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LIFE HISTORY, NYMPHAL FEEDING  
AND SECONDARY PRODUCTION OF  
EPHEMEROPTERA AND PLECOPTERA  
FROM SOUTHERN IBERIAN  
PENINSULA



Historia de vida, alimentación ninfal y productividad secundaria de  
Ephemeroptera y Plecoptera del sur de la Península Ibérica

**MANUEL JESÚS LÓPEZ RODRÍGUEZ**



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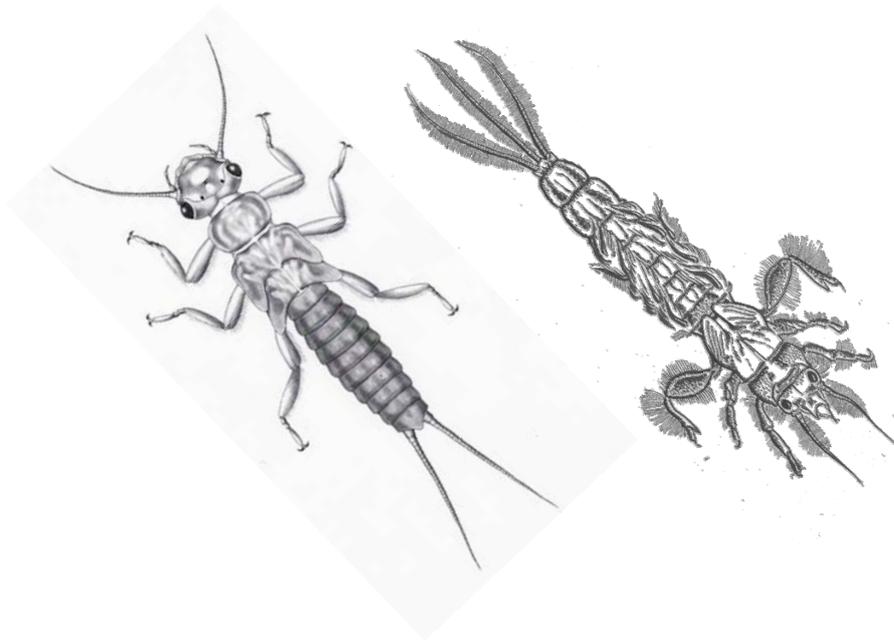


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Plecoptera del sur de la Península Ibérica**

Memoria presentada por D. Manuel Jesús López Rodríguez para optar al grado de Doctor  
por la Universidad de Granada

Vº Bº Directores

Fdo.: Dr. Javier Alba Tercedor

Fdo.: Dr. José Manuel Tierno de Figueroa

El Doctorando

Fdo.: Manuel Jesús López Rodríguez

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A mi familia

A Eu



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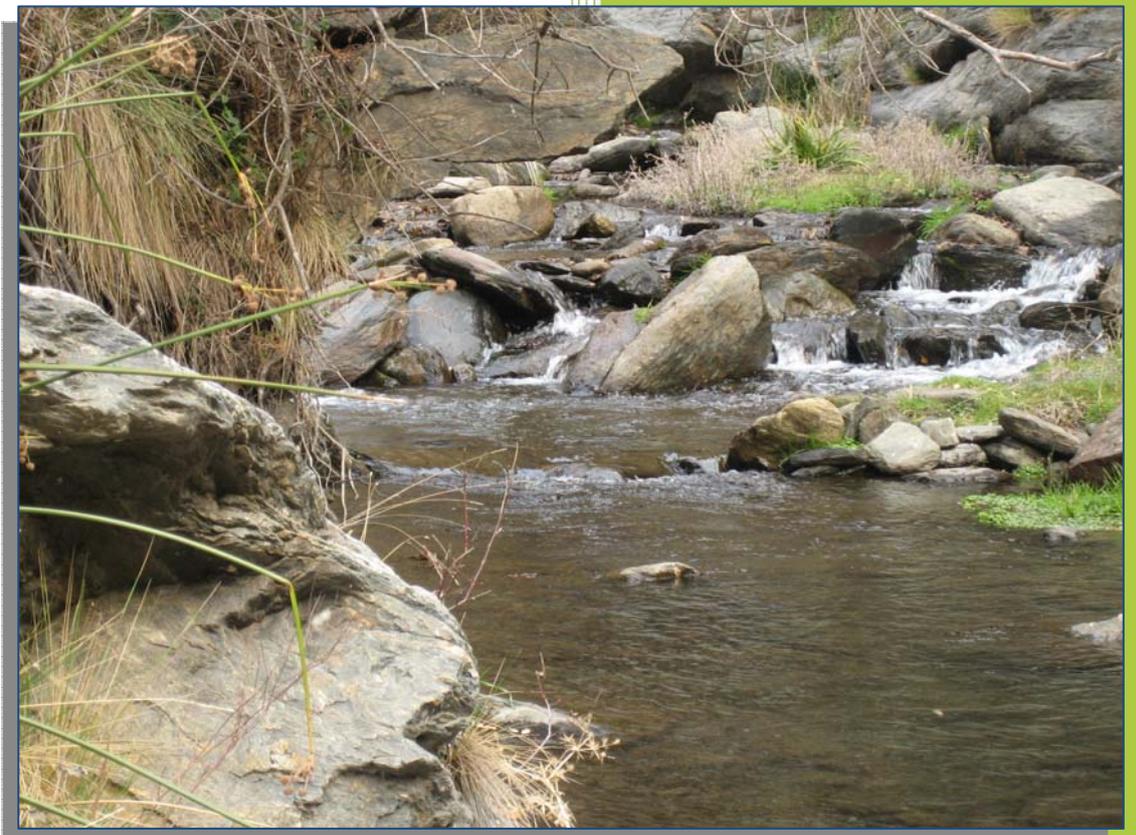
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## RESUMEN





La presente Tesis Doctoral pretende ser una aportación al conocimiento de la biología ninfal de los efemerópteros (Ephemeroptera) y plecópteros (Plecoptera) del sur de la Península Ibérica. Durante los años que ha durado se han realizado diferentes tipos de trabajos encaminados a profundizar en determinados aspectos de la biología de estos insectos acuáticos, principalmente en lo relativo a sus ciclos de vida, crecimiento, relación de este con la temperatura, alimentación de las fases juveniles y productividad secundaria de las mismas.

El primero de ellos (correspondiente al capítulo 2) se encaminó al estudio de cuatro taxa de efemerópteros [*Serratella ignita* (Poda, 1761), *Serratella spinosa nevadensis* (Alba-Tercedor, 1981), *Alainites muticus* (Linnaeus, 1758) y *Baetis alpinus* Pictet, 1843] y dos de plecópteros [*Amphinemura triangularis* (Ris, 1902) y *Capnioneura mitis* Despax, 1932] que poseían poblaciones en dos arroyos de alta montaña de Sierra Nevada (Granada, sur de la Península Ibérica) situados a diferentes altitudes (el arroyo Poqueira a 1540 m s.n.m. y el arroyo Válor a 1840 m sn.m.). Así pues, los regímenes de temperatura de ambos ríos diferían entre sí, y por tanto estos arroyos se mostraban como dos medios ideales para realizar un estudio sobre el efecto de la altitud, y por ende de la temperatura, en los ciclos de vida de dichas especies. De igual modo se estudió la alimentación de todas las especies en los diferentes medios. Así pues encontramos que, en el caso de *S. ignita* y *S. spinosa nevadensis*, los ciclos se veían alterados en relación a su duración y fenología, pero no en cuanto a su voltinismo, siendo en ambos arroyos univoltinos. En el caso de *A. muticus* la población del arroyo Poqueira fue univoltina, mientras que la del arroyo Válor fue bivoltina. Las poblaciones de *B. alpinus* se comportaron de forma opuesta, la del arroyo Poqueira con un ciclo bivoltino y la del arroyo Válor con uno univoltino. En el caso de los plecópteros, tal comparación no fue posible debido a que en el arroyo Poqueira, probablemente como consecuencia de la llegada masiva de limo, no se capturaron suficientes ninfas de los primeros estadíos como para sacar conclusiones. Se realizó, asimismo, un estudio de la influencia de la temperatura sobre el crecimiento (a partir de los grados-día acumulados por las

ninfas durante su desarrollo). Esto se llevó a cabo tan sólo en aquellas poblaciones en las que se pudo calcular una cohorte media. En dos de los taxa estudiados, *S. ignita* y *S. spinosa nevadensis*, esta relación fue positiva. En *A. triangularis* se observó que existía crecimiento incluso en aquellos meses en los que la temperatura del arroyo fue cercana a los 0 °C. En el caso de *C. mitis* hubo un importante período de crecimiento durante el otoño, coincidiendo con la entrada masiva de hojas en el arroyo, recurso importante en la dieta de esta especie. Con respecto a la alimentación de las poblaciones estudiadas, el detritus fue el componente mayoritario de su dieta, salvo en el caso de *B. alpinus* en el arroyo Poqueira, que se alimentó principalmente de diatomeas. Ambas poblaciones de *C. mitis* se alimentaron de detritus procedente de hojas fragmentadas, por lo que se comportaron principalmente como fragmentadoras. Existieron diferencias en las dietas de las poblaciones dependiendo del arroyo, así como cambios en relación con su tamaño. Esto muestra que el empleo de categorías a niveles supraespecíficos (en cuanto a tipos de alimentación o pertenencia a grupos funcionales se refiere) no es en muchas ocasiones adecuado, como tampoco lo es su extrapolación a otros niveles. Este capítulo ha sido previamente publicado con la siguiente referencia: López-Rodríguez, M.J., Tierno de Figueroa, J.M. y Aba-Tercedor, J. (2008). Life history and larval feeding of some species of Ephemeroptera and Plecoptera (Insecta) in the Sierra Nevada (Southern Iberian Peninsula). *Hydrobiologia*, 610(1): 277-295.

En una fase más avanzada de la tesis nos planteamos el estudio de medios temporales, para lo cual escogimos un río estacional de Sierra Morena, el Río Despeñaperros (Jaén, sur de la Península Ibérica), del cual se conocía bastante bien su fauna macroinvertebrada, principalmente en lo relativo a los plecópteros. En él nos planteamos dos estudios principales, que después se convertirían en tres: el estudio de las especies de plecópteros supuestamente depredadoras (pertenecientes a la superfamilia Perloidea), y el estudio de aquellas detritívoras y/o fitófagas (representantes de la superfamilia Nemouroidea). El tercero de los estudios, y que corresponde al capítulo siete de la presente Tesis, fue el estudio comparativo de una especie de efemeróptero, *S. ignita*, que se encontraba en este río y en otro del que hablaremos más adelante.

En el estudio de los plec6pteros Perloidea del R6o Despe1aperros (cap6tulo tres) escogimos tres especies, todas ellas pertenecientes a la familia Perlodidae y supuestamente depredadoras: *Guadalgenus franzi* (Aubert, 1963), *Hemimelaena flaviventris* (Pictet, 1842) e *Isoperla curtata* Nav6s, 1924. De esta 6ltima se desconoc6an las ninfas, por lo que fueron descritas previamente, descripci6n que aparece en el Ap6ndice I del cap6tulo tres, que est6 en prensa con la referencia siguiente: L6pez-Rodr6guez, M.J., Tierno de Figueroa, J.M. y Aba-Tercedor, J. (2008). Description of the nymph of *Isoperla curtata* Nav6s, 1924 (Insecta, Plecoptera). *Zoologica Baetica*, en prensa. As6 pues, estudiamos su ciclo de vida, su alimentaci6n, e incorporamos el estudio de la productividad secundaria, por ser esta un fiel reflejo de muchas otras caracter6sticas del ciclo de vida de las especies, tal y como el crecimiento, la din6mica poblacional, la adaptabilidad de la especie al medio, etc. Encontramos dos tipos principales de estrategias: la que presentaban *H. flaviventris* e *I. curtata*, que se trataba de un ciclo univoltino con per6odo de vuelo primaveral corto justo antes de la sequ6a del r6o, y la que present6 *G. franzi*, que ten6a un ciclo semivoltino, pasando el per6odo de sequ6a en el estadio ninfal, y tambi6n con un per6odo de vuelo primaveral corto. La estrategia presente en las dos primeras especies, *a priori*, parece ser la m6s adaptativa, por pasar el per6odo de sequ6a en el estadio de huevo, que es m6s resistente a condiciones de desecaci6n. Sin embargo, *G. franzi* tambi6n present6 una elevada adaptabilidad a este tipo de medios, haciendo frente a la falta de agua mediante el enterramiento en la zona hiporr6ica y, probablemente, mediante una quiescencia durante el desarrollo ninfal, puesto que el crecimiento fue nulo en este tiempo. Todo esto se vio apoyado por los resultados del estudio de la productividad secundaria de todas ellas, la cual fue bastante alta comparada con especies filogen6ticamente cercanas tanto en r6os temporales como permanentes. Pero lo m6s sorprendente vino de la mano del estudio de su dieta ninfal. Como dec6amos, estas especies han sido casi siempre catalogadas como depredadoras, generalizaci6n que se hace extensiva a la superfamilia Perloidea y, concretamente, a la familia Perlodidae. Sin embargo, nuestro estudio revel6 que, de las tres, tan s6lo *H. flaviventris* era una verdadera depredadora. Las otras dos especies fueron principalmente raspadoras, y se alimentaron principalmente de diatomeas, muy

abundantes en el río. El componente animal de la dieta de *I. curtata* fue casi nulo, si bien en el caso de *G. franzi* sí se encontró en mayor porcentaje, pero siempre en menor medida que las diatomeas. Realizamos, asimismo, un estudio de la preferencia de presas por las distintas especies a lo largo de su desarrollo ninfal (aunque, por lo anteriormente dicho, en *I. curtata* este no fue significativo). En él hallamos que la mayoría de las veces preferían los dípteros Chironomidae, aunque dicha preferencia cambió a lo largo del desarrollo. De nuevo nuestros resultados ponen de manifiesto la falta de precisión de los modelos generales en relación a la alimentación, y su ineficacia en grupos supuestamente tan bien delimitados como son los depredadores.

En el estudio sobre los plecópteros Nemouroidea (capítulo 4) analizamos los ciclos de vida, la alimentación ninfal y la productividad secundaria de dos taxa [*Tyrrhenoleuctra* sp. Consiglio, 1957) y *Brachyptera vera cordubensis* Berthélemy & Baena, 1984] que presentaron estrategias diferentes como adaptación al medio estacional. Su desarrollo ninfal tuvo lugar principalmente durante los meses de otoño e invierno, presentaron un ciclo de vida univoltino, y pasaron el período seco en diferentes estadios. En el caso de *Tyrrhenoleuctra* sp., esto tuvo lugar durante la fase ninfal, muy probablemente mediante alguna forma de latencia. Esta población presentó un largo período de vuelo y una gran amplitud de tamaños durante todo su desarrollo, lo cual es típico de fauna de aguas temporales. Los huevos que pudimos obtener en el laboratorio eclosionaron casi instantáneamente tras la puesta, indicando un cierto grado de ovoviviparismo. De este modo pudimos describir la ninfa de primer estadio. Por otro lado, *B. vera cordubensis* probablemente pasó la época de sequía en forma de huevo, con una fase de latencia durante el desarrollo embrionario (probablemente de diapausa). Dicho desarrollo tuvo lugar muy rápidamente, concentrándose en los meses justo después de la llegada de agua al río. Esto se vio reflejado en la alta productividad de este taxón, así como de *Tyrrhenoleuctra* sp., y sobre todo en su alto reemplazo de biomasa (ratio productividad/biomasa de la cohorte media), ligeramente superior al valor máximo señalado para los invertebrados acuáticos). Al estudiar la dieta observamos que *Tyrrhenoleuctra* sp. ingería principalmente detritus, mientras que *B. vera cordubensis* se alimentaba principalmente de diatomeas, si bien en ambos casos consumían

otros componentes de forma más o menos elevada. En este caso no se observaron grandes cambios en la dieta en relación al tamaño.

Volviendo de nuevo a aguas permanentes, los estudios de la última fase de la tesis los llevamos a cabo en un río de Sierra de Huétor (Granada, sur de la Península Ibérica), el Río Fardes. El primero de ellos (capítulo 5) se centró en dos especies de efemerópteros pertenecientes a la familia Leptophlebiidae [*Habrophlebia eldae* Jacob & Sartori, 1984 y *Paraleptophlebia submarginata* (Stephens, 1835)]. Estas especies presentaron un ciclo de vida parecido en duración, si bien desplazado en una con respecto a la otra. Así pues, en los meses en los que estaban presentes las ninfas de gran tamaño de una especie lo estaban las pequeñas de la otra. Al estudiar la alimentación observamos que, en ambas especies, las ninfas de pequeño tamaño se alimentaban principalmente de detritus, mientras que las más grandes incorporaban significativamente una mayor cantidad de materia orgánica particulada gruesa. Al estudiar su amplitud de nicho ecológico y su solapamiento de nicho en relación a la dieta pudimos constatar que la amplitud era muy pequeña en ambas especies y el solapamiento era casi total. Esta puede ser la razón de la segregación en la fenología de su ciclo, una cierta exclusión competitiva. Este hecho se vio reflejado también en su productividad secundaria, mucho mayor en *P. submarginata*, si bien esto está también en relación a su tamaño, bastante mayor y, por tanto, con mucha mayor biomasa.

En este mismo río realizamos también un estudio de la biología ninfal de un plecóptero [*Leuctra geniculata* (Stephens, 1836)] y un efemeróptero [*Ephemera danica* Müller, 1764], ambos habitantes del medio intersticial con hábitos cavadores. La especie *L. geniculata* presenta una serie de expansiones en las antenas (las cuales la distinguen de las otras especies pertenecientes al género *Leuctra*) con las cuales se ayuda a la hora de cavar. Por su parte, *E. danica* construye pequeños túneles en los cuales se aloja y donde crea corrientes de agua mediante el movimiento de sus branquias. La primera presentó un ciclo de vida univoltino, con una emergencia más o menos sincrónica. El período de incubación del huevo fue más largo de lo anteriormente señalado por otros autores, y durante él se acumularon más grados-día que en estudios previos de esos mismo autores, por lo que es posible que existiera una

diapausa embrionaria en esta población. En el caso de *E. danica*, el ciclo tardó unos 22 meses en completarse, tratándose por tanto de una población claramente semivoltina. El ciclo de esta especie ha sido estudiado por varios autores en distintas regiones, mostrándose relativamente variable en cuanto a su duración dependiendo de las condiciones ambientales. En el estudio de la alimentación ninfal de ambas especies se observó que el principal componente de sus dietas fue el detritus, si bien la materia orgánica particulada gruesa también tuvo una importante representación dentro de sus respectivas dietas. La productividad secundaria en las dos especies fue alta, ligeramente mayor en *L. geniculata*, lo cual apoya el hecho de que especies de grupos diferentes hagan un uso adecuado de un mismo hábitat, en este caso el medio intersticial.

Para finalizar los estudios propiamente dichos, nos planteamos una investigación comparativa entre poblaciones de *S. ignita* del Río Fardes (de régimen permanente) y del Río Despeñaperros (de régimen estacional), para así poder sacar algunas conclusiones acerca de la influencia de la sequía sobre especies concretas. Este estudio lo planteamos en el marco de las posibles condiciones futuras que pueden acaecer en los medios dulceacuícolas de latitudes medias como consecuencia del cambio climático global en el que posiblemente estemos inmersos. A parte de aumentos de temperatura y cambios en los modelos atmosféricos globales y regionales, se prevé un cambio progresivo de medios acuáticos permanentes a temporales, como efecto de los cambios en los patrones de precipitaciones. Así pues, estos dos ríos se mostraban como los medios naturales perfectos para realizar un estudio comparativo en este sentido, por tratarse de dos medios fluviales con características físico-químicas bastante parecidas. De igual modo se compararon dichos datos con los procedentes de estudios anteriores en Sierra Nevada (capítulo 2). El ciclo de *S. ignita* en el río permanente duró sólo cuatro meses, con un crecimiento ninfal bastante rápido, mientras que en el río estacional su duración fue de cinco meses, con un largo período de desarrollo embrionario que, probablemente, sufrió una fase de diapausa. Durante este período las ninfas acumularon aproximadamente la misma cantidad de grados-día. En ambos casos se alimentaron principalmente de detritus, si bien en la población del Río Despeñaperros las diatomeas también fueron un importante componente

de la dieta de la especie, aunque detectamos cambios en relación al tamaño. La productividad y biomasa fueron mayores en la población del río permanente, si bien en la del río estacional también fue muy elevada, lo cual indica una alta adaptabilidad de la especie a ambos ambientes. Estos resultados muestran que, para especies como *S. ignita*, existe toda una serie de características especialmente favorables a la hora de habitar ambientes más o menos inhóspitos como lo pueden ser las aguas temporales, tal y como la existencia de una diapausa embrionaria y un período de incubación largo, un desarrollo ninfal corto, etc., lo cual podría hacer que, en el caso de que dichas especies se vieran sometidas a estas supuestas condiciones futuras de mayor sequía, estas se vieran favorecidas en detrimento de algunas otras más estenoicas.

Por último, a partir de los resultados obtenidos a lo largo de estos años de duración de la Tesis, hemos recogido una serie de conclusiones que se pueden extraer de una visión integral de los mismos (capítulo 8). Como Anexo a la Tesis presentamos los datos preliminares de efemerópteros y plecópteros del proyecto en el cual se enmarca la misma ("Euro-Limpacs", an Integrated Project addressing the impact of climate change on European freshwater ecosystems, GOCE-CT-2003-505540), los cuales han sido expuestos tan sólo como resultados, sin pararnos a discutirlos por tratarse de datos puntuales, de fuentes muy diversas y obtenidos a partir de estudios de dispar rigor y profundidad, muchas veces encaminados a otros fines. Asimismo se adjuntan sendas listas de referencias bibliográficas recopiladas para tal fin.



## CHAPTER 1. General introduction





The present Ph.D. Thesis is focused in several aspects of two orders of aquatic insects, Ephemeroptera and Plecoptera, greatly represented in lotic, and in a lower concern in lentic, freshwaters, with some taxa also living in other types of habitats, such as, for instance, acid or brackish waters (Hynes, 1976, Brittain, 1982). They are found all around the world except Antarctica and some oceanic islands (Barber-James *et al.* 2008; Fochetti and Tierno de Figueroa, 2008). In Table 1 are summarized the families of both orders present in the Iberian Peninsula.

<b>Ephemeroptera</b>	<i>Superfamily Baetiscoidea</i>
	Prosopistomatidae
	<i>Superfamily Baetoidea</i>
	Baetidae
	Siphonuridae
	<i>Superfamily Caenoidea</i>
	Caenidae
	<i>Superfamily Ephemerelloidea</i>
	Ephemerellidae
	<i>Superfamily Ephemeroidea</i>
	Ephemeridae
	Polymitarcyidae
	Potamanthidae
	<i>Superfamily Heptagenioidea</i>
Heptageniidae	
Isonychiidae	
Oligoneuriidae	
<i>Superfamily Leptophlebioidea</i>	
Leptophlebiidae	
<b>Plecoptera</b>	<i>Superfamily Perloidea</i>
	Perlidae
	Perlodidae
	Chloroperlidae
	<i>Superfamily Nemouroidea</i>
	Taeniopterygidae
	Nemouridae
Capniidae	
Leuctridae	

Table 1. Iberian families and superfamilies of mayflies and stoneflies (after Alba-Tercedor and Jáimez-Cuéllar, 2003 and Tierno de Figueroa *et al.*, 2003)

In this introductory chapter we give a general overview on the biology of both orders, with a brief account of the natural history of these insects, including the biological characteristics of their life cycle stages. It is important to know that we do not pretend to make an exhaustive literature compilation or review of these aspects, and so exceptions to the general patterns are usually not considered. Afterwards, we focus on three aspects that are the main topics of the present Ph.D. Thesis: life histories, nymphal feeding and secondary production. We explain all the terms that are broadly used throughout this work, and to expose the frame in which this study has been developed, making especial reference to studies carried out in the Iberian Peninsula. We finish this section with the principal objectives and aims of this work, and a brief justification of its structure.

## 1.1. MAYFLIES

The order Ephemeroptera, or mayflies, contains over 3000 extant species, grouped in 42 families and more than 400 genera, and dates from the late Carboniferous or early Permian periods, about 290 mya (Brittain and Sartori, 2003; Barber-James *et al.*, 2008). They are the most primitive of the pterygote insect orders, and form, together with Odonata (dragonflies and damselflies), the group Paleoptera, but authors do not yet agree in if it is a monophyletic or paraphyletic taxon [see Gillott (2005) and Gullan and Cranston (2005) for a wide discussion]. This two groups share a series of features, such as that they cannot fold the wings back against the body when at rest, they retain the anterior median wing vein, the netlike arrangement of wing veins (they possess many crossveins), considerable change from juvenile to adult form, and the aquatic juvenile stage (Gillott, 2005), though the latter has evolved independently in several insects orders. Nevertheless, the emerging view that hexapods are closely related to branchiopod suggests that insects probably originated in freshwater, and so the aquatic stages would be ancestral [see Marden (2008)]. Mayflies are unique among the insects in that they have a winged stage between the nymph and the adult, the so called subimago. It differs from the imago in that the subimago present opaque to translucent wings, fine cilia in the outer and hind edges of

them, the body surface is covered by microtrichia, the caudal filaments are clothed with small hairs, the forelegs and the caudal filaments are shorter (especially in males), and the male genitalia, and sometimes also the eyes, are not full size (Edmunds and McCafferty, 1988). The imaginal moult takes place usually some hours after the emergence of the sub-imago (Alba-Tercedor, 2005). Thus, the life cycle of a mayfly is composed by an egg stage, a nymph, a subimago and an imago, being the egg and the nymph those of longer duration (Figure 1.1).

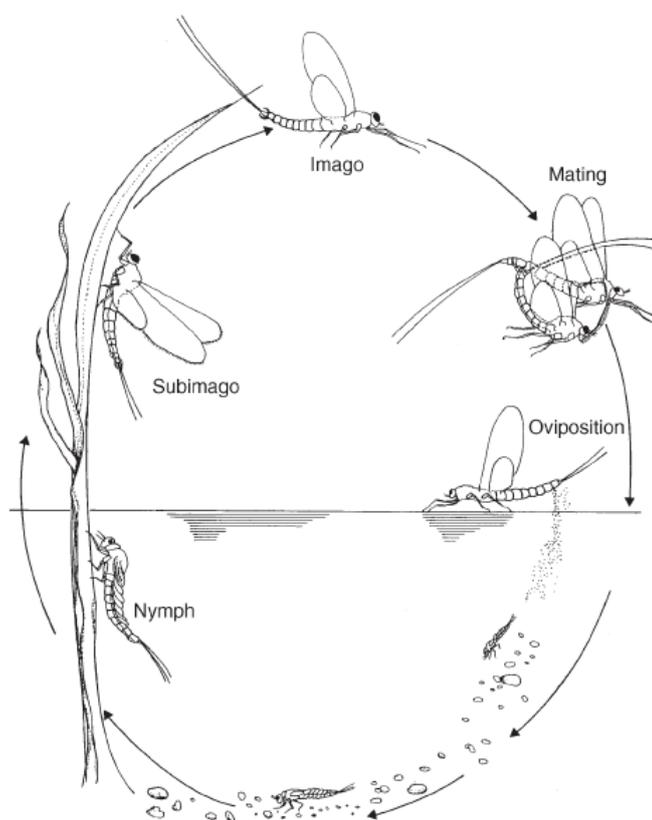


Figure.1.1. Life cycle of a mayfly (from Brittain and Sartori, 2003).

The imago is the reproductive and dispersive phase. Its life is very short (from hours to days), and it does not even feed. Instead, it has modified its gut as an aerostatic organ for improving its floatability on air. Mating usually takes place in flight, inside swarms or leks (groups of flying individuals), mainly composed by males (Alba-Tercedor *et al.*, 2006). Females interrupt into the swarms and males try to copulate with them. The latter possess the forelegs directed forward, like a net, for catching the female. In relation with the fact that males flight up and down inside the leks, they have developed an especial kind of eyes, the turban eyes,

which are projected upward, in order to have a better and wider vision for finding mate (Alba-Tercedor *et al.*, 2006). After mating, females lay down the eggs in the water in several ways, depending on the species. Hence, some release the eggs in small groups, dipping their abdomen into the water, others release a single batch that separates on contact with water, some goes underwater and lay their eggs directly on stones, and some others even throw the eggs from the air (Elliott and Humpesch, 1980; Brittain, 1982, Alba-Tercedor *et al.*, 2006). Oviposition almost always occurs after characteristic dances (Needham *et al.*, 1935). Parthenogenesis exists among some mayfly species, and may be obligatory or non-obligatory, although the low level of hatching success in the latter makes it of little importance in population dynamics (Brittain, 1982). Eggs are very different in morphology, and present different kinds of attachment structures in order to avoid their drift downstream, as well as gelatinous membranes. They are useful in phylogenetic studies (e.g. Koss and Edmunds, 1974; Dominguez and Cuezco, 2002), as has been also pointed out for spermatozoa (e.g. Soldán, 1979). The great majority of mayflies are oviparous, and so ovoviviparous species are rare. The egg development duration ranges greatly from species to species, and is mainly dependent on temperature (Elliott and Humpesch, 1980; Humpesch, 1984). Egg diapause has been proved for few species (Brittain, 1982; Clifford, 1982). The nymphal hatching use to take place within certain ranges of temperature, more or less restricted depending on the species. Hatching time in many species, as well as hatching success, depends on temperature, although intraspecific differences have been found (Elliott and Humpesch, 1980; Humpesch, 1984). Nymphs moult several times before reaching the pre-emergence size [from 10 to 50 instars have been pointed out, but this number does not appear to be constant neither for a particular species (Brittain, 1982; Brittain and Sartori, 2003, Alba-Tercedor *et al.*, 2006)]. Sexual dimorphism exists, and is more patent in more developed nymphs. Growth depends mainly on temperature, but also in other factors, such as nutrition or water flow. The great majority of mayfly nymphs are detritivores and/or herbivores, with carnivory restricted to few species (Brittain, 1982). Due to their occurrence usually in high number and their small size, they are common prey for other aquatic invertebrates and vertebrates. Nymphs also play an important role as in-stream

dispersive agents, due to they can move downstream by drifting, downward to the hyporheic zone, to the lateral parts of the stream, and even upstream (Elliott *et al.*, 1988). When completing their development, which usually occur in one year or less (although some species live up to three years), pre-emergent nymphs usually go up to the water surface or crawl to some stones or plants to do the pre-imaginal moult (Brittain and Sartori, 2003).

## 1.2. STONEFLIES

Stoneflies belong to the order Plecoptera, a group of uncertain relation within the Neoptera (Gillot, 2005; Gullan and Cranston, 2005). It comprises approximately 3500 extant species belonging to 286 genera and 16 families, and is known from the Lower Permian, some 260 mya (Fochetti and Tierno de Figueroa, 2008). They are usually associated with well oxygenated, cold water. The stonefly life cycle is composed by an egg, a nymph, and an adult stage (Figure 1.2).

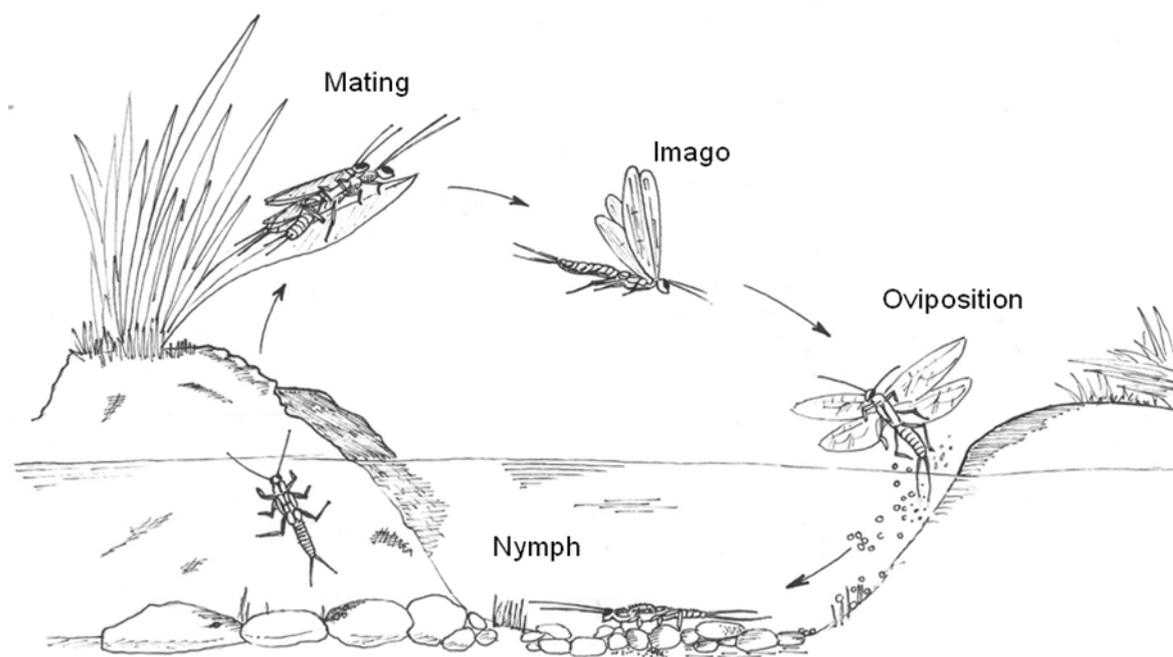


Figure.1.2. Life cycle of a stonefly<sup>1</sup>.

<sup>1</sup> Original drawing by Alejandro de la Rosa.

The main roles of the adults are also reproduction and dispersion, despite their relatively low flight, and so dispersal, capacity. The duration of the imaginal stage goes from several days to several weeks (Hynes, 1976; Tierno de Figueroa *et al.*, 2003), during which adults must find mate. They do this by direct searching and encounter, but frequently helped by the employing of vibrational communication (Stewart, 1994, 2001). Thus, they can establish duets between males and females, in which the male emits some kind of vibration and the females respond, in order to give information to the male of its position. Sometimes this mechanism is used by the female for discriminating among males of different “quality”. This communication may be by drumming, rubbing or tremulation, i.e., generating vibrations when touching with the abdomen on the substrate, dragging the substrate with the abdomen, vibrating the whole body and transmitting the vibrations through the legs to the substrate, or by a combination of several of these types. In some species the production of vibrations are aided by specialized structures. After encounter, copulation happens usually on stones or vegetation, and can be in three different positions (Tierno de Figueroa *et al.*, 2003): in parallel, in *X* or in *V*. Male displacement attempts are common in some species (Tierno de Figueroa, 2003), and mating balls of several males trying to copulate with a single female has been described in a few taxa (Tierno de Figueroa *et al.*, 2006). During the adult life, individuals may feed on several kinds of food, mainly on lichens, pollens, Cyanobacteria, etc., but some big-sized species do not ingest food during the imaginal life (Tierno de Figueroa *et al.*, 2003). Females lay down the eggs in groups, more rarely isolate, on the surface of the water. They fall down on stones of the riverbed, where they fix with specialized structures or gelatinous membranes, or sometimes they burrow partially into the substrate. The egg morphology, particularly in some groups as Perloidea, is useful in taxonomical studies (e.g. Tierno de Figueroa and Sánchez-Ortega, 1999), although spermatozoa ultrastructure is more useful in phylogenetical studies (e.g. Fausto *et al.*, 2003). Parthenogenesis has been recorded for this order, as well as ovoviviparity (Hynes, 1976), although they are not common. Egg diapause is frequent as mechanism of life cycle synchronization and avoidance of adverse conditions, although direct

development is widespread. The duration of the incubation period is related with temperature, as well as the hatching success (Tierno de Figueroa *et al.*, 2003). First instars nymphs of most species are similar and so difficult to identify (Hynes, 1976). The number of moults for completing the nymphal development varies from species to species, but it is in the range of 12 to 33, usually being different between males and females (Tierno de Figueroa *et al.*, 2003). Sexual dimorphism also exists in this group, and it is more patent in well developed nymphs of the biggest species. Growth depends mainly on temperature and nutrition. The majority of species are adapted to cold waters, although some of them may live in relatively warm waters. The species that develop during winter may grow even at 0°C, being relatively temperature independent (Brittain, 1990). Nymphal diapause appears in members of some families, also as a mechanism for avoiding adverse conditions (usually high temperature or desiccation) (Hynes, 1976; Zwick, 1980). During the nymphal development, and depending on the species, individuals may feed on detritus, green algae, diatoms, etc., and also on other animals. Thus, predator stoneflies are common and main agents of population regulation in freshwater ecosystems, together with some other taxa (Allan, 1983). The length of the nymphal stage is about one year or less, although some species may expend up to three years in it, sometimes more (Hynes, 1976). During it, nymphs may drift downstream due to the water current, but they also compensate this by actively moving upstream. Nymphs also reach the lateral and hyporheic zones, sometimes associated with a particular phase of their development. When the emergence period arrives, mature nymphs climb to stones or plants to complete the imaginal moult, period in which predation risk is higher. Stoneflies are basic components of the freshwater food webs, also serving as food for other animals, such as fishes or birds (Tierno de Figueroa *et al.*, 2003).

### **1.3. LIFE HISTORIES OF EPHEMEROPTERA AND PLECOPTERA**

The life cycle of a species is the sequence of morphological stages and physiological processes through which an individual passes during its life, and

that links one generation to the next (Butler, 1984). The components of this cycle will be the same for all members of a species, and can generally be described qualitatively. Thus, the life cycle of a mayfly includes the egg, nymphal, subimaginal and imaginal stages, and that of a stonefly comprises the egg, nymphal and imaginal stages. In contrast, the qualitative and quantitative details of the variable events that are associated with the life cycle make up a life history, which can vary between individuals or populations of one species (Butler, 1984). In this case, it concerns to the duration of stages, the growth, the hatching, etc. In order to determine the life history of a given species, researchers try to follow the development of a single cohort (Giller and Malmqvist, 1998), i.e., the development of a group of individuals that were born at the same time, or in practice the individuals derived from one reproductive period over a short period of time (Elliott & Humpesch, 1980). This would be equivalent to follow a single individual during its whole life cycle, or better, all individuals coming from the same batch. This must be differentiated of generation, which would group all the individuals that share approximately the same developmental stage at a given time, but may behave of different manner during the development. This would be like if we follow to different individuals coming from different batches, which may grow at different rates. Hence, a generation may be composed by several cohorts. This is usually reflected in a wide size spread that is more obvious in mayflies than in stoneflies.

Two aspects of life history patterns are especially important: phenology and voltinism. Phenology involves both the seasonal timing of life-cycle processes and their population synchrony, while voltinism refers to the frequency with which a life cycle is completed (Butler, 1984; Giller and Malmqvist, 1998). Regarding the latter, mayflies and stoneflies species [although it would be better to say populations, due to variations appear as a consequence of several factors, (Butler, 1984)] can be classified in the following groups:

- 1) *Univoltine*. This category groups those species (populations) with a single generation per year, with generally no overlapping between generations. This is the most common pattern in mayflies and stoneflies, and is present in species of almost every family.

- 2) *Multivoltine*. This group of species (populations) would complete the entire cycle more than one time per year. Thus, this kind of cycles involves several generations. Depending on if they complete it two times, three times, etc., this pattern is called bivoltine, trivoltine, etc. This type of life cycle is more common in mayflies than in stoneflies (Brittain, 1990), in which is restricted to very few species (Hynes, 1976). Typical multivoltine mayfly species from the Iberian Peninsula belong to the family Baetidae or Caenidae (Clifford, 1982).
- 3) *Merovoltine*. These species (populations) would complete the life cycle in more than one year. They are called semivoltine species if they do this in two years. In the Iberian Peninsula, this pattern is more frequent in stoneflies, mainly belonging to the families Perlidae and Perlodidae, but is also present in a family of mayflies, the Ephemeridae. Species with this type of cycle usually present a bigger size, increasing their individual fecundity (Brittain, 1990). It is relatively frequent that populations of typical univoltine species show merovoltine life cycles in low temperature conditions.

A difficult to classify type of life cycle is that known as “cohort splitting”. This concept makes reference to intrapopulation variability in life cycle duration. It occurs when a portion of a cohort reaches the adult stage within one season but the remainder is forced to add some more time to complete its development (Butler, 1984). Some examples occur in mayflies and stoneflies. For instance, Alba-Tercedor (1990) found in *Ephemera danica* Müller, 1764 that part of a population from Southern Iberian Peninsula developed in one year, while the other part lasted two years. Although in the Iberian Peninsula this pattern of cohort splitting has not been clearly identified for stoneflies, it has been recently cited for a Central European population of *Protonemura intricata* (Ris, 1902) in a constant temperature stream (Kozáčeková *et al.*, 2008).

A great approach to the life cycles classification was that of Hynes (1970), who recognized three main types of life cycles in temperate streams, with several subdivisions. These three groups are:

- 1) *Non-seasonal cycles*. It is presented by species (populations) in which all stages are present at all times, usually as a consequence of their long nymphal development (merovoltine).
- 2) *Slow seasonal cycles*. In them, the eggs begin to hatch soon after laying and growth occurs over a long period to maturity nearly a year later. In some species with this kind of cycles there is a very long hatching period, with steady recruitment over a lengthy period, and usually the cycle spans the winter.
- 3) *Fast seasonal cycles*. They present a fast growth period after a long egg diapause or one, or more, intermediate generations.

As mentioned before, several subdivisions appear within each category, and so detailed observation to them is needed in order to classify the cycles, due to intermediate types are usually found.

Several other authors have attempted to classify life cycles from exhaustive revisions, mainly of mayflies, such as Landa (1968) in Central Europe, Sowa (1975) in the Carpathians, and Clifford (1982), who made a broad review of life cycles and voltinism literature mainly from Northern Hemisphere species, which has been considered also when discussing the cycles of the species studied by us in the present work. Due to their extension, they will not be treated in this Introduction, but they will be explained in detail when cited.

The variations that originate different types of life history patterns can be grouped in two sets of factors (Giller and Malmqvist, 1998):

- 1) Intrinsic factors, such as physiology, morphology, and behaviour, which tend to restrict life history traits within a certain genetically or phylogenetically determined ranges.
- 2) Extrinsic factors, such as temperature, photoperiod, nutrition, degree of habitat permanence and presence of other taxa (Sweeney, 1984).

Hence, for a given species, what makes that different kinds of cycles occur in different conditions are mainly the extrinsic factors. Life histories of mayflies and stoneflies are extremely variable depending mainly on the environmental

conditions. The main factors that affect them are temperature, nutrition and photoperiod, although others, such as dissolved oxygen, pH, water current, predation, competition, etc., are also of great importance (Sweeney, 1984).

Temperature, together with nutrition, is the principal factor affecting growth and development of mayflies and stoneflies. The relative importance of temperature and nutrition are difficult to separate, because temperature affects metabolism and quantity and quality of ingested food (Sweeney, 1984). Most species possess a certain interval over and below which development is not possible. In general, mayflies have a wider range than stoneflies (Brittain, 1990). This affects both the egg and the nymphal stages, being their duration inversely proportional to the temperature in most cases (Elliott and Humpesch, 1980; Humpesch, 1984). The majority of experimental studies of the influence of temperature on the egg or nymphal development have been carried out at constant temperatures, but the effect of fluctuating temperatures, as occurs in nature, is uncertain, and may differ from species to species (Brittain, 1990). Thus, the number of day-degrees (or degree-days), calculated by summing daily mean temperatures above 0 °C, provides a useful measure of the cumulative temperature that an organism experiences, and can often predict the nymphal development period of a population (Sweeney, 1984; Giller and Malmqvist, 1998; Allan and Castillo, 2007). Usually, these day-degrees are calculated taking into account a developmental threshold temperature above which there is no development, but this requires experimental studies at constant temperatures (Gullan and Cranston, 2005). Nevertheless, when this information is not available (and mainly on mayflies and stoneflies, which usually have a very low developmental threshold temperature), the above mentioned sum of daily mean temperatures above 0 °C provides a good representation of day-degrees (Lee *et al.*, 2008). In relation with temperature, it is usually observed that life histories vary with altitude and latitude. At higher altitudes and latitudes, development uses to be slower, and several phases of the life cycle, such as emergence or egg hatching, are delayed in relation to lower altitudes and latitudes. This is because at higher altitudes or latitudes the individuals of a species probably expend more time in reaching the same day-degrees or optimum temperature.

Photoperiod is also an important factor controlling insect life histories, due to its predictability and its reliable signalling of forthcoming seasonal changes, especially in temperate regions (Sweeney, 1984). It has its main effect on the nymphal stages, and especially in constant thermal regime headwater streams. Constant temperature not reliably signal the seasonal changes that are critical to certain life history stages, and under these conditions it is probable that photoperiod provide a more reliable and informative signal than temperature (Sweeney, 1984), as has been observed in a Central European population of *P. intricata* developing in a constant temperature stream (Kozáčková *et al.*, 2008). Nevertheless, for some species also in no constant temperature streams photoperiod seems to be the most important factor unleashing emergence, while for others it is the temperature, as demonstrated Tierno de Figueroa *et al.* (2001) for different Plecoptera species in Southern Iberian Peninsula. Thus, some authors think that the photoperiod is the most important factor regulating the emergence period, acting as causal stimulus and that, after it, temperature determines the local emergence dates (Kuusela, 1976). This idea contrasts with that stated by other authors that point temperature as the maximum responsible on regulating the emergence moment, at least on hemimetabolous insects (Sweeney, 1984). Nebeker (1971) obtained different emergence models for different species according to the relative importance of photoperiod and temperature causing the emergence, and proposed the following three main types of emergence patterns: 1) where temperature is the main influence on the time emergence occurs, even though photoperiod may be the initial stimulus, 2) where photoperiod is the main regulator, and 3) where the photoperiodic stimulus is modified: emergence occurs at about the same time in a same area but is delayed by colder temperatures, for instance at high elevations.

A key factor in the phenology of life histories is the presence or not of a diapause stage, which usually takes place as a consequence of adverse conditions (extremely high or even low temperatures, drought, etc.). We must differentiate between diapause and quiescence, two different forms of dormancy. Diapause is a programmed (delayed) response with suppressed development lasting longer than the adverse conditions, combined with adaptive physiological changes (mainly in the nymphal stages), and quiescence is an immediate direct

response to a limiting factor that takes place concurrently with the adverse conditions (Danks, 1987). Diapause may be obligatory, when occurs at a fixed time regardless of varied environmental conditions, or facultative. Obligatory diapause is relatively common in univoltine insects to extend a short life cycle to one full year (Gullan and Cranston, 2005). Both, diapause and quiescence, may happen either in the egg or in the nymphal stages, but nymphal diapause has been reported only for stoneflies, not for mayflies, allowing a greater ability to survive adverse conditions, such as high summer temperatures or drought, and to adjust life cycle timing to take advantage of optimal conditions (Brittain, 1990). This mechanism is essential in temporary streams from temperate regions, where the resilience of the drought period is one of the main factors conditioning life histories. Thus, typical aquatic insects inhabiting these environments present several other adaptations, such as high powers of dispersal, rapid growth, short lifespan, small size, and opportunistic/generalistic feeding, although some studies would suggest otherwise (Williams, 2006), as also will be pointed out in the following chapters.

In the Iberian Peninsula several studies regarding life cycles have been carried out (for a complete reference list see Annex I), although they are scarce (and usually based on a few number of species) if we compare them with those of other parts of Europe. Mostly of them have been developed in Southern Iberian Peninsula, such as: Alba-Tercedor (1981, 1983, 1984, 1990a, 1990b), Sánchez-Ortega (1986); Sánchez-Ortega and Alba-Tercedor (1988, 1990, 1991), Aguayo-Corraliza *et al.* (1991), Ferreras-Romero and Agüero-Pelegrín (1994), Perán *et al.* (1999), Agüero-Pelegrín and Ferreras-Romero (2002), López-Rodríguez and Tierno de Figueroa (2004, 2005b, 2006), and Navarro-Martínez *et al.* (2007).

