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Study of Antioxidant Defense in Four Species of Perloidea (Insecta, Plecoptera)

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The aim of the present work is to conduct a comparative study of oxidative states in the nymphs of four species of Plecoptera belonging to the superfamily Perloidea: *Perla marginata* (Panzer, 1799) (family Perlidae), *Guadalgenus franzi* (Aubert, 1963), *Isoperla curtata* Navás, 1924, and *Isoperla grammatica* (Poda, 1761) (family Perlodidae) in relation to their ecological and biological characteristics. For this, the activity of the following antioxidant enzymes was determined: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione transferase (GST), and DT-diaphorase (DTD), together with lipid peroxidation. Glucose 6-phosphate dehydrogenase (G6PDH) was also determined. The four species studied were selected based on significant ecological and biological differences. The results obtained when studying different indicative parameters of the oxidative state of the nymph of different species showed that each has an important enzymatic antioxidant potential, and that differences among species are conditioned by the duration of the nymphal development period more than by whether they come from permanent or temporary habitats. Thus, Plecoptera, although traditionally considered as typical inhabitants of permanent waters, seem to have sufficient variability in physiological mechanisms, together with behavioral and ecological adaptations, to cope with potentially unfavorable conditions that may occur in temporary waters.

Key words: Plecoptera, antioxidant enzymes, oxidative stress, nymphal stage, ecological adaptation

INTRODUCTION

The generation of reactive oxygen species (ROS) in living cells is offset by the ability of the organism's antioxidant defence systems to buffer them. Imbalances caused either by excessive free radical generation or reduced efficiency of antioxidant defence systems would lead to the accumulation of free radicals and associated oxidative damage to macromolecules.

Studies of different antioxidant defenses in insects began in the early 1990s (Ahmad and Pardini, 1990). Though when compared with mammals, these studies are scarce, it has been demonstrated that there exists an antioxidant defense system similar to that of other extant organisms (Peng et al., 1986; Ahmad and Pardini, 1990; Aucoin et al., 1991; Ahmad, 1992; Bi and Felton, 1995; Choi et al., 1999; Barbehenn, 2002; Berra et al., 2004; Parker et al., 2004; Kim et al., 2007; Datkhile et al., 2009).

The study of the oxidative state of animals in general and insects in particular may be a tool to indicate physiological peculiarities in metabolic activity, which is directly related with ROS production (Ahmad and Pardini, 1990) and dietary habits (Barbehenn, 2002, 2003; Kim et al., 2007). Moreover, some studies have showed a correlation of oxidative state with developmental age of the individual (Nickla et al., 1983; Sohal and Allen, 1986). Thus, because insects are animals with mainly short life cycles, the study of the evolution of the oxidative state with age might provide in-depth knowledge of the relation between oxidative stress and age (Nickla et al., 1983; Sohal and Allen, 1986).

Furthermore, antioxidant defense level may suffer changes in animals exposed to stressing conditions, and so can be used as a biochemical marker. Biomarkers in aquatic species are regarded as important elements for detecting the presence of pollutants and changes in environmental factors (temperature, pH, oxygen content, etc) (Berra et al., 2004; Niemi and McDonald, 2004; Bonada et al., 2006).

Studies on oxidative stress have been carried out in some insect orders, such as Diptera, Orthoptera, and Lepidoptera (Ahmand and Pardini, 1990; Barbehenn, 2003; Datkhile et al., 2009). Berra et al. (2004) presented data on the activity of antioxidant enzymes for several macroinvertebrates, including two Plecoptera families (Perlidae and Leuctridae), from two Italian rivers. Their results emphasized significant differences among taxa (at the family level or higher) concerning the variability in specific activity of most enzymes. Nevertheless, variability among species belonging to same family can also be expected.

Plecoptera is a small order of hemimetabolous insects with more than 3,500 described species (Fochetti and Tierno de Figueroa, 2008). They constitute a numerically and ecologically significant component in running waters of

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all sizes, all over the world (Zwick, 2004), playing different ecological roles as primary and secondary consumers, and constituting, together with some other aquatic insects, the main component of the diet of many temperate freshwater fishes, and particularly of those with commercial and recreational value (Resh and Rosenberg, 1984; Fochetti and Tierno de Figueroa, 2008). Despite being externally very homogeneous, the scarce literature on this taxon shows that Plecoptera present great diversity in their anatomy, ultrastructure, biochemistry, physiology, and ecology (Tierno de Figueroa et al., 2003). They have further been used as biogeographical indicators (Zwick, 2003) and in evolutionary research [for instance, in the evolution of insect wings (eg. Thomas et al., 2000; Marden et al., 2000) and respiratory proteins (eg. Amore and Fochetti, 2009)], and they are frequently employed as bioindicators of water quality; the highest values as indicators of good ecological and environmental quality are normally assigned to this group (Fochetti and Tierno de Figueroa, 2008). Moreover, they are probably one of the most endangered groups of insects. In fact, due to the growing pollution of watercourses and to the strict ecological needs of Plecoptera, many stonefly species are reduced to small isolated populations and some others have already gone extinct (Fochetti and Tierno de Figueroa, 2006).

