Chapter 9

RELATIONSHIP BETWEEN POLLUTION AND FLUCTUATING ASYMMETRY IN A POLLUTION-TOLERANT CADDISFLY *Hydropsyche exocellata* (TRICHOPTERA, INSECTA).

INTRODUCTION

Aquatic macroinvertebrates have been widely used as indicators of pollution in rivers and streams (e.g., Hynes, 1960; Reynoldson, 1984; Cairns & Pratt, 1993), and are part of most of protocols to assess water quality over the world. Because their sensitivity to pollution, caddisfly have been used in many cases to assess water quality (Malicky, 1981; Usseglio-Polatera & Bournaud, 1989; Resh, 1992; de Moor, 1999; Stuijfzand *et al.*, 1999; Bonada *et al.*, Chapter 8) at different taxonomical levels and looking at different responses. Thus, they have been used at order level in some multimetric approaches (e.g., EPT index — Barbour *et al.*, 1999) or included in some biological indexes at family or even species level (see Resh, 1992). Less frequent, although increasing, are the studies performed at individual level. In that sense it is interesting to point out the works using deformities (e.g., Décamps *et al.*, 1973; Petersen & Petersen, 1983; Camargo, 1991; Vuori, 1995; Vuori & Kukkonen, 2002), changes in colour patterns (Chapely et al., 1997), morphological asymmetries (e.g., Clarke, 1993; Hogg et al., 2001; Bonada & Williams, 2002) or toxicity tests (e.g., Greve *et al.*, 1998). Because biological indexes only confer information about the presence or absence of taxa in a site, without including the condition of the population of those taxa, biomarkers give us extra information about the effect of environmental pollutants to organisms (Peakall & Walker, 1994). In that sense, it has been considered that these studies may be useful in conservation ecology because knowing the population status of one species in front a pollutant may be a tool to avoid their disappearance if disturbance increases (Clarke, 1995).

Clarke (1993) pointed out the need to assess water quality using techniques focused in developmental processes at individual level. It is widely accepted that developmental stability of individuals (i.e., the ability to develop properly in the face of genetic and environmental stresses that tend to upset development —*sensu* Watson & Thornhill, 1994) may be affected by genetic or environmental factors (e.g., Van Valen, 1962; Clarke, 1992; Palmer & Strobeck, 1992). Developmental stability has been widely measured using Fluctuating asymmetry (FA) (i.e., random and small deviations from perfect bilateral symmetry in morphological traits) (e.g., Clarke, 1992). Thus, if high environmental stress yields a low developmental stability, this is measured as a high level of fluctuating asymmetry. Consequently, FA may be used as a cost-effective measure of environmental stress (e.g., Leary & Allendorf, 1989; Drover et al., 1999; Cuervo, 2000; Hogg et al., 2001). Recent studies conclude that Antisymmetry (AA) and in some cases Directional asymmetry (DA) can be also a measure of development stress (Graham *et al.*, 1993; Kark, 2001).

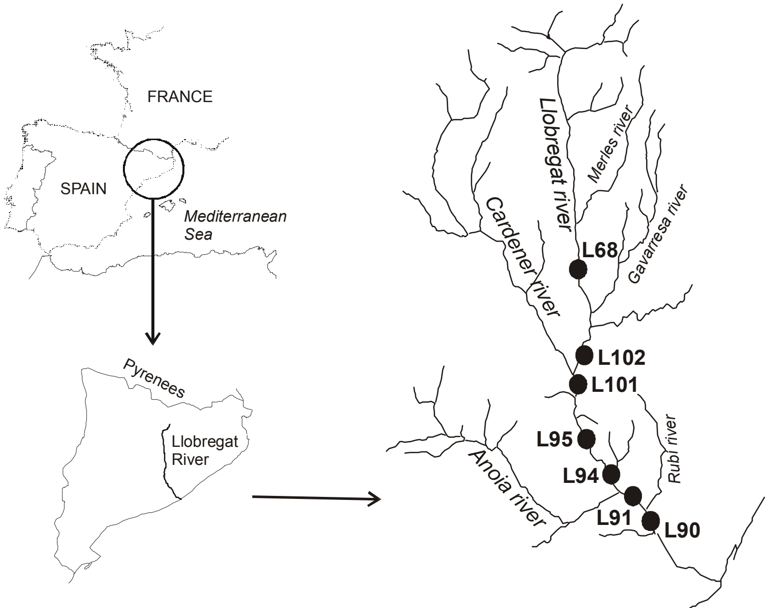
The three asymmetries (FA, AA, and DA) affecting organisms may be distinguished looking at the distribution frequencies of the measured values of a morphological trait comparing right and left side (R_i-L_i) (e.g., Van Valen, 1962; Parsons, 1990; Palmer & Strobeck, 1986). Directional Asymmetry (DA) is present in morphological character when the differences between the right and the left sides of the body (R-L differences) are normally distributed with a mean significantly different from zero. Antisymmetry (AA) occurs when the R-L differences are platykurtic or bimodal distributed, with a mean about zero. And finally, fluctuating Asymmetry (FA) is demonstrated when the R-L differences are normally distributed with a mean of zero in the ideal case (Van Valen, 1962; Palmer, 1994) although some leptokurtic distributions can be also admitted (see Palmer & Strobeck, 1992).