#### **1.4. NYMPHAL FEEDING OF EPHEMEROPTERA AND PLECOPTERA**

Aquatic insects in general, and mayflies and stoneflies in particular, seems to be generalist or omnivorous, although some of them, during their entire nymphal development or just during some phase of it, present a more or less

specialized feeding. Some of these insects present modified mouthparts in order to better obtain the resource on which they feed on (Giller and Malmqvist, 1998). Cummins (1973) developed a system of classification of insects (although it has been used also for other invertebrates) in functional feeding groups (FFG) attending to the food type and acquisition, that has been actualized afterwards several times, the most recent just a few years ago (Merritt and Cummins, 2006). These groupings have been used for several authors, such as Tachet *et al.* (1980, 2000) for classifying entire genus or families. The summarized classification in FFG and a brief explanation is shown in the following lines:

- *Shredders*: those insects that feed on coarse particulate organic matter (CPOM) by chewing it. They may be herbivores or detritivores. According to Tachet *et al.* (1980, 2000), these would group to the mayfly families Ephemeridae, Polymitarcidae, Potamanthidae, part of Prosopistomatidae, Oligoneuriidae, part of Caenidae, part of Siphonuridae, part of the Ephemerellidae and Leptophlebiidae, and the stonefly families Nemouridae, Capniidae, Leuctridae, and part of the Taeniopterygidae.
- *Filterer-collectors*: those that collect fine particulate organic matter (FPOM) from the water current. They are mainly detritivores. Depending on the authors (see Wallace and Merritt, 1980), some members of the families Ephemeridae, Oligoneuridae, Siphonuridae, Heptageniidae or Leptophlebiidae could be representatives of this group, but no one stonefly present this kind of feeding.
- *Gatherer-collectors*: those that feed on FPOM directly from the sediments. They are also detritivores. In accordance with Tachet *et al.* (1980, 2000), this would group at part of the mayflies of the family Caenidae, part of Siphonuridae, Baetidae, part of Ephemerellidae and part of Leptophlebiidae, and no one stonefly. Nevertheless, other authors have pointed out that species of different Nemouroidean stonefly families feed on detritus (Monakov, 2003).
- *Scrapers*: insects that feed on periphyton (i.e., attached algae and associated material, such as fungi and bacteria) by scraping it. Thus,

they are herbivores. Following to Tachet *et al.* (1980, 2000), this group would be represented by part of the family Prosopistomatidae and Heptageniidae within mayflies, and by some members of the family Taeniopterygidae within stoneflies.

- *Piercers-herbivores*: these are insects that suck the contents of algal cells with modified mouthparts, but these FFG is not represented neither in mayflies nor in stoneflies.
- *Predators*: are those carnivore insects that ingest wholes animals or parts of them. Within the mayflies there some Prosopistomatidae and Baetidae predators, and within the stoneflies we found them on the families Perlidae, Perlodidae and Chloroperlidae (Tachet *et al.*, 1980, 2000).

As we have seen, traditionally this classification has been used for grouping different species belonging to the same genus or family under the same FFG, using for that purpose the scarce information available for some representative of those wider groups, and so extrapolating this information to the whole set of species. When studying the feeding behaviour of concrete species it has been noted that some of these groupings are not well established, as we will also show during the following chapters. Ontogenetic shifts in food acquisition also make difficult the classification into a single FFG.

Some of the main resources available on streams for mayflies and stoneflies are detritus, biofilm on stones and leaves surfaces, composed mainly by attached algae (such as diatoms), fungi and bacteria, algae, Cianobacteria, leaves and other vegetal remains (that would form the CPOM group), in a lower concern pollen, and, of course, other animals. The term detritus is used for naming all forms of nonliving organic carbon, including fallen leaves, the waste products and carcasses of animals, fragments of organic material of unknown origin, and organic compounds (Allan and Castillo, 2007). Nevertheless, in our studies, we have used detritus as synonym of fine particulate organic matter (FPOM), because in this manner we can differentiate between species that feed on detritus (and so are gatherer-collectors) and those that feed on leaf remains (that are shredders). Detritus is very heterogeneous from the nutritional point of

view. In fact, dead organic material to many detritivores acts more as a substratum for microorganisms, which are their main food (Otto and Svensson, 1981).

The quality for the organism of the different food resources differs greatly among them, and also depending on the enzymatic complexes of each species, but little information exists about assimilation rates, digestive processes and several other aspects of nutrition on mayflies and stoneflies. Some species need to feed continuously in order to assimilate the food, and some mayflies eat their own faeces from the anus, probably for increasing the digestion efficiency (Brittain, 1982).

In the Iberian Peninsula, works on mayflies and stoneflies feeding are scarce (see Annex I). Following we present those realized in our study area, the Southern Iberian Peninsula, all of them on stoneflies: López-Rodríguez and Tierno de Figueroa (2004, 2005a, 2005b, 2006, 2008), Navarro-Martínez *et al.*, (2007), and Bo *et al.*, (2008).

## 1.5. SECONDARY PRODUCTION OF EPHEMEROPTERA AND PLECOPTERA

Secondary production (P) is defined as the formation of heterotrophic biomass (B) through time (Benke, 1993). Thus, annual secondary production is the sum of all biomass produced by a population during one year (Benke and Huryn, 2006). This is usually expressed in mass units per area per time ( $\text{mg dry mass}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ ). It is important to note that biomass is a measure of presence of living tissue mass in a population at one instant, while production is a flow, the flow rate of biomass produced through time (Benke, 1984).

An important parameter in secondary production studies is the turnover rate, or P/B rate (units are  $\text{time}^{-1}$ , i.e.,  $\text{year}^{-1}$  if we calculate annual P/B, but also monthly P/B or daily P/B may be calculated), which is a weighted mean value of biomass growth rates of all individuals in the population (Benke and Huryn, 2006), i.e., how much biomass is produced per unit of biomass. For example, if production (P) is equal to biomass (B) then the same biomass that is produced by the population is lost by mortality or migration. High P/B rates usually are

associated with fast life cycles and rapid individual growth rates (Huryñ and Wallace, 2000). This would be as saying that low “biomass” would “produce” high quantities of it very rapidly. Annual P/B values for individual populations seems to vary from less than 0.1 to more than 200 (Huryñ and Wallace, 2000). On the other hand, cohort P/B ratio reflects the production of a population over its life span divided by the mean biomass over this same time period, and usually ranges between three and eight, being generally near five.

Secondary production is a function of growth rate [apart from biomass, migration (emigration and immigration), recruitment, mortality, etc.], and so is influenced by the same factors that control it, mainly temperature and nutrition. In fact, there seem to be great differences between different functional feeding groups (see Benke, 1993), being the filterer-collectors the group with the highest values. In fact, estimation of secondary production coupled with examination of diet can provide valuable insight into energy flux through individual species (Allan and Castillo, 2007).

There are two main groups of techniques for calculating secondary production: cohort techniques, that may be used when it is possible to follow a single cohort through time, and noncohort techniques, that should be used in more complex life cycles when, for instance, several size classes inhabit at the same time. Within the cohort techniques we found different methods (although we will not go deeper into this topic), as the increment-summation method, the instantaneous growth method, the removal-summation method or the Allen curve method [see Benke and Huryñ (2006) for explanations]. The most important noncohort methods are the instantaneous growth method and the size-frequency method (both may be used also in populations with identifiable cohorts). The latter is the method most widely used for calculating secondary production. This, linked to the fact that can be used both as a cohort and noncohort technique, made us choose it as our preferred method. When using this method is important to make a correction for developmental times shorter or longer than a year, due to it was originally designed for an average cohort of one year length. That is what is called the cohort production interval (CPI), and represents the number of days (or months) from hatching to final size (Benke, 1979). Thus a CPI of 210 (or 7) indicates that the developmental time of an average cohort (from hatching to

maturity) is 210 days (or 7 months). We have preferred the use of months throughout this work, due to the nature of our data (monthly samplings) and because any time unit can be used in the correction (Benke, 1984). Thus, the annual (corrected) secondary production is calculated multiplying the uncorrected production by  $12/\text{CPI}$  (using months, if not it would be  $365/\text{CPI}$  using days). For perfect univoltine populations, with a CPI equal to 12, the uncorrected and annual production will be the same. For multivoltine populations the annual secondary production will be higher than the uncorrected one, and the opposite applies for merovoltine populations.

In conclusion, secondary production is the most comprehensive measure of success for a population because it is a composite of several other components of success: density, biomass, individual growth rate, reproduction, survivorship, and development time (Benke, 1993). Thus, it is an important clue of the fitness of the species under certain conditions.

In the Iberian Peninsula a few works have focused on secondary production of mayflies (see Annex I), but no one exists on stoneflies. Previously to the present work, no studies on Ephemeroptera and Plecoptera have been made regarding this topic in Southern Iberian Peninsula.

## **1.6. OBJECTIVES AND STRUCTURE OF THE PH.D. THESIS**

The main objectives of the present Ph.D. Thesis are:

- 1) To study the effects of altitude and temperature on the life histories of mayflies and stoneflies.
- 2) To assess the strategies that these insects have to face with drought.
- 3) To study the possible interactions between species in terms of competition for trophic resource utilization.
- 4) To examine the use of resources for burrower species of mayflies and stoneflies inhabiting and exploiting the same habitat.

- 5) To evaluate how certain species can behave and respond under the possible future conditions estimated within the global climate change scenario.
- 6) To estimate the feeding behaviour of mayflies and stoneflies, their possible differences between streams, their possible ontogenetic shifts and if they fit or not into some functional feeding groups.
- 7) To assess and discuss the different patterns of secondary production of several species with different life history strategies, feeding behaviour, and under different conditions.

The Thesis has been structured in nine chapters plus a final Annex. The first one is the present General introduction, where we have focused on the main general aspects that will be basic for better understanding the following chapters.

In chapter 2 we study how the altitude, and consequently temperature, affects life cycles of mayflies and stoneflies. Furthermore, we compare nymphal feeding between different populations of the same species to know possible differences within a stream and between populations. For this purpose we have focused in selected species of mayflies [*Serratella ignita* (Poda, 1761), *Serratella spinosa nevadensis* (Alba-Tercedor, 1981), *Alainites muticus* (Linnaeus, 1758) and *Baetis alpinus* Pictet, 1843] and stoneflies [*Amphinemura triangularis* (Ris, 1902) and *Capnioneura mitis* Despax, 1932] that inhabit in two streams from the Sierra Nevada mountains (Poqueira stream, 1540 m a.s.l., and Válor stream, 1840 m a.s. l. in Granada province, Southern Spain). These streams are situated at different altitudes, and so present different thermal regime. Thus, this is a perfect frame for studying the effect of the altitude, and so of the temperature, on the life histories of the species that are common to both streams. We also study their feeding habits and trophic behaviour in order to detect possible differences among species in the same stream, and between populations of both streams.

The following two chapters (3 and 4) are developed in a typical Mediterranean seasonal stream of the Sierra Morena mountains (Río Despeñaperros, 560 m a.s.l., in Jaén province, Southern Spain), representing an environment little studied in relation to the biology of stoneflies. There we assess how mayflies and stoneflies cope with the seasonal drought period and their

suitability to this environment. Furthermore, within stoneflies, we have chosen two groups of individual regarding feeding, one group traditionally classified as predators and one thought to be detritivores-herbivores, in order to study how they behave under these conditions.

In chapter 3 we study the life histories, nymphal feeding and secondary production of three species of Perlodea stoneflies [*Guadalgenus franzi* (Aubert, 1963), *Hemimelaena flaviventris* (Pictet, 1842) and *Isoperla curtata* Navás, 1924]. They are traditionally grouped as predators, but our study shows that this classification, at least in the type of habitat where we do the study, may not be very appropriate for some species. Furthermore, we find very different and interesting life strategies for coping with the seasonality of the stream, and we use the secondary production study to assess their fitness to it. As an Appendix to this chapter we present the description of the nymph of *I. curtata*, due to it is an essential preliminary step for being able to study its biology.

Chapter 4 is dedicated to two other stonefly taxa inhabiting the same stream (*Tyrrhenoleuctra* sp. Consiglio, 1957 and *Brachyptera vera cordubensis* Berthélemy & Baena, 1984). They belong to the Nemouroidea, the other big superfamily of Iberian stoneflies. Their biology is relatively unknown, and one of them, *Tyrrhenoleuctra* sp., presents a certain degree of ovoviviparity. Thus, in this chapter we also describe the first instar nymphs of this species. We describe and discuss their life cycle, which present several adaptations to the seasonality, their nymphal feeding and their secondary production, and relate all to the environment where they live.

In chapters 5 and 6 we return again to permanent waters and study several aspects of the nymphal biology of mayflies and stoneflies inhabiting a stream from the Sierra de Huétor (Río Fardes, 1200 m a.s.l., in Granada province, Southern Spain).

In chapter 5 we study the possible interspecific interactions, in relation to trophic resource utilization, between two close related species of mayflies, for assessing if competitive pressures exist. Hence, we focus on two cohabiting mayfly species of the family Leptophlebiidae [*Habrophlebia eldae* Jacob and Sartori, 1984 and *Paraleptophlebia submarginata* (Stephens, 1835)]. We study their life history, nymphal feeding and secondary production in order to assess

possible interactions between them. Thus, we also analyze their niche breadth and niche overlap in terms of food resources utilization to see if a possible competition between them could appear or have appeared previously, and to be the cause of their differences in terms of life cycle timing, food acquisition in relation with size, etc.

In chapter 6 we deepen on the nymphal biology of two burrower species, to know how they utilize the same habitat and if they share the same trophic resources or not. Concretely, we study a mayfly and a stonefly species [*Ephemera danica* Müller, 1764 and *Leuctra geniculata* (Stephens, 1836)] for seeing how two species, belonging to two different genera, exploit the same habitat. For that purpose we analyze their life histories, nymphal feeding and secondary production, to discuss the use that each one makes of this habitat and their fitness.

In chapter 7 we evaluate how the future predicted conditions due to the effect of global climate change can affect to species with particular characteristics, as well as to assess if these species will be able to survive under these conditions. Thus, this chapter includes two types of habitats, permanent and seasonal streams, used as models of the current and possible future climate conditions. We pretend to make an approximation to the assessment of the effects of predicted future climatic scenarios on the biology of some species. We have selected the mayfly species *S. ignita* because is present in both streams, widely distributed throughout Europe, and could be representative of some life strategies also present in other species. Hence, we establish a comparison between the life histories, nymphal feeding and secondary production of the two populations and hypothesize how these supposed future conditions may affect to species such as the studied by us.

Finally, chapter 8 is a synthesis of the results found throughout the previous chapters, giving a wider vision of them, and chapter 9 summarizes the main conclusions that can be drawn from each one of the studies and results that we have obtained during these past years.

As an Annex we have included some preliminary results of the project “Euro-Limpacs”, a European Integrated Project for the assessment of the impacts of global climate change on freshwater ecosystems (GOCE-CT-2003-505540) in

which this Ph.D. Thesis has been framed, and some comments on them. Particularly, we have selected for this annex the autoecological information regarding Ephemeroptera and Plecoptera from the Iberian Peninsula. Due to the data come from different sources and is sometimes punctual and proceed from studies focused on other aims, we do not discuss them further.

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**CHAPTER 2. Life history and nymphal feeding of some species of Ephemeroptera and Plecoptera (Insecta) in Sierra Nevada (Southern Iberian Peninsula)**





**ABSTRACT**

For the study carried out in this chapter, two high mountain streams from Sierra Nevada (Southern Spain) were monthly monitored along an annual cycle, measuring and analyzing physical and chemical parameters. Temperature was hourly registered. Life cycles and nymphal feeding of four mayfly species [*Serratella ignita* (Poda, 1761), *Serratella spinosa nevadensis* (Alba-Tercedor, 1981), *Alainites muticus* (Linnaeus, 1758) and *Baetis alpinus* Pictet, 1843] and two stonefly species [*Amphinemura triangularis* (Ris, 1902) and *Capnioneura mitis* Despax, 1932] were studied. Some species changed their strategies (growth rate, life cycle duration and/or voltinism) in accordance with different conditions (depending on the altitude and, consequently, on the temperature). The gut content data show that species fed mainly on detritus (as gatherer-collectors), except *B. alpinus* that behaved as scrapers in one of the streams and *C. mitis* that was mainly shredder. Differences in the nymphal diet of the same species in different streams, and even along its life, support the idea of not using general models to extrapolate the function of a species from a higher taxonomical level.

**KEYWORDS:** mayflies, stoneflies, nymphal biology, temperature, Southern Spain.

## 2.1. INTRODUCTION

A life cycle is defined as the sequence of morphological stages and physiological processes that link one generation to the next. The components of this cycle will be the same for all members of a species, and can generally be described qualitatively. In contrast, the qualitative and quantitative details of the variable events that are associated with the life cycle make up a life history, which can vary between individuals or populations of one species (Butler, 1984; Humpesch, 1984). These variations are consequences of intrinsic factors, such as physiology, morphology and behaviour, and of extrinsic factors, such as: temperature, photoperiod, nutrition, degree of habitat permanence and relations with other animals (Giller and Malmqvist, 1998). Within the latter, food and temperature are the primary factors determining aquatic insect growth and life histories (Sweeney, 1984). This makes that, when the environmental conditions are different, the life histories change and adapt to a particular situation. Such plasticity is found not only in different species, but also in different populations of the same species, and even within the population. This can be seen in the wide range of life history strategies. Some examples are the presence of a dormancy period (diapause, which is fixed in the life cycle, or quiescence, which appear mainly during adverse conditions), changes in the growth rate, or number of generations per year (Giller and Malmqvist, 1998).

This study focuses in four Ephemeroptera species, belonging to two different families: Ephemerellidae [*Serratella ignita* (Poda, 1761), *Serratella spinosa nevadensis* (Alba-Tercedor, 1981)] and Baetidae [*Alainites muticus* (Linnaeus, 1758), and *Baetis alpinus* Pictet, 1843], and two Plecoptera species, belonging also to two families: Nemouridae [*Amphinemura triangularis* (Ris, 1902)] and Capniidae (*Capnioneura mitis* Despax, 1932). These species (except the ssp. *S. spinosa nevadensis*) are widespread along the West Palearctic region or in Western Europe (in the case of *C. mitis*), which allows comparing our data with several other studies carried out in other geographical localities. The study was conducted in two high mountain streams of the Sierra Nevada (Southern Iberian Peninsula), with similar conditions to that found in other streams of more septentrional European localities. Furthermore, these species were also studied several years ago in Sierra Nevada (Alba-Tercedor, 1983, 1986, 1990a, 1990b;

Sánchez-Ortega, 1986; Sánchez-Ortega and Alba-Tercedor, 1990) giving the possibility of having a wider vision of possible changes occurred since then.

Thus, the aim of this work was to study the life cycles, growth, and feeding of these species, as well as the factors that influence them, and to make a comparison with previous available results.

## 2.2. MATERIALS AND METHODS

Samplings were carried out in two high mountain streams from Sierra Nevada (Granada, Southern Spain): Poqueira stream (UTM 30SVF691942, 1540 m a.s.l.) and Válor stream (UTM 30SVF911009, 1840 m a.s.l.). They were visited monthly from December 2004 to December 2005. The Válor stream could not be reached in February 2005 due to snow accumulation.

The Poqueira stream belongs to the Guadalfeo river basin (Southern Iberian Peninsula) and, in the studied site, receives water from a watershed of approximately 33.25 km<sup>2</sup>. It is a siliceous first order stream (at 10 x 10 km resolution). Along the year its width varied between 2.18 m and 6.75 m, and its depth between 0.05 m and 0.60 m. According to the IHF index categories (Pardo *et al.*, 2004), the substrate was composed mainly by more than 10% of blocks and stones, more than 10% of gravels, and less than 10% of sands, the latter mainly in pools and shores. There was also some mud in the shores. The submerged vegetation was scarce and mainly composed by *Potamogeton* sp., mosses and cyanobacteria masses. The riparian vegetation was composed by *Salix* spp., *Crataegus* sp., *Rubus* sp. and *Adenocarpus* sp.

The Válor stream belongs to the Adra river basin (South-Eastern Iberian Peninsula), and receives water from a watershed of 6 km<sup>2</sup> in the study site. It is also a siliceous first order stream (at 10 x 10 km resolution). During the sampling period the width ranges from 0.43 m to 2.20 m, and the depth fluctuated between 0.05 and 0.25 m. Its substrate was mainly composed by more than 10% blocks and rocks, more than 10% pebbles and gravels, less than 10% of sands and less than 10% of lime and mud. Patches of mosses were common in rapids. Riparian vegetation was dominated by *Salix* spp., *Adenocarpus* sp., *Populus* sp. and some Poaceae.

Physical parameters, such as dissolved oxygen, conductivity and discharge, were recorded *in situ* every sampling campaign, and one litre of water was collected and cold preserved in order to determine some chemical parameters in the laboratory (pH, ammonium, phosphates, nitrates, nitrites, sulphates, chlorides, alkalinity, suspended solids, calcium and magnesium content, hardness and turbidity). Temperature was recorded hourly with the aid of a submerged thermometer datalogger sited in the studied streams (HOBO<sup>®</sup> Water Temp Pro, 0.001 °C accuracy). These data were used in order to calculate the accumulated day-degrees of each stream per month.

Organisms were collected by a 300 µm mesh size kick net, and preserved with formalin (4%). In the laboratory, samples were sieved with a 150 µm mesh size sieve in order to remove the excess of formalin and fine detritus. After this, the six studied species were sorted out.

For mayflies, total length was measured with the aid of an ocular micrometer mounted on a microscope. For Baetidae, developmental stage were counted following Alba-Tercedor (1983), where stage I means recently hatched nymphs and stage VII means mature nymphs. For stoneflies, pronotum width and femur length (at the outer side of the right hind femur, when possible) were measured. As these two measures were highly correlated in all Plecoptera species, we used pronotum width in order to represent their life cycles graphically. For Ephemeroptera, we used developmental stage for Baetidae and total length for Ephemerellidae.

In order to study the gut content, each specimen was introduced in a vial with Hertwigs' liquid and heated in an oven at 65°C for 20-24 hours, following the methodology of Bello and Cabrera (1999) used in other studies of nymphal feeding (e.g. Derka *et al.*, 2004; López-Rodríguez and Tierno de Figueroa, 2006; Tierno de Figueroa *et al.*, 2006; Fenoglio *et al.*, 2008). By using a microscope with an ocular micrometer, it was estimated the percentage of the absolute gut content (at 40x), as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400x), as the area occupied for each component within the total gut content.

For the life cycles study, FiSAT II software (Gayaniilo *et al.*, 2002) was used, and for statistical analysis, STATISTICA software (StatSoft, 2005) was

employed. To decide about the suitability of data and to select what statistical test to run, we followed Guisande González *et al.* (2006).

None of the data analyzed agreed with the normality assumption needed to use parametric statistics, so non-parametric analyses were chosen.

For the physicochemical characterization of the streams, mean, standard deviation and minimum and maximum were computed (Table 2.1). In order to detect differences in the physicochemical conditions between the two studied streams, a Kolmogorov-Smirnov's test (K-S) was run. In both cases, February and April were not considered. In the first case, it was due to in the Válor stream there were no data for this month. For April, there were not data because of technical problems.

	Poqueira stream					Válor stream				
	N	Mean	S.D.	Min.	Max.	N	Mean	S.D.	Min.	Max.
<b>pH</b>	10	7.849	0.356	7.110	8.270	10	7.109	0.687	6.230	8.250
<b>Ammonium (mg/l)</b>	10	0.019	0.027	0.000	0.090	10	0.012	0.020	0.000	0.062
<b>Phosphates (mg/l)</b>	10	0.010	0.009	0.000	0.024	10	0.017	0.018	0.000	0.060
<b>Nitrates (mg/l)</b>	10	0.089	0.061	0.000	0.177	10	0.134	0.083	0.000	0.284
<b>Nitrites (mg/l)</b>	10	0.007	0.011	0.000	0.030	10	0.002	0.003	0.000	0.007
<b>Sulphates (mg/l)</b>	10	9.476	7.446	1.370	27.010	10	4.301	3.667	0.910	12.200
<b>Chlorides (mg/l)</b>	10	41.250	5.000	31.950	46.900	10	52.070	11.303	35.500	80.400
<b>Alkalinity (meq/l)</b>	10	0.816	0.215	0.400	1.140	10	0.314	0.257	0.120	0.980
<b>Ss (mg/l)</b>	10	9.580	14.850	0.600	48.900	10	17.296	37.614	0.800	122.600
<b>Ca (mg/l)</b>	10	20.480	7.647	7.200	29.600	10	12.320	7.334	4.800	25.600
<b>Mg (mg/l)</b>	10	6.415	3.412	1.944	10.692	10	3.256	3.031	0.486	8.748
<b>Hardness (mg CaCO<sub>3</sub>/l)</b>	10	77.544	31.516	25.980	115.919	10	44.166	29.366	21.987	99.930
<b>Turbidity (NTU)</b>	10	2.588	5.310	0.000	17.330	10	2.479	3.587	0.000	11.640
<b>O<sub>2</sub> (% sat)</b>	10	87.900	7.992	70.000	97.000	10	88.500	8.449	70.000	98.000
<b>O<sub>2</sub> (mg/l)</b>	10	8.470	1.291	6.500	10.100	10	7.760	1.018	6.100	9.000
<b>Temperature (°C)</b>	8827	9.102	4.424	0.079	18.319	8830	8.823	4.043	0.273	18.224
<b>Conductivity (µS/cm)</b>	10	107.640	13.302	84.200	123.900	10	33.110	2.586	29.900	38.200
<b>Discharge (m<sup>3</sup>/s)</b>	10	0.199	0.162	0.060	0.494	10	0.055	0.036	0.007	0.115

Table 2.1. Physicochemical characterization of the sampling sites.

To assess differences in the temperature regimes of both streams, a Wald-Wolfowitz runs test (W-W) was used. Growth was studied in relation to accumulated day-degrees (Table 2.2) by selecting those species and streams in

which a clear growth period could be identified. Growth was estimated for each month as the difference between the mean size of the species in a given month and the mean size in the previous month. Daily mean growth was calculated dividing such growth by the days between the two sampling dates. Mean growth rate was also estimated as the species percentage of growth per day. In several species some periods with negative growth appear, corresponding to the widely accepted decrease in mean size of the Ephemeroptera and Plecoptera populations at the end of the life cycle (Sweeney and Vannote, 1978; Vannote and Sweeney, 1980; Tierno de Figueroa and Sánchez-Ortega, 2004).

	Monthly accumulated day-degrees	
	Poqueira stream	Válor stream
January-February	87.628	102.597
February-March	91.435	204.106
March-April	255.040	168.061
April-May	268.831	283.341
May-June	342.409	326.479
June-July	490.641	466.145
July-August	451.854	424.220
August-September	433.216	408.771
September-October	338.796	310.289
October-November	340.095	300.030
November-December	209.597	191.939
<b>Total</b>	<b>3309.542</b>	<b>3185.977</b>

Table 2.2. Monthly accumulated day-degrees in each sampling site (months represent periods between sampling dates).

For the study of diet, mean, standard deviation and minimum and maximum were calculated for each single species. For studying the variations of the diet along the growth period (i.e., in relation to the size), a Gamma correlation test was run.

### 2.3. RESULTS

Both streams showed similar patterns in temperature regimes (Figure 2.1). However, significant differences in the thermal regime of the two streams were found ( $W-W Z = -29.178$ ;  $p < 0.05$ ). Water temperature of the site located at lower altitude, in the Poqueira stream, was higher (mean =  $9.10 \pm 4.42$  °C) than in the

Válór stream (mean=  $8.82 \pm 4.04$  °C) (Figure 2.1). Both studied sites had similar physicochemical conditions (Table 2.1), although in some variables (pH, chlorides, alkalinity and conductivity) significant differences were found (K-S  $p < 0.05$ ).

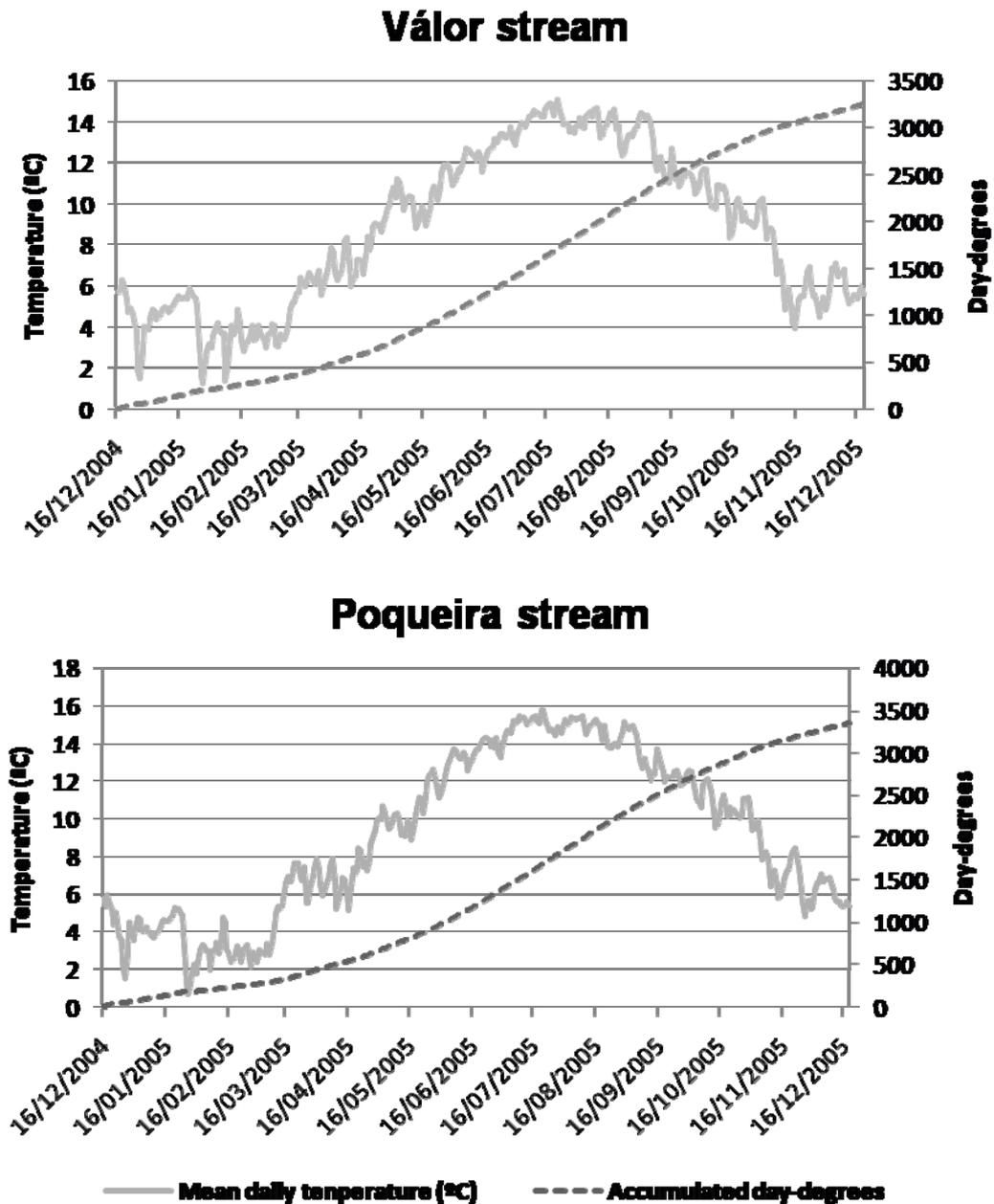


Figure 2.1. Mean daily temperature and accumulated day-degrees at the sampling sites.

2.3.1. *Serratella ignita*

The species showed a univoltine cycle, both in Poqueira and Válor streams (Figure 2.2), with several cohorts. Nymphs were present since June to August in the Poqueira stream, and since May to August in the Válor stream, i.e., for three months in the former and for four months in the latter, although some of them were also found punctually in October and January at the Poqueira stream and September at Válor stream. Mature nymphs were found during July-August, when oviposition occurred. Laid eggs remained in the stream during the whole autumn and winter, until the next spring.

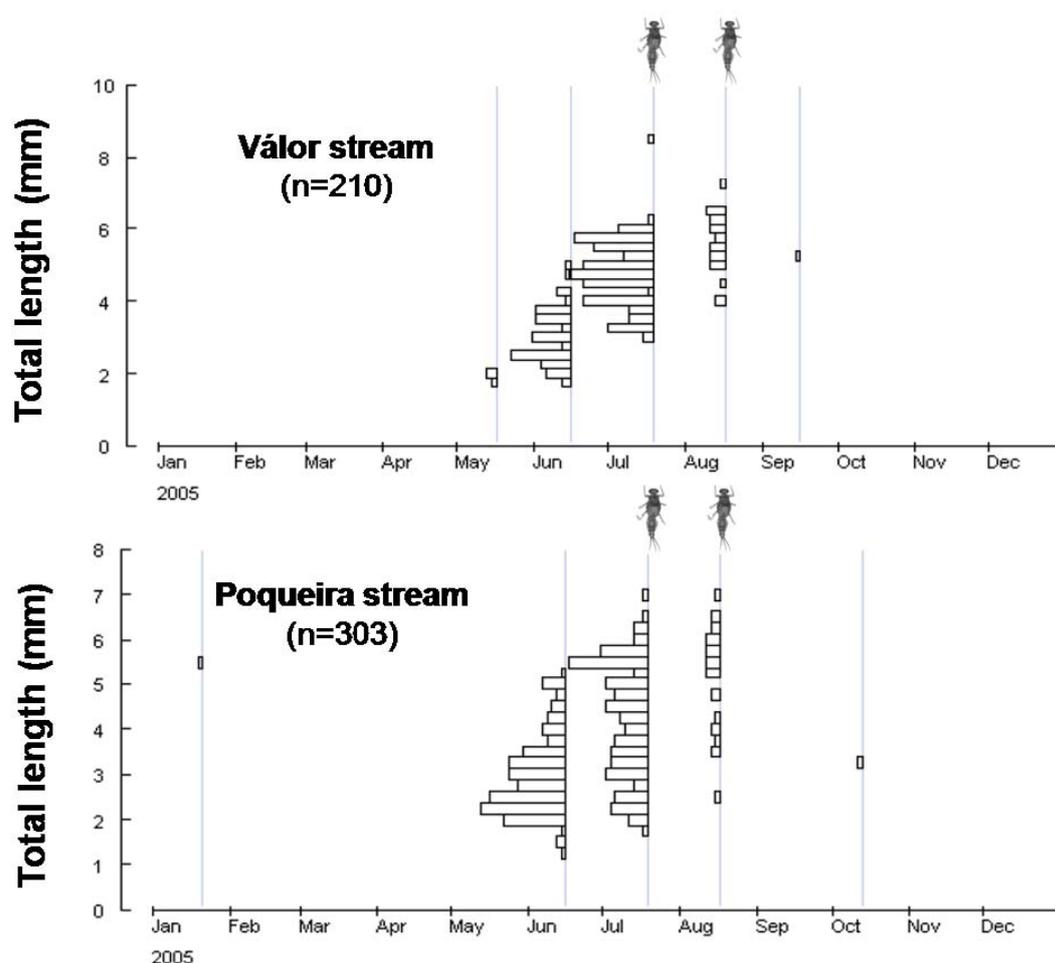


Figure 2.2. Life history of *S. ignita* in the two sampling sites. Presence of mature nymphs is marked with a nymph draw.

Growth was higher in the Poqueira stream in the periods when accumulated day-degrees were higher, showing a relation between growth rate and temperature (Table 2.3). In Válor no clear pattern can be concluded.

The most abundant component of the diet was detritus, both in Poqueira and Válor, so the species behaved as gatherer-collector, although an important scraper function was also detected, mainly in the Poqueira stream, due to the relatively high quantity of epilithic and epiphytic diatoms found in the gut (Table 2.4). When analyzing the changes in the diet in relation with the nymphal size, it was observed that detritus consumption was reduced in favour of other components (Table 2.6).

### 2.3.2. *Serratella spinosa nevadensis*

This species had a univoltine life cycle, which lasted four months (from April to July) in the Poqueira stream and seven months (from the end of March to September) in the Válor stream (Figure 2.3). As its co-generic species, there were several cohorts. Mature nymphs were found just in July in the Poqueira stream, and from June to September in the Válor stream. After oviposition, eggs remained in the stream until the next spring.

As occurred in *S. ignita*, the higher the accumulated day-degrees, the higher the growth rate in the Poqueira stream (Table 2.3). In Válor there was not a clear relation between growth and accumulated day-degrees. In the second middle of the cycle, it appeared a period with negative growth rate, probably by chance in the collection of individuals and possibly related to that many individuals had emerged in the previous month. Thus, we considered this case as a null growth period.

In the two streams, the main component found in the gut was detritus (Table 2.4). Thus, this species could be catalogued as gatherer-collector, although it could play a certain role also as scraper, and even as shredder in the Válor stream, due to the approximately equal presence of material coming from the biofilm (epilithic and epiphytic diatoms, hyphae) and phyllidia. The detritus intake in the Poqueira increased with increasing size, while in Válor decreased (Table 2.6).

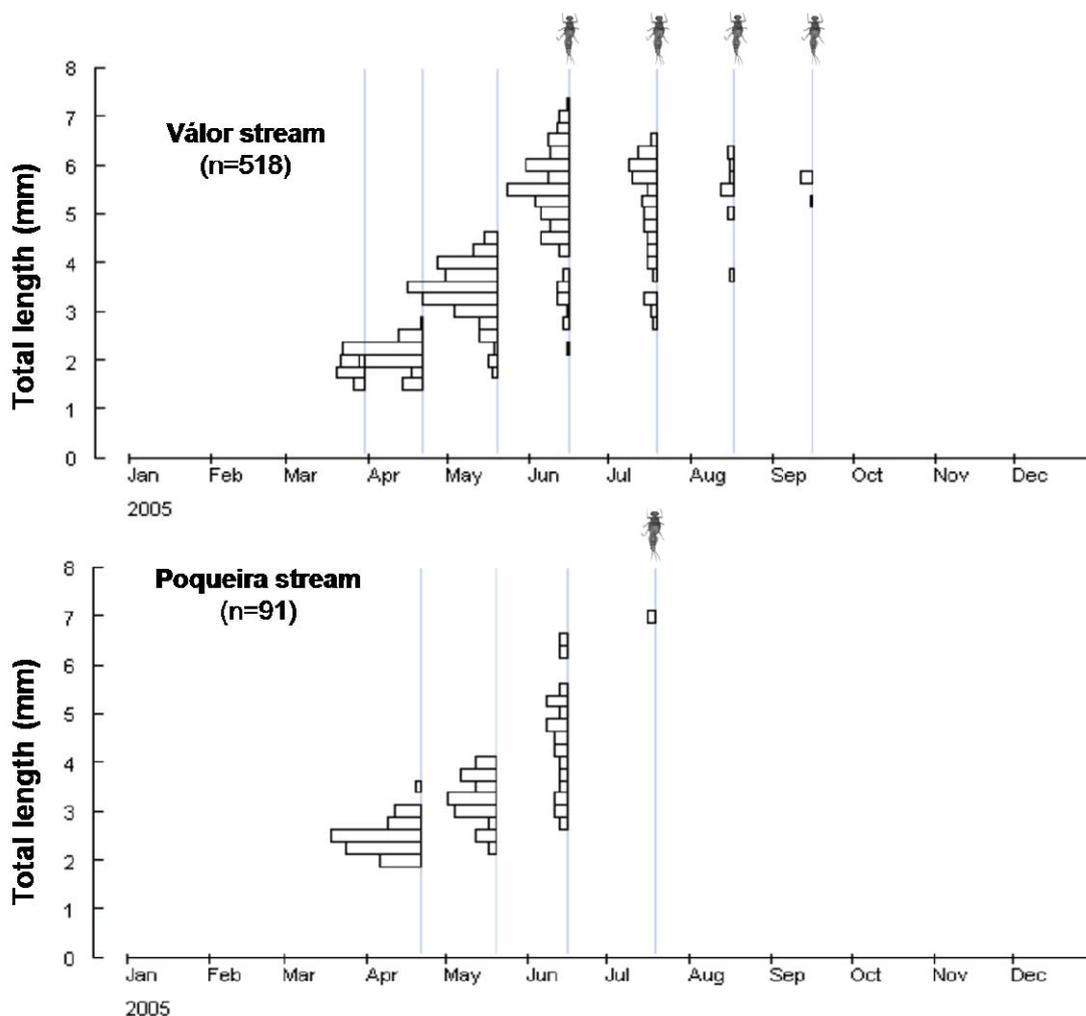


Figure 2.3. Life history of *S. spinosa nevadensis* in the two sampling sites. Presence of mature nymphs is marked with a nymph draw.

### 2.3.3. *Alainites muticus*

This species showed a univoltine life cycle in the Poqueira stream and a bivoltine cycle in the Válor stream, with several cohorts in both cases (Figure 2.4). Almost every nymphal stage (*sensu* Alba-Tercedor, 1983) was present during all the year, mainly in Válor. Mature nymphs were present in May in the Poqueira stream and in May, July, August and September in the Válor stream.

In both streams, the species behaved mainly as a gatherer-collector (the detritus was the most consumed resource), but it acted also as scraper, principally in the Poqueira stream, where a high quantity of epilithic and epiphytic diatoms was found (Table 2.4). The detritus was less consumed for bigger

nymphs from Poqueira (in Válor there was not a significant correlation), while simultaneously the other components were more frequently found in the gut (Table 2.6).

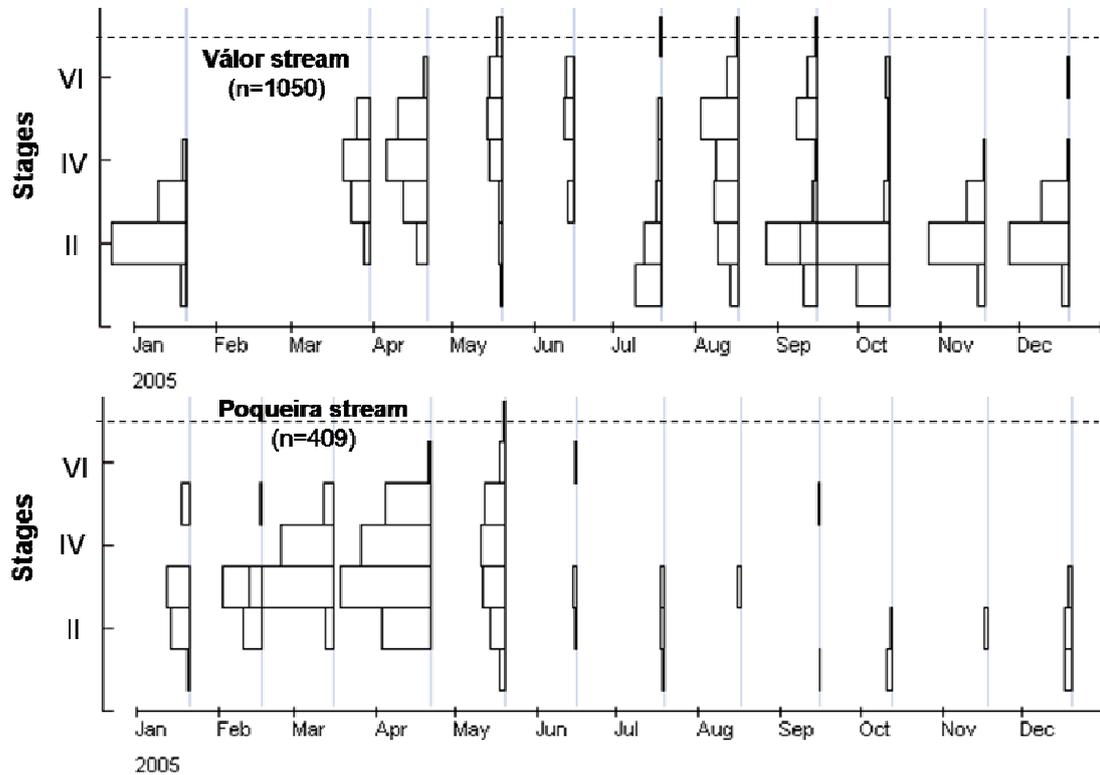


Figure 2.4. Life history of *A. muticus* in the two sampling sites. Dot line signs the VII stage (i.e., mature nymphs).

Species	Stream	Date	Period (days)	Growth (mm)	Mean daily growth (mm/day)	Mean growth rate (%/day)	Day-degrees (accumulated)
<i>S. ignita</i>	Poqueira	15/06/05 - 18/07/05	34	1.374	0.040	1.705	490.641
		18/07/05 - 16/08/05	29	0.996	0.034	1.449	451.854
	Válor	19/05/05 - 15/06/05	28	1.039	0.037	1.003	326.479
		15/06/05 - 18/07/05	33	1.782	0.054	1.459	466.145
		18/07/05 - 16/08/05	29	0.879	0.030	0.819	424.220
		16/08/05 - 15/09/05	30	*	*	*	408.771
<i>S. spinosa nevadensis</i>	Poqueira	21/04/05 - 19/05/05	29	0.804	0.028	0.601	268.831
		19/05/05 - 15/06/05	27	1.164	0.043	0.934	342.409
		15/06/05 - 18/07/05	33	2.646	0.080	1.738	490.641
	Válor	30/03/05 - 21/04/05	23	0.392	0.017	0.412	168.061
		21/04/05 - 19/05/05	28	1.317	0.047	1.139	283.341
		19/05/05 - 15/06/05	27	1.855	0.069	1.663	326.479
		15/06/05 - 18/07/05	33	**	**	**	466.145
		18/07/05 - 16/08/05	29	0.329	0.011	0.275	424.220
		16/08/05 - 15/09/05	30	0.237	0.008	0.192	408.771
		<i>A. triangularis</i>	Válor	15/09/05 - 12/10/05	28	0.088	0.003
12/10/05 - 17/11/05	36			0.077	0.002	0.241	300.030
17/11/05 - 19/12/05	32			0.108	0.003	0.378	191.939
20/01/05 - 30/03/05	70			0.350	0.005	0.562	306.703
30/03/05 - 21/04/05	22			0.189	0.009	0.967	168.061
21/04/05 - 19/05/05	28			0.078	0.003	0.312	283.341
<i>C. mitis</i>	Válor			15/09/05 - 12/10/05	28	0.076	0.003
		12/10/05 - 17/11/05	36	0.246	0.007	1.500	300.030
		17/11/05 - 19/12/05	32	0.133	0.004	0.914	191.939
		20/01/05 - 30/03/05	70	*	*	*	306.703
		30/03/05 - 21/04/05	22	*	*	*	168.061

Table 2.3. Growth, daily mean growth, mean growth rate and day-degrees for each species, for the two sampling sites and months. For Ephemeroptera employed measures are total length, and for Plecoptera pronotum width. \* Means period in which there was a negative growth, coinciding with the end of the cycle. \*\* Means period in which there was a negative growth in the middle of the cycle. See "Materials and methods" for details.

#### 2.3.4. *Baetis alpinus*

The life cycle of *B. alpinus* was probably bivoltine in the Poqueira stream, but due to the problems mentioned in the "Material and methods" section for sampling in Válor during February, we were not able to clearly determine if in this stream the cycle was univoltine or bivoltine (Figure 2.5). Nymphs of several stages were present almost the whole year in both cases. Mature nymphs were

found in February, April and May in the Poqueira stream, and in January, March and April in the Válor stream.

In the Poqueira stream, this species fed mainly on epilithic and epiphytic diatoms and detritus, being scraper and gatherer-collector, but in Válor the principal component of the diet was the detritus, followed by the epilithic and epiphytic diatoms (Table 2.4). Bigger nymphs consumed less amounts of detritus (Table 2.6).

