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The microhabitat preferences of water beetles in four rivers in Ourense province, Northwest Spain

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ABSTRACT

The microhabitat preferences of water beetles in four rivers in Ourense province, Northwest Spain

We analysed the microhabitat preferences of water beetle species in four rivers in Northwest Spain. In each river, we sampled 5 sites with different types of substrate. These sites were characterised in situ according to the predominant material type (macrophytes, moss, pebbles and sand). The occurrence of a substrate preference was verified from a comparative study of species richness and abundance among different microhabitats. The differences in abundance and richness between substrates and in the abundance of each species were tested with an ANOVA. The similarity between microhabitats was tested with non-metric multidimensional scaling (NMDS), and the correlation between fauna and substrates was verified with a correspondence analysis (CA). We observed different species distribution patterns, and these patterns reflected the microhabitat preference of each species. Both the ecological parameters and the correspondence analysis indicated that the preferred substrate for most of the species was moss, followed by pebbles.

Key words: Water beetles, substrates, preferences, rivers, Galicia.

RESUMEN

Preferencias de microhábitats en coleópteros acuáticos de cuatro ríos en la provincia de Ourense, Noroeste de España

Se analiza la preferencia de microhabitat de especies de coleópteros acuáticos en cuatro ríos del noroeste de España. En cada río se muestrearon 5 puntos en diferentes tipos de sustrato caracterizados in situ en función del tipo de material predominante (macrófitas, musgo, cantos-gravas y arena). La preferencia de sustrato fue verificada mediante un estudio comparado de riqueza y abundancia de especies entre los diferentes microhábitats. Las diferencias entre los diferentes sustratos para la abundancia y riqueza, así como para la abundancia de cada especie fueron testadas mediante un análisis ANOVA. La similitud entre microhábitats fue testada mediante un NMDS, mientras que la correlación entre la fauna y los sustratos, se verificó a partir de un análisis de correspondencias (CA). Se observaron diferentes patrones de distribución de las especies según su preferencia por determinados microhábitats. Tanto los parametros ecológicos de riqueza y abundancia como el análisis de correspondencias indican que el sustrato preferido por la mayoría de las especies fue el musgo, seguido de los cantos-gravas.

Palabras clave: *Coleópteros acuáticos, sustratos, preferencias, ríos, Galicia.*

INTRODUCTION

The study of river microhabitats is the key to understanding the structure of the assemblages inhabiting them and the correlation between species and the environment. In addition, the structure of communities and the ecological interactions that occur depend on the environmental variables (Illies & Botosaneanu, 1963) and the longitudinal gradient (Vannote *et al*., 1980; Minshall *et al*., 1985).

In studies of microhabitat association, it is important to consider the biotic and abiotic factors that determine the structure of aquatic communities. In this sense, inorganic and organic substrates are very important because they define microdistributions (Lloyd & Sites, 2000).

Aquatic beetles are considered to be good water quality indicators within these faunistic communities (García-Criado et al., 1999). These insects are widely distributed in running water (Smith *et al*., 2007), where they play an important role in trophic chains (Merritt & Cummins, 1996). Trophic diversity is a factor that makes beetles abundant and dominant in most freshwater environments, occupying different microhabitats formed by different substrates of the riverbed. In this sense, it is important to understand the interactions between these organisms and the environment where they live.

Several authors have used different groups of invertebrates as a model for analysing substrate preferences in rivers (Sheldon & Haick, 1981; Baptista *et al*., 2001; Crosa & Buffagni, 2002; Urbanic *et al*., 2005), but few previous studies have used water beetles as a model. Among these studies, the work of Lloyd & Sites (2000), who studied the association of three species of microhabitat Dryopoidea in the Missouri River (USA), can be highlighted.

The lack of such research in rivers in the Iberian Peninsula has motivated us to conduct the current study, whose purpose is to verify a possible correlation between the presence of water beetle species and the substrate by evaluating the degree of microhabitat preference shown by these species.

MATERIALS AND METHODS

Study area

This study was conducted in four rivers (Deva, Cadós, Tuño and Fragoso) located in southern Galicia, NW Spain (Fig. 1). The Deva and Tuño

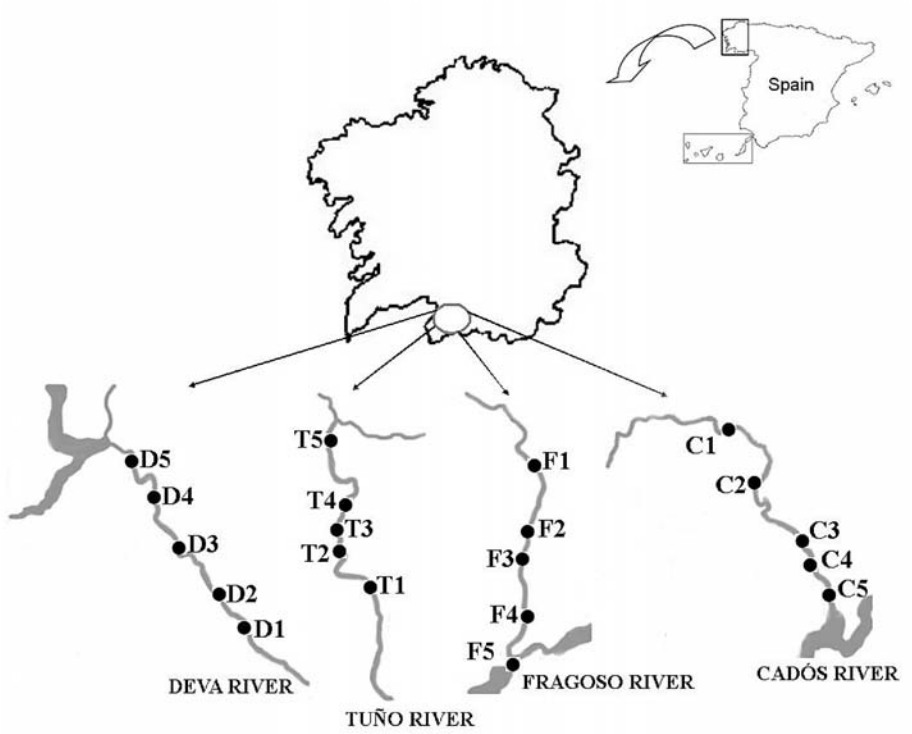

Figure 1. Location of the study area and sampling sites in the four rivers. *Localización del área de estudio y los puntos de muestreo en los cuatro ríos.*

Table 1. List of sampling sites with geographic data and coordinates. *Listado de los puntos de muestreo con los datos geográficos y coordenadas.*

River	Sampling site	Code	Altitude	UTM X	UTM Y
	Corbelle	C1	931	5813723869	46567385538
	Seoane	C2	768	5828905421	46550904208
Cadós	Pontenova	C3	697	5848731952	46529329982
	Xordos	C4	637	5848873810	46517486312
	Aguas abajo Central Cadós	C5	555	5857333375	46504693937
	Parada do Monte	F1	747	5788134605	46520147192
	Ponte do Groicio	F2	563	5791387083	46492544893
Fragoso	Ponte Abeleda	F3	545	5787222948	46482072459
	Requeixo	F4	419	5788295007	46453921633
	Grou	F5	410	5783543400	46438692400
	Santa Eufemia	T1	630	5808542671	46652495525
	Trasmiras	T2	486	5804987064	46670315590
Tuño	Pena Avegosa	T3	484	5805068379	46673247037
	O Outeiro	T4	384	5811502776	46690564631
	Ponte Madeiros	T5	298	5796436444	46718032278
	Retortoirio	D1	552	5757845968	46628952012
	Lavandeira	D2	329	5741678949	46652779154
Deva	Pena do Bugallo	D3	192	5729910636	46665118534
	Pontedeva	D4	93	5716285314	46686694927
	Ponte do Cantiño	D5	97	5712389185	46702078806

belong to the Miño and Arnoia river basins, respectively, whereas the Cadós and Fragoso belong to the Limia river basin. Table 1 shows the list of sampling sites with their names, codes (used below to identify the sampling sites), the river, altitude and UTM coordinates. The Tuño and Deva Rivers flow on schist materials, in which the predominant minerals belong to the group of silicates with high levels of silica, whereas the Fragoso and Cadós Rivers flow on materials that consist fundamentally of granite (Río Barja *et al.*, 1996). The dominant vegetation in the study area includes *Corylus avellana*, *Ulmus minor* Mill, *Fraxinus angustifolia* Vahl, *Alnus glutinosa* Gaertn and *Cornus sanguinea* L. These species form a riparian forest that is usually well structured.

Sampling methods

To perform this study, we selected 20 sampling sites, five in each river, distributed along the river course. We sampled all substrates present in the selected section of the river that were characterised by the predominant material. We identified a total of four substrate types: moss, macrophytes, sand and pebbles. Data were collected in eight sampling fields during one year, between July 2001 and June 2002, with two collections in each season, for a total of 160 samples.

The fauna was collected with a Surber net, 25 cm squared and 60 cm deep with 0.5 mm mesh, one sample for each substrate. The samples were fixed in the field with 4 % formaldehyde and taken to the laboratory. The specimens were identified according to standard procedures in entomology, using a stereomicroscope, a binocular microscope and different reference works, including Franciscolo (1979), Valladares (1988), Angus (1992), Prost *et al.* (1992), Gayoso *et al.* (1997) and Tachet *et al.* (2002). After the specimens were identified, they were preserved in 70° alcohol and deposited in the scientific collection of the Laboratory of Aquatic Entomology at Vigo University.

In addition, we measured several physical and chemical parameters in situ: water temperature, dissolved oxygen, pH, conductivity and total dis-

Table 2a. Abundance of species in the four substrate types and species codes in figure 3 (selected species in CA). *Abundancia de las especies en los cuatro tipos de sustrato y código de las especies en la figura 3 (especies seleccionadas en el CA).*

FAMILY	SPECIES	CODE	MA	MO	PE	SA
Haliplidae	*Haliplus lineatocollis*		0	0	1	0
Gyrinidae	*Orectochilus villosus*	24	12	5	35	7
Dytiscidae	*Oreodytes sanmarkii alienus*	21	0	1	5	2
	Hydroporus nigrita		0	0	0	1
	Stictotarsus bertrandi	22	1	0	6	1
	Deronectes ferruginens	23	2	4	6	5
	Graptodytes fractus		0	0	1	0
	Hydroglyphys geminus		0	1	2	0
	Scarodytes halensis		0	0	1	0
	Yola bicarinata bicarinata		0	1	1	1
	Nebrioporus carinatus		0	0	0	1
Hydrophilidae	*Anacaena lutescens*	26	2	8	1	0
Hydrochidae	*Hydrochus angustatus*	27	8	13	1	1
Helophoridae	*Helophorus flavipes*	30	1	2	0	1
Hydraenidae	*Hydraena iberica*	14	29	324	163	15
	Hydraena corinna	15	80	872	201	18
	Hydraena brachymera	16	65	542	122	4
	Hydraena testacea	17	28	338	42	4
	Hydraena hispanica	18	0	137	48	2
	Hydraena sharpi	19	12	393	21	1
	Hydraena barrosi		0	8	0	0
	Hydraena stussineri		0	2	0	0
	Hydraena unca		0	0	3	0
	Hydraena minutissima		0	1	0	0
	Ochthebius legionensis		0	1	0	0
	Ochthebius heydeni	20	1	14	0	0
	Limnebius lusitanus		0	1	1	0
	Limnebius evanescens		0	1	0	0
Elmidae	*Elmis aenea*	1	823	7059	375	116
	Elmis maugetii	2	148	1076	95	29
	Elmis rioloides	3	167	4067	210	46
	Elmis perezi	4	82	1326	50	21
	Limnius volckmari	5	31	11	180	131
	Limnius perrisi carinatus	6	62	52	485	231
	Limnius opacus	7	40	28	48	9
	Oulimnius bertrandi	8	75	475	211	70
	Oulimnius rivularis	9	35	133	41	162
	Oulimnius troglodites	10	6	91	198	90
	Oulimnius perezi		0	3	0	0
	Esolus angustatus	11	2	10	31	34
	Esolus parallelepipedus	12	10	16	170	37
	Dupophilus brevis	13	289	135	1508	334
	Stenelmis canaliculatus		0	0	1	0
Dryopidae	*Dryops luridus*	25	17	62	0	0
Scirtidae	*Cyphon* sp.	28	5	12	7	0
	Elodes sp.	29	5	23	6	1
Curculionidae	*Bagous* sp.		2	0	0	1

Table 2b. Physical, chemical and habitat variables of the rivers studied. *Variables físicas, químicas y del hábitat de los ríos estudiados.*

Variable	Mean ± standard deviation	Minimum	Maximum
Width m	5.94 ± 1.92	2.12	10.95
Depth m	0.87 ± 3.21	0.19	25.50
Altitude m	491.36 ± 215.94	93	931
Distance of Source m	11 012.56 ± 3748.09	4636	18 400
Stream Velocity m^2	3.18 ± 2.06	0.10	9.17
Temperature °C	10.44 ± 3.08	4.22	15.62
pH	6.69 ± 0.32	4.59	7.26
Conductivity $\mu S\ cm^{-1}$	0.42 ± 0.61	0.14	5.66
Dissolved oxygen %	103.98 ± 17.51	6.49	139.90
Dissolved oxygen mg/l	11.99 ± 8.11	5.43	82.07
TSS mg/l	22.11 ± 12.99	5.54	66.50

solved solids. We also measured several habitat parameters: width, depth, stream velocity, altitude and distance from the source (Table 2a and b).

Data analysis

We performed a series of statistical analyses to assess the microhabitat preferences of the water beetles found in the samples. For this purpose, we analysed the assemblage as a whole as well as each species.

The structure of the assemblage was evaluated for species richness (S) and abundance (N) for each substrate. These indices were selected because they potentially portray important characteristics of assemblages. An analysis of variance (one-way ANOVA) was used to test for significant differences between the four substrates in both richness measures and each species. The homogeneity of variance was tested with Levene's test. The ANOVA was conducted with SPSS version 19.

To determine the degree of correlation between the different water beetle species and the microhabitats colonised, we performed a correspondence analysis (CA). Prior to the CA, we refined the data, eliminating species present in 5 % or less of the samples. The selected species appear with a code in Table 2. The CA was conducted with CANOCO 4.5 (Ter Braak & Šmilauer, 2002).

Finally, the similarity between sites was evaluated with non-metric multidimensional scaling (NMDS). This analysis generated a similarity matrix between substrates for different sampling sites. For this purpose, we used the Bray-Curtis similarity index for the standardised data ($\log n$).

Table 3. Mean, SD and ranges of richness and abundance for the four different substrates. *Media, desviación estandar y rango de la riqueza y abundancia en los cuatro diferentes sustratos.*

Measures	Mean ± SD	Minimum	Maximum
Richness S			
Macrophytes	7.9 ± 6.3	0	19
Moss	16 ± 3.8	9	22
Pebbles	14.3 ± 3.8	8	22
Sand	6.7 ± 4.8	0	13
Abundance N			
Macrophytes	102 ± 160.3	0	505
Moss	862.4 ± 717.7	28	2986
Pebbles	213.9 ± 207.4	20	824
Sand	68.8 ± 92.0	0	319

RESULTS

Richness and abundance

We studied a total of 25 406 specimens belonging to 47 species of water beetles assigned to three families of Adephaga (Gyrinidae, Haliplidae, Dytiscidae) and eight Polyphaga (Helophoridae, Hydrochidae, Hydrophilidae, Hydraenidae, Elmidae, Dryopidae, Scirtidae and Curculionidae). The Hydraenidae and Elmidae were the families that were best represented in the study area, with 14 and 15 species, respectively (Table 2a).

Table 3 shows the mean, standard deviation (SD), maximum and minimum values of richness

Table 4. Significant ANOVA values ($p \leq 0.001$) for richness and abundance measures and species abundance with substrate types as factors. *Valores significativos (*$p \leq 0.001$*) de ANOVA para las medidas de riqueza y abundancia y la abundancia de especies con tipo de sustrato como factor.*

Measures	F	p
Richness S	18.68	0.000
Abundance N	18.72	0.000
Elmis aenea	26.42	0.000
Elmis rioloides	9.24	0.000
Limnius perrisi carinatus	9.56	0.000
Hydraena corinna	15.55	0.000
Hydraena brachymera	6.77	0.000
Hydraena testacea	9.30	0.000
Dryops luridus	10.05	0.000

(S) and abundance (N) in each substrate measured in the 20 sampling sites during the annual cycle.

Both the species richness and abundance of aquatic beetles in each type of substrate and in the 20 sampling sites indicated that moss is the preferred substrate. This preference is especially evident from the abundance values, which are substantially higher in moss than in the other substrates: 3441 in the Cadós, 3401 in the Fragoso, 2429 in the Tuño and 7977 in the Deva. Moss was also the substrate with the highest species richness. Although we did not observe clear evidence of dominance, we observed higher species richness on moss in all rivers: 26 species in the Cadós, 27 in the Fragoso, 21 in the Tuño and 29 in the Deva.

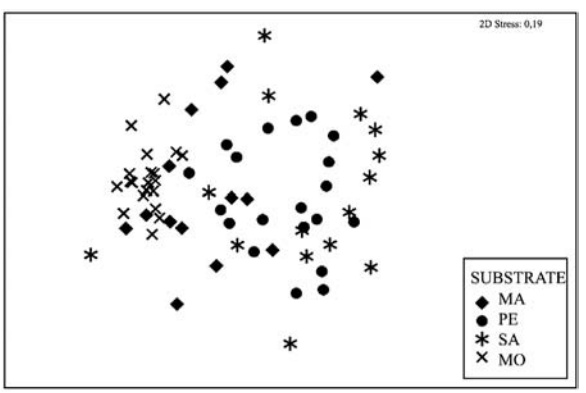

Figure 2. Non-metric multidimensional scaling (NMDS) based on species composition in the different substrates: MA (macrophytes) MO (moss), PE (pebbles) and SA (sand) in the 20 sampling sites. *Escalamiento multidimensional amétrico (NMDS) basado en la composición de especies en los diferentes sustratos MA (macrófitas) MO (musgo), PE (cantos-gravas) y SA (arena) en los 20 puntos de muestreo.*

Richness and abundance showed considerable variability among substrates as demonstrated by ANOVA. We also found significant variability ($p \leq 0.001$) among substrates in the abundance of seven species (Table 4).

The variation in species composition was high (Fig. 2), and the ordering of the sites remained constant during the year. A NMDS among substrates showed that moss (MO) represented a grouping that was relatively separate from the others, especially from sand (SA) and gravel (PE), which appeared to be correlated.

Figure 3 shows the results of the CA. The eigenvalues for axes I-IV were 0.566, 0.295, 0.249 and 0.195, respectively. The correlations for axes III and IV were low ($r < 0.5$), and only axes I and II were used for data interpretation. The cumulative percentage of variance explained for the species-habitats relation was 58.6 % for the first two axes. The first two canonical axes were significant, as shown by a Monte Carlo permutation test ($p = 0.002$). An overall Monte Carlo test also gave a significance of $p = 0.002$.

A large number of species appeared to be correlated with moss, including most species in the genera *Hydraena* and *Elmis*, *Ochthebius heydeni* and *Dryops luridus*, whereas *L. volckmari*, *L. perrisi carinatus* and *Oreodytes sanmarkii alienus* appeared to show an affinity for pebbles.

DISCUSSION

According to the results obtained, based on both the ecological parameters of richness and abundance and the correlation analysis, moss is the preferred substrate for most of the studied species of beetles. Note that several authors have documented the preference of species of aquatic beetles for microhabitats formed primarily by moss (Fernández-Díaz, 2003). According to Passos *et al.* (2003), substrates formed primarily by moss harbour an abundance of water beetles because they offer an abundant food source to herbivorous species.

Moss is associated with different species of Elmidae and Hydraenidae. The ANOVA demonstrated that most of these species also showed

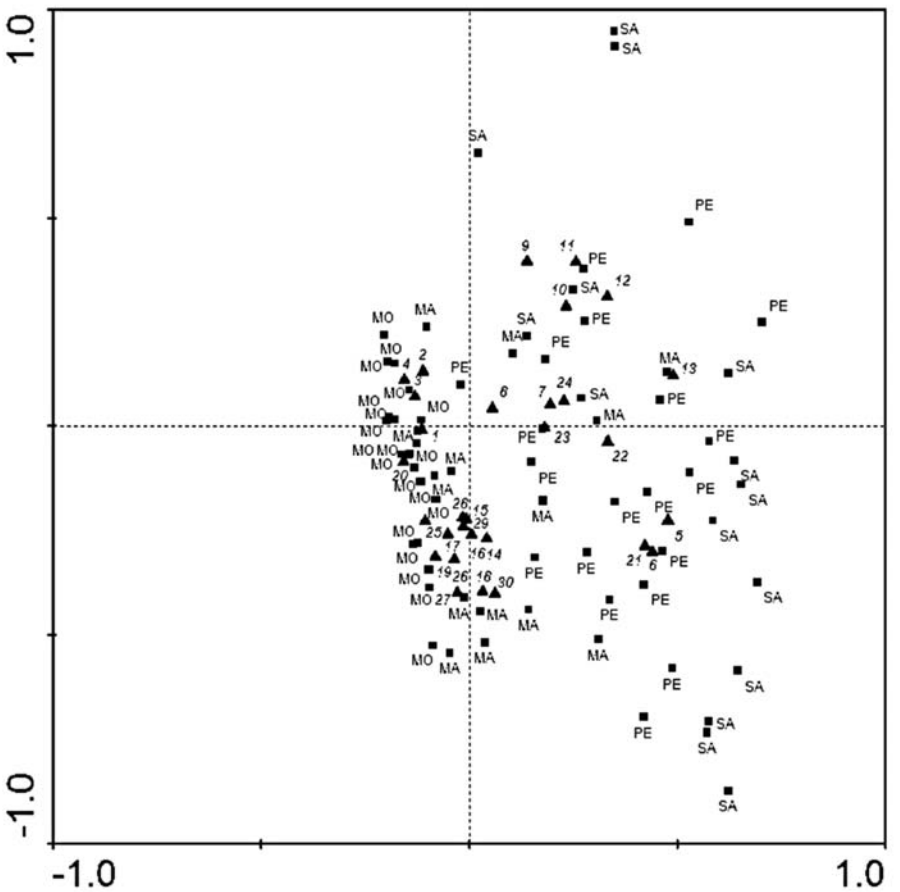

Figure 3. Correspondence analysis (CA) of the species in different microhabitats in the four rivers. *Análisis de Correspondencias (CA) de las especies en los distintos biotopos en los cuatro ríos.*

significant variability between substrates. This result confirms that the differing abundance of these species on different substrates may be indicative of microhabitat preferences.

The second most important substrate was found to be pebbles, which appear to be associated with a number of species. These species include *Limnius volckmari*, *Limnius perrisi carinatus* and *Oreodytes sanmarkii alienus*. These results are consistent with the findings of Garrido (1990), Sáinz-Cantero & Alba-Tercedor (1991), Gayoso *et al*. (1997) and Fernández-Díaz (2003).

The NMDS also found significant differences between the distributions of species on different substrates, especially moss, pebbles and sand. Pebbles and sand appear to be correlated on the side of the scaling diagram opposite that of the moss samples. The moss samples are close to each other in the diagram and form a separate group. This result may imply that the species that prefer this substrate are different from those that prefer the other substrates.

An analysis of the results by families shows that the family Hydraenidae exhibits a clear association of *H. brachymera, H. corinna, H. sharpi* and *H. testacea* with moss substrate. These results are consistent with the results of other studies conducted in different regions of the Iberian Peninsula. Specifically, Sainz-Cantero *et al*. (1987) have shown that *H. testacea* is primarily associated with moss, and Aguilera & Gerendas (1995) have found that moss is preferred by other species of the genus *Hydraena*, e.g., *H. sharpi.* In a study of

the Órbigo River (Leon) by García-Criado *et al.* (1999), *H. hispanica* was captured primarily in moss. However, according to Fernández-Díaz (2003), this species was primarily associated with particular substrates (gravel, stones and sand) and to a lesser extent with macrophytes. In this study, *H. hispanica* appeared to show a preference for moss, as was observed in the Cadós and Fragoso Rivers. However, *H. iberica* appears to be associated with pebbles and moss, as previously shown by Valladares (1989), Fernández-Díaz (2003) and Aguilera & Gerend (1995).

In the Elmidae, the four species in the genus *Elmis* appeared to prefer a moss substrate. According to Sainz-Cantero & Tercedor Alba (1991), several *Elmis* species are very common on mosses and filamentous algae. These data are also consistent with the results of Fernández-Díaz (2003), who has shown that 3 species of *Elmis* (*E. aenea*, *E. rioloides* and *E. maugetii maugetii*) prefer moss. In this context, Berthelemy (1966) and Gayoso *et al.* (1997) consider that *E. aenea* is the *Elmis* species that shows the greatest affinity for a moss substrate.

The species of *Oulimnius* investigated in this study appeared to show no particular preference for any of the substrates tested. The exception was *O. bertrandi*, which was related to moss. Fernández-Díaz (2003) has linked *O. bertrandi* to moss, macrophytes, stone and sand but with a greater abundance on moss, as also shown by Gayoso *et al*. (1997).

Two species of *Limnius*, *L. volckmari* and *L. perrisi carinatus* appeared to be associated with pebbles, as previously observed by Sáinz-Cantero & Alba-Tercedor (1991), Gayoso *et al.* (1997) and Fernández-Díaz (2003).

D. brevis showed a preference for pebbles and macrophytes, as previously indicated by Gayoso *et al*. (1997) and Fernández-Díaz (2003). *E. parallepepidus* and *E. angustatus* were associated with pebbles and sand. According to Olmi (1969) and Fernández-Díaz (2003), these species are related to pebble substrates and submerged vegetation.

This study has enabled us to determine certain patterns of distribution of water beetle species in the rivers studied as a function of microhabitat. Note that moss was the preferred substrate for most species, particularly for the families Elmidae and Hydraenidae. A possible explanation for this preference is that the substrate provides stability, protection and food for these species, which appear to have adapted to fast-current areas covered primarily by this type of substrate (Nilsson, 1996). In this sense, this study highlights the importance of knowing the structure of rivers, which are composed of different microhabitats, each with its own characteristics and ecological functions. In this way, we can adequately conserve aquatic habitats and thus facilitate the maintenance of their biodiversity.

REFERENCES

AGUILERA, P. & R. GEREND. 1995. El género *Hydraena*, 1794 (Col., Hydraenidae) en el conjunto del Sistema Ibérico (España). *Zapateri, Revista Aragonesa de Entomología*, 5: 63–74.

BAPTISTA, D. F., D. F. BUSS, L. F. M. DORVILLÉ & J. L. NESSIMIAN. 2001. Diversity and habitat preference of aquatic insects along the longitudinal gradient of the Macaé river basin, Rio de Janeiro, Brazil. *Revista Brasileira de Biologia*, 61(2): 249–258.

BERTHELEMY, C. 1966. Recherches écologiques et biogeographiques sur les Plécoptères et Coléoptères d'eau courante (*Hydraena* et Elminthidae) des Pyrénées. *Annales de Limnologie*, 2 (2): 224–458.

CROSA, G. & A. BUFFAGNI. 2002. Spatial and temporal niche overlap of two mayfly species (Ephemeroptera): the role of substratum roughness and body size. *Hydrobiologia*, 474: 107–115.

FERNÁNDEZ-DÍAZ, M. 2003. *Estudio faunístico y ecológico de los coleópteros acuáticos (Adephaga y Polyphaga) en la cuenca del río Avia (Ourense, NO España): Distribución espacial y temporal*. Tesis de Licenciatura, Universidad de Vigo. 146 pp.

GARCÍA-CRIADO F., C. FERNÁNDEZ ALÁEZ & M. FERNÁNDEZ-ALÁEZ. 1999. Environmental variables influencing the distribution of hydraenidae and Elmidae assemblages (Coleoptera) in a moderately-polluted river basin in northwestern Spain. *European Journal of Entomoogyl*, 96: 37–44.

GARRIDO, J. & C. E. SÁINZ CANTERO. 2004. Diversidad de coleópteros acuáticos en la Península

del Barbanza (Galicia, NW España) (Coleoptera, Adephaga y Polyphaga). *Nouvelle Revue Entomologique*, 21(1): 49–64.

GARRIDO, J. & I. MUNILLA. 2008. Aquatic Coleoptera and Hemiptera assemblages in three coastal lagoons of the NW Iberian Peninsula: assessment of conservation value and response to environmental factors. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18 (5): 557–569.

GAYOSO, A., J. M. SANTAMARÍA & J. C. OTERO. 1997. Contribución al conocimiento de la familia Elmidae Curtis, 1830 (Coleoptera, Dryopoidea) en Galicia (NW Península Ibérica). *Nova Acta Científica Compostelana, (Bioloxía)*, 7: 193–206.

ILLIES, J. & L. BOTOSANEANU. 1963. Problèmes et méthodes de la classification et de la zonation écologique des eaux courantes considérées surtout du point de vue faunistique. *Mitteilungen-Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 12: 1–57.

LLOYD, F. & R. W. SITES. 2000. Microhabitat associations of three species of Dryopoidea (Coleoptera) in an Ozark Stream: a comparison of substrate, and simple and complex hydraulic characters. *Hydrobiologia*, 439: 103–114.

MERRITT, R. W. & K. W. CUMMINS. 1996. *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt Publishing Co. Dubuque, Iowa. 862 pp.

MINSHALL, G. W., K. W. CUMMINS, R. C. PETERSEN, C. E. CUSHING, D. A. BRUNS, J. R. SEDELL & R. L. VANNOTE. 1985. Developments in stream ecosystem theory. *Canadian Journal of Fisheries and Aquatic Sciences*, 42: 1045–1055.

NILSSON, A. N., 1996. *Aquatic Insects of North Europe. A Taxonomic Handbook. Volume 1: Ephemeroptera, Plecoptera, Heteroptera, Megaloptera, Neuroptera, Coleoptera, Trichoptera and Lepidoptera*. Apollo Books, Stenstrup. 274 pp.

OLMI, M. 1969. Notizie ecologiche su *Esolus angustatus* (Ph. Muller) con considerazioni sinonimiche (Coleoptera, Dryopoidea, Elminthidae). *Bollettino della Societa Entomologica Italiana*, 99-101(7-8): 123–132.

PASSOS, M. I. S., J. L. NESSIMIAN & L. F. M. DORVILLE. 2003. Life strategies in an elmidae (Insecta: Coleoptera: Elmidae) community from first order stream in the Atlantic Forest, southeastern Brazil. *Acta Limologica Brasiliensia*, 15(2): 29–36.

PÉREZ-BILBAO, A. & J. GARRIDO. 2008. Diversidad de coleópteros acuáticos en las Gándaras de Budiño (zona LIC, Red Natura 2000) (Pontevedra, España). *Bulletin de la Societé Entomologique de France*, 113: 343–350.

RIBERA, I. 2000. Biogeograophy and conservation of Iberian water beetles. *Biological Conservation*, 92: 131–150.

RICHTER, B. D., D. P. BRAUN, M. A. MENDELSON & L. L. MASTER. 1997. Threats to imperilled freshwater fauna. *Conservation Biology*, 11(5): 1081–1093.

RÍO-BARJA, F., G. GÓMEZ, F. J. FUMEGA & J. A. ÁLVAREZ. 1996. *Galicia. Xeografía. Tomo XX. A Limia, Val de Monterrei e as Frieiras, Miño central ourensán e depresións e serras sudorientais*. Hércules ediciones. A Coruña. 505 pp.

SÁINZ-CANTERO, C. E., A. SÁNCHEZ ORTEGA & J. ALBA TERCEDOR. 1987. Distribución y autoecología de Hydraenidae (Col) en Sierra Nevada (España). *Boletin de la Asociación Española de Entomología*, 11: 355–365.