Studies relating FA with changes in the environment has increased in the last decade in many areas in Ecology (see review in Hogg et al., 2001). Some of the studies performed have been focused in aquatic macroinvertebrates relating FA with water quality variables (e.g., Groenendijk *et al.*, 1998; Dobrin & Corkum, 1999; Drover et al., 1999; Hardersen *et al.*, 1999; Hogg et al., 2001; Servia, 2001) or biological interactions (e.g., the effect of larval density in *Culex* sp. by Mpho et al., 2000 or the effect of a parasite in *Gammarus pulex* by Alibert et al., 2002). Most of these studies have been performed using midges (e.g., Clarke et al., 1995; Groenendijk *et al.*, 1998; Servia, 2001), Ephemeroptera (Dobrin & Corkum, 1999), Plecoptera (Hogg et al., 2001; Heteroptera (Drover et al., 1999); Crustacea (Savage & Hogarth, 1999; Alibert et al., 2002), or Odonata (Hardersen et al., 1999; Hardersen & Frampton, 1999; Hardersen, 2000) Overall, significant relationships between pollution variables and FA are reported, although it has been noticed that negative results in FA are rarely published (Dobrin & Corkum, 1999). However, although knowing its adequacy in water quality studies, few works have been performed looking at the asymmetries in caddisflies (but see Bonada & Williams, 2002). The present study try (1) to determine if levels of fluctuating asymmetry in the tolerant caddisfly *Hydropsyche exocellata* increase downstream a river system as a consequence of pollution using a large set of morphological traits and (2) to indicate possible chemical factors implied.

METHODOLOGY

Sampling area

The Llobregat basin with a drainage basin of 4948 km^2 is located in the northeast Spain (Figure 1). The main channel (the Llobregat River) flows from 1360 m to the sea in 145 km. The area is subjected to a mediterranean climate with an annual mean precipitation between 950 mm in headwaters to 550 mm in lowland reaches (Prat *et al.*, 1984; González *et al.*, 1985). It presents a dominant calcareous geology although some sedimentary deposits are found near the mouth (see Robles *et al.*, in press). Except for riparian zones and some isolated areas, sclerophyllous and evergreen trees and shrubs mainly compose basin vegetation. As in other mediterranean basins, the Llobregat has been largely affected by human activities as agriculture, cattle, urbanization, salinization by mining activity, water abstraction and regulation... affecting drastically the ecological status of the main river and tributaries (Prat *et al.*, 1984; 1997; 1999; 2000; 2001; 2002; González *et al.*, 1985). The first chemical and biological quality studies performed in the Llobregat basins date back from the late 70's (Prat *et al.*, 1984). During the 90's the construction of several water treatment plants along the Llobregat river and tributaries and a salt-collector improved substantially the chemical and biological water quality, allowing the survival of several macroinvertebrates in the lowland reaches (Prat *et al.*, 1997; 1999; 2000; 2001; 2002).



	number of specimens	
US	L68	9
	L102	25
MS	L101	12
	L95	30
	L94	30
DS	L91	30
	L90	29

Figure 1. Sampling area. The location of sampling sites in Llobregat River, and the number of specimens measured for sites are presented. Groups of sites used to compare levels of asymmetry and pollution are also presented (Upstream sites: US; Midstream sites: MS; Downstream sites: DS).

Sampling sites and procedure

Several reasons make *Hydropsyche exocellata* an ideal species to test the effect of pollution on the fluctuating asymmetry of larvae. *H. exocellata* has been considered as a pollution-tolerant species by several authors (e.g., Higler & Tolkamp, 1983; González del Tánago & García de Jalón, 1984; González *et al.*, 1985; Gallardo, 1994; Gallardo-Mayenco *et al.*, 1998; Usseglio-Polatera & Bournaud, 1989; Bonada *et al.*, Chapter 8), and when it is present it has been found with high abundances (Soler & Puig, 1999). It presents a variable life cycle from two to several generations per year (e.g., Tachet & Bournaud, 1981; García de Jalón, 1986; Soler & Puig, 1999) ant therefore specimens from the last instar can be found along the year (Soler & Puig, 1999; Vieira-Lanero, 2000).