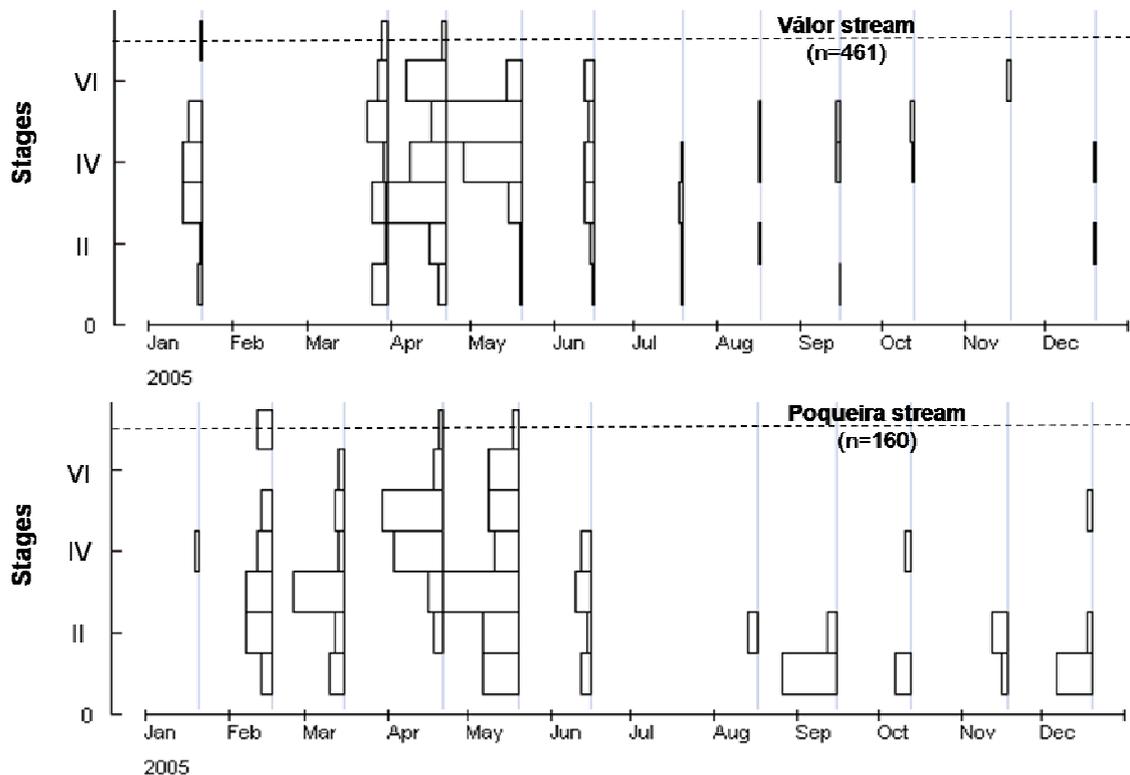


Figure 2.5. Life history of *B. alpinus* in the two sampling sites. Dot line signs the VII stage (i.e. mature nymphs).

Species	Stream		% absolute	% detritus	% diatoms	% hyphae	% fungi spores	% pollen	% phyllidia	FFG
<i>S. ignita</i>	Poqueira	N	275	261	261	261	261	261	261	<b>Collector-Gatherer/Scrapper</b>
		Mean	60.150	82.830	13.830	1.540	0.550	0.780	0.510	
		SD	28.390	26.560	24.640	2.720	1.400	1.410	5.620	
		Min-Max	0-100	4-100	0-90	0-15	0-12	0-10	0-88	
	Válor	N	184	175	175	175	175	175	175	<b>Gatherer-collector/Scrapper</b>
		Mean	68.420	83.360	1.470	6.650	1.680	2.680	4.530	
		SD	27.000	13.630	4.110	8.160	2.030	2.810	9.670	
		Min-Max	0-100	20-100	0-30	0-98	0-15	0-20	0-70	
<i>S. spinosa nevadensis</i>	Poqueira	N	89	86	86	86	86	86	86	<b>Gatherer-collector/Scrapper</b>
		Mean	66.520	88.030	10.100	0.940	0.480	0.350	0.090	
		SD	20.410	19.310	19.150	1.920	1.500	0.860	0.630	
		Min-Max	10-100	3-100	0-95	0-10	0-10	0-5	0-5	
	Válor	N	382	366	366	366	366	366	366	<b>Gatherer-collector/Scrapper/Shredder</b>
		Mean	64.330	90.700	0.760	2.960	0.980	0.980	3.180	
		SD	24.280	14.790	2.340	3.950	2.170	1.960	9.280	
		Min-Max	0-100	0-100	0-20	0-20	0-25	0-15	0-90	
<i>A. muticus</i>	Poqueira	N	335	330	330	330	330	330	330	<b>Gatherer-collector/Scrapper</b>
		Mean	53.809	70.460	26.620	1.240	0.810	0.830	0.050	
		SD	17.524	24.110	24.250	1.970	2.800	2.010	0.480	
		Min-Max	0-100	0-100	0-100	0-15	0-40	0-15	0-5	
	Válor	N	906	881	881	881	881	881	881	<b>Gatherer-collector/Scrapper</b>
		Mean	55.010	88.230	6.910	2.950	0.950	0.870	0.040	
		SD	18.910	13.530	12.500	3.290	1.810	1.720	0.410	
		Min-Max	0-100	0-100	0-100	0-25	0-15	0-12	0-6	
<i>B. alpinus</i>	Poqueira	N	150	130	130	130	130	130	-	<b>Scrapper/Gatherer-collector</b>
		Mean	34.570	43.190	53.430	1.400	1.780	0.120	-	
		SD	21.170	38.080	37.550	7.330	8.820	0.580	-	
		Min-Max	0-80	0-100	0-100	0-80	0-80	0-5	-	
	Válor	N	402	339	339	339	339	339	339	<b>Gatherer-collector/Scrapper</b>
		Mean	34.380	71.720	24.810	1.990	1.020	0.440	0.020	
		SD	25.780	31.020	30.760	2.980	4.660	1.160	0.290	
		Min-Max	0-100	0-100	0-100	0-25	0-50	0-10	0-5	

Table 2.4. Nymphal gut contents of the studied Ephemeroptera species in the two sampling sites. Bold letter in FFG column (Functional Feeding Group) represents the most important functional group into which the species can be assigned.

### 2.3.5. *Amphinemura triangularis*

Due to the lack of small nymph collections along several months in the Poqueira stream and the low number of collected nymphs in general, we do not comment the life cycle in this site, although data appears in Figure 2.6. In the Válor stream, the cycle was univoltine, with nymphs present from September to May, when mature nymphs started appearing.

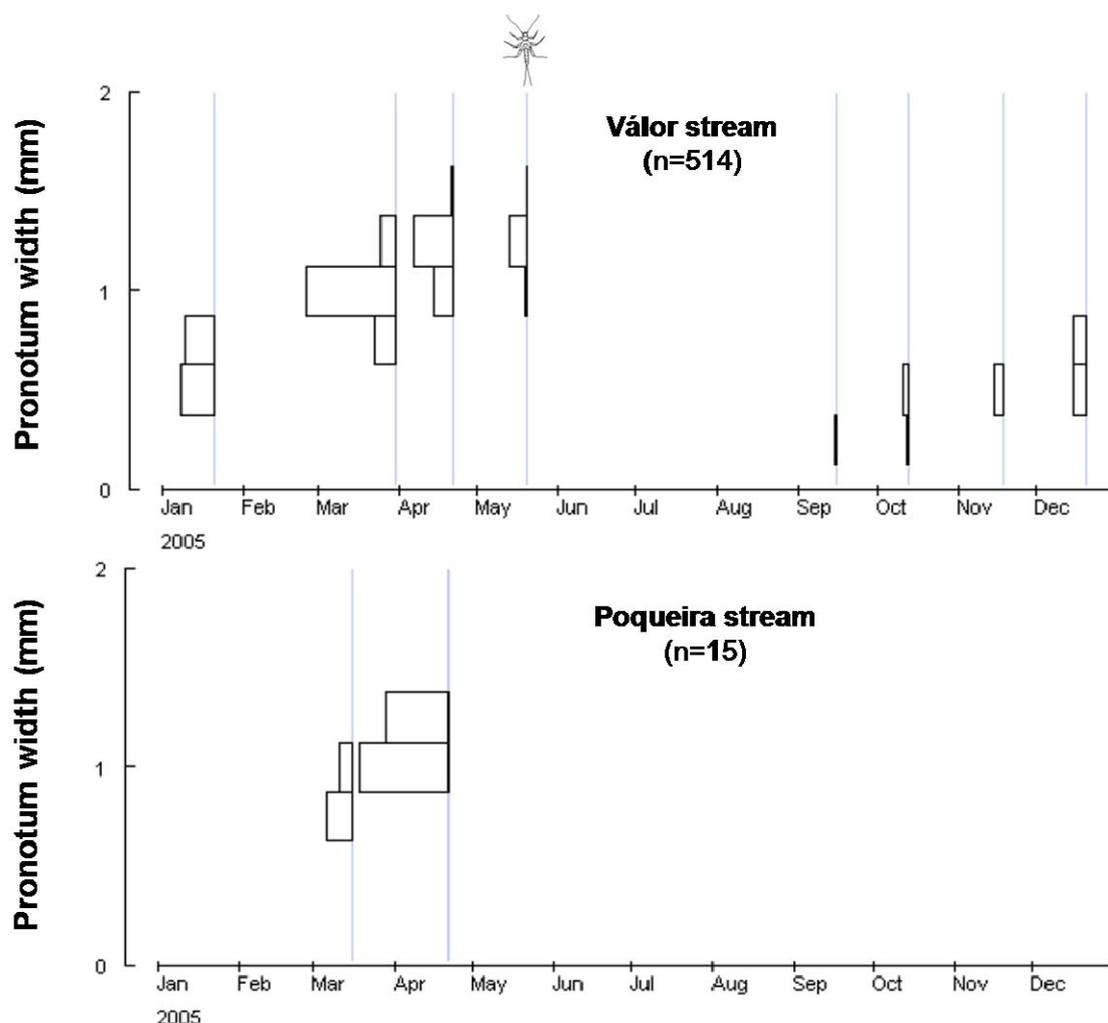


Figure 2.6. Life history of *A. triangularis* in the two sampling sites. Presence of mature nymphs is marked with a nymph draw.

When studying the growth of the species in relation with accumulated day-degrees in the Válor stream no patterns could be inferred from the results (Table 2.3). As can be seen, there were periods with high growth rate and low accumulated day-degrees and vice versa.

The diet of this species, both in Poqueira and Válor, was mainly composed by detritus, being the other components more scarcely found (Table 2.5). Therefore, this species could be grouped principally as gatherer-collector. Bigger nymphs in the Válor stream fed more on detritus than smaller ones (Table 2.6).

### 2.3.6. *Capnioneura mitis*

The cycle in the Poqueira stream is not commented due to the lack of information about the earlier stages and the low number of collected nymphs, as occurred for *Amphinemura triangularis*. In Válor this species had a univoltine life cycle, with nymphs present from September to April, although those appearing in March and April were very scarce (Figure 2.7). Mature nymphs were found in November and December.

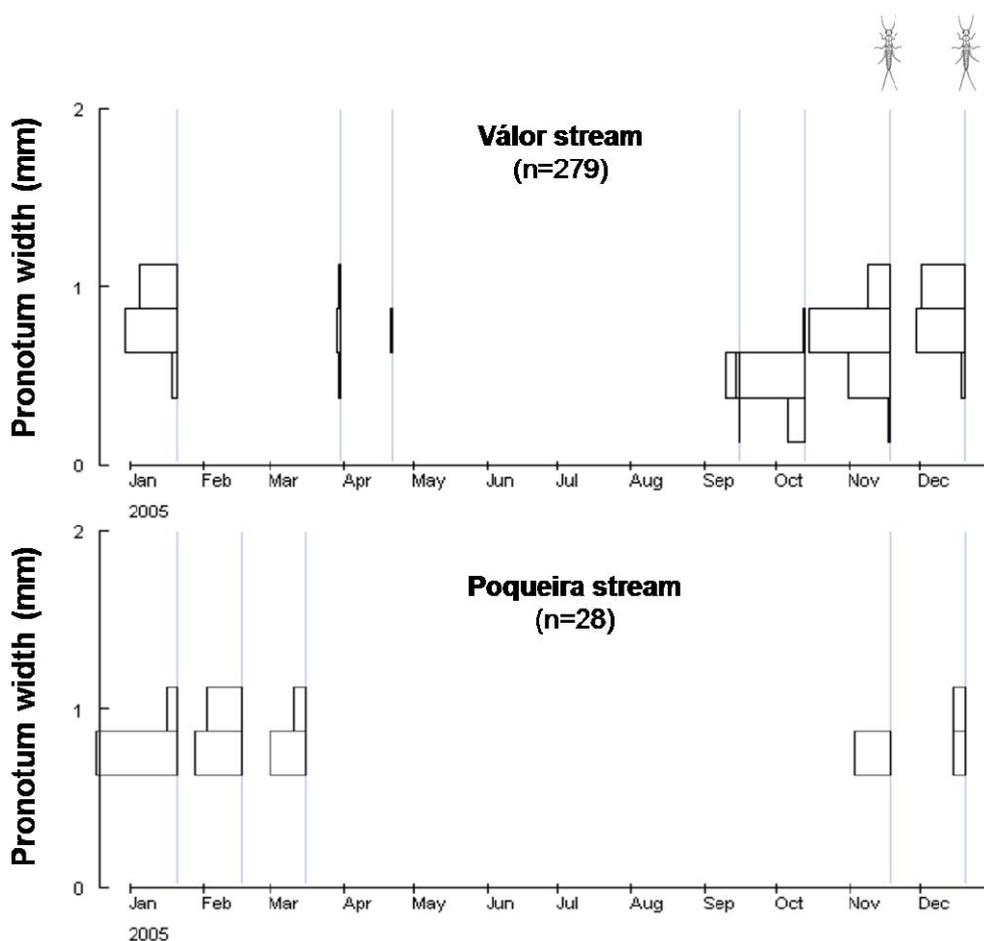


Figure 2.7. Life history of *C. mitis* in the sampling sites. Presence of mature nymphs is marked with a nymph draw.

The study of the growth of this species in Válor did not show any relationship with accumulated day-degrees (Table 2.3).

In *C. mitis*, the main component found in the gut was the detritus, but this came from fragmented leaves (Table 2.5). Thus, the species could be catalogued as shredder. Nevertheless, in the Poqueira stream, there were a high amount of epilithic and epiphytic diatoms in the gut, indicating that this species was also an important scraper in this site. In the Válor stream there was a decrease in detritus intake of bigger nymphs (Table 2.6).

Species	Stream		% absolute	% detritus	% diatoms	% hyphae	% fungi spores	% pollen	% phyllidia	FFG
<i>A. triangularis</i>	Poqueira	<b>N</b>	14	10	10	10	10	10	10	<b>Gatherer-collector/Scraper</b>
		<b>Mean</b>	43.570	90.500	5.800	0.800	0.200	0.200	2.500	
		<b>SD</b>	31.340	14.710	12.900	1.620	0.630	0.630	7.910	
		<b>Min-Max</b>	0-95	60-100	0-40	0-5	0-2	0-2	0-25	
	Válor	<b>N</b>	453	305	305	305	305	305	305	<b>Gatherer-collector/Scraper</b>
		<b>Mean</b>	41.610	94.630	0.830	2.350	1.110	0.280	0.810	
		<b>SD</b>	32.670	10.130	3.820	6.200	4.740	0.770	4.260	
		<b>Min-Max</b>	0-100	15-100	0-50	0-70	0-50	0-6	0-40	
<i>C. mitis</i>	Poqueira	<b>N</b>	28	16	16	16	16	16	-	<b>Shredder/Scraper</b>
		<b>Mean</b>	38.570	64.000	32.440	2.620	0.690	0.250	-	
		<b>SD</b>	37.340	34.800	35.450	3.140	1.700	0.580	-	
		<b>Min-Max</b>	0-100	8-100	0-90	0-10	0-5	0-2	-	
	Válor	<b>N</b>	250	185	185	185	185	185	185	<b>Shredder/Scraper</b>
		<b>Mean</b>	45.340	85.160	5.740	5.260	2.320	1.420	0.100	
		<b>SD</b>	34.080	16.330	10.740	10.500	7.050	2.480	0.820	
		<b>Min-Max</b>	0-100	0-100	0-80	0-100	0-80	0-20	0-10	

Table 2.5. Nymphal gut contents of the studied Plecoptera species in the two sampling sites. Bold letter in FFG column (Functional Feeding Group) represents the most important functional group into which the species can be assigned.

Species	Stream	% detritus	% diatoms	% hyphae	% fungi spores	% pollen	% phyllidia
<i>S. ignita</i>	Poqueira	-0.272*	0.243*	0.453*	0.449*	0.369*	0.672*
	Válor	-0.339*	-0.045	0.350*	0.052	0.300*	0.379*
<i>S. spinosa nevadensis</i>	Poqueira	0.328*	-0.391*	-0.178	0.135	-0.240	0.224
	Válor	-0.253*	0.092	0.185*	0.240*	0.296*	0.596*
<i>A. muticus</i>	Poqueira	-0.156*	0.122*	0.183*	0.244*	0.176*	0.369
	Válor	-0.034	-0.043	0.122*	0.115*	0.325*	0.198
<i>B. alpinus</i>	Poqueira	-0.154*	0.210*	-0.115	-0.048	-0.152	-
	Válor	-0.101*	0.067	0.151*	0.215*	0.232*	0.762*
<i>A. triangularis</i>	Poqueira	0.310	-0.130	0.043	0.556*	-0.556*	0.250
	Válor	0.098*	-0.168*	-0.138*	-0.177*	-0.119	0.530*
<i>C. mitis</i>	Poqueira	0.000	0.000	-0.450*	-0.706*	-0.437	-
	Válor	-0.245*	0.225*	0.180*	0.082	0.221*	0.400

Table 2.6. Correlations between nymphal size (total length for Ephemeroptera and pronotum width for Plecoptera) and diet components in the two study sites. Values marked with \* are significant at  $p < 0.05$ .

## 2.4. DISCUSSION

### 2.4.1. Life histories

#### 2.4.1.1. *Serratella ignita*

Due to the wide distribution of the species along the Palearctic region, many authors have studied it. Maitland (1965) describes in Scotland a life cycle for this species similar to the one we found: annual, with eggs lying dormant over winter, and hatching in April and May. Nymphs develop rapidly thereafter to emerge and lay the eggs in July and August. Hynes (1970) pointed out that eggs would wait for approximately 6 months to hatch. The nymphs would experiment a rapid growth during summer, with some of them remaining small several months after the flight period, as occurs in the Poqueira stream. In the Central Pyrenees, the cycle is more extended, with nymphs present throughout all the year, and adults flying from April to October (Lavandier and Dumas, 1971). In Southern England, nymphs are present in all months except October (Bass, 1976). Jazdzewska (1980) found in a population of a Polish stream a univoltine cycle, with nymphs

from the end of March to September, similar, but wider, to those found by us. In a study carried out in Central Wales, Cowx *et al.* (1984) found that *S. ignita* passed from being present in the stream from May to September in one year, to being present from April to August in the next, after a drought period. Zelinka (1984) describes the cycle as univoltine, with eggs passing through a winter diapause and hatching at the end of May. Nymphs grow very fast, until they emerge in July-August (with some subimagines collected in September). In Great Britain, the species is also univoltine, overwintering in the egg stage. Its growth is very fast, with about 85% of the nymphs developing in about three months (Elliott, 1967, 1978). Small nymphs are also found in October and November long after the flight period, although these nymphs probably do not survive the winter. In the warmer waters of Southern England, nymphs are found during the whole year, making possible the existence of two separate, winter and summer, generations. The main flight period is between April and September, although adults have been cited in January. In some populations, parthenogenetic eggs have been recorded (Elliott *et al.*, 1988), although their development is slower and they have less hatching success (Elliott and Humpesch, 1980). Deván (1993) also found a univoltine life cycle in two localities of Slovakia, with a development period of four months, from May to August, similar to that found in our study.

The studied cycle is similar to that drawn by Alba-Tercedor (1990a) also in the Poqueira stream, although in that survey nymphs were only present from the beginning of July to the end of August, with mature individuals found in August. In the same study, nymphs were collected in other streams with different temperature regimes, showing a more expanded cycle, but always univoltine, and with an emergence period going from late June to September. The author suggested the presence of a failed second generation in some studied streams [as previously was pointed out by Pleskot (1959), Hynes (1961), Elliott (1967, 1978), Wise (1980) and Rosillon (1986)]. Nevertheless, this second generation was not present in the Poqueira stream, as it is not in our study. Under other environmental conditions in Northern Iberian Peninsula, the cycle lasts eight, nine or ten months, with first nymphs appearing in autumn and emergence occurring in spring and summer (Riaño *et al.*, 1997; González *et al.*, 2000).

In our study area we found a single generation with several cohorts. Small nymphs would hatch in spring. Humpesch (1984) found in *S. ignita* that there are

intraspecific differences in the temperature range within which eggs hatch, and that the duration of hatching decreases with increasing temperature, that would explain the short hatching period found in our survey, and the almost coincidence of it in the studied streams, due to in both cases coincided with the rising of temperatures.

Hatching was quite synchronous, despite the presence of some nymphs in autumn and winter, mainly in the Poqueira stream. These nymphs would not survive the winter, as pointed out by several authors (see Elliott *et al.*, 1988; Alba-Tercedor, 1990a). In our study area, hatching started before in the Válor, the stream with a lower average temperature (Table 2.2).

Growth would take place rapidly during the spring and summer, and the flight period would occur in July and August in Poqueira and Válor respectively. The species would spend the rest of the year in the egg stage, probably passing through a diapause period, as pointed out by Sowa (1975) and Böhle (1972). The latter suggested that diapause was obligatory in Germany, but not in other European populations. Nevertheless, Elliott (1978), in an experimental study, did not find a diapause in a population of the English Lake District, so more studies should be carried out in order to clarify what happened during this period.

According to Hynes (1970), its cycle could be classified as “fast seasonal”, given that growth was very fast during the few months that the nymphs were present in the streams. Following Landa’s (1968) classification, it would fit within the “A2” category mentioned for some populations of the same species in Central Europe by the author, i.e., species in which, after oviposition, the eggs remain in diapause until the spring or summer of the next year. It could be also classified under the “B1” category of Sowa (1975), in which are grouped those species with a long egg development period and diapause occurring during it.

#### 2.4.1.2. *Serratella spinosa nevadensis*

In several streams of Sierra Nevada (the Poqueira stream included), Alba-Tercedor (1990b) found a more expanded cycle, going from October to the end of June, and with an increase in growth during spring and summer. This coincides with the period of growth of the population in the Poqueira stream in our study.

Thus, hatching would occur within a few months, indicating an approximately synchronous hatching period. Mature nymphs appeared before in the Válor stream than in the Poqueira. In the first the flight period seemed to be longer, going from June to September. Oviposition took place in those months. The species would pass the rest of the year probably in a diapause stage, as pointed out by Alba-Tercedor (1990b).

This was also a “fast seasonal” cycle (Hynes, 1970), and would fulfil the characteristics of the “A2” class pointed out by Landa (1968) for Central European species, and the “B1” mentioned by Sowa (1975).

As can be observed from the present results, *S. ignita* and *S. spinosa nevadensis* cycles almost do not overlap, i.e., nymphs of the same size belonging to different species are not found at the same time. This has been also found within populations of *S. ignita* and *Ephemerella mucronata* (Bengtsson, 1909) of Central Europe (Sowa, 1975). Such differences in the life history, as egg biology or timing of hatching, may be a cue factor for avoiding resource competition (Elliott, 1995; Giller and Malmqvist, 1998).

#### 2.4.1.3. *Alainites muticus*

There are several studies focused on this species along Europe. Ulfstrand (1968) studied it in North Sweden and found a univoltine cycle, overwintering in the egg stage. In the French Pyrenees, the species presents three generations that overlap (Thibault, 1971). Elliott *et al.* (1988) recorded a bivoltine life cycle in the British Isles, with a winter generation and a fast-growing summer generation, coinciding with that found in our study in the Válor population. In the North western of Slovakia, Deván (1992) found a univoltine life cycle, from September to August, with a long flight period, going from the end of April to August. More recently, in a study carried out in a Prealpine stream of the Italian Alps, Erba *et al.* (2003) found a univoltine life cycle, with a probable egg diapause from June to October or even December. It is likely that all these differences reflect the differences in the conditions of the streams where the studies were carried out.

Alba-Tercedor (1986) pointed out, from data coming from several streams in the Sierra Nevada range (where the Poqueira stream was included), a three generations cycle for the species: the first going from October to February-March,

the second from March to April-May, and the third from May-June to July-August. Nevertheless, in the Poqueira stream, it had two generations (Alba-Tercedor, 1986: Figure 8) as we found in this study. There were small nymphs from the beginning of October to March, when they grew. Mature nymphs appeared from the beginning of June to the end of August, coinciding with the beginning of the emergence in our study.

In the Poqueira stream we could differentiate a single generation, probably hatching in late summer and beginning of autumn (September or October), growing during winter and spring, and emerging around May. Nymphs were also present in other months, but in a lower quantity, probably representing intrapopulation differences in the hatching time or slow growth of the nymphs of first instars. This could be also interpreted as a second generation that would have not success. In the Válor stream we could distinguish two generations: the first would start hatching in September-October. Nymphs would grow during the winter, and emergence would take place around May. Eggs would be laid in this month and hatched nymphs would grow very fast in summer, coinciding with a high temperature period. This second generation emerged from July to September. Mature nymphs of this generation were smaller than those belonging to the first, due to their rapid and short growth period (mean mature nymph size for the first generation =  $6.96 \pm 0.44$  mm, maximum =  $7.56$  mm; mean mature nymph size for the second generation =  $5.46 \pm 0.71$  mm, maximum =  $6.28$  mm), supporting a bivoltine life cycle.

The species presented a “fast-seasonal” cycle (F1 type) in the Poqueira stream, according to Hynes (1970), owing to the rapid development of the nymphs, which hatch in autumn and mature in spring. In the Válor stream, the cycle was “slow-seasonal” for the winter generation and “fast-seasonal” for the summer generation. Following Landa’s (1968) classification, this species belongs partially to the “A1” class (as recorded by the author) in the Poqueira stream (i.e. the eggs hatch approximately one month after oviposition and nymphs grow until emergence occurs in spring or summer, depending on the altitude), and to the “B1” class in the Válor stream (two generations in the year, one long and one fast growing). In Sowa’s (1975) classification, the species in the Poqueira stream did not fall within any clear category. In the Válor stream it would belong to the “C2” category (species with two generations, one going from the end of summer to

spring, and another developing very fast in summer), as pointed out by the author for the Central European populations.

#### 2.4.1.4. *Baetis alpinus*

In Europe it is possible to find a wide diversity in the life histories of this species. Thus, Lavandier (1988) found that it had a univoltine cycle at 1920 m a.s.l. and a semivoltine at 2190 m a.s.l., and higher mature nymphs in the latter populations. In Poland, the life cycle shows also a marked plasticity, being bivoltine at low altitudes and univoltine or bivoltine at higher ones (Kukula, 1997). A study carried out along a glacial stream of the Austrian Alps showed that there was a different size distribution at the snow-covered and the snow-free sites. Under the snow, development was slower. Nymphs were found throughout the year, but the highest densities were recorded at the open site in winter (Schütz *et al.*, 2001). In the Italian Alps, *B. alpinus* has a trivoltine or bivoltine life cycle depending on environmental conditions, such as probably the temperature or the hydrological instability (Erba *et al.*, 2003).

Alba-Tercedor (1986) described three generations in one year for the same species in the Poqueira stream: the first going from February to May, the second from August to October and the third from November to January, approximately.

We found first stage nymphs during nearly every month, indicating an asynchronous hatching and/or slow growth of the smallest nymphs, and probably several cohorts, as pointed out by Humpesch (1979). Mature nymphs started appearing in February in the Poqueira stream, and in January in the Válor stream, probably showing an advance of the flight period in Válor. Thus, in the Poqueira stream we could distinguish two generations. The first would grow slowly throughout the autumn (from September to December, approximately) and faster in January. It would emerge in February, and oviposition would take place within a few weeks. The eggs would hatch almost immediately, originating the second generation, which would grow until April-May, when emergence would occur. These two generations were, as occurred in the case of *A. muticus* in the Válor stream, different in mature nymph size (mean mature nymph size for the first generation =  $7.57 \pm 0.64$  mm, maximum = 8.22 mm; mean mature nymph size

for the second generation = $6.83 \pm 0.94$  mm, maximum =7.50 mm). In the Válor stream, due to the absence of data in February, we could have two possibilities: 1) a univoltine cycle, with one generation hatching at the end of summer and growing until January, when emergence started and lasted until April, or 2) a bivoltine cycle, with the first generation going from the end of summer to January, and a second fast growing generation, with eggs laid on January that would hatch and grow very fast during winter, with emergence taking place in March and April. The first hypothesis is supported by the fact that mature nymphs of the supposed first generation were not higher than those of the second generation (mean mature nymph size for the first generation = $7.58 \pm 0.68$  mm, maximum =8.06 mm; mean mature nymph size for the second generation = $8.48 \pm 1.06$  mm, maximum =9.56 mm). Furthermore, the months in which the second generation would develop are those in which the minimum temperatures of all the year are recorded (Figure 2.1). In addition, in the literature, we find references to the change of a bivoltine cycle to a univoltine one when we go up in altitude (e.g., Landa, 1968; Sowa, 1975), that would support our findings in Sierra Nevada.

If we compare the cycle in Alba-Tercedor (1986) and that found in this study we can observe a reduction of one generation after more than two decades, which could be the reflection of changes in the environmental conditions, mainly in temperature, but due to the low number of temperature records in Alba-Tercedor's study, no reliable conclusions can be drawn.

We can classify this cycle in the Poqueira stream as "slow-seasonal" for the winter generation and "fast-seasonal" for the summer generation, and "fast-seasonal" (F1 type) in the Válor stream (Hynes, 1970). Following Landa's (1968) classification, this species belongs either to the "A1" class or to the "B1" class, depending on the altitude. Sowa (1975) classifies this species into the group "C2", coinciding with the type of cycle of the Poqueira stream. In Válor this species would not fit into any particular category.

#### 2.4.1.5. *Amphinemura triangularis*

No small nymphs were caught in the Poqueira stream. This site suffered a great input of sludge since September that made possible the existence of migratory movements of the smaller nymphs to other parts of the stream or other

mesohabitats in order to avoid this unfavourable situation, as pointed out in some studies (e.g., Chutter, 1969). These changes in substrate may act selectively on individual species and on individual life history stages (Lehmkuhl, 1979), and would be responsible of this lack of information.

The only study of the life history of this species in the rest of Europe is that of Raušer (1963) in the former Czechoslovakia, where the cycle was more extended, going from May to April, when the flight period began.

This species was also studied in the Poqueira stream by Sánchez-Ortega (1986), who found a univoltine life cycle with small nymphs in November and growing until June. This author supposed that the hatching period started at the end of the spring or at the beginning of summer, and lasted until the end of autumn. Nevertheless, nymphs of first stages were not collected in that study. In Sierra de Huétor, a mountain range next to Sierra Nevada, the cycle of the species lasted seven months, from October to April, with a higher growth period from February to April (López-Rodríguez and Tierno de Figueroa, 2004).

In the Válor stream we could clearly differentiate a single cohort, with a very synchronous hatching time, taking approximately two months. Mature nymphs began emerging in May, coinciding with the flight period recorded in the same river by Tierno de Figueroa *et al.* (2001).

As can be seen, in the different studies mature nymphs are always found in the spring months (from April to June). Thus, differences in the life cycle are mainly related with the time the eggs spend for hatching. The population studied by Raušer (1963) seems to hatch immediately after being laid, while the populations from Southern Iberian Peninsula remain in the riverbed during the summer months. It could be possible that during this period the species passed through a diapause (in order to avoid high temperatures).

Following the classification of Hynes (1970), this life cycle could be classified as “fast-seasonal”, although it was not clear that a diapause during the egg stage occurred.

#### 2.4.1.6. *Capnioneura mitis*

Sánchez-Ortega and Alba-Tercedor (1990) found in other streams from Sierra Nevada mountains a univoltine cycle, going from October, when the first

nymphs appear (although these authors pointed out that these nymphs did not belong to the first instars), to March. This would coincide with our data, but we did find nymphs of first instars in October (including some nymphs with no completely developed compound eyes). It is also pointed out that the species present an egg diapause [as also recorded by several authors for other species of the same genus (Lavandier, 1975; Navarro-Martínez *et al.*, 2007)]. This is likely in the population studied by us, and would allow the species to avoid the high temperatures of the summer.

In the Poqueira stream, nymphs belonging to the first instars were not caught for the same reasons mentioned for *A. triangularis*, so no actual pattern can be derived from these data. In Válor the cycle is univoltine. Nymphs of the first instars were found from September to November, but mainly in October, indicating a relatively synchronous hatching. Some nymphs remained in the stream until March-April, probably representing the nymphs that came from the eggs that hatched last. Mature nymphs appeared in November, coinciding with the beginning of the flight period described by Tierno de Figueroa *et al.* (2001) in this site. The occurrence of mature nymphs already in autumn indicated a very fast growth, completing their development in only three to four months.

This cycle can be catalogued as “fast-seasonal” according to Hynes (1970).

#### 2.4.2. Growth patterns and their relationships with temperature

Only for *S. ignita* and *S. spinosa nevadensis*, both in the Poqueira stream, there is a clear positive relation between growth and accumulated day-degrees. This relationship between growth and temperature has been also pointed out by different authors (e.g. Alba-Tercedor, 1981; Brittain, 1982; Huryn, 1996; Fenoglio *et al.*, 2005). In all the other cases this relation does not appear, maybe due to the influence of some other natural factors on growth. Furthermore, in the period in which the Válor stream reached nearly 0 °C (February-March), *A. triangularis* experienced a high growth rate. In fact, Illies (1952a) indicated that, in this genus, the temperature does not influence the nymphal growth, and that the emergence takes place in a constant manner. In general, this author pointed out that growth in Ephemeroptera is stopped by cold weather, while the developmental

temperature threshold of Plecoptera is very low, even near 0 °C (Raušer, 1962). This was also signed in Central Norway for some species of Ephemeroptera, which passed a great part of their nymphal development period under the ice (Arnekleiv, 1996). In the case of *C. mitis*, which feeds mainly on leaf remains, there is an important growth period coinciding with the autumn, probably due to the input of good quality food in the stream.

#### 2.4.3. Feeding behaviour

Detritus was the main component in all studied species except in *B. alpinus* in the Poqueira stream, where the epilithic and epiphytic diatoms were the principal component, agreeing with the scraper-gatherer feeding behaviour found by Galas and Dumnicka (2003) for this species. The general dominance of detritus instead of diatoms in all the other species does not agree with the pattern found in an experimental study carried out by Rosillon (1988), who observed that starved nymphs of *S. ignita* prefer a diatom-rich diet rather than a detritus diet, and that at a given temperature, growth rate was significantly higher on the diatom diet than on the detritus diet.

Moss phyllidia were particularly important in some cases, such as in *S. ignita* and *S. spinosa nevadensis*, both in Válor stream, in relation with the percentage found in other species and/or places. This could be related to the high density of moss present in the Válor stream and in the particular mesohabitats where the animals were caught. From studies on several Ephemerelellidae species, some authors have pointed out that the consumption of high quantities of mosses would have some kind of benefit but without specifying which one (Hawkins, 1985).

Some individuals of each species, except *A. triangularis*, had some punctual animal remains in their guts (as pointed for *S. ignita* by Riaño *et al.*, 1997), probably representing accidental ingestion when feeding on other sources. Nevertheless, these items did not represent a significant part of the diet in any case.

From a functional point of view, most species have an important role as gatherer-collectors, and some of them as scrapers too. Only *C. mitis* is mainly shredder (in the sense of Palmer and O'Keefe, 1992), although in the Poqueira

stream it acts as scraper in a lower concern. In other studies, Elliott *et al.* (1988) classified to *A. muticus* as scraper-gatherer and to *S. ignita* as gatherer-collector, similar to that found by Murphy and Giller (2000).

The feeding behaviour of both *Serratella* species was quite similar. Thus, they have not coincident life cycles, but a displacement of one cycle with respect to the other, avoiding in this way a resource competition between the two species. The existence of temporal segregation between close related species has been associated to a mechanism of resource competition avoidance in many aquatic insect groups (Illies, 1952b; Danks, 1979; Butler, 1984). We will treat this topic more deeply with two other species in chapter five.

When analyzing the possible correlation between nymphal size and percentage of each component found in the guts, it was observed that the general trend is to feed less in detritus, and to incorporate another kind of components when nymphs grow, except for *S. spinosa nevadensis* in the Poqueira stream and *A. triangularis* in the Válor stream, where there is a tendency to increase the detritus percentage (although in the case of *A. triangularis* the correlation was extremely low). Most nymphs that feed on moss phyllidia do it when they are bigger. In fact, as Arnekleiv (1996) pointed out, small changes in feeding may result in considerable changes of mesohabitats, allowing different habitats opportunities to the nymphs of different sizes.

We found differences by comparing the components of the diet of species in both studied streams. It may reflect differences in availability of a given resource. This agrees with Coffman *et al.* (1971), who suggested that availability of food is the dominant factor influencing composition of the diet.

## 2.5. CONCLUSIONS

There is a great plasticity in the species life histories and strategies for coping with the environmental conditions. This reflects the intrinsic factors that also play an important role on their success, together with the extrinsic factors, such as temperature, photoperiod, nutrition, etc. (Giller and Malmqvist, 1998). Temperature affects the growth rate of some species more than others, as occurs with *S. ignita* and *S. spinosa nevadensis* in the Poqueira stream. For mayflies,

the environmental factors, such as temperature, seems to be more determinant than for stoneflies, in which the life history patterns are more stable. We may conclude this by observing the life histories found by some authors (also in Sierra Nevada or in other mountain ranges) and those found by us in two streams of the same mountains, with different temperature conditions. As shown, some species of mayflies (e.g. *A. muticus* and *B. alpinus*) suffered great ontogenetic changes (passing from univoltine to bivoltine, and some of them having several cohorts in a very short period) depending on the stream (and consequently on the temperature). In the stoneflies this comparison was not possible due to the lack of data from the Poqueira stream. However, by comparison with others studies, it is clear that temperature does not affect them changing the number of generations, but just advancing, delaying or displacing the cycle some months. For mayflies in general, if we take into account the time, and we compare with studies carried out in the same stream several years ago, we realize that this plasticity keep on being higher. This high plasticity in the life history of the species is one of the more important factors that preadapt them to different environments, and allow them to be widespread in the Palearctic region, as they are indeed. Thus, once again, the use of general patterns at species or higher taxonomical levels result inadequate. It is not appropriate to use the information coming from a given population to assume that the species as a whole behaves in the same manner due to differences in the environmental conditions determine their life histories.

In relation to the feeding behaviour, no general pattern (at genus or family level) can be used to catalogue a given species in a particular site. Furthermore, as we have seen, several changes in the diet may occur in the same population, so generalization such as their grouping in functional feeding groups (FFG), even for a single species, must be taken with caution. One of the major inconvenient of the classification following FFG's is that spatial, developmental and temporal dietary variability makes very difficult the actual sorting of macroinvertebrate taxa into FFG's (King *et al.*, 1988; Mihuc, 1997), as has been also shown in the present study.

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**CHAPTER 3. Life strategies of three Perlodidae species  
(Insecta: Plecoptera) in a Mediterranean seasonal stream of  
Southern Iberian Peninsula**





**ABSTRACT**

Some aspects of the nymphal biology and ecology of three Perlodidae species [*Guadalgenus franzi* (Aubert, 1963), *Hemimelaena flaviventris* (Pictet, 1842) and *Isoperla curtata* Navás, 1924] are studied in a seasonal stream in Southern Iberian Peninsula. The different strategies that they present are greatly conditioned by the particular characteristics of the environment that they inhabit, i.e., aestival drought and relatively warm temperatures, although differences among them are detected. The cycle of *G. franzi* is semivoltine, probably with a nymphal quiescence when the stream is dry. On the other hand, *H. flaviventris* and *I. curtata* exhibit relatively short univoltine life cycles, overlapping but slightly advanced in the latter. Both pass the dry period in the egg stage, probably with a diapause phase. Growth rate is maximum previous to emergence for the univoltine species, and just previously and after the drought for *G. franzi*. The three species show a relatively short flight period compared with other species from seasonal streams. Differences in feeding habits are detected among them: *G. franzi* and *I. curtata* are mainly scrappers, feeding on diatoms, while *H. flaviventris* is mainly predator. Some changes are detected in diet in relation with size, and different prey electivity patterns are noted for the different species, although they mainly prefer Chironomidae, principally when smaller in the case of *G. franzi*. Secondary production is high for all the species in comparison with other stonefly species from both temporary and permanent waters. In fact, these three Plecoptera species have a relatively high biological success in this habitat, constraining for other aquatic insects.

**KEYWORDS:** Stoneflies, life history, secondary production, feeding, temporary stream, Southern Spain.

### 3.1. INTRODUCTION

Stoneflies are aquatic insects typically rheophyls and cold stenotherms, i.e., they are found on clean, fast-flowing waters with relatively low temperatures and high dissolved oxygen content (Fochetti and Tierno de Figueroa, 2008). Temporary waters are, *a priori*, unsuitable habitats for them due to the adverse conditions that they present. Nevertheless, stoneflies face with and adapt to this constraining environment, more similar during the drought period to that found on terrestrial habitats than that of the typical streams and rivers (Williams, 2006). They do this in several ways: they can avoid drought in the adult stage by migration, reaching permanent waters in the immature stages, burrowing deep into the substrate reaching the hyporheic zone, modifying their life history in order to fit the immature development within the wet period, and/or developing a dormancy stage (Boulton *et al.*, 1998; Williams, 2006). During the wet period the main factors that control growth are temperature and food supplies (Hynes, 1970; Cummins and Klug, 1979). Each species has its own temperature optimum of development, and this contribute to the characteristic succession of species in the community. Regarding feeding, typical faunas of temporary waters seem to be opportunistic/generalistic, although comprehensive dietary studies are rare (Williams, 2006). This kind of information together with food-web interactions, when combined with data about secondary production, gives an improved understanding of the structure and function of both communities and ecosystems (Huryn and Wallace, 2000). Moreover, secondary production is the most comprehensive measure of success for a population because it is a composite of several other components of success: density, biomass, individual growth rate, reproduction, survivorship, and development time (Benke, 1993). Thus, in seasonal streams such as that studied by us, it is an important clue of the fitness of the species to the environment.

The aim of this work is to analyze, in the context of a seasonal stream of Southern Iberian Peninsula, the life history, feeding behavior and secondary production patterns of three Plecoptera species typical of temporary waters belonging to the family Perlodidae: *Guadalgenus franzi* (Aubert, 1963), an endemism of the Iberian Peninsula, *Hemimelaena flaviventris* (Pictet, 1842), a species distributed by the Iberian Peninsula and Northern Africa, and *Isoperla*

*curtata* Navás, 1924, also endemic of the Iberian Peninsula (Tierno de Figueroa *et al.*, 2003). We integrate all the information mentioned and discuss the different strategies that they present and their adaptations to this particular environment, comparing our data with the previously available information. Due to their restricted distribution, few authors have studied these species. Thus, we also compare our results with other studies of similar species of Perlodidae in seasonal streams, and punctually in permanent ones.

### 3.2. MATERIALS AND METHODS

We collected samples monthly, from November 2006 to June 2007, in Río Despeñaperros (Sierra Morena, Jaén, Spain; UTM: 30SVH558476, 560 m a.s.l.), a Mediterranean type seasonal stream, during the period in which it carried water. During the summer and part of the autumn, the stream was completely dry, and no pool remained. In mid April, due to an extreme event of raining, the stream suffered a flood, so this sample had to be taken afterwards in April. In June, previous to the drought of the stream, we collected samples biweekly. We visited the stream regularly prior to the arrival of the water in order to detect the exact moment when this happened. Furthermore, for recording the hourly temperature of the water, we placed a datalogger in the riverbed (HOBO<sup>®</sup> Water Temp Pro, 0.001 °C accuracy). From its records we know that the water arrived at the end of October (Figure 3.1).

We recorded *in situ* physical parameters, such as dissolved oxygen, conductivity and discharge, every sampling campaign, and we collected one liter of water that was cold preserved and transported to the laboratory in order to have a physicochemical characterization of the studied site (Table 3.1). During the sampling period, the width of the stream varied from 2.95 to 5.35 m, and the depth ranged from 0.04 to 0.31 m, except in the flood event, when it was not possible to measure them. The substrate was mainly composed by approximately 85% of block and stones, 10% of gravels and 5% of sands and silt. There were some branches and trunks on the riverbed. During spring and summer there were abundant Ranunculaceae and *Nasturtium* sp. Mosses were absent in the sampling site. The riparian vegetation was abundant at both sides of the stream,

mainly represented by *Nerium oleander*, *Fraxinus* sp., *Berberis* sp., Poaceae, Umbelliferae and Compositae.

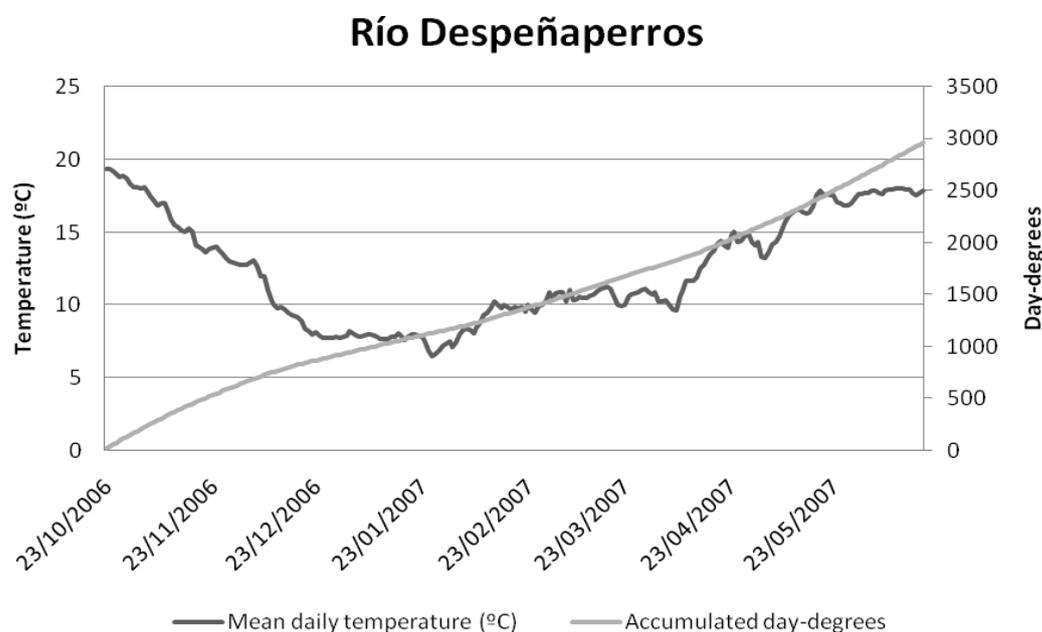


Figure 3.1. Mean daily temperature and accumulated day-degrees during the wet period.

	N	Mean	S.D.	Min.	Max.
pH	9	8.24	0.34	7.61	8.79
Ammonium (mg/l)	9	0.65	1.84	0.00	5.55
Phosphates (mg/l)	9	0.01	0.01	0.00	0.03
Nitrates (mg/l)	9	0.03	0.04	0.00	0.11
Nitrites (mg/l)	9	0.04	0.05	0.00	0.13
Sulfates (mg/l)	9	22.55	7.26	10.14	33.24
Chlorides (mg/l)	9	49.10	8.70	36.40	68.25
Alkalinity (meq/l)	9	39.85	7.86	24.64	50.02
Ss (mg/l)	9	4.02	4.17	0.60	12.60
Ca (mg/l)	9	65.96	12.80	50.40	88.00
Mg (mg/l)	9	29.70	7.77	20.90	40.34
Hardness (mg CaCO <sub>3</sub> /l)	9	286.94	54.13	213.87	379.77
Turbidity (NTU)	9	1.33	0.87	0.40	3.20
O <sub>2</sub> (% sat)	9	69.11	23.75	13.00	92.00
O <sub>2</sub> (mg/l)	9	7.03	2.73	1.10	9.70
Temperature (°C)	5908	12.54	3.86	5.90	25.87
Conductivity (µS/cm)	9	454.89	69.43	359.00	553.00
Discharge (m <sup>3</sup> /s)	9	0.10	0.11	0.00	0.32

Table 3.1. Physicochemical parameters of the sampling site.

The total macroinvertebrate community was collected with a Surber sampler (0.09 m<sup>2</sup> area and 250 µm mesh size). We took six replicates including the different mesohabitats of the sampling station. Animals were preserved in 4% formalin and brought to the laboratory, where they were sieved with a 150 µm mesh size in order to remove the excess of formalin and fine detritus. The stoneflies were sorted out and identified at species level. The rest of the animal community was also sorted out and identified at family level, except for Ostracoda, Hydracarina, Nematomorpha, Nematoda and Copepoda (Table 3.2).

