Revista Colombiana de Entomología 37 (2): 305-312 (2011)

Seasonal size distribution of *Anacroneuria* (Plecoptera: Perlidae) in an andean tropical river

Distribución estacional del tamaño de *Anacroneuria* (Plecoptera: Perlidae) en un río tropical andino

HUGO BOHÓRQUEZ[1], GLADYS REINOSO[2], and GIOVANY GUEVARA[3*]

Abstract: Total body length and head capsule width (mm) were used as biometric characteristics in discriminating between size-frequency estimates of three *Anacroneuria* morphospecies, from 28 sampling sites along a tropical river subject to bimodal variations in water level in Central Colombia. A total of 344 specimens grouped into four size classes were collected. The highest abundance of *Anacroneuria* larvae was observed during the dry season and there was a higher abundance of small-sized individuals (body length <12mm). Overall, the highest abundance of stonefly larvae was recorded between 1000 to 1600m.a.s.l. Conductivity and pH showed a significant correlation with the total abundance of preimaginal stoneflies. Plecoptera size distribution patterns could be useful in assessing which stonefly size-spectra are susceptible to disturbance in tropical rivers and in evaluating their population dynamics.

Key words: Neotropics. Andes. Biometry. Body Size. Tropics.

Resumen: Se utilizaron el largo total y el ancho de la capsula cefálica como rasgos biométricos para estimar la frecuencia de tamaño de tres morfo-especies de *Anacroneuria* (Insecta: Plecoptera) registrados en 28 estaciones de muestreo ubicadas longitudinalmente en un río tropical del centro de Colombia sometido a un régimen de precipitación bimodal. Se coleccionaron 344 especímenes que fueron agrupados en cuatro clases de tamaño. La mayor abundancia de larvas se registró durante la estación seca con una mayor presencia de individuos "pequeños" (tamaño corporal <12mm). Considerando ambas épocas hidrológicas, la mayor abundancia de plecópteros ocurrió entre 1000 y 1600m.s.n.m. La conductividad y el pH del agua mostraron una correlación significativa con la abundancia de plecópteros pre-emergentes. Los patrones de distribución de tamaño de larvas de Plecoptera constituyen una herramienta valiosa para establecer la vulnerabilidad de los estadios larvales frente al efecto de la perturbación en ríos tropicales y para evaluar su dinámica poblacional.

Palabras clave: Neotrópico. Andes. Biometría. Tamaño Corporal. Trópico.

Introduction

The order Plecoptera, is a group of hemimetabolous insects with more than 3497 species described worldwide, of which 474 species are Neotropical (Fochetti and Tierno de Figueroa 2008), but recently 32 out of 508 valid names were considered questionable by Froehlich (2010). This order is an important component in lotic systems, both in terms of abundance and in terms of ecological significance (Zwick 2004; McLellan and Zwick 2007; Stark *et al.* 2009). In comparison to their temperate counterparts, tropical stoneflies are poorly understood (Sheldon and Theischinger 2009), and regional and local species lists are extremely incomplete. One reason for the paucity of systematics and ecological studies of tropical river Plecoptera is that identification of tropical species is difficult for non-specialists (Suhaila and Che Salmah 2011).

In Colombia, the order Plecoptera is represented by two families: Perlidae with three genera (*Anacroneuria, Macrogynoplax* and *Klapalekia*; Tamaris-Turizo *et al.* 2007; Zúñiga *et al.* 2007) and, Gripopterygidae with one genus (*Claudioperla,* Barreto *et al.* 2005; Zúñiga *et al.* 2009). *Anacroneuria* Klapálek, 1909 (Plecoptera: Perlidae) is the largest Neotropical genus with more than 300 species (Froehlich 2003; Bispo *et al.* 2005; Tomanova and Tedesco 2007). Its distribution extends from the southern United States to northern Argentina (Stark 2001; Froehlich 2004). Sixty-one species are reported from Colombia alone (Zúñiga *et al.* 2007). Nevertheless, there is no published information on stoneflies from the Tolima province, Central Colombia, except for the first record of the Gripopterygidae family in the Páramo ecosystem (Barreto *et al.* 2005), a special biogeographic zone at the highest altitudes of the Andean region in northern South America (Hofstede 1995). Despite their importance as water quality indicators, stoneflies have received little attention in Colombia and the peer-reviewed literature is scarce (Stark *et al.* 2009). The life histories and trophic interactions of stonefly larvae are not well-known even though they play an important ecological role in the freshwater ecosystems (Bohórquez *et al.* 2006; Tamaris-Turizo *et al.* 2007; Gamboa *et al.* 2009).