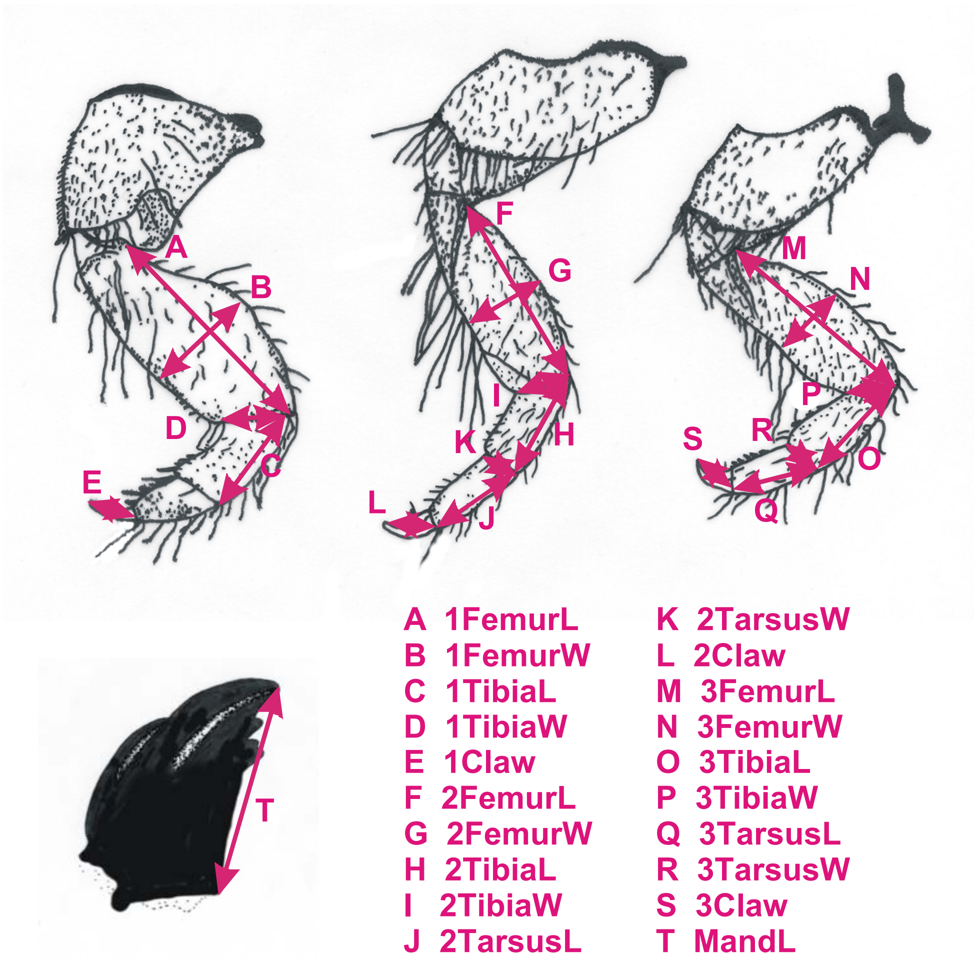


Figure 2. Measured traits on the first, second and third leg, respectively from left to right, and the mandible.

Larvae of *H. exocellata* were obtained from 7 sites in Llobregat River in summer 2000 (Figure 1). These localities have been grouped in three groups to facilitate comparisons and to increase sample size, differing in chemical and biological variables: upstream sites, midstream sites, downstream sites. These groups have been defined according to the limit of distribution of *H. exocellata* in Llobregat River. The upper localities (US: L68 and L102) present a better chemical and biological quality compared with downstream reaches, although L102 is slightly affected by salinity than L68 (Prat *et al.*, 1997; 1999; 2000; 2001; 2002). Before midstream sites (MS; L101, L95, L94), the Llobregat river receives the Cardener, which is highly influenced by salinity because the presence of several salt deposits and mining activities (Prat *et al.*, 1984), affecting notoriously the water quality of Llobregat river. In the lower parts (DS: L91, L90), Llobregat River is influenced by Anoia and Rubí rivers carrying out a high organic and industrial pollution despite the recent improvement on water quality (Prat *et al.*, 2002). Chemical data from spring and summer was obtained from Prat *et al.* (2002).

Larvae were collected in the field during summer. Ideally, 30 last instar individuals (1.250-1.950 mm head width, Soler & Puig, 2000) were analyzed for each sampling site (see data in Figure 1), although because of low population abundances this number was lower in some localities. A total of 20 bilateral morphological traits were selected to test the presence of FA and to relate it with pollution variables (Figure 2). Pair legs and mandibles were dissected in a slide with glycerin to facilitate the proper orientation of each piece to be measured. Left and right pieces from each trait were measured separately, under a stereoscope provided with a micrometer with an accuracy of 0.019 mm at the maximum magnification possible. Missing or damaged pieces (e.g., claws of entire legs) were not measured. Because FA might be highly biased by measurement error (e.g., Palmer, 1994; Merilä & Bjorklund, 1995; Björklund & Merilä, 1997), a subsample of 30 individuals selected at random was measured twice one day apart. Measurement error for each trait was detected with a two-way mixed-model ANOVA using sides as a fixed factor and individual as a random one (Palmer & Strobeck, 1986). Accordingly, when the interaction between side-individual is significant (i.e., $MS_{error}<MS_{effect}$) it can be assumed that no measurement error was done.

RESULTS

Selecting FA traits

Three main characteristics have been identified to bias FA measures, and therefore should be considered in any FA study: measurement error, other kinds of asymmetry, and allometry (see Palmer, 1994). Results from measurement error analysis indicated that only the 1Claw and the 3TibiaL characteristics presented a high measurement error ($p>0.05$). These two traits were deleted from the analysis to avoid bias in the FA evaluation.