During the sampling campaign, adults of the three species were collected beating the vegetation with an entomological net and picking them directly from the stones. These individuals were preserved in 70% ethanol for establishing the flight period together with the information obtained from collection of mature nymphs.

To study the life cycle, we first measured total length and pronotum width of 30 nymphs using the micrometer of a binocular microscope. Measures were standardized by putting every individual between two slides. Due to these two measures were highly correlated (Gamma correlation higher than 0.77 for every case,  $p < 0.05$ ), we used total length. Every collected nymphs were distributed in 1 mm length size classes. The representation of the life cycle by mean of size-frequency graphs was made by using FiSAT II software (Gayanilo *et al.*, 2002). We calculated mean growth among two sampling dates as the mean length of the species in one month minus the mean length of the species in the prior month.

Due to the nymphs of *I. curtata* were unknown, they were previously described. The description appears as an Appendix of this chapter.

Secondary production was evaluated using the size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke, 1979; Benke and Huryn, 2006), due to there were nymphs of different size classes inhabiting at the same time. Estimation of nymphal biomass was made according to the equation:

$$DW = a X^b$$

or, in natural logarithmic form:

$$\ln DW = \ln a + b \ln X$$

Taxon	16/11/06	15/12/06	17/01/07	12/02/07	12/03/07	26/04/07	17/05/07	04/06/07	18/06/07
Libellulidae	0.00	0.00	0.00	1.85	0.00	0.00	0.00	0.00	1.85
Gerridae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.22
Veliidae	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Notonectidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	7.41
Glossosomatidae	0.00	1.85	1.85	0.00	0.00	0.00	0.00	0.00	0.00
Hydrophilidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.56	1.85
Hydropsychidae	0.00	3.70	9.26	7.41	1.85	0.00	3.70	40.74	0.00
Limnephilidae	0.00	0.00	0.00	7.41	1.85	0.00	0.00	0.00	0.00
Psychomyiidae	0.00	1.85	0.00	0.00	0.00	0.00	0.00	0.00	1.85
Rhyacophilidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.85	0.00
Dytiscidae (larvae)	33.33	0.00	5.56	3.70	0.00	0.00	3.70	7.41	1.85
Dytiscidae (adult)	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Elmidae (larvae)	85.19	122.22	94.44	27.78	1.85	24.07	24.07	50.00	12.96
Elmidae (adult)	9.26	5.56	11.11	16.67	35.19	12.96	9.26	0.00	1.85
Halplidae (larvae)	0.00	3.70	5.56	1.85	0.00	0.00	0.00	0.00	12.96
Hydraenidae (adult)	1.85	0.00	0.00	1.85	0.00	0.00	0.00	24.07	0.00
Scirtidae (adult)	9.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anthomyidae (larvae)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.41	0.00
Anthomyidae (pupae)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	3.70
Athericidae	0.00	7.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ceratopogonidae	0.00	0.00	1.85	0.00	0.00	0.00	5.56	11.11	0.00
Chironomidae (pupae)	1.85	1.85	0.00	3.70	0.00	1.85	7.41	170.37	142.59
Dixidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.70	0.00
Limoniidae	3.70	1.85	20.37	55.56	44.44	59.26	16.67	20.37	0.00
Simuliidae (pupae)	1.85	0.00	0.00	0.00	0.00	0.00	0.00	3.70	0.00
Tabanidae	20.37	29.63	22.22	18.52	14.81	14.81	33.33	27.78	3.70
Tipulidae	0.00	3.70	0.00	0.00	0.00	1.85	1.85	1.85	16.67
Dugesiiidae	42.59	142.59	46.30	53.70	9.26	22.22	64.81	116.67	5.56
Haplotaenidae	0.00	0.00	0.00	0.00	0.00	0.00	1.85	0.00	0.00
Glossiphoniidae	12.96	25.93	7.41	18.52	3.70	7.41	5.56	11.11	9.26
Hydrobiidae	0.00	0.00	0.00	0.00	0.00	1.85	0.00	0.00	0.00
Neritidae	0.00	0.00	0.00	0.00	0.00	1.85	0.00	0.00	0.00
Planorbidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.41
Gammaridae	0.00	0.00	0.00	1.85	0.00	0.00	0.00	1.85	0.00
Ostracoda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hydracarina	0.00	0.00	3.70	0.00	0.00	0.00	0.00	3.70	0.00
Nematomorpha	0.00	0.00	0.00	0.00	0.00	1.85	0.00	0.00	0.00
Chironomidae (larvae)	418.52	50.00	68.52	188.89	42.59	20.37	455.56	1846.30	2642.59
Simuliidae (larvae)	661.11	16.67	20.37	12.96	0.00	0.00	972.22	5953.70	0.00
Ephemeroptera	0.00	7.41	31.48	38.89	83.33	140.74	1000.00	4100.00	227.78
Plecoptera	972.22	461.11	396.30	416.67	292.59	366.67	62.96	66.67	0.00
Ancylidae	338.89	307.41	151.85	303.70	151.85	227.78	272.22	164.81	0.00
Lumbriculidae	151.85	220.37	81.48	75.93	103.70	162.96	677.78	303.70	259.26
Nematoda	0.00	0.00	0.00	0.00	0.00	3.70	0.00	0.00	0.00

Table 3.2. Densities (ind·m<sup>-2</sup>) of the different taxa collected in the different sampling dates.

where  $DW$  = individual dry weight,  $X$  = total length,  $a$  = constant, and  $b$  = slope of the regression.

For constructing the regression line, formalin preserved specimens were measured, dried at 60 °C for 24 hours and placed in a desiccator during 1 hour. After this, they were weighed to the nearest 0.000 mg using a Mettler mod. M3 microbalance. For *G. franzi* and *H. flaviventris* we used 30 individuals, and for *I. curtata* we used 29.

In the diet study, we used the same individuals employed for correlating pronotum width and total length. The smallest nymphs were analyzed following the methodology proposed by Bello and Cabrera (1999), as in other studies of aquatic insect nymphal feeding (e.g. Tierno de Figueroa *et al.*, 2006; López-Rodríguez and Tierno de Figueroa, 2006; Navarro *et al.*, 2007; Bo *et al.*, 2008; Fenoglio *et al.*, 2008). Each individual was introduced in a vial with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. After this, they were mounted on slides for its study under the microscope. Larger nymphs were dissected through the ventral right side of the thorax in order to remove the gut. The food was spread out on a slide for removing the prey, if present, and the rest contents were mounted in Hertwigs' liquid. In both cases, the percentage of the absolute gut content (at 40x), as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400x), as the area occupied for each component within the total gut content, were estimated using the microscope with an ocular micrometer. Mean, standard deviation, minimum and maximum, presence (i.e., number of individuals which gut contained a given item) and percentage of presence (i.e., proportion of individuals in which a given item is found with respect to the total number of studied individuals) were calculated. The species were classified into functional feeding groups (FFG) according to food sources and mechanisms of food acquisition (Cummins, 1973; Merritt and Cummins, 2006).

For studying the correlation between size of the nymphs and gut contents, 30 nymphs (when there was enough number of nymphs) were measured per month (0.01 mm accuracy). For studying the possible election of prey for the species, the Ivlev's index was used (Ivlev, 1961):

$$E = (r_i - p_i) / (r_i + p_i)$$

where  $r_i$  = relative abundance of a particular taxon in the diet

$p_i$  = relative abundance of the same taxon in the benthic community.

The index ranges from -1 to 1. A value of -1 means total avoidance, 1 indicates preference and 0 indicates indifference.

For statistical analysis, STATISTICA software (StatSoft, 2005) was employed. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For the election of the proper statistical tests we followed Guisande González *et al.* (2006).

### 3.3. RESULTS

#### 3.3.1. Life histories

The life cycle of *G. franzi* was semivoltine in the study area, with two different generations inhabiting at the same time (Figure 3.3). The first generation (represented by the smallest nymphs in Figure 3.2) was present in the benthos from November to May. In June, although there was still water in the stream, no one nymph was collected, probably because they migrated to the hyporheic zone. The generation corresponding to the second year of the cycle was present from November to the end of April, when adults started flying. The flight period of the species expanded to June, when water started disappearing. In these two months, the eggs were laid down in the last ponds that predominated in the stream. Eggs probably remained in the stream without hatching to the next wet period, because nymphs collected in November belonged to first stages. The highest growth rate of nymphs occurred in the months prior and after the drought (Figure 3.3). No growth was observed during the dry period. Thus, the sizes of the nymphs of the smallest generation in May were similar to that belonging to the biggest generation collected in November. The development needed approximately 4477.67 day-degrees for its entire completion, but we did not find significant correlations between growth and day-degrees ( $p > 0.05$ ).

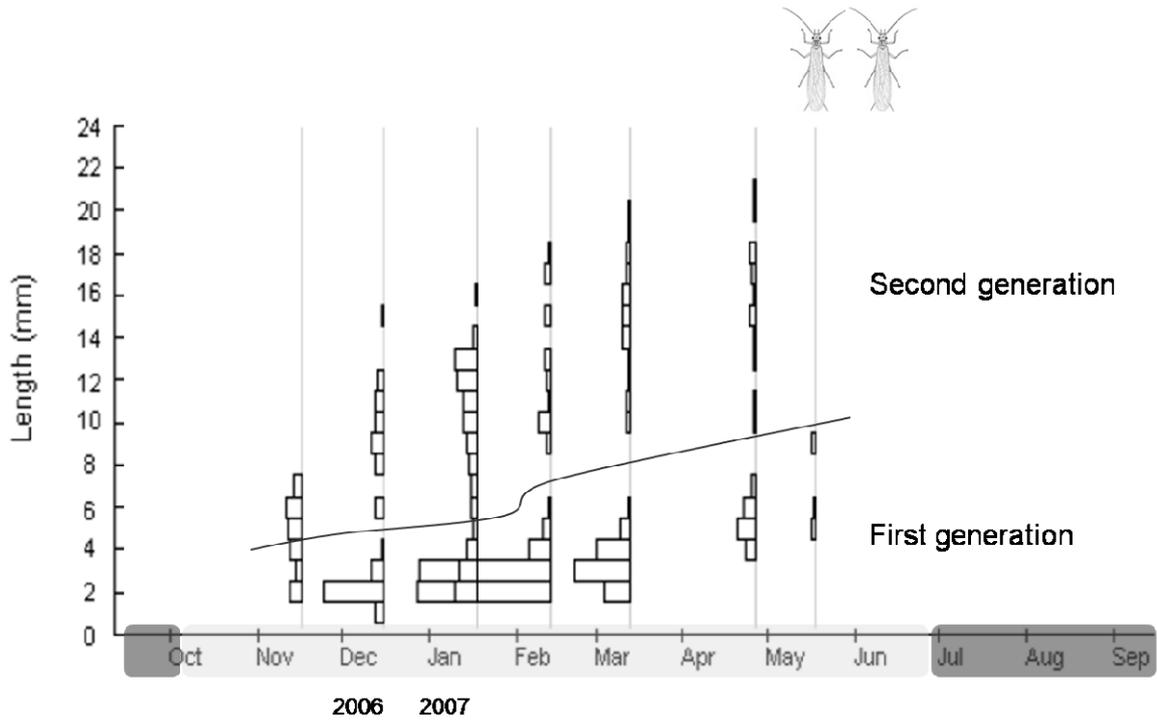


Figure 3.2. Size-frequency graph representing the life cycle of *Guadalgenus franzi* (N= 498). Mature nymphs are marked with a nymph draw, and adults are represented by a macropterous adult draw. The period in which the stream was dry is marked darker in the horizontal axis.

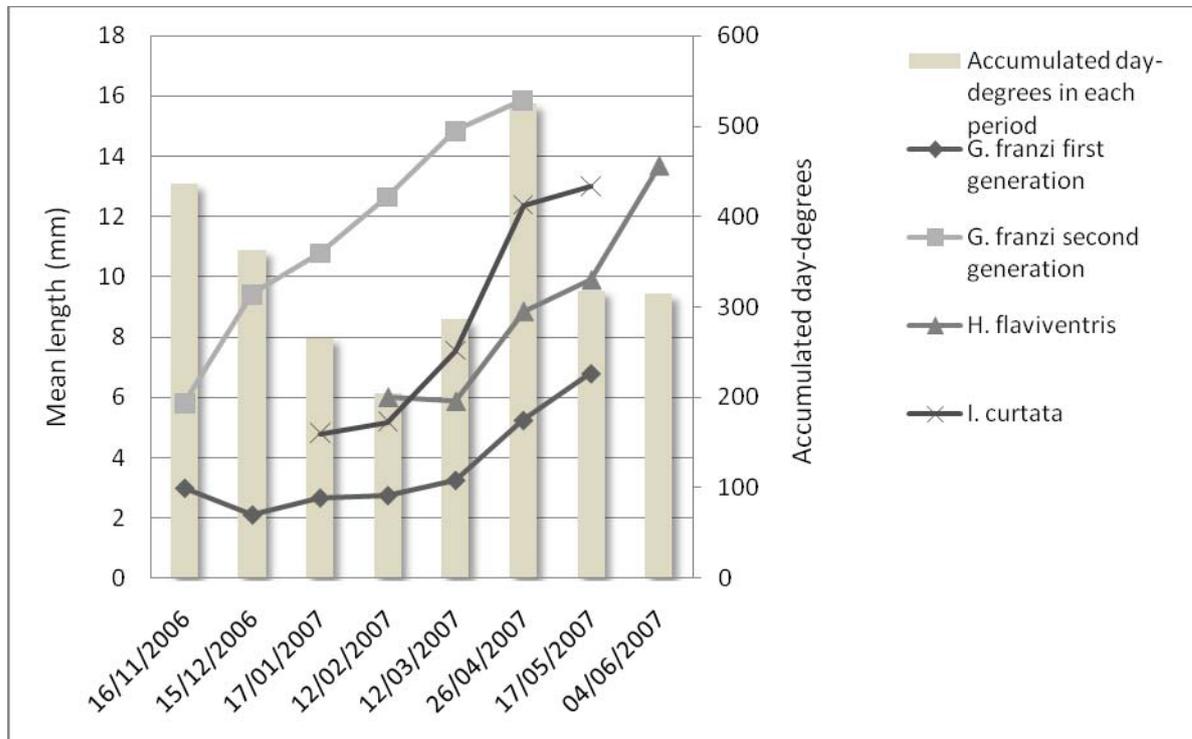


Figure 3.3. Growth patterns of the studied species and accumulated day-degrees during the interval between two consecutive sampling dates.

On the other hand, *H. flaviventris* presented a univoltine life cycle, with nymphs collected in the benthos from February to June (Figure 3.4). Mature nymphs (with black wingpads) were found from final April to June, and adults were collected from May to June. After the reproduction period, eggs were laid down in the water and they probably hatched at the beginning of the next wet period, after a possible dormancy period. We did not find the smallest nymphs. There were nymphs belonging to several size classes cohabiting at the same time. As can be seen from the life cycle, the development of this species was quite fast (approximately five-six months) taken into account its mature nymphal size. We observed the presence of eggs in mature nymphs of April and June. Growth was very fast, mainly in May-June, prior to the drought (Figure 3.3). In the period in which the nymphs were present in the benthos, they accumulated 1647.69 day-degrees during their entire development, although we did not find significant correlations between growth and day-degrees ( $p > 0.05$ ).

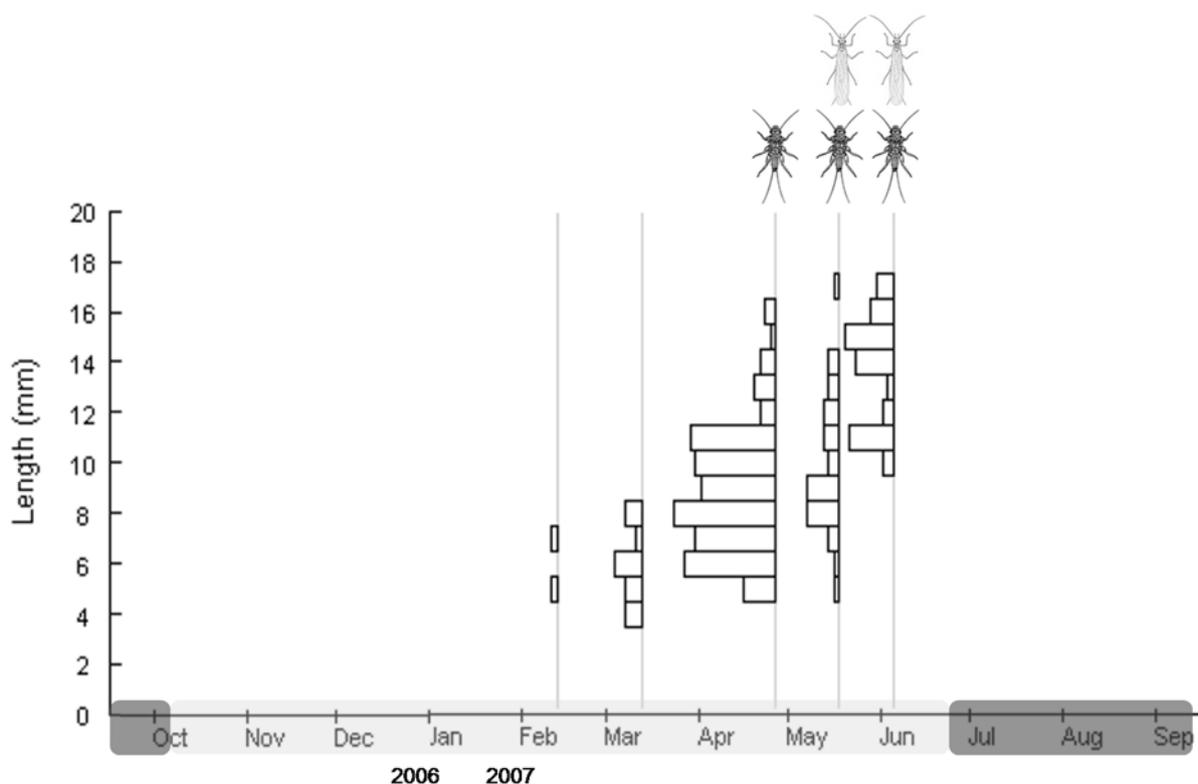


Figure 3.4. Size-frequency graph representing the life cycle of *Hemimelaena flaviventris* (N= 197). Mature nymphs are marked with a nymph draw, and adults are represented by a macropterous adult draw. The period in which the stream was dry is marked darker in the horizontal axis.

The life cycle of *I. curtata* was also univoltine, although a little in advance in relation to *H. flaviventris*, with nymphs present in the benthos from January to May (Figure 3.5). Mature nymphs were found in May, and adults were collected in May and June. The eggs, as in the prior species, would probably remain without hatching until the next wet period, maybe passing through a dormancy phase. Nymphs likely hatched prior to the first collections, due to no first instars nymphs were captured. In *I. curtata* the growth was also very fast (Figure 3.3), taking place after 1696.29 accumulated day-degrees. We neither found significant correlations between growth and day-degrees ( $p > 0.05$ ).

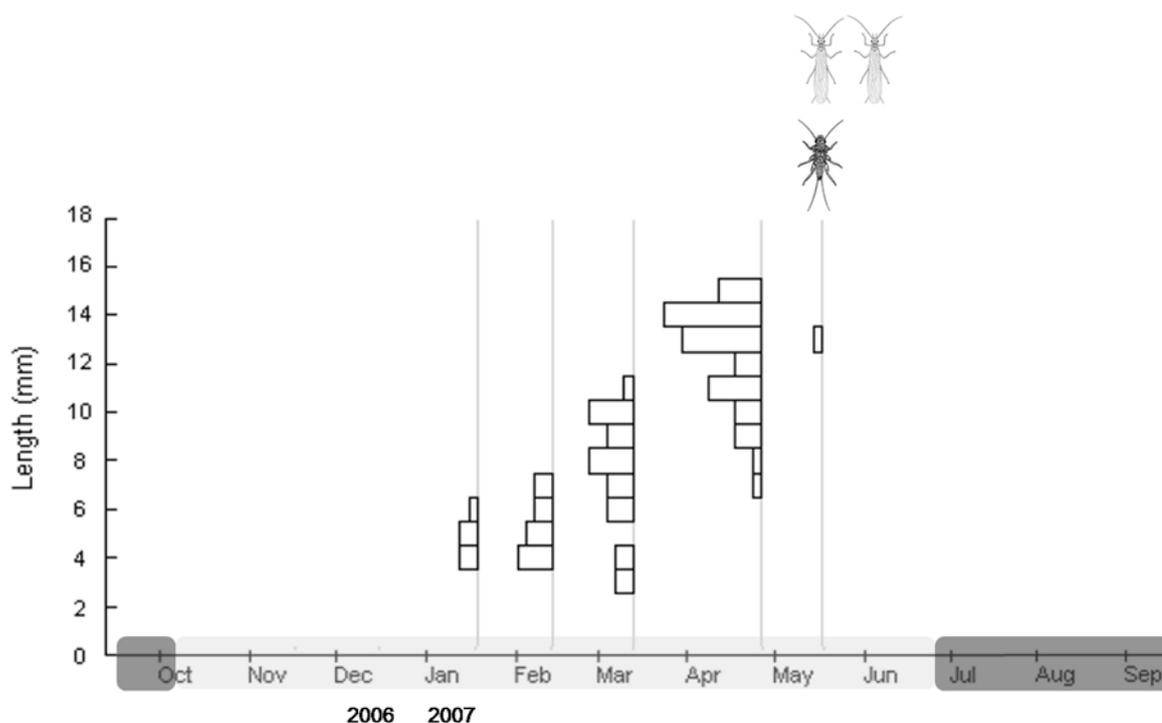


Figure 3.5. Size-frequency graph representing the life cycle of *Isoperla curtata* (N= 83). Mature nymphs are marked with a nymph draw, and adults are represented by a macropterous adult draw. The period in which the stream was dry is marked darker in the horizontal axis.

### 3.3.2. Feeding

The three studied species fed mainly on epilithic diatoms, the great majority belonging to the genus *Melosira* (Sánchez-Castillo, University of Granada, personal communication), followed by detritus (in terms of both percentage of occupied area and percentage of presence) (Table 3.3). In the case of *H. flaviventris*, the one which fed more on animal matter, some of these

non-animal items could come from the gut of its prey, but when analyzing the gut content of the individuals without any prey, we realized that the main found components were also the diatoms and the detritus (Table 3.4). The presence of prey in the guts of *G. franzi* and *I. curtata* was extremely low, similar or even lower to that of coarse particulate organic matter (CPOM), for instance (Table 3.3). Contrary, in *H. flaviventris* we noted that there was a high percentage of individuals with prey in their guts, and that the number of prey ingested for some of them was actually high, reaching to more than 40 ingested prey in some individuals.

	<i>Guadalgenuis franzi</i>						<i>Hemimelaena flaviventris</i>						<i>Isoperla curtata</i>					
	N	Mean	SD	Min-Max	Pres.	% pres.	N	Mean	SD	Min-Max	Pres.	% pres.	N	Mean	SD	Min-Max	Pres.	% pres.
% absolute	180	31.25	28.97	0-100	-	-	99	33.03	32.54	0-100	-	-	50	44.10	37.32	0-100	-	-
% detritus	175	19.26	22.52	0-95	144	82.29	68	26.07	29.57	0-95	61	89.71	47	22.70	21.77	0-100	43	91.49
% diatoms	175	78.27	23.82	0-100	174	99.43	68	71.50	30.58	0-100	67	98.53	47	76.66	21.81	0-100	46	97.87
% hyphae	175	0.18	0.68	0-5	15	8.57	68	0.03	0.17	0-1	2	2.94	47	0.11	0.43	0-2	3	6.38
% fungi spores	175	0.12	0.54	0-4	10	5.71	68	0.04	0.36	0-3	1	1.47	47	0.13	0.74	0-5	2	4.26
% CPOM	175	0.65	2.17	0-15	20	11.43	68	0.94	3.37	0-20	8	11.76	47	0.38	1.07	0-5	6	12.77
% Cyanobacteria	175	0.87	6.14	0-70	19	10.86	68	0.04	0.36	0-3	1	1.47	47	0.02	0.15	0-1	1	2.13
% pollen	175	0.09	0.52	0-5	7	4.00	68	0.01	0.12	0-1	1	1.47	-	-	-	-	-	-
<b>N Chironomidae</b>	175	0.19	0.67	0-5	22	12.57	68	2.90	6.37	0-28	29	42.65	47	0.06	0.25	0-1	3	6.38
<b>N Simuliidae</b>	175	0.08	0.36	0-3	10	5.71	68	2.81	6.74	0-40	26	38.24	-	-	-	-	-	-
<b>N Plecoptera</b>	175	0.07	0.28	0-2	12	6.86	-	-	-	-	-	-	-	-	-	-	-	-
<b>N Ephemeroptera</b>	175	0.01	0.08	0-1	1	0.57	68	0.13	0.42	0-2	7	1.47	47	0.02	0.15	0-1	1	2.13
<b>N Ancyliidae</b>	175	0.07	0.60	0-7	4	2.29	-	-	-	-	-	-	-	-	-	-	-	-
<b>N Lumbriculidae</b>	175	0.01	0.08	0-1	1	0.57	-	-	-	-	-	-	-	-	-	-	-	-
<b>N Hydracarina</b>	-	-	-	-	-	-	68	0.01	0.12	0-1	1	1.47	-	-	-	-	-	-
<b>N Ostracoda</b>	-	-	-	-	-	-	-	-	-	-	-	-	47	0.02	0.15	0-1	1	2.13
<b>N unidentifiable animal matter</b>	175	0.05	0.22	0-1	8	4.57	68	0.04	0.21	0-1	3	4.41	47	0.02	0.15	0-1	1	2.13
<b>FFG</b>	<b>Scraper/Gatherer-collector/Predator</b>						<b>Predator/Scraper/Gatherer-collector</b>						<b>Scraper/Gatherer-collector/Predator</b>					

Table 3.3. Nymphal gut contents of the studied species in the sampling site. Bold letter in FFG column (Functional Feeding Group) represents the most important functional group into which the species can be assigned. [Pres. = presence]

	N	Mean	SD	Min-Max	Presence	% presence
% detritus	18	18.28	22.59	0-60	18	100.00
% diatoms	18	81.06	22.40	40-100	18	100.00
% hyphae	-	-	-	-	-	-
% fungi spores	-	-	-	-	-	-
% CPOM	18	0.61	1.97	0-8	18	100.00
% Cyanobacteria	-	-	-	-	-	-
% pollen	18	0.06	0.24	0-1	18	100.00

Table 3.4. Nymphal gut contents of the individuals of *Hemimelaena flaviventris* without prey in their guts.

When we analyzed the correlation between total length and percentage of food items in the gut (Table 3.5), we observed that in *G. franzi* there was a decrease in the ingestion of Chironomidae and an increase in that of Ancyliidae and Plecoptera when bigger. We did not find a significant correlation when studying the number of prey and the length of the individuals. In *H. flaviventris* we found that it incremented the ingestion of Simuliidae and Chironomidae. There was also a positive correlation, although very low, between total length and number of prey found in its gut (Gamma correlation = 0.34,  $p < 0.05$ ). In the case of *I. curtata* we observed that there was a decrease in the ingestion of hyphae, and also a positive, low correlation between total length and number of prey ingested (Gamma correlation = 0.51,  $p < 0.05$ ), but this result must be interpreted with caution due to the low number of prey found in the gut. Although in this species there was a perfect positive correlation between the presence of Ostracoda in the gut and the total length, this represented the presence of an only one Ostracoda in the gut of a big size nymph, probably ingested accidentally when collecting detritus.

	<i>Guadalgenu</i> <i>franzi</i> total length (mm)	<i>Hemimelaena</i> <i>flaviventris</i> total length (mm)	<i>Isoperla</i> <i>curtata</i> total length (mm)
% detritus	0.05	0.13	0.03
% algae	-0.05	-0.10	-0.03
% hyphae	-0.03	-0.07	-0.75*
% fungi spores	0.03	-0.85	0.12
% CPOM	0.19	0.14	0.00
% Cyanobacteria	-0.09	-0.18	-0.74
% pollen	0.17	-0.76	-
<b>N Chironomidae</b>	-0.23*	0.25*	0.01
<b>N Simuliidae</b>	0.20	0.44*	-
<b>N Plecoptera</b>	0.41*	-	-
<b>N Ephemeroptera</b>	0.29	0.19	0.91
<b>N Ancyliidae</b>	0.65*	-	-
<b>N Lumbriculidae</b>	0.86	-	-
<b>N Hydracarina</b>	-	-0.52	-
<b>N Ostracoda</b>	-	-	1.00*
<b>N unidentifiable animal matter</b>	-0.10	-0.64*	0.77

Table 3.5. Gamma correlations between total length and the percentage of the different food items in the studied species. Values marked with an asterisk are significant at  $p < 0.05$ .

The Ivlev's electivity index of *G. franzi* (Figures 3.6 and 3.7) showed that the nymphs belonging to the first generation preferred Chironomidae and avoided Plecoptera and Ephemeroptera (although mayflies were found in the gut only in one month). Simuliidae were preferred first and avoided later. Nymphs of the second generation preferred Ancyliidae, Chironomidae (although in a lesser concern than in the first generation nymphs), Simuliidae and Lumbriculidae, the latter two just found punctually. Plecoptera were indistinctly preferred or avoided depending on the month.

In the case of *H. flaviventris* (Figures 3.6 and 3.7), Chironomidae were preferred at the beginning of its life cycle and avoided at the end. Simuliidae were firstly avoided and later preferred, and Ephemeroptera were always greatly avoided. Hydracarina showed an Ivlev's index value of one, i.e. total preference, but this was only an artifact produced by the ingestion of one Hydracarina by an individual in a month when no Hydracarina had been recorded in the community.

In *I. curtata* we found a preference for Ostracoda, Chironomidae and Ephemeroptera (Figures 3.6 and 3.7), but these results should be taken with caution due to the low number of individuals that acted as predators.

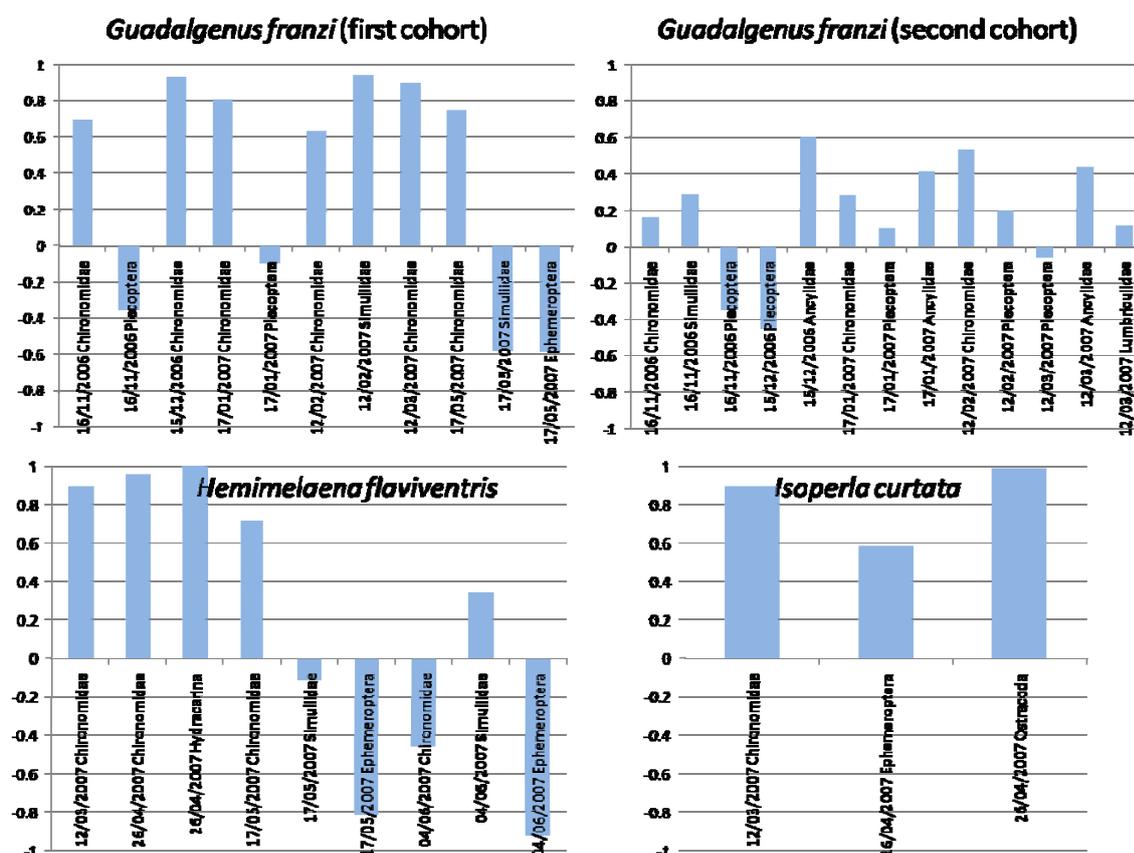


Figure 3.6. Ivlev's electivity index of the studied species in each month.

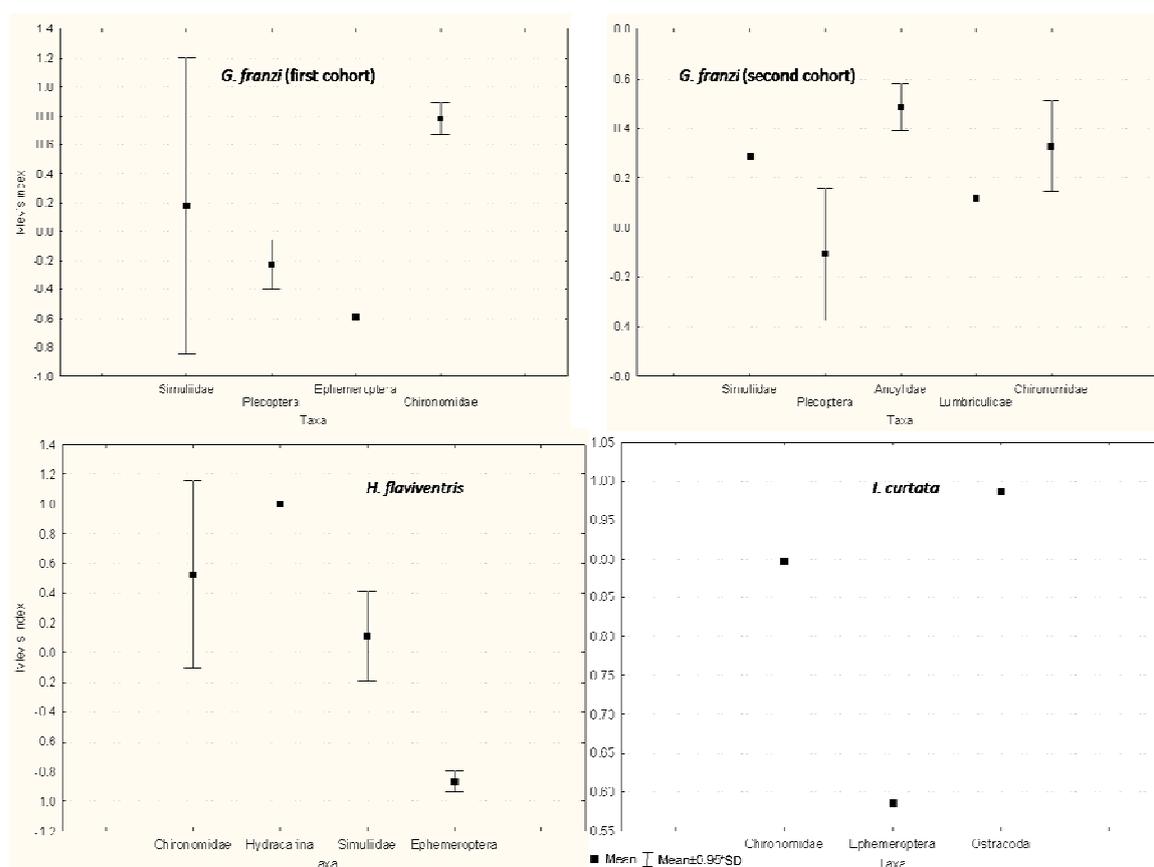


Figure 3.7. Mean Ivlev's index for each studied species.

### 3.3.3. Secondary production

In the studied species, dry weight ( $DW$ ) was related to body length ( $X$ ) by the following equations:

$$\ln DW = -5.31 + 2.89 \ln X, (r^2 = 0.95, F_{1,28} = 513.57, p < 0.05) \text{ for } G. \text{ franzi}$$

$$\ln DW = -4.74 + 2.68 \ln X, (r^2 = 0.94, F_{1,28} = 421.40, p < 0.05) \text{ for } H. \text{ flaviventris}$$

$$\ln DW = -4.80 + 2.48 \ln X, (r^2 = 0.71, F_{1,27} = 65.33, p < 0.05) \text{ for } I. \text{ curtata.}$$

Production values and parameters are summarized in Table 3.6. Thus, *G. franzi* presented an annual secondary production of  $13.03 \text{ gDWm}^{-2}\text{year}^{-1}$ , based on a cohort production interval (CPI) of 13 months, with a cohort production/biomass rate (P/B) equal to 4.91. In the case of *H. flaviventris*, the annual secondary production was  $21.72 \text{ gDWm}^{-2}\text{year}^{-1}$ , corrected by a CPI of 5

months, and the cohort P/B ratio equal to 3.14. For *I. curtata*, the annual secondary production was equal to  $6.09 \text{ gDWm}^{-2}\text{year}^{-1}$ , with a CPI of 5 months and a cohort P/B ratio of 2.49.

Species	Secondary production (gDWm <sup>-2</sup> )	CPI (months)	Annual secondary production (gDWm <sup>-2</sup> year <sup>-1</sup> )	Annual P/B (year <sup>-1</sup> )	Cohort P/B
<i>Guadalgenus franzi</i>	14.11	13	13.03	4.53	4.91
<i>Hemimelaena flaviventris</i>	9.05	5	21.72	7.54	3.14
<i>Isoperla curtata</i>	2.54	5	6.09	5.97	2.49

Table 3.6. Secondary production parameters of the studied species.

### 3.4. DISCUSSION

#### 3.4.1. Life histories

The life cycle of *G. franzi* was non-seasonal, taking two years for its entire development and with different generations inhabiting at the same time [according to Hynes (1970)]. A similar life cycle was found for this species in two seasonal streams of the same mountain range by Agüero-Pelegrín and Ferreras-Romero (2002), who hypothesized that nymphs of the first generation survived the dry period burrowing into the hyporheic zone. In a perlodid species of North America, Dieterich and Anderson (1995) found, in a temporary stream, a life cycle also comparable to the one described by us. In our study, growth in the dry period was almost null, as shown in Figure 3.3, so a dormancy phase seems to be the most probable strategy. There are two types of dormancy forms known to exist in Plecoptera: diapause, that it is a programmed (delayed) response with suppressed development lasting longer than the adverse conditions, and quiescence, that is an immediate direct response to a limiting factor that takes place concurrently with the adverse conditions (Danks, 1987). Both types may happen either in the egg or in the nymphal stages. Nymphal diapause is not very common among Plecoptera in general and among Perlodidae in particular (Harper and Hynes, 1970; Pugsley and Hynes, 1985; Stewart and Stark, 2002), so quiescence could be the actual strategy of this species. This is supported by several facts: first, in our study nymphs began to disappear at the same time in which drought began and reappeared with the water arrival; if diapause usually

precedes and lasts the adverse conditions (Danks, 1987), nymphs must disappear and reappear just previously and afterwards to the adverse conditions and not simultaneously. Second, nymphal diapause in Plecoptera has been reported in the first stages of development (e.g. Harper and Hynes, 1970), and in *G. franzi* the drought arrives when is mid-size. Finally, it has been proved for other aquatic insects that when they dehydrate as a consequence of high temperatures, they enter in a quiescence period in which they can support extremely high temperatures (Hinton, 1951, 1960), what could be a proper strategy in *G. franzi*. Thus, a possible hypothesis could be that the mid-developed nymphs of this species migrated to the hyporheic zone when drought began and there either remained in the wet zone within it or dehydrated within the substrate interstices (as a consequence of the summer high temperatures) and stayed until the next wet period, in a similar form as pointed out by Harper and Hynes (1970). This is also supported by the fact that we found nymphs in the first samplings, when they would have rehydrated and reactivated their development. Moreover, nymphs of the end of the first (smallest) generation and those of the beginning of the second (biggest) generation were approximately equal in size, indicating that no growth occurred during the summer drought. After this period, nymphs of the second generation grew twice of those of the first generation, reaching their maximum size in April, when the flight period started. After mate finding and copulation, females probably laid the eggs on May-June, just before the stream dried out. Thus, the eggs must also pass through a drought period, either with a normal development or with an embryonic diapause stage. Eggs of Systellognatha, group to which *G. franzi* belongs, possess typically an anchor plate which adheres firmly to the substrate (Hynes, 1976), but in this species, as well as in some others, it is not present (Tierno de Figueroa *et al.*, 2003). The lack of anchor structure in other temporary water perlodid species has been related with their facility to slide down into the substrate, avoiding the exposure to the air when the stream becomes dry (Berthélemy, 1973; Tierno de Figueroa *et al.*, 1998).

Both *H. flaviventris* and *I. curtata* had a “fast-seasonal” life cycle [following the classification of Hynes (1970)]. The entire cycle would take some additional months apart from those recorded by us by the presence of the nymphs in the benthos, if we take into account that we did not collect the smallest nymphs of the

two species. Alouf (1989) showed that the recently hatched nymphs of *Isoperla libanica* Aubert, 1964 in a seasonal stream migrated to the hyporheic zone and appeared in the benthos later. This could be the reason why we did not collect small nymphs neither of *H. flaviventris* nor of *I. curtata*. Furthermore, the life cycle found by this author coincides widely with that found by us for *I. curtata*, with nymphs collected from February to May. If we observe Figure 3.3 we could extrapolate the mean growth pattern of both species and to estimate, with a certain error margin, that the hatching must have taken place one or two months before our collections. During the dry period, eggs probably passed through a diapause period, as has been found in other Perlodidae species (Hynes, 1976), although this must be confirmed experimentally. As shown in other *Isoperla* species, they can remain in diapause up to seven-eight months (Lillehammer *et al.*, 1989). If this was the case in *I. curtata*, the eggs laid down on May-June would hatch from December to February, what would coincide with the beginning of the life cycle hypothesized by us. Something similar could also occur in *H. flaviventris*. A possible adaptation to cope with the drought and the high temperatures of the summer in this species could be, as in the case of *G. franzi*, the absence of the anchor plate in the eggs that could help them to slide down into deeper zones of the riverbed (Berthélemy, 1973; Tierno de Figueroa *et al.*, 1998). If an embryonic diapause existed in both species, its rupture must be very asynchronic, due to there were nymphs of many size classes inhabiting at the same time. Hynes (1976) pointed out that several species of *Isoperla* have a large size spread among nymphs and a fairly short emergence period, as in our case, although the author associated this strategy to the carnivorous behavior of the nymphs. In our case, another factor that may contribute for this spread could be the marked sexual dimorphism in size (mainly found in mature nymphs of *H. flaviventris* in June, when males and females could be clearly differentiated). Nevertheless, this can be a key strategy in temporary waters to face with unpredictable droughts, as shown by Dieterich and Anderson (1995). These authors proposed also a prolonged adult emergence for species inhabiting temporary streams, but in our data we did not find it. A reason for this can be that the studied species are spring species, in which emergence takes place few weeks before the drought of the stream. Thus, a better strategy could be a synchronic emergence and a short flight period for a rapid mate finding and

oviposition. This last hypothesis can be supported by the fact that the other Plecoptera species with earlier emergence present in the studied stream, as *Tyrrhenoleuctra* cf. *minuta* (Klapálek, 1901), *Brachyptera vera* Berthélemy and González del Tánago, 1983 or *Capnioneura gelesae* Berthélemy and Baena, 1984, showed a relatively long flight period (Chapter 4 and personal observations).

In no one of the studied species there was a significant correlation between growth and day-degrees. This is probably because for some stonefly species growth is relatively temperature independent within a certain temperature interval, although this is specially marked in those species in which nymphal development takes place during winter at low temperatures (Hynes, 1970; Brittain, 1990). The species studied here grew during winter but, due to the thermal characteristics of the stream (Figure 3.1, Table 3.1), they were not exposed to very low temperatures. In addition, we found that *H. flaviventris* and *I. curtata* accumulated almost the same number of day-degrees, although the life cycle of the former was delayed one month with respect to the latter. This may point out an equal day-degree requirement for the complete development of both species, though rearing laboratory experiments are needed to confirm this hypothesis.

#### 3.4.2. Feeding

It is usually accepted that Perlodidae (and also Perlidae) are the major macroinvertebrate predators in stream ecosystems (Merrit and Cummins, 1996), although vegetal matter can be also important for some species and/or during certain stages of their lives (Stewart and Stark, 2002). Nevertheless, from our data we can conclude that *G. franzi* and *I. curtata* were mainly primary consumers, while *H. flaviventris* was an actual predator. As we have seen before, *H. flaviventris* also consumed great amounts of non-animal matter, mainly diatoms, but when it acted as a predator, it ingested great quantities of prey. Thus we can say that *G. franzi* and *I. curtata* were scrapers and gatherer-collectors, with also a less important role as predators (principally in the former). A similar trophic behavior was also found by Frison (1935), Shapas and Hilsenhoff (1976) and by Jop and Szczytko (1984) for other species of *Isoperla*,

in the latter cases with a great amount of diatoms present in the diet. As pointed out by Stewart and Stark (2002), the food habits of *Isoperla* are highly diverse, being predator, detritivorous-herbivorous or omnivorous. On the other hand, *H. flaviventris* was a voracious predator that, when it did not prey, it acted also as a scraper and gatherer-collector. The latter species was also studied by Azzouz and Sánchez-Ortega (2000), who found that Chironomidae were the main consumed prey, with also a great proportion of vegetal matter (mainly diatoms), principally on the smallest nymphs.

If we take into account the percentage of presence of each prey in the gut, we realize that the three studied species ingested mainly Chironomidae. Some of the prey found in their guts were present punctually, such as Ephemeroptera and Lumbriculidae in *G. franzi*, Hydracarina in *H. flaviventris* and Ephemeroptera and Ostracoda in *I. curtata*.

As shown in Table 3.5, there were some changes in the diet of the three species in relation with size. Such ontogenetic shifts on diet have been also found in other species of Perlodidae stoneflies (Fuller and Stewart, 1977; Jop and Szczytko, 1984; Malmqvist *et al.*, 1991; Azzouz and Sánchez-Ortega, 2000; Fenoglio *et al.*, 2005; Céréghino, 2006). This was also reflected in the electivity index of the studied species that changed along the sampling period. Nevertheless we must take into account that in *H. flaviventris* and *I. curtata* we missed the smallest stages due to they were probably in the hyporheic zone, so we did not know their feeding habits. In the case of the most carnivorous species, *H. flaviventris*, we noted that the preference of prey along the cycle varied, indicating a generalist predator condition. This was also the rule in *G. franzi* that, when predator, fed on the most abundant prey (in order of abundance in the community: Simuliidae, Chironomidae, Ephemeroptera, Plecoptera, Lumbriculidae and Ancyliidae). This species would feed on diatoms and detritus and, when found some of these animals, it would prey on them. For *I. curtata* no conclusion can be drawn at this respect due to the scarcity of prey ingested. The generalist condition of the studied species is also supported by the fact that all of them, when they do not prey, fed on diatoms and detritus, probably the most abundant resources in the stream. This generalist behavior for aquatic insects in general was already pointed out by Cummins (1973), indicating that they are polyphagous and lack specialization in food intake.

Changes on diet composition along the life cycle can be a consequence of prey size and/or mobility. Predator aquatic invertebrates often are indiscriminating in their diets, capturing whatever they encounter that is small enough to subdue (Allan and Castillo, 2007). We noted that in *G. franzi* and *H. flaviventris* the smallest stages preferred mainly Chironomidae (Figure 3.6), which are small and slow-moving prey, and rejected bigger or more mobile ones, such as Plecoptera, Ephemeroptera or Simuliidae (in this case just because of their big size). Likewise, some of these prey, e.g. Simuliidae, are preferred by bigger size nymphs. Such a trend in Plecoptera of eating Chironomidae in early instars and then broadening the size of the ingested prey has been pointed out by several authors (e.g. Allan and Castillo, 2007).