Biometric studies are important because several biological, physiological and ecological properties can be extracted from specific body dimensions (body length or head width; Beer-Stiller and Zwick 1995; Krasnov *et al.* 1996; Klingenberg and Spence 1997; Zwick 2003). The morphological characteristics of aquatic insect larvae can be influenced by biotic and abiotic factors (Crosa and Buffagni 2002; Tomanova and Tedesco 2007). Altitude, temperature, seasonality, competition and predation are key factors regulating insect abundances in aquatic environments (Brittain 1983; Saltveit *et al.* 1994; Céréghino and Lavandier 1998; Céréghino *et al.*

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2002). Worldwide, biometric research on plecopterans has mainly involved obtaining linear measurements to determine growth in immature stages (larvae) for life-cycle and secondary production analyses (e.g., Sheldon 1969, 1980; Schmidt and Tarter 1985; Lillehamnur *et al.* 1989; Townsend and Pritchard 1998; Fenoglio *et al.* 2007; Principe 2008). Similarly, studies of morphometric differences (except taxonomic descriptions) in larvae and adults of the *Anacroneuria* genus and their relationships to environmental factors have been highlighted for some tropical rivers (Tomanova and Tedesco 2007; Fenoglio *et al.* 2007; Cressa *et al.* 2008; Gamboa and Arrivillaga 2010).

In tropical rivers, precipitation and discharge play an important role in structuring the benthic community (Silveira *et al.* 2006; Wantzen *et al.* 2006). These rivers and their aquatic communities are conditioned by rainfall during the rainy season (Turcotte and Harper 1982; Flecker and Feifarek 1994; Jacobsen and Encalada 1998; Buss *et al.* 2004; Jacobsen 2004; Bispo *et al.* 2006). Rivers in the Andean region of Colombia are also highly influenced by seasonal rainfall, normally occurring during two wet seasons, with sporadic spates even during the "dry" season (Restrepo and Kjerfve 2000; Arias *et al.* 2007).

The present study was carried out in the Prado River Basin, which is a valuable tributary of the Magdalena River, the most important river system in Colombia (Galvis and Mojica 2007). The objectives of this study were to measure the variation in body size of *Anacroneuria* larvae across sampling sites of varying in altitude during two contrasting seasons (dry and wet); to identify morphological differences caused by these temporal and spatial parameters; and to determine whether this life-history characteristic varies with elevation and season.