The aim of the present work is to compare the oxidative states of the nymphs of four Plecoptera species belonging to the superfamily Perloidea: *Perla marginata* (Panzer, 1799) (family Perlidae), *Guadalgenus franzi* (Aubert, 1963), *Isoperla curtata* Navás, 1924 and *Isoperla grammatica* (Poda, 1761) (family Perlodidae) in relation to their ecological and biological characteristics. For this purpose, the activity of the following antioxidant enzymes will be determined: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione transferase (GST), DT-diaphorase (DTD), together with lipid peroxidation. The activity of glucose 6-phosphate dehydrogenase (G6PDH), as an enzyme implicated in supporting reducing power due to its intervention on the NADPH synthesis, will also be determined. These four species were selected given their significant ecological and biological differences with respect to temporality of the habitat (permanent vs. seasonal), duration of nymphal development, nymphal feeding type, presence/absence of adult feeding, mature nymphal size, and stage in which spermatogenesis occurs (Table 1). Thus, it is hoped that valuable information will be obtained regarding the relationship between the ecological and biological characteristics of particular species and their oxidative state, which will allow us better to understand the degree of adaptation of stoneflies to different habitats and conditions. Knowledge of the oxidative state under natural condition will serve as the starting point for future work seeking to explain the interaction between abiotic and biotic factors in this insect group.

MATERIALS AND METHODS

Collection locations and samplings

Nymphs of the four species studied (*Perla marginata*, *Guadalgenus franzi*, *Isoperla grammatica*, and *I. curtata*) were collected in three streams from the Southern Iberian Peninsula: Río Dílar (Sierra Nevada, Granada) coordinates: 37°4′3.14″N 3°34′30.26″W, Río Aguas Blancas (between Sierra Nevada and Sierra de Huétor, Granada) coordinates: 37°13′23.92″N 3°24′30.25″W, and Río Despeñaperros (Sierra Morena, Jaén) coordinates: 38°22′21.97″N 3°30′27.87″W. Río Dílar and Río Aguas Blancas are permanent streams sited at 900 and 1140 m.a.s.l., respectively. Río Despeñaperros is sited at 560 m.a.s.l., and has a seasonal regime, becoming dry during summer. All three streams were well preserved, at least in the sampling sites, and did not present significant contamination levels. Physico-chemical data and collection dates are presented in Table 2. Water temperature, conductivity, dissolved oxygen, turbidity, and pH were recorded in situ.

Nymphs were collected with a Surber sampler (0.09 m^2, 250-μm mesh size), identified in situ and immersed in liquid nitrogen for their transport to the laboratory. All individuals were collected in the final stages of their nymphal development. This makes it possible to establish comparisons between species with different life cycle durations, although they were collected in different months.

Treatment of samples

Animal samples (approximately 2–8 individuals/sample depending on size; five replicates per species) were homogenized in ice-cold buffer (100 mM Tris–HCl, 0.1 mM EDTA and 0.1% triton X-100 (v/v), pH 7.8) at a ratio of 1:4 (w/v). Homogenates were centrifuged at 30000 × *g* for 30 min in a Centrikon H-401 centrifuge.

Table 1. Biological and ecological data of the studied species. Information from Sánchez-Ortega and Alba-Tercedor (1990, 1991), Tierno de Figueroa and Sánchez-Ortega (1999), Fausto et al. (2002), Tierno de Figueroa et al. (2003), Bo et al. (2008), López-Rodríguez et al. (2009), and unpublished data.

	P. marginata	*G. franzi*	*I. grammatica*	*I. curtata*
Family	Perlidae	Perlodidae	Perlodidae	Perlodidae
Stream regime	Permanent	Seasonal	Permanent	Seasonal
(Stream name)	(R. Dílar)	(R. Despeñaperros)	(R. Aguas Blancas)	(R. Despeñaperros)
Duration of nymphal development (months)	~ 34	~ 13	~ 10	~ 6
Duration of life cycle (egg → adult) (months)	~ 36	~ 24	~ 12	~ 12
Nymphal feeding	Carnivorous	Omnivorous	Omnivorous	Omnivorous
Adult feeding	No	Probably scarce or absent	Yes	Yes
Size of mature nymph (mm)	Big (up to 33)	Medium (up to 21)	Small (up to 13)	Small (up to 15)
Spermatogenesis	Completed during the nymphal stage	No data available	Completed during the adult stage	Probably completed during the adult stage

Table 2. Characteristics of the sampling sites.