It is outstanding that large nymphs of *G. franzi* ingested Ancyliidae. They are very slow-moving, but their shells would make them a non-attractive prey. Nevertheless they were preyed several times along the life cycle of this species (Figure 3.6). It is possible that, when *G. franzi* was eating diatoms [that are also ingested by Ancyliidae (Monakov, 2003)], it encountered them and engulfed them, perhaps accidentally. This was likely also the case of some other prey found in its diet, as Lumbriculidae, or in other species diet, such as Hydracarina in *H. flaviventris* or Ostracoda in *I. curtata*. This unpremeditated carnivory may provide high-quality protein needed by many invertebrates to complete their life cycles (Anderson, 1976).

### 3.4.3. Secondary production

We calculated the secondary production of the stages present on benthos of the three species. Thus, we used a CPI of 13 months for *G. franzi*, and of 5 months both for *H. flaviventris* and *I. curtata* (Table 3.6).

The three studied species showed intermediate to low values of annual secondary production and annual P/B if we take into account the minimum and maximum values compiled by Huryn and Wallace (2000) for stream macroinvertebrates. *H. flaviventris* showed the highest value, followed by *G. franzi* and *I. curtata*. The cohort P/B value of *G. franzi* is actually close to five, that is the value more usually found on freshwater invertebrates, and the values of the

other species are in the range generally pointed out of two to eight (Benke, 1993).

Though intermediate when compared with other macroinvertebrates in general, *G. franzi* and *I. curtata* showed relatively high values of annual production in comparison with other scrapers, and also *H. flaviventris* when compared with other predators, but annual P/B values could be considered as the most frequently found (Benke, 1993). The great majority of the production of the *G. franzi* and *I. curtata* was probably due to the diatom rich diet. In *H. flaviventris* diatoms contributed also greatly, but animal matter was more important. We have neither data about the assimilation efficiencies of the different components found on their guts nor information on the enzymatic complexes that may act on the digestion of these species. Nevertheless, if we would proceed as in Benke and Wallace (1980) for estimating the amount of production derived of each component [but with a net production efficiency of 40%, as assumed by Benke and Jacobi (1994)] we would notice that the data obtained would support the high contribution of the diatoms to the annual production (higher than 70% for *G. franzi* and *I. curtata*). The animal matter would account for an extremely low percentage, except in *H. flaviventris*, in which this would be the main component contributing to annual production. We do not present these results detailed here because little or nothing is known about the digestion process in these species and perhaps too many assumptions should be made.

If we compare the values of annual production found by other authors who studied *Isoperla* sp. or other Perlodidae (e.g. Short and Ward, 1980; Lavandier, 1982; Jop and Szczytko, 1984; Stewart and Stark, 2002; Céréghino, 2006), we realize that the values found in our study are higher, even if we compare with populations of permanent waters. Few works have recorded secondary production in temporary streams, so comparison is difficult. Chadwick and Huryn (2007), in a study carried out in different habitats of an intermittent-stream system, recorded low values of annual secondary production for the entire macroinvertebrate community and justified them on the basis of the absence of large-bodied taxa. In our case, the three studied species are large-bodied, which could contribute to the highest values found by us. If we compare, for instance, our data with the values of 5.07 to 7.14 DWgm<sup>-2</sup>year<sup>-1</sup> of annual secondary production found by Jop and Stewart (1987) for a stonefly assemblage of 13

species, we can confirm that species inhabiting in Río Despeñaperros showed relatively high values. This may reflect an actual success of the species if we take into account (as mentioned in the introduction section) that secondary production is a measure of such success for a population (Benke, 1993).

### 3.5. CONCLUSIONS

Stoneflies are not common on temporary waters, although they have developed several strategies to occupy an important niche in these habitats too. The most commonly cited are small size (that implies a faster development), egg or nymphal diapause, high fecundity, staggered hatching of long-diapause eggs, and opportunistic/generalistic feeding (Jacobi and Cary, 1996; Williams, 1996, 2006). The species studied by us partially fulfill some of the typical characteristics of temporary waters fauna. Both *H. flaviventris* and *I. curtata* presented a probable long embryonic diapause and staggered hatching of eggs, a fast nymphal development, and a generalistic feeding, but they present big size nymphs. On the other hand, *G. franzi* presented also a dormancy phase in the nymphal stages during the drought period, due to its long, semivoltine life cycle. In this species this strategy, *a priori*, could seem to be less adaptive, because eggs in embryonic diapause are probably the stage in which species more easily face with drought (Hynes, 1970). Nevertheless, values of annual secondary production showed that this species, as well as the two others, live successfully in this habitat.

Regarding feeding, we found great differences between species. If we would make a gradient of herbivory to carnivory, we would situate to *I. curtata* as the first one, followed by *G. franzi*, and finally *H. flaviventris* as the more carnivorous. The two extremes of this gradient are the species that presented a more similar life cycle, partially overlapped. This could be the reason of the differences in the food habits: a competitive segregation. Nevertheless, this would not explain why *G. franzi* behaved as an herbivorous more than as a predator, because this species would not find competitors (among stoneflies) during the part of the cycle that did not coincide with the presence of the other species. Therefore, this behavior must be the result of an

opportunistic/generalistic feeding in this species, and possibly also in *I. curtata*, that would take advantage of the high abundant resource that supposed the diatoms in the stream. This was also reflected in the high use that *H. flaviventris* made of this resource when it did not act as a predator. From a general point of view, and as we have seen, since the majority of species appear to be generalists, statements about feeding habits are subject to considerable variation and require qualification with regard to habitat- and age-specific differences (Cummins, 1973). Thus, the present study supports that, as established by Stewart and Stark (2002), the feeding habits of an unstudied species cannot be inferred from the placement of a genus or higher taxon in a generalized grouping based on studies of congeners.

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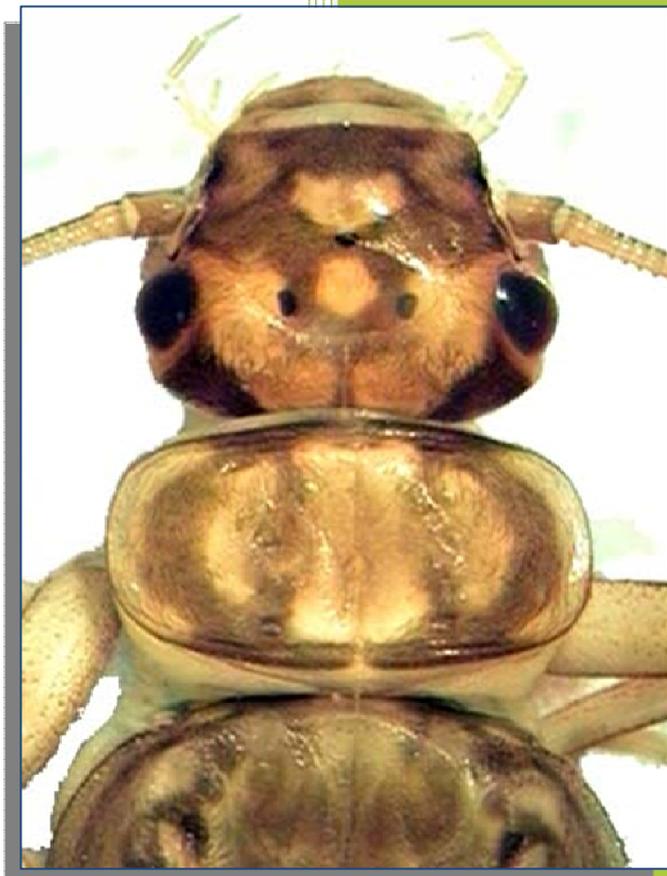
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APPENDIX I. Description of the nymph of *Isoperla curtata*  
Navás, 1924 (Insecta: Plecoptera)





The genus *Isoperla* Banks, 1906, with more than a hundred species along the Holarctic Region (Tierno de Figueroa *et al.*, 2003), is composed by 45 European species (Fochetti and Tierno de Figueroa, 2006).

From a morphological point of view, the species of *Isoperla* as a whole only can be correctly identified using the morphology of penial armature, and the scales forming it, in adult males. Nevertheless, the nymphs of some species can be distinguished in base of some characters (Consiglio, 1980). This can be particularly useful in concrete areas where stonefly community is known from studies on adults.

In Southern Iberian Peninsula there are five species of *Isoperla*: *I. bipartita* Aubert, 1963, *I. grammatica* (Poda, 1761), *I. nevada* Aubert, 1952, *I. pallida* Aubert, 1963, and *I. curtata* Navás, 1924 (Tierno de Figueroa *et al.*, 2003). The latter is the only species in which the nymph is not yet described.

*I. curtata* is an endemic but widely distributed species in the Iberian Peninsula, presenting a relatively high degree of polymorphism (Aubert, 1956).

For describing the nymph of this species, we employed 10 individuals from a monthly sampling program in Río Despeñaperros, Sierra Morena (Jaén, Spain), UTM: 30SVH558476, 560 m a.s.l. All individuals were collected in April and May 2007. Sampling station is characterized by temperatures ranging from 5.90 °C to 25.87 °C. Substrate is composed mainly by pebbles and cobbles. In this point, mean oxygen saturation is  $69.11 \pm 23.75$  % and mean oxygen concentration is  $7.03 \pm 2.73$  mg/l. Mean conductivity is  $454.89 \pm 69.43$   $\mu$ S. Caudal varies from 0.00 to 0.32 m<sup>3</sup>/s, depending on the rainfall, given that this stream has a mainly pluvial regime.

Studied nymphs of this species reach a relatively big size (body length: 11.2 to 14.8 mm. for mature nymphs). Body color is mainly yellowish with brown ornamentation. All the body is covered by abundant hairiness.

The head, with a characteristic design (Figure Ap1A), presents a light round spot surrounded by the three ocelli and other spot in front of the anterior ocellus [resembling the head designs of *I. rivulorum* (Pictet, 1842) and *I. insularis* (Morton, 1930) (see Consiglio, 1980: pp. 17, figures 19b and 19c)]. This last spot has a characteristic pentagonal shape. Other clear areas occupy the posterior

part of the head, bordering the compound eyes and reaching the antennae base, medially divided by a dark line. Antennae are yellow, with darker basal segments. According with literature, *I. curtata* head design is different from *I. grammatica* and *I. nevada* (see designs of Sánchez-Ortega and Alba-Tercedor, 1990: pp. 91, figure 5f; pp. 94, figure 6f) and *I. bipartita* (see description of Aubert, 1963), and resembles to *I. pallida* according to that pointed out by Aubert (1963).

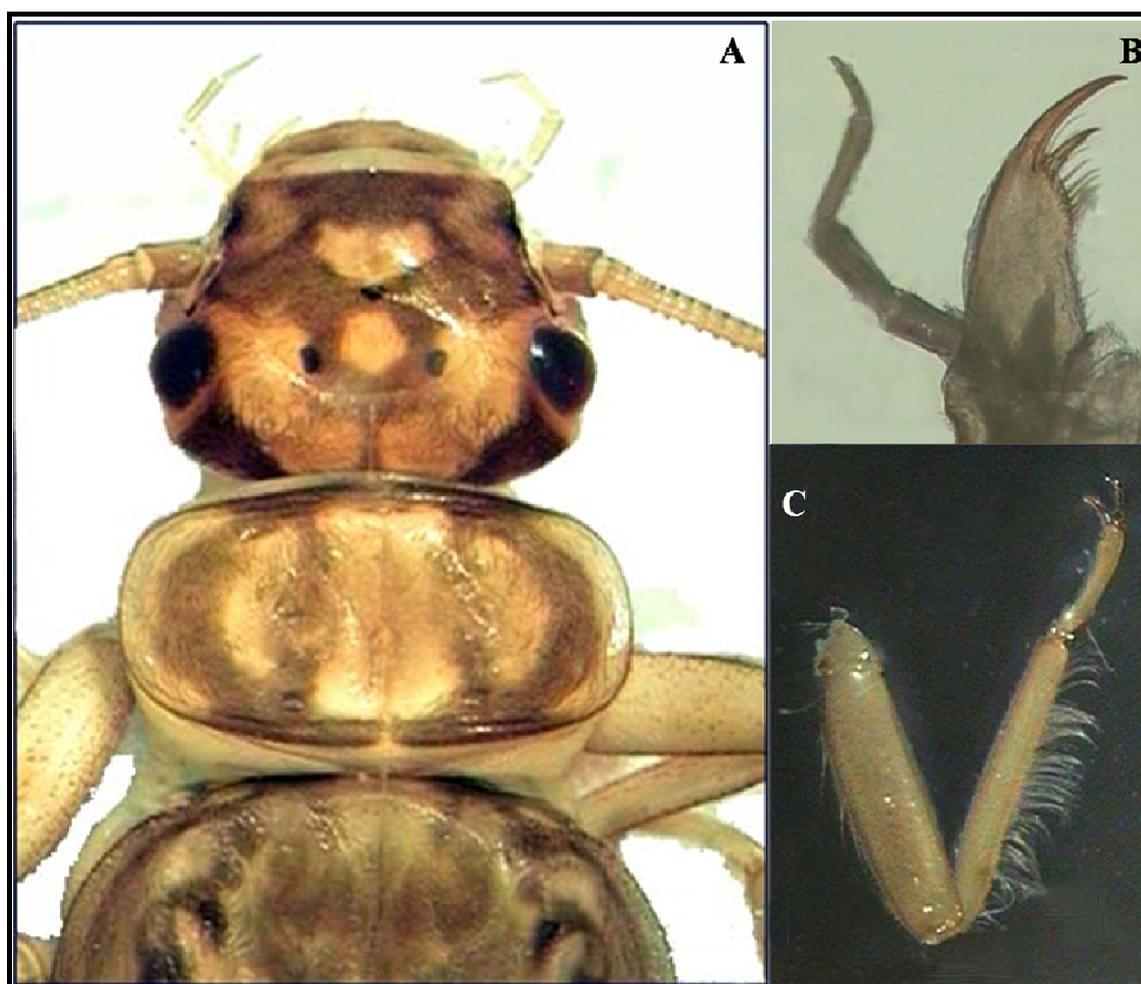


Figure Ap1. *Isoperla curtata* nymph: A) head and pronotum; B) maxilla; C) leg.

The maxilla (Figure Ap1B) has a relatively long lacinia and long and thick bristles in the apical part and shorter ones in the medial part. As head design, the lacinia of this species is more similar to the one of *I. rivulorum* (see Consiglio, 1980: pp. 18, figure 20b) than those of other species inhabiting in Southern Iberian Peninsula, at least *I. grammatica* and *I. nevada* (see Consiglio, 1980: pp. 18, figure 20a; Rupperecht, 1984: pp. 89, figure 7c; Sánchez-Ortega and Alba-Tercedor, 1990: pp. 91, figure 5i and pp. 94, figure 6g).

The pronotum (Figure Ap1A) is elliptic, with a medial clear area and two smaller kidney-shaped ones in the laterals, resembling that of *I. grammatica* (Consiglio, 1980: pp. 17, figure 18a; Sánchez-Ortega and Alba-Tercedor, 1990: pp. 91, figure 5f).

The leg has a silky fringe (with long bristles) in the outer femur, tibia and tarsus (Figure Ap1D).

The abdomen (Figures Ap2A and Ap2B) presents a characteristic pattern, dorsally bi-color and yellowish in the ventral and lateral parts. Each urite presents dorsally a clear medial area with a tear-shaped dark spot, surrounded by a dark contour. Bristles in the posterior edge of each tergum are around 1/8 of the urite length in the mature nymphs, considerable smaller than the 1/3 or 1/4 pointed for *I. grammatica* (Sánchez-Ortega and Alba-Tercedor, 1990).

Cerci present a crown of smaller bristles around each article and a longer bristles fringe in the distal part (Figures Ap2C and Ap2D).

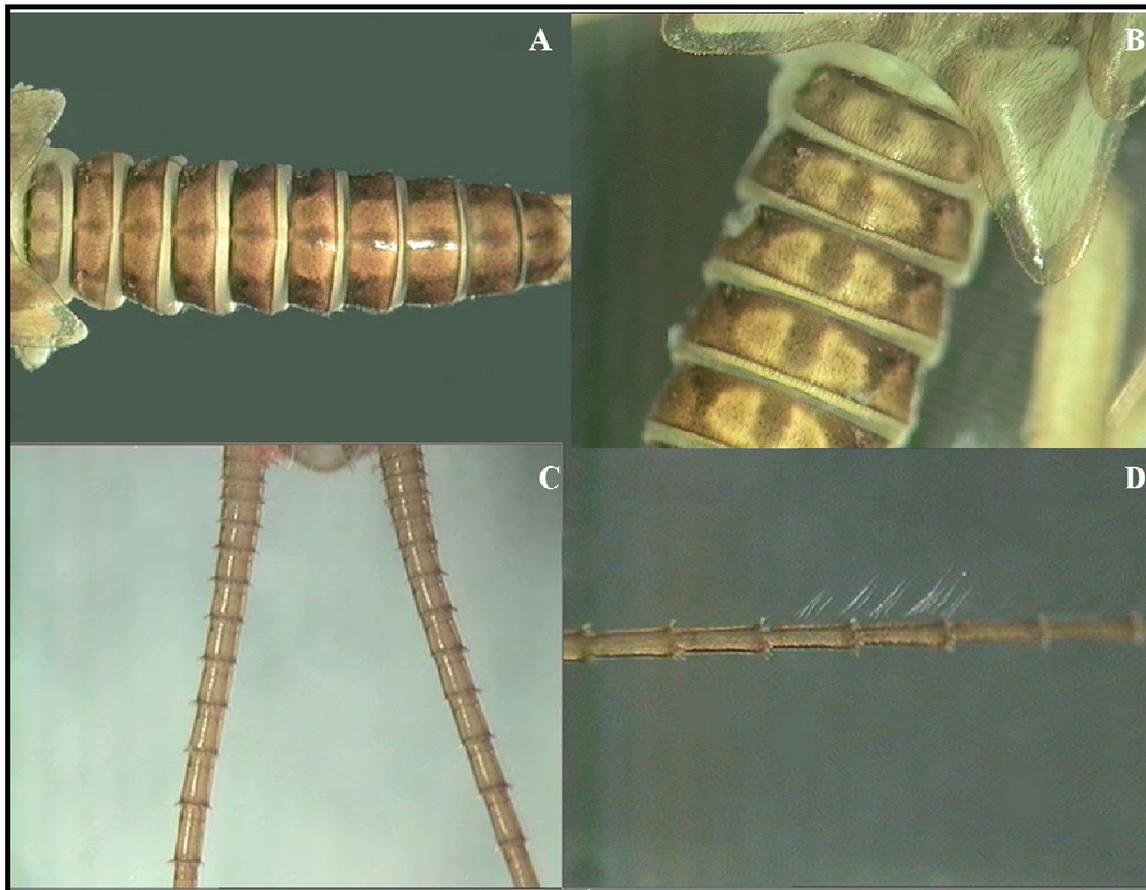


Figure Ap2. *Isoperla curtata* nymph: A) and B) abdomen; C) and D) cerci.

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**CHAPTER 4. Life history, feeding and secondary production of two Nemouroidea species (Insecta: Plecoptera) in a temporary stream of Southern Iberian Peninsula**





**ABSTRACT**

In the present chapter we study some ecological aspects of two taxa of Nemouroidean stoneflies (*Tyrrhenoleuctra* sp. Consiglio, 1957 and *Brachyptera vera cordubensis* Berthélemy and Baena, 1984) from a seasonal stream from Southern Europe. *Tyrrhenoleuctra* sp. shows a univoltine life cycle with a nymphal dormancy during the drought, and long flight period. The eggs of this taxon hatch rapidly after been laid, indicating a certain degree of ovoviviparism. The first instar nymph is described for the first time. On the other hand, *B. vera cordubensis* shows a fast seasonal univoltine life cycle with an egg dormancy. The feeding behaviour study reveals that *Tyrrhenoleuctra* sp. can be catalogued mainly as gatherer-collector while *B. vera cordubensis* can be classified mainly as scraper, although neither of them belong uniquely to a single functional group. No significant changes are detected in the diet in relation to the size; only in *B. vera cordubensis* a higher ingestion of CPOM is detected when larger. Regarding secondary production, although both taxa present a similar biomass, it is outstanding that *B. vera cordubensis* has a higher secondary production and a very high cohort P/B ratio (exceeding the upper limit usually pointed out for aquatic invertebrates), probably related to its fast development.

**KEYWORDS:** *Tyrrhenoleuctra* sp., *Brachyptera vera cordubensis*, seasonal stream, life cycle, growth, trophic behaviour, secondary production, Southern Spain.

#### 4.1. INTRODUCTION

Nemouroidea constitutes a superfamily of Plecoptera including five families and more than 1500 species (Fochetti and Tierno de Figueroa, 2008). The biology of this group has been studied mainly in permanent waters, showing a wide variety of life strategies: life cycle ranging from multivoltine (e.g. *Nemurella pictetii* Klapálek, 1900) to merovoltine [e.g. *Pachyleuctra benllochi* (Navás, 1917)], with predominance of the univoltine ones, both fast seasonal and slow seasonal; feeding mechanisms (scrappers, shredders, gatherer-collectors); reproductive behaviours [oviparous or ovoviviparous, the latter scarcer but cited, for example, in some *Capnia bifrons* (Newman, 1838) populations], etc. (see Tierno de Figueroa *et al.*, 2003a). The species inhabiting temporary streams have been less studied, despite that the constraining characteristics of these environments favour the development of particular strategies. As pointed out by Giller and Malmqvist (1998), drought is a fundamental conditional factor in the growth and development of the aquatic insects that live in this type of rivers and streams. Thus, fast seasonal life cycles with egg or nymphal dormancy periods, ovoviviparity cases, eggs with peculiar membranes, or asynchronic growth (reflected on high levels of adult polymorphism) are some of the strategies previously detected in temporary water species (Harper and Hynes, 1970; Berthélemy, 1973; Hynes, 1976; Zwick, 1980; Puig *et al.*, 1990; Jacobi and Cary, 1996). Nevertheless, new studies in other species can help to understand the use of this habitat by an animal group usually adapted to cold, oxygenated and permanent waters.

In the present study, we selected two taxa of Nemouroidea which nymphal biology is almost completely unknown: *Tyrrhenoleuctra* cf. *minuta* (Klapálek, 1901) and *Brachyptera vera cordubensis* Berthélemy and Baena, 1984.

*Tyrrhenoleuctra* is a Western Mediterranean stonefly genus composed by a complex of species that recently has begun to be clarified despite to the inexistence of morphological differences (Fochetti *et al.*, 2004, in press). The studied population could belong to *T. minuta* species, according with its distribution. The information on the *Tyrrhenoleuctra* biology is scarce. Different degree of ovoviviparity and the existence of nymphal diapause have been pointed out for *T. tangerina* (Navás, 1922) and *T. minuta* (Berthélemy, 1973).

Data on nymphal feeding show that this taxon behaves mainly as detritivorous (Tierno de Figueroa *et al.*, 2003b). *Tyrrhenoleuctra* usually inhabits temporary streams (including isolating pools), spanning from low (almost the sea level) to high altitude (1500 m a.s.l.) and its flight period expands from final winter to spring, although it is variable (Tierno de Figueroa *et al.*, 2003a).

*Brachyptera vera* Berthélemy and González del Tánago, 1983 is an endemism from the Iberian Peninsula that includes two subspecies, one of them, *B. vera cordubensis* present in our study area. No data are available regarding the biology of this species; only a winter flight period has been pointed out for it (Tierno de Figueroa *et al.*, 2003a).

The aim of this work is to increase the knowledge on nymphal biology (life cycle, growth, feeding behaviour and secondary production) of both species living in a seasonal stream. This information will be also useful for a better understanding of the adaptations of Nemouroidea species to this harsh habitat.

## 4.2. MATERIALS AND METHODS

The study was conducted in Río Despeñaperros (Sierra Morena, Jaén, Spain; UTM: 30SVH558476, 560 m a.s.l.), a Mediterranean seasonal stream. We collected samples monthly from November 2006 to June 2007, during the period in which it carried water, except in June, when we collected them biweekly, due to the nearness of the drought. We did not detect any pools during the dry period. It was not possible to sample on mid April due to a flood caused by an extremely high raining period, so this sample was taken at the end of the month. By mean of visiting the stream regularly we detected that the water arrived at the end of October, when the first pools started appearing. Moreover, we placed a datalogger in the riverbed (HOBO<sup>®</sup> Water Temp Pro, 0.001 °C accuracy) for recording the temperature hourly (Figure 4.1). Prior to the arrival of the water, the datalogger was exposed to the atmosphere, showing a great oscillation in temperature records. This oscillation started being absorbed when water arrived, so we could establish the beginning of the wet period, with a low margin of error, in October 23<sup>rd</sup> to 25<sup>th</sup>.

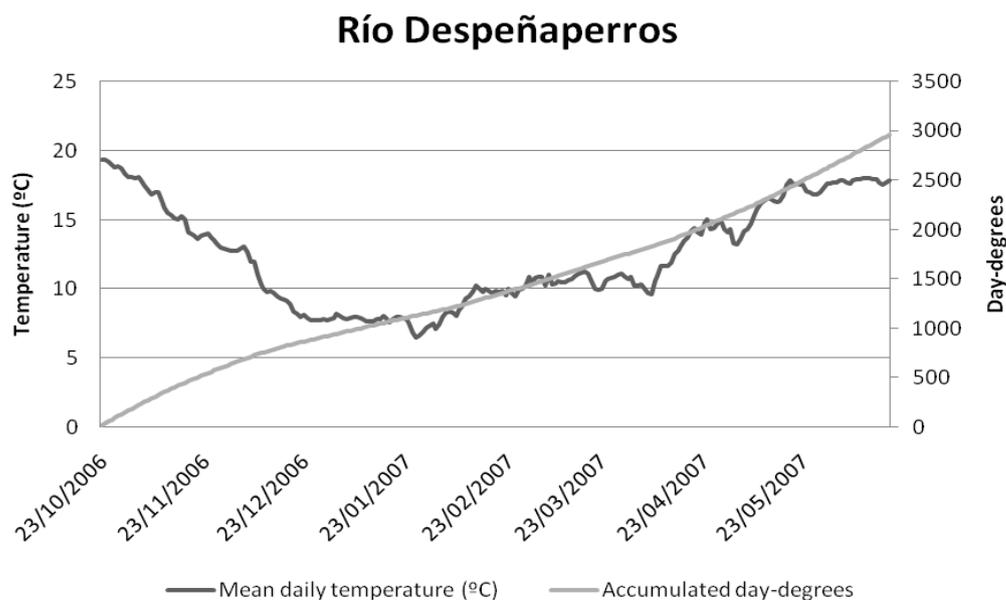


Figure 4.1. Mean daily temperature and accumulated day-degrees during the wet period.

We recorded *in situ* physical parameters, such as dissolved oxygen, conductivity and discharge, every sampling campaign. We also collected one liter of water that was cold preserved and transported to the laboratory for additional physicochemical characterization (Table 4.1).

	N	Mean	S.D.	Min.	Max.
pH	9	8.24	0.34	7.61	8.79
Ammonium (mg/l)	9	0.65	1.84	0.00	5.55
Phosphates (mg/l)	9	0.01	0.01	0.00	0.03
Nitrates (mg/l)	9	0.03	0.04	0.00	0.11
Nitrites (mg/l)	9	0.04	0.05	0.00	0.13
Sulfates (mg/l)	9	22.55	7.26	10.14	33.24
Chlorides (mg/l)	9	49.10	8.70	36.40	68.25
Alkalinity (meq/l)	9	39.85	7.86	24.64	50.02
Ss (mg/l)	9	4.02	4.17	0.60	12.60
Ca (mg/l)	9	65.96	12.80	50.40	88.00
Mg (mg/l)	9	29.70	7.77	20.90	40.34
Hardness (mg CaCO <sub>3</sub> /l)	9	286.94	54.13	213.87	379.77
Turbidity (NTU)	9	1.33	0.87	0.40	3.20
O <sub>2</sub> (% sat)	9	69.11	23.75	13.00	92.00
O <sub>2</sub> (mg/l)	9	7.03	2.73	1.10	9.70
Temperature (°C)	5908	12.54	3.86	5.90	25.87
Conductivity (µS/cm)	9	454.89	69.43	359.00	553.00
Discharge (m <sup>3</sup> /s)	9	0.10	0.11	0.00	0.32

Table 4.1. Physicochemical parameters of the sampling site.

This stream has a typical Mediterranean typology, with a width during the sampling period that varied from 2.95 to 5.35 m, and a depth that ranged from 0.04 to 0.31 m. The substrate was mainly composed by approximately 85% of block and stones, 10% of gravels and 5% of sands and silt. There were some branches and trunks on the riverbed. During the spring and summer there were abundant Ranunculaceae and *Nasturtium* sp. Mosses were absent in the sampling site. The riparian vegetation was abundant at both sides of the stream, mainly represented by *Nerium oleander*, *Fraxinus* sp., *Berberis* sp., Poaceae, Umbelliferae and Compositae.

We collected samples by using a Surber sampler (0.09 m<sup>2</sup> area and 250 µm mesh size). For including the different mesohabitats, we took six replicates in each sampling date. Animals were preserved in 4% formalin and brought to the laboratory, where they were sieved with a 150 µm mesh size sieve in order to remove the excess of formalin and fine detritus. The stoneflies were sorted out and identified at species level.

We measured total length and pronotum width of 30 individual each month using the micrometer of a binocular microscope (0.01 mm accuracy). Measures were standardized by putting every individual between two slides. Due to the two measures were highly correlated (Gamma correlation higher than 0.76 for every case,  $p < 0.05$ ), we used total length for representing the life cycles of the studied species. All the collected individuals were classified into 1 mm intervals. The size-frequency graphs representing the life cycles were made with FiSAT II software (Gayanilo *et al.*, 2002).

Secondary production was calculated using the size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke, 1979; Benke and Huryn, 2006), due to no cohort could be clearly identified. Estimation of nymphal biomass was made according to the equation:

$$DW = a X^b$$

or, in natural logarithmic form:

$$\ln DW = \ln a + b \ln X$$

where  $DW$  = individual dry weight,  $X$  = total length,  $a$  = constant, and  $b$  = slope of the regression.

For estimating the regression equation, 30 formalin preserved specimens of each species were measured, dried at 60 °C for 24 hours and placed in a desiccator during 1 hour. After this, they were weighed to the nearest 0.000 mg using a Mettler mod. M3 microbalance.

The diet study was performed according to the methodology used by Bello and Cabrera (1999), as in other studies of stonefly feeding (e.g. Tierno de Figueroa *et al.*, 2006; López-Rodríguez and Tierno de Figueroa, 2006; Navarro-Martínez *et al.*, 2007; Fenoglio *et al.*, 2008). We used the same 30 individuals measured for studying the correlation between total length and pronotum width. Each individual was introduced in a vial with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. After this, they were mounted on slides for its study under the microscope. We estimated the percentage of the absolute gut content (at 40x), as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400x), as the area occupied for each component within the total gut content, using the microscope with an ocular micrometer. Mean, standard deviation, minimum and maximum were calculated. The species were classified into functional feeding groups (FFG) according to food sources and mechanisms of food acquisition (Cummins, 1973; Merritt and Cummins, 2006). We also studied the correlation between size of the nymphs and gut contents.

During the sampling campaign, adults of the two taxa were collected beating the vegetation with an entomological net and picking them directly from the stones. Some of these individuals were preserved in 70% ethanol for establishing the flight period together with the information obtained from collection of mature nymphs. Some adults were brought alive to the laboratory with the aim of obtaining egg clutches. Eggs obtained in laboratory were maintained in Petri dishes with stream water at room temperature.

For statistical analysis, STATISTICA software (StatSoft, 2005) was employed. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For the election of the proper statistical tests we followed Guisande González *et al.* (2006).

### 4.3. RESULTS

#### 4.3.1. Life histories

We collected nymphs of *Tyrrhenoleuctra* sp. in the benthos from November to final April, with a maximum of individuals in November, just after the arrival of the water (Figure 4.2). Mature nymphs were found on November, January and February, and adults were collected from December to March. It is outstanding the spread of the nymphs sizes along the sampling period.

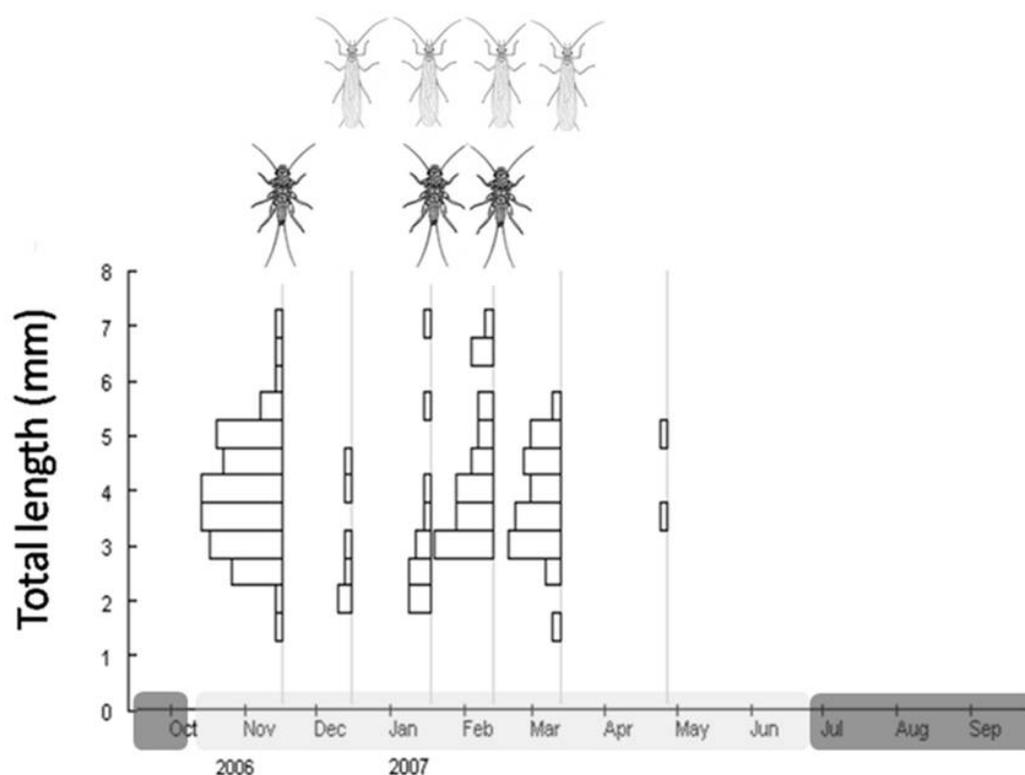


Figure 4.2. Size-frequency graph representing the life cycle of *Tyrrhenoleuctra* sp. (N= 200). Mature nymphs are marked with a nymph draw, and adults are represented by a macropterous adult draw. The period in which the stream was dry is marked darker in the horizontal axis.

Some of the adults were brought to the laboratory, where we put them in pots, both males and females, in order to let them copulate. We obtained two clutches of two different females, which were collocated in water. Microscope examination showed that the nymphs were fully developed within the eggs, with distinguishable eyespots. After few hours, first instar nymphs started appearing.

They were unpigmented, had not ocelli, and presented four cercal and nine antennal segments (Figure 4.3). They had a low number of bristles in the conjunction of every cercal segments. Interocelar distance varied from 0.12 to 0.13 mm, and total length ranged from 0.47 to 0.77 mm.



Figure 4.3. First instar nymph of *Tyrrhenoleuctra* sp. (life individual)

Observing the growth along the sampling period (Figure 4.4), we noticed that there was a relatively high growth rate from December to February, and also from March to the end of April, but in this case, due to the low number of individuals found in April, it is not significant. According with Figure 4.4, no relationship between growth and accumulated day-degrees was found.

In *B. vera cordubensis*, nymphs were present in the benthos from November to February (although very scarce in the last month) (Figure 4.5). Small nymphs were collected in November and December. We found the greatest quantity of small nymphs in the latter, but at the same time we collected both mature nymphs and adults. Mature nymphs were also found on January and February. As in the previous taxon, there was a high spread in the size of the nymphs.

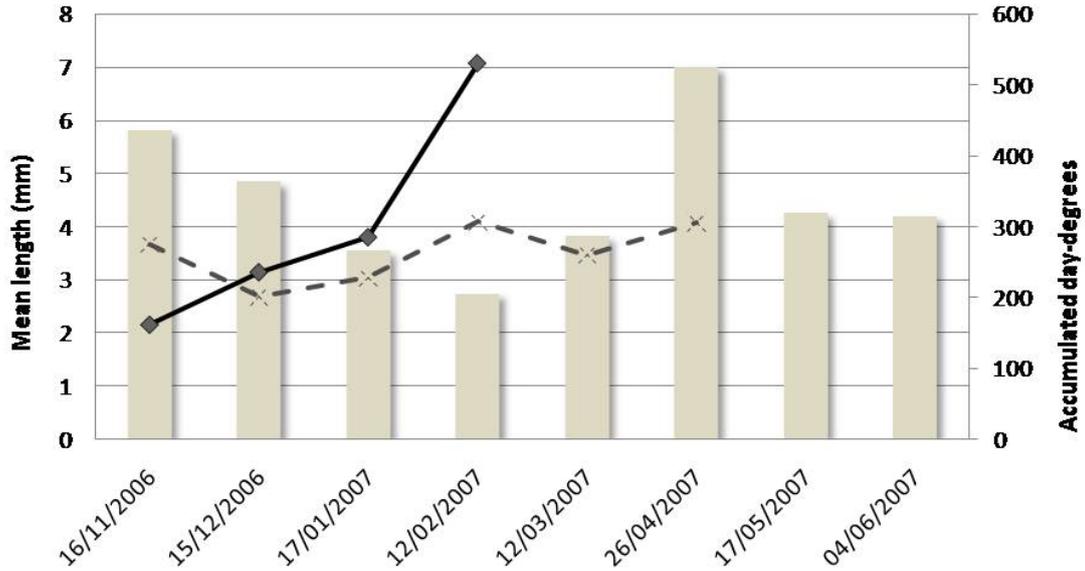


Figure 4.4. Growth patterns of *Tyrrhenoleuctra* sp. (grey dotted line) and *Brachyptera vera cordubensis* (black line) and histograms of the accumulated day-degrees during the interval between two sampling dates.

The growth of this taxon was very fast. Mature nymphs of January and February were probably the same nymphs found in great quantities in November and December (Figure 4.4), indicating an extremely fast growth rate. In this case there is a negative relation between day-degrees and growth.

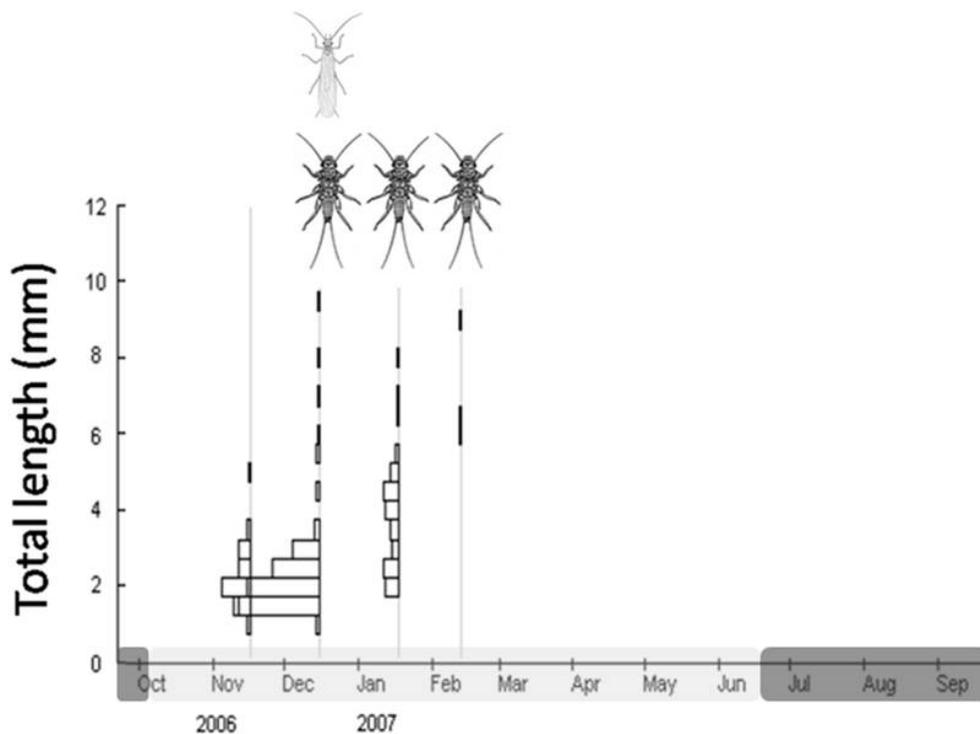


Figure 4.5. Size-frequency graph representing the life cycle of *Brachyptera vera cordubensis* (N= 204). Mature nymphs are marked with a nymph draw, and adults are represented by a macropterous adult draw. The period in which the stream was dry is marked darker in the horizontal axis.

### 4.3.2. Feeding

When we analyzed the gut contents of the studied taxa we observed that both fed mainly on detritus and diatoms, being the detritus more important in the diet of *Tyrrhenoleuctra* sp. and the diatoms in the diet of *B. vera cordubensis* (Table 4.2). Thus, we can catalogue to *Tyrrhenoleuctra* sp. as mainly gatherer-collector, but also with an important role as scraper. On the other hand, *B. vera cordubensis* was mainly scraper, also acting as gatherer-collector in a lesser concern. We found other components in the gut, such as Cyanobacteria, coarse particulate organic matter (CPOM) and fungal remains, but they were ingested less frequently.

Species		% absolute	% detritus	% diatoms	% hyphae	% fungi spores	% CPOM	% Cyanobacteria	FFG
<i>Tyrrhenoleuctra</i> sp.	N	104	77	77	77	77	77	77	Gatherer-collector/scraper
	Mean	43.09	52.96	38.12	1.78	0.18	2.96	4.25	
	SD	30.08	31.92	32.71	8.08	0.48	6.28	13.49	
	Min-Max	0-100	0-100	0-98	0-70	0-2	0-40	0-90	
<i>B. vera cordubensis</i>	N	95	75	75	75	75	75	75	Scraper/Gatherer-collector
	Mean	32.89	25.91	65.48	0.33	0.03	0.99	7.12	
	SD	27.83	34.03	38.06	1.44	0.16	3.91	19.12	
	Min-Max	0-100	0-100	0-100	0-10	0-1	0-25	0-90	

Table 4.2. Nymphal gut contents of the studied species in the sampling site. FFG = Functional Feeding Group.

If we correlate the size of the nymphs with the percentage of each component found in the gut we observe that in *Tyrrhenoleuctra* sp. there is no changes in food intake in relation to size. Nonetheless, in *B. vera cordubensis* there is a positive correlation between size and percentage of CPOM found in the gut (Table 4.3).

	<i>Tyrrhenoleuctra</i> sp. total length (mm)	<i>B. vera cordubensis</i> total length (mm)
% detritus	-0.01	-0.07
% diatoms	0.02	0.02
% hyphae	0.16	0.04
% fungi spores	0.10	0.10
% CPOM	0.11	0.52*
% Cyanobacteria	0.00	0.06

Table 4.3. Gamma correlations between total length and the percentage of the different food items in the studied species. Values marked with an asterisk are significant at  $p < 0.05$ .

### 4.3.3. Secondary production

In the studied taxa, dry weight ( $DW$ ) was related to body length ( $X$ ) by the following equations:

$\ln DW = -5.365 + 2.44 \ln X$ , ( $r^2 = 0.89$ ,  $F_{1,28} = 233.63$ ,  $p < 0.05$ ) for *Tyrrhenoleuctra* sp.

$\ln DW = -6.704 + 3.59 \ln X$ , ( $r^2 = 0.82$ ,  $F_{1,28} = 129.05$ ,  $p < 0.05$ ) for *B. vera cordubensis*.

We calculated the secondary production of both taxa during the sampling period (Table 4.4), so we used a cohort production interval (CPI) of 6 in *Tyrrhenoleuctra* sp. and of 4 in *B. vera cordubensis*. The former presented an annual secondary production of 280.78 mgDWm<sup>-2</sup>year<sup>-1</sup> with a cohort production/biomass rate (P/B) equal to 3.20. In the case of the latter, the annual secondary production was 1470.68 mgDWm<sup>-2</sup>year<sup>-1</sup> and the cohort P/B ratio equal to 8.74.

Species	Secondary production (mgDWm <sup>-2</sup> )	CPI (months)	Annual secondary production (mgDWm <sup>-2</sup> year <sup>-1</sup> )	Annual P/B (year <sup>-1</sup> )	Cohort P/B
<i>Tyrrhenoleuctra</i> sp.	140.39	6	280.78	6.40	3.20
<i>B. vera cordubensis</i>	490.23	4	1470.68	26.21	8.74

Table 4.4. Secondary production parameters of the studied species.

## 4.4. DISCUSSION

### 4.4.1. Life histories

In the study area, *Tyrrhenoleuctra* sp. seems to show a univoltine life cycle (Figure 4.2), in which growth occurs mainly during the wet period, with a possible nymphal dormancy during the drought period. As mentioned in the results section, we found first instar nymphs just some hours after the females laid down the eggs, indicating the oviposition of embryonated eggs, as has been cited by Berthélemy (1973) for this genus. This same author pointed out that species

belonging to the genus *Tyrrhenoleuctra* present a nymphal diapause, particularly after the fifth moult in *T. tangerina*. In our study area, some kind of dormancy would allow that the nymphs would pass part of the drought period in a resting stage, probably buried in the hyporheic zone. The species flight period was extended, from November to March. This long flight period (four to five months length) is a strategy commonly pointed out for temporary water faunas (Williams, 2006) and would allow the survival of the population in the case of an unpredictable drought. As a consequence of the long flight period (that implies a long period of oviposition), nymphs would be in a different stage of development when the adverse conditions arrive, because no synchronization was detected. Thus, they possibly go into a dormancy stage with different sizes that would provoke the wide size range that we found at the beginning of the wet period. So, the dormancy period probably is a quiescence controlled by environmental factors that in the study area could be photoperiod and/or temperature (because no nymphs appeared in the last two months of the wet period). The existence of a nymphal dormancy period in different instars have been recorded for *Capnia bifrons*, although in this case the author pointed it out as diapause, that usually undergo dormancy on fourth or fifth instars, but sometimes in third, sixth or even seventh instars (Khoo, 1964). Finally, taking into account that *Tyrrhenoleuctra* sp. nymphs that underwent dormancy were slightly developed, growth must be fast, lasting only a few months to be completed.

In *B. vera cordubensis* we detected a “fast-seasonal” life cycle according to the classification of Hynes (1970). We collected a very high number of first stages individuals during November and December. In the latter there were also mature nymphs and adults, probably indicating that nymphal development started some months before. From the presence of both mature nymphs and adults (although the latter were scarcely collected) we can say that this species has a relatively long flight period of approximately three months. After mating and oviposition, eggs would remain in the stream until the beginning of the next wet period, probably in egg diapause. Another possible strategy in this species could be the existence of a nymphal dormancy, as in the previous taxon. In Taeniopterygidae, nymphal diapause has been detected in the third, fourth, fifth or sixth instars (Harper and Hynes, 1970). Because we did not collect small nymphs during several months before the arrival of the drought, it is more

probable that the species has evolved for passing the drought in the egg stage. As pointed out by Hynes (1970), eggs in embryonic diapause are probably the stage in which species more easily face with drought. Furthermore, such a dormancy period has already been recorded for other species of the *Brachyptera* genus (Khoo, 1964; Harper and Hynes, 1970; Neumann, 1992). Nevertheless, egg diapause probably broke some time before the first collections and some nymphs were living in the hyporheic zone until the water table reached the surface due to the arrival of the rain. Though there is a great spread in the nymphal size, a most abundant cohort can be detected, corresponding to the one that finished its development in January and February. Thus, the growth of this cohort would be extremely fast, taking approximately 4 months. Such a rapid growth has been also detected in other species inhabiting temporary streams, including some Taeniopterygidae species as *Brachyptera galeata* Koponen, 1949 (four months) (Alouf, 1989) or *Rhabdiopteryx christinae* Theischinger, 1975 (also four months) (López-Rodríguez and Tierno de Figueroa, 2006).