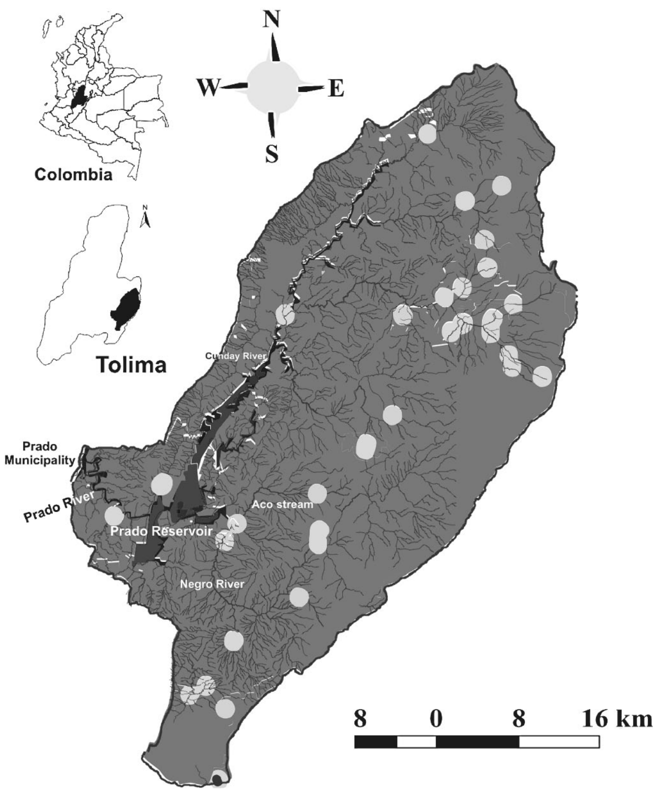

Figure 1. Location of the Prado River Basin in Tolima Province (Colombia). The distribution of *Anacroneuria* larvae in watershed networks is shown by yellow circles.

Materials and Methods

Study Area. The Prado River Basin is located in central Colombia, in the Department of Tolima (3°45'N - 74°56'W; Fig. 1), in the upper Magdalena Valley, between the Central Range and the Eastern Range of the Colombian Andes. The basin covers 170.000ha, with an annual mean temperature of 23.4°C and annual mean precipitation of 1896mm associated with two rainy seasons, one from March to May and the second between October and December (see Canosa and Pinilla 2001; Bohórquez *et al.* 2006; Guevara *et al.* 2009). Selected sampling sites ranged from 280 to 2260m.a.s.l.

Sampling of stonefly larvae. Sampling of *Anacroneuria* larvae was carried out over ten consecutive days at 28 sampling sites, during two contrasting seasons, August-September (dry season) and November 2005 (wet season). Stonefly larvae were collected using a hand-net (250µm mesh) and a Surber net (30 x 30cm, 250µm mesh) and then stored in 75% ethanol. All samples were pooled for each sampling site.

In the laboratory, body length (BL; from the front of the head to the tip of the abdomen), head capsule width (HCW; across the compound eyes), and mesothoracic wing pad length (WL; from the tip of the wing pad to the paramedial contact point with the mesonotum) of each specimen (*sensu* Nesterovitch and Zwick 2003) were measured to the nearest 0.01mm using a stereomicroscope (Olympus, 10X). We sorted the collected larvae into three different *Anacroneuria* morphospecies (M1, M2, M3; see Bohórquez *et al.* 2006) related to variations in body size and pigmentation, among other morphological traits. Intrastadial sizes characterized by differences in the shape of the margins, the orientation and the pigmented pattern of hind wingpads, i.e., rounded, pronounced and darkish, were traced by assigning larvae to one of four arbitrary size classes (obtained from frequency histograms), given that estimation of larval instars is difficult for stoneflies because of their molting times (Otsuki and Iwakuma 2008). Therefore, body length measurements for each morphospecies were used to construct size-frequency distributions pooled into four size classes (mm). Selected physical, chemical and microbiological parameters were recorded at each sampling site and later analyzed following methodologies proposed by APHA-AWWA-WEF (Clesceri *et al.* 1999).

The relationships between total larval abundance and elevation or physicochemical variables were analyzed by means of Spearman Rank Correlation test, due to lack of normality. We used linear regressions to test the relationships between the body length and head capsule width measurements. Since morphospecies 3 (M3) abundance was low during each sampling period (4 during the dry season, 2 during the wet season), we combined the data from both seasons when fitting linear regressions. All analyses were performed using STATISTICA version 7 (StatSoft 2004) considering a significance of 5% (α=0.05).