SÁINZ-CANTERO, C. E. & J. ALBA-TERCEDOR. 1991. Los Polyphaga acuáticos de Sierra Nevada (Granada, España). Coleoptera: Hydraenidae, Hydrophilidae, Elmidae, Dryopidae). *Boletín de la Asociación española de Entomología*, 15: 171–198.

SEGURA, M. O., A. A. FONSECA-GESSNER & M. O. TANAKA. 2007. Composition and distribution of aquatic Coleoptera (Insecta) in low order streams in the state of São Paulo, Brazil: Influence of environmental factors. *Acta Limnologica Brasiliensa*, 19(3): 247–256.

SHELDON, A. L. & R. A. HAICK. 1981. Habitat selection and association of stream insects: a multivariate analysis. *Freshwater Biology*, 11: 395–403.

URBANIC, G., M. J. TOMAN, & C. KRUSNIK. 2005. Microhabitat type selection of caddisfly larvae (Insecta: Trichoptera) in a shallow lowland stream. *Hydrobiologia*, 541: 1–12.

VALLADARES, L. F. 1989. Los Palpicornia acuáticos de la provincia de León. II. *Hydraena* Kugelann, 1794 y *Limnebius* Leach., 1815. (Coleoptera: Hydraenidae). *Boletín de la Asociación española de Entomología*, 13: 313–330.

VANNOTE, R. L., G. W. MINSHALL, K. W. CUMMINS, J. R. SEDELL & C. E. CUSHING. 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37: 130–137.

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Evaluation of the ACR SmartButton thermometer and a low-cost protective case for continuous stream temperature measurement

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ABSTRACT

Evaluation of the ACR SmartButton thermometer and a low-cost protective case for continuous stream temperature measurement

Water temperature is a common variable of interest in stream ecology studies. In this paper, the performance of the ACR SmartButton thermometer and a low-cost protective case were evaluated for stream temperature measurement. The accuracy of the SmartButtons at 0, 10 and 15 °C was well within the ± 1 °C range specified by the manufacturer. For 50-60 % of the readings performed, the error was greater than the ± 0.2 °C correction factor accepted for meteorological temperature measurements. However, the observed level of accuracy is most likely sufficient for most biological applications if the loggers are calibrated against a reference standard. The metallic case that we used had a negligible effect on temperature measurements and offers a reliable way to protect the SmartButton during use in small streams.

Key words: Water temperature, river, stream, digital thermometer, methodology, calibration.

RESUMEN

Evaluación de los termómetros ACR SmartButton y de una carcasa protectora de bajo coste para la medición en continúo de la temperatura del agua en ríos

La temperatura del agua es una variable de interés en los estudios de ecología fluvial. En este trabajo, se evalúa el rendimiento de los termómetros ACR SmartButton y de una carcasa protectora de bajo coste para la medida de la temperatura en ríos. La precisión de los SmartButton a 0, 10 y 15 °C se encuentra dentro del rango de ± 1 °C que especifica el fabricante. El error observado en las lecturas sobrepasa el factor máximo de corrección de ± 0.2 °C que se acepta para la medida meteorológica de la temperatura en el 50-60 % de los datos recogidos. Sin embargo, la precisión observada es probablemente suficiente para otras aplicaciones biológicas, si los termómetros se calibran frente a una referencia estándar. Las carcasas protectoras que hemos utilizado tienen un efecto despreciable en las medidas de temperatura y representan un método seguro para instalar los SmartButtons en arroyos.

Palabras clave: *Temperatura del agua, río, arroyo, termómetro digital, metodología, calibración.*

INTRODUCTION

Temperature is a common variable of interest in stream studies because it influences in-stream processes (*e. g.*, organic matter decomposition, Stout, 1989) and the distribution, physiology and behaviour of stream biota (Wehrly *et al.*, 1998; Lewis *et al.*, 2000; Dunham & Chandler, 2001). Various methods are currently used for measuring temperature in aquatic systems, including Raman backscatter distributed temperature sensing (Selker *et al.*, 2006; Tyler *et*

al., 2009), infrared remote sensing (Wawrzyniak *et al.*, 2012) and ground-based thermography (Cardenas *et al.*, 2008; Tonolla *et al.*, 2010). However, the use of digital thermometers with datalogging capability is still the most affordable and common method for monitoring water temperature (*e. g.*, Malard *et al.*, 2001; Johnson *et al.*, 2005; Herb & Stefan, 2011). Attempts to assess stream temperature across large regions have been limited by the high equipment and travel costs associated with maintaining a large number of thermometers spread out over many sites (Wehrly *et al.*, 1998), but the recent development of small, more affordable sensors has resulted in an increased number of individual datasets collected by private and public organisations (*e. g.*, Lewis *et al.*, 2000). The utility of such collective efforts may be limited, however, if variation in measurement quality prevents the comparison of multiple datasets from different sources. A situation may develop in which large datasets exist but the information that can be extracted from them is not reliable (the data-rich-but-information-poor syndrome; Ward *et al.*, 1986; Timmerman *et al.*, 2010). The information in multi-source water temperature datasets will be useful if, among other things, it is credible and the users perceive it to be accurate, valid and of high quality (Cash *et al.*, 2003). In addition to data acquisition, efforts should be directed towards documenting and assuring the quality of stream temperature measurements.

Standards for the quality of environmental temperature measurements have been established by the World Meteorological Organization (WMO, 2008). However, it is not economical to use thermometers that meet these requirements directly. Typically, less expensive thermometers are used by calibrating the thermometer in the laboratory and applying correction factors to the collected data as needed. Calibration involves comparison of the thermometer readings with a standard to determine how closely the instrument matches the standard. Calibration standards for many environmental variables (*e. g.*, concentrations of chemicals in water) can be easily produced in the laboratory for measurement (*e. g.*, a calibration curve for a spectrophotometer). In contrast, the standard scale for temperature measurement is based on the thermodynamic state of various substances at triple point or freezing point equilibrium as measured with a platinum resistance thermometer (Preston-Thomas, 1990). Because the reproduction of this scale for routine calibration is not feasible, a need arises to calibrate against another thermometer that is traceable back to the international temperature standard. Performing periodic calibrations of the thermometers against a reference standard is also useful for detecting those that become defective due to malfunction or age (Dunham *et al.*, 2005).

In addition to the issue of calibration, the use of temperature dataloggers in streams requires making decisions about the installation of the sensor in the field. Installation of a thermometer in a stream commonly requires the use of a protective case to avoid physical damage to the instrument. The case can be either a part of the instrument itself or a commercially manufactured or individually constructed enclosure. Water-resistant sensors and cases that allow the flow of water through the case are preferred for making measurements in aquatic systems because the temperature of air trapped inside a watertight case equilibrates with the surrounding water too slowly and causes a time lag in measurements (Dunham *et al.*, 2005). The material and colour of the case are also important (Dunham *et al.*, 2005). Some materials, such as wood and plastic, are poor conductors of heat, which can lead to differences between the temperature measurements and actual temperatures. In the case of colour, metallic and white surfaces are preferred because dark surfaces result in increased heating of the thermometer when exposed to sunlight. In any case, testing of the protective case is required to ensure that the case does not interference with the temperature measurements (Hubbart *et al.*, 2005).

In this study, we evaluated the use of the ACR SmartButton thermometer (ACR Systems Inc., 2010) for continuous water temperature measurement and tested the effects of a new protective case on temperature measurements. We also proposed a protocol for the calibration of the thermometer and the correction of sensor temperature data.

MATERIALS AND METHODS

Reference standard measurement for temperature

The current accepted international standard for temperature measurements is the International Temperature Scale of 1990 (Preston-Thomas, 1990; ITS-90, 1999), which establishes the temperature values for various substances at triple point or freezing equilibrium. As an example, standards within the range −40 to 30 °C, which are significant for measuring environmental temperatures, include the triple point of mercury (−38.8344 °C), the triple point of water (+0.01 °C) and the freezing point of gallium (+29.7646 °C).

A calibration must be performed against a thermometer that is traceable back to this standard scale. As a reference thermometer, we used an ASTM 63C mercury thermometer (measuring range −8.0-32.0 °C, resolution 0.1 °C). For this thermometer, the supplier provides a certificate from a calibration laboratory that gives correction factors at 0, 10.0, 20.0 and 30.0 °C.

ACR button thermometer

The ACR SmartButton datalogger (17.35 mm diameter × 5.89 mm height) has a stainless steel case and a weight of 4 g (ACR Systems Inc., 2010). The measuring element is a silicon thermistor that has an operational range of −40 °C to 85 °C with a stated accuracy of ± 1.0 °C from −30 °C to 45 °C and a resolution of 0.5 °C. The SmartButton stores up to 2048 temperature measurements and the sampling interval can be programmed from 1 to 255 minutes. If measurements are recorded at 1-hour intervals, which would detect stream maximum daily temperature within ± 1 °C with a probability of 98 % (Dunham *et al.*, 2005), the SmartButton has the storage capacity to continuously record measurements of stream water temperature for 85 days.

Accuracy tests

All accuracy tests were performed using water in a plastic tray that was placed over a 2-cm polystyrene plate for isolation. In the first accuracy test, the ice bucket method (Dunham *et al.*, 2005; Hubbart *et al.*, 2005) was used by adding ice to the plastic tray. The second and third accuracy tests were performed in an INFOR Multitron incubator with target temperatures of 15 and 10 °C, respectively. In the accuracy tests, the temperature of the water in the tray was measured at 15-minute intervals with the reference mercury thermometer. Based on the observations from the accuracy tests, we developed a method for routine calibration of the SmartButton and calculated discrete values (−1.0, −0.5, 0.0, +0.5 and +1.0 °C) to correct sensor readings (see Appendix).

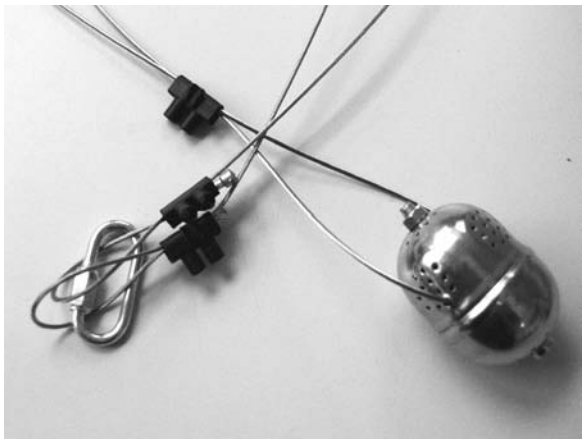

Figure 1. Completed protective case with stainless steel wire attached. *Carcasa protectora terminada con el cable de acero inoxidable.*

Construction and testing of the protective case

The protective case was constructed from a stainless steel tea filter (Fig. 1). The two halves of the tea filter were separated, and a hole was drilled into the top of each piece with a 6-mm metal drill bit. The two halves were held together with a stainless steel screw (6-mm diameter, 40-mm length) and two bolts. The screw was fixed to one half of the filter case with one of the bolts such that the other half could slide on and off the screw for opening and closing the case. The sliding half was secured in position with the second bolt.

The anchorage cable was made with stainless steel wire. We used bicycle brake wires because they are conveniently riveted in one ending. The

wire was passed through two of the small holes in the filter and an electrical connector was use to secure the case in the center of the wire. Two other electrical connectors make the ending wire loops that fix the case in the field. Each set of case and attachment wire was supplied with a galvanized iron karabiner (5 or 6 mm thickness) that allows fixing easily the thermometer to the roots and branches that are found in the stream.

To test for a possible effect of the protective case on temperature measurements, two incubations were performed in a 5-L bucket filled with water. The first incubation test was performed in the field, and the second one was performed in an incubation chamber held at a constant temperature (11 °C). Four ACR SmartButtons were labelled (T1, T2, T3 and T4) and programmed for data acquisition at 5-minute intervals. Each incubation lasted for two weeks. During the first week, all four thermometers were incubated without a protective case. During the second week, T1 and T2 were incubated without a protective case and T3 and T4 were each wrapped in small plastic Ziploc bag (13 × 7 cm) and incubated inside a protective case. The data from thermometers T1 and T2 from the second week were used to estimate the response of thermometers T3 and T4 without the case. The estimates were calculated with linear regressions that used the temperature measurements from thermometers T3 and T4 as the dependent variables and the mean values of the temperature measurements from thermometers T1 and T2 as the predictor variable. The data collected on the first and last day of the incubations were discarded so that only the data from the days in which the whole daily temperature cycle was measured were used in the analysis.

In addition, to test for the effect of the correction factors on measurements obtained under field conditions, six thermometers were placed in a small stream for 36 hours, and the mean, minimum and maximum temperatures of each thermometer were calculated before and after applying the correction factors obtained from the calibration test. The thermometers used in this test were selected to represent the range of error observed in the accuracy tests. Thus, two thermometers had error values < −0.5 °C, two thermometers had error values between −0.5 and 0.5 °C and the remaining two thermometers had error values > 0.5 °C.

Statistical analysis

The SmartButton mean temperatures and the reference temperatures from the accuracy tests were compared using one sample t-tests, and the raw and corrected temperature measurements were compared using paired t-tests. The variance of the raw measurements and the variance of the corrected measurements were compared with an F test. The comparison analyses followed Zar (2010). Least square linear regressions were performed following Montgomery *et al.* (2001) to determine the effects of the protective case on temperature measurements. All statistical analyses were performed with R (R Development Core Team, 2011).

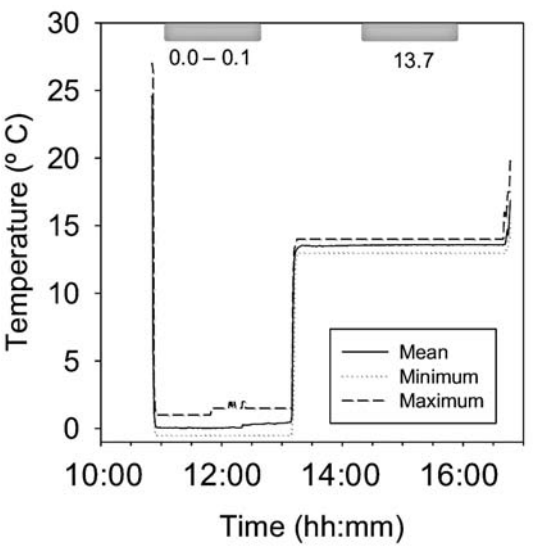

Figure 2. Temperatures measured with SmartButtons (n = 35) during the first and second calibration tests (mean, minimum and maximum temperatures are shown). Gray bands indicate the data that were used for the analysis of the accuracy of the SmartButtons and the reference thermometer measurements. *Temperaturas medidas con los Smartbuttons (*n = *35) durante la primera y la segunda prueba de calibración (se muestran la temperatura media, mínima y máxima). Las bandas grises indican los periodos que se utilizaron para analizar la precisión de los SmartButtons y la temperatura medida con el termómetro de referencia.*

Table 1. Error values for temperature measurements taken with SmartButton thermometers in the three accuracy tests before and after corrections were applied (target temperature: temperature at which the thermometer is to be calibrated; incubator setting: temperature that is set manually using the incubator thermostat; reference temperature: temperature measured with the reference mercury thermometer placed inside the incubator). *Error de las medidas de temperatura recogidas con SmartButtons en las tres pruebas de precisión antes y después de aplicar correcciones (temperatura objetivo: temperatura a la que queremos calibrar los termómetros; ajuste de la incubadora: temperatura que introducimos manualmente en el termostato de la incubadora; temperatura de referencia: temperatura medida con el termómetro de mercurio de referencia dentro de la incubadora).*

	Test 1 n **= 3185**		**Test 2** n **= 3185**		**Test 3** n **= 1325**	
Target temperature (°C)	0.00		15.00		10.00	
Incubator/bath setting (°C)	0.00[a]		15.00		11.20	
Reference temperature (°C)						
Mean ± SD	0.06 ± 0.05		13.70 ± 0.00		9.60 ± 0.00	
Uncorrected sensor readings						
$x < -1.0$ °C	0	0.0 %	0	0.0 %	0	0.0 %
$-1.0 \leq x < -0.5$ °C	592	18.6 %	224	7.0 %	58	4.4 %
$-0.5 \leq x \leq 0.5$ °C	2456	77.1 %	2961	93.0 %	1267	95.6 %
$0.5 < x \leq 1.0$ °C	86	2.7 %	0	0.0 %	0	0.0 %
$x > 1.0$ °C	51	1.6 %	0	0.0 %	0	0.0 %
Corrected sensor readings						
$x < -1.0$ °C	0	0.0 %	0	0.0 %	0	0.0 %
$-1.0 \leq x < -0.5$ °C	76	2.4 %	1	0.0 %	15	1.1 %
$0.5 < x \leq 1.0$ °C	3060	96.0 %	3184	100.0 %	1310	98.9 %
$-0.5 \leq x \leq 0.5$ °C	49	1.5 %	0	0.0 %	0	0.0 %
$x > 1.0$ °C	0	0.0 %	0	0.0 %	0	0.0 %

[a] A bath filled with ice and water was used (0.00 °C is the melting point of water).

RESULTS

Accuracy tests

The reference temperature varied between 0.0 and 0.3 °C during the ice bucket incubation, but only the measurements from the period in which the temperature oscillated between 0.0 and 0.1 °C were used for the accuracy test (Fig. 2, Table 1). In the second and third accuracy tests, the reference temperature held constant at 13.7 °C (Fig. 2, Table 1) and 9.7 °C (not shown, Table 1). In both cases, the reference temperature was lower than the target temperature and also differed from the thermostat setting of the incubator (Table 1).

The error values for the sensor readings in the accuracy tests are presented in Table 1. During the ice bucket incubation, 3185 temperature measurements were taken, of which 51 (1.6 %) differed from the reference temperature by more than 1 °C, 678 (21.3 %) differed from the reference temperature by between 0.5 and 1.0 °C and 2456 (77.1 %) differed from the reference temperature by less than 0.5 °C. During the accuracy test at 13.7 °C, 3185 measurements were taken. Of these measurements, none differed from the reference temperature by more than 1.0 °C, but 224 (7.0 %) differed from the reference temperature by between 0.5 and 1.0 °C and 2961 (93.0 %) differed from the reference temperature by less than 0.5 °C. During the accuracy test at 9.7 °C, 1325 measurements were taken, of which none differed from the reference temperature by more than 1 °C, but 58 (4.3 %) differed from the reference temperature by between 0.5 and 1.0 °C and 1267 (95.6 %) differed from the reference temperature by less than 0.5 °C.

The mean values of the temperature measurements taken with the SmartButtons in the accuracy tests differed slightly from the reference temperatures (Table 2); however, a significant difference between the reference and mean measured temperature (Student's t, $p < 0.05$) was only observed in the second accuracy test. The

error values for the SmartButton measurements ranged from −0.13 to 0.07 °C and the repeatability of the measurements (calculated as the standard deviation of the error) ranged from 0.25 to 0.37 °C, which indicated that 95 % of the SmartButton mean temperatures were within ± 0.73, ± 0.49 and ± 0.53 of the reference temperature for the first, second and third accuracy test, respectively.

Based on the accuracy tests, we calculated correction factors for each sensor that ranged from −1 to 1 °C (Appendix). After the corrections were applied, the percentage of measurements that were within 0.5 °C of the reference temperature increased from 77.1 to 96.0 % for the first accuracy test, from 93.0 to 100.0 % for the second accuracy test and from 95.6 to 98.9 % for the third accuracy test, indicating that the error in the sensor readings decreased (Table 1). The corrected mean temperatures from the SmartButtons (Table 2) were significantly different (paired Student's t-test, $p < 0.001$) from the reference temperatures for the three accuracy tests. The standard deviations of the measurements decreased significantly (F test, $p < 0.01$) after the corrections were applied. The standard deviations ranged from ± 0.04 to ± 0.07 °C, indicating that 95 % of the mean temperatures values from the SmartButtons were within ± 0.15 °C of the mean temperature for the first and second accuracy tests and within ± 0.09 °C for the third accuracy test. The calibration process increased the repeatability of the measurements and the error values were maintained within ± 0.25 °C of the reference temperatures. The significant differences between the SmartButton measurements and the reference temperatures after the correction were a result of the higher repeatability and lower variance of the SmartButton measurements.

Incubation tests

During the first week of the field incubation, temperature varied between 4 and 14 °C (Fig. 3A, Table 3), and the mean temperature according to the measurements of SmartButtons T1 and T2 was 9.78 °C. Differences between the SmartButtons that displayed the lowest and highest temperature were equal to or less than 0.5 °C for 96.7 % of the measurements ($n = 7752$) and equal to or less than 1.0 °C for 99.8 % of the measurements ($n = 7752$). The data collected during the field incubation were used to build linear models showing the relationships between the data from T3 and T4 (y) and the mean values of the data from T1 and T2 (x):

$$T3, y = 0.99x + 0.01,\ r^2 = 0.99,\ p < 0.001 \quad (1)$$

$$T4, y = 1.01x + 0.20,\ r^2 = 0.99,\ p < 0.001 \quad (2)$$

In the second week of the field incubation (Fig. 3B, Table 3), the temperature measurements varied between 3 and 14 °C, and the mean temperature was 8.34 °C, approximately 1.5 °C lower than in the first week. Differences

Table 2. Temperature (mean ± standard deviation, °C) measured with the SmartButtons in the three accuracy tests before and after corrections were applied. *Temperatura (media ± desviación estándar, °C) medida con los SmartButtons en las tres pruebas de precisión antes y después de aplicar correcciones.*

	Test 1 ***n* = 35**	**Test 2** ***n* = 35**	**Test 3** ***n* = 25**
Reference temperature (°C)			
Mean ± SD	0.06 ± 0.05	13.70 ± 0.00	9.60 ± 0.00
Measured temperature (°C)			
Mean ± SD	0.07 ± 0.37	13.57 ± 0.25	9.67 ± 0.27
Error ± SD	0.01 ± 0.37	−0.13 ± 0.25	0.07 ± 0.27
Corrected temperature (°C)			
Mean ± SD	0.03 ± 0.07	13.52 ± 0.07	9.51 ± 0.04
Error ± SD	−0.03 ± 0.07	−0.18 ± 0.07	−0.09 ± 0.04

between the thermometers that displayed the lowest and highest temperature were equal to or less than 0.5 °C for 96.3 % of the measurements (n = 7752) and equal to or less than 1.0 °C for 100 % of the measurements (n = 7752). We estimated that the use of the protective case increased the temperature measurements of the SmartButtons by 0.05-0.10 °C (Table 3). The chamber incubations (Fig. 3C and 3D) confirmed the field observations, showing that the effect of the case on the temperature measurements was negligible (Table 3). In summary, the differences in measurements among the thermometers in the incubation tests were within 0.5 °C, which

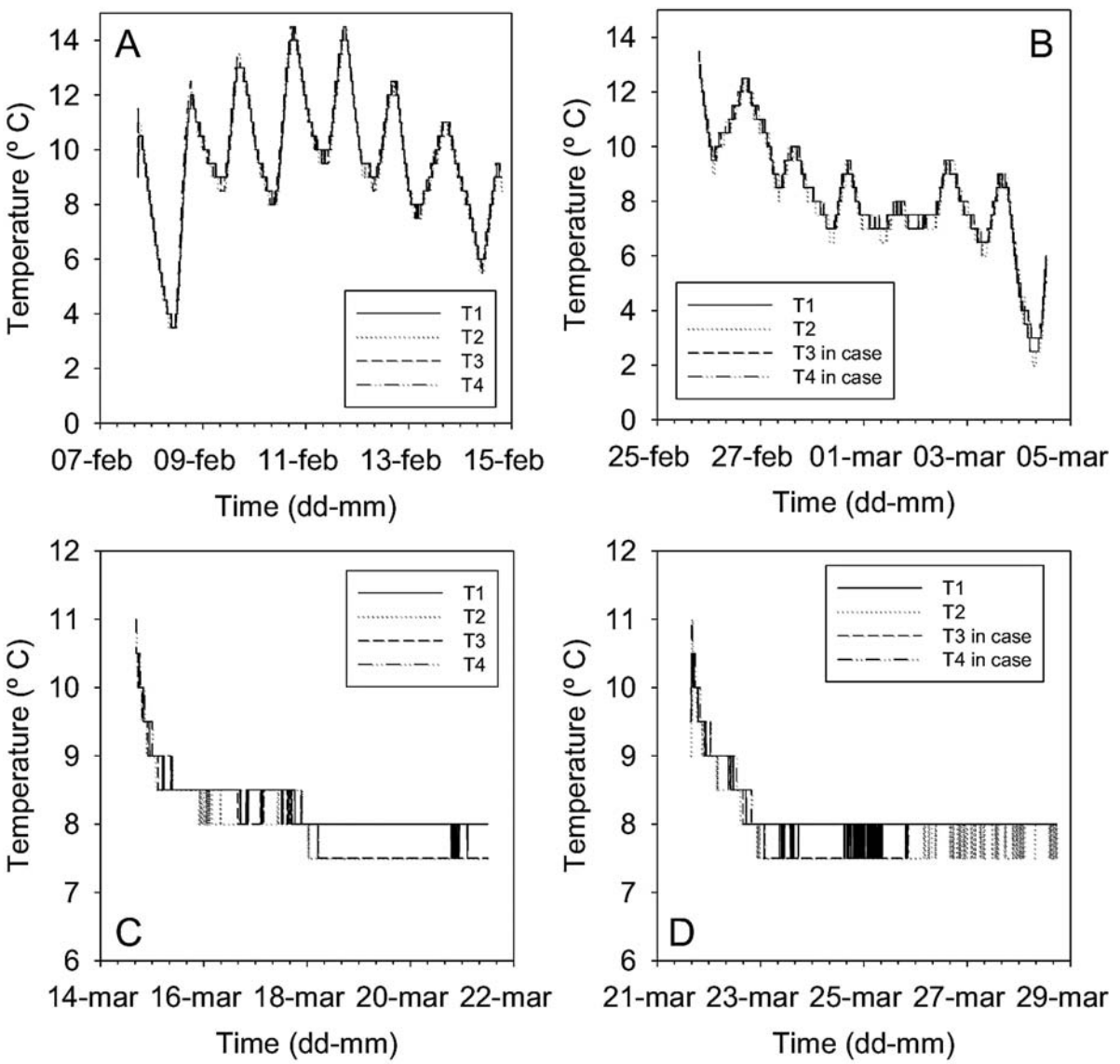

Figure 3. Temperatures measured with SmartButtons (n = 4) during the incubation tests (A, first week of field incubation; B, second week of field incubation; C, first week of chamber incubation; D, second week of chamber incubation). *Temperaturas medidas con los SmartButtons (*n = *4) durante los experimentos de incubación (A, primera semana de incubación en el campo; B, segunda semana de incubación en el campo; C, primera semana de incubación en cámara fría; D, segunda semana de incubación en cámara fría).*

Table 3. Temperature (mean ± standard deviation, °C) measured during the incubation experiments and estimation of the effect of the protective cases (T1 and T2 are control thermometers incubated without protective cases, T3 and T4 are test thermometers incubated without a protective case during the first week and inside a protective case during the second week). *Temperatura (media ± desviación estándar, °C) medida durante los experimentos de incubación y estimación del efecto de las carcasas protectoras (T1 y T2 son los termómetros de control incubados sin carcasas protectoras, T3 y T4 son los termómetros de prueba incubados sin carcasas protectoras durante la primera semana y dentro de las carcasas protectoras durante la segunda semana).*

Field incubation	**T1 (°C)**	**T2 (°C)**	**T3 (°C)**	**T4 (°C)**
First week	10.05 ± 2.29	9.51 ± 2.29	9.62 ± 2.28	9.91 ± 2.30
Second week	8.50 ± 1.55	8.28 ±1.26	8.47 ± 1.51	8.70 ± 1.53
Estimated values[1]	—	—	8.37 ± 1.54	8.65 ± 1.56
Case effect	—	—	+0.10	+0.05
Chamber incubation	**T1 (°C)**	**T2 (°C)**	**T3 (°C)**	**T4 (°C)**
First week	8.28 ± 0.45	7.89 ± 0.53	8.00 ± 0.59	8.33 ± 0.57
Second week	8.13 ± 0.47	7.84 ± 0.52	7.96 ± 0.48	8.19 ± 0.49
Estimated values[1]	—	—	7.87 ± 0.56	8.23 ± 0.46
Case effect	—	—	+0.11	+0.04

[1]Estimated with linear models (1) and (2).

Table 4. Temperature (mean ± standard deviation, °C) measured with calibrated and uncalibrated SmartButtons in a small stream over a 36-hour period (n = 6). *Temperatura (media ± desviación estándar, °C) medida con SmartButtons calibrados y sin calibrar en un arroyo durante 36 horas (*n = *6).*

	Mean temperature (°C)	**Minimum temperature (°C)**	**Maximum temperature (°C)**
Uncorrected	15.16 ± 0.22	14.67 ± 0.26	15.58 ± 0.20
Corrected	15.00 ± 0.12	14.50 ± 0.00	15.42 ± 0.22

was similar to the resolution of the SmartButtons. Furthermore, the protective cases did not interfere with the temperature measurements.

During the stream test, temperature in the stream was fairly constant and varied between 14.5 and 15.5 °C (Fig. 4). The difference between the thermometers that displayed the lowest and the highest temperature was 0.5 °C for 90 % of the measurements (n = 355) and 1.0 °C for 10 % of the measurements (n = 355). After the correction factors were applied, these differences decreased to 0.0 °C for 38 % of the measurements and 0.5 °C for 62 % of the measurements. However, there were no significant differences (paired Student's t-test, $p > 0.05$) in the mean, minimum and maximum temperatures from the SmartButtons before and after applying the correction factors (Table 4). After the corrections were applied, the standard deviations of the

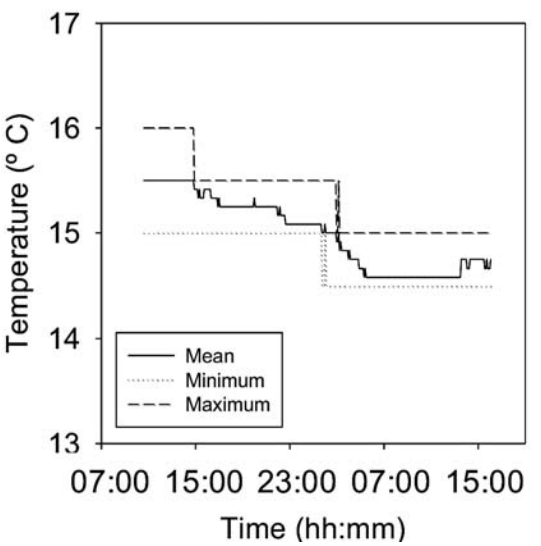

Figure 4. Temperatures measured with Smart Buttons (n = 6) during incubation in a stream (mean, minimum and maximum temperatures are shown). Correction factors have been applied to the sensor readings. *Temperaturas medidas con los SmartButtons (*n = *6) durante su incubación en un arroyo (se muestran la temperatura, media, mínima y máxima). Se han aplicado los factores de corrección a las lecturas de los sensores.*

mean and minimum temperatures decreased, an indication of improved repeatability; however, the difference was only significant (F test, $p < 0.001$) for the minimum temperature values. In contrast, the standard deviation of the maximum temperature values increased after the

calibration factors were applied, although the difference was not significant (F test, $p > 0.05$).