The rest of traits were used to check asymmetry. For each individual and trait we calculate the signed difference between left and right side (R_i-L_i). From the resulting data set, outliers (e.g., presence of deformities) were omitted to avoid distortions in FA detection (Palmer, 1994). To evaluate the viability to group sites (i.e., US, MS, DS), a non-parametric Kruskal-Wallis test was applied to $|R\text{-}L|$ values of each trait and group. Because for each group none of the traits displayed significant differences ($p>0.05$), we considered these groups independently.

Several tests were performed with each group to detect FA from DA and AA: skewness, kurtosis, t-test, and visual observations from (R_i-L_i) distributions (see Annex 1 for results). Test for skewness and kurtosis have been considered very useful to distinguish Fluctuating Asymmetry (FA) from the other asymmetries, providing information about how a distribution departures from normality (Palmer & Strobeck, 1992). Any trait displayed Antisymmetry (AA), as kurtosis was positive for all cases. Directional Asymmetry (DA) were detected only for two traits: MandL for all group of sites and 1FemurW for downstream sites (DS) group. However, because 1FemurW only displayed DA in one occasion, we have assumed the possible presence of and Type I error. Therefore, FA was established for all traits except mandible (MandL); as the mean of the (R_i-L_i) were not different from 0 ($p>0.05$) and kurtosis were positive in all cases. However, kurtosis values were very high, being more typical of a leptokurtic than normal distribution, but this does not invalidate the assignation of the morphological traits measure to FA .

Once FA-traits are detected and because FA can be influenced by trait size (Palmer & Strobeck, 1986; Leung, 1988), r-Pearson correlations between $|R_i\text{-}L_i|$ respect $(R_i\text{-}L_i)/2$ were performed for each FA trait. Results indicated that asymmetry in 1TibiaA ($r=-0.111$, $p=0.038$), 2Claw ($r=-0.202$, $p=0.003$), 3FemurW ($r=-0.189$, $p=0.001$) and 3Claw ($r=-0.210$, $p=0.004$) were significant associated with size, but having low correlation coefficient. Although size-dependency traits can be treated using specific FA-indexes (see Palmer, 1994), to facilitate data analysis only traits showing independency with size were retained.

To plot and compare levels of FA between sites, FA1 index (FA1=mean$|R\text{-}L|$) was applied (see Palmer, 1994). Differences between groups of sites were carried out applying a non-parametric Kruskal-Wallis test using $|R_i\text{-}L_i|$ differences for each group of sites (Palmer & Strobeck, 1986). To check the extend of the influence of pollution on the asymmetry, FA levels were correlated with chemical variables using r-Pearson correlations.

Changes of asymmetry between sites and relationships with pollution

Overall, a downstream increase of asymmetry is presented by all traits, except in few cases (e.g., in 2FemurW). In some cases the extent of changes of FA1 downstream is different depending on the trait. All measured features from the first leg increase proportionally from upstream (US) to downstream (DS) sites. Besides, some traits from the second leg traits increase only from US sites to MS sites (midstream sites), whereas others in the third leg do it between MS and DS. Measured features from the first leg reach the maximum level of FA1 in downstream reaches compared with second and third leg traits. Patterns observed in Figure 3 agree with results from Kruskal-Wallis test (Table 2). Thus, for example, all traits present significant differences between US and DS sites, whereas only few traits are significant between US vs. MS or MS vs. DS sites. Some of the traits from the first and second leg display a significant increase of asymmetries between upstream and midstream sites, whereas others, mainly from the third leg, present lower asymmetries in middle than downstream sites (Table 2).

Table 2. Results from the Kruskal-Wallis non-parametric test (*p<0.05, **p<0.001).

Comparison	Trait	$\chi2$	p-value	
US vs MS	**1FemurL**	6.669	0.0098	**
	1FemurW	4.3738	0.0365	*
	1TibiaL	1.8946	0.1687	
	2FemurL	0.8212	0.3648	
	2FemurW	9.6574	0.0019	**
	2TibiaL	5.7959	0.0161	*
	2TibiaW	1.5358	0.2152	
	2TarsusL	10.9316	0.0009	**
	2TarsusW	4.0861	0.0432	*
	3FemurL	1.5358	0.2152	
	3TibiaW	1.0874	0.297	
	3TarsusL	0.1035	0.7476	
	3TarsusW	2.4779	0.1155	

Comparison	Trait	$\chi2$	p-value	
MS vs DS	**1FemurL**	0.8353	0.3607	
	1FemurW	3.8319	0.0503	
	1TibiaL	15.1879	0.0001	**
	2FemurL	3.0854	0.079	
	2FemurW	0.0533	0.8173	
	2TibiaL	3.743	0.053	
	2TibiaW	1.1584	0.2818	
	2TarsusL	0.0234	0.8784	
	2TarsusW	4.7999	0.0285	*
	3FemurL	2.2922	0.13	
	3TibiaW	4.7068	0.03	*
	3TarsusL	10.6543	0.0011	**
	3TarsusW	6.444	0.011	*