Results

Abundance and physicochemical variables. During the dry season, there were three-fold more plecopteran larvae than

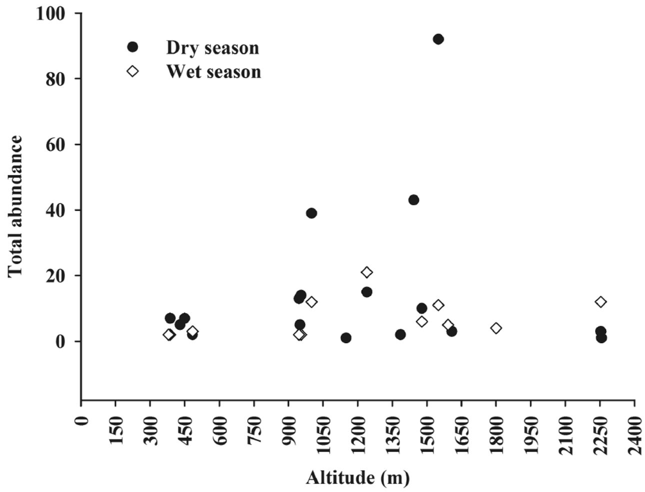

Figure 2. Scatter plot of the total abundance of *Anacroneuria* larvae against elevation in the Prado River Basin (Tolima, Colombia) during 2005. Note the contrasting total numbers of individuals between dry and wet seasons.

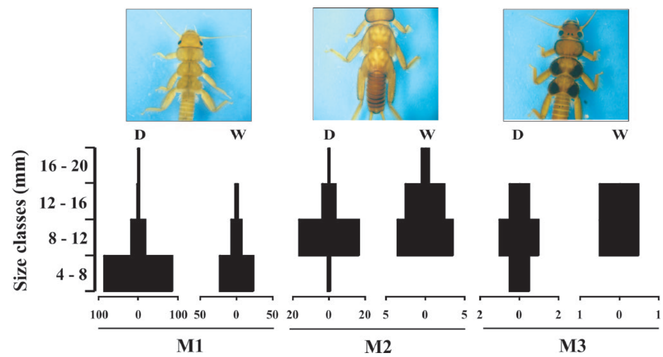

Figure 3. Size-frequency distribution (mm) of the body length of Plecoptera larvae in the Prado River Basin in central Colombia. Values of the X-axis are proportional to the total abundance during each season. D=Dry season, W=Wet season. M=morphospecies: specimens that have been sorted and classified as distinct according to their shape and morphological characteristics, although unidentified and unsexed; M1: morphospecies 1, M2: morphospecies 2, M3: morphospecies 3. Main intrastadial changes are shown in the upper part.

during the rainy season. The total abundance was significantly correlated with elevation during the wet season (n=12; r=0.66, P=0.018), but not during the dry season (n=17; r=0.08, P>0.05). The highest larval abundance was registered between 1000 to 1600m.a.s.l. (Fig. 2).

The physicochemical variables, water temperature, air temperature, pH, conductivity, total hardness, and total and fecal coliforms, were highest during the dry season. On the contrary, conductivity, dissolved oxygen and O_2 saturation percentage displayed higher values during high water level conditions (Table 1). The pH and conductivity variables showed a significant correlation with total abundance during the dry season (n=17; r=-0.58, P=0.014; r=-0.57, P=0.015, respectively), while only conductivity was marginally significant during the wet season (n=12; r=-0.57, P=0.047).

Measurements of body size. We measured 344 *Anacroneuria* larvae, of which 262 were collected during the dry season and 82 during the wet season. Larvae varied in size, representing a wide range of growth phases (Fig. 3). Body size ranged from 4 to 20mm. In all cases, larval stages were more abundant during the dry season, with a great abundance of the smallest larvae, i.e., <12mm in body length (Fig. 3). However, we did not find a significant relationship between total body length and altitude, or any physical and chemical parameter.

The smallest head capsule width was 1.08mm, and the maximum was 3.95mm. We found a relationship between total body length and head capsule width in every morphospecies and according to season. This relationship was strongly isometric (Fig. 4) and linear, with the following equations being highly significant for all morphospecies: **M1** (dry and wet season; r^2=0.98, P<0.001 and r^2=0.91, P<0.001); **M2** (r^2=0.81, P<0.001 and r^2=0.69, P<0.001); **M3** (r^2=0.80, P<0.016). Larvae of all morphospecies exhibited continuous variation of both HCW and WL and could not be grouped precisely into instars based on either one of these variables.

