ciclo semivoltino de las especies* Boyeria irene *(superior) y* Coenagrion mercuriale *(inferior). El cuadrado pequeño representa la mediana, la caja el rango entre el cuartil uno (25 %) y el tres (75 %), las barras el rango de valores no atípicos, el asterisco los valores extremos y el círculo los atípicos.*

The three species of the family Elmidae (*D. brevis*, *L. perrisi carinatus* and *L. volckmari*) have semivoltine cycles as reported by Berthélemy & Olmi (1978). Other authors (Bertrand, 1954; Nilsson, 1996) have also reported that the larvae of riffle beetles appear mostly during the winter in freshwater streams and that many species of this family can live in the larval state for two years. In the family Noteridae, the life cycle is univoltine with overwintering adults and with larvae during the summer in the north of Europe (Nilsson, 1996). In our study, we confirmed this cycle for *N. laevis* in a southern region (Fig. 2). Another life cycle that coincided with existing literature was that of the water strider *A. najas* (Fig. 2). This species has a univoltine cycle as indicated by Nieser *et al.* (1994). The single species of backswimmer captured in this study, *A. sardeus*, also has a univoltine cycle, with the last larval instars appearing in December and with overwintering adults.

In temperate regions, most species of Odonata have univoltine cycles, but some species can have a second generation in the same year if the conditions are favourable. Otherwise, their development can last several years (D'Aguilar *et al.*, 1987). In this study, all dragonfly species had univoltine or semivoltine cycles. According to Askew (2004), the growth rate of Odonata larvae depends on the temperature. Dragonflies are not the only insects affected by this variable. Water temperature is one of the most important parameters that influence the development of aquatic insects (Nilsson, 1996; Cayrou & Céréghino, 2005; Braune *et al.*, 2008; Haidekker & Hering, 2008). We found a significant correlation between water temperature and the total body length of two species: the coleopteran *N. laevis* ($R = 0.92$, $p < 0.05$) and the dragonfly *B. irene* ($R = -0.73$, $p < 0.05$). *N. laevis* presents a positive correlation, with the last larval instars being captured during the months with the highest temperatures (summer). As mentioned above, this data confirms what Nilsson (1996) proposed for this species in a northern region. However, the body length of *B. irene* was negatively correlated with water temperature. The last larval instars were collected during the winter, when the temperature was lower. We consider *B. irene* a "summer species" (*sensu* Corbet, 1954), with larvae growing slowly during the winter without diapause. Ferreras-Romero (1997) also described this type of cycle for this species in the south of Spain.

This study has served to analyse the life cycles of several species of aquatic insects in a southern area of the Atlantic biogeographical region. Some of these life cycles, including those of *N. laevis*, *A. najas* and *B. irene*, have already been reported in other regions; thus, we have confirmed what has been proposed by other authors. We can conclude that the "Gándaras de Budiño" wetland contains important communities of aquatic insects that must be conserved.

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