Comparison	Trait	$\chi2$	p-value	
US vs DS	**1FemurL**	10.0501	0.0015	**
	1FemurW	11.4181	0.0007	**
	1TibiaL	16.4244	0.0001	**
	2FemurL	4.8875	0.0271	*
	2FemurW	10.3234	0.0013	**
	2TibiaL	12.2243	0.0005	**
	2TibiaW	3.9541	0.0468	*
	2TarsusL	11.3539	0.0008	**
	2TarsusW	10.506	0.0012	**
	3FemurL	5.2878	0.0215	*
	3TibiaW	6.4159	0.0113	*
	3TarsusL	5.0385	0.0248	*
	3TarsusW	8.8747	0.0029	**

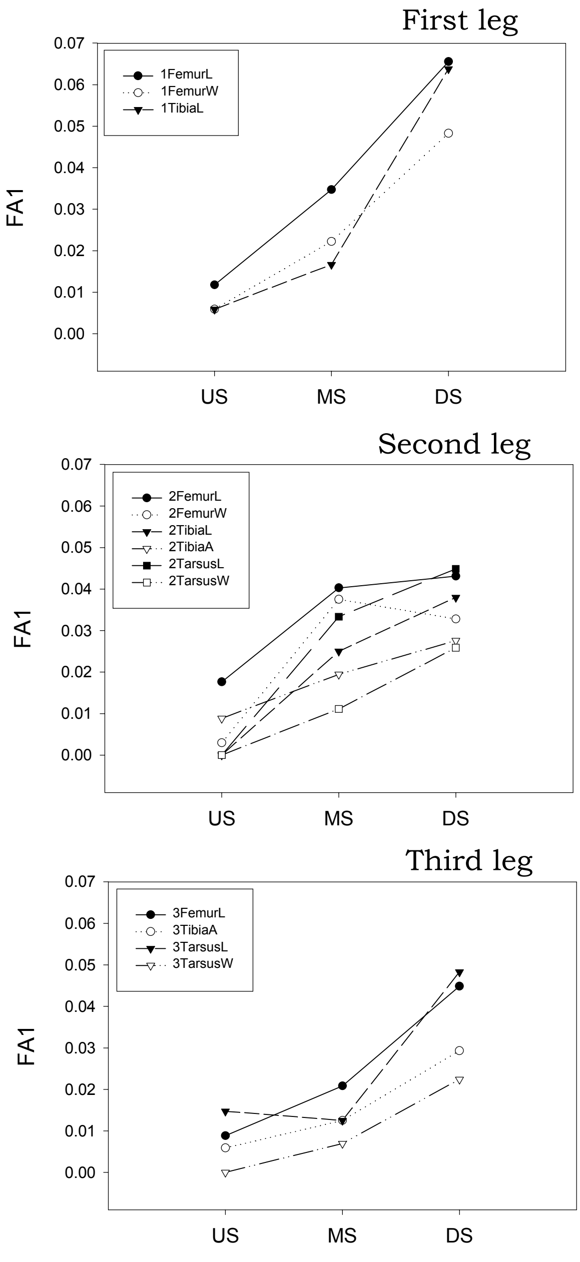


Figure 3. Levels of FA1 index between upper, middle and downstream sites. Each plot refers to traits measured in first, second and third leg respectively.

Similarly to levels of FA1, chemical parameters measured change downstream (Figure 4). Different patterns are observed for different variables. Values of suspended solids are similar between US and MS, but higher in DS sites. On the other hand, ammonium, P-phosphates, chloride and conductivity increase between upstream and middle reaches, remaining more or less constant in downstream reaches. It can not be observed differences downstream in N-nitrates and oxygen concentrations.