Stream (sampling date)	Temperature	Conductivity	Dissolved oxygen	pH	Turbidity	Collected species
R. Dílar (15-I-2009)	4.5°C	242 μS/cm^2	110%	Slightly basic	No data	*P. marginata*
R. Despeñaperros (24-IV-2009)	13.3°C	277 μS/cm^2	80%	Slightly basic	307 NTU	*G. franzi* *I. curtata*
R. Aguas Blancas (22-IV-2009)	10.65°C	498 μS/cm^2	105%	Slightly basic	518 NTU	*I. grammatica*

After centrifugation, the supernatant was collected and frozen at –80°C until analysed.

Analytical methods

All enzymatic assays were carried out at 25 ± 0.5°C using a PowerWavex microplate scanning spectrophotometer (Bio-Tek Instruments, USA) in duplicate in 96-well microplates (UVStar®, Greiner Bio-One, Germany). The enzymatic reactions were initiated by the addition of the tissue extract, except for SOD, for which xanthine oxidase was used. The specific assay conditions were as follows.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decrease of H_2O_2 concentration at 240 nm according to Aebi (1984). The reaction mixture contained 50 mm potassium phosphate buffer (pH 7.0) and freshly prepared 10.6 mM H_2O_2.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured spectrophotochemically by the ferricytochrome c method using xanthine/xanthine oxidase as the source of superoxide radicals. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome c and 0.024 IU ml^{-1} xanthine oxidase. One activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome c reduction rate measured at 550 nm (McCord and Fridovich, 1969).

Glutathione peroxidase (GPX; EC 1.11.1.9) activity was measured following the method of Flohé and Günzler (1984). A freshly prepared glutathione reductase solution (2.4 U ml^{-1} in 0.1 M potassium phosphate buffer, pH 7.0) was added to a 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM sodium azide, 0.15 mM NADPH and 0.15 mM cumene hidroperoxide. After the addition of 1 mM GSH (reduced glutathione), the NADPH-consumption rate was monitored at 340 nm.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed as described by Calberg and Mannervik (1975), with some modifications, by measuring the oxidation of NADPH at 340 nm. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.63 mM NADPH, and 0.15 mM GSSG.

Glutathione transferase (GST; EC 2.5.1.18) activity was determined by the method of Habig et al. (1974) adapted to microplate. The reaction mixture consisted of 0.1 M phosphate buffer (pH 6.5), 1.2 mM GSH and 1.23 mM solution of 1-chloro-2,4- dinitrobenzene in ethanol were prepared just before the assay. GST activity was monitored at 340 nm by the formation of glutathione-CDNB-conjugate.

DT-diaphorase (DTD; EC 1.6.99.2) activity was measured as described by Sturve et al. (2005). The reaction mixture contained 50 mM Tris-HCl (pH 7.3), 50 μM DCPIP (2,6-dichlorophenol indophenol) and 0.5 mM NADH. Control reaction was measured by the addition of distilled water instead of sample extract. DTD activity was defined as the rest between sample and control DCPIP reduction.

With the exception of SOD, for which the arbitrary units have already been mentioned, for enzymatic activities, one unit of activity is defined as the amount of enzyme required to transform 1 μmol of substrate/min under the above assay conditions.

The protein content of the supernatant solutions was determined by the Bradford method (1976), using bovine serum albumin as standard.

Lipid-peroxidation levels were determined by quantifying the concentration of thiobarbituric-acid-reacting substances (TBARS), expressed as nmol malondialdehyde (MDA) per g tissue, according to Buege and Aust (1978).

All biochemicals, including substrates, coenzymes, and purified enzymes, were obtained from Roche (Mannheim, Germany) or Sigma Chemical Co. (USA). All other chemicals came from Merck (Darmstadt, Germany) and were of the reagent grade.

Statistical analysis

Results are expressed as means ± SE. Data were analyzed by a one-way analysis of variance (ANOVA), after testing normality for every variable (Kolmogorov-Smirnov $P > 0.05$). Significant differences among means ($P \leq 0.05$) in the same species were determined by the Duncan's multiple-range test (Duncan, 1955). For the analysis of the dependence between two variables, the Pearson correlation coefficient was estimated and the data were adjusted by linear regression. Data analyses were performed using a SPSS version 13.0 for Windows software package.

RESULTS

Results of the specific activities of different antioxidant defense enzymes (SOD, CAT, GPX, GR, GST and DTD) in the studied species are shown in Fig. 1. These results show that values found in antioxidant enzymes SOD, GPX, GST and DTD are very similar in *I. grammatica* and *I. curtata*, with a significantly higher activity for CAT and GR activity in *I. grammatica*.