DISCUSSION

The results of our analyses indicate that the accuracy of the SmartButtons is well within the ± 1.0 °C accuracy quoted by the manufacturer (ACR Systems Inc., 2010). Given a similar range of temperatures to that which we found in small streams in our geographical area, the sensor readings are within ± 0.5 °C of the actual water temperature 90 % of the time. Based on this study, the mean error of the SmartButtons falls within the ± 0.1 °C of variation from the correction factor for each 10°C range that is considered acceptable by the World Meteorological Organization (WMO, 2008). However, the standard deviation of the error values indicated that 50 to 60 % of the thermometers require a correction factor that is outside the ± 0.2 °C range accepted by the World Meteorological Organization (WMO, 2008) for meteorological measurements. Therefore, the SmartButtons are not suitable for regular meteorological use. However, their low cost and small size, combined with the ease of programming data collection and retrieving data, makes them highly attractive for other environmental uses. The repeatability of the measurements taken with the SmartButtons and the accuracy of the sensor readings are significantly improved by calibrating the sensors against a traceable reference standard. Our observations suggest that the degree of accuracy obtained through calibration will likely suffice for most biological applications. In the case that greater accuracy is required, Hubbart *et al*. (2005) proposed a screening method for discarding thermometers with relatively low accuracy. For SmartButtons, this would most likely mean discarding approximately 40 % of a batch of new SmartButtons to use only thermometers that do not require corrections. This information should be taken into account when calculating the costs of acquiring the equipment.

Although the ice bucket method is an accepted method for the calibration and screening of thermometers (Dunham *et al*., 2005; Hubbart *et al*., 2005), our experience suggests that it is difficult to maintain a constant ice bath temperature, and therefore, we have discarded this method as an option for the routine calibration of SmartButtons. In our case, shifts in the temperature of the ice bath seem to have occurred as a function of the overall temperature of the laboratory. Using an incubator that has been demonstrated to maintain a constant temperature is preferred over the ice bucket method. Based on the results of our accuracy tests, we propose a calibration method for the SmartButtons. We used an INFOR Multitron incubator, which has a transparent lid that is very convenient for checking the temperature of the reference thermometer during calibration without opening the equipment. Additionally, calibration against a certified thermometer must be performed independently of the calibration method (WMO, 2008). Performing a calibration of the incubator helped to reduce uncertainty during the calibration of the SmartButtons (*e. g*., by making it easier to find the thermostat setting for a given target temperature). However, the thermostat setting, the incubator thermometer readings and the temperature inside the incubator differed slightly even after calibration of the incubator. A record of the reference temperature during calibration and the correction factors for each thermometer should be kept for documenting the conversion from field measurements to the final corrected dataset.

Our short field incubation in a stream showed that the calibration of the SmartButtons improved the repeatability of the measurements but did not improve detection of maximum temperatures. These results suggest that the SmartButton might function differently when cooling down or heating up. Similarly, Hubbart *et al*. (2005) observed differences in the performance of small button thermometers at low and high temperatures. Temperature measurements by semiconductor thermometers (or thermistors) are based on the change of electrical resistance of a measuring element (a silicon sensor in the case of the SmartButton). The passage of electricity through the measuring element produces heat and self-heating of the thermometer causes the

temperature of the instrument to become higher than the temperature of its surroundings. This self-heating effect is greater in small thermistors than in large ones (WMO, 2008). The impact of self-heating on the quality of temperature measurements is probably negligible for most environmental applications, but special care should be taken if obtaining maximum stream temperature data is important for fulfilling the objectives of a project (*e. g*., determining habitat suitability for salmonids).

Water leakage was not a problem in our laboratory tests, although leakage appears to have caused malfunctioning of loggers and data loss with other thermistor models (Wolaver & Sharp, 2007). Coating the thermometer with plastic is an alternative if waterproofing the thermometer is necessary, although the coating interferes with temperature measurements if the thermometers are directly exposed to the sun (Roznik & Alford, 2012). Before introducing the SmartButtons into the metallic protective cases, we wrapped each of them in a small Ziploc bag for additional protection from dirt and for convenient handling (*e. g.*, the bag is easily labelled with a permanent marker). This method can also be used with thermistors that are not waterproof because the size of the resulting package is not much bigger than the instrument itself.

The protective case that we used provides a reliable way to protect the SmartButtons during in-stream use at a cost of approximately 6 Euros per case, which is lower than commercial metallic cases. The effect of the case on temperature measurement was negligible and was similar in magnitude to the effects that have been observed for other cases (*e. g*., Malard et al., 2001). However, we observed that silt tends to accumulate inside the case, so these cases are not suitable for temperature measurements in streams that transport large loads of fine sediment. The design of the case and the anchoring system has been improved through several field trials. In a recent study we used 50 cases, of which 3 were lost due to human vandalism and one was lost due to breakage at the anchoring point. To date, we have not lost any sensors due to failure of the anchoring cable or damage to the case itself.

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REFERENCES

ACR SYSTEMS INC. 2010. ACR SmartButton temperature datalogger. Product specifications. http://www.acrsystems.com/products/smartbutton/. Accessed on July 2011.

CARDENAS, M. B., J. W. HARVEY, A. I. PACKMAN & D. T. SCOTT. 2008. Ground-based thermography of fluvial systems at low and high discharge reveals potential complex thermal heterogeneity driven by flow variation and bioroughness. *Hydrological Processes*, 22: 980–986.

CASH, D. W., W. C. CLARK, F. ALCOCK, N. M. DICKSON, N. ECKLEY, D. H. GUSTON, J. JÄGER & R. B. MITCHELL. 2003. Knowledge systems for sustainable development. *Proceedings of the National Academy of Science*, 100: 8086–8091.

DUNHAM, J. B. & G. L. CHANDLER. 2001. *Models to predict suitable habitat for juvenile bull trout in Washington State.* Washington Bull Trout Habitat Study. Rocky Mountain Research Station, Boise, Idaho, US. 75 pp.

DUNHAM, J., G. CHANDLER, B. RIEMAN & D. MARTIN. 2005. *Measuring stream temperature with digital data loggers: a user's guide*. USDA Forest Service, Gen. Tech. Rep. RMRS-GTR-150WWW. Rocky Mountain Research Station, Boise, Idaho, US. 15 pp.

HERB, W. R. & H. G. STEFAN. 2011. Modified equilibrium temperature models for cold-water streams. *Water Resources Research*, 47: W06519.

HUBBART, J., T. LINK, C. CAMPBELL & D. COBOS. 2005. Evaluation of a low-cost temperature measurement system for environmental applications. *Hydrological Processes*, 19: 1517–1523.

ITS-90. 1999. International Temperature Scale of 1990. http://www.its-90.com/. Accessed on July 2011.

JOHNSON, A. N., B. R. BOER, W. W. WOESSNER, J. A. STANFORD, G. C. POOLE, S. A. THOMAS & S. J. O'DANIEL. 2005. Evaluation of an inexpensive small-diameter temperature logger for documenting ground water-river interactions. *Ground Water Monitoring and Remediation*, 25: 68–74.

LEWIS, T. E., D. W. LAMPHEAR, D. R. MCCANNE, A. S. WEBB, J. P. KRIETER & W. D. CONROY. 2000. *Regional assessment of stream temperatures across Northern California and their relationship to various landscape-level and site-specific attributes*. Forest Science Project. Humboldt State University Foundation, Arcata, California, US. 15 pp.

MALARD, F., A. MANGIN, U. UEHLINGER & J. WARD. 2001. Thermal heterogeneity in the hyporheic zone of a glacial floodplain. *Canadian Journal of Fisheries and Aquatic Sciences*, 58: 1319–1335.

MONTGOMERY, D. C., E. A. PECK & G. C. VINING. 2001. *Introduction to linear regression analysis*. Wiley & Sons, New York. 641 pp.

PRESTON-THOMAS, H. 1990. The International Temperature Scale of 1990 (ITS-90). *Metrologia*, 27: 3–10.

R DEVELOPMENT CORE TEAM. 2011. R: *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/. Accessed on November 2011.

ROZNIK, E. A. & R. A. ALFORD. 2012. Does waterproofing Thermochron iButton dataloggers influence temperature readings? *Journal of Thermal Biology*, 37: 260–264.

SELKER, J., N. VAN DE GIESEN, M. WESTHOFF, W. LUXEMBURG & M. B. PARLANGE. 2006. Fiber optics opens window on stream dynamics. *Geophysical Research Letters*, 33: L24401.

STOUT, R. J. 1989. Effects of condensed tannins on leaf processing in mid-latitude and tropical streams: a theoretical approach. *Canadian Journal of Fisheries and Aquatic Sciences*, 46: 1097–1106.

TIMMERMAN, J. G., E. BEINAT, K. TERMEER & W. COFINO. 2010. Analyzing the data-rich-but-information-poor syndrome in Dutch water management in historical perspective. *Environmental Management*, 45: 1231–1242.

TONOLLA, D., V. ACUÑA, U. UEHLINGER, T. FRANK & K. TOCKNER. 2010. Thermal heterogeneity in river floodplains. *Ecosystems*, 13: 727–740.

TYLER, S. W., J. S. SELKER, M. B. HAUSNER, C. E. HATCH, T. TORGERSEN, C. E. THODAL & S. G. SCHLADOW. 2009. Environmental temperature sensing using Raman spectra DTS fiber-optic methods. *Water Resources Research*, 45: W00D23.

WARD, R. C., J. C. LOFTIS & G. B. MCBRIDGE. 1986. The "Data-rich but Information-poor" syndrome in water quality monitoring. *Environmental Management*, 10: 291–297.

WAWRZYNIAK, V., H. PIÉGAY & A. POIREL. 2012. Longitudinal and temporal thermal patterns of the French Rhône River using Landsat ETM+ thermal infrared images. *Aquatic Sciences*, 74: 405–414.

WEHRLY, K. E., M. J. WILEY & P. W. SEELBACH. 1998. Landscape-based models that predict July thermal characteristics of lower Michigan rivers. Michigan Department of Natural Resources, Fisheries Research Report No. 2037, Ann Arbor, Michigan, US.

WMO. 2008. *Guide to meteorological instruments and methods of observations. World Meteorological Organization No*. 8, 7th edition. Geneva. 681 pp.

WOLAVER, B. D. & J. M. SHARP Jr. 2007. Thermochron iButton: Limitation of this inexpensive and small-diameter temperature logger. *Ground Water Monitoring and Remediation*, 27: 127–128.

ZAR, J. H. 2010. *Biostatistical analysis*. 5th. Ed. Prentice Hall, Upper Saddle River, New Jersey. 944 pp.

APPENDIX: Calibration method for the ACR SmartButtons

MATERIALS

Plastic tray filled with water

2 cm polystyrene plate

ASTM 63C thermomether with calibration bulletin (certified reference thermometer)

Incubator INFOR Multitron

PROCEDURE

1. Program the SmartButtons for data collection at 2 minute intervals.
2. Set-up the incubator for a reference temperature of interest.
3. Place the SmartButtons and the reference thermometer in the plastic tray and introduce the plastic tray in the incubator over a 2 cm polystyrene plate. Make sure that the scale of the reference thermometer can be read through the glass door of the incubator. Wait 2 hours for temperature equilibration.
4. Check the temperature of the reference thermometer at 15 minute intervals (Tr). Collect data for 2 hours. The reference temperature should not change during this period.
5. Download data and calculate the mean temperature for each SmartButton for the two hours period (Ts).
6. Calculate the difference between each SmartButton and the reference thermomether, $D = Ts - Tr$.

CORRECTION FACTORS

Correction factors are calculated as a function of D:

$D > 1.0$	Probable malfunction (discard thermometer)
$0.75 < D < 1.0$	-1
$0.25 < D < 0.75$	-0.5
$-0.25 \leq D \leq 0.25$	0 (no correction required)
$-0.75 \leq D < -0.25$	0.5
$-1.0 \leq D < -0.75$	1
$D < -1.0$	Probable malfunction (discard thermometer)

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The first record of the European catfish *Silurus glanis* Linnaeus, 1758 in the Guadalquivir River basin

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ABSTRACT

The first record of the European catfish *Silurus glanis* Linnaeus, 1758 in the Guadalquivir River basin

This paper describes the first record of the European catfish *Silurus glanis* Linnaeus, 1758, introduced into the Guadalquivir River basin. One individual of the species was captured in the Iznájar Reservoir (Córdoba, Spain) in July 2011. Most likely, the illegal introduction of the species is due to anglers that brought the fish from other Iberian river basins, where the species has been present since 1974.

Key words: Siluridae, Guadalquivir River basin, first record, exotic fish.

RESUMEN

Primera cita del siluro europeo Silurus glanis Linnaeus, 1758 en la cuenca del río Guadalquivir

Esta es la primera cita de siluro europeo Silurus glanis *Linnaeus, 1758, introducido en la cuenca del río Guadalquivir. Un individuo de esta especie fue capturado en el embalse de Iznájar (Cordoba, España) en julio de 2011. La introducción ilegal de esta especie probablemente está causada por pescadores y procede de otras cuencas de la península Ibérica donde la especie está presente desde 1974.*

Palabras clave: *Siluridae, cuenca del río Guadalquivir, primera cita, especie exótica.*

INTRODUCTION

In Spain, the presence of exotic freshwater fish species is one of the most important negative factors affecting the survival of the native fish species (Clavero & García-Berthou, 2005). Currently, approximately 25 exotic fish species are naturalised on the Iberian Peninsula (Elvira & Almodovar, 2001), and the list is still growing. *Silurus glanis* (Siluridae) is native to the North, Baltic, Black, Caspian and Aral Sea drainages but has been introduced and translocated throughout Europe and the Balkhash basin (Kazakhstan) (Kottelat & Freyhoff, 2007). The species was introduced on the Iberian Peninsula in 1974 at Mequinenza-Ribarroja Reservoir in the Ebro River basin (Doadrio, 2002). Later, it was introduced in a reservoir in the Tajo River basin (Doadrio, 2002) and in the Susqueda and Sau Reservoirs (Ter River basin) (Carol *et al*., 2003). Finally, Benejam *et al*. (2007) described the presence of *S. glanis* in La Baells Reservoir in the Llobregat River basin.

METHODS

The specimen was captured on July 2011 in Iznájar Reservoir (Córdoba), located on the Genil River (37°16′35″ N, 4°23′10″ W). This is the largest reservoir in the Guadalquivir River basin, with 981.12 hm^3 of capacity and 2125.72 ha of surface area. The individual was captured on a fishing rod during a field survey program developed by the Regional Environmental Agency and was identified following Kobayakawa (1989) and Kottelat & Freyhof (2007).

RESULTS AND DISCUSSION

The specimen that was caught was scaleless, with one pair of long maxillary barbels (145 mm) and two pairs of shorter mental barbels (35 mm). The fish presented one dorsal fin (I, 4), one pair of pectoral fins (I, 14), one pair of pelvic fins (I, 11), one anal fin (I, 89) and a caudal fin (16). It was dark in colour, with pale spots around the ventral zone. The caudal fin was very long and emarginated. The specimen was registered in the Ichthyological Collection of the Zoology Department of the University of Córdoba (Spain).

This report is the first official record of the species in the Guadalquivir River watershed. Nevertheless, several unofficial comments and videos posted on the Internet suggest that *S. glanis* has been present in the Iznájar Reservoir since at least 2008. The Guadalquivir is the fifth river basin on the Iberian Peninsula affected by the presence of this species. However, *S. glanis* continues its expansion due to its popularity likely among anglers.

The captured specimen had a total length of 490 mm and a total weight of 0.877 kg. The maximum length recorded for the species is 5 m and the maximum weight 300 kg, but individuals are usually approximately 2 m in length and weigh approximately 80 kg (Kottelat & Freyhoff, 2007). The Iznájar Reservoir fish community consists of two native species (*Luciobarbus sclateri*, *Pseudochondrostoma willkommii*) and five exotic species (*Alburnus alburnus*, *Cyprinus carpio*, *Gambusia holbrooki*, *Lepomis gibbosus* and *Micropterus salmoides*). According to Copp *et al*. (2009), the risk that *S. glanis* poses to the native fauna includes disease transmission, predation and the possible modification of the food web structure. Moreover, this exotic species could affect the water quality in the reservoirs through its effects on the food chain (Carol, 2007). Due to its predatory feeding habits, *S. glanis* is a threat to the abundance and survival of native fish and other vertebrates (i.e., amphibians, birds and small mammals) (Kottelat & Freyhoff, 2007).

Because the Iberian ichthyofauna has evolved in the absence of native piscivorous fishes, the many endemic fish species have developed no mechanisms to escape from the introduced piscivorous species. Therefore, piscivorous exotic fishes could affect Iberian fishes more than the fishes of other geographic areas. However, Copp *et al*. (2009) suggest that *S. glanis* exerts trophic pressure on native fish if human impacts are present. The ecological impact of this introduced species on the native fauna is still unknown, but it is highly likely that the introduced species has an effect on the native cyprinids of the Iberian Peninsula (Carol *et al*., 2009). It has been shown that the abundance of aquatic birds is lower in reservoirs in which *S. glanis* occurs. This finding suggests the occurrence of an ecological impact on the community (Carol *et al*., 2009). More research is required to assess the specific ecological impacts that *S. glanis* could be producing in Iberian riverine ecosystems.

REFERENCES

BENEJAM, L., J. CAROL, J. BENITO & E. GARCÍA-BERTHOU. 2007. On the spread of the European catfish (*Silurus glanis*) in the Iberian Peninsula: first record in the Llobregat river basin. *Limnetica*, 26(1): 169–171.

CAROL, J. 2007. *Ecology of an invasive fish (Silurus glanis) in Catalan reservoirs*. PhD Thesis. Universitat de Girona. 120 pp.

CAROL, J., L. BENEJAM, Q. POU, L. ZAMORA & E. GARCÍA-BERTHOU. 2003. Primera citació de brema blanca (*Abramis bjoerkna*) a Catalunya i

noves introduccions de peixos exòtics (*Alburnus alburnus, Sander lucioperca* i *Silurus glanis*) en diverses conques catalanes. *Butlletí de la Institució Catalana d'Història Natural.*, 71: 135–136.

CAROL, J., L. BENEJAM, J. BENITO & E. GARCÍA-BERTHOU. 2009. Growth and diet of European cafish (*Silurus glanis*) in early and late invasion stages. *Fundamental and Applied Limnology*, 174(4): 317–328.

CLAVERO, M. & E. GARCÍA-BERTHOU. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution*, 20(3): 110.

COPP, G. H., J. R. BRITTON, J. CUCHEROUSSET, E. GARCÍA-BERTHOU, R. KIRK, E. PEELER & S. STAKENAS. 2009. Voracious invader or benign feline? A review of the environmental biology of European catfish *Silurus glanis* in its native and introduced ranges. *Fish and Fisheries*, 10: 252–282.

DOADRIO, I. 2002. *Atlas y Libro Rojo de los Peces Continentales de España*. Madrid. Dirección General de Conservación de la Naturaleza. 364 pp.

ELVIRA, B. & A. ALMODOVAR. 2001. Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st Century. *Journal of Fish Biology*, 59 (Supplement A): 323–331.

KOBAYAKAWA, M. 1989. Systematic revision of the catfish genus *Silurus*, with description of a new species from Thailand and Burma. *Japanese Journal of Ichthyology*, 36(2): 155–170.

KOTTELAT, M. & J. FREYHOF. 2007. *Handbook of European freshwater fishes*. Cornol, Switzerland. Publications Kottelat. 646 pp.

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Feeding ecology of the Ruivaco *Achondrostoma oligolepis*, a Portuguese endemic cyprinid fish

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ABSTRACT

Feeding ecology of the Ruivaco *Achondrostoma oligolepis*, a Portuguese endemic cyprinid fish

This study assessed the feeding ecology of the ruivaco *Achondrostoma oligolepis*, a Portuguese endemic resident cyprinid fish whose dietary habits are virtually unknown. Samples were taken seasonally in three medium-sized rivers representing a gradient of temporality. The stomach contents of 97 individuals (42-126 mm total length, TL) were analysed. Although there was no significant overall variation in diet composition between rivers, differences were found among seasons. A broad range of food categories was identified, although a smaller subset of primarily detritus (77.6 %) and plant material (18.4 %) constituted the base diet. Of the animal prey, Coleoptera and Diptera were the most prevalent, occurring in 13.2 % and 9.8 % of the fish, respectively, and were consumed mainly in the spring. Based on the observed diet composition and feeding strategy, the ruivaco could be considered a generalist, foraging on the most abundant and available prey.

Key words: Feeding strategies, native species, diet composition, seasonal variability, Portugal.

RESUMEN

Ecología alimentaria del Ruivaco* Achondrostoma oligolepis*, un pez ciprinideo endémico de Portugal

*Este estudio evaluó la ecología alimentaria del ruivaco (*Achondrostoma oligolepis*), un ciprínido endémico de Portugal cuyos hábitos alimenticios son prácticamente desconocidos. Las muestras fueron recogidas estacionalmente, en tres ríos medianos representando un gradiente de temporalidad. Los contenidos estomacales de 97 individuos (42-126 mm de longitud total, LT) fueron analizados. Aunque en general no hubo variación significativa en la composición de la dieta entre ríos, sí se encontraron diferencias entre las estaciones. Se identificó una amplia gama de categorías de alimentos, aunque un subconjunto más pequeño conformó la base de la dieta, encontrándose principalmente dominada por detritus (77.6 %) y material vegetal (18.4 %). Los coleópteros y dípteros fueron los grupos más frecuentes entre las presas animales, siendo encontrados en el 13.2 % y el 9.8 % de los peces respectivamente, y consumidos principalmente en primavera. Con base en la composición de la dieta y en la representación de la estrategia trófica, el ruivaco puede ser considerado como generalista, alimentándose de las presas más abundantes y disponibles.*

Palabras clave: *Ecología trófica,* Achondrostoma oligolepis, *composición de la dieta, variabilidad estacional, Portugal.*

INTRODUCTION

Examining the feeding ecology of fishes is important for evaluating the ecological role that these species play in a broader context and understanding their position in the food web structure (Allan & Castillo, 2007). Additionally, assessing the diet of fishes is important for aquatic management. The European Water Framework Directive (European Union, 2000), a framework to assess ecological integrity of rivers and streams across Europe, has developed numerous indices of biological integrity for fish. These indices frequently include metrics for measuring the position of individual fish in the food chain, which consequently show significant responses to disturbance (e. g., Pont *et al.*, 2007). However, while the trophic ecology of some Iberian fish species is based on expert judgment, there is little support from ecological data, particularly for small-sized cyprinids. There is still a large gap in information about seasonal and size-related feeding habits. Thus, quantifying the diet composition of such species is extremely important, not only to improve knowledge of species ecology but also to obtain more accurate estimates of indices of biological integrity.

The ruivaco *Achondrostoma oligolepis* (Robalo, Doadrio, Almada & Kottelat, 2005) is a small-sized (< 150 mm TL) cyprinid endemic to Portuguese rivers. Its distribution covers the central and northern river basins of Portugal, occupying a great variety of lotic systems from cold-water and permanent streams to warm-water and intermittent watercourses (Ferreira *et al.*, 2007a). This species normally inhabits moderate to slow-flowing waterways with sandy or gravel substratum and aquatic vegetation and is generally considered tolerant to stream degradation (Santos *et al.*, 2004; Ferreira *et al.*, 2007b). It reaches sexual maturity in the second year of life and reproduces from April to June. There are studies that focus on the genetic variability (Robalo *et al.*, 2007) and spatial distribution of this species at different spatial scales (Ferreira *et al.*, 2007a), but none have addressed feeding ecology. This information could prove extremely useful for other small cyprinid species (*Achondrostoma spp.*), such as the endangered Western ruivaco *Achondrostoma occidentale* (Robalo, Almada, Sousa-Santos, Moreira & Doadrio, 2005) (Robalo *et al.*, 2008) whose feeding habitats are poorly understood.

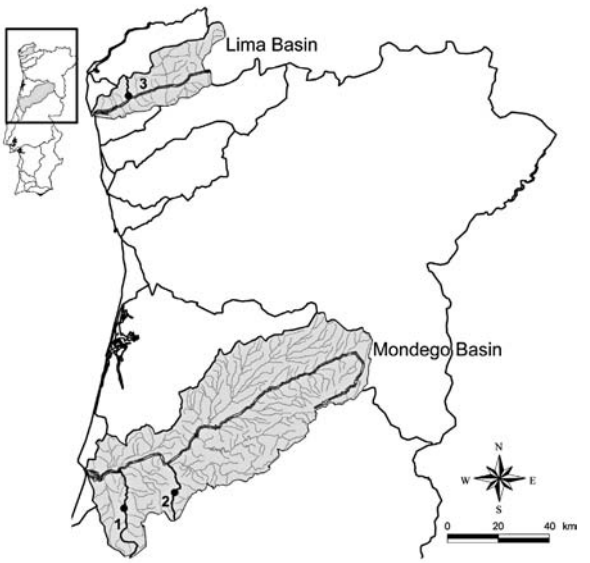

Figure 1. Map of the study area, showing the location of the Arunca (1), Corvo (2) and Estorãos (3) rivers. *Mapa del área de estudio, mostrando la localización de los ríos Arunca (1), Corvo (2) y Estorãos (3).*

This study aimed to address the seasonal feeding ecology of the ruivaco at sites with distinct environmental characteristics in central and northern Portugal. Specifically, the following questions were asked: (a) What are the main food items consumed by the ruivaco?; (b) Are there differences in the diet composition of the ruivaco among sites, seasons, and size-classes, and if so, what are the most important dietary items?; and (c) To what trophic guild could the ruivaco be assigned?

METHODS

Study area

The ruivaco individuals were captured at three sites in the Arunca, Corvo and Estorãos rivers (Fig. 1). These rivers were chosen to reflect a gradient of intermittency from seasonal to

perennial. The Arunca is a seasonally intermittent, medium-sized (length = 60 km) river in the Mondego River basin, W Portugal. The climate is Mediterranean pluviseasonal oceanic with most of the rainfall (mean annual value c. 800 mm) between October and March. There is virtually no flow in the warmest months of July and August when large sections of river turn into a series of unconnected temporary pools (intermittent reaches *sensu* Gasith & Resh, 1999). The Arunca is a shallow (mean depth = 24.7 cm), low-gradient river with an abundance of riffle and pool habitats and sparse aquatic vegetation. Stream-bed materials are heterogeneous, mainly dominated by sand, gravel and pebbles, with silting in pool habitats. Continuous riparian vegetation, mainly willow *Salix spp*. and common alder *Alnus glutinosa* gallery forest, border the river channel. The Corvo is a medium-sized (length = 40 km) tributary of the Mondego River and is intermediate in its degree of desiccation and mean annual precipitation (1100 mm). It is relatively deep (mean depth = 62.7 cm) with a predominantly silt-sandy substratum that lacks well-defined pool-riffle sequences. The river channel is bordered by semi-fragmented riparian vegetation with irrigated agriculture in the uplands. The availability of cover for fish along both rivers is generally high, mainly in the form of logs, twigs, undercut banks and root masses. Apart from the ruivaco, other species, such as the Iberian barbel *Barbus bocagei* (Steidachner), the Iberian straight-mouth nase *Pseudochondrostoma polylepis* (Steindachner) and the Northern Iberian chub *Squalius carolitertii* (Doadrio), also occur in these rivers. The Estorãos is a small-sized (length = 18 km) perennial river in the Lima River basin, NW Portugal. Climate is temperate oceanic with relatively high run-off due to a mean annual rainfall of 1800 mm. It is a lowland river with well-defined riffle sections that are interconnected with pool habitats. While the riffles are abundant, the pool habitats predominate. The substratum is mainly composed of silt and sand with abundant riparian and aquatic vegetation. Riparian vegetation is continuous on both banks and consists mainly of English oak (*Quercus robur*), common alder (*Alnus glutinosa*), willow (*Salix* spp.) and poplar (*Populus* spp.). There are no major pollution sources in the river. Other fish species recorded at the study site included the Northern Iberian chub and the brown trout *Salmo trutta* (L.).

Fish sampling

Fish sampling was conducted two (Estorãos River) to three (Arunca and Corvo rivers) times at each site during daylight hours in the spring, the beginning of the fish spawning season and seasonal plant growth (April 2007); the summer, the period of minimum flow (July 2007); and the autumn, the beginning of the wet season periods (October 2007). No sampling was conducting during floods in the winter because high water levels prevented efficient sampling. At each site, a 150 m-long reach was electrofished (DC, 200–400 V, Hans Grassl model IG-200) in accordance with CEN (2003) standards, encompassing repeating habitat types (riffles, pools). After sampling, all the collected individuals were identified, counted and measured (TL to the nearest mm). Most individuals were returned alive to the river. However, whenever possible, a sample of 15 individuals of the ruivaco that represented the size range in each collection was retained for diet analysis. They were euthanised immediately with an overdose of benzocaine and transported on ice to the laboratory. The specimens were fixed in 4 % formaldehyde solution for 48 h and then preserved in 70 % ethanol solution.

Diet analysis

In the laboratory, individuals were dissected and contents of the gastrointestinal tract were removed. Only fish whose gastrointestinal tract was > 75 % full were selected. When available, fish with empty guts were replaced randomly with other individuals. Diet items were initially identified to the lowest readily recognisable *taxon*, usually family, under a dissecting microscope (up to 50× magnification) or a high-power microscope (100-400× magnification), weighted and assigned to 14 food categories: detritus, plant material, Diptera, Trichoptera, Coleoptera,

Hymenoptera, Odonata, Ephemeroptera, Lepidoptera, Mollusca, Nematoda, zooplankton, fish eggs and clutches, and other prey (i.e., unidentified macroinvertebrate remains).

Benthos Availability

To support data on the diet composition of the ruivaco, food availability was assessed in the same fish sampling sites by sampling benthic macroinvertebrate communities. Samples were taken along a 50 m-long stretch in accordance with the official Portuguese protocol for macroinvertebrate sampling (INAG, 2008). The sampling area was selected to cover the greatest possible diversity of habitats, including riffles and areas of deposition. Type and extent of habitats were visually estimated. Six 1 m-long sampling units of the most representative habitats (stones, sand and silt, boulders (> 256 mm), submerged plants and algae) were taken using a 0.25 m × 0.25 m handnet. The composite sample was placed in a labelled plastic flask and fixed in situ using 4 % formaldehyde. In the laboratory, samples were washed, sieved, sorted and identified to the lowest recognisable *taxon*, usually family, by a low-power stereo-microscope and later assigned to order level in accordance with resource-use data (i.e., diet analysis). Data are expressed as relative abundance or number of each food category.

Data analysis

Three metrics for the description of stomach contents were used to evaluate the importance of each food category (*i*): frequency of occurrence (F_i, %), prey abundance (A_i, %), and prey-specific abundance (P_i, %) (Amundsen, 1995; Amundsen *et al.*, 1996), which were defined according to the following equations:

$$F_i = 100\,(N_i\,N^{-1}) \quad (1)$$

$$A_i = 100\,(\Sigma\,S_i\,\Sigma\,S_t^{-1}) \quad (2)$$

$$P_i = 100\,(\Sigma\,S_i\,\Sigma\,S_{ti}^{-1}) \quad (3)$$

where N_i is the number of individuals with item *i* in gastrointestinal contents, N is the total number of individuals, S_i is the stomach content (weight) composed of item *i*, S_t is the total stomach content (weight) of all stomachs in the entire sample, and S_{ti} is the total stomach content in only those fish with prey *i* in their stomach. To test for significant differences in the abundance (A_i) of food categories among sites, a non-parametric Kruskal-Wallis test was employed. When an overall significant effect was detected, Tukey post hoc tests were then used for pairwise comparisons. In addition, the trophic diversity of the sampled populations was calculated according to the Shannon-Wienner diversity index ($H' = -\Sigma p_i \cdot \log_2 p_i$, p_i-proportion of prey item *i* among the total weight of preys), which provides a relatively objective indication of niche breath (Marshall & Elliott, 1997). Low values indicate diets dominated by few items, which we then define as specialists, and high values indicate diets dominated by many items, which we define as generalists. In the present study, diets with values greater than 2 were considered high, whilst values lower than 1 were considered low (Encina *et al.*, 2004). In order to evaluate diet specialisation, the evenness index ($E = H'$ ($H'^{-1}_{\max}$)) was computed. Values close to 0 indicate a stenophagous diet, whereas an index closer to one points to a euryphagous diet. The relative importance of each food category among sites and seasons was also assessed by calculating the Index of Relative Importance (*IRI*) (Windell, 1971), which is described by the equation:

$$IRI = (F_i\,A_i)^{0.5} \quad (4)$$

Significant differences in diet composition among seasons were investigated using a randomisation method the ANOSIM analysis (Clarke & Warwick, 1994). ANOSIM operates on a resemblance matrix and is similar to a standard univariate ANOVA but does not require normality or homoscedasticity of data. This method employs the *R* statistic to assess the existence of significant differences between the predetermined groups for a given factor (e.g., season). *R* ranges between −1 and +1, where 0 indicates completely random grouping. For each season, a Similarity Percentages analysis

(SIMPER) was performed to determine which food categories contributed most to the differences among the seasons.