#### 4.4.2. Feeding

As mentioned before, *Tyrrhenoleuctra* sp. behaved mainly as gatherer-collector, although with a great role as scraper too. A high amount of detritus in the guts was already pointed out by Tierno de Figueroa *et al.* (2003b) in a study of the feeding of all the species belonging to this genus. This would not support the classification of Leuctridae within a functional feeding group [for instance, Tachet *et al.* (1980) classified it as shredders] because, as shown by the great quantity of diatoms ingested, it is not possible to assign it to a single group since the presence of different types of food implies that no single feeding mechanism is involved (Stewart and Stark, 2002).

*B. vera cordubensis* would be mainly scraper. Similar feeding patterns were found for other *Brachyptera* species in North Africa by Azzouz and Sánchez-Ortega (2000). This can be a consequence of the filelike structure that this genus present in the galea, apparently used for scraping stones (Hynes, 1976). We found a positive correlation between CPOM content in the diet of this species and size, that can be related with the higher shredding power of bigger nymphs with reinforced mandibles. This subspecies would fulfil the category

assigned to its entire family (Tachet *et al.*, 1980), although, as in the previous case, non exclusive category can be assigned to it (Stewart and Stark, 2002).

Thus, for both taxa we found a generalist feeding behaviour, dominated by some components, but that included several others, as it is generally accepted for aquatic insects (Cummins, 1973).

#### 4.4.3. Secondary production

The annual secondary production of *B. vera cordubensis*, the species with a shorter life cycle, is significantly higher than that of *Tyrrhenoleuctra* sp., despite their biomass in the studied site is not very different. This is also shown in the extremely high value of the cohort P/B ratio of *B. vera cordubensis*, meaning that it produces more than eight times its biomass in each cohort. This value is even out of the range traditionally considered as normal for aquatic invertebrates (Benke, 1993; Huryn and Wallace, 2000), and probably is a consequence of the extremely fast development of this taxon in the studied site.

The obtained values on annual secondary production and annual P/B ratio of *Tyrrhenoleuctra* sp. do not differ from the range usually recorded for other Leuctridae species from European permanent waters (Krno, 1996, 1997; Krno and Šporka, 2003), and would correspond to the most frequent values found for gatherer-collectors (Benke, 1993). In the case of *B. vera cordubensis* these values are greatly higher than those reported for other Taeniopterygidae (e.g. Krno, 1997). If we consider the main functional feeding group in which this taxon can be included, the values of annual secondary production and annual P/B of the studied population belong to an interval little represented by aquatic invertebrates (Benke, 1993). This is due to scrapers, although often feeding on a high quality food, generally do not have especially high P/B values, as many of them are relatively large (Benke, 1993). In our study the species has both small size and fast growth, making the annual P/B actually high.

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**CHAPTER 5. Comparative study of the nymphal biology of *Habrophlebia eldae* and *Paraleptophlebia submarginata* as two coexisting Leptophlebiidae (Insecta: Ephemeroptera) in a Mediterranean stream from Southern Iberian Peninsula**





**ABSTRACT**

In this chapter we study the life history, nymphal feeding and secondary production of two leptophlebiid mayfly species [*Habrophlebia eldae* Jacob and Sartori, 1984 and *Paraleptophlebia submarginata* (Stephens, 1835)] that cohabit at the same site and present a very high niche overlap in terms of trophic resources. We discuss the possible effect of the interaction of one species on the other. They present a life cycle similar in duration, but displaced with respect to the other. Thus, when they coincide at the same time, they present different sizes. Annual secondary production of *P. submarginata* was much higher than that of *H. eldae*, and presented a quite similar annual P/B ratio, but slightly higher in *P. submarginata*. The study of the gut contents reveals that they are mainly detritivores, but when bigger they feed also on CPOM. They present an almost total niche overlap in terms of food acquisition, but the previously mentioned shift in trophic resources utilization makes possible that, due to no similar size classes of each species are present at the same time, niche segregation exists between the two species. This could be the consequence of previous episodes of competition between them, though now this competition is clearly reduced.

**KEYWORDS:** Mayflies, *Habrophlebia eldae*, *Paraleptophlebia submarginata*, life cycle, feeding, secondary production, niche overlap, Southern Spain.

## 5.1. INTRODUCTION

Life history of freshwater invertebrate species is conditioned by several factors that can be grouped into two broad classes, intrinsic factors (such as morphology, physiology, behaviour, etc.) and extrinsic factors (as temperature, photoperiod, nutrition, degree of habitat permanence and presence of other taxa) (Giller and Malmqvist, 1998). Regarding the latter, abiotic factors are determinant in species distribution and abundances, but interactions among taxa also play an important role (Allan and Castillo, 2007), and may have a reflection in the life history of the species. One of them can be the temporal separation of life cycles that is, particularly in mayflies, one of the most common mechanisms permitting coexistence among closely related species (Brittain, 1982), but other factors, such as nutrition, fecundity, predation pressure, and size are also important in allowing it (Brittain, 1972, 1980). In fact, closely related species that perform a similar trophic function may temporally separate growth and adult emergence within the same stream reach (Hauer and Stanford, 1982, 1986). The result of this coexistence may also affect secondary production dynamics of the taxa (Benke, 1984). In this context we have studied two mayfly species, *Habrophlebia eldae* Jacob and Sartori, 1984 and *Paraleptophlebia submarginata* (Stephens, 1835), both belonging to the Leptophlebiidae family, that coexist in the same site. The former is distributed in the South-Western of Europe, while the latter is widely present in Europe, the East-Palaearctic region and the Near East (Thomas and Belfiore, 2004). We have analyzed their life cycle, nymphal feeding habits and secondary production, and have estimated the niche breadth and overlap of both species in terms of trophic resources utilization, for trying to assess to what concern the life history of one species is affected by the presence of the other.

## 5.2. MATERIALS AND METHODS

The study was carried out in Río Fardes (Sierra de Huétor, Granada, Spain; UTM: 30SVG465413, 1200 m a.s.l.), a typical Mediterranean stream with permanent regime. In the sampling site, the width varied from 1.15 to 3.02 m

during the sampling period, and the depth ranged from 0.07 to 0.27 m. The substrate was mainly represented by a 50% of sands, 35% of pebbles and 15% of mud. Submerged vegetation was composed by *Nasturtium* sp. and Characeae. The riverside vegetation was abundant and principally represented by Juncaceae or Ciperaceae, *Salix* sp, Poaceae, *Equisetum* sp., *Mentha* sp. and some *Carduus* sp.

Samplings were carried out monthly from May 2006 to April 2007. A datalogger (HOBO® Water Temp Pro, 0.001 °C accuracy) was placed in the riverbed for registering the temperature hourly, and so calculating the accumulated day-degrees between two sampling dates (Figure 5.1). Every sampling date we recorded physical parameters *in situ* (oxygen, conductivity and discharge) and one litre of water was transported cold preserved to the laboratory for analyzing some physicochemical parameters (Table 5.1).

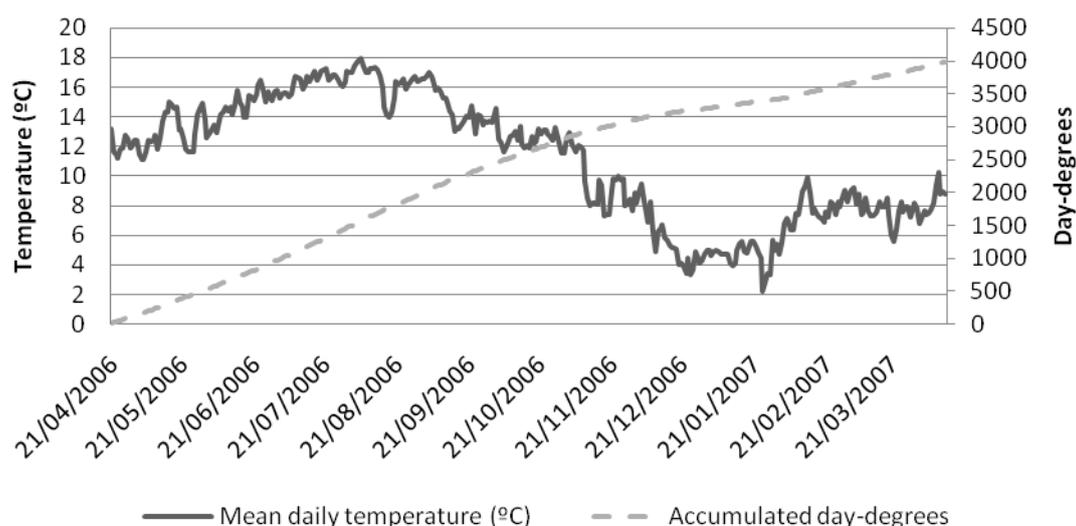


Figure 5.1. Mean daily temperature and accumulated day-degrees at the sampling site during the sampling period.

Individuals of both species were collected with a Surber sampler (0.09 m<sup>2</sup> area and 250 µm mesh size). Six replicates were taken for representing the different mesohabitats of each sampling site. They were preserved in 4% formalin and carried to the laboratory, where they were sieved with a 150 µm mesh size sieve in order to remove the excess of formalin and fine detritus. Afterwards, organisms were sorted out and identified at species level.

	N	Mean	S.D.	Min.	Max.
<b>pH</b>	12	8.05	0.46	7.03	8.61
<b>Ammonium (mg/l)</b>	12	0.01	0.01	0.00	0.02
<b>Phosphates (mg/l)</b>	12	0.01	0.01	0.00	0.05
<b>Nitrates (mg/l)</b>	12	0.01	0.01	0.00	0.05
<b>Nitrites (mg/l)</b>	12	0.50	1.01	0.03	2.85
<b>Sulfates (mg/l)</b>	12	27.25	20.70	2.43	61.98
<b>Chlorides (mg/l)</b>	12	21.37	9.94	7.10	39.05
<b>Alkalinity (meq/l)</b>	12	51.04	21.73	31.96	114.68
<b>Ss (mg/l)</b>	12	18.78	52.66	1.00	185.80
<b>Ca (mg/l)</b>	12	78.13	49.44	3.90	140.00
<b>Mg (mg/l)</b>	12	43.50	18.12	20.90	82.62
<b>Hardness (mg CaCO<sub>3</sub>/l)</b>	12	374.11	106.57	95.76	461.66
<b>Turbidity (NTU)</b>	12	2.31	1.74	0.00	6.51
<b>O<sub>2</sub> (% sat)</b>	12	85.08	5.43	76.00	95.00
<b>O<sub>2</sub> (mg/l)</b>	12	8.11	0.74	7.10	9.20
<b>Temperature (°C)</b>	8571	11.13	4.40	0.25	20.39
<b>Conductivity (µS/cm)</b>	12	428.08	102.73	104.00	474.00
<b>Discharge (m<sup>3</sup>/s)</b>	12	0.11	0.06	0.05	0.27

Table 5.1. Physicochemical parameters at the sampling site.

Each month we measured the total length and pronotum width of 30 individual (when possible) using the micrometer of a binocular microscope (0.01 mm accuracy). Due to these two measures were highly correlated (Gamma correlation > 0.86 in both species), we used total length for representing the life cycles of the studied species. All the collected individuals were classified into 1 mm intervals. Measures were standardized by putting every individual between two slides. We used FiSAT II software (Gayaniilo *et al.*, 2002) for generating the size-frequency graphs representing the life cycles.

Growth was calculated each month as the weighted mean of nymphal total length. Mean was weighted by the number of individuals in each size class.

The diet study was performed according to the methodology used by Bello and Cabrera (1999), as in other studies of Ephemeroptera nymphal feeding (e.g. Fenoglio *et al.*, 2008). We used the same 30 nymphs measured every month for correlating total length and pronotum width. Each individual was introduced in a vial with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. After this, they were mounted on slides for its study under the microscope.

We estimated the percentage of the absolute gut content (at 40x), as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400x), as the area occupied for each component within the total gut content, using the microscope with an ocular micrometer. Mean, standard deviation, minimum and maximum were calculated. From these data the species were classified into functional feeding groups (FFG) according to food sources and mechanisms of food acquisition (Cummins, 1973; Merritt and Cummins, 2006). We also studied the correlation between size of the nymphs and percentage of the different gut contents.

We used the Levins' index (Levins, 1968) for niche breadth, with the Hurbert's standardization (Hurbert, 1978), to assess if the studied species were more of less generalist. The scale of the standardized index varies between 0 and 1. The higher the value is, the higher the niche breadth, which indicates a more generalist condition. The Levins' index ( $B$ ) and the Hurbert's standardization ( $B_A$ ) were calculated as shown below:

$$B = 1 / (\sum p_j^2)$$

$$B_A = (B-1) / (n-1)$$

being  $p_j$  = proportion of items in the diet that are of food category  $j$

$n$  = number of possible resource states, evaluated as the whole resources observed in the gut content of all the individuals studied for each species.

We also calculated the niche overlap between the two studied species, in relation with food resources, by using the Simplified Morisita Index proposed by Horn (1966):

$$C_H = [2\sum_i^n p_{ij} \cdot p_{ik}] / [\sum_i^n p_{ij}^2 + \sum_i^n p_{ik}^2]$$

where  $C_H$  = Simplified Morisita Index of niche overlap between species  $j$  and  $k$ ,

$p_{ij}$  = proportion resource  $i$  is of the total resources used by species  $j$ ,

$p_{ik}$  = proportion resource  $i$  is of the total resources used by species  $k$ .

The index ranges from 0 (no overlap) to 1 (total niche overlap).

Secondary production was calculated by mean of the size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke, 1979; Benke and Huryn, 2006), due to the presence of many size classes at the same date. Estimation of nymphal biomass was made according to the equation:

$$DW = aX^b$$

or, in natural logarithmic form:

$$\ln DW = \ln a + b \ln X$$

where  $DW$  = individual dry weight,  $X$  = total length,  $a$  = constant, and  $b$  = slope of the regression.

For calculating the regression equations, 29 and 30 formalin preserved specimens of *H. eldae* and *P. submarginata* respectively were measured, dried at 60 °C for 24 hours and placed in a desiccator during one hour. After this, they were weighed to the nearest 0.000 mg using a Mettler mod. M3 microbalance.

For statistical analysis, STATISTICA software (StatSoft, 2005) was employed. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For the election of the proper statistical tests we followed Guisande González *et al.* (2006).

### 5.3. RESULTS

We captured nymphs of *H. eldae* from November to August. This species presented a univoltine life cycle, with egg hatching starting in November and mature nymphs captured on final June and July, with an average developmental time of approximately nine months (Figure 5.2). During this period, the main cohort accumulated 2622.81 day-degrees. Development took place between a mean daily temperature of 2 and 18°C (Figure 5.1). Growth was null during the

winter months, and rapidly increased at the beginning of spring and mainly in summer (Figure 5.3).

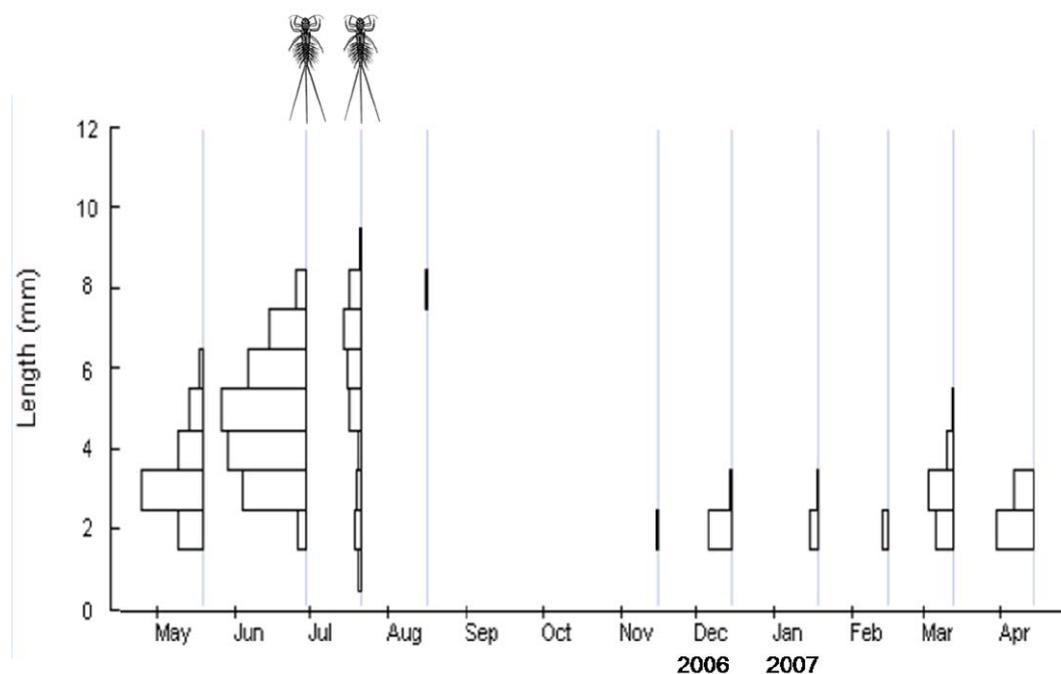


Figure 5.2. Size-frequency graph representing the life cycle of *Habrophlebia eldae* at the sampling site (N= 588). Nymph drawings represent presence of mature nymphs, with black wingpads.

The life cycle of *P. submarginata* was also univoltine, with nymphs present in the stream from May to April, probably representing several cohorts. Nymphal development of the main cohort lasted approximately nine months (from May to January). Egg hatching started in May and the maximum nymphal size was reached in January, probably representing the beginning of the flight period, although no mature nymphs were recorded in this case (Figure 5.4). After this peak, some smaller nymphs were still captured from February to April. The total amount of accumulated day-degrees by the main cohort from May to January was 3363.67. Development also occurred between a mean daily temperature of 2 and 18°C. Growth was approximately constant from May to January (Figure 5.3).

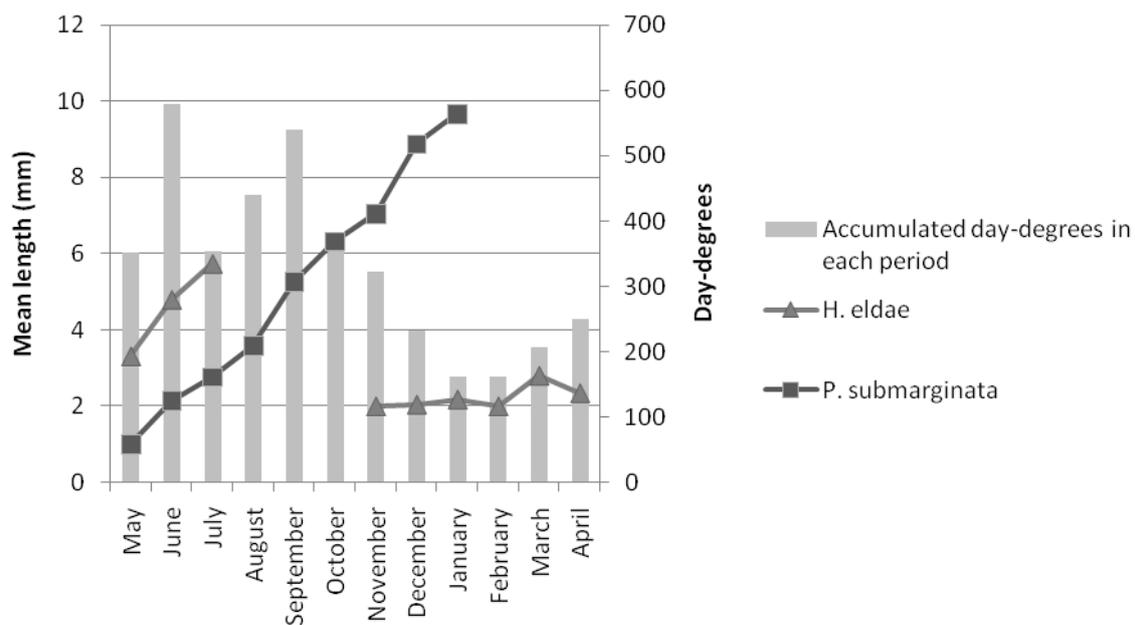


Figure 5.3. Growth patterns of *Habrophlebia eldae* and *Paraleptophlebia submarginata* and accumulated day-degrees between two consecutive sampling dates during the sampling period.

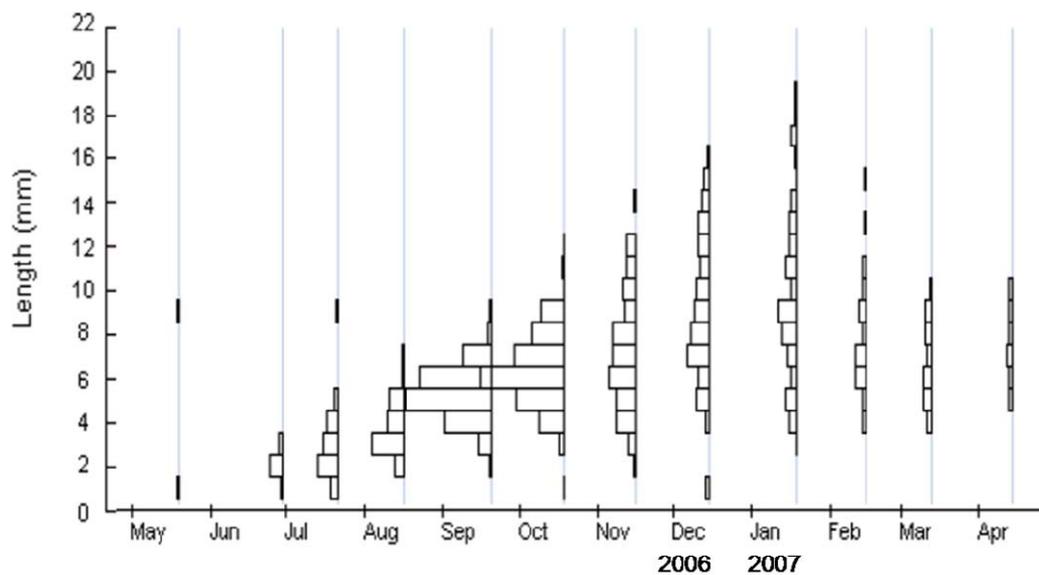


Figure 5.4. Size-frequency graph representing the life cycle of *Paraleptophlebia submarginata* at the sampling site (N=980).

Both species fed mainly on detritus and, in a very lesser concern, on coarse particulate organic matter (CPOM). Some other components were little represented in both species guts (Table 5.2). When we studied the correlation between percentage of gut contents and size we observed that in *H. eldae* detritus was less consumed by bigger nymphs, which incorporated a greater quantity of pollen, hyphae, CPOM and fungi spores (Table 5.3). On the other hand, *P. submarginata* also decreased the intake of detritus when bigger and increased the percentage of hyphae, CPOM and fungi spores. When we studied the niche breadth of both species we observed that they had very low values ( $B_A = 0.046$  for *H. eldae* and  $B_A = 0.055$  for *P. submarginata*). The niche overlap between both species in terms of trophic resources was almost total, with a Simplified Morisita Index ( $C_H$ ) equal to 0.99.

	<i>H. eldae</i>				<i>P. submarginata</i>			
	N	Mean	SD	Min-Max	N	Mean	SD	Min-Max
% absolute	196	59.08	27.10	0-100	294	66.62	23.20	0-100
% detritus	176	89.40	14.51	5-100	284	87.62	13.42	0-100
% diatoms	176	1.29	6.96	0-88	284	0.14	1.00	0-10
% hyphae	176	0.45	1.10	0-6	284	0.96	2.83	0-40
% fungi spores	176	0.45	1.11	0-8	284	0.39	1.10	0-10
% CPOM	176	7.43	9.33	0-60	284	9.30	9.99	0-50
% pollen	176	0.43	1.13	0-6	284	1.02	3.23	0-20
<b>FFG</b>	<b>Gatherer-collector/shredder</b>				<b>Gatherer-collector/shredder</b>			

Table 5.2. Nymphal gut contents of *Habrophlebia eldae* and *Paraleptophlebia submarginata* at the sampling site.

	<i>H. eldae</i> total length (mm)	<i>P. submarginata</i> total length (mm)
% absolute	-0.23*	0.08
% detritus	-0.47*	-0.47*
% diatoms	0.14	0.26
% hyphae	0.51*	0.48*
% fungi spores	0.37*	0.30*
% CPOM	0.51*	0.41*
% pollen	0.62*	-0.05

Table 5.3. Gamma correlations between total length and the percentage of the different food items (animal matter not included) in *Habrophlebia eldae* and *Paraleptophlebia submarginata*. Values marked with an asterisk are significant at  $p < 0.05$ .

Regarding secondary production of both species, we obtained the following equations linking dry weight ( $DW$ ) and body length ( $X$ ):

$$\ln DW = -6.07 + 3.05 \ln X, (r^2 = 0.92, F_{1,27} = 312.82, p < 0.05) \text{ for } H. eldae$$

$$\ln DW = -5.17 + 2.71 \ln X, (r^2 = 0.94, F_{1,28} = 477.04, p < 0.05) \text{ for } P. submarginata$$

We estimated the cohort production interval (CPI) of both species in 9 months, representing in each case an average cohort. The annual secondary production of *H. eldae* was equal to 2.08 gDWm<sup>-2</sup>year<sup>-1</sup>, while the one of *P. submarginata* was 27.36 gDWm<sup>-2</sup>year<sup>-1</sup> (Table 5.4). The cohort production/biomass ratio (P/B) was equal to 5.23 for *H. eldae* and to 6.13 for *P. submarginata*. Both species presented also an approximately similar annual P/B (6.97 and 8.17 for *H. eldae* and *P. submarginata* respectively).

Species	Secondary production (gDWm <sup>-2</sup> )	CPI (months)	Annual secondary production (gDWm <sup>-2</sup> year <sup>-1</sup> )	Annual P/B (year <sup>-1</sup> )	Cohort P/B
<i>H. eldae</i>	1.56	9	2.08	6.97	5.23
<i>P. submarginata</i>	20.52	9	27.36	8.17	6.13

Table 5.4. Secondary production parameters of *Habrophlebia eldae* and *Paraleptophlebia submarginata* at the sampling site.

## 5.4. DISCUSSION

The two studied species presented a univoltine life cycle, “slow-seasonal” following the classification by Hynes (1970). The hatching period of *H. eldae* seems to be relatively prolonged. Nymphs of first instars hatching during winter did not grow in this period (Figure 5.3), probably due to the low temperatures (Figure 5.1). After this, when temperatures started rising, growth rate was high. From the records of mature nymphs we estimate that the flight period was short and concentrated in final June and July. The only one nymph captured in August would represent an individual with delayed development. On the other hand, the hatching period of *P. submarginata* started in May and June, although further recruitment seemed to happen throughout the nymphal development (Figure 5.4).

This was reflected in the presence of mid-size nymphs after the peak of January (when emergence probably started), representing individuals of several cohorts. Growth of the main cohort was almost constant during the nymphal development (Figure 5.3), without clear differences between the period of the nymphal development that took place in spring-summer and the one that took place during autumn-winter. Thus, growth in this species seems to be relatively independent of the temperature.

A similar life cycle was found for *P. submarginata* by Alba-Tercedor (1981) in a close mountain system stream with similar characteristics. This author found nymphs from October to June, with a peak in size in February and March, although this author did not find mature nymphs until final April and June, and they were smaller in size to those of the peak. This supports that, in our study, flight period started after January. Landa (1968) also found a univoltine life cycle in central European populations of this species, but with nymphs reaching maturity in autumn, and continuing growing until the spring, when the adults emerged. This author classified to *P. submarginata* in the “A1” group, where are grouped winter species that develop during autumn and winter and with adults in spring, but our population would not completely fit on this or other group. Sowa (1975) pointed out that nymphs of this species were present in central European streams from August to April-May, when emergence occurred, and grouped it in the category “B2”, with species that grow during autumn and winter, though again our population would not fit on this group. In the British Isles this species presents also a univoltine life cycle with overwintering nymphs, and adults are present from April to July (Elliott and Humpesch, 1983; Elliott *et al.*, 1988), although in southern France the flight period expands from March to November (Lavandier and Dumas, 1971). Welton *et al.* (1982), in a experimental stream with temperatures oscillating between 8.1 and 16.8 °C, found a life cycle of seven months, with a higher growth rate during summer, and with an uncertain number of cohorts as in our studied population.

In relation with the feeding behaviour of the studied species, both were gatherer-collectors, feeding mainly on detritus, with a minor role as shredder of CPOM (Table 5.2). Pleskot (1953) already had pointed out that *P. submarginata* fed collecting detritus, as has been cited for other leptophlebiids (Elliott *et al.*,

1988), and concretely for other *Paraleptophlebia* spp. (Shapas and Hilsenhoff, 1976; Mattingli, 1987). We also observed that when the nymphs were bigger they fed less on detritus and more on other components, such as CPOM and fungi, probably due to their higher shredding power. It is outstanding that when the percentage of CPOM ingested is higher, the percentage of fungi hyphae and spores is also higher. This is probably due to they constitute, together with bacteria, the biofilm present in the leaves surface, which conditions this difficult to assimilate resource and contribute substantially to shredder nutrition (Allan and Castillo, 2007).

When we analyzed the niche breadth in terms of trophic resources we found that both species had very low values, indicating that they fed on few resources (mainly detritus, but also CPOM). Moreover, they showed a very high niche overlap in terms of food resources. So, they fed on the same few resources (detritus and CPOM), and they shared this behaviour, creating a proper situation for competing on food acquisition. Nevertheless, as when they cohabited they were in different developmental stages, and so presented different sizes, it is probable that the possible resource competition would be lower, due to one species fed mainly on detritus (because, as mentioned before, smaller nymphs fed on it) when the other fed mainly on CPOM.

On the other hand, there was a great difference in the values of annual secondary production of the studied species, being much higher in *P. submarginata*, in spite of having a similar life cycle duration and not being so different in number of captured individuals. This is probably related to its considerable bigger size. Nevertheless, if we observe the annual and the cohort P/B we realize that they were also higher, although only slightly, in *P. submarginata*, indicating that this species had a higher biomass turnover than *H. eldae*. The annual secondary production of *H. eldae* fit on the range of values reported for leptophlebiids (see González *et al.*, 2003), but that of *P. submarginata* is greatly higher.

Some aspects of the biology of close related species coexisting in the same site may be modified as consequence of direct competition on food and/or space. The relationship between competition and niche overlap is complex (Holt, 1987), and trophic niche overlap does not always imply competition, due to

resources may not be limiting for populations (Abrams, 1980), but is a good index of resource sharing between species. In our study two close related species inhabited in the same site, and also had low values of niche breadth and a high niche overlap, reflected in the approximately same use of food resources that both presented. As it was previously noted, there is no overlap of size classes between species at the same time, and it seems that the fitness of one of them, *P. submarginata*, is slightly higher than that of the other, taking into account the secondary production analysis results. Thus, it would be possible that the displacement of the nymphal development of one species with respect to the other was favoured by a lower competition for food resources. Considering that the same size classes in both species fed on the same resources, it could be possible that an interspecific competition between them would occur if their nymphal development would coincide. The pointed shifts in diet composition among size classes can result in considerable changes in microhabitats, enabling a different habitat choice among the different size classes of both species, as pointed out by Baekken (1981). This author considered this to reduce the interaction between nymphal stages within the same species and between two coexisting detritivores species, as it is our case. Such explanation could be also applied for interpreting the great numbers of nymphs with a wide size distribution that we found (Arnekleiv, 1996). These phenological differences in life histories have been also pointed out for several other aquatic insects (Giller and Malmqvist 1998). Our results also support the conclusions drawn by González *et al.* (2003) that pointed out that two coexisting species of leptophlebiids presented a low competition, and that hypothesized that if the species studied by them would coexist at the same site, temporal segregation in resource use would reduce interspecific competition.

A clear effect of one species on other is illustrated by Brittain (1982), who found that two *Leptophlebia* spp., which present essentially similar life cycles throughout Europe, displaced their life cycles “out of step” when they occurred together. These two species also presented differences in size at maturity, as it is our case. Thus, although the requirements of the species studied by us, in terms of developmental temperature needs, food utilization, etc., are surely the main factors that explain the different life cycle strategies that they present in the study

area, we cannot ignore the effect that a possible interaction between them would have had on their biology. Although now the possible competition between these two species has been reduced in the ways previously discussed, it is possible that these strategies are the consequence of ancient episodes of competition among them. This is difficult to establish from field data, and further studies under controlled conditions could throw light on this topic.

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**CHAPTER 6. Life history of two burrowing aquatic insects in Southern Iberian Peninsula: *Leuctra geniculata* (Insecta: Plecoptera) and *Ephemera danica* (Insecta: Ephemeroptera)**





**ABSTRACT**

Burrowing is a common behavioural adaptation of lotic freshwater invertebrates to avoid the effects of current. In this chapter we have focused in two burrower aquatic insects, the stonefly *Leuctra geniculata* (Stephens, 1836) and the mayfly *Ephemera danica* Müller, 1764, inhabiting and adapted to the interstitial environment. We have studied their life cycles and their relation with temperature and day-degrees, feeding and secondary production. The stonefly presented a univoltine life cycle of eight months, with an egg incubation period longer than previously reported. The possibility of an egg diapause stage is discussed. The mayfly was semivoltine, completing its nymphal development in 22 months. Both species fed mainly on detritus, but also ingested a high quantity of CPOM and some other minor components. Annual secondary production in both species was relatively high, being higher in the stonefly.

**KEYWORDS:** Stonefly, *Leuctra geniculata*, mayfly, *Ephemera danica*, life cycle, feeding, secondary production, Southern Spain.

## 6.1. INTRODUCTION

Current is the most significant characteristic of running waters (Hynes, 1970; Giller and Malmqvist, 1998), and so acts as an evolutive pressure on animals living on them. Freshwater invertebrates respond to it in several related ways. One of them is by mean of morphological adaptations, such as flattened forms, streamlining, the developing of attachment structures or mechanisms (such as suckers, hooks, silk or sticky secretions), etc. As a complement of them several behavioural adaptations exist, such as the reduction or avoidance of the flow exposure (Hynes, 1970). Thus, many aquatic insects spend at least part of their life deep in the substratum, constituting the stygobiotic fauna. Furthermore, this burrowing macroinvertebrates perform an important role because their activities help to redistribute particles and fluid in sediment-water interface, strongly influencing the physical, chemical and microbiological proprieties of the sedimentary deposits in which they live (Charbonneau and Hare, 1998).

In this chapter we have focused the study on two burrowing species of aquatic insects, *Leuctra geniculata* (Stephens, 1836) (Plecoptera, Leuctridae) and *Ephemera danica* Müller, 1764 (Ephemeroptera, Ephemeridae). The stonefly *L. geniculata* is widespread distributed in Central, West and South Europe (including the British Islands) and northern Africa (Tierno de Figueroa *et al.*, 2003; Fochetti and Tierno de Figueroa, 2004), and the mayfly *E. danica* is widely distributed in the West Palearctic region (Thomas and Belfiore, 2004). Both burrow deeply under stones and/or into the substrate (Consiglio, 1980; Elliott *et al.*, 1988; Hynes, 1976), and so present morphological adaptations to live there. In *L. geniculata* the main modifications are those of the antennae. Lestage (1920) has suggested that the peculiar processes on the antennae, which distinguish nymphs of this species from others of the genus, are an adaptation for burrowing. The mayfly *E. danica* posses modified mouthparts and particular head and prothoracic processes that would help in burrowing, and is hairy, which would help to keep fine particles of the surroundings out of its body, in order to prevent smothering (Hynes, 1970). This species builds U-shaped tunnels and creates water current with the aid of its gills (Eastham, 1939; Ladle and Radke, 1990).

The aim of this study is to increase the knowledge of some aspects of the nymphal biology of these two burrowing species in a stream where both coexist, such as their life cycles and the influence of temperature on them, nymphal feeding and secondary production, and discuss the results with the existing literature when available.

## 6.2. MATERIALS AND METHODS

The study was carried out in Río Fardes (Sierra de Huétor, Granada, Spain; UTM: 30SVG465413, 1200 m a.s.l.), a typical Mediterranean stream with permanent regime. In the sampling station, the width varied from 1.15 to 3.02 m during the sampling period, and the depth ranged from 0.07 to 0.27 m. The substrate was mainly represented by a 50% of sands, 35% of pebbles and 15% of mud. Submerged vegetation was composed by *Nasturtium* sp. and Characeae. The riverine vegetation was abundant and principally represented by Juncaceae or Ciperaceae, *Salix* sp, Poaceae, *Equisetum* sp., *Mentha* sp. and some *Carduus* sp.

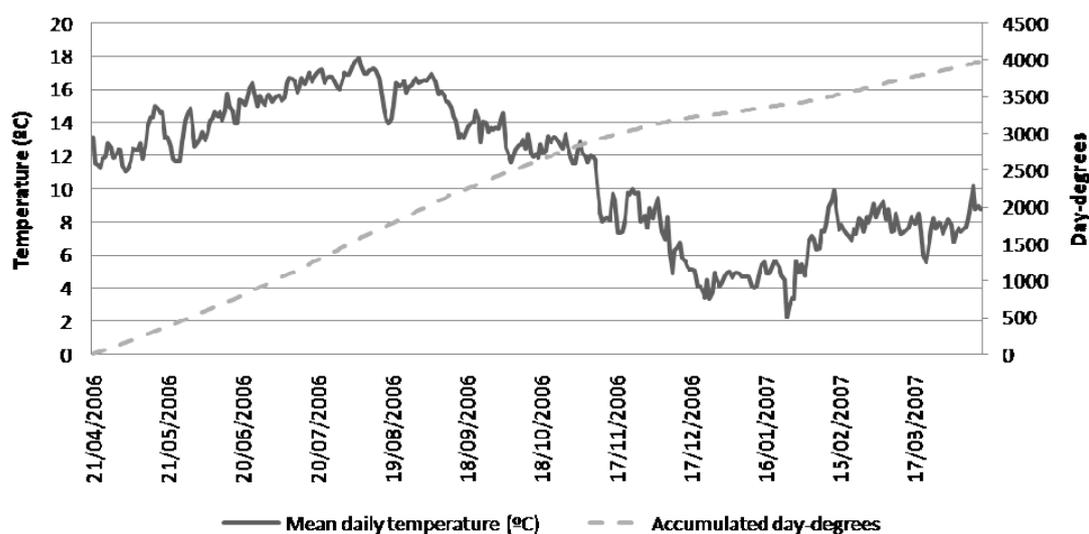


Figure 6.1. Mean daily temperature and accumulated day-degrees at the sampling site during the sampling period.

Samplings were carried out monthly from May 2006 to April 2007. A datalogger (HOBO® Water Temp Pro, 0.001 °C accuracy) was placed in the riverbed for registering the temperature hourly, and so calculating the accumulated day-degrees between two sampling dates (Figure 6.1). Every sampling date we recorded physical parameters *in situ* (oxygen, conductivity and discharge) and one litre of water was transported cold preserved to the laboratory for analyzing some physicochemical parameters (Table 6.1).

	N	Mean	S.D.	Min.	Max.
pH	12	8.05	0.46	7.03	8.61
Ammonium (mg/l)	12	0.01	0.01	0.00	0.02
Phosphates (mg/l)	12	0.01	0.01	0.00	0.05
Nitrates (mg/l)	12	0.01	0.01	0.00	0.05
Nitrites (mg/l)	12	0.50	1.01	0.03	2.85
Sulfates (mg/l)	12	27.25	20.70	2.43	61.98
Chlorides (mg/l)	12	21.37	9.94	7.10	39.05
Alkalinity (meq/l)	12	51.04	21.73	31.96	114.68
Ss (mg/l)	12	18.78	52.66	1.00	185.80
Ca (mg/l)	12	78.13	49.44	3.90	140.00
Mg (mg/l)	12	43.50	18.12	20.90	82.62
Hardness (mg CaCO <sub>3</sub> /l)	12	374.11	106.57	95.76	461.66
Turbidity (NTU)	12	2.31	1.74	0.00	6.51
O <sub>2</sub> (% sat)	12	85.08	5.43	76.00	95.00
O <sub>2</sub> (mg/l)	12	8.11	0.74	7.10	9.20
Temperature (°C)	8571	11.13	4.40	0.25	20.39
Conductivity (µS/cm)	12	428.08	102.73	104.00	474.00
Discharge (m <sup>3</sup> /s)	12	0.11	0.06	0.05	0.27

Table 6.1. Physicochemical parameters at the sampling site.

Individuals of both species were collected with a Surber sampler (0.09 m<sup>2</sup> area and 250 µm mesh size), removing the substrate with the aid of a rake reaching an approximate mean depth of 20 cm. Six replicates were taken for representing the different mesohabitats of each sampling site. They were preserved in 4% formalin and carried to the laboratory, where they were sieved with a 150 µm mesh size sieve in order to remove the excess of formalin and fine detritus. Afterwards, organisms were sorted out and identified at species level.

Each month we measured the total length and pronotum width of 30 individual (when possible) using the micrometer of a binocular microscope (0.01

mm accuracy). Due to these two measures were highly correlated (with a Gamma correlation of 0.91 for *L. geniculata*, of 0.94 for *E. danica*;  $p < 0.05$  in both cases), we used total length for representing the life cycles of the studied species. All the collected individuals were classified into 1 mm intervals. Measures were standardized by putting every individual between two slides. We used FiSAT II software (Gayaniilo *et al.*, 2002) for generating the size-frequency graphs representing the life cycles.

Growth was calculated each month as the weighted mean of nymphal total length. Mean was weighted by the number of individuals in each size class.

The diet study was performed according to the methodology used by Bello and Cabrera (1999), as in other studies of aquatic insects feeding (e.g. Tierno de Figueroa *et al.*, 2006; Fenoglio *et al.*, 2008). We used the same 30 individuals per months previously measured for the study of the correlation between pronotum and total length. Each individual was introduced in a vial with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. Afterwards, they were mounted on slides for its study under the microscope. We estimated the percentage of the absolute gut content (at 40x), as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400x), as the area occupied for each component within the total gut content, using the microscope with an ocular micrometer. Mean, standard deviation, minimum and maximum were calculated. From these data, when possible, the species were classified into functional feeding groups (FFG) according to food sources and mechanisms of food acquisition (Cummins, 1973; Merritt and Cummins, 2006). We also studied the correlation between size of the nymphs and percentage of the different gut contents.

Secondary production was calculated by mean of the size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke, 1979; Benke and Huryn, 2006), due to the presence of many size classes at the same date. Estimation of nymphal biomass was made according to the equation:

$$DW = aX^b$$

or, in natural logarithmic form:

$$\ln DW = \ln a + b \ln X$$

where  $DW$  = individual dry weight,  $X$  = total length,  $a$  = constant, and  $b$  = slope of the regression.

For calculating the regression equation, 30 formalin preserved specimens of each species were measured, dried at 60 °C for 24 hours and placed in a desiccator during one hour. After this, they were weighed to the nearest 0.000 mg using a Mettler mod. M3 microbalance.

For statistical analysis, STATISTICA software (StatSoft, 2005) was employed. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For the election of the proper statistical tests we followed Guisande González *et al.* (2006).

## 6.3. RESULTS

### 6.3.1. *Leuctra geniculata*

The life cycle of *L. geniculata* expanded from February to September, taking approximately eight months to complete the nymphal development (Figure 6.2). It presented a clear univoltine pattern. The flight period, estimated from mature nymph records, took place in August and September. After mating and oviposition, eggs would remain in the hyporheic zone up to five months, when hatching would start. Growth was relatively constant, with a slight increase in it during the last months, coinciding with the emergence (Figure 6.3). For completing its nymphal development, *L. geniculata* accumulated a total amount of 2884.30 day-degrees, and growth took place between an approximately mean daily temperature of 5.5 and 18 °C.

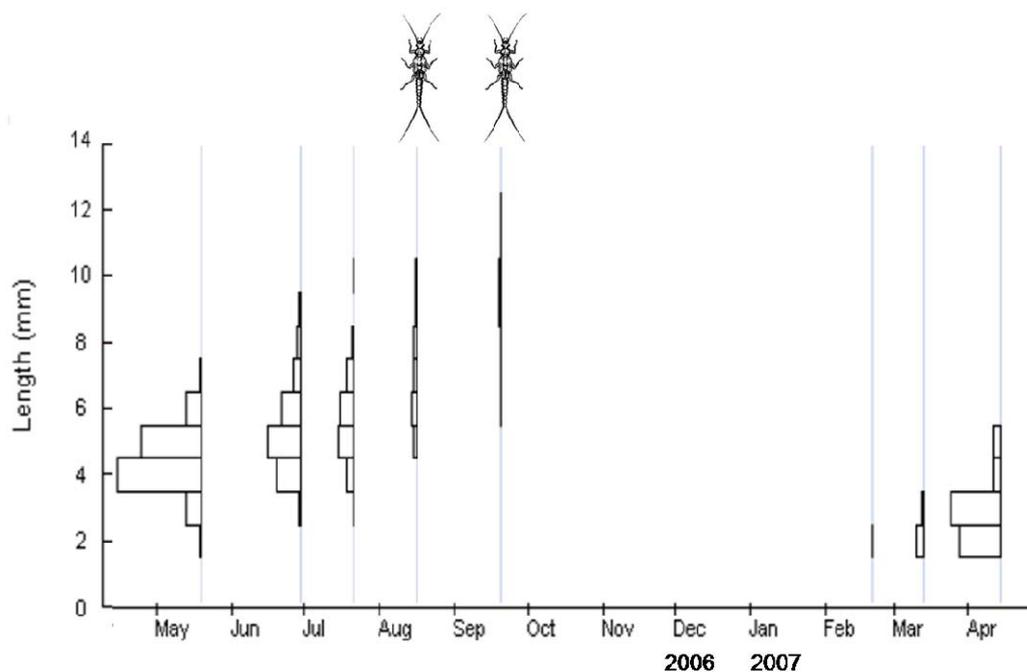


Figure 6.2. Size-frequency graph representing the life cycle of *Leuctra geniculata* at the sampling site (N= 1631). Presence of mature nymphs are marked with a nymph draw.

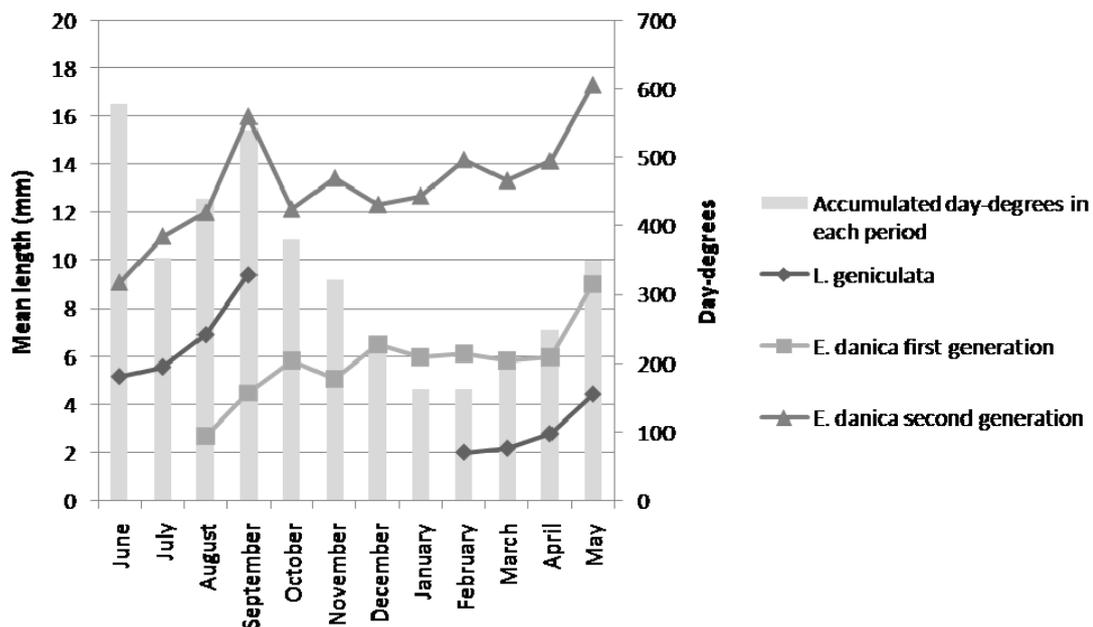


Figure 6.3. Growth patterns of *Leuctra geniculata* and *Ephemera danica* and accumulated day-degrees between two consecutive sampling dates during the sampling period.