P. marginata shows higher activity in the antioxidant enzymes SOD and GST, although the difference is only significant for GST, in comparison with the remaining species. *G. franzi* shows higher activity for CAT and GPX, and a lower activity of DTD and GST than the rest of species.

G6PDH activity (Fig. 1) is similar in every species except *P. marginata*, in which it is significantly lower.

Fig. 2 shows the total antioxidant capacity, i.e. the sum of the different antioxidant enzyme activities of each species. *P. marginata* has a higher total antioxidant activity, followed by *G. franzi*. Finally the value for *I. grammatica* is lower than that of *G. franzi* and higher than that of *I. curtata*.

Regarding the levels of lipid peroxidation (Fig. 3) *G. franzi* and *Isoperla* spp. maintain lower levels of this, while *P. marginata* show significantly higher values.

From Fig. 4 it can be appreciated that a lower concentration of corporal soluble proteins are found in *P. marginata* than in the other studied species.

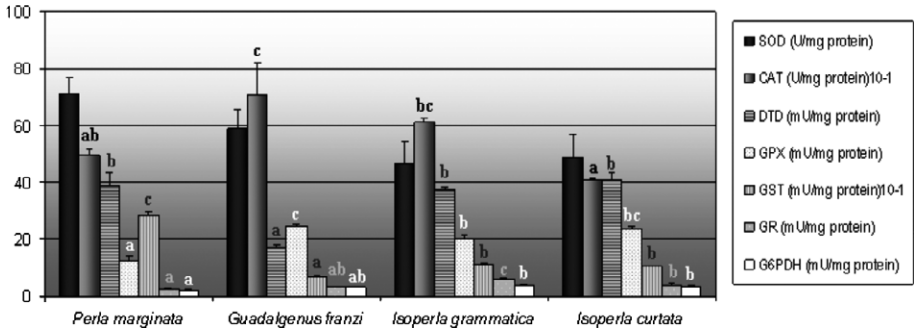

Fig. 1. Enzyme specific activity of superoxide dismutase, (SOD), catalase (CAT), diaphorase (DTD), glutathione peroxidase (GPX), glutathione transferase (GST), glutathione reductase (GR), and glucose 6 phosphate dehydrogenase (G6PDH) in the four Plecoptera species studied (a, b, c, d: significant differences among species for a same enzyme, $P \leq 0.05$).

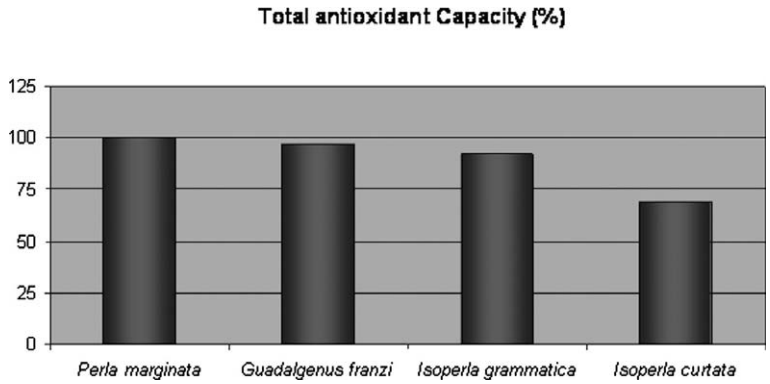

Fig. 2. Total antioxidant capacity (the sum of the different antioxidant enzyme activities of each species, assigning a value of 100% to the species with the highest value, and a percentage relative to this to other species) in four studied Plecoptera species.

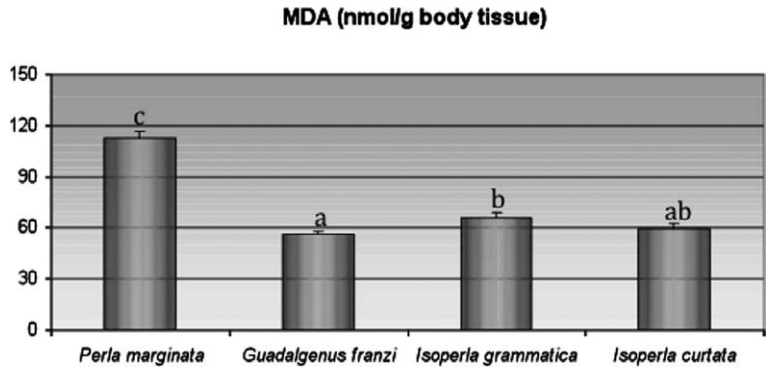

Fig. 3. Lipid peroxidation expressed as malondialdehyde levels (MDA) in the four studied Plecoptera species (a,b,c,d: significant differences among species, $P \leq 0.05$).