Discussion

Larvae occurrence. Our results suggest that the greatest abundances of larger larvae from M2 and M3 of *Anacroneuria* spp. during the dry season corresponded to individuals near emergence (pre-emergent), although it has been suggested that some Colombian *Anacroneuria* emerge throughout year, thereby displaying possible non-seasonality (Tamaris-Turizo *et al.* 2007). In other regions of the country emergence peaks are related to the transition between dry and rainy seasons (Zúñiga *et al.* 2003). Also, some mature *Anacroneuria* larvae (last instar) reaching the adult stage have been found to be of smaller size (M. del C. Zúñiga, pers. com.). Various insects, hemi- as well as holometabolous, emerge at successively smaller sizes throughout the emergence period (Nesterovitch and Zwick 2003). Although a frequency distribution of measurements was constructed to visualize the number of larval instars, the limits of each instar were defined by lower frequencies fitted to one of four size classes. We associated the instars of each morphospecies with the development, shape and coloration of wing pads (e.g., Roessler and Zamora 1997). Our results indicate the existence of at least four larval instars for these morphospecies. However, we strongly recommend carrying out thorough periodical sampling over a year and fitting the WL : HCW ratio against the HCW, as suggested by Beer-Stiller and Zwick (1995) to determine voltinism and identify "real" instars.

Zwick (2003) argued that in Plecoptera, the first small rudiments of wingpads appear in the antepenultimate instar, and the definite shape is reached during subsequent molts. We sorted morphs according to biometric characters (mainly wingpads) and color similarities, and identified and grouped them according to stonefly congeneric specie, which unquestionably under-represents the actual species richness for the river and the region. For instance, we report the presence of at least three species which are probably correlated to the three morphs, but a minimum of 58 species have been described in Colombia based on adult collections (Zúñiga *et al.* 2007). Since most ecological and biological studies ideally involve aquatic insects in the adult stage (mainly males), it is neces-

Table 1. Physical, chemical and microbiological parameters at 28 sampling sites along the Prado River Basin recorded during two contrasting seasons in 2005.