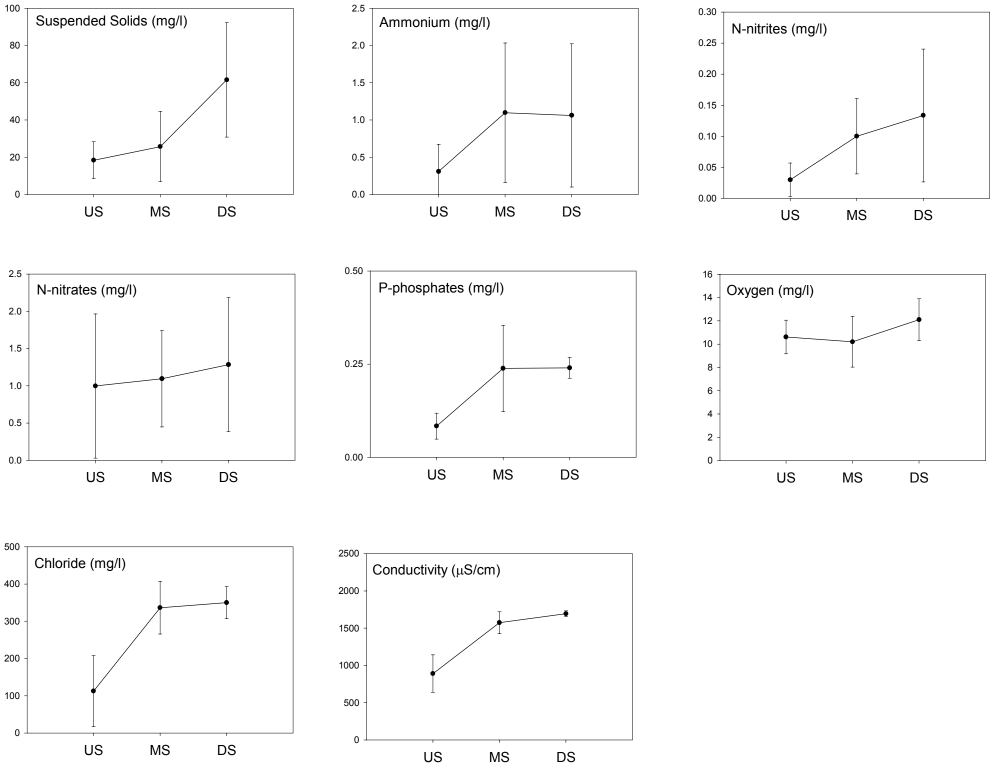


Figure 4. Mean and standard deviations of chemical variables between upper, middle and downstream reaches.

Comparing Figure 3 and 4, it appears that the increase of FA1 is related to some chemical parameters but not to others. When r-Pearson correlations are performed between chemical variables and measured traits, positive correlations result in all cases (see Table 3). However, only in few cases, significant values were obtained. Thus, suspended solids are related to an increase of asymmetry in all features except for second leg femur. Almost all traits are significantly correlated with salinity (i.e., chloride and conductivity). High concentrations of P-phosphates appear also correlated with most of the traits of the second leg, but not for the others.

Table 3. Pearson correlation coefficients (r) and p-values associated between measured traits and chemical parameters. Significant correlations are presented in bold.

		1FemurL	1FemurW	1TibiaL	2FemurL	2FemurW	2TibiaL	2TibiaW
Suspended Solids	*r-Pearson*	**0.635**	**0.646**	**0.673**	0.412	0.28	**0.536**	**0.581**
	p-value	**0.006**	**0.005**	**0.003**	0.101	0.276	**0.027**	**0.014**
Ammonium	*r-Pearson*	0.323	0.306	0.218	0.424	0.427	0.394	0.37
	p-value	0.206	0.232	0.4	0.09	0.088	0.118	0.144
N-nitrites	*r-Pearson*	0.487	0.475	0.402	0.503	0.454	0.521	0.514
	p-value	0.092	0.101	0.173	0.08	0.119	0.068	0.072
N-nitrates	*r-Pearson*	0.135	0.135	0.132	0.104	0.084	0.122	0.129
	p-value	0.619	0.617	0.625	0.701	0.757	0.651	0.635
Chloride	*r-Pearson*	**0.681**	**0.652**	**0.493**	**0.84**	**0.828**	**0.8**	**0.762**
	p-value	**0.003**	**0.005**	**0.044**	**0**	**0**	**0**	**0**
Oxygen	*r-Pearson*	0.291	0.306	0.363	0.093	0.002	0.191	0.233
	p-value	0.258	0.232	0.152	0.723	0.993	0.462	0.369
Conductivity	*r-Pearson*	**0.78**	**0.753**	**0.596**	**0.906**	**0.875**	**0.885**	**0.854**
	p-value	**0**	**0**	**0.012**	**0**	**0**	**0**	**0**
P-phosphates	*r-Pearson*	0.54	0.513	0.368	**0.684**	**0.682**	**0.646**	**0.612**
		0.107	0.129	0.296	**0.029**	**0.03**	**0.044**	**0.06**

		2TarsusL	2TarsusW	3FemurL	3TibiaW	3TarsusL	3TarsusW
Suspended Solids	*r-Pearson*	**0.491**	**0.634**	**0.658**	**0.666**	**0.652**	**0.662**
	p-value	**0.046**	**0.006**	**0.004**	**0.004**	**0.005**	**0.004**
Ammonium	*r-Pearson*	0.409	0.324	0.284	0.262	0.112	0.274
	p-value	0.103	0.205	0.269	0.31	0.67	0.288
N-nitrites	*r-Pearson*	0.519	0.488	0.458	0.44	0.303	0.45
	p-value	0.069	0.091	0.115	0.133	0.314	0.123
N-nitrates	*r-Pearson*	0.116	0.134	0.136	0.135	0.118	0.135
	p-value	0.669	0.62	0.617	0.618	0.664	0.617
Chloride	*r-Pearson*	**0.822**	**0.683**	**0.613**	**0.572**	0.292	**0.594**
	p-value	**0**	**0.003**	**0.009**	**0.016**	0.255	**0.012**
Oxygen	*r-Pearson*	0.154	0.29	0.324	0.339	0.396	0.331
	p-value	0.556	0.26	0.205	0.183	0.116	0.194
Conductivity	*r-Pearson*	**0.901**	**0.782**	**0.715**	**0.675**	0.392	**0.697**
	p-value	**0**	**0**	**0.001**	**0.003**	0.12	**0.002**
P-phosphates	*r-Pearson*	**0.666**	0.541	0.478	0.441	0.185	0.461
		0.035	0.106	0.163	0.202	0.609	0.18