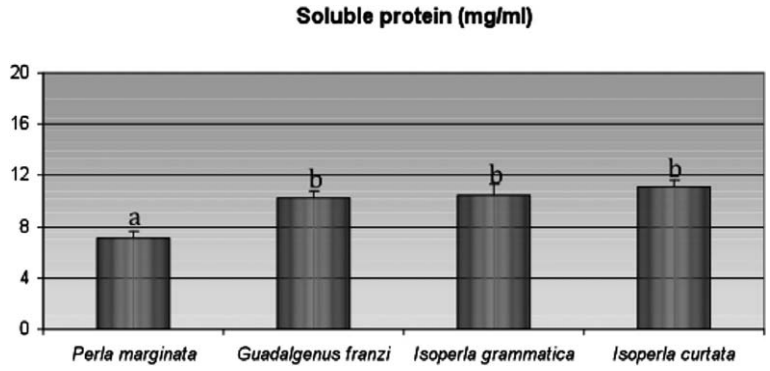

Fig. 4. Total body soluble protein concentration in the four Plecoptera species studied (a,b,c,d: significant differences among species, $P \leq 0.05$).

DISCUSSION

Our results show that the four stonefly species share an important antioxidant enzymatic pool, mainly in relation to the level of SOD and especially CAT. It should be emphasized that the values of CAT activity are higher than those reported in previous studies using the same methodology in other taxa, such as in different fish tissues (sturgeon and trout) (Trenzado et al., 2006; Furné et al., 2008) and in *Artemia* sp. (Díaz et al., 2009).

The SODs are one of the first enzymatic defenses against ROS, which are primarily generated by aerobic metabolism. The SOD enzymes work by converting negatively charged oxygen molecules to hydrogen peroxide (H_2O_2) subsequently reduced by CAT and GPX, to H_2O. Parker et al. (2004) suggest a general pattern of SOD defence for metazoans that includes the full repertoire of mitochondrial, cytoplasmic, and extracellular SODs. In this study we found higher, although not statistically significant, SOD activity in *P. marginata* and *G. franzi* than in the other species studied.

CAT is the most important scavenger of H_2O_2 produced in the mitochondria and peroxisomes at a higher steady-state concentration. This enzyme shows dual activity (Chance et al., 1979): catalase activity when two molecules of H_2O_2 are the substrate, and peroxidase activity when one molecule of H_2O_2 and one of alcohol are the substrate. The catalase gene is well-studied in *Drosophila melanogaster* (Orr et al., 1990), *Anopheles gambiae* (DeJong et al., 2007), *Apis melifera* (Corona and Robinson, 2006), *Bombyx mori* (Yamamoto et al., 2005), and *Protaetia brevitarsis* (Kim et al., 2007). Bibliographic data on Plecoptera are almost non-existent. The only data for Perlidae and Leuctridae, at family level, are available showing for the former (the only belonging to the superfamily object of this study) high values of this enzymatic activity (Berra et al., 2004). In general, it seems that insects show high CAT activity (Ahamad and Pardini, 1990; Ramasarma, 2007; Kim et al., 2007).

In contrast, we found low levels of GPX and GR in the studied stonefly species, supporting the results of previous studies on other insects. Mathews et al. (1997) suggested hat GPX was absent in insects. Sohal and Allen (1986) considered that the increase of oxidated glutathion with age in *D. melanogaster* was due to the low level of GR. Finally, Cervera et al. (2004) and Xie et al. (2009) also supported the presence of low levels of GR in insects. Our results suggest that high levels of CAT coincide with low levels of GPX and GR in insects.

It seems that, in insects, SOD and CAT are the two main enzymes implicated in antioxidant protection. Datkhile et al. (2009) found an increase in the activity of these enzymes for defending *Chironomus ramosus* subjected to ionizing radiation, while there was no response either in the activity of GPX or GR.

GSTs are a group of enzymes that catalyze the conjugation of organic molecules. They have an electrophylic reactive centre with a glutathion thiol group that can convert a lipophylic molecule and make it hydrosoluble, enabling its elimination. High GST activity would indicate resistance to contaminant agents (Clark, 1989). In relation to this, *P.*

marginata showed not only a high SOD activity, but also the highest GST activity among the species studied, despite the fact that it was collected at lower temperatures (Table 2). This species presents a long three-year life cycle in which some processes that occur during the adult life in the other species, must happen during the nymphal stage (Table 1).

P. marginata has also higher values of DTD than *G. franzi*, the other long-lived species discussed in this study (Table 1). The antioxidant function of DTD comes from its capacity to reduce quinones to hydroquinones by accepting two electrons from NADH or NADPH. Hydroquinones are more stable components, and can produce conjugates with glutathion through the action of GST, promoting its further excretion. In fact, DTD has been proposed as marker of the oxidative state of the quinones in fishes (Bagnasco et al., 1991). Studies carried out by Sturve et al. (2005) pointed out that in the trout liver exists a similar behaviour to that of mammals in the induction process of DTD exposed to pro-oxidant agents, such as certain contaminants.