Feeding strategy was determined using Costello's (1990) graphical method, as modified by Amundsen *et al.* (1996). These diagrams are based on two-dimensional representation where each point represents the frequency of occurrence (F_i) and the prey-specific abundance (P_i). Prey importance (rare prey will be located near the lower left corner of the graph and dominant prey near the upper right corner) and feeding strategy (most points at the bottom of the graph reflect generalisation and most points at the top reflect specialisation) can thus be evaluated by examining the distribution of points along the diagonals and axes of the diagram. In addition, the relationship between feeding strategy and between- or within-phenotype contributions to the niche width is also represented. Points located at the lower right corner represent a high within-phenotype component, whereas points located at the upper left corner represent a high between-phenotype component (Amundsen *et al.*, 1996). Statistical analyses were carried out with the STATISTICA (StatSoft Inc., 2000) and PRIMER packages (Clarke & Warwick 1994).

RESULTS

Stomach contents of 97 ruivaco specimens (42-126 mm TL) were analysed. Although the species consumed a large spectrum of food categories, the diet base was primarily detritus (F_i = 77.6 %) and plant material (F_i = 18.4 %) (Table 1). Of all the animal prey items recorded, Coleoptera and Diptera were the most frequent, occurring in

Table 1. Frequency of occurrence (F_i, %) and abundance (A_i, %) (weight) of food categories found in the stomachs of the ruivaco in the Arunca, Corvo and Estorãos rivers. Significant differences among rivers were searched by Kruskal-Wallis tests (H). The results of a posteriori Tukey test are indicated. Rivers (A-Arunca; C-Corvo; E-Estorãos) with the same lowercase letter are not significantly different; *** $P < 0.001$, ns-not significant. *Frecuencia de ocurrencia (*F_i*, %) y abundancia (*A_i*, %) (peso) de los tipos de presa encontrados en los estómagos de los ruivacos en los ríos Arunca, Corvo y Estorãos. Las diferencias estadísticas entre los ríos fueron registrados por las pruebas de Kruskal-Wallis (*H*). Resultados de una prueba a posteriori de Tukey se indican. Ríos (A-Arunca, C-Corvo, E-Estorãos) con la misma letra minúscula no son significativamente diferentes; *** P < 0.001, ns-no significativo.*

			Arunca (N = 34)		Corvo (N = 38)		Estorãos (N = 25)				
Food categoty	Acronym		F_i (%)	A_i (%)	F_i (%)	A_i (%)	F_i (%)	A_i (%)	H	P	Tukey test
Detritus	DET		64.7	27.6	92.1	77.9	76.0	44.3	18.6	***	A^a C^b E^{ab}
Plant material	VEG	Vegetal debris,seed, filamentous and vascular algae, macrophytes	20.6	27.2	17.9	9.1	16.8	11.1	0.7	ns	
Diptera	DIP	Chironomidae, Ceratopogonidae, Limoniidae, Simuliidae	11.8	26.9	10.1	8.9	7.3	24.0	1.4	ns	
Trichoptera	TRI	Philopotamidae, Glossosomatidae, Rhyacophilidae, Hydropsychidae	4.4	2.6	3.9	0.4	2.0	0.4	1.3	ns	
Coleoptera	COL	Elmidae	11.8	5.6	11.8	0.8	16.0	17.0	0.3	ns	
Hemynoptera	HYM	Formicidae	5.9	0.4	2.6	0.0	0.0	0.0	1.7	ns	
Odonata	ODO	Gomphidae, Aeshnidae	4.4	1.6	1.3	0.2	2.0	0.0	1.5	ns	
Ephemeroptera	EFE	Baetidae, Heptageniidae, Ephemeridae	4.9	4.5	7.9	0.8	1.3	0.0	3.6	ns	
Lepidoptera	LEP		0.0	0.0	2.6	0.1	0.0	0.0	1.6	ns	
Mollusca	MOL	Hydrobiidae, Planorbidae	8.8	3.2	5.3	0.7	0.0	0.0	3.7	ns	
Nematoda	NEM		0.0	0.0	0.0	0.0	8.0	0.0	5.8	ns	
Zooplankton	ZOO	Cladocera, Cyclopoida	0.0	0.0	3.9	1.0	0.0	0.0	3.1	ns	
Fish eggs and clutches	EGG		1.5	0.0	1.3	0.2	14.0	0.2	8.1	ns	
Other prey	OTH	Unidentified macroinvertebrate remains	38.2	0.4	18.4	0.1	28.0	3.0	3.0	ns	
Trophic diversity (H')			2.42		1.21		2.01				
Eveness diversity (E)			0.70		0.33		0.60				

13.2 % and 9.8 % of the fish and accounting for 7.8 % and 19.9 % of the abundance (A_i), respectively. Trichoptera, Odonata and Ephemeroptera were also found in the stomachs of the ruivaco but were less important ($A_i < 3$ %). Unidentified macroinvertebrate remains (categorised as "Other prey") occurred in a fair percentage of stomachs ($F_i = 28.2$ %) but were consumed in very low proportions (*c.* $A_i = 1$ %).

Overall, there was no difference in diet composition among rivers as shown by non-significant spatial variation in abundance (Kruskal-Wallis test, $p > 0.05$) in 13 out of 14 food categories (Table 1). Only detritus was more frequently consumed in the Corvo than the Arunca (Tukey test, $p < 0.001$). The trophic diversity of the ruivaco was relatively high, particularly in the Arunca and the Estorãos ($H' = 2.42$ and 2.01, respectively), as was its evenness index ($E = 0.70$ and 0.60, respectively), which indicates a euryphagous diet. In the Corvo, diversity was lower ($H' = 1.21$, $E = 0.33$) as a result of a high proportion of consumed detritus ($A_i = 77.9$ %).

Relative abundance of benthic macroinvertebrates in the Arunca and the Corvo varied across seasons, particularly in the case of Diptera and Ephemeroptera, which were the most abundant potential forms of prey available to the ruivaco in the spring (both categories > 75 %), decreasing in following seasons (Table 2). The same groups were also found to be the most abundant (55-65 %) in the Estorãos, although their availability did not change markedly between spring and summer.

The importance of different food categories was found to differ across seasons (Fig. 2). In the Arunca, diet was dominated by detritus and plant material in summer (*IRI* = 56.9 % and 83.1 %, respectively) and autumn (78.3 % and 26.6 %, respectively), with Mollusca consumption (35.9 %) increasing in the latter. Conversely, animal prey items, such as Diptera (86.3 %), Coleoptera (26.3 %) and Ephemeroptera (21.2 %), were mainly consumed in the spring, whereas detritus (4.0 %) was eaten only marginally. In the Corvo, detritus consumption was important throughout all seasons (94.8 % in summer and 82.3 % in autumn), although with a lower proportion was consumed in the spring (52.7 %) when Diptera (68.9 %) took the lead in this species' diet. Ephemeroptera (18.7 %) and Coleoptera (9.4 %) were also consumed during this period. In the Estorãos, the spring diet was highly dominated by detritus (96.3 %), with Diptera (16.2 %) and fish eggs (5.6 %) of less importance. In the summer, detritus consumption (31.9 %) was replaced by a more diverse diet of vegetation (42.5 %), Coleoptera (34.6 %) and Diptera (33.1 %).

These results were confirmed by the pairwise analysis of similarity (ANOSIM) and SIMPER tests. These showed that differences in diet com-

Table 2. Relative abundance (number) of benthic macroinvertebrates found in the Arunca, Corvo and Estorãos rivers across the different sampling seasons. *Abundancia relativa (número) de macroinvertebrados bentónicos que se encontraron en los ríos Arunca, Corvo y Estorãos en diferentes estaciones de muestreo.*

	Arunca			Corvo			Estorãos	
Food category	spring	summer	autumn	spring	summer	autumn	spring	summer
Diptera	50.9	13.5	23.5	32.1	24.5	14.9	36.2	25.4
Trichoptera	1.8	10.6	17.3	4.9	46.4	29.4	6.7	7.7
Coleoptera	0.2	13.2	14.7	0.5	3.7	0.0	12.0	13.3
Hemiptera	0.2	0.0	0.3	0.0	1.8	0.6	0.0	3.2
Hemynoptera	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Odonata	0.2	0.8	5.9	0.9	1.8	4.6	11.5	3.9
Ephemeroptera	45.7	38.4	3.6	58.8	14.9	18.1	28.7	32.9
Lepidoptera	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6
Megaloptera	0.0	0.9	1.3	0.0	1.2	5.4	1.6	0.0
Mollusca	1.0	22.6	32.7	0.3	2.8	16.6	0.0	0.0
Nematoda	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Plecoptera	0.0	0.0	0.7	2.5	2.9	10.4	3.3	12.0

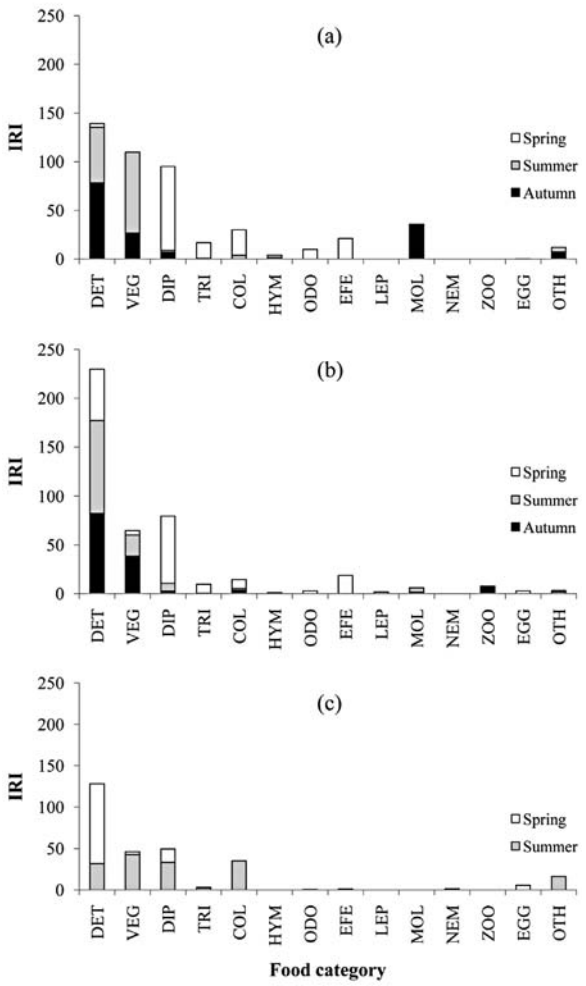

Figure 2. Index of relative importance (IRI) (%) presented in a stack of the different food categories in the diet of the ruivaco *Achondrostoma oligolepis*. (a) Arunca River; (b) Corvo River; (c) Estorãos River. *Índice de Importancia Relativa (IRI) (%), presentado en fichas, de las diferentes categorías de presas en la dieta del ruivaco* Achondrostoma oligolepis*. Ríos (a) Arunca; (b) Corvo; (c) Estorãos.*

position among seasons were greatest in the Arunca, particularly between spring and the remaining seasons (ANOSIM, $R > 0.75, P < 0.01$) (Table 3). In the Estorãos and the Corvo, the degree of separation was also significant, but to a lesser extent ($R < 0.40$, $P < 0.01$) in the latter. In this case, only differences in diet composition between summer and autumn were found to be non-significant ($R = 0.007$, $P > 0.05$). The results of the SIMPER analysis comparing food categories consumed between seasons agree with patterns observed in previous analyses (Table 4).

Table 3. ANOSIM pairwise R statistics for diet composition of the ruivaco among seasons in each of the three studied rivers. ***$P < 0.001$; **$P < 0.01$. *Estadísticas R de ANOSIM para la composición de la dieta del ruivaco entre estaciones en cada uno de los tres ríos estudiados.* ***P < 0.001; **P < 0.01.*

River	Seasons	R	*P*
Arunca			
	spring vs summer	0.953	***
	spring vs autumn	0.919	***
	summer vs autumn	0.371	***
Corvo			
	spring vs summer	0.284	**
	spring vs autumn	0.337	***
	summer vs autumn	0.007	
Estorãos			
	spring vs summer	0.481	***

Table 4. Average similarity (%) on the diet composition of the ruivaco within different seasons for each of three studied rivers and corresponding breakdown contributions (%). Food acronyms are listed in Table 1. *Similitud media (%) en la composición de la dieta en las distintas estaciones para cada uno de los tres ríos estudiados y contribuciones correspondientes (%). Siglas de los elementos de la dieta están en la Tabla 1.*

		Similarity	
Season	Rivers	Average (%)	Contribution (%)
Spring			
	Arunca	41.5	DIP (62.8), COL (23.6), EFE (6.6)
	Corvo	44.1	DET (67.0), DIP (22.1), EFE (8.0)
	Estorãos	71.9	DET (94.5)
Summer			
	Arunca	56.3	VEG (71.2), DET (27.9)
	Corvo	60.7	DET (88.7), VEG (9.8)
	Estorãos	26.7	VEG (48.4), COL (23.3), DET (18.4)
Autumn			
	Arunca	43.6	DET (79.4), VEG (16.0)
	Corvo	53.3	DET (77.6), VEG (20.2)
	Estorãos	—	—

In the Arunca, average similarity in diet among seasons ranged between 41.5 % and 56.3 %, with detritus and plant material contributing over 90 % of the similarity in summer and autumn. On the contrary, in the spring, Diptera consumption alone contributed over 60 % of the average similarity. The results were similar for the Corvo, with detritus and plant material both accounting for more than 95 % of the average similarity

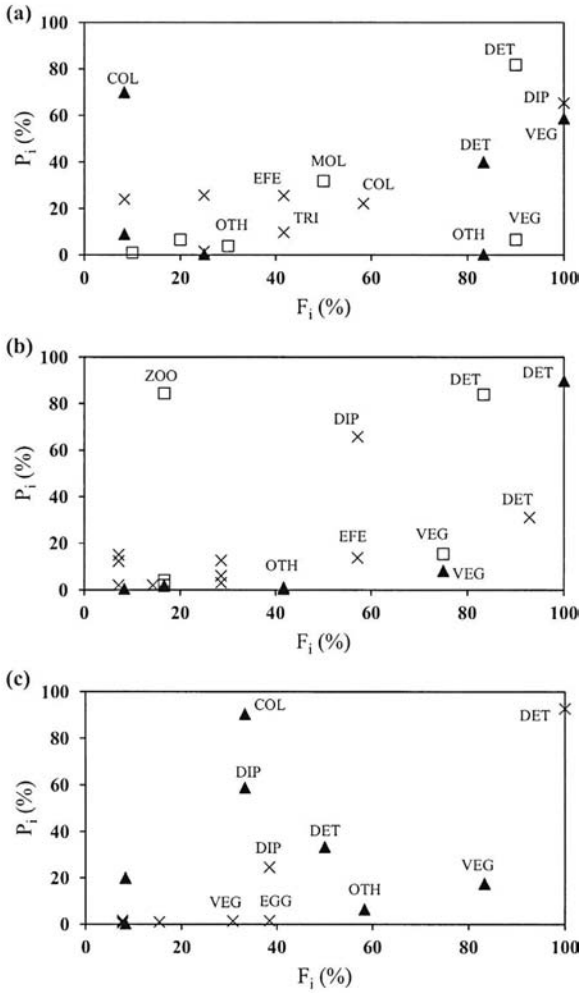

Figure 3. Costello's graphic method modified by Amundsen (1996) for all food categories in the diet of the ruivaco in Arunca (a), Corvo (b) and Estorãos (c) rivers. (×) Spring; (▲) Summer; (□) Autumn. Note that acronyms for rare food categories (i.e., located in the lower left corner) were omitted for clarity. *Método gráfico de Costello modificado por Amundsen (1996) para todas las categorías de presas en la dieta del ruivaco en los ríos Arunca (a), Corvo (b) y Estorãos (c). (×) Primavera; (▲) Verano; (□) Otoño.*

(range: 44.1 %-60.7 %) in summer and autumn. As in the Arunca, Diptera was also an important component of diet composition during spring with a moderate contribution of 22.1 %. In the Estorãos, the maximum contribution of a single item was 94.5 % (detritus in the spring). This was the only item contributing to the average similarity of 71.9 %. During summer, a decrease in the proportion of detritus (18.4 %) in the diet was observed along with an increase in the proportions of plant material (48.4 %) and Coleoptera (23.3 %) to the average similarity (26.7 %).

Analysis of feeding strategy plots, according to Costello's graphical method as modified by Amundsen, seems to indicate an overall generalist feeding strategy in the three studied rivers. Most points representing food categories are located at the bottom of the graph (P_i (%) < 40 and 5 < F_i (%) < 90), thereby reflecting generalization (Fig. 3). Nonetheless, a frequent (F_i (%) > 80) and high (P_i (%) > 80) seasonal consumption of particular food items in some rivers could denote temporary differentiation in diet. As shown by previous results, detritus was the dominant food item in the Arunca and the Corvo in summer and autumn, whereas Diptera was dominant in spring. In the Estorãos, detritus was the most frequent and abundant food category consumed in the spring, but both prey, including Coleoptera and Diptera, and plant material were consumed in greater abundance in summer. The overall absence of points located at the upper left corner of the diagrams indicates that a high between-phenotypic contribution was unlikely to occur.

DISCUSSION

This analysis of ruivaco feeding habits revealed a diet composed largely of detritus and plant material, although it also showed ingestion of a broad range of less frequently consumed items, such as benthic macroinvertebrates. The importance of detritus and plant material has been highlighted for other native Iberian species, particularly cyprinids (Encina *et al.*, 1999), as these materials often represent the only available food resources in fluctuating environments and periods of food shortage (Magalhães, 1992; Encina *et al.*, 2004). This is the case with the Arunca and the Corvo, which seasonally experience seasonal periods of extended water scarcity and where the highest contribution of detritus has been observed in this species' diet. The consumption of detritus and plant material permits a considerable drop in the cost of searching for food (Collares-Pereira *et al.*, 1996) despite the fact that their assimilation

by fish and nutritional value are low compared to animal items. However, with the exception of detritus, there was no significant variation in diet composition between rivers, although pronounced seasonal variations were detected and may denote an opportunistic behaviour involving the use of locally abundant food resources.

The significant variation in diet composition observed across seasons may reflect the availability of different items in the environment and their accessibility (Greenberg *et al.*, 1997). In the Arunca and the Corvo, the contribution of detritus and plant material to the diet of ruivaco was highest in summer and autumn with virtually no consumption of animal prey items. Animal prey items, particularly Diptera and Ephemeroptera, however, were consumed in quantity and composed the main forage base in spring in both rivers. These seasonal shifts in food consumption are likely related to seasonal fluctuations in abundance of aquatic macroinvertebrates (Ribeiro *et al.*, 2007). Accordingly, the relative abundance of Diptera – an order that is of great importance to fish because of its high caloric content and low mobility that facilitates its capture (Easton & Orth, 1992) – is greater in spring and decreases by early autumn (Magalhães, 1993). The results in this study conform to this pattern of abundance of benthic macroinvertebrates in the Arunca and the Corvo in spring (> 50 %), with subsequent decreases by autumn (< 25 %), lowering the likelihood of consumption by fish. During periods of flooding, biological resources are destroyed. The high torrential flow, lower temperature and scarce food availability are unfavourable to fish. At the end of winter, after living in harsh environmental conditions, trophic resources are used for somatic energy storage (Encina & Granado-Lorencio, 1997a). This energy can be used later in spring during gonad maturation and reproduction (Encina & Granado-Lorencio, 1997b). Similar results were found for a "sister" species, *A. arcasii*, in other Iberian rivers, where the importance of detritus in the diet decreased when availability of macroinvertebrate prey increased (Lobón-Cerviá & Rincón, 1994). This could be a feeding strategy for fish species that inhabit fluctuating environments. Conversely, in the Estorãos, the availability of the animal prey Diptera and Coleoptera was equally abundant throughout spring and summer, although their consumption was more important in summer. It is possible that the colder and wetter climate of the Estorãos may have induced a delay in the emergence of several groups of prey that have aerial adult stages, such as Ephemeroptera and Diptera, which preferentially occurs in the summer when environmental conditions are more favourable. Bonada *et al.* (2007) presented similar findings when they examined biological trait differences in benthic macroinvertebrates between Mediterranean and temperate rivers. Analysis of feeding strategy plots with food item points mainly located at the bottom of the diagrams suggests that the ruivaco adopts a generalist feeding strategy in all of the studied rivers (Noble *et al.*, 2007). This result is consistent with other studies in Iberia (Magalhães, 1993; Valladolid & Przybylski, 1996) and highlights the versatility of feeding habits of species that have evolved in seasonally fluctuating environments. However, some food categories, such as detritus, plant material and Diptera, seem to contribute more to diet, as they are positioned in the upper right corner of the diagram. At the same time, the overall absence of points in the upper left corner of the diagrams suggests the absence of a high between-phenotypic component, i.e., there was no evidence that some of prey have been consumed by a few specialised individuals. It is clear that further studies addressing the influence of sex and size-related microhabitat use on a seasonal and diet basis are needed to clarify and advance the knowledge about the mechanisms that regulate food-resource use by this species.

Taken together, results from the present study suggest that the ruivaco is a generalist fish that feeds opportunistically according to food availability. This plasticity allows the species to inhabit different environments with apparently no costs for its life history, particularly in harsh, seasonally fluctuating rivers. The assignment of the trophic guild to the target species, as presented in this study, is essential not only for an improved understanding of their feeding habits but also as a basis to assess food habits of other small-size cyprinids (*Achon-*

drostoma spp.), particularly the critically endangered Western ruivaco, for which knowledge of their trophic ecology remains poor and scarce.

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REFERENCES

ALLAN, J. D. & M. M. CASTILLO. 2007. *Stream ecology: structure and function of running waters* (2nd ed.). Dordrecht. Springer. 388 pp.

AMUNDSEN, P. A. 1995. Feeding strategy of Arctic charr (*Salvelinus alpinus*): general opportunist, but individual specialist. *Nordic Journal of Freshwater Research*, 71: 150–156.

AMUNDSEN, P. A., H. M. GABLER & F. J. STALDVIK. 1996. A new approach to graphical analysis of feeding strategy from stomach contents data-modification of the Costello (1990) method. *Journal of Fish Biology*, 48: 607–614.

BONADA, N., S. DOLÉDEC & B. STATZNER. 2007. Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. *Global Change Biology*, 13: 1658–1671.

CEN 2003. *Water quality: sampling of fish with electricity*. European Standard EN 14011:2003. European Committee for Standardization. Brussels.

CLARKE, K. R. & R. M. WARWICK. 1994. *Change in marine communities: an approach to statistical analysis and interpretation*. Plymouth Marine Laboratory. Plymouth. 88 pp.

COLLARES-PEREIRA, M. J., M. J. MARTINS, A. M. PIRES, A. M. GERALDES & M. M. COELHO. 1996. Feeding behaviour of *Barbus bocagei* assessed under a spatio-temporal approach. *Folia Zoologica*, 45: 65–76.

COSTELLO, M. J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. *Journal of Fish Biology*, 36: 261–263.

EASTON, R. S. & D. J. ORTH. 1992. Ontogenetic diet shifts of age-0 smallmouth bass (*Micropterus dolomieu* Lacepède) in the New River, West Virginia, USA. *Ecology of Freshwater Fish*, 1: 86–98.

ENCINA, L. & C. GRANADO-LORENCIO. 1997a. Seasonal variations in condition and energy content of somatic and reproductive tissues of *Chondrostoma polylepis willkommi*. *Folia Zoologica* 46 (Suppl. 1), 123–133.

ENCINA, L. & C. GRANADO-LORENCIO. 1997b. Seasonal changes in condition, nutrition, gonad maturation and energy content in barbel, *Barbus sclateri*, inhabiting a fluctuating river. *Environmental Biology of Fishes*, 50: 75–84.

ENCINA, L., V. CASTAÑO, B. GARCÍA & M. GIL. 1999. Ecología trófica del barbo (*Barbus sclateri*) en cuatro embalses del Sur de España. *Limnetica*, 17: 95–105.

ENCINA, L., A. RODRÍGUEZ-RUIZ & C. GRANADO-LORENCIO. 2004. Trophic habits of the fish assemblage in an artificial freshwater ecosystem: the Joaquín Costa reservoir, Spain. *Folia Zoologica*, 53: 437–449.

EUROPEAN UNION 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities*, L327: 1–72.

FERREIRA, T., L. SOUSA, J. M. SANTOS, L. REINO, J. OLIVEIRA, P. R. ALMEIDA & R. CORTES. 2007a. Regional and local environmental correlates of native Iberian fish fauna. *Ecology of Freshwater Fish*, 16: 504–514.

FERREIRA, T., J. OLIVEIRA, N. CAIOLA, A. DE SOSTOA, F. CASALS, R. CORTES, A. ECONOMOU, S. ZOGARIS, D. GARCÍA-JALÓN, M. ILHÉU, F. MARTINEZ-CAPEL, D. PONT, C. ROGERS & J. PRENDA. 2007b. Ecological traits of fish assemblages from Mediterranean Europe and their responses to human disturbance. *Fisheries Management and Ecology*, 14: 473–481.

GASITH, A. & V. H. RESH. 1999. Streams in Mediterranean climate regions: abiotic influences and

biotic responses to predictable seasonal events. *Annual Review of Ecology Evolution and Systematics*, 30: 51–81.

GREENBERG, L. A., E. BERGMAN & A. G. EKLOV. 1997. Effects of predation and intraspecific interactions on habitat use and foraging by brown trout in artificial streams. *Ecology of Freshwater Fish*, 6: 16–26.

INAG, I. P. 2008. *Manual para a avaliação biológica da qualidade da água em sistemas fluviais segundo a Directiva Quadro da Água: protocolo de amostragem e análise para os macroinvertebrados bentónicos*. Ministério do Ambiente, Ordenamento do Território e do Desenvolvimento Regional. Instituto da Água, I.P. Lisboa.

LOBÓN-CERVIÁ, J. & P. A. RINCÓN. 1994. Trophic ecology of red roach (*Rutilus arcasii*) in a seasonal stream; an example of detritivory as a feeding tactic. *Freshwater Biology*, 32: 123–132.

MAGALHÃES, M. F. 1992. Feeding ecology of the Iberian cyprinid *Barbus bocagei* Steindachner, 1865 in a lowland river. *Journal of Fish Biology*, 40: 123–133.

MAGALHÃES, M. F. 1993. Feeding of an Iberian stream cyprinid assemblage: seasonality of resource use in a highly variable environment. *Oecologia*, 96: 253–260.

MARSHALL, S. & M. ELLIOTT. 1997. A comparison of univariate and multivariate numerical and graphical techniques for determining inter- and intraspecific feeding relationships in estuarine fish. *Journal of Fish Biology*, 51: 526–545.

NOBLE, R. A., I. G. COWX, D. GOFFAUX & P. KESTEMONT. 2007. Assessing the health of European rivers using functional ecological guilds of fish communities: standardising species classification and approaches to metric selection. *Fisheries Management and Ecology*, 14: 381–392.

PONT, D., B. HUGUENY & C. ROGERS. 2007. Development of a fish-based index for the assessment of river health in Europe: the European Fish Index. *Fisheries Management and Ecology*, 14: 427–439.

RIBEIRO, F., R. L. ORJUELA, M. F. MAGALHÃES & M. J. COLLARES-PEREIRA. 2007. Variability in feeding ecology of a South American cichlid: a reason for successful invasion in mediterrraneantype rivers? *Ecology of Freshwater Fish*, 16: 559–569.

ROBALO, J. I., I. DOADRIO, V. C. ALMADA & M. KOTTELAT. 2005. *Chondrostoma oligolepis*, new replacement name for *Leuciscus macrolepidotus* Steindachner, 1866 (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 16: 47–48.

ROBALO, J. I., V. C. ALMADA, A. LEVY & I. DOADRIO. 2007. Re-examination and phylogeny of the genus Chondrostoma based on mitochondrial and nuclear data and the definition of 5 new genera. *Molecular Phylogenetics and Evolution*, 42: 362–372.

ROBALO, J. I., C. S. SANTOS, I. DOADRIO & V. C. ALMADA, V. 2008. Threatened fishes of the world: *Achondrostoma occidentale* Robalo, Almada, Sousa-Santos, Moreira & Doadrio 2005 (Cyprinidae). *Environmental Biology of Fishes*, 83: 347.

SANTOS, J. M., F. N. GODINHO, M. T. FERREIRA & R. CORTES. 2004. The organisation of fish assemblages in the regulated Lima basin, Northern Portugal. *Limnologica*, 34: 224–235.

STATSOFT INC. 2000. STATISTICA for Windows (computer program manual). StatSoft, Inc. Tulsa.

VALLADOLID, M. & M. PRZYBYLSKI. 1996. Feeding relations among cyprinids in the Lozoya River (Madrid, central Spain). *Polish Archives of Hydrobiology*, 43: 213–223.

WINDELL, J. T. 1971. Food analysis and rate of digestion. In: *Methods for assessment of fish production in fresh waters*. W. E. Ricker (ed.): 197–203. Blackwell. Oxford.

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Endozoochory of seeds and invertebrates by migratory waterbirds in Oklahoma, USA

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ABSTRACT

Endozoochory of seeds and invertebrates by migratory waterbirds in Oklahoma, USA

Given their abundance and migratory behavior, waterbirds have major potential for dispersing plants and invertebrates within North America, yet their role as vectors remains poorly understood. We investigated the numbers and types of invertebrates and seeds within freshly collected faecal samples (n = 22) of migratory dabbling ducks and shorebirds in November 2008 in two parts of Lake Texoma in southern Oklahoma. Killdeer *Charadrius vociferus* were transporting a higher number and diversity of both plants and invertebrates than the green-winged teal *Anas carolinensis*. Ten plant taxa and six invertebrate taxa were identified to at least genus level, although viability was not confirmed for most of these taxa. Bryozoan statoblasts (from four species not previously recorded from Oklahoma) were especially abundant in killdeer faeces, while the ostracod *Candona simpsoni* was detected as a live adult in torpor in the teal faeces. Cyperaceae and Juncaceae were the most abundant plant families represented and *Cyperus strigosus* seeds germinated after extraction from killdeer faeces. This snapshot study underlines the importance of waterbirds as vectors of passive dispersal of many organisms and the need for more research in this discipline.

Key words: Dispersal ability, Endozoochory, Bryozoa, *Plumatella*, *Charadrius vociferus*, *Anas carolinensis*, Ostracoda, Cyperaceae.