DISCUSSION

The high level of kurtosis and the high skewness values found in some characters, even though the mean of R-L differences were not different from zero, may be related to the precision of the measurement system used (see Cuervo, 2000). A lower precision system may only display big differences in R-L measures and overlook the small ones, whereas in very precise systems rarely an individual displays in a trait a R-L of 0 although the global mean is not different of 0. However, independently of the measurement technique used, Palmer & Strobeck (1992) pointed out that leptokurtic distributions might be obtained as a result of a mix of individuals with low and high FA or a mix of individuals with FA and AA, beeing very difficult to discern both situations. However, Leung & Forbes (1997) modeling FA found that leptokurtic distributions may be possible and therefore be subjected to environmental stress, as the ideal FA does. Likely, a repercussion of the use of a less precise measurement method is an underestimation of the real level of FA in a population, although that is not a problem when levels of FA in a population are used to compare with others or to relate them to environmental variables.

Hydropsyche exocellata is a very pollution tolerant caddisfly in the Iberian Mediterranean area (González del Tánago & García de Jalón, 1984; Millet & Prat, 1984; Gallardo, 1994; Bonada *et al.*, Chapter 8). This species has been found in very saline environments in the south of Spain, until 8400 μS/cm (Gallardo, 1994). However, few is known about the status of the populations subjected to different water pollution levels. In our study we have observed that although *H. exocellata* is able to survive to relatively high pollution levels, the developmental stability is lower downstream with the increasing of pollution. From all measured chemical variables, salinity and suspended solids appear to influence the asymmetry of almost all traits, whereas phosphates only affects to specific characters. The large number of morphological characters and environmental variables measured in this study support the suggestion made by several authors that to determine the relationship between asymmetry and pollution, a large set of variables, as FA can not be detected by all environmental stressors individually (Leary & Allendorf, 2000; Clarke *et al.*, 1995; Hogg *et al.*, 2001). In that sense, Clarke *et al.* (1995) pointed out that FA is the result of the combined effect of several environmental variables rather than single ones. For example in a study in the asymmetry of adults of *Hexagenia rigida* in Canadian lakes, Dobrin & Corkum (1999) did not find a relationship between PCB concentrations in lakes and FA in mayflies. Consequently, they pointed out that the relationship between FA and PCB could be masked by the effects of other non-measured factors. Similarly, Hogg *et al.* (2001) looking at the effect of small temperatures shifts on meristic traits on *Nemoura trispinosa* did not found significant results. One of the explanations provided by the authors is that it might be possible that a high FA in control sites was present by non-measured stressors which effects are unable to separate from the temperature.

Few is known about the mechanisms that enhance development instability under environmental stress and how this is translated to asymmetries in the individuals (but see Emlen, 1993). Consequently, difficulties are found to interpret why some chemical variables are related to FA instead of others, and to discern if there is a direct of an indirect effect of the factor over the development. Suspended solids, salinity and phosphates are the most significant variables to explain an increase of FA in *H. exocellata* in Llobregat River.

When populations are compared, one of the difficulties is to select the characters to be used and to know if results will vary depending on the character (Leary & Allendorf, 1989; Lajus, 2001). Therefore it is necessary to use multiple traits to obtain reliable results (Watson & Thornhill, 1994), although it has been observed reviewing literature that is unlikely that the positive relationship between FA and stressors depend on the number of traits analyzed (Hogg *et al.*, 2001). Consequently, although it is recommended to use several traits to test for FA in relation to stress, our results suggest that at least for *H. exocellata*, few traits may be enough. This could simplify the harsh work of measure FA, and make the method more efficient, effective, easy to use and low in cost to be applied in biomonitoring programs, as it has pointed out by Clarke (1993). In that sense, fluctuating asymmetry has been identified as an easy and efficient method to assess the population status before its extinction by increasing environmental stress (Leary & Allendorf, 1989; Clarke, 1993, 1994, 1995), and therefore in that sense, it could be a useful tool for a proper management of aquatic systems.

In the lower parts of the Llobregat river, *H. exocellata* can survive although an increase of asymmetry is detected compared with upper reaches. Our data suggest that fluctuating asymmetry could be used as an early warning system of the disappearance of this species, which has been considered very important for river processes (see Soler & Puig, 1999). Because a high biological stress of larvae have been identified in lower Llobregat reaches, the presence of this species in these localities may be instable. It would seem improbable that larvae can disappear because to the increase of the high asymmetry itself, unless that functional traits are highly affected constraining the survival of larvae or the moulting to another instar. More work should be done to detect asymmetry in functional characters. Moreover, experimental work is needed to quantify the pollution threshold from where the population developmental patters are highly instable.

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Annex 1. Values of mean (R_i-L_i), skewness and kurtosis with standard errors. Results from the t-test and the p-value associated are also presented.