P. marginata could require higher GST and DTD activity to cope with an extremely long aquatic development period. Moreover, this species is not able to enter into quiescence to withstand unfavourable conditions as, for example, *G. franzi* can do (Hynes, 1970; Tierno de Figueroa et al., 2003; López-Rodríguez et al., 2009).

Regarding this difference, we must underline the fact that *G. franzi* has the lowest values of detoxificant enzymes GST and DTD among the species studied. This could be related to the fact that it is the only species studied here in which nymphs can enter quiescence in response to adverse stream conditions (López-Rodríguez et al., 2009).

G6PDH is a cytosolic enzyme that catalyzes the conversion of glucosa-6-phosphate to 6-phosphategluconate with the contemporary generation of NADPH from $NADP^+$. In this study it was found an important specific activity of this enzyme in the four studied species, being lower in *P. marginata*. Berra et al. (2004) noted a positive correlation between the activity of GST and the activity of NADPH-generating enzymes (G6PDH, malic enzyme and isocitric dehydrogenase) in some insect groups (e.g. Diptera), and pointed out that G6PDH activity is very low or undetectable in most taxa, including Plecoptera. Nevertheless, we detected activity in the four species studied, but no relation between G6PDH and GST activities. The lack of any correlation between both enzymes is not a determining factor, as G6PDH is an enzyme in the pentose phosphate pathway, which plays roles in different metabolic processes.

In the present study, total antioxidant capacity (Fig. 2), as defined and used in previous comparative studies (Trenzado et al., 2006), reveals that the two species with long life cycles and larger body size (Table 1) are those with the highest values, mainly due to the increased activity of SOD and CAT (Fig. 2), as stated above. During the nymphal stage these species must accumulate reservoirs for the adult life when scarce or no food is ingested. This may lead to a higher metabolic rate, and so a higher requirement for antioxidant protection (Ahamad and Pardini, 1990). In fact, the accumulation of lipid energetic reservoirs might explain why *P. marginata*, with a longer nymphal development period, presents the highest levels of lipid peroxidation. We did not determine lipid corporal composition in the species studied, but we did determine the soluble protein corporal composition. As shown in Fig. 4, *P. marginata* has a diminished level relative to the other species. It is well known that there is an inverse relationship between protein content and corporal lipids, which would indicate a higher lipid level in *P. marginata*.

Both *Isoperla* species have shorter nymphal developmental periods than those of *P. marginata* and *G. franzi*, and they present very similar antioxidant enzyme systems; with the exception of CAT, there are no outstanding differences between these two taxa.

In conclusion, the results of our comparative analysis of different parameters indicative of the oxidative state of nymphs of the Plecoptera species studied indicate that all species show an important enzymatic antioxidant potential, and that differences found among species seem to be mainly due to the duration of the nymphal developmental period, instead of being due to their permanent or temporary habitats. These results support previous data on high population densities and secondary production values found for the two temporary species in the same stream (López-Rodríguez et al., 2009). Thus, Plecoptera, although traditionally considered typical inhabitants of permanent waters (Graf et al., 2009), seem to have sufficient physiological defenses, together with behavioral and ecological adaptations, to cope with the potentially unfavorable conditions that may occur in temporary waters. Future researches on this topic under different conditions and in different nymphal developmental stages will improve our knowledge about this insect group, and will contribute to answer some of the open questions previously sketched.

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REFERENCES

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105: 121–126

Ahmad S (1992) Biochemical defence of pro-oxidant plant allelochemicals by herbivorous insects. Biochem Syst Ecol 20: 269–296

Ahmad S, Pardini RS (1990) Mechanisms for regulating oxygen toxicity in phytofagous insects. Free Rad Biol Med 8: 401–413

Amore V, Fochetti R (2009) Present knowledge on the presence of hemocyanin in stoneflies (Insecta: Plecoptera). Aquat Insects 31 (suppl 1): 577–583

Aucoin RR, Philogene BJR, Arnason JT (1991) Antioxidant enzymes as biochemical defenses against phototoxin-induced oxidative stress in three species of herbivorous Lepidoptera. Arch Insect Biochem Physiol 16: 139–152

Bagnasco M, Camoirano A, De Flora S, Melodia F, Arillo A (1991) Enhanced liver metabolism of mutagens and carcinogens in fish living in polluted seawater. Mutat Res Lett 262: 129–137

Barbehenn RV (2002) Gut-based antioxidant enzymes in a polyphagous and a graminivorous grasshopper. J Chem Ecol 28: 1329–1347

Barbehenn RV (2003) Antioxidants in grasshoppers: higher levels defend the midgut tissues of a polyphagous species than a graminivorous species. J Chem Ecol 29: 683–702