RESUMEN

Endozoocoria de semillas e invertebrados por aves acuáticas migratorias en Oklahoma, USA

*Debido a su abundancia y a sus migraciones, las aves acuáticas tienen un gran potencial como dispersores de plantas e invertebrados en el Norte de América, pero su papel como vectores apenas se ha estudiado. Se investigó la cantidad y diversidad de invertebrados y semillas presentes en heces frescas (*n = *22) de patos nadadores y limícolas recogidas en noviembre 2008 en dos zonas del Lago Texoma en el sur de Oklahoma. El chorlitejo colirrojo* Charadrius vociferus *transportó un número y diversidad de plantas y de invertebrados más alto que la cerceta americana* Anas carolinensis*. Se identificaron diez taxones de plantas y seis de invertebrados, al menos a nivel de género, aunque para la mayoría de los taxones no se comprobó su viabilidad. Los estatoblastos de briozoos fueron especialmente abundantes en las heces de chorlitejos, mientras que en las heces de una cerceta se recuperó vivo un ejemplar adulto del ostrácodo* Candona simpsoni*. Las familias Cyperaceae y Juncaceae fueron las más representadas entre las semillas recuperadas, y las semillas de* Cyperus strigosus *germinaron tras extraerse de las heces. Este breve estudio subraya la importancia de las aves acuáticas como vectores de dispersión para muchos organismos, y la necesidad de más investigaciones en esta línea.*

Palabras clave: *Capacidad de dispersión, endozoocoria, Bryozoa,* Plumatella, Charadrius vociferus, Anas carolinensis, *Ostracoda, Cyperaceae.*

INTRODUCTION

There is a growing appreciation of the role of waterbirds as vectors for dispersal of aquatic plants and invertebrates (Green *et al.*, 2002; Frisch *et al.*, 2007; Brochet *et al.*, 2009; van Leeuwen *et al.*, 2012) and the importance of this process for maintaining connectivity between populations in isolated waterbodies (Amezaga *et al.*, 2002; Figuerola *et al.*, 2005). Passive dispersal via birds is likely to play a vital role in colonization of wetlands in response to habitat creation (e.g. reservoirs, Havel *et al.*, 2005), habitat restoration (McKinstry & Anderson, 2002; Badosa *et al.*, 2010) or to global change (Brochet *et al.*, 2009). Nevertheless, there remain surprisingly few empirical studies that quantify such dispersal in the field. In North America, there have been recent efforts to study the role of dabbling ducks as vectors of seeds (Holt-Mueller & van der Valk, 2002; Wongsriphuek *et al.*, 2008, see also Powers *et al.*, 1978). Furthermore, Proctor and coworkers (Proctor, 1964, 1968; Proctor *et al.*, 1967; de Vlaming & Proctor, 1968) conducted the first detailed investigation of the capacity of waterbirds to disperse seeds and invertebrates that are able to survive passage through the avian gut (endozoochory). Several crustaceans were hatched from faeces recovered from dabbling ducks shot in Texas (Proctor 1964). In Texas, 71 killdeer (*Charadrius vociferous*) were shot at different times of the year and 27 of them contained intact seeds in the gizzard, mainly of *Amaranthus* and *Polygonum*. Experiments with captive birds demonstrated that these seeds can survive gut passage in a viable state (deVlaming & Proctor 1968).

In this paper we present a snapshot study of endozoochory of plants and invertebrates by dabbling ducks and shorebirds in Oklahoma. Our objectives were to identify as far as possible those taxa present in an apparently viable condition in avian faeces and to compare the potential rates of dispersal recorded in killdeer and the green-winged teal *Anas carolinensis*. These bird species were selected on an opportunistic basis.

STUDY AREA AND METHODS

Lake Texoma is the 12th largest reservoir in the USA by volume and was created by the construction of Denison Dam in the 1940s (Sublette 1955). Fresh samples of faeces were collected from two areas in the Lake Texoma system. On 7 November 2008, six samples were collected from Killdeer along the lakeshore at the Cumberland Pool at Tishomingo National Wildlife Refuge (TNWR, 34°11′19″N, 96°38′53″W). On 11 November, three samples from Killdeer and 11 from green-winged teal were collected from a pond in TNWR (34°11′27″N, 96°38′57″W) that was adjacent to the lake and only separated from it by a road. In addition, two samples were collected from a mixed flock of *A. carolinensis* and Mallard *A. platyrhynchos* on 8-9 November, 37 km to the south along the shore of Lake Texoma (33°52′39″N, 96°48′27″O) and adjacent to the University of Oklahoma Biological Station (UOBS). The size of droppings corresponded to those of Mallard.

Duck faeces were collected from spots where flocks (monospecific with the above exception) were observed roosting out of the water. After pinpointing flocks with a telescope, one person remained with the telescope to guide a second person into the correct spot to look for faeces. Killdeer were observed feeding individually and allowed a close approach before flushing, facilitating collection of fresh faeces. Any part of the dropping in contact with soil was removed with a penknife and discarded to avoid contamination. Droppings collected were separated by at least 1 m. Given the numbers of birds present, it is unlikely that any individual bird was sampled more than once. Each dropping was sealed in a plastic tube, then transported immediately to the UOBS for processing.

Extraction of dispersed organisms

Faecal samples were stored in the dark in a refrigerator at 2 °C until sieving. Between 9 and 17 November, we washed faeces through stacked 841 μm, 420 μm and 62 μm sieves using distilled water. Fresh weight for the amount of sieved

sample was 0.43 ± 0.043 g (mean ± s.e., n = 11) for teal, 0.64 ± 0.13 g (n = 9) for killdeer, 3.47 ± 1.30 g (n = 2) for mallard. Items retained on the sieves were placed in sample trays and inspected using a binocular microscope, removing intact invertebrate propagules or plant seeds that were then sorted and placed in tubes with a mineral water. Some invertebrates or eggs were placed in water from Lake Texoma (previously filtered through a 25 μm sieve) in Petri dishes at room temperature and any daily development observed.

A representative sample of the seeds extracted were sent for identification to the National Wetlands Research Center (NWRC), where they were stored moist in the refrigerator and compared to images, written descriptions and a seed collection. Seeds of all types that had a total $n > 1$ were photographed using a Sony DKC-ST5 camera mounted on a Nikon SM2-2T stereo microscope and sent to Dr. Charles R. Gunn, retired Director of the U.S. National Seed Herbarium, who provided further identifications. Identifications were confirmed at the Seed Herbarium and Arboretum library by Dr. Joseph H. Kirkbride, Jr. of the U. S. National Arboretum. Seed taxa were determined to the most detailed taxonomic classification possible. Photographs of seeds are available on request. In May 2010, seeds were planted in flats of Jiffy Seed Germination Mix and placed in a greenhouse with a maximum daytime temperature of 27 °C and a minimum night time temp of 23 °C for three months.

The number of seeds or invertebrates detected per sample and the number of taxa were compared between killdeer and green-winged teal using Mann-Whitney U tests conducted in Statistica 6.0 (StatSoft, Inc. 2000).

RESULTS

Invertebrates

Killdeer faeces contained significantly more invertebrates than teal (Mann-Whitney U test, n = 11, 9, U = 19.5, p = 0.02) and significantly more invertebrate taxa (U = 21.0, p = 0.03). The most abundant organisms were the statoblasts of colonial plumatellid bryozoans, which were only found in killdeer (Table 1). Fourteen of the *Plumatella* statoblasts were identified as follows; four *P. fungosa*, eight *P. reticulata* and two *P. vaihiriae*. In two samples, *P. reticulata* was found to co-occur with either *P. fungosa* or *P. vaihiriae*. Some *Plumatella* statoblasts were still inside fragments of bryozoan colonies, showing that they had been ingested when birds were feeding on the colonies themselves.

The one ostracod recovered from teal was found to be alive, even though it had been kept in the fridge within the faeces for 6 days before

Table 1. Invertebrate propagules and adults recorded in waterbird faecal samples. Listed are the number of samples with invertebrates (WI), the total number of invertebrates (TI) and the maximum number of in any given sample (Max). Only propagules or adults that were apparently intact are included. *Propágulos y adultos de invertebrados registrados en las muestras de heces. Se presenta el número de muestras con invertebrados (WI), el número total de invertebrados (TI) y el número máximo en una sola muestra (Max). Se incluyen solamente los propágulos o adultos aparentemente intactos.*

Invertebrate type	GW Teal (n = 11)			Killdeer (n = 9)			Total (n = 20)		
	WI	TI	Max	WI	TI	Max	WI	TI	Max
Plumatella statoblast				6	42	25	6	42	25
Cristatella statoblast				1	1	1	1	1	1
Daphnia ephippia				1	1	1	1	1	1
Ostracoda[1]	1	1	1	1	2	2	2	3	2
Other unidentified eggs (3 Types)	4	6	2	3	27	20	7	33	20
Total	4	7	2	8	73	32	12	80	32

[1] adults or subadults.

extraction. It was first seen moving 5 h after extraction and was still alive two days later, before fixation in alcohol. This individual was later identified as *Candona simpsoni* (adult or subadult, probably female, no eggs present).

General faecal contents showed that other food items for teal included aquatic insects such as Coleoptera larvae and gastropods. Killdeer faeces contained aquatic Coleoptera adults and terrestrial insects.

Plant seeds

Killdeer faeces contained significantly more seeds than teal ($U = 4.5$, $p = 0.0001$) and significantly more plant taxa ($U = 7$, $p = 0.0005$). A total of 166 seeds were recovered from 13 taxa. Overall, 38 % of seeds recovered were Cyperaceae and 43 % Juncaceae (Table 2).

Only three seeds of one type germinated when germination runs were commenced six months after seed extraction. Seedlings were transplanted into 4″ pots and grown until fruiting and identified as *Cyperus strigosus*. A voucher specimen of this species was placed in the National Wetlands Research Center herbarium.

Strands of filamentous algae in an apparently healthy state (i. e. green with intact cellular structure) were recorded in seven samples from teal and four from killdeer, but were not further identified.

DISCUSSION

Despite the relatively small sample size, potential endozoochory was demonstrated for six invertebrate taxa and 10 plant taxa that were identified to at least genus level. Given the shortage of similar studies, our results represent a significant advance in the understanding of passive dispersal of plants and invertebrates by migratory waterbirds in North America. However, there is some overlap in the seeds we recorded from killdeer faeces and those recorded by de Vlaming & Proctor (1968) in killdeer gizzards, which included *Eleocharis* and *Amaranthus*. Together with many other seed types, *C. strigosus* and *E. palustris* have previously been reported in the gizzards of green-winged teal in Virginia (Perry, 1981). The dispersal of considerable numbers of Cyperaceae and Juncaceae seeds has been recorded in other field studies of waterfowl faeces (Holt-Mueller & Van der Valk, 2002; Green *et al.*, 2008; Brochet *et al.*, 2010a) and appears to be a very frequent process.

Although we observed killdeer to transport more seeds and invertebrates than teal, the oppo-

Table 2. Intact seeds recorded in waterbird faecal samples. Shown are the number of samples with intact seeds (WS), total number of seeds (TS) and maximum number in any given sample (Max). *Semillas intactas registradas en las muestras de heces. Se presenta el número de muestras con semillas intactas (WS), el número total de semillas (TS) y el número máximo en una sola muestra (Max).*

Plant family	Taxon	Mallard ($n = 2$)			GW Teal ($n = 11$)			Killdeer ($n = 9$)			Total ($n = 22$)		
		WS	TS	Max	WS	TS	Max	WS	TS	Max	WS	TS	Max
Cyperaceae	*Eleocharis palustris*	1	44	44	1	1	1				2	45	44
	Cyperus strigosus							4	13	5	4	13	5
	Schoenoplectus sp.							2	5	4	2	5	4
Juncaceae	*Juncus* sp.				3	3	1	6	68	28	9	71	28
Polygonaceae	*Persicaria* cf. *persicaria*	1	4	4							1	4	4
Ranunculaceae	*Ranunculus* sp.							1	1	1	1	1	1
Amaranthaceae	*Amaranthus* sp.							1	1	1	1	1	1
Urticaceae	*Parietaria* cf. *pensylvanica*							2	7	7	2	7	6
Xyridaceae	*Xyris* sp.							2	2	2	2	2	1
Poaceae	*Panicum* cf. *capillare*							1	1	1	1	1	1
Unidentified (3 Taxa)					1	3	3	4	13	11	5	16	9
Total		2	48	44	4	7	3	9	111	34	15	166	44

site might be found in different locations or dates, owing to the great spatial-temporal variability to be expected in the frequency of dispersal of different taxa by waterbirds (e.g. Figuerola *et al.*, 2003). The closely related Eurasian teal *A. crecca* is a major seed vector in Europe (Figuerola *et al.*, 2002; Brochet *et al.*, 2009, 2010a) and diet studies in the literature suggest that green-winged teal are often likely to disperse large numbers of seeds and invertebrate propagules such as cladoceran ephippia (Perry, 1981; Johnson, 1995; Frisch *et al.*, 2007).

Teal, mallards and killdeer have an extensive range and make long-distance migratory movements capable of dispersing plants and invertebrates over hundreds of kilometres or more (Johnson, 1995; Jackson *et al.*, 2000; Viana *et al.*, 2012). Teal are only present in the study area during the winter and migration periods, whereas killdeer are resident and likely to be important as vectors of dispersal between waterbodies throughout the annual cycle.

Studies in captivity show the retention times of seeds in the gut of killdeer (Proctor *et al.*, 1967; Proctor, 1968) and the Eurasian teal (Pollux *et al.*, 2005; Brochet *et al.*, 2010b) are sufficient for long-distance dispersal over hundreds of km, when ingestion occurs prior to migratory movements. The same applies to bryozoan statoblasts and other invertebrate propagules (Charalambidou *et al.*, 2003; Sánchez *et al.*, 2012).

The plant taxa dispersed by birds in this study all have broad geographical ranges across North America spanning from Texas to Canada (http://plants.usda.gov/). Darwin (1859) argued that such broad ranges are likely to be largely a consequence of the capacity to disperse via migratory birds. All the *Plumatella* species recorded are widespread in North America (T. Wood pers. comm.).

We failed to germinate many seeds, perhaps because of the long delay between faeces collection and the commencement of germination runs. Previous studies show that seeds of plants closely related to those recorded here retain high germinability after passage through the gut of dabbling ducks or shorebirds (de Vlaming & Proctor, 1968; Sánchez *et al.*, 2006; Wongsriphuek *et al.*, 2008; Brochet *et al.*, 2010b; Figuerola *et al.*, 2010).

The relationships between the movement patterns of waterfowl and genetic patterns of *Daphnia* and *Cristatella* populations across North America suggest that gene flow mediated by waterfowl is important for these organisms (Figuerola *et al.*, 2005). Owing to the presence of external hooks, it has previously been assumed that *C. mucedo* statoblasts disperse on feathers on the outside of birds (epizoochory). However, the recent observation of large numbers of *C. mucedo* statoblasts in waterfowl gizzards (Mouronval *et al.*, 2007) and our observation of a statoblast in killdeer faeces suggests that endozoochory may be more important. Recent studies suggest that endozoochory is much more important for seed dispersal by dabbling ducks than epizoochory (Brochet *et al.*, 2010a). However, epizoochory via waterbirds is likely to be important for some seeds and invertebrates (Vivian-Smith & Stiles, 1994; Figuerola & Green, 2002; Green & Figuerola, 2005).

Killdeer appear to be particularly good vectors of propagules found close to the shoreline, both above and below the waterline. These shorebirds do not generally feed at depths of more than a few cm (Jackson *et al.*, 2000) and are likely to have ingested most of the bryozoan statoblasts while feeding on colonies growing on stones or shoreline vegetation. Bryozoans are little known organisms likely to have been overlooked in many studies of waterbird diet, but are also consumed by dabbling ducks (Taylor, 1978). Although this is the first study to confirm the endozoochory of statoblasts in North America, the presence and viability of *Plumatella* statoblasts in waterfowl faeces has previously been documented in Europe and Australia (Figuerola *et al.*, 2004; Green *et al.*, 2008; Brochet *et al.*, 2010c) and seems likely to be a cosmopolitan process. Because little attention has been paid to freshwater bryozoans, the four species we recorded have not previously been recorded from Oklahoma (T. Wood pers. comm.).

The transport of torpid ostracod adults or subadults by the closely related Eurasian teal was also recently reported (Frisch *et al.*, 2007) and such transport by waterfowl seems likely to be widespread. Proctor (1964) found a green-

winged teal shot in Texas to be transporting viable eggs of *Cypridopsis vidua* and although the endozoochory of ostracod eggs also appears to be a frequent process (Green *et al.*, 2008; Brochet *et al.*, 2010c), the transport of live adults has additional consequences previously overlooked in the literature. In a manner analogous to the endozoochory of insects that live inside plant seeds (Hernández, 2011), parasites and commensalists of ostracods can also be dispersed between water bodies within the adult ostracods found in the avian gut. These might include, for example, helminths that parasitize fish but use ostracods as intermediate hosts (Marcogliese, 1995).

Our results illustrate the value of field studies of endozoochory by waterfowl and shorebirds and underline the importance of birds in the metacommunity ecology of aquatic plants and invertebrates (Amezaga *et al.*, 2002). More extensive studies are recommended, to identify those organisms which are readily dispersed and clarify which groups are likely to be dispersal limited. Such information is useful to managers because it would allow predictions of which taxa will colonize new habitats via waterbirds e.g. after wetland restoration and which can move northwards at a sufficient rate to compensate for climate change.

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REFERENCES

AMEZAGA, J. M., L. SANTAMARÍA & A. J. GREEN. 2002. Biotic wetland connectivity-supporting a new approach for wetland policy. *Acta Oecologica*, 23: 213–222.

BADOSA, A., D. FRISCH, A. ARECHEDERRA, L. SERRANO & A. J. GREEN. 2010. Recovery of zooplankton diversity in a restored Mediterranean temporary marsh in Doñana National Park (SW Spain). *Hydrobiologia*, 654: 67–82.

BROCHET, A. L., M. GUILLEMAIN, H. FRITZ, M. GAUTHIER-CLERC & A. J. GREEN. 2009. The role of migratory ducks in the long-distance dispersal of native plants and the spread of exotic plants in Europe. *Ecography*, 32: 919–928.

BROCHET, A. L., M. GUILLEMAIN, H. FRITZ, M. GAUTHIER-CLERC & A. J. GREEN. 2010a. Plant dispersal by teal (*Anas crecca*) in the Camargue: duck guts are more important than their feet. *Freshwater Biology*, 55: 1262–1273.

BROCHET, A. L., M. GUILLEMAIN, M. GAUTHIER-CLERC, H. FRITZ & A. J. GREEN. 2010b. Endozoochory of Mediterranean aquatic plant seeds by teal after a period of desiccation: Determinants of seed survival and influence of retention time on germinability and viability. *Aquatic Botany*, 93: 99–106.

BROCHET, A. L., M. GAUTHIER-CLERC, M. GUILLEMAIN, H. FRITZ, A. WATERKEYN, A. BALTANAS & A. J. GREEN. 2010c. Field evidence of dispersal of branchiopods, ostracods and bryozoans by teal (*Anas crecca*) in the Camargue (southern France). *Hydrobiologia*, 637: 255–261.

CHARALAMBIDOU, I., L. SANTAMARÍA & J. FIGUEROLA. 2003. How far can the freshwater bryozoan *Cristatella mucedo* disperse in duck guts? *Archiv Fur Hydrobiologie*, 157: 547–554.

DARWIN, C. 1859. *On the Origin of Species by Means of Natural Selection*. Murray, London.

DE VLAMING, V. & V. W. PROCTOR. 1968. Dispersal of aquatic organisms: viability of seeds recovered from the droppings of captive killdeer and mallard ducks. *American Journal of Botany*, 55: 20–26.

FIGUEROLA, J. & A. J. GREEN. 2002. How frequent is external transport of seeds and invertebrate eggs by waterbirds? A study in Donana, SW Spain. *Archiv Fur Hydrobiologie*, 155: 557–565.

FIGUEROLA, J., A. J. GREEN & T. C. MICHOT. 2005. Invertebrate eggs can fly: Evidence of

waterfowl-mediated gene flow in aquatic invertebrates. *American Naturalist*, 165: 274–280.

FIGUEROLA, J., I. CHARALAMBIDOU, L. SANTAMARÍA & A. J. GREEN. 2010. Internal dispersal of seeds by waterfowl: effect of seed size on gut passage time and germination patterns. *Naturwissenschaften*, 97: 555–565.

FIGUEROLA, J., A. J. GREEN, K. BLACK & B. OKAMURA. 2004. Influence of gut morphology on passive transport of freshwater bryozoans by waterfowl in Donana (southwestern Spain). *Canadian Journal of Zoology*, 82: 835–840.

FRISCH, D., A. J. GREEN & J. FIGUEROLA. 2007. High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences*, 69: 568–574.

GREEN, A. J. & J. FIGUEROLA. 2005. Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. *Diversity and Distributions*, 11: 149–156.

GREEN, A. J., J. FIGUEROLA & M. I. SÁNCHEZ. 2002. Implications of waterbird ecology for the dispersal of aquatic organisms. *Acta Oecologica*, 23: 177–189.

GREEN, A. J., K. M. JENKINS, D. BELL, P. J. MORRIS & R. T. KINGSFORD. 2008. The potential role of waterbirds in dispersing invertebrates and plants in arid Australia. *Freshwater Biology*, 53: 380–392.

HAVEL, J. E., C. E. LEE & M. J. VANDER ZANDEN. 2005. Do reservoirs facilitate invasions into landscapes? *Bioscience*, 55: 518–525.

HERNÁNDEZ, A. 2011. Internal dispersal of seed-inhabiting insects by vertebrate frugivores: a review and prospects. *Integrative Zoology*, 6: 213–221.

HOLT-MUELLER, M. & A. G. VAN DER VALK. 2002. The potential role of ducks in wetland seed dispersal. *Wetlands*, 22: 170–178.

JACKSON, B. J. & J. A. JACKSON. 2000. Killdeer (*Charadrius vociferus*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/517

JOHNSON, K. 1995. Green-winged Teal (*Anas crecca*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu/bna/species/193

MARCOGLIESE, D. J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. *Reviews in Fish Biology and Fisheries*, 5: 336–371.

MCKINSTRY, M. C. & S. H. ANDERSON 2002. Creating wetlands for waterfowl in Wyoming. *Ecological Engineering*, 18: 293–304.

MOURONVAL, J. B., M. GUILLEMAIN, A. CANNY & F. POIRIER. 2007. Diet of non-breeding wildfowl Anatidae and Coot *Fulica atra* on the Perthois gravel pits, northeast France. *Wildfowl*, 57: 68–97.

PERRY, M. C. & F. M. UHLER. 1981. Asiatic Clam (*Corbicula manilensis*) and Other Foods Used by Waterfowl in the James River, Virginia. *Estuaries*, 4: 229–233.

POLLUX, B. J. A., L. SANTAMARÍA & N. J. OUBORG. 2005. Differences in endozoochorous dispersal between aquatic plant species, with reference to plant population persistence in rivers. *Freshwater Biology*, 50: 232–242.

POWERS, K. D., R. E. NOBLE & R. H. CHABRECK. 1978. Seed distribution by waterfowl in southwestern Louisiana. *Journal of Wildlife Management*, 42: 598–605.

PROCTOR, V. W. 1964. Viability of crustacean eggs recovered from ducks. *Ecology*, 45: 656–658.

PROCTOR, V. W., C. R. MALONE & V. L. DEVLAMING. 1967. Dispersal of aquatic organisms: viability of disseminules recovered from the intestinal tract of captive Killdeer. *Ecology*, 48: 672–676.

PROCTOR, V. W. 1968. Long-distance dispersal of seeds by retention in digestive tract of birds. *Science*, 160: 321–322.

SÁNCHEZ, M. I., A. J. GREEN & E. M. CASTELLANOS. 2006. Internal transport of seeds by migratory waders in the Odiel marshes, south-west Spain: consequences for long-distance dispersal. *Journal of Avian Biology*, 37: 201–206.

SÁNCHEZ, M. I., F. HORTAS, J. FIGUEROLA & A. J. GREEN. 2012. Comparing the potential for dispersal via waterbirds of a native and an invasive brine shrimp. *Freshwater Biology*, 57: 1896–1903.

SUBLETTE, J. E. 1955. The physico-chemical and biological features of Lake Texoma (Denison Reservoir), Oklahoma and Texas: a preliminary study. *The Texas Journal of Science*, 7: 164–182.

TAYLOR, T. S. 1978. Spring foods of migrating Blue-winged Teals on seasonally flooded impoundments. *Journal of Wildlife Management*, 42: 900–903.

VAN LEEUWEN, C. H. A., G. VAN DER VELDE, B. VAN LITH & M. KLAASSEN. 2012. Experimental quantification of long distance dispersal potential of aquatic snails in the gut of migratory birds. *Plos One*, 7(3): e32292. doi:10.1371/journal.pone.0032292

VIANA, D. S., L. SANTAMARÍA, T. C. MICHOT & J. FIGUEROLA. 2012. Migratory strategies of waterbirds shape the continental-scale dispersal of aquatic organisms. *Ecography*, 35: 001–009 (EV).

VIVIAN-SMITH, G. & E. W. STILES. 1994. Dispersal of salt marsh seeds on the feet and feathers of waterfowl. *Wetlands*, 14, 316–319.

WONGSRIPHUEK, C., B. D. DUGGER & A. M. BARTUSZEVIGE. 2008. Dispersal of wetland plant seeds by mallards: Influence of gut passage on recovery, retention, and germination. *Wetlands*, 28, 290–299.

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La comunidad de coleópteros y hemípteros acuáticos de un arroyo costero cantábrico (norte de España): composición, variación estacional e influencia de los factores ambientales

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ABSTRACT

Aquatic Coleoptera and Hemiptera assemblages of a Cantabrian coastal stream (North Spain): composition, seasonal variation and environmental factors

The aquatic Coleoptera and Hemiptera assemblages have been studied in a short Cantabrian coastal stream (La Llantada, Asturias, North Spain). We aim to know the assemblage composition and to detect the environmental factors that affected it, considering seasonal changes (samplings in spring and autumn during two annual cycles). Most (89 %) of the identified species (thirty-three of Coleoptera and eleven of Hemiptera) shows a high range distribution. It is remarkable the presence of some Southern species (*Hydrochus grandicollis, Dryops striatellus, Velia caprai bertrandi*). *Chaetarthria simillima* (Coleoptera: Hydrophilidae) is recorded for the second time in the Iberian Peninsula.

The results show a clear decrease of richness towards the mouth that seems to be related to the decrease of water quality. Seasonal changes of assemblage composition are related to organic pollution, particularly during the months of summer with low rainfall and greater anthropic impact. CCA shows that most species of both groups respond negatively to high nutrient pollution, mainly total nitrogen and ammonium. A small group associated to samples with a high load of nutrients is detected and can be considered tolerant to pollution, for instance the water beetles *Hydroporus vagepictus, Stictotarsus duodecimpustulatus, Helophorus minutus, Helophorus brevipalpis* and *Octhebius dilatatus.*

Key words: Aquatic Hemiptera, aquatic Coleoptera, faunistics, coastal stream, seasonality, environmental variables, North Spain.

RESUMEN

La comunidad de coleópteros y hemípteros acuáticos de un arroyo costero cantábrico (norte de España): composición, variación estacional e influencia de los factores ambientales

*Se ha estudiado la comunidad de coleópteros y hemípteros acuáticos en un pequeño arroyo costero cantábrico (La Llantada, Asturias). Se pretende conocer su composición e identificar los factores ambientales que influyen en ella, teniendo en cuenta los cambios estacionales (muestreos en primavera y otoño durante dos años consecutivos). La mayoría (89 %) de las 33 especies de coleópteros y 11 de hemípteros identificadas muestran una amplia distribución geográfica. Pese a ello, destaca la presencia de especies meridionales (*Hydrochus grandicollis, Dryops striatellus, Velia caprai bertrandi*). Se señala el segundo registro ibérico de* Chaetarthria simillima *(Coleoptera: Hydrophilidae).*

Los resultados muestran un claro gradiente de disminución de la riqueza hacia la desembocadura del arroyo que parece estar relacionado con el descenso de la calidad del agua. La variación estacional de la composición de la comunidad se ve afectada por el efecto de la contaminación orgánica, especialmente durante los meses de verano, cuando disminuyen las precipitaciones y el impacto antrópico es mayor. El ACC muestra que la mayoría de las especies de ambos grupos responden negativamente a elevadas concentraciones de contaminantes, principalmente nitrógeno total y amonio. Se detecta un pequeño grupo de especies asociadas a muestras con una fuerte carga de nutrientes y que pueden considerarse tolerantes a la contaminación, como los coleópteros Hydroporus vagepictus, Stictotarsus duodecimpustulatus, Helophorus minutus, Helophorus brevipalpis *y* Ochthebius dilatatus.

Palabras clave: *Coleópteros acuáticos, hemípteros acuáticos, faunística, arroyo costero, estacionalidad, variables ambientales, Norte de España.*

INTRODUCCIÓN

Las comunidades biológicas de los pequeños arroyos costeros del norte de la Península Ibérica constituyen un hábitat acuático muy poco estudiado, debido al pequeño tamaño de estos sistemas y a la escasa variedad de sus hábitats. En particular, la costa cantábrica es una de las áreas de la Península Ibérica con menor conocimiento de la fauna de hemípteros y especialmente de coleópteros acuáticos, ya que con la información actualizada por Sánchez-Fernández *et al*. (2011) esta zona, y en general toda la costa cantábrica, es una de las menos estudiadas a nivel peninsular. A la singularidad de estos ecosistemas y el escaso conocimiento de estos grupos faunísticos, hay que añadir el incremento de la presión humana sobre gran parte de las zonas costeras peninsulares. El estudio de estos arroyos ofrece la posibilidad de evaluar el efecto que provocan los factores antrópicos sobre estos medios de aguas corrientes de pequeña entidad, a priori mucho más vulnerables.

El presente trabajo se engloba en un estudio más amplio que tiene como objetivos la valoración de la calidad ambiental de una playa arenosa del norte de España, y del arroyo que en ella desemboca, y el análisis de cómo influyen los aportes de agua dulce en dicha playa en dos épocas del año: antes y después del verano (Mazé *et al*. 2011). En principio, estas dos épocas presentan diferencias importantes ya que durante los meses de verano, además de una menor precipitación, en la zona aumenta considerablemente la población humana debido al turismo.

En este estudio se profundiza en dos grupos de macroinvertebrados acuáticos, los coleópteros y los hemípteros, con identificación a nivel de especie. Ambos son dos grupos abundantes y diversos (Foster, 1987; Eyre & Foster, 1989; Foster *et al*., 1990; Millán *et al.*, 2002; Carbonell *et al.*, 2011). Sobre todo para los coleópteros, existen numerosos estudios que señalan su valor como indicadores de biodiversidad y del estado de conservación de los medios acuáticos, ya que cumplen los criterios propuestos para ser considerados como tales (Ribera & Foster, 1993; Pearson, 1994; Eyre *et al*., 2005; Sánchez-Fernández *et al.*, 2004). El valor indicador de los coleópteros acuáticos ha sido comprobado por diferentes autores (García-Criado & Fernández-Aláez, 1995; García-Criado *et al*., 1999; Benetti *et al*., 2007; Fernández-Díaz *et al*., 2008), incluso en el análisis de la contaminación por actividades mineras (García-Criado & Fernández-Aláez, 2001). Los hemípteros parecen ser peores indicadores por ser un grupo menos rico en especies y con mayores rangos de distribución, pero también han sido incluidos en ocasiones en este tipo de análisis (Eyre & Foster, 1989; Savage, 1996; Garrido & Munilla, 2007; Carbonell *et al.*, 2011).