	Altitude (m)	Dry season										Wet season									
		WT	AT	pH	T	C	TH	DO	% DO	TC	FC	WT	AT	pH	T	C	TH	DO	% DO	TC	FC
1	2257	17.5	22.9	7.22	1.10	39.5	17.00	5.0	69.40	7	0	16.4	13	6.44	2.27	51	23.84	7.9	81.9	8	0
2	2254	14.2	25.2	7.62	3.60	47.2	18.00	4.9	68.00	3	1	15.3	12.8	6.01	4.5	49.6	38	7	97.2	10	0
3	1800	17.7	28.3	4.30	1.67	27.7	13.00	6.0	83.30	20	2	17.5	16.1	5.02	7.04	18.5	69.3	7.6	77	30	7
4	1703	20.7	21.2	3.85	5.82	171.0	4.00	7.7	84.70	3	0	17.7	18.3	5.14	2.62	14.3	10.58	5.7	79.1	15	1
5	1608	15.8	19.5	6.01	77.00	59.1	30.00	8.3	87.50	7	1	17.5	16.3	6.37	119	40.3	16.35	6.6	91.6	15	4
6	1592	17.8	18.9	5.34	1.17	11.0	5.20	6.9	95.80	9	0	20	17.7	6.03	1.55	13	8.27	4.8	66.6	7	1
7	1550	17.4	23.2	6.70	0.82	2.0	8.00	4.9	68.06	10	0	18	15.9	4.96	0.7	25.1	48.3	6.3	87.5	39	3
8	1478	18.2	18.9	6.62	6.50	20.0	12.00	4.4	61.00	40	0	23.9	20	6.39	4.6	11.6	4.81	5.6	77	5	1
9	1443	22.0	18.5	5.68	3.33	20.3	33.85	4.8	66.60	18	5	22	18.5	5.68	3.33	20.3	33.85	4.8	66.6	18	5
10	1441	19.2	23.3	5.15	1.07	105.0	10.40	6.5	90.20	9	0	22.7	20.2	6.06	1.5	15.7	14.62	6.1	84.7	10	0
11	1386	17.8	21.6	7.43	1.76	88.5	44.00	6.0	83.33	110	0	19.5	17.7	6.36	3.03	66.3	0	5	69.4	65	6
12	1240*	19.0	22.3	6.53	2.58	14.4	27.00	5.0	68.00	10	0	23.2	18	5.27	3.11	17	25.9	6.5	90.2	23	4
13	1240	16.7	20.0	7.07	0.97	21.4	12.00	6.2	86.11	50	0	19.4	17	5.23	1.39	28.7	51.34	6	83.3	21	6
14	1150	20.7	27.1	7.28	2.00	172.1	81.00	4.5	62.50	10	0	23.2	21.2	6.36	5.7	56.2	29.8	7.3	87.5	24	2
15	1000*	21.6	27.3	7.04	2.01	50.5	21.00	6.7	93.06	30	0	26.1	21.6	6.27	12.7	37.6	20.7	4.8	66.6	16	1
16	1000	21.1	23.9	7.05	2.10	46.1	19.00	6.3	87.50	31	1	26.1	21.8	6.68	3.43	42	19.61	5.1	70.8	25	2
17	969	20.5	23.2	6.53	3.20	19.0	14.00	5.4	75.00	14	0	25.3	21.3	6.56	2.05	14.6	16.15	6	83.3	3	2
18	955	22.0	35.3	6.74	4.15	21.7	15.00	5.4	75.00	17	0	22.7	20.2	6.49	0.22	14	15.7	4.9	68	13	0
19	950	19.0	19.2	7.68	1.50	290.0	73.00	4.4	61.11	80	0	19.3	18.5	8.53	20.5	95.6	48.27	7.2	86.1	13	27
20	946	21.8	31.0	6.57	0.12	15.6	12.00	5.7	79.17	12	0	21.4	22.4	6.54	1.66	11.6	13.65	6.9	95.8	6	0
21	484	25.0	31.6	7.70	2.92	49.5	29.00	5.9	81.94	11	0	27.6	24.1	6.62	10.2	19.8	12.5	6.1	84.7	31	0
22	450	29.0	24.7	7.23	2.57	93.3	43.00	4.9	69.00	160	200	27.7	23	6.96	82.6	54.9	30.2	5.8	80	12	20
23	430	25.1	31.6	6.92	11.50	27.2	20.00	5.7	79.00	120	0	26.1	24.9	7.6	12.8	35.4	17.5	7.2	72	9	1
24	429	23.6	24.7	6.85	1.10	27.1	20.00	5.4	72.00	115	5	23.3	23	7	0.32	23.7	15.4	7.1	98.6	7	3
25	387	24.7	31.2	7.64	5.80	262.0	74.00	6.6	91.00	20	10	30.9	23.7	6.9	0.96	91.3	34.03	7.2	72	5	7
26	380	23.4	25.0	7.00	3.40	51.7	24.00	5.7	79.00	60	0	26.4	24.3	7.05	14.4	61.4	29	6.3	87.5	2	3
27	308	24.5	24.4	6.35	7.32	62.7	32.88	6.1	85.00	530	270	24.5	24.4	6.35	7.32	62.7	32.88	6.1	85	530	270
28	289	28.1	26.1	7.47	4.30	57.4	36.00	5.5	72.00	50	0	28.2	26.7	7	5.45	64.5	28.85	6.1	84	190	400

WT = water temperature (°C); AT = air temperature (°C); T = turbidity (NTU); C = conductivity (μScm^{-1}); TH = Total Hardness (mgl^{-1} $CaCO_3$); DO = dissolved oxygen (mgl^{-1}); %DO = percentage dissolved oxygen; TC = total coliforms (CFU $100ml^{-1}$); FC = fecal coliforms (CFU $100ml^{-1}$). *Different sampling points.