The nymphs of *L. geniculata* fed mainly on detritus, but also ingested a great quantity of coarse particulate organic matter (CPOM) (Table 6.2). The other components found on their guts were scarcely represented. Less than 2 % of the

studied individuals contained some animal remains or sand. Some changes in diet composition appeared when nymphs were bigger. Thus, both detritus and diatoms decreased in bigger nymphs, and CPOM, pollen and Cyanobacteria increased (Table 6.3).

	<i>Leuctra geniculata</i>				<i>Ephemera danica</i>			
	N	Mean	SD	Min-Max	N	Mean	SD	Min-Max
% absolute	206	65.26	29.82	0-100	303	83.40	15.93	0-100
% detritus	182	73.23	26.85	10-100	299	81.20	13.58	8-100
% diatoms	182	1.10	2.79	0-20	299	0.05	0.33	0-4
% hyphae	182	0.89	1.96	0-20	299	0.55	1.21	0-10
% fungi spores	182	0.70	3.25	0-41	299	0.30	1.09	0-10
% CPOM	182	24.27	25.37	0-88	299	17.64	12.75	0-65
% pollen	182	0.20	0.81	0-6	299	0.03	0.22	0-2
% Cyanobacteria	182	0.02	0.18	0-2	299	0.02	0.18	0-2

Table 6.2. Nymphal gut contents of *Leuctra geniculata* and *Ephemera danica* at the sampling site.

	<i>L. geniculata</i> total length (mm)	<i>E. danica</i> total length (mm)
% absolute	0.06	0.24*
% detritus	-0.16*	-0.47*
% diatoms	-0.40*	-0.14
% hyphae	-0.05	0.07
% fungi spores	0.06	-0.18*
% CPOM	0.21*	0.53*
% pollen	0.28*	0.04
% Cyanobacteria	0.86*	-0.37

Table 6.3. Gamma correlations between total length and the percentage of the different food items (animal matter not included) in *Leuctra geniculata* and *Ephemera danica*. Values marked with an asterisk are significant at  $p < 0.05$ .

Dry weight ( $DW$ ) was related to body length ( $X$ ) by the following equation:

$$\ln DW = -6.39 + 3.10 \ln X, (r^2 = 0.95, F_{1,28} = 492.87, p < 0.05)$$

Production parameters are summarized in Table 6.4. The annual secondary production of *L. geniculata* was  $7.40 \text{ gDWm}^{-2}\text{year}^{-1}$ , and the cohort production/biomass ratio ( $P/B$ ) was 6.77. In our calculations we used a cohort production interval (CPI) of 8 months.

Site	Secondary production (gDWm <sup>-2</sup> )	CPI (months)	Annual secondary production (gDWm <sup>-2</sup> year <sup>-1</sup> )	Annual P/B (year <sup>-1</sup> )	Cohort P/B
<i>Leuctra geniculata</i>	4.93	8	7.40	10.15	6.77
<i>Ephemera danica</i>	10.24	22	5.59	2.80	5.13

Table 6.4. Secondary production parameters of *Leuctra geniculata* and *Ephemera danica* at the sampling site.

### 6.3.2. *Ephemera danica*

The mayfly *E. danica* presented a semivoltine life cycle, with two generations inhabiting at the same time (Figure 6.4). Nymphs hatched approximately in August, after the peak of annual temperature, and grew during 22 months to achieve the mature stage in May, almost two years later. Eggs laid by individuals emerging in May developed during two months for hatching on August. Growth was irregular throughout the nymphal development (Figure 6.3). This species accumulated 7033.71 day-degrees during its nymphal development, that happened between a mean daily temperature of 2 and 18 °C.

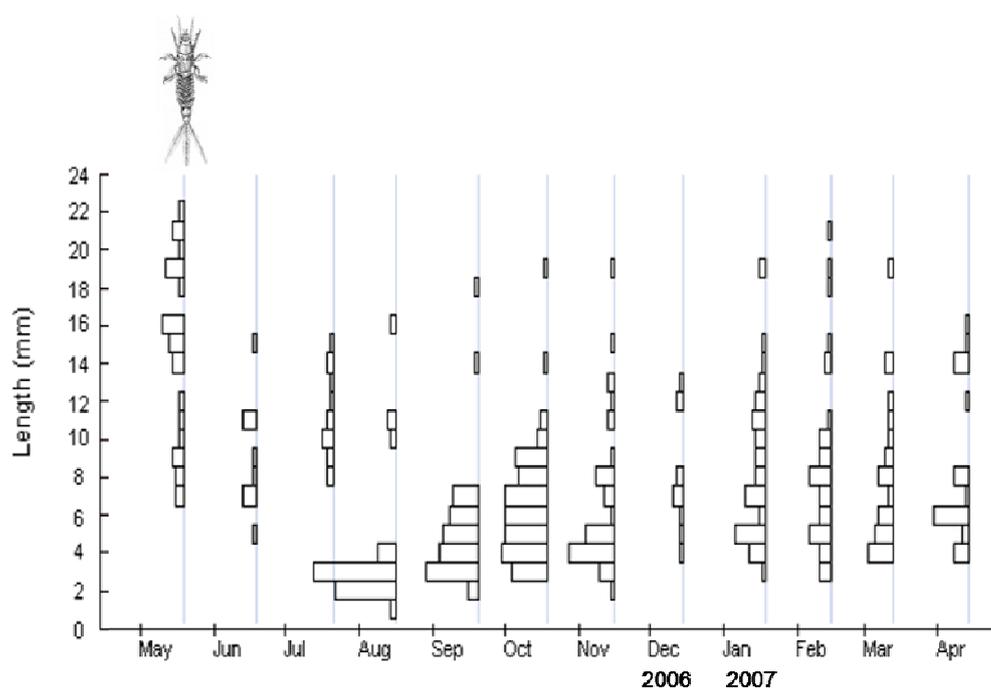


Figure 6.4. Size-frequency graph representing the life cycle of *Ephemera danica* at the sampling site (N= 442). Presence of mature nymphs are marked with a nymph draw.

This species also fed mainly on detritus, with a high ingestion of CPOM too, and other components present in a lesser concern (Table 6.2). As in the case of the prior species, we found some animal remains and sand in the gut of some nymphs, but these were represented in less than 2 % and 9% of the studied individuals respectively. Some ontogenetic shifts appeared in bigger nymphs too: a decreasing percentage of detritus and fungi spores and an increase in the ingestion of CPOM by bigger nymphs (Table 6.3).

Dry weight ( $DW$ ) and body length ( $X$ ) were related by the following equation:

$$\ln DW = -6.48 + 3.05 \ln X, (r^2 = 0.95, F_{1,28} = 503.95, p < 0.05)$$

The annual secondary production of *E. danica* was  $5.59 \text{ gDWm}^{-2}\text{year}^{-1}$  and the cohort P/B was 5.13. Taking into account the life cycle of this species we used a cohort production interval (CPI) of 22 months.

#### 6.4. DISCUSSION

The stonefly *L. geniculata* presented a typical univoltine life cycle that could be catalogued as “fast-seasonal” following Hynes’ (1970) classification, although this category requires the presence of a diapause stage during the embryonic development that in our population was not completely confirmed. Eggs started hatching approximately synchronically on February and March, coinciding with the beginning of the rising of temperatures in the stream (Figure 6.1). The temperature during this period coincided with that pointed out as optimum temperature for egg hatching for this species by Elliott (1987a) in an experimental study (with constant temperatures) in Great Britain, although this author pointed out that egg would hatch after accumulating 369 day-degrees, and in our case the eggs accumulated 1261.88 day-degrees. This fact could be related with the effect of natural fluctuating temperatures on egg development (Brittain, 1990), or, more probably, to be a consequence of the presence of a diapause stage in the studied population, as an adaptation to synchronize its

nymphal development with the favorable growing season, and for avoiding the low temperatures of winter. In fact, although egg hatching happened in February-March at relatively low temperatures, nymphal growth was greater from April-May (Figure 6.3), when temperatures were higher (Figure 6.1). Nevertheless, more studies focused on embryonic development are needed in this population to clarify it. Nymphal development was fairly synchronic, with little spread among size classes, and so an approximately clear cohort could be identified. Mature nymphs were collected just in two months (August and September), indicating a short emergence period and a flight period inside the limits of this species in the Iberian Peninsula (Tierno de Figueroa *et al.*, 2003). In fact, previous studies on adult stoneflies in the same mountain system showed an early autumnal flight period for this species (Luzón-Ortega *et al.*, 1998; López-Rodríguez *et al.*, 2004). A similar, univoltine life cycle was found by Ferreras-Romero and Agüero-Pelegrín (1994) in the Sierra Morena, a mountain system from South-Western Iberian Peninsula, although they hypothesized that some of the nymphs could remain in the stream a second winter, probably in quiescence, and emerge the following spring. Elliott (1987b) found also a univoltine life cycle but longer, with nymphal development lasting from October to August-September, with the smallest nymphs approximately coinciding in size with those found by us at the end of winter. These data could support the possible existence of an embryonic diapause in our study area that would explain the longer egg period found by us. Several authors have studied the life cycle of this species in several parts of its distribution (Hynes, 1941; Neveu *et al.*, 1979; Pařil *et al.*, 2008), always confirming the univoltinism. This pattern of univoltinism in our study area is also supported by the existence of a regular flight period in different years (Luzón-Ortega *et al.*, 1998; López-Rodríguez *et al.*, 2004; present data for 2006).

The life cycle of *E. danica* in the studied site was clearly semivoltine, “non-seasonal” following Hynes’ (1970) classification. It fit on the “A1” category of Sowa’s (1975) classification, where are placed species with semivoltine life cycle, short hatching period (one-two months) and long nymphal development (more than 20 months). Within Landa’s (1968) classification it can be catalogued within the “C1” group, where are all the mayfly species with a two year cycle. Eggs started hatching on August, just two months after oviposition by adults. Nymphs

grew during 22 months before reaching the mature stage. Growth was less evident during the winter period, and greater during spring and summer, when temperatures were higher (Figures 1 and 3). Egg hatching was approximately synchronic, but spread in nymphal size increased with time, mainly due to sexual dimorphism, which is more patent in bigger nymphs. Alba-Tercedor (1990) found a semivoltine pattern in which part of a population from Southern Iberian Peninsula developed in one year, while the other part lasted two years. The life cycle of the two year cohort was similar, but slightly delayed, to the found by us, probably due to the different thermal regime of the stream. A univoltine life cycle has been also pointed out by Aguayo-Corraliza *et al.* (1991) in a South-Western Iberian Peninsula stream. Several other studies have focused on the life cycle of *E. danica* throughout Europe (see Alba-Tercedor, 1990 for a review; Otto and Svensson, 1981; Tokeshi, 1985; Elliott *et al.*, 1988), indicating variations in its duration, from one to three years, and even variations among the same population, with different cohorts of the same generation lasting different time to complete the entire development (one-two years or two-three years).

Regarding feeding, both species are mainly detritivorous, with also an important role as fitophagous (Table 6.2). The ingestion of detritus by *L. geniculata* was already noted by Jones (1949), who pointed out that this species fed also on leaf fragments and green algae, although the amount of these components was very small. A similar result was found in a population of North Africa, where nymphs ingested mainly detritus but also vegetal matter (diatoms and algae), which supposed up to 30% of the diet (Azzouz and Sánchez-Ortega, 2000). Hynes (1941) indicated that this species showed a higher preference for algae than other species belonging to its genus, but this is not supported by our results, due to very few nymphs ingested diatoms. Thus, we can classify to *L. geniculata* as gatherer-collector, with also an important role as shredder.

In relation with *E. danica*, several studies on feeding coincides in that this species ingest mainly detritus [see Elliott *et al.* (1988) for a review]. The way of acquisition of this detritus is a more discussed matter. Otto and Svensson (1981) and Ladle and Radke (1990) pointed out that this species uses its forelegs to filter a water current produced by the respiratory movements of the gills through its burrow. Thus, the species would be filterer-collector, as pointed out by

Wallace and Merritt (1980) for some ephemerid mayflies. Other authors classify this species as gatherer-collector (Merritt and Cummins, 2006), so its assignation to a main FFG remains unclear. Apart from this, in our study the species also showed an important role as shredder of leaves that probably would obtain from the benthos, out of its tunnel.

Animal remains were found in the gut of few nymphs of both species, but its low proportion indicates accidental ingestion, probably when collecting detritus. This was also found by Hynes (1941) for *L. geniculata*. The same reason can be argued for the sand found in their guts.

In relation with the secondary production of *L. geniculata*, no previous data are available for what we know. In this stream we recorded a relatively high annual secondary production if we compare with other species of macroinvertebrates (Hurn and Wallace, 2000) and within gatherer-collectors, with also a high annual P/B (Benke, 1993).

Annual secondary production of *E. danica* was extremely similar to that obtained by Tokeshi (1985) in a British population ( $5.58 \text{ gDWm}^{-2}\text{year}^{-1}$  of this author's study *versus*  $5.59 \text{ gDWm}^{-2}\text{year}^{-1}$  in our study), although the annual P/B ratio was slightly higher in our population. If we compare our results with that compiled by Lee *et al.* (2008), we observe that the annual secondary production of our study is greater than that of the majority of multivoltine and univoltine species, including some members of the *Ephemera* genus. Poepperl (2000) also estimated the secondary production of two ephemerid species, finding lower values than ours. In relation to the FFG of this species in our study, both if we consider it as filterer-collector or gatherer-collector, the annual secondary production is relatively high, although the annual P/B would be among the more common values (Benke, 1993).

If we compare one species with the other, taking into account that the diet is approximately the same, we observe that both have similar values of annual secondary production despite of having very different life cycle duration. Also, the cohort P/B is similar and around 5, the most typical value for freshwater invertebrates (Benke, 1993). Nevertheless we observe that the annual production and the cohort P/B are slightly higher in *L. geniculata*, probably conditioned by

the life cycle duration, due to species with a two year life cycle exhibit lower P/B ratios than those with a one year or shorter life cycle (Waters, 1977).

In conclusion, we can see that these two species, belonging to different taxonomic group, make an optimum utilization of the same habitat. They greatly differ on life cycle duration, but share approximately the same diet and population dynamics (in terms of biomass production), maybe as a consequence of a convergence for exploiting the available resources.

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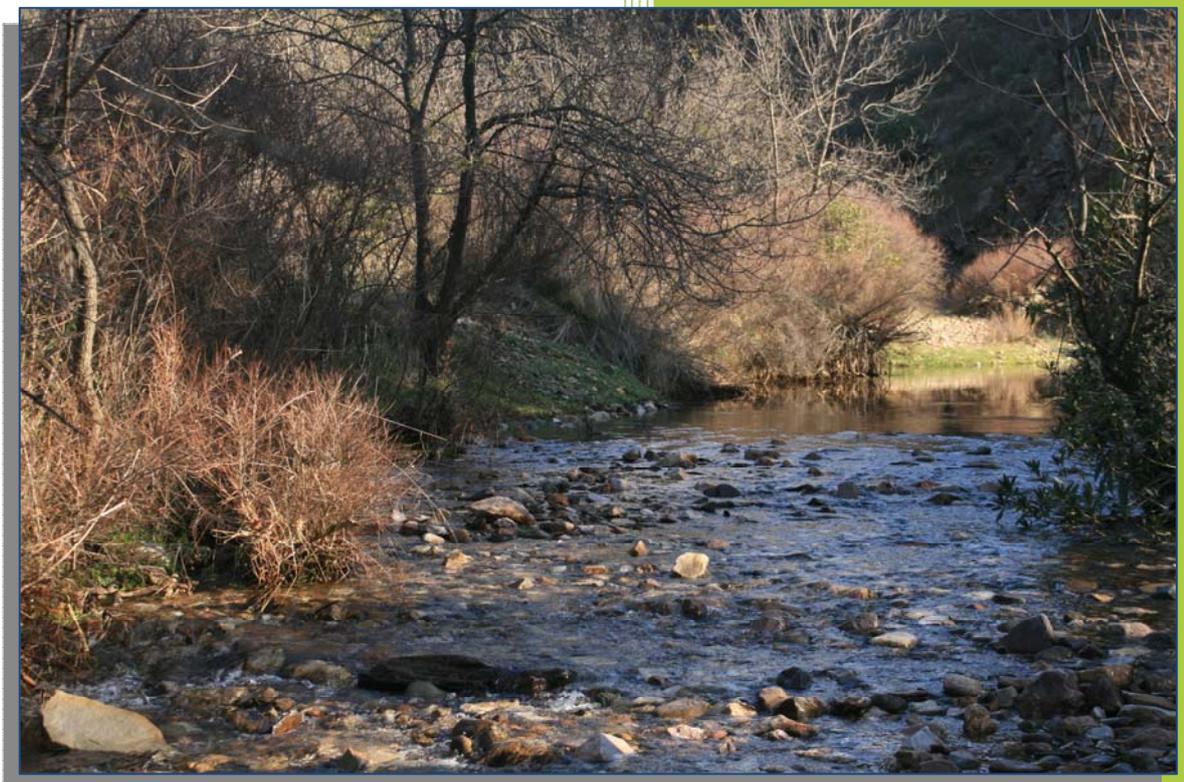
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**CHAPTER 7. Comparing permanent and temporary streams from Southern Iberian Peninsula: a case study on the life history of *Serratella ignita* (Insecta: Ephemeroptera), with some considerations about the effects of global climate change**





**ABSTRACT**

In mid latitudes as the Mediterranean ones, one of the consequences of the global climatic change will be a progressive shift of permanent to temporary water courses, altering the life histories of the present species or making them to disappear. Here is presented a study on the life history of a mayfly species, *Serratella ignita* (Poda, 1761), in two Mediterranean streams, one permanent and one seasonal. The research focuses on important parameters, such as life cycle, nymphal growth, trophic behaviour, biomass and secondary production. The life cycle shows a fast seasonal univoltine pattern during four (in the permanent stream) and five (in the seasonal one) months of nymphal growth, with a long egg stage with diapause. The accumulated day-degrees during the nymphal growth were similar between streams. Both populations behave mainly as gatherer-collector, but the one from the seasonal stream has also an important role as scraper. Animal matter was also consumed for nymphs from both populations. Gut content analysis shows that both *S. ignita* populations are opportunist/generalist and present changes in diet in relation with size. Biomass and secondary production were notably higher in the permanent stream, but they were also high in the seasonal one. These data show that *S. ignita* presents a high fitness in both habitats. Thus, our results support the idea that species such as this will cope the supposed future global climate change scenario maintaining, or even increasing, their populations at the expense of more stenoecic species.

**KEYWORDS:** *Serratella ignita*, mayfly, life cycle, feeding, secondary production, Southern Spain.

## 7.1. INTRODUCTION

It is widely accepted that the increase of carbon dioxide and other greenhouse gases in the atmosphere contributed to an increase in temperature over the past century, and that additional warming is expected to occur during the present century, as well as regional and seasonal variations in precipitation (In Allan and Castillo, 2007). The direct effects on aquatic ecosystems will be due to changes in temperature and flow regimes (Allan *et al.*, 2005). Future climatic scenarios predict more frequent and extended droughts, especially in the mid-latitudes (Boulton and Lake, 2008), and so a progressive shift from permanent to temporary water bodies, as a consequence of global climate change. This will be more evident in Mediterranean-type streams, which flow is mainly conditioned by the rainfall, and that exhibits a strong seasonal and annual variability (Gasith and Resh, 1999). Drought is a very severe disturbance because it results in community changes and may introduce different stresses on the ecosystem functioning (Grimm, 1993). Other consequences of this global change will be the rising of temperatures that will also affect greatly lotic ecosystems by, for example, declining the oxygen solubility, and so affecting species with high oxygen requirements (Grimm, 1993). Phenology and life history changes are means, apart from migration, of coping with higher temperature and associated changes in food quality (Sweeney *et al.*, 1992), and also with drought. The way this extended dry periods can alter life histories of organisms can be harmful, but also punctually beneficial, increasing the overall fitness of certain species (Ladle and Bass, 1981; Harper and Peckarsky, 2006). Within this particular context, at the species level, some characteristics are particularly advantageous under these future climatic conditions, such as long egg development, asynchronous egg hatching and nymphal development, temperature independence and flexibility in the life cycle, as well as a generalist condition (Brittain, 2008).

Permanent and temporary waters differ greatly in the factors that control the development and behaviour of the species that inhabit on them. Sometimes is difficult to make general assumptions on factors that control life histories in different habitats, such as permanent and seasonal streams, due to the strategies we found are related to some species that are not present in both

environments. In our study we have taken as a model a species of mayfly that is widely represented in these two type of habitats, *Serratella ignita* (Poda, 1761) (Ephemeroptera, Ephemerellidae), and also greatly distributed throughout the Palaearctic region (Thomas and Belfiore, 2004). This allows drawing conclusions on several aspects of its life history on both environments, such as growth and the influence of temperature on it, feeding, and success under some given conditions, estimated from the secondary production of the populations. Using the permanent stream as a model of the current climatic conditions and the seasonal one as a model of possible future conditions, we can draw some consequences of this new scenario on species such as that studied by us.

Thus, the aim of this chapter is to make a comparison between permanent and temporary waters (as models of current and future climatic conditions), using for it a mayfly species that is widely represented in this two type of habitats, discussing the influence of abiotic and biotic factors on its life history.

## 7.2. MATERIALS AND METHODS

The study was performed in two Mediterranean streams with different hydrological regime: Río Despeñaperros (Sierra Morena, Jaén, Spain; UTM: 30SVH558476, 560 m a.s.l.), a seasonal stream that carried water from the end of October 2006 to June 2007 (afterwards it dried out completely), and Río Fardes (Sierra de Huétor, Granada, Spain; UTM: 30SVG465413, 1200 m a.s.l.), a permanent one. Río Despeñaperros presented a width during the wet period that ranged from 2.95 to 5.35 m, and a depth that varied from 0.04 to 0.31 m. The composition of the substrate was approximately 85% of blocks and stones, 10% of gravels and 5% of sands and silt. There were some branches and trunks on the riverbed. During the spring and summer there were abundant Ranunculaceae and *Nasturtium* sp. Mosses were absent in the sampling station. The riparian vegetation was abundant at both sides of the stream, mainly represented by *Nerium oleander*, *Fraxinus* sp., *Berberis* sp., Poaceae, Umbelliferae and Compositae. On the other hand, Río Fardes had a width of 1.15 to 3.02 m during the sampling period, and the depth varied from 0.07 to 0.27 m. The substrate was

mainly represented by a 50% of sands, 35% of pebbles and 15% of mud. Mosses were absent, but *Nasturtium* sp. and Characeae were abundant. The riverine vegetation was well represented by Juncaceae or Ciperaceae, *Salix* sp, Poaceae, *Equisetum* sp., *Mentha* sp. and some *Carduus* sp.

Macroinvertebrate monthly samplings were carried out with a Surber sampler (0.09 m<sup>2</sup> area and 250 µm mesh size) from May 2006 until April 2007. Six replicates were taken for representing the different mesohabitats of each sampling site. They were preserved in 4% formalin and carried to the laboratory, where they were sieved with a 150 µm mesh size sieve in order to remove the excess of formalin and fine detritus. Afterwards, organisms were sorted out and identified at species level. In Río Despeñaperros, monthly samplings were carried out just during the wet period (from the end of October 2006 to June 2007), doing a biweekly sampling in June, due to the nearness of the drought period. In mid April it was not possible to sample because of a flood caused by an extreme high raining period, but we sampled it at the end of this month.

A datalogger (HOBO<sup>®</sup> Water Temp Pro, 0.001 °C accuracy) was placed in the riverbed for registering the temperature hourly, and so calculating the accumulated day-degrees between two sampling dates (Figure 7.1). Every sampling date we recorded physical parameters *in situ* (oxygen, conductivity and discharge) and collected one litre of water that was transported cold preserved to the laboratory for analyzing some physicochemical parameters (Table 7.1). Both streams presented very similar physicochemical characteristics, with only significant differences in nitrates, chlorides and hardness (Kolmogorov-Smirnov test,  $p < 0.05$ ).

Each month we measured the total length and pronotum width of 30 nymphs of *Serratella ignita* using the micrometer of a binocular microscope (0.01 mm accuracy). Due to these two measures were highly correlated (with a Gamma correlation of 0.88 in Río Despeñaperros population, and 0.87 in Río Fardes population, respectively;  $p < 0.05$  in both cases), we used total length for representing the life cycles of the studied species. All the collected individuals were classified into 0.5 mm intervals. Measures were standardized by putting every individual between two slides. We used FiSAT II software (Gayaniño *et al.*, 2002) for generating the size-frequency graphs representing the life cycles.

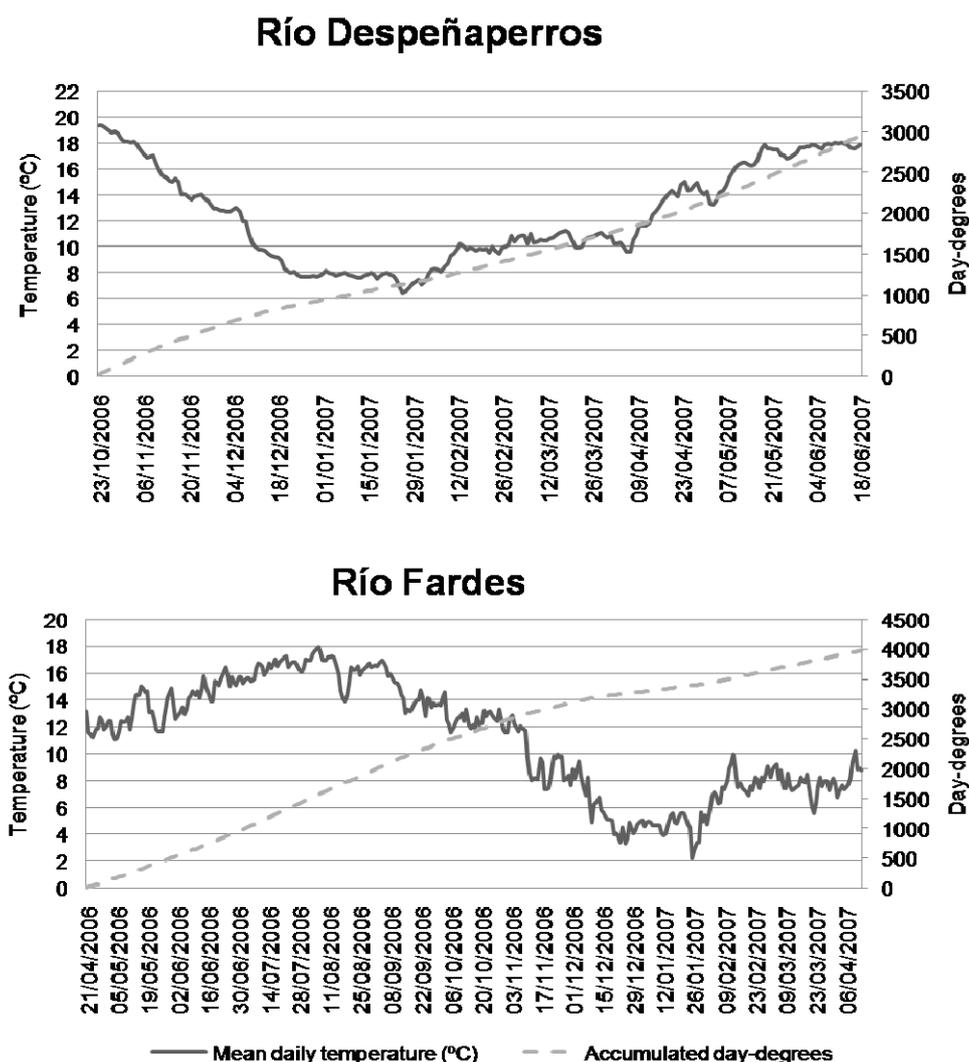


Figure 7.1. Mean daily temperature and accumulated day-degrees in both sampling sites during the sampling period.

Growth was calculated each month as the weighted mean of nymphal total length. Mean total length was weighted by the number of individuals in each size class.

The diet study was performed according to the methodology used by Bello and Cabrera (1999), as in other studies of mayflies feeding (e.g. Fenoglio *et al.*, 2008). We used the same 30 nymphs previously measured for the study of the correlation between pronotum width and total length. Each individual was introduced in a vial with Hertwigs' liquid and heated in an oven at 65 °C for approximately 24 hours. Afterwards, they were mounted on slides for its study

under the microscope. We estimated the percentage of the absolute gut content (at 40x), as the total area occupied by the content in the whole digestive tract, and the relative gut content (at 400x), as the area occupied for each component within the total gut content, using the microscope with an ocular micrometer. Animal matter was quantified by number of consumed individuals (from isolated sclerotized parts, i.e. heads, legs, etc.) and not by the occupied area. For non animal matter, mean, standard deviation, minimum and maximum were calculated. From these data, the species were classified into functional feeding groups (FFG) according to food sources and mechanisms of food acquisition (Cummins, 1973; Merritt and Cummins, 2006). We also studied the correlation between size of the nymphs and percentage of the different gut contents.

	Río Despeñaperros					Río Fardes				
	N	Mean	S.D.	Min.	Max.	N	Mean	S.D.	Min.	Max.
pH	9	8.24	0.34	7.61	8.79	12	8.05	0.46	7.03	8.61
Ammonium (mg/l)	9	0.65	1.84	0.00	5.55	12	0.01	0.01	0.00	0.02
Phosphates (mg/l)	9	0.01	0.01	0.00	0.03	12	0.01	0.01	0.00	0.05
Nitrates (mg/l)	9	0.03	0.04	0.00	0.11	12	0.01	0.01	0.00	0.05
Nitrites (mg/l)	9	0.04	0.05	0.00	0.13	12	0.50	1.01	0.03	2.85
Sulfates (mg/l)	9	22.55	7.26	10.14	33.24	12	27.25	20.70	2.43	61.98
Chlorides (mg/l)	9	49.10	8.70	36.40	68.25	12	21.37	9.94	7.10	39.05
Alkalinity (meq/l)	9	39.85	7.86	24.64	50.02	12	51.04	21.73	31.96	114.68
Ss (mg/l)	9	4.02	4.17	0.60	12.60	12	18.78	52.66	1.00	185.80
Ca (mg/l)	9	65.96	12.80	50.40	88.00	12	78.13	49.44	3.90	140.00
Mg (mg/l)	9	29.70	7.77	20.90	40.34	12	43.50	18.12	20.90	82.62
Hardness (mg CaCO <sub>3</sub> /l)	9	286.94	54.13	213.87	379.77	12	374.11	106.57	95.76	461.66
Turbidity (NTU)	9	1.33	0.87	0.40	3.20	12	2.31	1.74	0.00	6.51
O <sub>2</sub> (% sat)	9	69.11	23.75	13.00	92.00	12	85.08	5.43	76.00	95.00
O <sub>2</sub> (mg/l)	9	7.03	2.73	1.10	9.70	12	8.11	0.74	7.10	9.20
Temperature (°C)	5908	12.54	3.86	5.90	25.87	8571	11.13	4.40	0.25	20.39
Conductivity (µS/cm)	9	454.89	69.43	359.00	553.00	12	428.08	102.73	104.00	474.00
Discharge (m <sup>3</sup> /s)	9	0.10	0.11	0.00	0.32	12	0.11	0.06	0.05	0.27

Table 7.1. Physicochemical parameters of the sampling sites.

Secondary production was calculated by mean of the size-frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke, 1979; Benke and Huryn, 2006), due to the presence of many size classes at the same date.

Estimation of larval biomass was made according to the equation:

$$DW = aX^b$$

or, in natural logarithmic form:

$$\text{Ln } DW = \text{Ln } a + b \text{ Ln } X$$

where  $DW$  = individual dry weight,  $X$  = total length,  $a$  = constant, and  $b$  = slope of the regression.

For calculating the regression equation, 29 formalin preserved specimens of each sampling site were measured, dried at 60 °C for 24 hours and placed in a desiccator during 1 hour. Afterwards, they were weighed to the nearest 0.000 mg using a Mettler mod. M3 microbalance. We calculated one equation for each population.

For statistical analysis, STATISTICA software (StatSoft, 2005) was employed. None of the variables studied were normally distributed, thus non-parametric statistics were used in all cases. For the election of the proper statistical tests we followed Guisande González *et al.* (2006).

### 7.3. RESULTS

#### 7.3.1. Life histories

The life cycle duration was five months in Río Despeñaperros and four months in Río Fardes, although in the latter two individuals were collected also in September and other two in December 2006. Thus, the life cycle was univoltine and presented several cohorts in both cases.

In Río Despeñaperros the life cycle started in February 2007 and ended in June 2007, previous to the drought period (Figure 7.2). Mature nymphs were collected in May and June, indicating a mainly spring flight period. The spread of

the nymph size was very high in the last months, with individuals of very different size classes coexisting at the same time. After mating and oviposition, eggs would remain in the stream until the next year. Thus, this species would pass in the egg stage up to seven months. For its entire development, the nymphs needed a total amount of 1647.69 day-degrees.

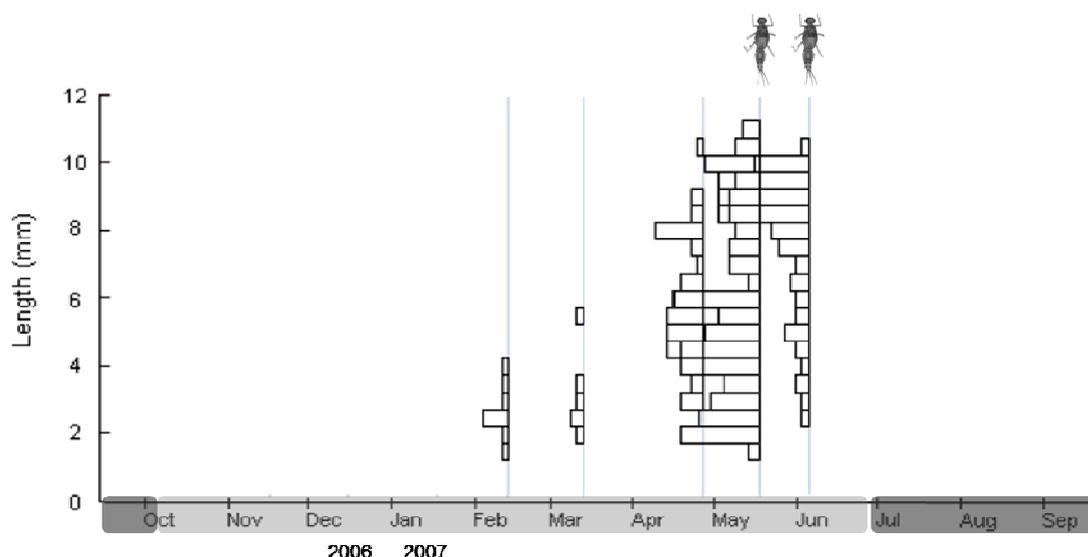


Figure 7.2. Size-frequency graph representing the life cycle of *Serratella ignita* in Río Despeñaperros (N= 295). Presence of mature nymphs is marked with nymph pictures. The period in which the stream was dry is marked darker in the horizontal axis.

In Río Fardes, nymphs were present in the stream from May to August 2006, with punctual presence in September (Figure 7.3). Only two nymphs were collected in December 2006. Mature nymphs were present from May to August, indicating a long fly period. There was also a great spread in the size of the nymphs, and an increasing mortality with the pass of the months. For reaching the adult stage, the nymphs accumulated a total of 1724.70 day-degrees.

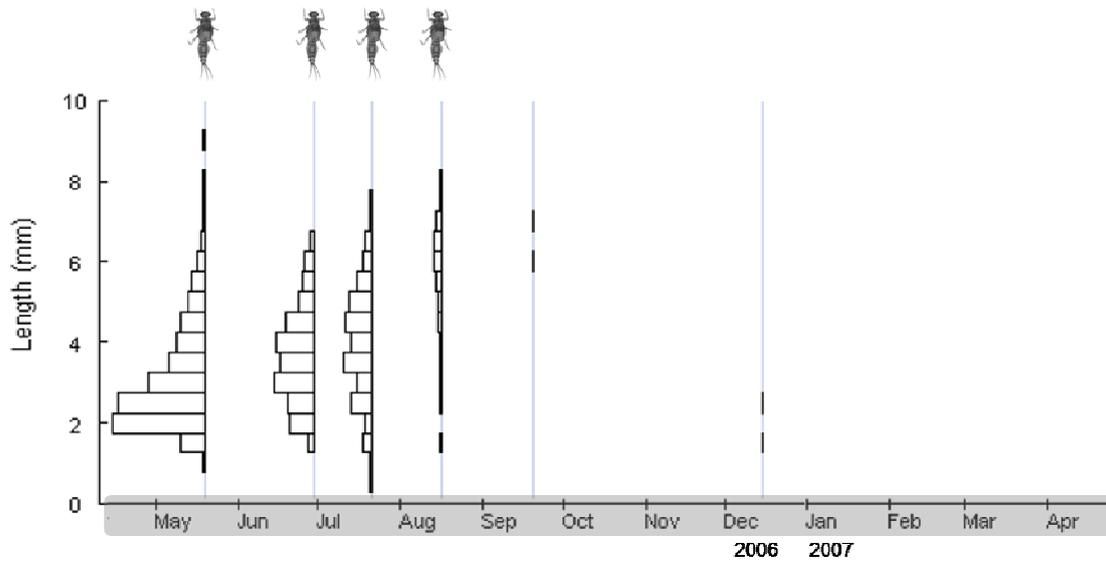


Figure 7.3. Size-frequency graph representing the life cycle of *Serratella ignita* in Río Fardes (N= 4865). Presence of mature nymphs is marked with nymph pictures.

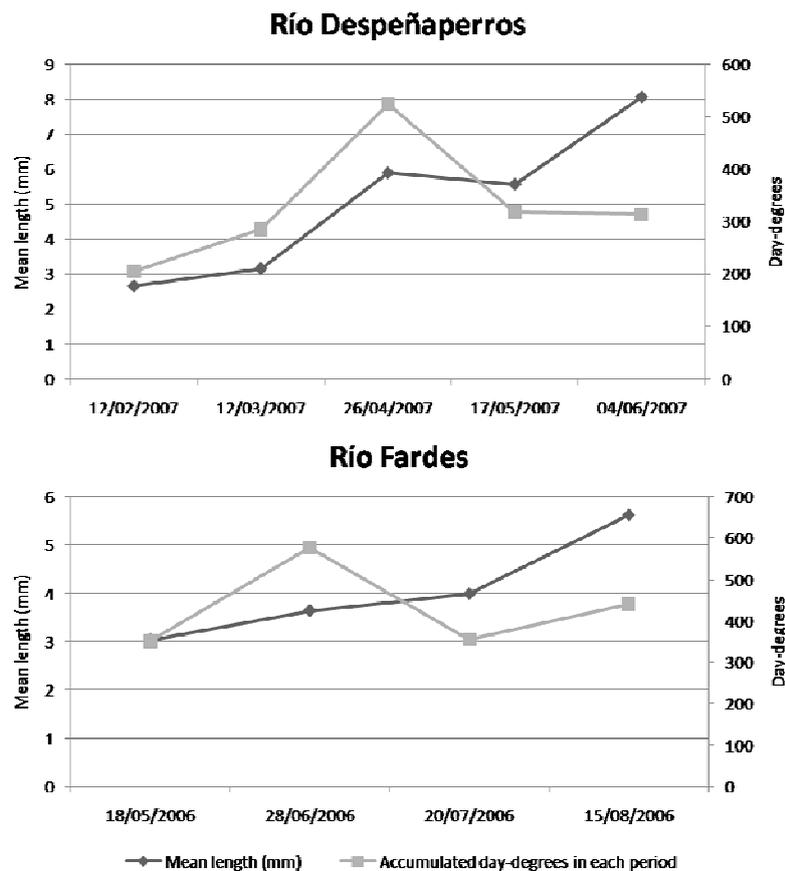


Figure 7.4. Growth pattern of *Serratella ignita* and accumulated day-degrees between two consecutive sampling dates in each stream.

When studying the relation between growth and accumulated day-degrees in each period, no significant correlation was found in none of the populations, although some trends can be observed during some parts of the nymphal development (Figure 7.4). In average, nymphal development took place between 10 and 18 °C in the Despeñaperros population, and between 11 and 18 °C in the Fardes population.

### 7.3.2. Feeding

Individuals of *S. ignita* of both populations fed mainly on detritus (Table 7.2), being the diatoms also greatly consumed in the Despeñaperros population. In both cases the species took a relatively low percentage of coarse particulate organic matter (CPOM), but in the case of the Fardes population this was the second more consumed resource. Some ontogenetic shifts were detected when correlating total length with the percentage of the different consumed resources (Table 7.3). In Despeñaperros population the percentage of hyphae decreased when bigger the nymphs, and the percentage of fungi spores and CPOM increased. In Fardes population the detritus was less consumed by bigger nymphs, which consumed more hyphae, fungi spores, CPOM and pollen.

	Río Despeñaperros				Río Fardes			
	N	Mean	SD	Min-Max	N	Mean	SD	Min-Max
% absolute	106	58.98	33.00	0-100	124	63.15	30.81	0-100
% detritus	93	60.52	31.53	5-100	114	88.30	12.36	10-100
% diatoms	93	29.83	30.65	0-95	114	2.37	4.74	0-25
% hyphae	93	0.19	0.49	0-2	114	0.71	1.25	0-5
% fungi spores	93	0.26	0.71	0-5	114	0.22	0.70	0-5
% CPOM	93	8.02	17.14	0-80	114	8.23	10.69	0-90
% pollen	93	0.68	2.23	0-20	114	0.22	0.68	0-4
% Cyanobacteria	93	0.44	2.81	0-25	-	-	-	-
<b>FFG</b>	<b>Gatherer-collector/scrapper</b>				<b>Gatherer-collector/shredder</b>			

Table 7.2. Nymphal gut contents of *Serratella ignita* in the sampling sites (animal matter has not been considered; see Methods). Bold letter in FFG column (Functional Feeding Group) represents the most important functional group into which the species can be assigned.

Individuals from both populations presented animal remains (Simuliidae and other non-identifiable animal matter), notably higher in the Despeñaperros population (9.43% of the studied individuals *versus* 2.42% of the Río Fardes studied individuals). These were always present in the gut together with non-animal matter. The presence of animal remains in the gut contents was not significantly correlated with the nymphal size ( $p > 0.05$  in both cases).

	<i>S. ignita</i> total length (mm) in Despeñaperros	<i>S. ignita</i> total length (mm) in Fardes
% absolute	-0.01	0.05
% detritus	-0.12	-0.31*
% diatoms	0.05	0.09
% hyphae	-0.29*	0.28*
% fungi spores	0.29*	0.33*
% CPOM	0.23*	0.33*
% pollen	0.12	0.26*
% Cyanobacteria	0.47	-

Table 7.3. Gamma correlations between total length and the percentage of the different food items (animal matter not included) in *Serratella ignita*. Values marked with an asterisk are significant at  $p < 0.05$ .

### 7.3.3. Secondary production

In *S. ignita* dry weight (*DW*) in each sampling site was related to body length (*X*) by the following equations:

$\text{Ln } DW = -6.22 + 3.40 \text{ Ln}X$ , ( $r^2 = 0.92$ ,  $F_{1,27} = 312.47$ ,  $p < 0.05$ ) for Río Despeñaperros population

$\text{Ln } DW = -6.19 + 3.35 \text{ Ln}X$ , ( $r^2 = 0.83$ ,  $F_{1,27} = 133.38$ ,  $p < 0.05$ ) for Río Fardes population

Production parameters appear in Table 7.4. The cohort production interval (CPI) was 5 in Río Despeñaperros and 4 in Río Fardes due to that only two nymphs were collected in September in the latter, not being significant. In Río Despeñaperros, *S. ignita* showed an annual secondary production of 11.21  $\text{gDWm}^{-2}\text{year}^{-1}$ , with a cohort production/biomass ratio (*P/B*) of 4.14. On the other

hand, in Río Fardes, the annual secondary production was  $48.95 \text{ gDWm}^{-2}\text{year}^{-1}$ , and the cohort P/B ratio was equal to 6.12.

Site	Secondary production ( $\text{gDWm}^{-2}$ )	CPI (months)	Annual secondary production ( $\text{gDWm}^{-2}\text{year}^{-1}$ )	Annual P/B ( $\text{year}^{-1}$ )	Cohort P/B
Río Despeñaperros	4.67	5	11.21	9.92	4.14
Río Fardes	16.32	4	48.95	18.37	6.12

Table 7.4. Secondary production parameters of *Serratella ignita* in the two sampling sites.

## 7.4. DISCUSSION

The two studied populations of *S. ignita* showed a very similar, short life cycle and a rapid growth, with the nymphal development lasting four or five months (in Fardes and Despeñaperros respectively), and little in advance in Río Despeñaperros, probably conditioned by the proximity of the drought at the end of the nymphal growth. In it, there was a particularly great spread in size in the last months, probably due to both sexual dimorphism and the recruitment of new hatched individuals. Some of these small nymphs probably did not have enough time to complete their development, due to the arrival of the drought period. The flight period occurred just before this, when the adults would oviposit. The eggs would remain in the hyporheos until the next year, probably passing the drought period and part of the wet period in a diapause stage. This stage has been already pointed out by several authors for this species in permanent streams (e.g. Maitland, 1965; Zelinka, 1984; Sowa, 1975; Böhle, 1972).

In Río Fardes, although it seems that the main growth occurs from May to August, probably corresponding to the main cohort, the presence of mature nymphs in the first months implies a rapid growth of some individuals that probably hatched some months before, but that were not collected by us.

Nymphs from December 2006 in Río Fardes were probably unsynchronized ones. The two nymphs collected in September corresponded to a few proportion of individuals that usually do not reach the normal mature nymphal size when emergence period occurs (Sweeney and Vannote, 1978; Vannote and Sweeney, 1980). The presence of nymphs several months after the

flight period (corresponding to a second failed generation) has been already pointed out by several authors (Pleskot, 1959; Hynes, 1961, 1970; Elliott, 1967, 1978; Alba-Tercedor, 1990; Chapter 2).

In both streams the species life cycle fulfil the characteristics of a “fast-seasonal” cycle according to Hynes’ (1970) classification. Within Landa’s (1968) classification it would be in the “A2” category corresponding to species with a long egg stage and a diapause during it, and that would hatch during spring or summer. Nevertheless, in Río Despeñaperros the cycle would be a little in advance due to the presence of a drought period during summer, and the diapause would occur mainly in this period. Following Sowa’s (1975) classification it would fit in the “B1” category that take into account the long egg development and the presence of a diapause stage during it.

The Río Fardes population presents a life cycle very similar to that found by the same authors in two close, high mountain streams of the Sierra Nevada, with different thermal regime (Chapter 2). In these higher sites, the species accumulated less day-degrees (1284.90 and 1500.19) along the nymphal development in comparison with Río Fardes stream, in spite of that it coincides on time (although in one of them, the one with less day-degrees accumulated by nymphs, the life cycle had a duration of only three months). Furthermore, in Río Despeñaperros, where the nymphs as a whole accumulated the highest amount of day-degrees in comparison with the other three streams, the nymphal development was the longest. It is expected that the development of the nymphs was faster in streams where the species reach before the necessary accumulated day-degrees, because the development of mayflies is temperature dependent (Brittain, 1990), but this is not completely supported by our data. This could be a consequence of the wide thermal requirements of the species during the nymphal stages (Belfiore, 1983). Thus, the life cycle could be mainly determined by the time the species passes in the egg stage, which seems to be a very important stage of the life cycle of this species, as well as in many others, given its duration (Clifford, 1982). Supporting that pointed out by Elliott and Humpesch (1980), we also found intraspecific differences in egg hatching in relation to temperature in both populations, reflected in the presence of small and mature nymphs throughout all the cycle, which would come from eggs that had

supported different temperatures. Nevertheless, the temperature at which hatching must take place is included in the range pointed out by Elliott and Humpesch (1980) and Humpesch (1984).

The presence of a diapause in these populations would not be an adaptation to particular conditions, such as drought, but would be a necessary prerequisite to complete the embryonic development, as pointed out by Böhle (1972) at least for some German populations. Thus, this would preadapt the species for living in changing and harsh environments, such as seasonal streams, and could contribute to its widespread distribution throughout the Palaearctic region. Nevertheless, in some other populations or under experimental conditions this diapause stage was not found (Böhle, 1972; Elliott, 1978), although this fact has been discussed (Elliott *et al.*, 1988).