Los principales objetivos del trabajo son conocer la composición de la comunidad de coleópteros y hemípteros acuáticos de un pequeño arroyo costero cantábrico, resaltar sus peculiaridades biogeográficas y estudiar su variación estacional. Finalmente se analiza la influencia de determinados factores ambientales sobre la composición de esta comunidad y los cambios estacionales que suceden en ella, cambios en los que pueden influir los impactos causados por las actividades humanas.

ÁREA DE ESTUDIO

El estudio se realizó en el arroyo de La Llantada (Asturias, norte de España). Se trata de un pequeño arroyo costero de menos de cuatro kilómetros de longitud situado en el concejo de Gozón. El arroyo discurre en dirección sur-norte entre praderías cantábricas hasta desembocar en la playa de Bañugues (Fig. 1), muy frecuentada por turistas especialmente en verano. El interés del área de estudio queda reflejado en su inclusión en el Paisaje Protegido de Cabo de Peñas (Decreto 80/95 de 12 de mayo de la Consejería de Medio Ambiente y Urbanismo del Principado de Asturias) y en la Red Natura 2000 como LIC Cabo de Busto-Luanco (ES0000318). La zona tiene un clima atlántico húmedo, con temperaturas suaves (media anual de 13 ^{o}C). La precipitación media en verano (de junio a septiembre) es de 65.2 mm (± 11.6), y 107.8 mm (± 16.4) durante el resto del año (datos obtenidos de los registros de precipitación entre 1971 y 2001 en el aeropuerto de

Ranón, próximo al área de estudio). Los dos años del periodo de muestreo (2004 y 2005) fueron relativamente secos durante el verano, la precipitación media fue de un poco más de 30 mm. En primavera de 2005 esta no excedió de 80 mm y fue considerablemente más baja en 2006, con 46 mm.

A priori la calidad del agua en el tramo más bajo del arroyo se ve afectada por el mal funcionamiento de la estación depuradora de aguas residuales, que está situada cerca de su desembocadura, especialmente durante los meses de verano, cuando el volumen de evacuación se incrementa.

Las tres estaciones de muestreo (R1, R2 y R3) están localizadas entre 43°37′10″ y 43°37′43″ latitud Norte, 5°48′31″ y 5°48′54″ longitud Oeste (Fig. 1). R1 y R2 están situadas aguas arriba de la estación depuradora que recoge el agua del colector del núcleo habitado y R3 está inmediatamente aguas abajo. La pendiente del tramo estudiado es suave, de 1.3 % desde R1 a R3. La proximidad de la fuente de contaminación a la playa donde desemboca el arroyo no permitió colocar más lejos la estación R3. Se describen a continuación las principales características de las estaciones de muestreo:

- R1: estación de cabecera situada a 1.5 km de su desembocadura en la playa, a 20 m de altitud. Es una zona rodeada de prados, con orillas bien conservadas, dominada por árboles de ribera: *Corylus avellana* y *Salix* spp. Existen varios tipos de flujo ya que se alternan zonas de corriente,

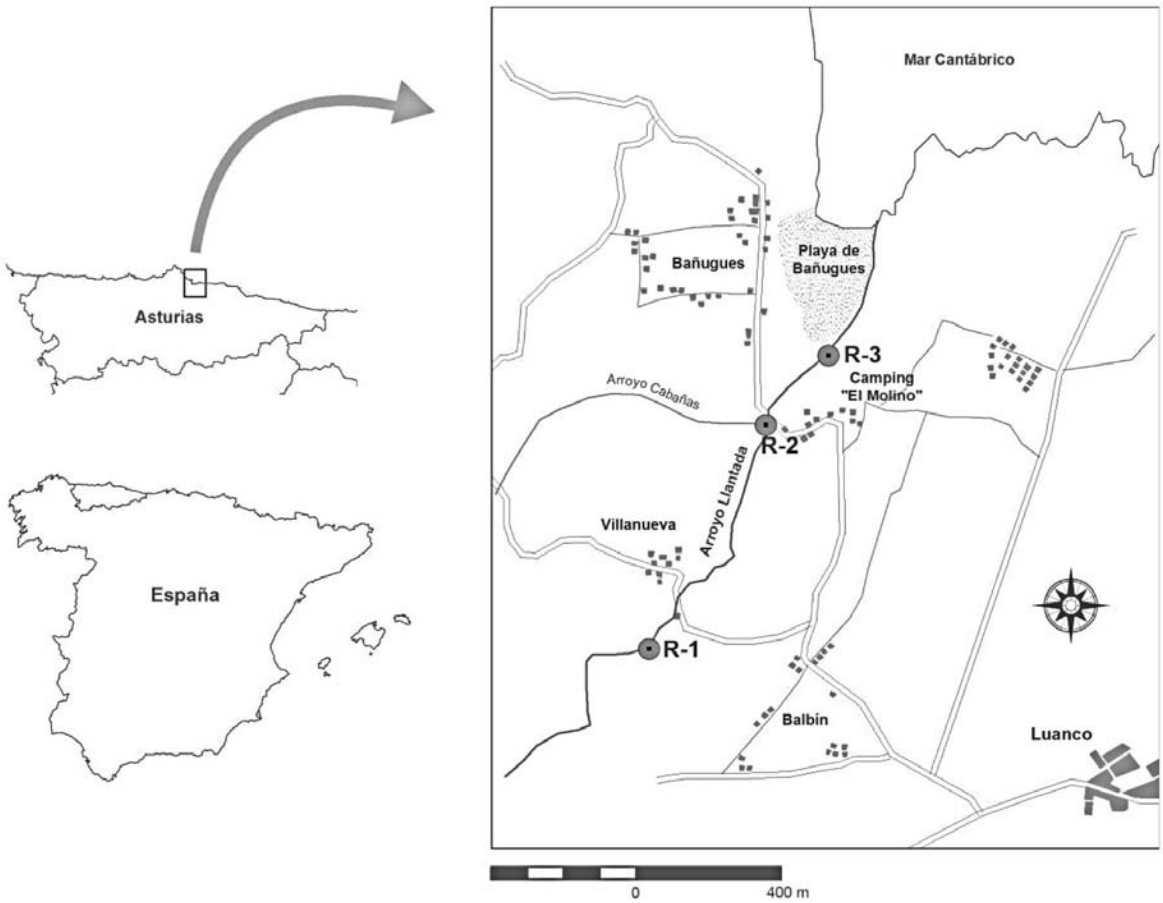

Figura 1. Localización del área de estudio: arroyo de La Llantada (Asturias, norte de España) y de las estaciones de muestreo. *Location of the study area: La Llantada stream (Asturias, North Spain) and samplings sites.*

tablas y pozas, estas últimas poco profundas. El principal componente del sustrato son las gravas y el limo. Litológicamente, el sustrato es una mezcla de suelos silíceos y calcáreos.

- R2: situada a 250 m de R1, aguas arriba de la estación depuradora, a 8 m de altitud. El tipo de flujo es intermedio entre R1 y R3. La vegetación de ribera es más escasa y la mayor parte de la superficie del agua está cubierta por vegetación emergente (*Typha*, *Phragmites*) y el sustrato es similar al de R1. Litológicamente es una zona donde dominan los depósitos calcáreos.
- R3: se sitúa a 100 m de la desembocadura en el mar y aguas abajo de la depuradora. La vegetación de ribera está casi exclusivamente representada por *Alnus glutinosa*. No existen zonas de corriente y abundan las pozas más o menos profundas. Aquí el cauce es más ancho que en las otras estaciones y el sustrato es fangoso. Litológicamente es una zona de depósitos litorales.

METODOLOGÍA

Muestreo

El muestreo se llevó a cabo durante dos ciclos anuales. Se visitaron las estaciones de muestreo a principios del otoño (mediados de septiembre de 2004 y 2005) y al final de la primavera (finales de mayo de 2005 y 2006), mencionados en este artículo como otoño y primavera, respectivamente. Mayo y septiembre son a priori los momentos más adecuados para detectar cambios en la dinámica de la contaminación del arroyo, con el periodo de vacaciones entre ambos, que es cuando la presión antrópica se incrementa en la zona. También hay una menor precipitación durante el periodo estival.

En cada muestreo se midieron las variables fisicoquímicas y microbiológicas del agua que figuran en la Tabla 1 y se utilizó la metodología recogida en el estudio de Mazé *et al.* (2011). Los coleópteros y hemípteros acuáticos fueron recogidos mediante una manga pentagonal de entomología acuática de 250 μm de luz de malla. La red fue colocada sobre el sustrato del arroyo y con la boca hacia la corriente, de forma que cuando las piedras, el limo y la vegetación eran removidos y "lavados" los invertebrados eran capturados dentro, incluidos los coleópteros y los hemípteros. Además, un colador de malla fina sirvió para capturar los ejemplares que flotaban al remover el sustrato y la vegetación asociada a las orillas. En cada estación de muestreo fueron prospectados los diferentes hábitats del arroyo durante un tiempo suficiente en el que más barridos no aportaban nuevas especies a la esta-

Tabla 1. Media ± desviación estándar de las variables medidas en las tres estaciones de muestreo. Se muestran los valores promedio por época del año y por estación. *Average ± standard error of the variables measured at the three sampling sites. Average values per season and per site are shown.*

	Otoño (n = 6)	Primavera (n = 6)	R1 (n = 4)	R2 (n = 4)	R3 (n = 4)
Temperatura agua (°C)	16.1 ± 1.1	15.8 ± 1.5	15.8 ± 1.5	16.0 ± 1.3	16.1 ± 1.2
Conductividad (μS/cm)	850 ± 21	730 ± 48	772 ± 83	794 ± 72	806 ± 79
Oxígeno disuelto (mg/l)	4.6 ± 1.3	6.8 ± 2.1	6.4 ± 1.5	5.9 ± 2.1	4.8 ± 2.6
pH	7.8 ± 0.3	7.7 ± 0.5	7.8 ± 0.4	7.7 ± 0.5	7.7 ± 0.4
Potencial redox (mV)	120.9 ± 55.1	128.3 ± 47.0	86.2 ± 56.2	151.6 ± 43.8	135.9 ± 24.7
Coliformes fecales (ufc/100 ml)	$2 \cdot 10^3 \pm 2 \cdot 10^3$	$40 \cdot 10^3 \pm 70 \cdot 10^3$	$3 \cdot 10^3 \pm 4 \cdot 10^3$	$5 \cdot 10^3 \pm 90 \cdot 10^3$	$10 \cdot 10^3 \pm 20 \cdot 10^3$
DBO_5 (mg O_2/l)	0.8 ± 0.8	12.5 ± 5.6	4.0 ± 3.6	7.8 ± 9.0	8.3 ± 9.0
DQO (mg O_2/l)	28.8 ± 11.7	145.1 ± 93.4	97.5 ± 109.3	94.3 ± 118.9	69.0 ± 39.3
SST (mg/l)	19.8 ± 8.5	47.7 ± 45.0	35.2 ± 37.7	35.1 ± 35.4	30.9 ± 39.6
SSV (mg/l)	18.7 ± 7.8	2.7 ± 3.0	10.9 ± 10.6	13.7 ± 12.6	7.6 ± 8.6
Nitrógeno total (mg N/l)	1.6 ± 2.1	5.0 ± 2.9	1.9 ± 1.7	3.8 ± 4.0	4.2 ± 3.2
Amonio (mg N/l)	0.2 ± 0.3	1.7 ± 2.6	0.1 ± 0.1	1.5 ± 2.3	1.4 ± 2.6
Nitrógeno orgánico (mg N/l)	1.4 ± 2.3	3.2 ± 1.0	1.8 ± 1.8	2.3 ± 2.0	2.8 ± 2.4
Fósforo total (mg/l)	0.0 ± 0.0	1.9 ± 1.9	0.6 ± 1.1	1.0 ± 1.5	1.2 ± 2.3

ción. Este método de muestreo semicuantitativo es uno de los más usados en este tipo de estudios en la Península Ibérica (Alba-Tercedor & Pujante, 2000; Sánchez-Fernández *et al.*, 2004).

Análisis de datos

La riqueza se refiere al número de especies de coleópteros y hemípteros acuáticos capturados en cada muestreo. No se ha hecho ningún tipo de corrección debido a que el esfuerzo de muestreo fue el mismo en todas las ocasiones.

Para las especies de coleópteros acuáticos se utilizaron las categorías biogeográficas propuestas por Ribera *et al*. (1998), junto con las modificaciones de Fery & Fresneda (2007). Para los hemípteros acuáticos fueron usadas las categorías biogeográficas propuestas por Nieser *et al*. (1994).

En cuanto a los datos fisicoquímicos, se calcularon los valores medios de las variables físicas, químicas y microbiológicas del agua en las tres estaciones de muestreo y en las dos épocas del año estudiadas. También se ha realizado un análisis de varianza de dos factores para determinar si hay diferencias entre estaciones de muestreo y entre épocas del año, y si existe interacción entre ellas, usando para ello las comparaciones post-hoc con el test de Tukey. La normalidad de los datos se analizó utilizando el test de Kolmogorov-Smirnov, usando una transformación logarítmica ($x + 1$) para los parámetros que no presentaban una distribución normal, cumpliendo todos ellos la homocedasticidad requerida. Para el análisis de correlación de estas variables fisicoquímicas se usó una correlación de Pearson. Estos análisis se realizaron utilizando el programa SPSS 15.0.

Se ha utilizado el índice de afinidad de Sørensen para comparar la similitud de la composición de las comunidades entre estaciones y épocas de muestreo (12 muestras). Con la matriz de similaridad se agruparon las comunidades mediante el método U.P.G.M.A. (*unweighted pair-groups method using arithmetic averages*), gráficamente representado por un dendrograma. El programa usado fue Community Analysis Package 3.0. Se utilizó un Análisis de Correspondencias Canónicas (ACC) para estudiar las relaciones entre la composición de la comunidad y los factores ambientales (Ter Braak & Van Tongeren, 1995). El ACC permite identificar los factores ambientales que más influyen en la comunidad de coleópteros y hemípteros acuáticos. Para comprobar que las variables estudiadas influyen en la ordenación de las muestras y especies, se realizó previamente un Análisis de Correspondencias sin tendencias (ACD) sin variables ambientales, del que se obtuvo una ordenación en el gráfico similar al ACD, lo que pone de manifiesto que son las variables ambientales las que condicionan la composición de la comunidad. La significación estadística de la ordenación de los ejes 1 y 2 fue determinada usando el test de Monte Carlo. Se llevó a cabo con el programa CANOCO 4.5 y para el análisis se utilizó el valor total de la abundancia y los mismos valores de las variables ambientales que para el ANOVA. Para evitar la distorsión que pudieran producir las especies raras, se han excluido del análisis aquellas representadas por un solo individuo (Garrido & Munilla, 2007; Fernández-Díaz *et al*., 2008).

RESULTADOS

Variables ambientales

En la Tabla 1 se muestran los valores medios de las variables fisicoquímicas y microbiológicas analizadas en primavera y otoño y en las tres estaciones de muestreo. En cuanto a la época del año, existen diferencias aparentes en varias variables: conductividad, sólidos en suspensión volátiles (SSV), nitrógeno total y los parámetros relacionados con el oxígeno (OD, DBO_5 y DQO). Otras como la temperatura y el pH presentan valores muy homogéneos en las dos épocas del año. A nivel espacial, algunas de las variables analizadas se comportan de manera similar a lo largo del eje longitudinal del arroyo; es el caso de la temperatura y el pH. Tampoco varían los valores SST, SSV y potencial redox. En cambio parece existir un incremento de las concentraciones de los principales indicadores de contaminación hacia la desembocadura: conductividad, nitrógeno total, ión amonio, nitrógeno orgánico, fósforo total y coliformes fecales. Al mismo tiempo se pro-

duce un descenso en la concentración de oxígeno disuelto y de otros parámetros relacionados con éste (DBO_5 y DQO).

El análisis ANOVA de dos vías (Tabla 2) solo muestra diferencias significativas entre las épocas del año para dos variables, conductividad y SSV; otras tres variables presentan diferencias cercanas a la significación ($p < 0.1$): nitrógeno total, oxígeno disuelto y fósforo total. Entre las estaciones de muestreo, solo la variable DBO_5 presenta diferencias significativas, ya que la estación R3 es significativamente diferente a las otras dos estaciones según el test de Tukey ($p < 0.05$).

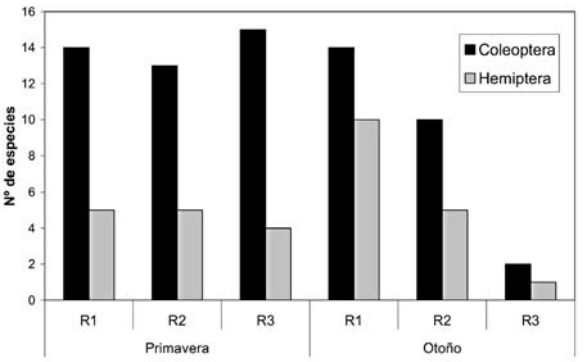

Figura 2. Riqueza total de especies en las tres estaciones de muestreo para cada época del año estudiada. *Total species richness in the three sampling sites for each studied season.*

Composición de la comunidad

Se han estudiado un total de 710 ejemplares adultos, 552 de coleópteros y 158 de hemípteros. Se han identificado 33 especies de Coleoptera y 11 especies de Hemiptera, que se relacionan en la Tabla 3. Para cada especie se indica el número de ejemplares capturado y el tipo biogeográfico, siguiendo las categorías que se indican en la metodología. Con un asterisco (*) se señalan las nuevas citas para Asturias, que corresponden a 12 especies de coleópteros y a una de hemípteros (*Velia caprai bertrandi)*. Las especies más abundantes fueron *Graptodytes varius*, *Ochthebius dilatatus* y *Helophorus brevipalpis* entre los coleópteros y *Sigara venusta* entre los hemípteros. Destaca la presencia de 12 especies (30 %) que se podrían calificar de "raras" ya que solo se ha capturado un ejemplar de cada una, a pesar de que se han llevado a cabo cuatro muestreos intensivos a lo largo de dos años.

Existe un claro gradiente de disminución de la riqueza entre la cabecera del arroyo y su desembocadura (Fig. 2). Esta reducción es más acentuada en los hemípteros, donde se pasa de 10 es-

Tabla 2. Comparaciones entre épocas del año y estaciones de muestreo analizadas mediante un ANOVA de dos vías (se presentan los valores de *F* y *p*). Las diferencias significativas ($p < 0.05$) se señalan en negrita. *Comparisons among seasons and sampling sites were analysed by two-way ANOVA (*F *and* p *values are given). Significant differences (*$p < 0.05$*) are in bold.*

	Épocas del año		Estaciones de muestreo	
	F	*p*	*F*	*p*
Temperatura agua	0.14	0.724	0.06	0.944
Conductividad	**21.62**	**0.004**	0.61	0.575
Oxígeno disuelto	4.46	0.079	1.15	0.379
pH	0.32	0.594	0.17	0.846
Potencial redox	0.01	0.945	1.20	0.364
Coliformes fecales	0.15	0.710	2.21	0.191
DBO_5	1.91	0.216	**20.47**	**0.002**
DQO	0.09	0.770	2.02	0.214
SST	1.35	0.289	0.01	0.986
SSV	**16.45**	**0.007**	0.43	0.668
Nitrógeno total	4.08	0.090	0.73	0.520
Amonio	1.14	0.327	0.60	0.580
Nitrógeno orgánico	2.76	0.148	0.30	0.753
Fósforo total	3.94	0.094	0.14	0.871

pecies en R1 a solo 3 en R3, que en los coleópteros, donde se pasa de 23 en R1 a 17 en R3.

La relación entre el número de especies de coleópteros y de hemípteros en el conjunto del arroyo objeto de estudio es de 3:1. Sin embargo, esta relación tiene un gradiente ascendente desde la cabecera hacia la desembocadura; así, en R1 la relación es de 2.3:1, en R2 se incrementa y es de 3.8:1, y, finalmente, en R3 el número de coleópteros respecto al de hemípteros es de 5.7:1.

Composición biogeográfica

En la tabla 3 se muestra la asignación de cada especie a su correspondiente corotipo. La composición biogeográfica muestra un claro predomi-

Tabla 3. Abundancia y tipo biogeográfico de las especies de coleópteros y hemípteros acuáticos capturadas en el arroyo de La Llantada. El asterisco indica las nuevas citas para Asturias. T = especie transibérica, N = especie iberoeuropea, S = especie iberoafricana, E = especie endémica. *Abundance and biogeographical categories of the aquatic Coleopteran and Hemipteran species captured in La Llantada stream. With asterisk the new records for Asturias. T = Trans-Iberian species, N = Northern species, S = Southern species, E = Iberian endemic species.*

	N.º capturas	Tipo biogeográfico
COLEOPTERA		
Gyrinidae		
Gyrinus urinator Illiger*	14	T
Haliplidae		
Haliplus lineatocollis (Marsham)	17	T
Noteridae		
Noterus laevis Sturm	1	T
Dytiscidae		
Laccophilus hyalinus (De Geer)	18	T
Hydroporus discretus (Fairmaire & Brisout)	1	T
Hydroporus tesellatus Drapiez	1	T
Hydroporus vagepictus Fairmaire & Laboulbène	6	E
Graptodytes flavipes (Olivier)	4	T
Graptodytes ignotus (Mulsant) *	15	T
Graptodytes varius (Aubé) *	150	T
Stictonectes epipleuricus (Seidlitz)	23	E
Stictotarsus duodecimpustulatus (Fabricius)	15	T
Agabus bipustulatus (Linnaeus)	1	T
Agabus brunneus (Fabricius) *	1	T
Hydrochidae		
Hydrochus grandicollis Kiesenweter *	4	T
Helophoridae		
Helophorus (Atractelophorus) brevipalpis Bedel	47	N
Helophorus (Rhopalhelophorus) minutus Fabricius *	10	N
Helophorus (Rhopalhelophorus) obscurus Mulsant	38	N
Hydrophilidae		
Chaetarthria simillima Vorst & Cuppen *	2	N
Anacaena (Anacaena) bipustulata (Marsham) *	7	T
Anacaena (Anacaena) lutescens (Stephens) *	39	T
Laccobius (Dimorpholaccobius) bipunctatus (Fabricius)	11	T
Laccobius (Dimorpholaccobius) ytenensis Sharp	12	T
Helochares (Helochares) lividus (Forster) *	4	T
Hydrophilidae (cont.)		
Coelostoma (Coelostoma) orbiculare (Fabricius)	1	N
Hydraenidae		
Hydraena testacea Curtis *	2	T
Octhebius (Asiobates) dilatatus Stephens	81	T
Octhebius (Asiobates) minimus (Fabricius)	1	N
Elmidae		
Elmis aenea (Müller)	12	N
Limnius perrisi carinatus (Pérez-Arcas)	1	E
Limnius volckmari (Panzer)	4	N
Dryopidae		
Dryops luridus (Erichson)	4	T
Dryops striatellus (Fairmaire & Brisout)*	5	T
HEMIPTERA		
Hydrometridae		
Hydrometra stagnorum (Linnaeus)	5	T
Veliidae		
Velia (Plesiovelia) caprai bertrandi Tamanini*	4	E
Geriidae		
Aquarius najas (De Geer)	20	T
Gerris (Gerris) lacustris (Linnaeus)	1	T
Corixidae		
Corixa panzeri Fieber	1	N
Hesperocorixa sahlbergi (Fieber)	7	T
Sigara (Pseudovermicorixa) nigrolineata (Fieber)	2	N
Sigara (Retrocorixa) venusta (Douglas & Scout)	104	N
Nepidae		
Nepa cinerea (Linnaeus)	1	T
Notonectidae		
Notonecta maculata Fabricius	12	T
Notonecta meridionalis Poisson	1	S

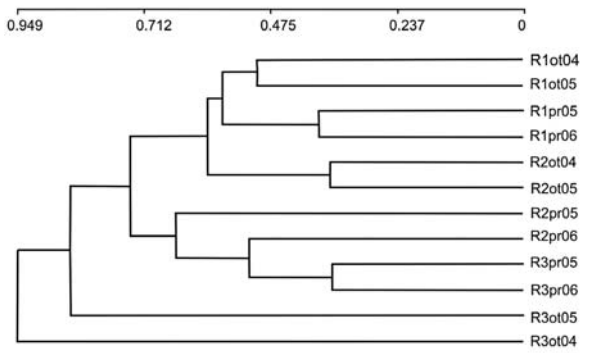

Figura 3. Análisis de afinidad mediante el índice cualitativo de Sørensen entre las muestras estudiadas. *Qualitative Sørensen index affinity between samples.*

nio de especies de amplia distribución, sobre todo transibéricas (64 %), seguidas por los elementos iberoeuropeos (25 %) y una representación muy reducida de elementos de distribución más restringida: una especie iberoafricana (2 %) y cuatro endémicas (9 %). Por órdenes, los coleópteros presentan un 67 % de especies transibéricas y un 24 % de iberoeuropeas, porcentajes muy similares a los de hemípteros (55 % de elementos transibéricos y 27 % de iberoeuropeos). Por último, los elementos iberoafricanos representan el 9 % de los hemípteros, y las especies endémicas el 9 % en ambos órdenes.

Llama la atención la presencia de especies consideradas meridionales como *Hydrochus grandicollis*, *Dryops striatellus* o *Velia caprai bertrandi*, el único endemismo ibérico de hemíptero capturado en el arroyo. Los coleópteros endémicos son *Hydroporus vagepictus*, *Stictonectes epipleuricus* y *Limnius perrisi carinatus*.

Variación espacial y temporal

El número total de especies capturadas es ligeramente superior en primavera que en otoño, 33 especies en primavera frente a 28 en otoño. Si diferenciamos por órdenes, se observa una mayor riqueza de coleópteros en primavera (25 especies frente a 18) y una mayor riqueza de hemípteros en otoño (10 especies frente a 8).

En el análisis de afinidad mediante el índice de Sørensen (Fig. 3) se pueden reconocer varios grupos de muestras con composiciones taxonómicas afines. En primer lugar, queda claramente delimitado un grupo con todas las muestras de la localidad R1. También se observa cómo las muestras de primavera de R2 y R3 constituyen otro grupo, mientras que las muestras de otoño de R2 forman un pequeño grupo más afín al grupo de R1 y que juntos constituyen un gran grupo. Las muestras de R3 en otoño difieren claramente de las restantes.

Influencia de los factores ambientales

Los dos primeros ejes del ACC explican el 45.3 % de la varianza acumulada en los datos de las especies y el 66.3 % de la varianza acumulada en la relación especies-variables ambientales. El test de Monte Carlo indica que solo el primero de los dos ejes fue significativo ($F = 1.736$, $p = 0.049$). Las variables correlacionados positivamente con el eje 1 son las tres formas del nitrógeno analizadas, con una r entre 0.86 y 0.71 y también el fósforo total ($r = 0.58$). Entre ellas destaca el nitrógeno total, que presenta lógicamente una alta correlación positiva con el amonio ($r = 0.78$, $p < 0.05$) y el nitrógeno orgánico ($r = 0.78$, $p < 0.05$). En el otro extremo del eje hay dos variables correlacionadas negativamente con el eje 1, los SSV ($r = -0.75$) y la conductividad ($r = -0.56$). La conductividad presenta una elevada correlación positiva con SSV ($r = 0.83$, $p < 0.05$). Asimismo la conductividad tiene una elevada correlación negativa con el oxígeno disuelto ($r = -0.82$, $p < 0.05$). Por otro lado, el eje 2 tiene dos variables correlacionadas negativamente, los SST ($r = -0.59$) y el oxígeno disuelto ($r = -0.55$).

El eje 1 discrimina las muestras de primavera hacia la parte positiva o muy cerca de esta, mientras que todas las muestras de otoño se encuentran en la parte negativa del gráfico (Fig. 4). En el extremo positivo del primer eje aparecen las muestras con una mayor concentración de nitrógeno (en diversas formas) y que condicionan la composición de la comunidad.

Existe un pequeño grupo de ocho especies de coleópteros asociadas al extremo positivo del primer eje y caracterizadas por proceder de muestras con una fuerte carga de nutrientes: *Ochthebius dilatatus*, *Helophorus obscurus*, *Anacae-*

na lutescens, Helophorus brevipalpis, Hydroporus vagepictus, Stictonectes duodecimpustulatus, Hydrochus grandicollis y *Helophorus minutus.* Por el contrario, la mayoría de las especies responden negativamente a altas concentraciones de nitrógeno, amonio, nitrógeno orgánico y fósforo total. Especies como *Graptodytes ignotus, Helochares lividus, Dryops striatellus, Graptodytes varius* o el hemíptero *Sigara nigrolineata* se localizan en la parte del eje asociada a una menor presencia de nutrientes indicadores de contaminación. En el segundo eje aparece una relación con el oxígeno disuelto y los SST, que agrupa principalmente a las especies *Laccobius bipunctatus, Anacaena bipustulata, Velia caprai bertrandi* o *Hydrometra stagnorum*. Al mismo tiempo se asocia con este eje la muestra R2pr05. Ninguna especie se asocia claramente con bajas concentraciones de oxígeno disuelto, si bien las dos muestras de otoño de R3 y una muestra de R2 aparecen en el extremo opuesto, asociadas a valores bajos de oxígeno.

DISCUSIÓN

El área de estudio se localiza en una zona para la que algunos autores (Sánchez-Fernández *et*

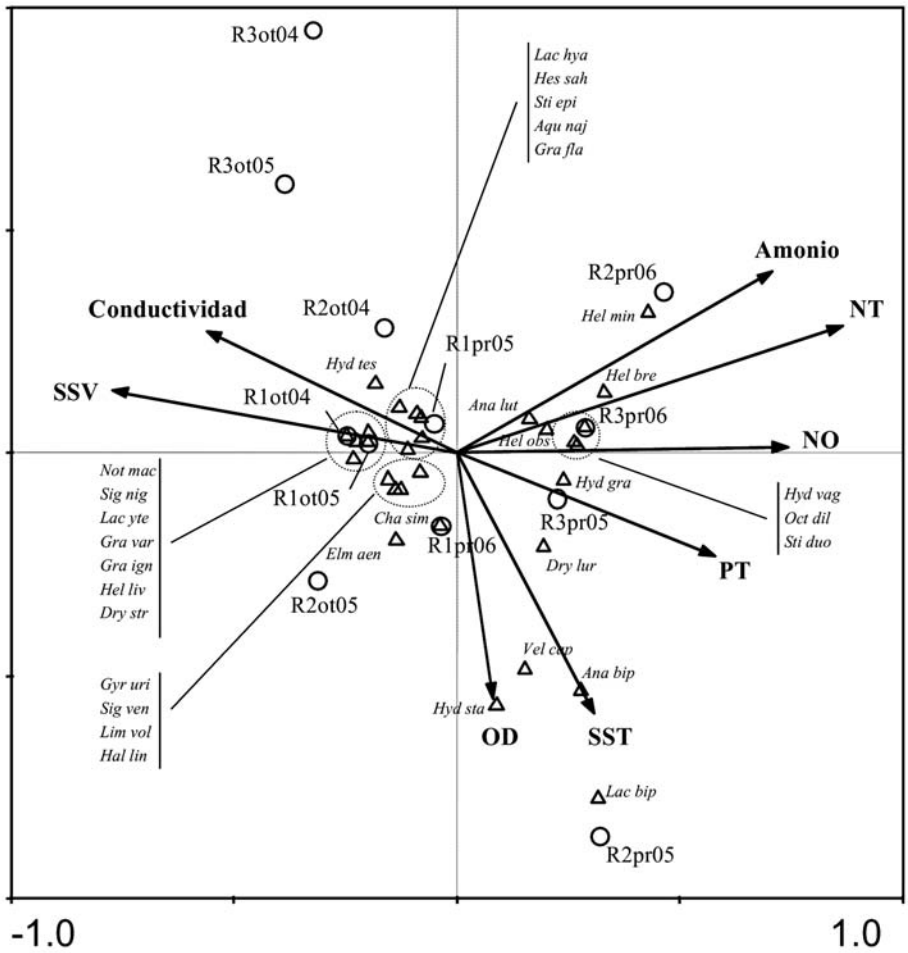

Figura 4. Resultado del Análisis de Correspondencias Canónicas (ACC) de las especies de coleópteros y hemípteros acuáticos respecto a los factores ambientales. Las flechas representan las variables fisicoquímicas, los círculos los muestreos y los triángulos las especies. *Results of the Canonical Correspondence Analysis (CCA) of the Coleopteran and Hemipteran species with regard to environmental factors. Arrows represent the physicochemical parameters, circles the sampling campaigns, and triangles the species.*

al., 2011) predicen una baja riqueza de coleópteros acuáticos a nivel peninsular. Sin embargo, teniendo en cuenta lo reducido del área de estudio (un único arroyo de pequeñas dimensiones con tres estaciones de muestreo) el número de especies inventariadas es notable. También hay que tener en cuenta que por sus características (bajo caudal, velocidad de la corriente escasa y sustrato preferentemente limoso) este arroyo carece de la mayoría de las especies de fauna reófila típicas de arroyos de montaña (especialmente especies de las familias Hydraenidae y Elmidae). Buena parte de la composición faunística del arroyo estudiado es típica de medios de aguas estancadas.