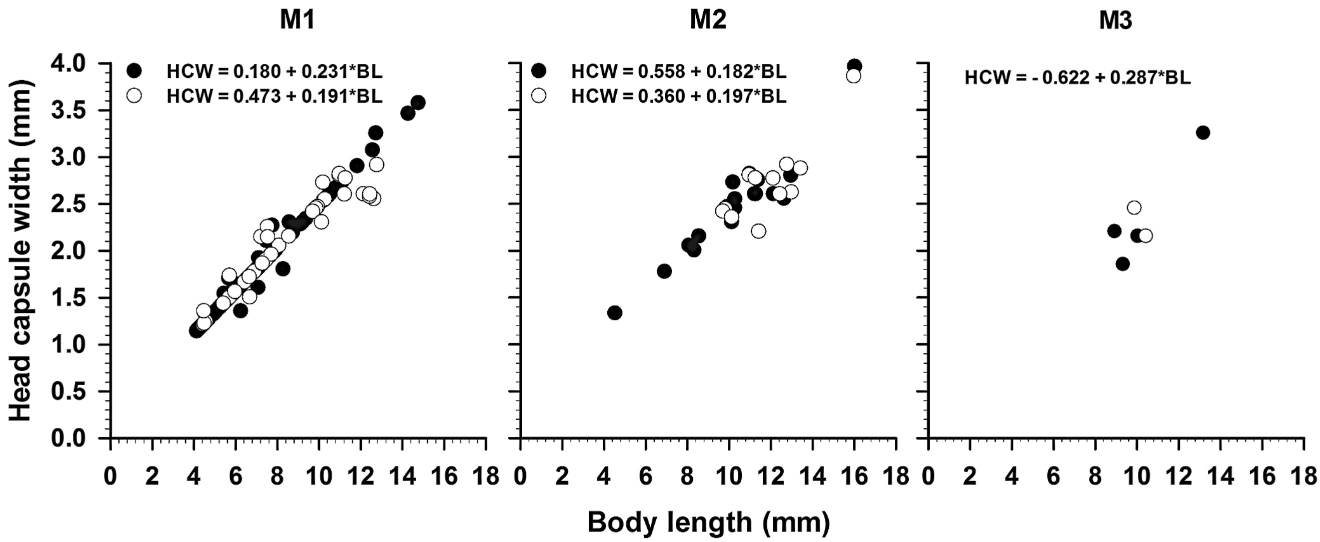

Figure 4. Relationship between body length (BL) and head capsule width (HCW) of three *Anacroneuria* morphospecies registered during contrasting seasons in the Prado River Basin (Tolima, Colombia). Regression equations are also shown. ●=dry season, ○=wet season.

sary to rear larvae in order to associate them with the adults (Hamada and Couceiro 2003). Therefore, more detailed studies are required in the region, beginning with adult collections, species identification and adult-larvae associations (rearing). These aspects are important for future life-cycle studies, nutrient fluxes, biodiversity and secondary production analyses in tropical rivers where the limnology remain understudied (Allan *et al.* 2006).

Alternation between dry and wet seasons. The entire Prado River Basin and network of streams experience two pronounced dry seasons (from January through March, and July through September) alternating with two rainy seasons from April to June and October to December, exhibiting notable seasonal precipitation, flow and discharge (Salazar *et al.* 2002; Guevara *et al.* 2009). During this bimodal water level variation, changes occur in the richness and abundance of benthos (Caupaz *et al.* 2006; Guevara *et al.* 2007a) and fish communities (Castro-Roa *et al.* 2007). This discharge in Andean rivers has been observed to be strongly seasonal (Allan *et al.* 2006). For instance, in rivers of the Colombian Andes subjected to high water-level conditions, a reduction in the abundance of invertebrates has been reported (Arias *et al.* 2007; Guevara *et al.* 2007b), but in northern Colombia, in the Sierra Nevada de Santa Marta Natural National Park, *Anacroneuria* larvae show similar distribution patterns (Tamaris-Turizo *et al.* 2007). Additionally, in tropical rivers the effects of predation by insectivorous fish on the population dynamics of *Anacroneuria* larvae are important (Tomanova and Tedesco 2007).