Berra E, Forcella M, Giacchini R, Marziali L, Rossaro B, Parenti P (2004) Evaluation of enzyme biomarkers in freshwater invertebrates from Taro and Ticino river, Italy. Ann Limnol-Int J Lim 40: 169–180

Bi JL, Felton GW (1995) Foliar oxidative stress in insect herbivory: Primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. J Chem Ecol 21: 1511–1530

Bo T, Fenoglio S, López-Rodríguez MJ, Tierno de Figueroa JM (2008) Trophic behaviour of two *Perlidae* species (Insecta, Plecoptera) in a Southern Spain river. Int Rev Hydrobiol 93: 167–174

Bonada N, Prat N, Resh VH, Statzner B (2006) Developments in aquatic insect biomonitoring: A comparative analysis of recent approaches. Ann Rev Entomol 51: 495–523

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 72: 248–254

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52: 302–310

Calberg L, Mannervik B (1975) Purification and characterization of the flavo enzyme glutathione reductase from rat liver. J Chem Ecol 250: 5475–5480

Cervera A, Maymo AC, Sendra M, Martínez-Pardo R, Garcera MD (2004) Cadmium effects on development and reproduction on *Oncopeltus fasciatus* (Heteroptera: Lygaeidae). J Insect Physiol 50: 737–749

Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59: 527–605

Choi J, Roche H, Caquet T (1999) Characterization of superoxide dismutase activity in *Chironomus riparius* Mg. (Diptera, Chironomidae) larvae - a potential biomarker. Comp Biochem Physiol C 124: 73–81

Clark AG (1989) The comparative enzymology of the glutathione s-transferases from non-vertebrate organisms. Comp Biochem Physiol B 92: 419–446

Corona M, Robinson GE (2006) Genes of the antioxidant system of the honey bee: annotation and phylogeny. Insect Mol Biol 15: 687–701

Datkhile KD, Mukhopadhyaya R, Dongre TK, Nath BB (2009) Increased level of superoxide dismutase (SOD) activity in larvae of *Chironomus ramosus* (Diptera: Chironomidae) subjected to ionizing radiation. Comp Biochem Physiol C 149: 500–506

DeJong RJ, Miller LM, Molina-Cruz A, Gupta L, Kumar S, Barillas-Mury C (2007) Reactive oxygen species detoxification by catalase is a major determinant of fecundity in the mosquito *Anopheles gambiae*. P Natl Acad Sci USA 104: 2121–2126

Díaz E, Furné M, Trenzado CE, García-Gallego M, Domezain A, Sanz A (2010) Antioxidant defences in the first life phases of the sturgeon *Acipenser naccarii*. Aquaculture 307: 123–129

Duncan DB (1955) Multiple range and multiple *F* test. Biometrics 11: 1–42

Fausto AM, Belardinelli M, Fochetti R, Tierno de Figueroa JM, Mazzini M (2002) Comparative spermatology in Plecoptera (Insecta). II. An ultrastructural investigation on four species of Systellognatha. Arthropod Struct Dev 31: 147–156

Flohé L, Günzler WA (1984) Assay of glutathione peroxidase. Methods Enzymol 105: 115–121

Fochetti R, Tierno de Figueroa JM (2006) Notes on diversity and conservation of the European fauna of Plecoptera (Insecta). J Nat Hist 40: 2361–2369

Fochetti R, Tierno de Figueroa JM (2008) Global diversity of stoneflies (Plecoptera; Insecta) in freshwater. Hydrobiol 595: 365–377

Furné M, García-Gallego M, Hidalgo MC, Morales AE, Domezain A, Domezain J, Sanz A (2008) Oxidative stress parameters during starvation and refeeding periods in Adriatic sturgeon (*Acipenser naccarii*) and rainbow trout (*Oncorhynchus mykiss*). Aquacult Nutr 15: 587–595

Graf W, Lorenz AW, Tierno de Figueroa JM, Lücke S, López-Rodríguez MJ, Davies C (2009) Distribution and ecological preferences of European freshwater organisms. Vol 2. Plecoptera. Ed by A Schmidt-Kloiber, D Hering, Pensoft Publishing, Sofia, Bulgaria

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases – the first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130–7139

Hynes HBN (1970) The ecology of running waters. University of Liverpool Press, Liverpool

Kim BY, Kim HJ, Lee KS, Seo SJ, Jin BR (2007) Catalase from the white-spotted flower chafer, *Protaetia brevitarsis*: cDNA sequence, expression, and functional characterization. Comp Biochem Physiol B 149: 183–190