La mayoría de los estudios realizados en Asturias se han llevado a cabo en zonas de montaña de la Cordillera Cantábrica y Picos de Europa (Sánchez-Fernández *et al*., 2011); en cambio, los estudios en zonas costeras son escasos y en la mayoría de los casos antiguos. Así, no sorprende que el número de nuevas especies citadas para Asturias sea de 13 (Tabla 3), todas menos una corresponden a especies de coleópteros. La existencia de un inventario y catálogo de hemípteros acuáticos de la provincia de Asturias (Fernández Bernaldo de Quirós, 1985) explica en gran medida el mejor conocimiento de este grupo respecto al de los coleópteros.

Tres citas de especies de coleópteros son aportaciones faunísticas de interés a nivel de la Península Ibérica. *Chaetarthria simillima* es una especie descrita recientemente que se distribuye por el centro y oeste de Europa, incluidas las Islas Británicas (Bratton, 2009). Hasta la actualidad solo existía un registro ibérico de esta especie en el norte de la provincia de León (Vorst & Cuppen, 2003). *Hydrochus grandicollis* se distribuye mayoritariamente por el sur peninsular (Valladares & Ribera, 1999), y en algunas localidades de la Meseta Norte (Valladares & Miguélez, 2004; Valladares *et al.*, 2000); su presencia en la costa cantábrica es pues destacable y parece indicar un área de distribución más amplia en zonas con características ambientales favorables. *Dryops striatellus* cuenta con muy escasas, y en general antiguas, citas ibéricas limitadas al área mediterránea, ya que está citada en las provincias de Badajoz, Girona, Huelva y sur de Portugal (Montes & Soler, 1986; Ribera & Aguilera, 1996; Millán *et al.*, 2005). La presencia en Asturias de *Ochthebius dilatatus* y *O. minimu*s también se señaló con el material de este estudio, pero estas citas han sido publicadas previamente (Valladares & Delgado, 2007). Destaca en este caso la singularidad del hábitat en que se ha localizado *Ochthebius minimus*, ya que se trata de una especie característica de charcas y humedales de meseta del centro peninsular, que aquí se ha capturado en un arroyo de la costa cantábrica.

En el arroyo de La Llantada conviven dos poblaciones de especies muy próximas, *Graptodytes varius* y *G. ignotus*, donde se han encontrado varios ejemplares con características intermedias, tres en R1 y dos en R2, los cuales no pueden ser atribuidos a una u otra especie con certeza. Estos ejemplares o bien son el resultado de una hibridación entre ambas especies o bien un complejo de formas polimórficas de la misma especie (Ribera *et al*., 1998).

La situación geográfica del área estudiada, su carácter no montañoso y su condición de medio de escasa corriente aportan una menor representación de elementos endémicos (Ribera & Vogler, 2000), y permiten explicar esta composición corológica. La endemicidad del 9 % en los coleópteros es baja frente al 22 % a nivel peninsular. El único hemíptero endémico de la zona (9 % del total) indica un nivel de endemismo normal para este grupo en el contexto peninsular (10 %).

No se cumple aquí la regla señalada por algunos autores (Millán *et al*., 2001) que observan un incremento en el número de especies de hemípteros en relación con el de coleópteros a medida que los medios están más contaminados. En el arroyo de La Llantada sucede lo contrario, la relación entre el número de coleópteros y hemípteros aumenta a lo largo del eje longitudinal del arroyo, el cual sufre un fuerte descenso en la calidad de sus aguas a medida que se acerca a su desembocadura, y que queda manifiesto en la disminución de los valores en los índices IBMWP e IASPT, en el número de familias y en la diversidad (Mazé *et al.*, 2011). Las diferencias de riqueza totales entre las tres estaciones muestreadas, y para ambos grupos, parecen seguir un patrón relacionado con el descenso de la calidad del agua.

La separación de las muestras de otoño de R3 que refleja el índice de Sørensen se debe, probablemente, a la mayor contaminación orgánica en este tramo, a la que se suma la influencia de la estacionalidad. La menor presión antrópica y la mayor precipitación en primavera explicarían la agrupación en el dendrograma de las muestras de R3pr con las muestras de R2pr. La estación R1, en la cabecera, queda bien delimitada en este análisis, con una comunidad faunística con menores impactos y menos influenciada por la estacionalidad. En general, las diferencias en la composición de la comunidad son más evidentes en las muestras de otoño, y especialmente en R3, mientras que las diferencias en primavera son menos evidentes.

La calidad del agua en el arroyo La Llantada está afectada por la contaminación de las actividades humanas, en especial durante los meses de verano, cuando disminuyen las precipitaciones y la incidencia del turismo es mayor. Los valores de temperatura del agua son homogéneos en las tres estaciones a causa de la climatología de la zona de estudio y la corta longitud del arroyo. El pH ligeramente básico podría ser debido a la disolución de materiales calcáreos del arroyo, ya que es homogéneo en las épocas y estaciones muestreadas. Sin embargo, el muestreo de otoño presenta valores significativamente más elevados en la conductividad y SSV, y aunque sin diferencias significativas se observa una disminución en la concentración de oxígeno disuelto, DQO y DBO_5. La conductividad es una variable que aumenta a lo largo del gradiente longitudinal de un cauce, pero también es un indicador de contaminación orgánica (Sandin & Hering, 2004), y dada la escasa longitud del mismo, parece ser un parámetro importante. Los valores de conductividad parecen responder a un patrón estacional, con una conductividad más elevada en periodos de bajo caudal en verano y otoño. En varios ríos del noroeste de España se ha señalado la conductividad como una variable que determina la comunidad de coleópteros por ser un buen indicador de contaminación, tanto por vertidos de aguas residuales (Paz, 1993), como por los efectos de las extracciones mineras (García-Criado & Fernández-Aláez, 2001). También se ha señalado la conductividad como una variable determinante en la distribución de las especies de hemípteros acuáticos (Carbonell *et al.*, 2011). En el gráfico del análisis ACC, la variable SSV sigue el mismo patrón que la conductividad y ambas son opuestas a variables como PT, SST y oxígeno disuelto. Sin embargo, no se han medido otras variables, como fertilizantes, que pudieran influir en las diferencias estacionales. Aunque no se observan diferencias importantes de conductividad en las tres estaciones, es posible que la salinidad que aportan temporalmente las mareas pueda influir en la estación R3. Se ha señalado la salinidad como una variable que reduce la riqueza de especies de coleópteros y hemípteros en otros humedales costeros del noroeste de España y a la que responden negativamente la mayoría de las especies (Garrido & Munilla, 2007). En consonancia con nuestros resultados, estudios similares señalan que la mayoría de especies de coleópteros responden negativamente a la contaminación por nitrógeno y otras variables como el hierro y el manganeso (Fernández-Díaz *et al*., 2008; Benetti & Garrido, 2010). También se ha señalado el oxígeno disuelto como una variable importante que determina que la comunidad de coleópteros sea más o menos reófila (Pérez-Bilbao & Garrido, 2009).

Diferentes estudios han relacionado la disminución de la riqueza de especies, la abundancia total y la diversidad con valores elevados de variables químicas (Prenda & Gallardo-Mayenco, 1996; Benetti & Garrido, 2010). Nuestros datos indican que la comunidad de coleópteros y hemípteros acuáticos del arroyo estudiado está influenciada por el grado de contaminación orgánica de sus aguas. Los impactos en el tramo bajo parecen ser la consecuencia del incremento de la población humana durante los meses de verano, la deficiente depuración de sus aguas y la disminución de las precipitaciones. Así, la mayoría de las especies responden negativamente a las variables indicadoras de contaminación y la riqueza de especies se reduce desde la cabecera a la desembocadura.

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BIBLIOGRAFÍA

ALBA-TERCEDOR, J. & A. PUJANTE. 2000. Running-water biomonitoring in Spain. Opportunities for predictive approach. In: *Assessing the Biological Quality of Freshwater RIVPACS and similar techniques.* Freshwater Biological Association: 207–216.

BENETTI, C. J., A. I. ALONSO & J. GARRIDO. 2007. Comparación de la comunidad de coleópteros acuáticos (Adephaga y Polyphaga) en dos cuencas hidrográficas con distinto grado de acción antropogénica (Pontevedra, NO de España). *Limnetica*, 26: 115–128.

BENETTI, C. J. & J. GARRIDO. 2010. The influence of water quality and stream habitat on water beetle assemblages in two rivers in northwest Spain. *Vie et milieu - Life and Environment*, 60: 53–63.

BRATTON, J. H. 2009. Further English and welsh records of *Chaetarthria*. *Latissimus*, 25: 13–14.

CARBONELL, J. A., C. GUTIÉRREZ-CÁNOVAS, D. BRUNO, P. ABELLÁN, J. VELASCO & A. MILLÁN. 2011. Ecological factors determining the distribution and assemblages of the aquatic Hemiptera (Gerromorpha & Nepomorpha) in the Segura River basin (Spain). *Limnetica*, 30: 59–70.

EYRE, M. D. & G. N. FOSTER. 1989. A comparation of Aquatic Heteroptera and Coleoptera communities as a basis for environmental and conservation assessments in static water sites. *Journal of Applied Entomology*, 108: 355–362.

EYRE, M. D., G. N. FOSTER & M. L. LUFF. 2005. Exploring the relationship between land cover and the distribution of water beetle species (Coleoptera) at the regional scale. *Hydrobiologia*, 353: 87–98.

FERNÁNDEZ BERNALDO DE QUIRÓS, C. 1982. Heterópteros acuáticos y semiacuáticos de Asturias (N. de España). I. Catálogo sistemático. *Boletín de la Real Sociedad Española de Historia Natural (Biología)*, 80 (3-4): 211–218.

FERNÁNDEZ-DÍAZ, M., C. J. BENETTI & J. GARRIDO. 2008. Influence of iron and nitrate concentration in water on aquatic Coleoptera community structure: Application to the Avia River (Ourense, NW. Spain). *Limnetica*, 27: 285–298.

FERY, H. & J. FRESNEDA. 2007. Los *Hydradephaga* (Coleoptera: Dytiscidae, Gyrinidae, Haliplidae, Noteridae, Paelobiidae) de la Península Ibérica e Islas Baleares de las colecciones J. Fresneda y H. Fery. *Boletín Sociedad Entomológica Aragonesa*, 41: 119–171.

FOSTER, G. N. 1987. The use of Coleoptera records in assesing the conservation status of wetlands. In: *The use of Invertebrates in site assessment for conservation.* M. Luff (ed.): 8–18. University Newcastle, Newcastle Upon Tyne.

FOSTER, G. N., A. P. FOSTER, M. D. EYRE & T. BILTON. 1990. Classification of water beetle assemblages in arable fenland and ranking of sites in relation to conservation value. *Freshwater Biology*, 22: 343–354.

GARCÍA-CRIADO, F. & M. FERNÁNDEZ-ALÁEZ. 1995. Aquatic Coleoptera (Hydraenidae and Elmidae) as indicators of the chemicals characteristics of water in the Órbigo River basin (N-W Spain). *Annales de Limnologie*, 31: 185–199.

GARCÍA-CRIADO, F., C. FERNÁNDEZ-ALÁEZ, & M. FERNÁNDEZ-ALÁEZ. 1999. Environmental variables influencing the distribution of Hydraenidae and Elmidae assemblages (Coleoptera) in a moderately-polluted river basin in northwestern Spain. *European Journal of Entomology*, 96: 37–44.

GARCÍA-CRIADO, F. & M. FERNÁNDEZ-ALÁEZ. 2001. Hydraenidae and Elmidae assemblages (Coleoptera) from a Spanish river basin: good indicators of coal mining pollution? *Archiv für Hydrobiologie*, 150 (4): 641–660.

GARRIDO, J. & I. MUNILLA. 2007. Aquatic Coleoptera and Hemiptera assemblages in three coastal lagoons of the NW Iberian Peninsula: assessment of conservation value and response to enviromental factors. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18 (5): 557–569.

MAZÉ, R. A., G. ANSOLA & L. F. VALLADARES. 2011. Water quality assessment of a small coastal

stream: use of different biological indices based on macroinvertebrates. *Vie et milieu-Life and Environment*, 61 (1): 27–34.

MILLÁN, A., J. L. MORENO & J. VELASCO. 2001. Estudio faunístico y ecológico de los coleópteros y heterópteros acuáticos de las lagunas de Albacete (Alboraj, Los Patos, Ojos de Villaverde, Ontalafia y Pétrola). *Sabuco*, 1: 43–94.

MILLÁN, A., J. L. MORENO & J. VELASCO. 2002. *Los coleópteros y heterópteros acuáticos y semiacuáticos de la provincia de Albacete. Catálogo faunístico y estudio ecológico.* Instituto de Estudios Albacetenses "Don Juan Manuel", de la Excma. Diputación Provincial de Albacete, Albacete.

MILLÁN, A., C. HERNANDO, P. AGUILERA, A. CASTRO. & I. RIBERA. 2005. Los coleópteros acuáticos y semiacuáticos de Doñana: Reconocimiento de su biodiversidad y prioridades de conservación. *Boletín Sociedad Entomológica Aragonesa*, 36: 157–164.

MONTES, C. & A. G. SOLER. 1986. *Lista faunística y bibliográfica de los coleópteros acuáticos Dryopoidea (Dryopidae & Elmidae) de la Península Ibérica e Islas Baleares.* Listas de la Flora y Fauna de las aguas continentales de la Península Ibérica n.º 13. Asociación Española de Limnología, Madrid.

NIESER, N., M. BAENA, J. MARTÍNEZ-AVILÉS & A. MILLÁN. 1994. *Claves para la identificación de los heterópteros acuáticos (Nepomorpha & Gerromorpha) de la Península Ibérica-Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira.* Asociación Española de Limnología. Madrid.

PAZ, C. 1993. *Hydradephaga (Coleoptera) en la cuenca del río Landro (NW Península Ibérica). Estudio faunístico y ecológico.* Tesis Doctoral. Universidad de Santiago de Compostela, España.

PEARSON, D. L. 1994. Selecting indicator taxa for quantitative assessment of biodiversity. *Philosophical Transactions of the Royal Society of London*, Series B, 345: 75–79.

PÉREZ BILBAO, A. & J. GARRIDO. 2009. Evaluación del estado de conservación de una zona LIC (Gándaras de Budiño, Red Natura 2000) usando coleópteros acuáticos como indicadores. *Limnetica*, 28 (1): 11–22.

PRENDA, J. & A. GALLARDO-MAYENCO. 1996. Self-purification, temporal variability and the macroinvertebrate community in small lowland Mediterranean streams receiving crude domestic sewage effluents. *Archiv für Hydrobiologie*, 136: 159–170.

RIBERA, I. & G. N. FOSTER. 1993. Uso de coleópteros acuáticos como indicadores biológicos (Coleoptera). *Elytron*, 6: 61–75.

RIBERA, I. & J. AGUILERA. 1996. Els estanys de Capmany: the missing Spanish pingo (or palsa) fens? *Latissimus*, 7: 2–6.

RIBERA, I., C. HERNANDO & P. AGUILERA, 1998. An annotated checklist of the Iberian water beetles (Coleoptera). *Zapateri Revista aragonesa de Entomología*, 8: 43–111.

RIBERA, I. & A. P. VOGLER. 2000. Habitat type as a determinant of species range sizes: the example of lotic-lentic differences in aquatic Coleoptera. *Biological Journal of the Linnean Society*, 71: 33–52.

SÁNCHEZ-FERNÁNDEZ, D., P. ABELLÁN, J. VELASCO & A. MILLÁN. 2004. Selecting areas to protect the biodiversity of aquatic ecosystems in a semiarid Mediterranean region using water beetles. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 14: 465–479.

SÁNCHEZ-FERNÁNDEZ, D., J. M. LOBO, P. ABELLÁN & A. MILLÁN. 2011. How to identify future sampling areas when information is biased and scarce: An example using predictive models for species richness of Iberian water beetles. *Journal for Nature Conservation*, 19: 54–59.

SANDIN, L. & D. HERING. 2004. Comparing macroinvertebrate indices to detect organic pollution across Europe: a contribution to the EC Water Framework Directive intercalibration. *Hydrobiologia*, 516: 55–68.

SAVAGE, A. A. 1996. Density dependent and density independent relationships during a twenty-seven year study of the population dynamics of the benthic macroinvertebrate community of a chemically unstable lake. *Hydrobiologia*, 335: 115–131.

TER BRAAK, C. J. F. & O. F. R. VAN TONGEREN. 1995. *Data Analysis in Community and Landscape Ecology*. Cambridge University Press. Cambridge.

VALLADARES, L. F. & J. A. DELGADO. 2007. Aportaciones al conocimiento faunístico de los géneros *Limnebius* Leach, 1815 y *Ochthebius* Leach, 1815 en la Península Ibérica (Coleoptera, Hydraenidae). *Graellsia*, 63: 333–338.

VALLADARES, L. F., J. A. DÍAZ & J. GARRIDO. 2000. Coleópteros acuáticos del Sistema Ibérico Septentrional (Coleoptera: Haliplidae, Gyrinidae,

Dytiscidae, Hydraenidae, Helophoridae, Hydrochidae, Hydrophilidae). *Boletín Asociación Española Entomología*, 24 (3-4): 59–84.

VALLADARES, L. F. & D. MIGUÉLEZ. 2004. Fauna actual de coleópteros y heterópteros acuáticos de la turbera fósil de Espinosa de Cerrato (Palencia). *Boletín Asociación española Entomología*, 28 (1-2): 71–88.

VALLADARES, L. F. & I. RIBERA. 1999. *Lista faunística y bibliográfica de los Hydrophiloidea acuáticos (Coleoptera) de la Península Ibérica e Islas Baleares*. Listas de la Flora y Fauna de las aguas continentales de la Península Ibérica, n.º 15. Asociación Española de Limnología.

VORST, O. & J. G. M. CUPPEN. 2003. A third Paleartic species of *Chaetarthria* Stephens (Coleoptera: Hydrophlidae). *Koleopterologische Rundschau*, 73: 161–167.

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Distribución y ecología de algunas especies de rodófitos (*Rhodophyta*) en la cuenca del río Ebro

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ABSTRACT

Distribution and ecology of some species of red algae (*Rhodophyta*) in the Ebro River Basin

The implementation of the Water Framework Directive (2000/60/CE) in the Ebro River Basin and the evaluation of the ecological status of rivers, lakes and wetlands are increasing the knowledge about the distribution and ecology of aquatic organisms, including the red algae (*Rhodophyta*). In this work we show new data concerning the distribution, habitat and environmental ranges of five species of *Rhodophyta* in the Ebro River Basin with scarce records: *Bangia atropurpurea, Chroodactylon ornatum, Chroothece rupestris, Compsopogon coeruleus* and *Thorea hispida*. The former three species showed an optimum development in calcareous mountain streams while the latter two species did in lower reaches where salinity and eutrophication increase.

Key words: Algae, *Rhodophyta*, Ebro, environmental ranges, geographic distribution.

RESUMEN

Distribución y ecología de algunas especies de rodófitos (*Rhodophyta*) en la cuenca del río Ebro

*La aplicación de la Directiva Marco del Agua (2000/60/CE) en la cuenca del Ebro y la consiguiente evaluación del estado ecológico de los ríos, lagos y humedales mediante indicadores biológicos (red CEMAS, Control del Estado de las Masas de Agua Superficiales), están permitiendo aumentar el conocimiento de la distribución y ecología de los organismos acuáticos, entre ellos las algas rojas (*Rhodophyta*). Fruto de este seguimiento, se presentan nuevos datos sobre la distribución, hábitat y ecología de cinco especies de rodófitos en la cuenca del Ebro de las que existen datos escasos:* Bangia atropurpurea, Chroodactylon ornatum, Chroothece rupestris, Compsopogon coeruleus *y* Thorea hispida. *Los resultados indican que las tres primeras tienen un desarrollo óptimo en ríos calcáreos de montaña, mientras que las dos últimas lo tienen en tramos medios y bajos donde aumenta la salinidad y la concentración de nutrientes.*

Palabras clave: *Algas,* Rhodophyta, *Ebro, rangos ambientales, distribución geográfica.*

INTRODUCCIÓN

Los rodófitos son un grupo de algas amenazado en Europa, propio de aguas blandas, claras y puras, siendo, en general, indicadores de buena calidad ecológica (Eloranta & Kwandrans, 2004). En el norte de Europa los rodófitos son frecuentes y abundantes, mientras que en el centro y sur de Europa son más escasos, reduciendo su distribución conocida a áreas montañosas donde los impactos humanos como la eutrofización y la regulación hidrológica mediante embalses, presas y canalizaciones son menores (Kwandrans & Eloranta, 2010). Debido a su ciclo de vida largo y complejo (Lee, 1980; van den Hoek *et al*., 1995), necesitan unas condiciones ambientales hidrológicamente estables, y son en general débiles competidores: en tramos altos compiten con los briófitos por el sustrato, mientras que en tramos medios y bajos, de aguas más eutróficas y turbias, compiten con los clorófitos filamentosos y las cianobacterias (Eloranta & Kwandrans, 2004). La predominancia de pigmentos como ficoeritrina y ficocianina les confiere colores muy llamativos como el rojo, violeta, azul, verde, marrón o grisáceo.

Desde la publicación del catálogo nacional de rodófitos (Álvarez-Cobelas, 1984), las aportaciones al conocimiento de este grupo algal han sido escasas y las más destacadas se publicaron hace dos décadas (p. ej. Llimona *et al*., 1985; Aboal, 1989; Sabater *et al*., 1989). Tampoco existen estudios extensivos previos sobre este grupo de algas que cubran toda la cuenca del Ebro. Durante los últimos años, la obligación de cumplir con los objetivos establecidos en la Directiva Marco del Agua (DMA) (Directiva 2000/60/CE) y su aplicación en los ríos de la Península Ibérica, ha dado lugar a un incremento del conocimiento de los diferentes componentes bióticos de nuestros ríos: macrófitos (macroalgas, briófitos y plantas vasculares), macroinvertebrados, diatomeas, peces y vegetación de ribera. Por otra parte, este seguimiento continuo durante varios años de la biota que habita nuestros ríos está permitiendo analizar variaciones temporales (estacionales e interanuales) en la distribución espacial de los diferentes organismos acuáticos, así como ampliar los rangos ecológicos de los mismos.

En relación con la flora acuática, en la cuenca del Ebro se viene aplicando el índice trófico denominado IVAM (Moreno *et al*. 2006), con el fin de evaluar el estado ecológico de los ríos. Este índice utiliza los organismos acuáticos autótrofos y macroscópicos identificados a nivel de género, entre los que se incluyen las algas rodófitas. Sin embargo, a nivel específico no se han realizado estudios recientes sobre la distribución y ecología de los rodófitos en la cuenca del Ebro, por lo que la aportación de nuevos datos sobre la presencia de especies de rodófitos resulta de gran interés florístico y ecológico.

En el presente trabajo se presenta la distribución, el hábitat y los rangos ambientales de algunas especies de rodófitos sobre las que existen escasas citas en la cuenca del Ebro o en la Península Ibérica: *Bangia atropurpurea*, *Chroodactylon ornatum*, *Chroothece rupestris*, *Compsopogon coeruleus* y *Thorea hispida*.

MATERIAL Y MÉTODOS

Durante los meses de mayo a septiembre en el periodo 2006-2011, se visitaron entre 211 (2006) y 362 (2009) estaciones de muestreo pertenecientes a las redes de control operativo, vigilancia y referencia de las masas de agua de la cuenca del Ebro, localizada en el noreste peninsular. Para ello, se seleccionaron tramos representativos de unos 100 m de longitud, donde se realizaron recorridos en zigzag aguas arriba y de orilla a orilla, recolectando todos los macrófitos visibles a simple vista. La abundancia de cada taxon se registró como el porcentaje de cobertura en el tramo de muestreo. De acuerdo con la metodología adoptada para el seguimiento del estado ecológico en las cuencas hidrográficas españolas, la mayoría de los muestreos fueron realizados entre finales de primavera y verano (Anexo I, material adicional disponible en www.limnetica.net/internet). El número de puntos de muestreo fue establecido siguiendo los criterios de delimitación de masas de agua definidos para la Directiva ("Identification of Water Bodies", Guidance document nº 2, Common Implementation Strategy for the Water Framework Directive), de forma que exista al menos un pun-

to de control por cada masa de agua. Según la regionalización fisiográfica oficial de los ríos españoles realizada por el Centro de Estudios Hidrográficos (CEDEX), los ríos de la cuenca del Ebro pertenecen a las siguientes tipologías: ríos mineralizados de baja montaña mediterránea (código 109); ríos de montaña mediterránea calcárea (112); ejes mediterráneo-continentales poco mineralizados (115); ejes mediterráneo-continentales mineralizados (116); grandes ejes en ambiente mediterráneo (117); ríos de montaña mediterránea silícea (111); ríos de montaña húmeda calcárea (126) y ríos de alta montaña (127).

Los organismos fueron fijados en formol al 4 % y se llevaron al laboratorio para ser analizados con la lupa binocular y el microscopio (CHE, 2005). En algunos casos (*Chroothece*) se tomaron muestras de limos y areniscas por encima del nivel del agua y se humedecieron para detectar la presencia de estas algas. Además del muestreo biológico, en cada visita se midieron algunos parámetros fisicoquímicos in situ mediante una sonda multiparamétrica YSI 556 MPS (pH, temperatura, conductividad y oxígeno disuelto) y se tomaron muestras de agua para analizar posteriormente en el laboratorio la concentración de nitrato, amonio y fosfato (fósforo reactivo soluble, PRS). Los análisis de agua se realizaron según los métodos y protocolos ITC-MMA EECC-1/06 (MMA, 2006). Una vez realizado el muestreo de cada una de las estaciones, se procedió a la desinfección del material y de los equipos de muestreo utilizados, de acuerdo con el protocolo establecido por la CHE (2007) para evitar la propagación del mejillón cebra y otros organismos. Las fotografías se realizaron con un microscopio Olympus BX50 equipado con un set Colorview II. Una parte del material se prensó y se conservó en pliegos de herbario que se depositaron en el herbario de la Universidad de Murcia (MUB-ALGAS).

Los rangos ambientales y preferencias ecológicas de las especies se describen mediante gráficos comparativos de cajas y líneas. Con el fin de comprobar estadísticamente las tendencias ambientales observadas, se realizó un análisis de correlaciones de Spearman entre las variables ambientales estandarizadas y los datos de presencia-ausencia de las especies. Los valores de los parámetros ambientales pertenecen a los datos oficiales de las redes de control biológico de la Confederación Hidrográfica del Ebro correspondientes a los años 2006-2011, así como a otras observaciones esporádicas realizadas en 2011 y 2012.

RESULTADOS

Durante el periodo de estudio se tomaron y procesaron en total 1350 muestras de macrófitos acuáticos, 264 de las cuales presentaron alguna de las 5 especies de rodófitos analizadas. En el Anexo I (www.limnetica.net/internet) se enumeran las localidades donde fueron recolectadas junto con la fecha de muestreo, abundancia, coordenadas UTM, la tipología fluvial oficial CEDEX y la provincia administrativa.

Distribución y hábitat

Bangia atropurpurea (Roth 1806) C. Agardh 1824 (Fig. 1) se recolectó en diferentes tipos de ríos, desde la alta montaña en los Pirineos hasta los tramos medios de algunos afluentes principales del Ebro, especialmente en las cuencas del Cinca y Jalón y sobre sustratos muy diversos: *Cladophora* sp., *Oedogonium* sp., *Potamogeton pectinatus*, musgos, piedras, roca madre, cemento de puentes, hilos de pescar, plásticos o tallos de *Phragmites australis* y *Rubus* sp., todos ellos presentes en zonas turbulentas y de elevada corriente. La profundidad osciló entre los 0 y 20 cm. En algunos tramos, como en el río Ésera en Graus, se ha observado sobre el mismo sustrato y lugar en años sucesivos.

Chroodactylon ornatum (C. Agardh) Basson 1979 es en la cuenca del Ebro una especie común en ríos calcáreos de montaña. Se ha detectado principalmente creciendo de forma epífita sobre *Cladophora* sp., sobre todo en talos con deposiciones de carbonatos y con presencia de cianobacterias como *Lyngbya* sp. y *Chamaesiphon* sp., así como de diatomeas (*Cocconeis* sp.). Con menor frecuencia, se ha observado creciendo sobre musgos, *Vaucheria* sp., *Oedogonium* sp., *Lemanea* sp., limos y fundas de tricópteros de la familia *Hydroptilidae*.