US	Mean	SE	Skewness	SE	Kurtosis	SE	t	p-value
1FemurL	0	0	2.42	0.41	15.5	0.8	0	1
1FemurW	-0.0062	0	-3.79	0.41	13.22	0.8	-1.438	0.161
1TibiaL	0.0071	0	3.51	0.44	11.18	0.85	1.441	0.161
1TibiaW	-0.0103	0	-0.29	0.43	1.45	0.84	-1.14	0.264
2FemurL	0	0.01	-1.48	0.44	7.17	0.85	0	1
2FemurW	-0.0033	0	-5.47	0.42	30	0.83	-1	0.326
2TibiaL	0	0	-	-	-	-	-	-
2TibiaW	-0.0034	0	-0.77	0.43	8.02	0.84	-0.571	0.573
2TarsusL	0	0	-	-	-	-	-	-
2TarsusW	0	0	-	-	-	-	-	-
2Claw	0.0111	0.01	3	0.71	9	1.39	1	0.347
3FemurL	0.012	0	2.49	0.46	4.56	0.9	1.809	0.083
3FemurW	-0.0035	0	-5.29	0.44	28	0.85	-1	0.326
3TibiaW	0.0086	0	3.14	0.48	8.6	0.93	1.447	0.162
3TarsusL	-0.0125	0.01	-1.98	0.47	6.86	0.91	-1.141	0.266
3TarsusW	0	0	-	-	-	-	-	-
3Claw	-0.01	0.01	-3.16	0.68	10	1.33	-1	0.343
MandL	0.4333	0.03	0.2	0.44	1.43	0.87	12.679	0

MS	Mean	SE	Skewness	SE	Kurtosis	SE	t	p-value
1FemurL	-0.0014	0	-1.17	0.28	4.83	0.56	-0.178	0.859
1FemurW	0.0085	0	0.27	0.28	1.45	0.56	1.514	0.135
1TibiaL	0.009	0	1.39	0.29	5.64	0.58	1.623	0.109
1TibiaW	-0.0061	0	-0.59	0.29	2.25	0.58	-0.839	0.375
2FemurL	0.02	0.01	3.86	0.29	23.99	0.58	1.275	0.207
2FemurW	0.0164	0	0.93	0.29	3.28	0.57	1.744	0.086
2TibiaL	0.009	0	2.65	0.29	12.66	0.58	0.948	0.347
2TibiaW	-0.0061	0	-0.93	0.29	0.18	0.58	-1	0.321
2TarsusL	0.021	0.01	1.58	0.31	4.17	0.62	1.997	0.051
2TarsusW	-0.007	0	-0.85	0.31	4.26	0.62	-1.427	0.159
2Claw	-0.0157	0.01	-1.14	0.38	1.98	0.74	-1.356	0.183
3FemurL	-0.005	0	-0.01	0.31	4.56	0.61	-0.685	0.496
3FemurW	0.0083	0	0.39	0.3	4.04	0.6	1.043	0.301
3TibiaW	-0.002	0	-0.12	0.33	2.94	0.66	-0.33	0.743
3TarsusL	0.006	0	3.1	0.33	17.19	0.66	0.771	0.444
3TarsusW	0.0063	0	1.38	0.34	6.66	0.68	1.353	0.183
3Claw	0.035	0.02	5.14	0.37	29.49	0.73	1.663	0.104
MandL	0.403	0.01	-0.76	0.29	1.4	0.58	26.823	0

DS	Mean	SE	Skewness	SE	Kurtosis	SE	t	p-value
1FemurL	0.0037	0.01	-1.26	0.32	8.25	0.63	0.204	0.839
1FemurW	0.029	0.01	1.62	0.32	5.11	0.63	2.466	0.017
1TibiaL	0.0267	0.01	0.04	0.31	2.09	0.62	1.936	0.058
1TibiaW	0	0.01	0.19	0.32	2.92	0.63	0	1
2FemurL	-0.0017	0.01	0.47	0.31	3.43	0.62	-0.168	0.868
2FemurW	0.0052	0	-0.51	0.31	1.7	0.62	0.651	0.517
2TibiaL	0	0.01	-1.49	0.31	5.91	0.62	0	1
2TibiaW	-0.0035	0	0.6	0.31	2.68	0.62	-0.468	0.642
2TarsusL	-0.0037	0.01	0.46	0.32	7.21	0.64	-0.256	0.799
2TarsusW	0.006	0	0.04	0.33	0.48	0.66	0.771	0.444
2Claw	-0.0128	0.01	-1.68	0.37	5.37	0.74	-0.819	0.418
3FemurL	0.0279	0.01	2.19	0.36	6.36	0.7	1.576	0.123
3FemurW	0.0133	0.01	0.42	0.35	0.2	0.69	1.182	0.244
3TibiaW	0.0071	0.01	0.4	0.36	0.66	0.71	0.684	0.498
3TarsusL	0.025	0.01	1.29	0.37	3.71	0.73	1.325	0.193
3TarsusW	-0.0025	0	0	0.37	0.17	0.74	-0.274	0.786
3Claw	-0.0166	0.01	-0.81	0.42	1.65	0.83	-1	0.326
MandL	0.3755	0.03	-1.54	0.33	9.16	0.66	12.139	0