If we observe the flight period of the two populations we realize that in the permanent stream is longer than in the seasonal one. *A priori* this could be seen as a contradiction with the typical patterns found in these types of environments (Dieterich and Anderson, 1995). Nonetheless, the presence of long flight period in species of aquatic insects inhabiting temporary waters that dry out during summer is not common in spring flight species (personal observations. See also Chapter 3 and 4 for a discussion on this topic), where a synchronic and short emergence period could be a better strategy in order to find mate before the drought period. Another typical characteristic of temporary water fauna is the small size, that would imply a faster development (Jacobi and Cary, 1996; Williams, 1996, 2006), but, once again, we do find the opposite in the studied populations. The seasonal stream population had a mean mature nymphal total length of  $9.39 \pm 0.89$  mm, while in the permanent stream population it was equal to  $6.02 \pm 0.53$  mm (see Figures 7.2 and 7.3). Furthermore, if we compare these data with that of the high mountain streams populations previously mentioned, we realize that the sizes are surprisingly similar between those populations and that of Río Fardes (the mean total length of the mature nymphs in those high mountain streams was  $6.10 \pm 0.47$  mm and  $6.10 \pm 0.17$  mm respectively). Another characteristic related to temporary water populations is the high fecundity (Jacobi and Cary, 1996; Williams, 1996, 2006), typical of *r* strategists that also usually present high population densities. Nevertheless, in the seasonal

stream, the total number of captured individuals was 295, while in the permanent stream it was 4865, both in an equal surface.

Regarding feeding behaviour, both populations can be catalogued as gatherer-collectors. In Río Despeñaperros, *S. ignita* would perform also an important function as scraper and, in a lesser concern, as shredder. The great amount of diatoms found in the gut contents of this population is probably related with their abundance in this site, and would suggest an opportunistic feeding condition, as usually pointed out for aquatic insects in general (Cummins, 1973; Monakov, 2003). The Fardes population would have also a minor role as shredder of CPOM, similar to that of the Despeñaperros one.

Despite not being quantified as occupied area, animal matter was not a main component of the gut content, and then it was not considered for the functional feeding group classification. The presence of some animal remains in the gut of these nymphs could be a consequence of accidental ingestion, as also suggested Riaño *et al.*, (1997), but the presence of several animals in the same gut could sign an active ingestion of them, at least occasionally. This probably unpremeditated carnivory may provide high-quality protein needed by many invertebrates to complete their life cycles (Anderson, 1976). In fact, the Simuliidae were one of the more abundant macroinvertebrates in the stream and could have been easily ingested by the nymphs when collecting detritus or scraping the diatoms on the stones surface, where the blackflies live. This would also support the opportunistic/generalistic feeding condition mentioned above (Cummins, 1973; Monakov, 2003) and by some authors particularly for temporary stream fauna (Jacobi and Cary, 1996; Williams, 1996, 2006).

As shown before, there were some changes in the gut content composition related with size. In both populations there was an increase in CPOM presence with size, probably related with the higher chewing capacity of higher nymphs, which would have more powerful mouthparts. This could be also related with the increase of fungi hyphae and spores in bigger nymphs (mainly in the Fardes population), due to the greatest part of them would be part of the biofilm on the leaves surface. This would lead to a higher efficiency in resource use because many aquatic insects obtain their nutrients from fungi and bacteria metabolism (Cummins, 1973).

In relation to the secondary production of the studied populations, both present very high values for being mainly gatherer-collectors species (Benke, 1993). It is notable the great amount of biomass produced by the Fardes population, almost four times higher than that of the Despeñaperros one. This was probably a consequence of the great number of individuals present in the former stream (N= 4865 *versus* N= 295 in Río Despeñaperros). Nevertheless, if we take into account the cohort P/B ratio the difference is not so marked. Both values are around five, which is the mean value usually found in freshwater invertebrates in general, but if we observe the annual P/B we can see that the values are relatively high for gatherer-collectors (Benke, 1993), although medium if we compare them with the rest of freshwater macroinvertebrates (Huryn and Wallace, 2000). Some authors have studied the secondary production of this species in other parts of Europe (see review in Elliott *et al.*, 1988; González *et al.*, 2000), providing a wide range of values for the species. Comparing with them, our values are very high, even that of the seasonal stream, what can be related to the fitness of the species to this habitat.

As has been pointed out by several authors (e.g. Butler, 1984; Hynes, 1970), the main factors controlling nymphal growth are temperature and food supplies. In temporary waters the water level is also important in regulating growth (Williams, 2006). As we have seen, the studied populations of *S. ignita* developed among a similar range of temperatures in both streams (between 10 and 18 °C in Río Despeñaperros and 11 and 18 °C in Río Fardes), and accumulated approximately the same quantity of day-degrees to complete the cycle, being slightly higher in the permanent one. On the other hand, both populations behaved mainly as gatherer-collectors, although the one of Despeñaperros fed also on diatoms, a more energetic and easily assimilable resource (Benke and Wallace, 1980). This should be reflected in a higher secondary production for the population of Río Despeñaperros, and also in a shorter life cycle, but this is not supported by our results. Contrary, we found a longer cycle and a lower secondary production in the seasonal stream, where the nymphs fed also in a more energetic resource. This can be related with the relatively harsh conditions imposed by the seasonality compared with those found in permanent streams. Nevertheless we found relatively high values of

secondary production in the Río Despeñaperros population, indicating that this population is well adapted to this environment, given that secondary production is the most comprehensive measure of success for a population (Benke, 1993). When comparing with the population of the permanent stream we realize that this fitness must be constrained by some environmental factor different to temperature and food, probably water level and oxygen content variations (Table 7.1). Thus, this comparative study gives a wider vision on the factors that control life histories and the success of the species in different environments, and may provide a natural experiment for assessing the conditions to which some species will probably be submitted under a future global climate change scenario, as well as how they will be able to evolve. Within this scene, with droughts occurring more frequently, some species, such as *S. ignita*, would achieve a relatively high fitness to the new conditions, as has been already pointed out by Ladle and Bass (1981). Furthermore, as we have mentioned previously, the typical characteristics of temporary waters fauna are present in the permanent stream population, what would also preadapt it to the supposed future conditions of seasonality. It is important to note that these conclusions can be only applied to a reduced group of species that, as the species studied here, presents a number of preadaptations to drought, as egg diapause, long egg development and/or a short life cycle. For others with longer cycles or without resistance stages, the consequences cannot be so gentle. Thus, the study of a higher number of species, representing different kind of adaptations and preadaptations, will let to know not only which species will probably disappear or will be threatened by global change, but also which ones could increase their populations at the expense of these.

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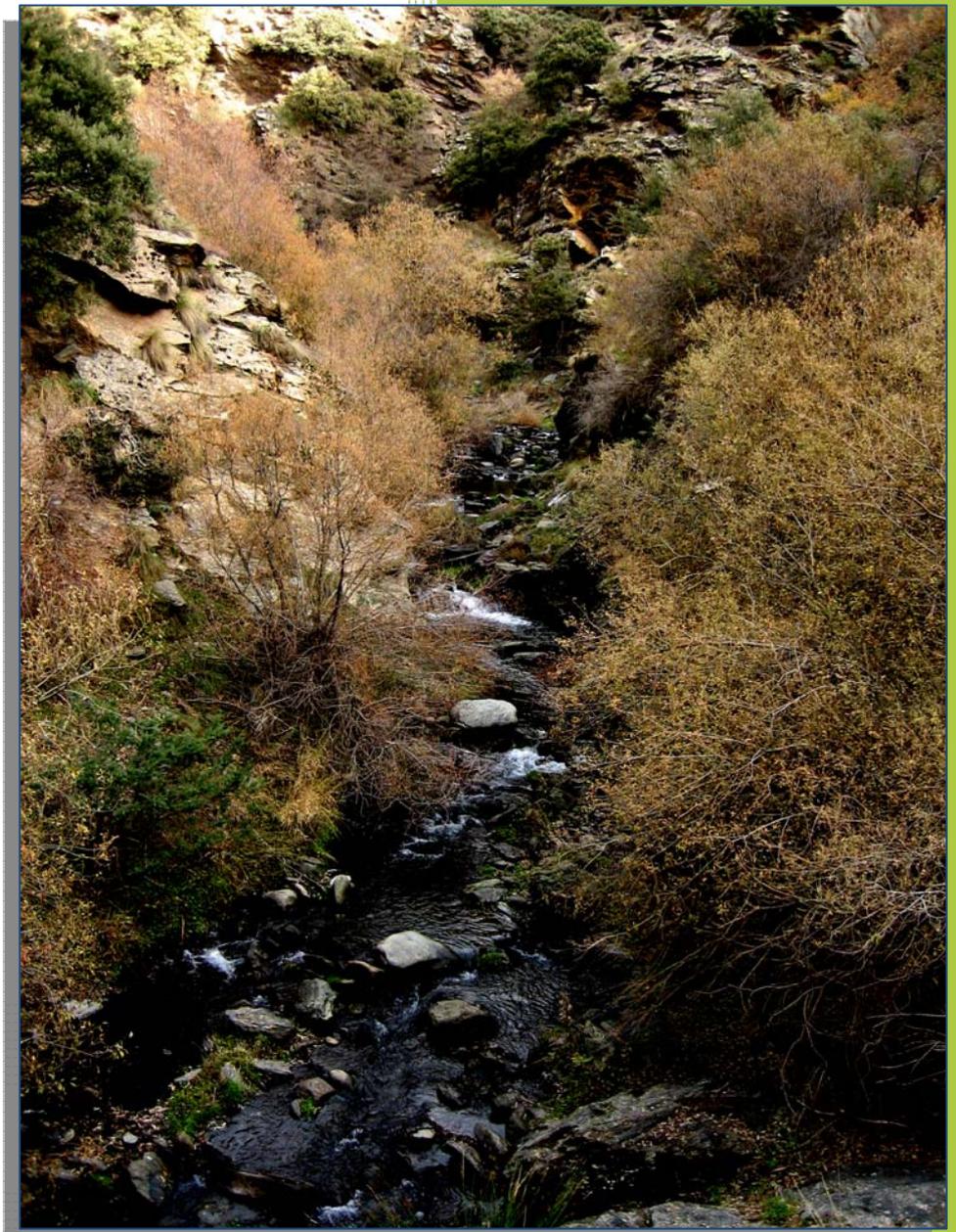
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## CHAPTER 8. Synthesis





Life histories present a great plasticity and are influenced by different factors such as temperature, nutrition, drought, competition among species or predation, etc. We have tried to determine the effect of several of these factors and to see until what concern they condition life histories, but sometimes this is difficult because they are not isolated and usually act synergically. To have a wide range of possibilities that let us to understand better the life histories of these aquatic insects groups, we have studied univoltine, bivoltine and semivoltine populations, and also detritivores, herbivores and carnivores species, sometimes under very different conditions (such as inhabiting different kinds of streams, both permanent and seasonal ones).

In chapter 2 we have seen how altitude, and accordingly temperature, conditions the life history of different species of mayflies and stoneflies. Though in this study it is not possible to compare between the stonefly populations of both streams (Poqueira and Válor) due to the low number of nymphs collected in one of them, the Poqueira stream, we compare them with the available literature, observing also several differences with respect to streams sited at other altitudes. In the case of mayflies, both *Serratella ignita* and *Serratella spinosa nevadensis* present a similar life cycle, but more expanded in time in the Válor stream, where mean annual temperature is lower. In the Poqueira stream there is a positive relation between accumulated day-degrees and growth. Probably, the more expanded nymphal development period in Válor stream is a consequence of the previously mentioned lower temperatures, which would make the nymphs expend more time in reaching their optimum temperature for completing their development. For other mayflies, such as *Alainites muticus* and *Baetis alpinus*, changes in voltinism occur when altitude increases. Two different strategies are present: *A. muticus* passes from being univoltine to bivoltine in upper reaches with lower temperatures, while *B. alpinus* changes from a bivoltine to a univoltine life cycle when goes up in altitude. The latter is the typical pattern that we found when we study populations at different altitudes, but the former seems to be the reflex of some other factor inherent to this population or related with the environmental conditions. The first option is the most probable because the same

species in other water courses from Sierra Nevada behaves as expected, i.e., reducing its number of generations when increasing in altitude.

In the case of stoneflies studied in chapter 2, nymphal development occurs during the coldest months of the year. *Amphinemura triangularis* even has a high growth rate during the months in which the stream reaches temperatures close to 0 °C. This points that these species are relatively temperature independent, i.e., that they are able to grow under low temperature conditions, contrary to that found in some mayflies, as previously mentioned. Nutrition also plays a major role in growth, as it is seen in *Capnioneura mitis* that experiences a high growth period after the input of good quality food in the stream. From the comparison with other studies we observe that the life cycles are delayed or advanced some months, but that do not change in voltinism.

In seasonal streams of temperate regions, mayfly and stonefly life histories are greatly conditioned by summer drought, as we have seen in populations living in Río Despeñaperros (chapters 3, 4 and 7). They present different strategies to cope with it. Some of them have long embryonic development periods, as occurs in *Brachyptera vera cordubensis* and *S. ignita*. This allows the population to pass the drought period in the egg stage, which is more resistant to drying conditions than the nymph. Another adaptation is to have a wide size spread, as presented, for instance, *Tyrrhenoleuctra* sp., *Hemimelaena flaviventris* or the above mentioned *S. ignita*. This guarantees that, if a non predictable drought period occurs, some individuals may reach the mature stage, and so reproduce. Another strategy is the presence of dormancy stages in some phases of the life cycle. The most common among mayflies and stoneflies is the embryonic diapause, which seems to be present in the studied population of *S. ignita*, as well as in several studied stoneflies. Nevertheless, we have seen in *Gualdalgenus franzi* and *Tyrrhenoleuctra* sp. that nymphal dormancy is relatively frequent in seasonal stream stoneflies. This allows semivoltine species such as the former to survive during the adverse conditions, and to complete their nymphal development properly.

We observed two main groups of species in this seasonal stream, those that fly mainly on spring and those that do it some months before (in autumn and winter). The spring species, conditioned by the nearness of the drought period, have a short flight period, and end development just previously to it, after a more

or less synchronized growth. On the other hand, autumnal-winter species present a longer recruitment and flight period, with individuals hatching and emerging over a long time.

Nonetheless, not all the characteristics typical of seasonal streams populations are present. For instance, it is usually reported that populations living in seasonal streams used to have smaller size than those of permanent ones, but, in our comparative study between Río Fardes and Río Despeñaperros (chapter 7) we have demonstrated that the population from the seasonal stream was bigger. In this research we also find that the population of the permanent stream has a much higher population density than in the seasonal stream, when the opposite is usually pointed out.

Thus, from the results of the secondary production analysis, we conclude that species living in seasonal streams are completely adapted to this relatively harsh environment and they do it in many different ways. But not only are the populations that live on these environments adapted to them. Some other populations, such as the one studied by us in chapter 7, are preadapted to drought conditions. In this chapter we have made a comparison between two populations of *S. ignita* in Río Fardes and Río Despeñaperros, and we have discussed that, if shifts from permanent to temporary streams would occur (as has been pointed out under the future climate scenario predicted by experts), species such as this, that present several preadaptations to drought, would survive to the new conditions, even possibly displacing to some others.

In Río Fardes we have also studied how two populations of different taxonomical groups, as mayflies and stoneflies, exploit the same habitat, in this case, the interstices of the substrate, where they burrow when are nymphs. Despite having different life cycle duration, *Leuctra geniculata* and *Ephemera danica* use the same food resources during their nymphal development. They feed mainly on detritus, but also on coarse particulate organic matter (CPOM) and other minor supplies. They change their diets in a more or less similar manner when they are bigger, decreasing the input of detritus and increasing that of CPOM. Nevertheless, it is possible that they obtain their food in different ways. It has been pointed out that *E. danica* obtains its food by actively filtering the water current created by its gills on the tunnel where it lives, while *L. geniculata* feed collecting detritus. From the secondary production study we see that both

species have similar population dynamics in terms of biomass generation, despite having different life cycle duration, which support the similar use of resources pointed above.

In addition, we have studied two close related mayfly species, *Habrophlebia eldae* and *Paraleptophlebia submarginata*, in Río Fardes too, searching for possible interactions between them (mainly regarding food acquisition). We observe that the life histories are segregated in time, avoiding the coincidence of nymphs of the same size at the same time. When we study the feeding habits of both populations we realize that they behave in the same manner: they are mainly detritivores but, when bigger, they incorporate more CPOM and less detritus to their diets. They have equal average cohort duration, but the previously mentioned life cycle segregation makes that the possible interaction between them is reduced. If we observe size at maturity, we can see that *P. submarginata* is bigger than *H. eldae*, which has been pointed out for other authors as typical from close related species entering in competition. This is also reflected in the much higher secondary production of *P. submarginata*, which indicates a higher success of this species.

Considering all the results presented in the previous chapters regarding feeding, we must underline that general patterns of feeding behaviour at high taxonomical levels many times do not fit with reality due to ontogenetic shifts and interpopulations differences in resource utilization. This is evidenced, for instance, when we compare between populations of the same species of mayflies and stoneflies from Poqueira and Válor stream (chapter 2), or observing the change of diet composition when the nymphs are bigger (e.g. chapter 5 and 6). The most outstanding case studied by us is that of the Perlodidae stoneflies (chapter 3), which are almost always categorized as predators but that, when we study their feeding habits, are sometimes more herbivorous than carnivorous. Even the most carnivorous species studied by us, *H. flaviventris*, feed greatly on non animal resources. This is probably a consequence of their generalist condition.

If we take into account the secondary production results we observe that populations from permanent streams studied by us are more productive than the one from the seasonal stream. Nevertheless, these results also show that seasonal stream populations are better adapted and present a higher fitness to

these conditions than what could be thought. These populations present secondary production values comparable to some from permanent stream populations that support their high adaptability to these conditions and that, probably, seasonal streams are not as constraining as we usually think.

Finally, we would like to note that several aspects of the biology and ecology of these animals are still unknown and more studies are needed to improve the knowledge. Laboratory studies on punctual phases or factors of the life of these insects surely will through light and help to solve some of the remaining uncertainties (in fact they are the base for the following phase of our research), but we do not must forget that the best place to study animals is their environment itself, and these studies should serve as a complement of field studies, that are the base knowledge from which we must build the rest.



## CHAPTER 9. Conclusions/Conclusiones





1. **Altitude, and consequently temperature**, has effects on life cycle duration and phenology of mayflies and stoneflies as follows:
  - a. *Changes in duration of life cycle* are more evident in mayflies. Longer nymphal development is associated with higher altitude (lower temperature) as observed in *Serratella ignita* and *Serratella spinosa nevadensis*.
  - b. *Changes in voltinism* also appear in mayflies. At higher altitude, *Baetis alpinus* shifts from bivoltine to univoltine life cycle (as expected), while in *Alainites muticus* occurs the opposite, probably related to some factors inherent to this population.
  - c. *Changes in phenology* are common both in stoneflies and mayflies. In higher areas (with lower temperature), species use to show an advance life cycle, as observed in *Amphinemura triangularis*, *Capnioneura mitis*, *Serratella ignita* and *Serratella spinosa nevadensis*.
2. **For coping with drought**, mayflies and stoneflies inhabiting in seasonal streams have evolved different strategies:
  - a. Existence of a *long embryonic development period*, sometimes with a possible diapause during it, as is the case of *Brachyptera vera cordubensis*, *Hemimelaena flaviventris*, *Isoperla curtata* and *Serratella ignita*.
  - b. Existence of *dormancy during the nymphal development period*, as pointed out in *Guadalgenus franzi* and *Tyrrhenoleuctra* sp.
  - c. *Short development period with fast growth rates under the favourable conditions*. This is common in all the studied species except *Guadalgenus franzi* that present a semivoltine life cycle.
  - d. *Short flight period in spring emergence species*, as is the case of *Guadalgenus franzi*, *Hemimelaena flaviventris*, *Isoperla curtata* and *Serratella ignita*.
  - e. *Long flight period and continuous recruitment* (reflected in the presence of a nymphal wide size spread during a great part of the

cycle) in the autumnal-winter species, as is the case of *Brachyptera vera cordubensis* and *Tyrrhenoleuctra* sp.

3. **Competitive pressures regarding food resources utilization** seem to be the cause of variations on the life histories and phenology of mayfly close related species, as occurs in *Habrophlebia eldae* and *Paraleptophlebia submarginata*. This possible competition is even greater when the species present a reduced niche breadth as in our study.
4. **As a consequence of a similar use of the same habitat and resources** by species belonging to different groups, **convergences** are observed in *Leuctra geniculata* (Plecoptera) and *Ephemera danica* (Ephemeroptera).
5. **In a future possible scenario of global climate change**, a progressive change from permanent to temporal waters will take place. Thus, species as *Serratella ignita*, preadapted to support drought also when living in permanent streams (with characteristics such as fast growth rates, embryonic diapause, long egg development or a long flight period) will increase its fitness in this new scenario.
6. **Regarding feeding behaviour**, general patterns at the genus or family level are not appropriate to group certain species in particular sites, which is reflected in:
  - a. *The existence of variations between populations* of the same species, as shown when comparing the diet of mayfly and stonefly species of Poqueira and Válor streams.
  - b. *The existence of ontogenetic shifts*, as detected in all the studied species except for *Tyrrhenoleuctra* sp. In general, detritus is more important in the diet of smaller nymphs.
  - c. *The existence of a generalist condition*, particularly demonstrated in our study in the seasonal stream. Thus, even well established groups such as the Perlodidae, traditionally classified as carnivorous, include species as *Guadalgenus franzi* and *Isoperla curtata* that mainly feed on non-animal resources.
7. **Species living in permanent stream species are more productive than those living on seasonal ones.** Nevertheless, the latter, in our study

area, also present a very high secondary production compared with close related species inhabiting permanent streams in other areas.

8. **Within the stoneflies, those feeding on animal matter** (*Hemimelaena flaviventris* and, in a lower concern, *Guadalgenus franzi*) **present a higher annual secondary production than those that feed more on other non-animal resources**, including the species of the permanent stream (such as *Leuctra geniculata*).
9. **Semivoltine populations** (*Guadalgenus franzi* and *Ephemera danica* in our study) **present the lowest annual P/B rate**, related with the greatest length of their nymphal development.
10. **Cohort P/B ratio**, both in mayflies and stoneflies from the study area, **always oscillates around five**, with some exceptions as the extremely high value found in *Brachyptera vera cordubensis*, probably related to its extremely high growth rate.

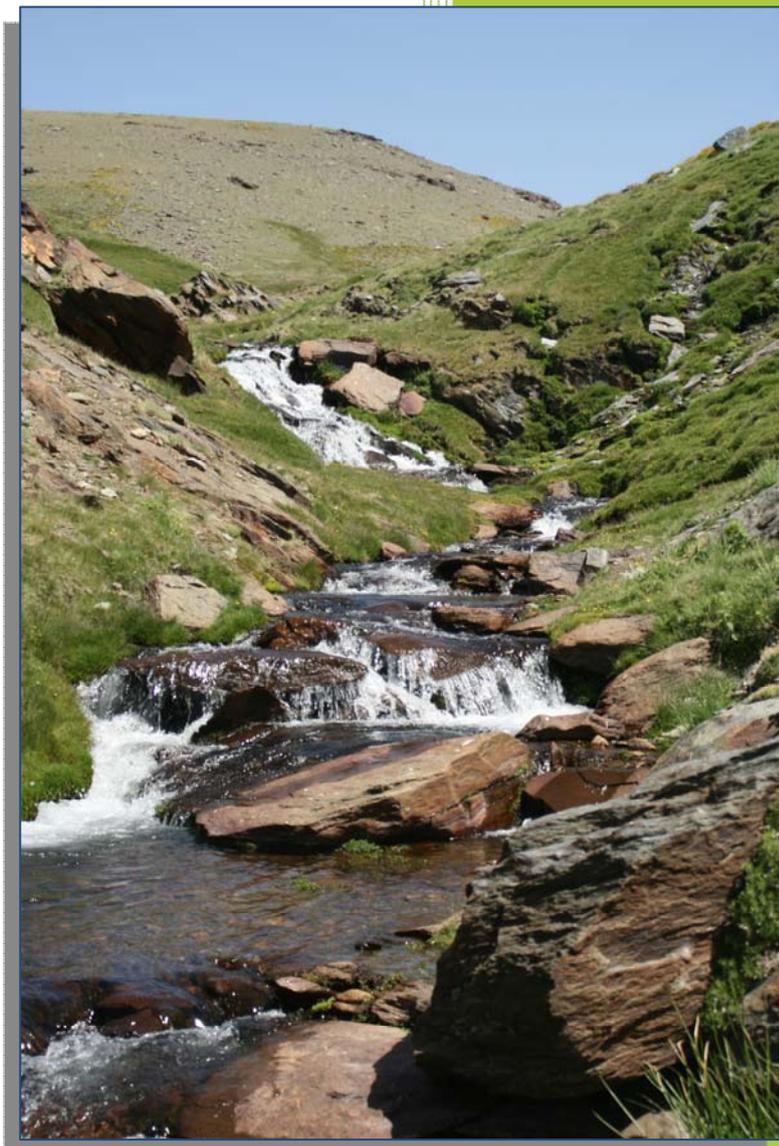
1. **La altitud, y consecuentemente la temperatura**, tiene efectos sobre la duración del ciclo de vida y la fenología de los efemerópteros y plecópteros de los siguientes modos:
  - a. *Los cambios en la duración del ciclo* son más evidentes en efemerópteros. Un desarrollo ninfal más extenso está asociado con mayores altitudes (menores temperaturas), como se observó en *Serratella ignita* y *Serratella spinosa nevadensis*.
  - b. *Los cambios en voltinismo* también aparecen en los efemerópteros. A mayor altitud, *Baetis alpinus* pasa de tener un ciclo bivoltino a uno univoltino (como se esperaba), mientras que en *Alainites muticus* ocurre lo contrario, probablemente en relación a algún factor inherente a esta población.
  - c. *Los cambios en fenología* son comunes tanto en plecópteros como en efemerópteros. En áreas más altas (con menor temperatura), las especies suelen mostrar un avance en el ciclo de vida, como se observó en *Amphinemura triangularis*, *Capnioneura mitis*, *Serratella ignita* y *Serratella spinosa nevadensis*.
2. **Para enfrentarse a la sequía**, los efemerópteros y plecópteros que habitan en arroyos estacionales han desarrollado diferentes estrategias:
  - a. La existencia de un periodo de desarrollo embrionario largo, a veces con una posible diapausa durante el mismo, como es el caso de *Brachyptera vera cordubensis*, *Hemimelaena flaviventris*, *Isoperla curtata* y *Serratella ignita*.
  - b. La existencia de un *estado de latencia durante el desarrollo ninfal*, como se señaló en *Guadalgenus franzi* y *Tyrrhenoleuctra* sp.
  - c. *Un corto periodo de desarrollo con elevadas tasas de crecimiento bajo las condiciones favorables*. Esto es lo común en todas las especies estudiadas a excepción de *Guadalgenus franzi*, la cual presenta un ciclo de vida semivoltino.
  - d. *Un período de vuelo corto en las especies de emergencia primaveral*, como es el caso de *Guadalgenus franzi*, *Hemimelaena flaviventris*, *Isoperla curtata* y *Serratella ignita*.

- e. *Un periodo de vuelo largo y reclutamiento continuo* (reflejado en la presencia de un amplio rango de tamaños durante gran parte del ciclo) en las especies de emergencia otoñal-invernal, como es el caso de *Brachyptera vera cordubensis* y *Tyrrhenoleuctra* sp.
3. **Las presiones competitivas en relación al uso de recursos alimenticios** parecen ser la causa de variaciones en las historias de vida y la fenología de especies de efemerópteros emparentadas, como ocurre en *Habrophlebia eldae* y *Paraleptophlebia submarginata*. Esta posible competición es incluso mayor cuando las especies presentan una reducida amplitud de nicho, como en nuestro estudio. Los cambios ontogenéticos en la composición de la dieta pueden ser una estrategia importante para evitar dicha competición, así como el desplazamiento del desarrollo ninfal de una especie con respecto a la otra. Así pues, si las mismas clases de tamaño de ambas especies no coinciden, como en las especies estudiadas, esta posible interacción se ve reducida.
  4. **Como consecuencia de un mismo uso de hábitat y recursos** por especies pertenecientes a diferentes grupos, se observan convergencias en *Leuctra geniculata* (Plecoptera) y *Ephemerella danica* (Ephemeroptera).
  5. **En un posible escenario futuro de cambio climático global** tendrá lugar un cambio progresivo de aguas permanentes a temporales. Así pues, especies como *Serratella ignita*, preadaptadas a soportar la sequía también cuando viven en aguas permanentes (con características tales como elevadas tasas de crecimiento, diapausa embrionaria, largo período de desarrollo del huevo o largo período de vuelo), incrementarán su éxito en este nuevo escenario.
  6. **En relación al comportamiento trófico**, los patrones generales a nivel de género o familia no son apropiados para agrupar a determinadas especies en lugares concretos, lo que se refleja en:
    - a. *La existencia de variaciones entre poblaciones* de la misma especie, como se muestra al comparar la dieta de efemerópteros y plecópteros de los arroyos Poqueira y Válor.
    - b. *La existencia de cambios ontogenéticos*, como se detectó en todas las especies estudiadas a excepción de *Tyrrhenoleuctra* sp. A

grandes rasgos, el detritus es más importante en la dieta de las ninfas más pequeñas.

- c. *La existencia de una condición generalista*, particularmente demostrada en el estudio del arroyo estacional. Por tanto, incluso grupos bien establecidos como los Perlodidae, tradicionalmente clasificados como carnívoros, incluyen especies como *Guadalgenus franzi* e *Isoperla curtata* que se alimentan principalmente de recursos no animales.
7. **Las especies que viven en arroyos permanentes son más productivas que las de arroyos estacionales.** De todos modos, las últimas, en nuestra área de estudio, también presentan una productividad secundaria muy elevada comparada con la de especies emparentadas que habitan arroyos permanentes de otras áreas.
8. **Dentro de los plecópteros, los que se alimentan de materia animal (*Hemimelaena flaviventris* y, en menor medida, *Guadalgenus franzi*) presentan una mayor productividad secundaria anual que aquellas que lo hacen en otros recursos de origen no animal**, incluyendo especies de arroyos permanentes (como *Leuctra geniculata*).
9. **Las poblaciones semivoltinas (*Guadalgenus franzi* y *Ephemera danica* en nuestro estudio) presentan la menor tasa P/B anual**, en relación a la mayor duración de su desarrollo ninfal.
10. **La razón P/B**, tanto en efemerópteros como en plecópteros de nuestra área de estudio, **siempre oscila alrededor de cinco**, con algunas excepciones como el valor extremadamente alto encontrado en *Brachyptera vera cordubensis*, probablemente en relación a su alta tasa de crecimiento.

**ANNEX I. Autoecological database of Iberian Ephemeroptera  
and Plecoptera**





This annex is a compilation of information regarding different autoecological aspects of mayflies and stoneflies from the Iberian Peninsula. It is part, although adapted, of a wider work in the frame of a European research project called “Euro-Limpacs”, an Integrated Project addressing the impact of climate change on European freshwater ecosystems (GOCE-CT-2003-505540). In it, the participant countries have made a compilation of information coming from the published literature, but also from “dark” literature and expert opinions, for the ecoregions defined by Illies (1978), in order to generate a wide database of the current knowledge on autoecological aspects of the European species. The list of consulted references appears as separate spreadsheets of this same Annex, each one with the code used in the “Source” column of each database variable. Hence, each variable is accompanied by a “Source” column, where we can see where the information comes from. The definitive version of the database will be accessible at [www.freshwaterecology.info](http://www.freshwaterecology.info), also with a printed version in a series of book that will be published under the series title “Distribution and Ecological Preferences of European Freshwater Organisms” (Schmidt-Kloiber, A. and Hering, D. eds.). Hereafter we will make a brief summary of the data presentation and coding in order to make this annex useful, but a wider explanation can be seen in Graf *et al.* (2008).

Species are alphabetically sorted by family, subfamily, genus and species. We have chosen the autoecological variables that are more useful in the context of this Ph.D. Thesis. The coding of the information has been done in different ways, depending on the variables. Following we present a summary of the variables used and their coding:

- Distribution according to Illies. We present the recorded presence of the species in the different ecoregions of Illies (1978). They are numbered from 1 (Iberian Peninsula) to 25, plus X and Y that represent North Africa and the Middle East respectively. Presence of one species in an ecoregion is recorded by the number of such ecoregion.

- Altitude WFD (Water Framework Directive). In this category we used a “presence/absence” (1/0) coding system to classify the species in altitude ranges according with the Water Framework Directive (2000).
- Rare species. Here are presented those species that are usually found in a very low number, assessed with the “presence/absence” coding system.
- Red list species. In this category are the species that appear in the Spanish Red List (Verdú and Galante, 2006). Again a “presence/absence” coding system was employed.
- Stream zonation presence. Here, the species are classified according to their presence in the different parts of the longitudinal profile of a stream or river. In this case we used a “10 points” coding system, in which these 10 points were distributed among the different categories where the species was present according to the literature. Thus, more points were given to the category where the species was more frequently recorded.
- Feeding types. In this variable, species are classified according to their food acquisition mode, also with a “10 points” system.
- Reproductive cycles per year. The species were classified into semivoltine, univoltine, bivoltine, etc., classes according to the length of their life cycle. A “one assignment” coding system was used, consisting in marked with “1” the category in which the species could be classified. In this case it was included in brackets the ecoregion to which the information is related, due to voltinism may change from zone to zone, depending on the altitude and latitude.
- Resistance/resilience to droughts. Also with a “one assignment” coding system, we tried to point out the possible strategies of the species for coping with drought, when available.
- Flight (emergence)-period I. In this category species are classified depending on if they have a short or long emergence period with a “one assignment” system. This does not mean that the species life is shorter or longer, but it is related to the duration of the period in which adults were captured.

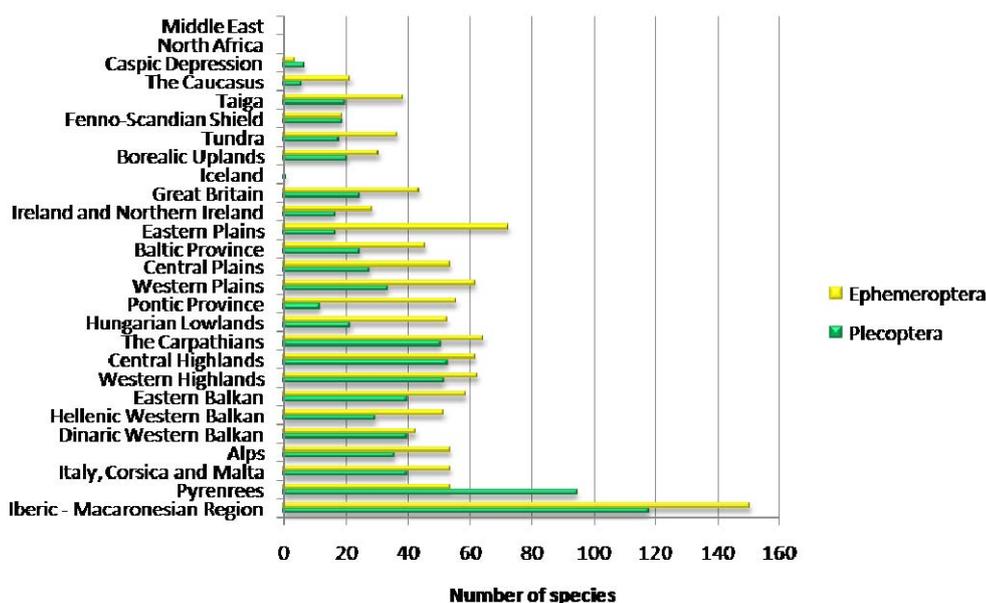
- Flight (emergence)-period II. In this case, the coding is a “10 points” system, distributed in seasons, according to the presence of the adults in them.
- Larval development cycle. Here, we used a “10 points” system for assessing the presence of nymphs in the different seasons of the year, with also a category for those species that are present during the whole year.

Caution must be taken when interpreting data, due to the information we present comes from the available data, and many times the absence of data (reported as “0”) can be a consequence of the lack of studies.

In the following lines we make a brief summary of the compiled data. No further discussion has been carried out due to the nature of the data. Many data come from punctual studies carried out on few species or on very concrete areas, and some are only anecdotal. Thus, this Annex pretends be more a bibliographic compilation of the previous knowledge about some aspects of the autoecology of mayflies and stoneflies species than a subject of discussion.

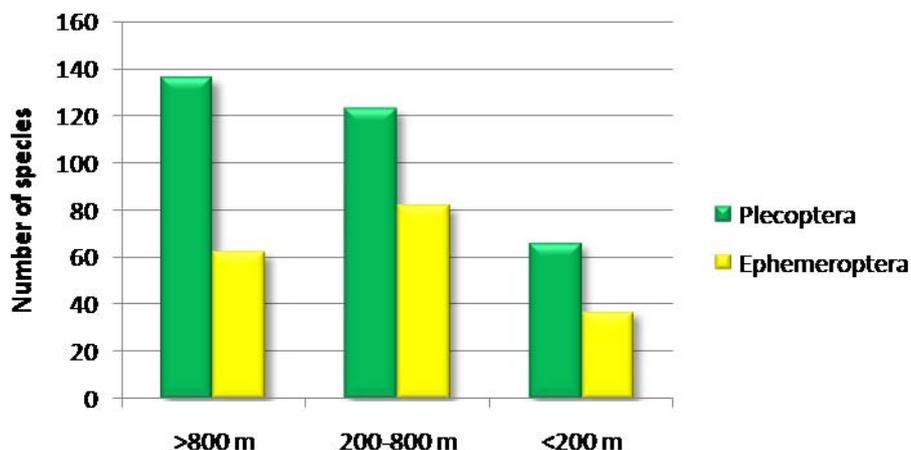
Regarding the ecoregional distribution, we observe that the maximum number of species of both orders is concentrated in the Iberic-Macaronesian region.

### Number of species per ecoregion



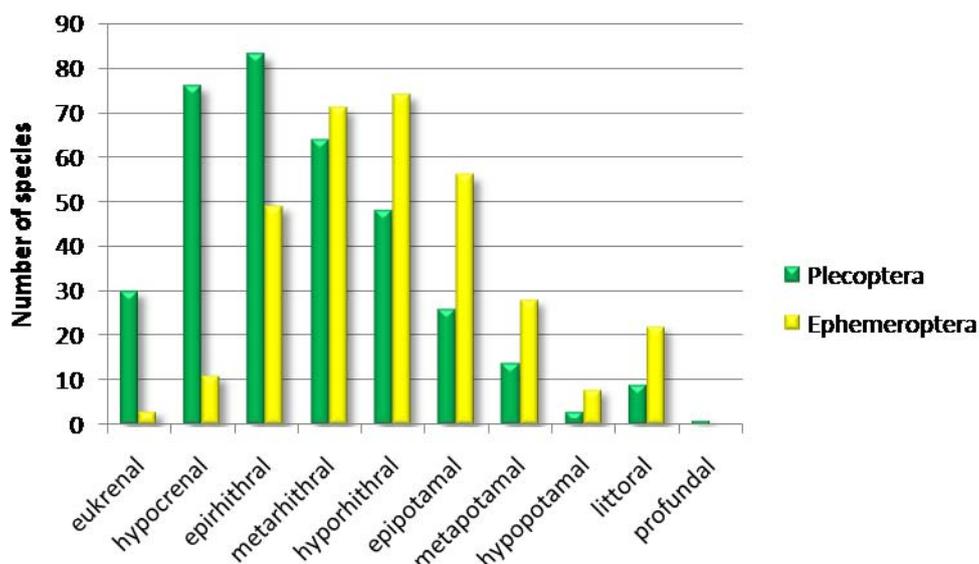
In the case of mayflies, these taxa are mainly found at mid-low altitudes, while stoneflies are principally present in high altitudes.

### Altitude WFD

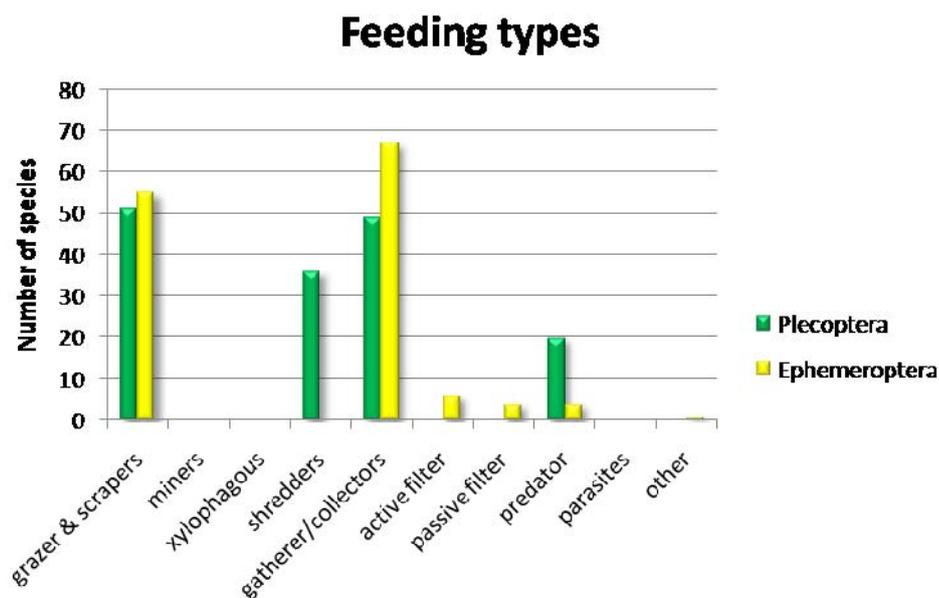


This is also reflected in the presence of the species along the longitudinal gradient of the streams and rivers, being mayflies more frequent at mid reaches and stoneflies at high ones, which is in accordance with the general ecological requirements of both orders.

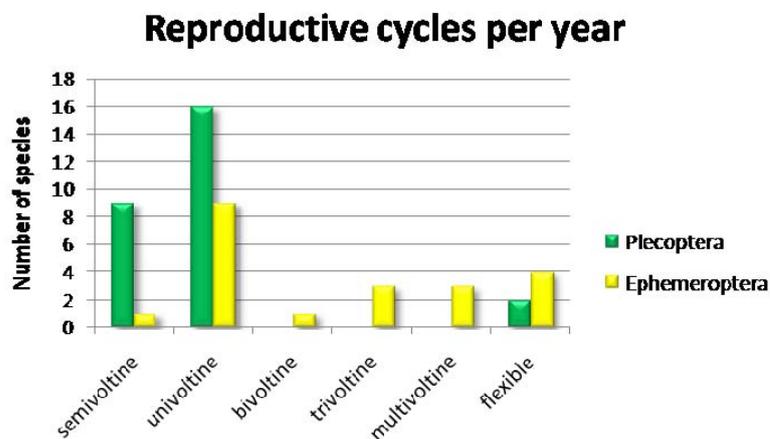
### Stream zonation presence



In relation with their feeding types, and from the literature available, most mayflies seem to be gatherer-collectors and scrapers, with a low number of species being filterers or predators. On the other hand, stoneflies have been more usually recorded as scrapers and gatherer-collectors, but there is a great number of species being shredders and few being predators, though notably more than mayflies.

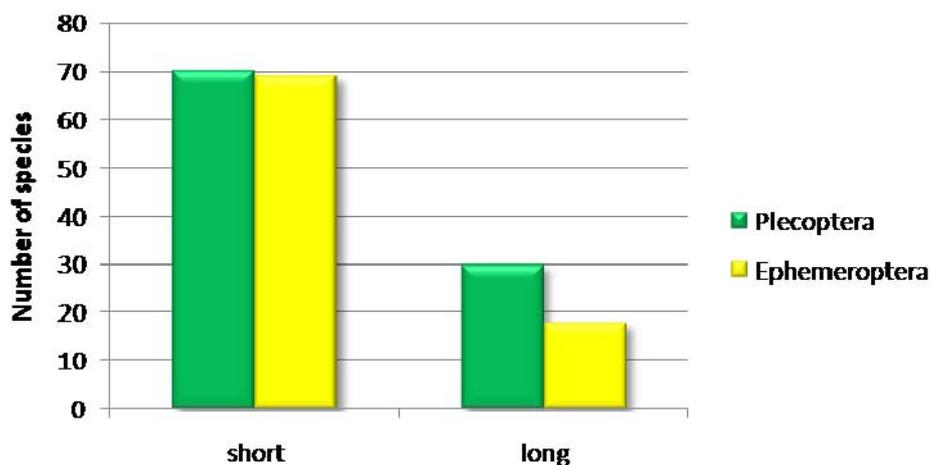


In both orders predominate univoltine species, being the multivoltine pattern relatively frequent in mayflies and absent in stoneflies, while the semivoltine one is more frequent in stoneflies and little represented in mayflies. Flexible cycles are found in both orders, probably depending on the environmental conditions.

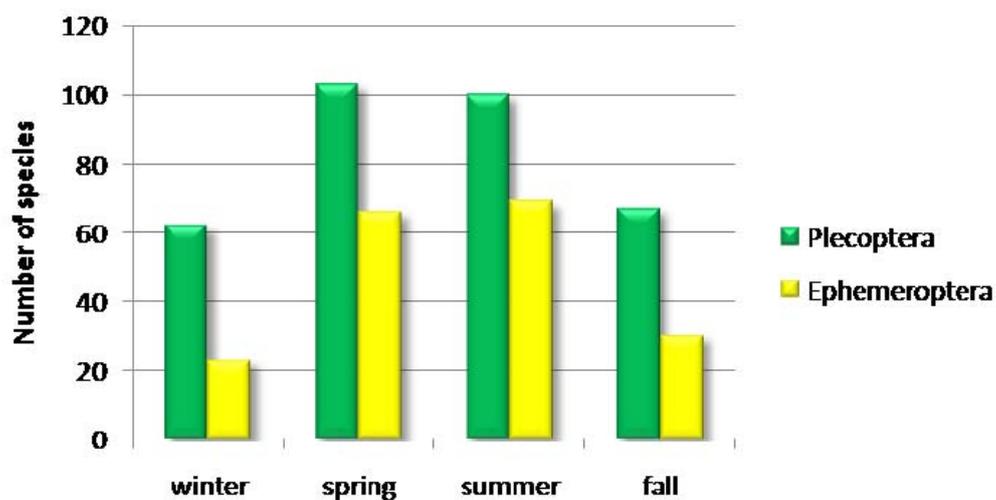


The flight period of both mayflies and stoneflies is mainly short and concentrated in the spring and summer months, but there is also a great quantity of species with autumn and winter flight periods.

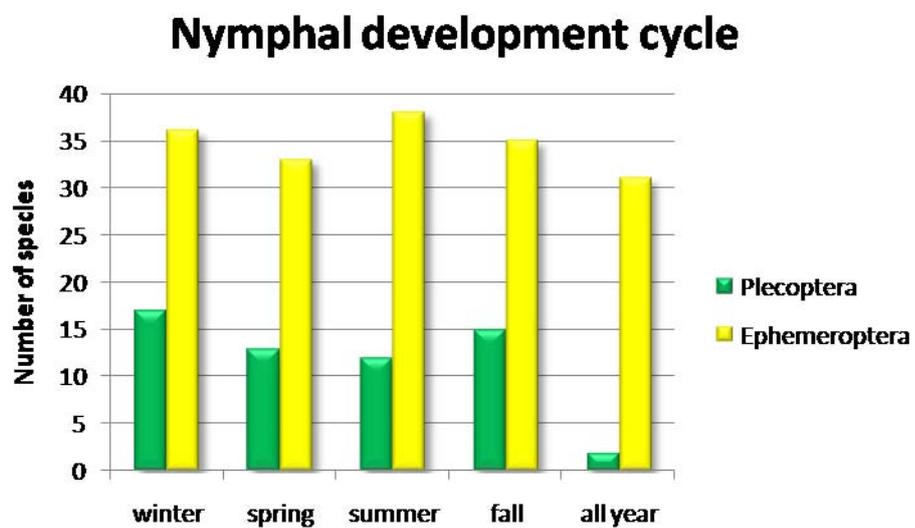
### Flight (emergence)-period I



### Flight (emergence)-period II



Finally, and in relation with the months that the nymphs are present in the stream or river, mayflies and stoneflies are found almost equally during all the seasons.



**LITERATURE CITED**

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