In general, tropical freshwater bodies are characterized by drastic variations in benthic community abundance during high water level conditions (Winemiller and Jepsen 1998; Jacobsen and Encalada 1998; Rincon and Cressa 2000; Wantzen 2003). Turcotte and Harper (1982) suggested that rainfall spates are the major factor regulating benthic densities in non-seasonal environments (Amazon drainage basin, Ecuador). The stonefly larvae abundance found during the wet season (November) was markedly different from that found during the dry season (August-September). The high precipitation registered during the second sampling period, November (wet season), increased discharge and consequently stream and river instability, altering the community structure by dislodging substrate and forcing drifting (Céréghino and Lavandier 1998; Céréghino *et al.* 2002). In a tropical river of Venezuela, *Anacroneuria* larvae were most abundant during the period of least precipitation (Pérez and Segnini 2005). These results are concomitant with a study of caddisfly larvae in the Coello River watershed, Central Colombia, where lowest abundances were recorded during the rainy season (Guevara *et al.* 2005, 2007b).

Physicochemical variables and altitude. Several physicochemical parameters varied in relation to seasonal hydrological variation in tropical rivers (Winemiller and Jepsen 1998). During extended wet periods, water temperature and conductivity tend to be lower, and dissolved oxygen concentrations tend to be higher. This tendency was observed in the majority of the variables measured in our study, which were related to seasonal changes in water level. However, only pH and conductivity were significantly correlated with abundance under low water level conditions suggesting a possible cause-and-effect relationship between these variables. During rising water events, conductivity appears to affect the total abundance of *Anacroneuria* larvae (Bücker *et al.* 2010). Throughout the drainage networks of the Prado River Basin, physicochemical attributes also are affected by human activities (e.g., agriculture, livestock, and cobble and sand extraction) mainly in lower altitude areas, generating different population responses in stoneflies to variations in altitude. Water temperature and dissolved oxygen decreased with altitude, while other measured variables were uncorrelated with elevation, as was reported for Ecuadorian streams (Jacobsen 2008). It is therefore necessary to explore with more detail the relationships among physicochemical variables, *Anacroneuria* species richness, abundance, emergence patterns, and altitude from headwater streams to the river's mouth.

In general terms, a decline in species richness with increasing altitude was observed (Von Ellenrieder 2007), as well as a decline in frequency of occurrence in certain species towards higher altitudes, without disappearing altogether (Jacobsen *et al.* 2003; Jacobsen and Brodersen 2008). Other species can disappear completely at different points along the altitude gradient, which affects their local and regional abundance (Romero-Alcaraz and Avila 2000).

Although the size of stoneflies in temperate regions seems to be correlated with environmental factors such as photoperiod, water temperature and season (Nesterovitch and Zwick 2003), our results suggest that environmental factors in the study area do not influence size or that these physicochemical parameters are not sufficiently different to affect larval size. Also, we did not find a significant relationship between body length and elevation, but it is possible that a further evaluation of pre-emergent *Anacroneuria* larvae may reveal significant correlations with altitude and/or any other physicochemical variable (e.g., Tomanova and Tedesco 2007; Cressa *et al.* 2008).

Acknowledgements

The authors would like thank to the anonymous reviewers for the very helpful suggestions and criticisms that improved the manuscript draft. All remaining errors and omissions are our responsibility. Thanks also to CORTOLIMA (regional environmental authority) and the University of Tolima for supporting this research. G. Guevara thanks the Austral University of Chile for the doctoral fellowships (MECESUP UCO0214 - AUS0703) and the Subcommittee of the Permanent Committee for Mayflies and Stoneflies Conferences for travel scholarships to attend the International Joint Meeting on Ephemeroptera and Plecoptera held in Stuttgart in June 2008.

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Recibido: 28-feb-2011 • Aceptado: 25-oct-2011