López-Rodríguez MJ, Tierno de Figueroa JM, Fenoglio S, Bo T, Alba-Tercedor J (2009) Life strategies of 3 Perlodidae species (Plecoptera, Insecta) in a Mediterranean seasonal stream of Southern Europe. J N Am Benthol Soc 28: 611–625

Marden JH, O'Donnell BC, Thomas MA, Bye JY (2000) Surface-skimming stoneflies and mayfiles: the taxonomic and mechanical diversity of two-dimensional aerodynamic locomotion. Physiol Biochem Zool 73: 751–764

Mathews MC, Summers CB, Felton GW (1997) Ascorbate peroxidase: A novel antioxidant enzyme in insects. Arch Insect Biochem 34: 57–68

McCord JM, Fridovich I (1969) Superoxide dismutase: an enzyme function for erythrocuprein. J Biol Chem 244: 6049–6055

Nickla H, Anderson J, Palskill T (1983) Enzymes involved in oxygen detoxification during development of *Drosophila melanogaster*. Experientia 39: 610–612

Niemi GJ, McDonald ME (2004) Application of ecological indicators. Annu Rev Ecol Evol S 35: 89–111

Orr EC, Bewley GC, Orr WC (1990) cDNA and deduced amino acid sequence of *Drosophila* catalase. Nucleic Acids Res 18: 3663

Parker JD, Parker KM, Keller L (2004) Molecular phylogenetic evidence for an extracellular Cu Zn superoxide dismutase gene in insects. Insect Mol Biol 13: 587–594

Peng T, Xu MA, Ayala MJ (1986) Irradiation-resistance conferred by superoxide dismutase: possible adaptive role of a natural polymorphism in *Drosophila melanogaster*. Proc Natl Acad Sci USA 83: 684–687

Ramasarna T (2007) Many faces of superoxide dismutase, originally known as erythrocuprein. Curr Sci 92: 184–191

Resh VH, Rosenberg DM (1984) The ecology of aquatic insects. Praeger Publishers, New York

Sánchez-Ortega A, Alba-Tercedor J (1990) Life cycles of some species of Plecoptera in Sierra Nevada (South of Spain). In "Mayflies and Stoneflies: Life history and Biology" Ed by IC Campbell, Kluwer Academic Publishers, Dordrecht, pp 43–52

Sánchez-Ortega A, Alba-Tercedor J (1991) The life cycle of *Perla marginata* and *Dinocras cephalotes* in Sierra Nevada (Granada, Spain) (Plecoptera: Perlidae). In "Overview and strategies of Ephemeroptera and Plecoptera" Ed by J Alba-Tercedor, A Sánchez-Ortega, The Sandhill Crane Press, Gainessville, pp 493–501

Sohal RS, Allen RG (1986) Relationship between oxygen metabolism, aging and development. Free Radical Bio Med 2: 117–160

Sturve J, Stephensen E, Forlin L (2005) Effects of redox cycling compounds on DT diaphorase activity in the liver of rainbow trout (*Oncorhynchus mykiss*). Comp Hepatol 4: 4

Thomas MA, Walsh KA, Wolf MR, McPherson BA, Marden JH (2000) Molecular phylogenetic analysis of evolutionary trends in stonefly wing structure and locomotor behavior. Proc Natl Acad Sci USA 97: 13178–13183

Tierno de Figueroa JM, Sánchez-Ortega A (1999) Imaginal feeding of certain Systellognathan stonefly species (Insecta, Plecoptera). Ann Entomol Soc Am 92: 218–221

Tierno de Figueroa JM, Sánchez-Ortega A, Membiela Iglesia P, Luzón-Ortega JM (2003) Plecoptera. In "Fauna Ibérica, vol 22"

Ed by Museo Nacional de Ciencias Naturales, CSIC, Madrid
Trenzado C, Hidalgo MC, García-Gallego M, Morales AE, Furné M, Domezain A, Domezain J, Sanz A (2006) Antioxidant enzymes and lipid peroxidation in sturgeon *Acipenser naccarii* and trout *Oncorhyndus mykiss*. A comparative study. Aquaculture 254: 758–767
Xie L, Flippin JL, Deighton N, Funk DH, Dickey DA, Buchwalter DB (2009) Mercury (II) bioaccumulation and antioxidant physiology in four aquatic insects. Environ Sci Technol 43: 934–940
Yamamoto K, Banno Y, Fujii H, Miake F, Kashige N, Aso Y (2005) Catalase, from the silkworn, *Bonbyx mori*: gene sequence, distribution, and overexpression. Insect Biochem Molec 35: 277–283
Zwick P (2003) Biogeographical Patterns. In "Encyclopedia of Insects" Ed by VH Resh, RT Cardé, Academic Press, Elsevier Science, Burlington, pp 94–103
Zwick P (2004) Key to the West Paleartic genera of stoneflies (Plecoptera) in the larval stage. Limnologica 34: 315–348

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