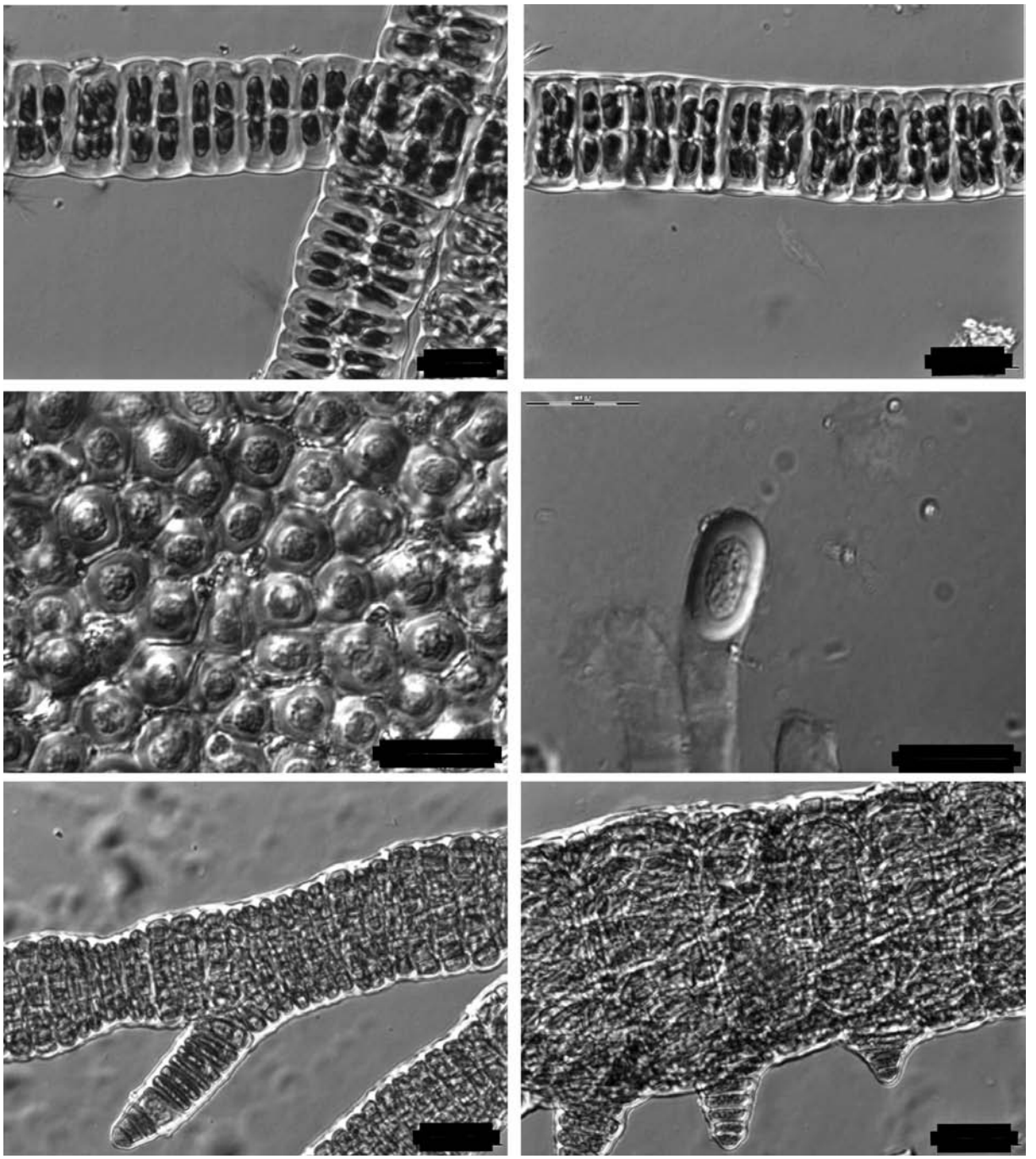

Figura 1. Arriba: filamentos multiseriados de *Bangia atropurpurea* en el río Alcanadre. Centro: vista superficial de una colonia de *Chroothece rupestris* y detalle de una célula con su pedúnculo en el río Arba de Riguel en Sádaba. Abajo: detalle de la corticación y de las ramas de *Compsopogon coeruleus* en el río Ebro en Pina de Ebro. La escala representa 50 μm en las imágenes de arriba y centro y 20 μm abajo. *Up: multiseriate filaments of* Bangia atropurpurea *in the Alcanadre River. Center: surface view of a colony of* Chroothece rupestris *and detail of a cell with its stem in the Arba de Riguel River in Sádaba. Down: detail of the cortication and the branches of* Compsopogon coeruleus *in the Ebro river at Pina de Ebro. The scale represents 50 microns (up and center) and 20 microns (down).*

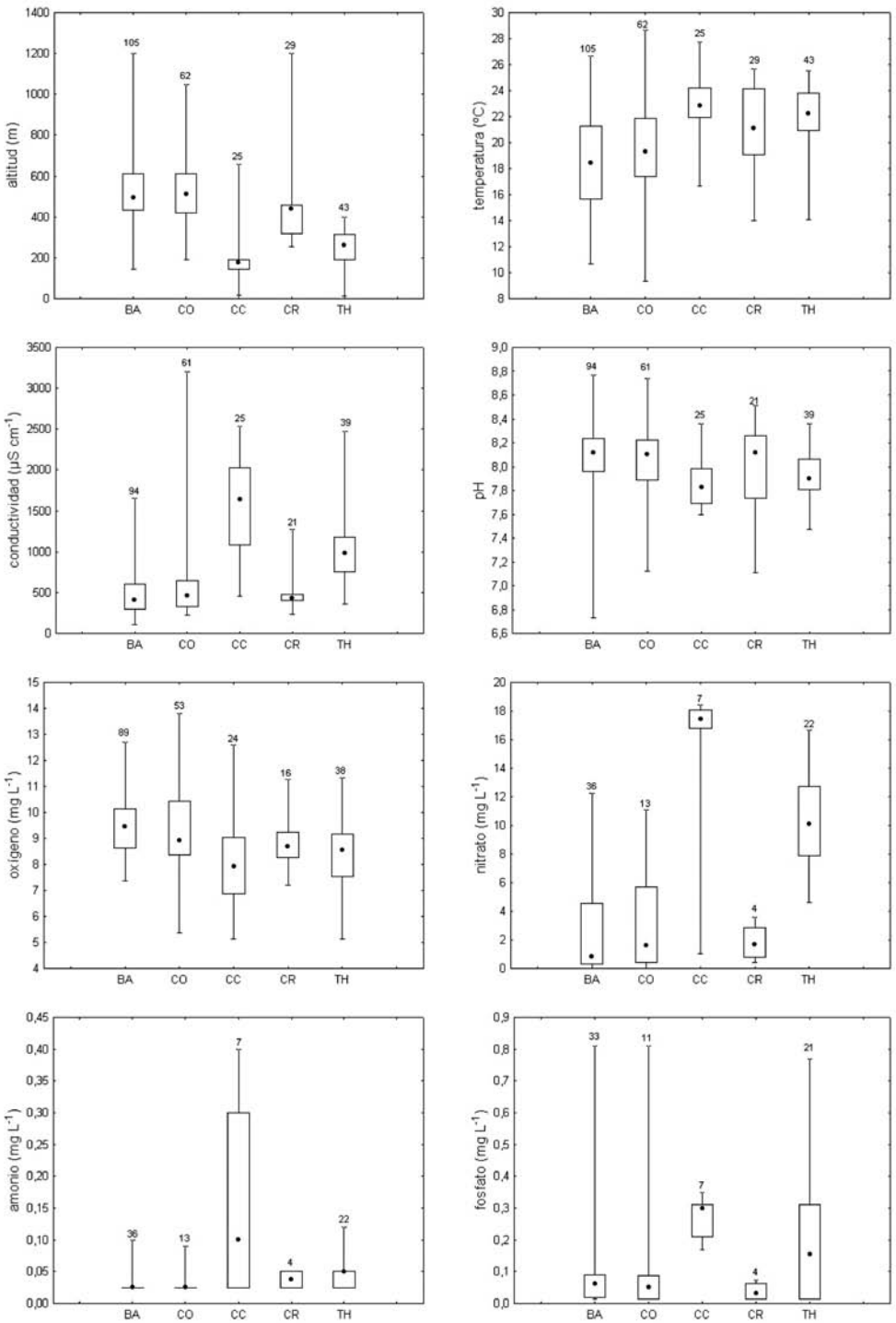

Figura 2. Gráficos comparativos de cajas y líneas de las cinco especies estudiadas para las ocho variables ambientales medidas. Los números encima de las líneas hacen referencia al número de casos (*n*). Los puntos representan la mediana; las cajas el 25 %-75 %; y la barra el Mín-Máx. BA: *Bangia atropurpurea*; CO: *Chroodactylon ornatum*; CC: *Compsopogon coeruleus*; CR: *Chroothece rupestris*; TH: *Thorea hispida*. *Comparative Box and whiskers graphs of the five studied species for the eight environmental variables measured. Numbers above whiskers mean the number of cases (*n*). Dots represent the median; boxes the 25 %-75 %; and bars Min-Max. BA:* Bangia atropurpurea*; CO:* Chroodactylon ornatum*; CC:* Compsopogon coeruleus*; CR:* Chroothece rupestris*; TH:* Thorea hispida.

Chroothece rupestris Hansgirg 1884 (Fig. 1) presentó una distribución reducida en la cuenca del Ebro. Se ha recolectado principalmente en ríos mineralizados de baja montaña calcárea, especialmente en los ríos Flumen y Alcanadre. Esta especie se encontró creciendo en sustratos diversos: sobre piedras sumergidas (epilítica), epífita de musgos y en taludes de las márgenes del río entre 15 y 50 cm por encima del nivel del agua. En general, las colonias presentaron formas semiesféricas o en tapetes, con un tamaño que osciló entre 3 mm y 3 cm.

Compsopogon coeruleus (Balbis) Montagne 1850 (Fig. 1) se halló en el eje principal del río Ebro y en tramos medios de los ríos Jalón (aguas abajo de la localidad de Alhama de Aragón, tramo con influencia de surgencias termales), Martín y Segre. Estos tramos presentaron un caudal elevado y una profundidad de la lámina de agua superior a los 50 cm. En el tramo medio del río Ebro, las aguas tuvieron una turbidez elevada durante la mayor parte del periodo de estudio, siendo difícil su visualización. Sin embargo, durante el año 2011, con caudales bajos y aguas más transparentes que en años anteriores, la especie se recolectó en nuevas localidades. Los talos filamentosos se encontraron creciendo sobre piedras del fondo entremezclados con *Cladophora* sp., o epífitos sobre *Cladophora* sp., *Potamogeton pectinatus* y *Myriophyllum* sp.

Thorea hispida (Thore) Desvaux 1818 presentó una distribución restringida, limitándose al río Ebro y a los tramos bajos de los ríos Arga, Aragón y Gállego. La especie prefiere aguas turbias de profundidad variable (desde 10 cm hasta más de 1 m) y se encontró creciendo sobre bloques y piedras cubiertas de sedimento, así como sobre el molusco invasor *Dreissena polymorpha*. En los años 2008 y 2011 la especie fue más frecuente, recolectándose ejemplares de longitud superior a 1 m. En ejemplares de longitud superior a 1.5 cm, se observaron estructuras reproductoras, detectándose la fase *Chantransia* sobre bloques y también epífita de *Potamogeton pectinatus*.

Respecto a la abundancia de las especies, en la mayoría de los casos cubrieron menos del 0.1 % de cauce, excepto en cinco ocasiones: *B. atropurpurea* alcanzó una cobertura del 50 % en el río Alcanadre en junio de 2010, mientras que *C. rupestris* cubrió entre el 1 y el 5 % del cauce en cuatro ocasiones, tres en junio (río Alcanadre en 2010 y 2011; río Arba de Riguel en 2009) y una en febrero (río Alcanadre) (Anexo 1. www.limnetica.net/internet).

Preferencias ecológicas

En la figura 2 se presentan los valores máximos y mínimos, así como el rango de óptimo desarrollo de las especies en la cuenca del Ebro, obtenidos durante el periodo de estudio para las 8 variables fisicoquímicas estudiadas (el intervalo comprendido entre los percentiles 25 y 75 incluye los valores ambientales medidos con más frecuencia para cada especie). *B. atropurpurea*, *C. ornatum* y *C. rupestris* son, en la cuenca del Ebro, especies típicas de montaña, con presencia en altitudes superiores a 1000 m y desarrollo óptimo entre 300-600 m, mientras que *C. coeruleus* y *T. hispida* se presentaron típicamente en tramos medios y bajos entre 100-300 m de altitud. Los valores de correlación entre las especies y la altitud fueron estadísticamente significativos excepto para *C. rupestris*, lo que indica una clara influencia de la altitud en la frecuencia de aparición de dichas especies (Tabla 1). De la misma forma, *B. atropurpurea*, *C. coeruleus* y *T. hispida* mostraron correlaciones significativas con la temperatura, la primera encontrando su óptimo en aguas más frías que las dos últimas.

En cuanto a la salinidad, todas las especies presentaron un rango amplio de tolerancia, pudiendo desarrollarse en aguas con valores de conductividad elevados, superiores a 1000 μS cm^{-1}. No obstante, el rango óptimo de desarrollo de *B. atropurpurea*, *C. ornatum* y *C. rupestris* se encuentra en torno a los 500 μS cm^{-1} de conductividad, mientras que *C. coeruleus* y *T. hispida* prefieren aguas con cierta salinidad, entre 1000-2000 μS cm^{-1}. Estas dos especies mostraron correlaciones positivas y significativas con la conductividad del agua (más frecuentes en aguas salinas), mientras que *B. atropurpurea* presentó una correlación negativa significativa (más frecuente en aguas dulces) (Tabla 1).

Respecto al pH, las cinco especies se desarrollaron óptimamente en aguas básicas, siendo *C.*

Tabla 1. Coeficientes de correlación de Spearman entre los parámetros ambientales y las especies de rodófitos estudiadas (***: $p < 0.001$, **: $p < 0.01$; *: $p < 0.05$). *Spearman's correlation coefficient between environmental parameters and species (***: $p < 0.001$, **: $p < 0.01$; *: $p < 0.05$).*

	Bangia atropurpurea		*Chroodactylon ornatum*		*Compsopogon coeruleus*		*Chroothece rupestris*	*Thorea hispida*	
altitud	0.3585	***	0.2491	***	−0.3649	***	ns	−0.4676	***
temperatura	−0.3240	***	ns		0.2820	***	ns	0.2562	***
pH	0.2853	***	ns		−0.2550	***	ns	−0.2274	***
conductividad	−0.3356	***	−0.1379	*	0.4113	***	ns	0.3623	***
oxígeno	0.2678	***	ns		−0.2069	**	ns	−0.1979	**
amonio	−0.3388	**	ns		0.2212	*	ns	0.3429	**
nitrato	−0.5160	***	ns		0.3997	***	ns	0.5023	***
fosfato	ns		ns		0.3567	**	ns	ns	

coeruleus y *T. hispida* más frecuentes en aguas con los valores de pH más bajos medidos. De hecho, ambas especies mostraron correlaciones negativas significativas con este parámetro (Tabla 1). Los valores óptimos de oxígeno disuelto para las cinco especies se encontraron por encima de 8 mg L^{-1}, si bien *C. ornatum*, *C. coeruleus* y *T. hispida* se han recolectado ocasionalmente en aguas con valores bajos, entre 5-6 mg L^{-1}. *C. coeruleus* y *T. hispida* presentaron correlaciones negativas significativas con el oxígeno disuelto, indicando su preferencia por aguas menos oxigenadas.

En cuanto a las condiciones tróficas, todas las especies, excepto *C. rupestris*, han sido recolectadas en aguas eutróficas en algún momento del periodo de estudio, lo que indica la tolerancia de estos rodófitos a la eutrofización (Fig. 2). *C. coeruleus* es la especie que presentó un desarrollo óptimo en las aguas más eutróficas, tolerando frecuentemente concentraciones de nitrato superiores a 16 mg L^{-1}, entre 0.1-0.3 mg L^{-1} de amonio y entre 0.2-0.3 mg L^{-1} de fosfato, y presentando además correlaciones positivas significativas con los tres nutrientes medidos (Tabla 1). En el extremo opuesto se encuentra *B. atropurpurea*, que mostró correlaciones negativas significativas con la concentración de nitrato y amonio, indicando su preferencia por las aguas oligomesotróficas. *C. ornatum* y *C. rupestris* presentaron también un desarrollo óptimo en aguas oligomesotróficas (Fig. 2), aunque no se obtuvieron correlaciones significativas con ninguno de los nutrientes analizados (Tabla 1). Finalmente, *T. hispida* presentó una alta correlación significativa con aguas ricas en nitrato, y aunque su correlación con el fosfato no fue significativa, se trata de una especie con desarrollo óptimo en aguas eutróficas con altos niveles de nitrato y fosfato (Fig. 2).

DISCUSIÓN

Bangia atropurpurea es una especie de aguas salobres y marinas, pero que ha invadido las aguas continentales como grandes ríos, canales y lagos de alta conductividad (Eloranta & Kwandrans, 2002; Sheath & Sherwood, 2002; Kwandrans & Eloranta, 2010). En España se trata de una especie escasamente citada, mientras que en Europa es un rodófito común en tramos altos de montaña (Sabater *et al.*, 1989). Nuestros resultados indican que en la cuenca del Ebro se trata de una especie frecuente, ya que ha sido recolectada en 66 localidades. En la cuenca del Ebro es propia de aguas dulces de montaña oligomesotróficas, aunque puede tolerar altos niveles de eutrofia en tramos inferiores (> 0.8 mg L^{-1} de fosfato).

Según Sheath (2003) *Chroothece* es un raro componente de arroyos, suelos húmedos y turberas. *C. rupestris* ocupa hábitats y sustratos diversos como rocas húmedas de aguas alcalinas en ambientes poco iluminados (Starmach, 1977; Eloranta *et al.*, 2011), superficies higropétricas de fuentes (Margalef, 1955) o epilíticas y sumergidas en arroyos alcalinos de corriente débil (Aboal, 1989; Sabater *et al.*, 1989). Se trata de una especie cuya distribución es poco conocida

en la península, por lo que se considera de interés su ampliación a 19 localidades más. Es conveniente comentar que la clave más actual disponible para la diferenciación entre las especies *C. rupestris* y *C. richtheriana* (Eloranta *et al*., 2011) utiliza el tamaño celular como carácter diagnóstico principal (15-18 μm para *C. richtheriana* y 9-15 μm *C. rupestris*) y secundariamente el hábitat (aguas algo salinas y sustratos limosos para *C. richtheriana*; aguas dulces y sustratos duros para *C. rupestris*). Las poblaciones estudiadas en la cuenca del Ebro presentaron un tamaño entre 10 y 25 μm, lo que incluiría a ambas especies, pero de acuerdo con los valores de conductividad propios de los arroyos calcáreos (485 μS cm^{-1} de valor medio, con un rango de 236-1268 μS cm^{-1}) y el tipo de hábitat (sumergida, epilítica) indica que se trataría de *C. rupestris*. Estas características biométricas y ecológicas de las poblaciones de la cuenca del Ebro son similares a las poblaciones de *C. rupestris* citadas para arroyos alcalinos de bajo caudal del norte y sur peninsular (9-20 μm de longitud celular y 250-1100 μS cm^{-1} de conductividad; Sabater *et al*., 1989; Aboal, 1989).

En el área de estudio, el epifitismo de *Chroodactylon ornatum* sobre *Cladophora* sp., los géneros epífitos acompañantes, así como los rangos ambientales, coinciden con lo indicado para distintas zonas geográficas (Starmach, 1966; Sheath & Morrison, 1982; Sheath & Sherwood, 2002; Wolowski *et al*., 2007). En la Península Ibérica es una especie eurioica que crece en aguas dulces o salinas (hasta 25 000 μS cm^{-1}), tanto oligotróficas como eutróficas (Aboal 1989; Sabater *et al*. 1989). En la cuenca del Ebro se ha recolectado principalmente en arroyos de agua dulce, aunque también ocasionalmente en tramos más salinos y eutróficos.

Compsopogon coeruleus es una especie ampliamente distribuida en arroyos cálidos templados y tropicales (Kwandrans & Eloranta, 2010). En la Península Ibérica las citas son escasas, creciendo principalmente en aguas salobres cerca de la costa o en ramblas salinas del sureste peninsular (Aboal, 1989; Sabater *et al*., 1989), y ocasionalmente en aguas dulces interiores (Busquets *et al*., 1985). En la cuenca del Ebro, *C. coeruleus* se ha recolectado en 16 localidades, correspondiendo con tramos medios caudalosos de aguas cálidas, alcalinas, mineralizadas y eutróficas.

En general, *T. hispida* es una especie que crece en ríos y arroyos europeos con aguas turbias y eutróficas, sobre piedras o arena (Eloranta & Kwandrans, 2007). Dos localidades ibéricas han sido previamente citadas para la especie: el Marjal de Pego-Oliva, Valencia (Tomás, 1981) y el río Ebro, Tarragona (Sabater *et al*., 1989). En el Marjal de Pego-Oliva, Egidos & Aboal (2003) citaron *Thorea violacea* en una surgencia de aguas subsalinas y cálidas (3500 μS cm^{-1}, 21.3 °C) y posteriormente se recolectó *T. hispida* en las partes bajas del río Bullent (Aboal, dat. inédt.). En el presente estudio se amplía su distribución a 22 sitios más, cuyas condiciones ambientales de aguas cálidas, alcalinas, bien oxigenadas, de conductividad elevada y alta concentración de nutrientes coinciden con las indicadas previamente por otros autores (Tomás *et al*., 1980; Sabater *et al*., 1989; Rott *et al*., 1999; Eloranta & Kwandrans, 2007).

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BIBLIOGRAFÍA

ABOAL, M. 1989. Contribución al conocimiento de las algas epicontinentales del SE de España II: Los rodófitos (*Rhodophyceae*). *Lazaroa*, 11: 115–122.

ÁLVAREZ-COBELAS, M. 1984. Catálogo de las algas continentales españolas. II. *Craspedophyceae*. *Cryptophyceae*, *Chrysophyceae*, *Dinophyceae*, *Euglenophyceae*, *Haptophyceae*, *Phaeophyceae*, *Rhodophyceae*, *Xanthophyceae*. *Acta Botanica Malacitana*, 9: 27–40.

BUSQUETS, J., M. C. PICADO & M. C. HERNÁNDEZ-MARINÉ. 1985. *Compsopogon coeruleus*

(Balbis) Montagne (*Rhodophyta, Bangiophyceae*). Ampliación de su área de distribución en la Península Ibérica. *Collectanea Botanica*, 16(1): 229–230.

CHE (Confederación Hidrográfica del Ebro) 2005. *Metodología para el establecimiento del Estado Ecológico según la Directiva Marco del Agua: Protocolos de muestreo y análisis*. Ministerio de Medio Ambiente.

CHE (Confederación Hidrográfica del Ebro). 2007. *Protocolo de desinfección de embarcaciones y equipos en masas de agua infectadas por mejillón cebra (Dreissena polymorpha)*. Ministerio de Medio Ambiente y Medio Rural y Marino.

EGIDOS, A. I. & M. ABOAL. 2003. *Thorea violacea* Bory de Saint-Vicent (*Thoreaceae, Rhodophyceae*) en una surgencia del Marjal de Pego-Oliva, Comunidad Valenciana. Nueva cita para la flora algal española. *Anales del Jardín Botánico de Madrid*, 60(1): 27–32.

ELORANTA, P. & J. KWANDRANS. 2002. Notes on some interesting freshwater *Rhodophyta* from Finland. *Algological Studies*, 105: 95–109.

ELORANTA, P. & J. KWANDRANS. 2004. Indicator value of freshwater red algae in running Waters for water quality assessment. *Oceanological and Hydrobiological Studies, International Journal of Oceanography and Hydrobiology*, 33(1): 47–54.

ELORANTA, P. & J. KWANDRANS. 2007. Freshwater Red Algae (*Rhodophyta*): Identification Guide to European Taxa, Particularly to Those in Finland. *Norrlinia*, 15: 1–103.

ELORANTA, P., J. KWANDRANS & E. KUSEL-FETZMANN. 2011. *Süsswasserflora von Mitteleuropa, Bd 7: Rhodophyta and Phaeophyceae*. Spektrum Akademischer Verlag. 155 pp.

KWANDRANS, J. & P. ELORANTA. 2010. Diversity of freshwater red algae in Europe. *International Journal of Oceanography and Hydrobiology*, 39: 161–169.

LEE, R. E. 1980. *Phycology*. Cambridge University Press, Cambridge.

LLIMONA, X., E. BALLESTEROS, M. BRUGUÉS, F. A. COMÍN, R. M. CROS, J. MOLERO, J. ROMERO, X. TOMÁS & F. TORRELLA. 1985. *Història Natural dels Països Catalans: Plantes Inferiors*. Tom 4. Enciclopèdia Catalana, S. A, Barcelona.

MARGALEF, R. 1955. Materiales para una flora de las algas del NE de España. VI. *Desmidiales, Rhodophyceae*. *Collectanea Botanica*, 4: 319–330.

MMA (Ministerio de Medio Ambiente). 2006. ORDEN MAM/3207/2006, de 25 de septiembre, por la que se aprueba la instrucción técnica complementaria MMA-EECC-1/06, determinaciones químicas y microbiológicas para el análisis de las aguas. ITC-MMA, EECC-1/06. *BOE*, 250: 36326–36440.

MORENO, J. L., C. NAVARRO & J. DE LAS HERAS. 2006. Propuesta de un índice de vegetación acuática (IVAM) para la evaluación del estado trófico de los ríos de Castilla-La Mancha: Comparación con otros índices bióticos. *Limnetica*, 25 (3): 821–838.

ROTT, E., P. PFISTER, H. VAN DAM, K. PALL, E. PIPP, N. BINDER & K. ORTLER. 1999. *Indikationslisten für Aufwuchsalgen. Teil 2: Trophieindikation und autökologische Anmerkungen, Bundesministerium für Land- und Forstwirtschaft.* Wasserwirtschaftkataster.

SABATER, S., M. ABOAL & J. CAMBRA. 1989. Nuevas observaciones de rodofíceas en aguas epicontinentales del NE y SE de España. *Limnetica*, 5: 93–100.

SHEATH, R. G. 2003. Red Algae. In: *Freshwater Algae of North America*. John D. Wehr & Robert G. Sheath (eds.): 197–224. Elsevier Science, San Diego, USA.

SHEATH, R. G. & M. O. MORISON. 1982. Epiphytes on *Cladophora glomerata* in the Great Lakes and St. Lawrence Seaway with particular reference to the red alga *Chroodactylon ramosum (= Asterocytis smargdina)*. *Journal of Phycology*, 18: 385–391.

SHEATH, R. G. & A. R. SHERWOOD. 2002. Phylum *Rhodophyta* (Red Algae). In: *The Freshwater Algal Flora of the British Isles: an identification guide to freshwater and terrestrial algae*. D. M. John, B. A. Whitton & A. J. Brook. (eds.): 123–143. Cambridge University Press, Cambridge.

STARMACH, K. 1966. *Homoeothrix crustacea* Woronichin i glony towarzyszace w górnym biegu Raby. *Acta Hydrobiologica*, 8: 309–320.

STARMACH, K. 1977. *Phaeophyta*-Brunatnice and *Rhodophyta*-Krasnorosty. *Flora Slodkowodna Polski* 14. Ed. Polska, Academia, Warszawa.

TOMÁS, X. 1981. *Thorea ramosissima* en un canal del litoral Valenciano. *Folia Botanica Miscellanea*, 2: 71–74.

TOMÁS, X., P. LÓPEZ, R. MARGALEF & F. A. COMÍN. 1980. Distribution and ecology of *Compso-*

pogon coeruleus (Balbis) Montagne (*Rhodophyta, Bangiophyceae*) in Eastern Spain. *Cryptogamie Algologie*, l(3): 179–186.

VAN DEN HOEK, C., D. G. MANN & H. M. JAHNS. 1995. *Algae. An Introduction to Phycology*. Cambridge University Press, Cambridge.

WOLOWSKI, K., J. KOWALSKA, & F. HINDÁK. 2007. *Chroodactylon ornatum* (*Rhodophyta, Porphyridiales*) occurring in Poland and Slovakia. *Biologia Bratislava*, 62(6): 646–649.

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The dominance of desmids in tropical monomictic lakes (SE Brazil)

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ABSTRACT

The dominance of desmids in tropical monomictic lakes (SE Brazil)

Disturbance is a concept used to explain the structure of communities. This concept emphasizes the effects of disturbance on the "break" of biomass stability in stable environments and the opportunities for the development of other species. The major disturbances in natural warm monomictic lakes include the contrasting effects of seasonal changes and long-term in thermal stability, as well as short-term changes in the mixing layer. This study investigated the effects of disturbance and abiotic environmental factors on the biomass of desmids, based on samples collected monthly from January 2002 through December 2006 in the limnetic region of two natural lakes, Carioca (small and shallow) and Dom Helvécio (large and deep), located in Rio Doce State Park, Minas Gerais State, southeast Brazil. At Lake Dom Helvécio, the extensive banks of aquatic macrophytes, the lake's dendritic shape and the period of clear water produced higher richness values and facilitated the occurrence of relatively large (maximum linear dimension > 20 μm) desmid species, primarily species belonging to *Staurastrum* and *Staurodesmus*. In contrast, less richness and a dominant group of relatively small (maximum linear dimension < 20 μm) desmid species, especially species belonging to the genus *Cosmarium*, were observed at Lake Carioca in conjunction with a sparse macrophyte cover. The lakes showed a seasonal thermal stratification characterized by high temperature (25-32 °C), thermal stability and higher desmid biomass. The stability of the epilimnetic desmid biomass was an indicator of the adaptive flexibility of the desmid species and the capacity of the biomass to recover after the mixing period.

Key words: Biomass stability, desmid, seasonal variability, thermal stability.

RESUMEN

Dominio de desmidiáceas en lagos monomíticos tropicales (SE Brasil)

La perturbación es un concepto utilizado para explicar la estructura de las comunidades, con énfasis en sus efectos sobre la "ruptura" de la estabilidad de la biomasa en ambientes estables y sobre las oportunidades para el desarrollo de nuevas especies. Entre las perturbaciones más importantes encontradas en lagos naturales monomíticos destaca el efecto de las variaciones estacionales y de largo plazo en la estabilidad térmica, así como los cambios a corto plazo en la profundidad de la mezcla. *En este trabajo se describe el efecto de la estratificación y mezcla del lago así como de otros factores abióticos sobre la biomasa de desmidiáceas en lagos tropicales. Los muestreos se realizaron mensualmente entre enero de 2002 y diciembre de 2006 en la región pelágica de dos lagos naturales monomíticos cálidos, Carioca (pequeño y poco profundo) y Dom Helvécio (grande y bastante profundo) ubicados en el Parque Estadual do Rio Doce (Estado Minas Gerais, SE Brasil). En el lago Dom Helvécio, las grandes acumulaciones de macrófitas acuáticas, la forma dendrítica del lago y el período de aguas transparentes influyeron de forma sinérgica en una mayor riqueza de especies y de tamaños grandes, mayores de 20 μm, siendo principalmente especies de* Staurastrum *y* Staurodesmus. *Sin embargo, en el Lago Carioca se observó una menor riqueza y el dominio de desmidiáceas de pequeño tamaño (< 20 μm), especialmente* Cosmarium. *Este último lago presentó también una cobertura escasa de macrófitas. Los lagos mostraron un patrón estacional caracterizado, durante la estratificación térmica, por el aumento de la temperatura (25-32 °C), estabilidad térmica y mayor biomasa de desmidiáceas.*

La estabilidad de la biomasa epilimnética fue indicativo de una flexibilidad adaptiva y recuperación después del período de mezcla.

Palabras clave: *Desmidiáceas, estabilidad de la biomasa, estabilidad térmica, variabilidad estacional.*

INTRODUCTION

Brook (1981) stated that the primary challenging questions associated with desmid ecology worldwide include the spatial and temporal distribution of the group and the multiplicity of factors controlling this distribution. In the past decades, relatively few advances have been made on these problems (Gerrath, 1993). Descriptive and experimental studies of desmid ecology in tropical regions are extremely scarce, and the need for such studies is great.

Desmids are important components of freshwater phytoplankton but very seldom play a major role in terms of biomass. For this reason, it is difficult to conduct investigations of characteristics related to the seasonal fluctuations of desmid biomass. Few desmid species have been found to occur in eutrophic environments (Gerrath, 1993; Coesel, 2001). Highly sensitive to environmental changes, desmids are considered good indicators of the ecological quality of water and of the saprobity level of different environments. They are commonly used as a tool for aquatic environment conservation and management (Coesel *et al*., 1978; Coesel, 2001; Ngearnpat & Peerapompisal, 2007).

Factors associated with the dominance of planktonic desmids

Desmids are frequently associated with periphytic and epiphytic communities, but the following hypotheses can explain the success of planktonic desmids in lakes and reservoirs:

1. Chemical factors: the distribution of desmids in continental waters is always related to the chemistry of the system (Woelkerling & Gough, 1976). Among the most important factors controlling desmid distributions are water conductivity, pH and CO_2 (Moss, 1972, 1973; Coesel, 1986, 1993; Coesel & Kooijman-van-Blokland, 1991; 1994).
2. Physical features associated with temperature and thermal stability: desmids are also extremely well adapted to turbulent environments (Brook, 1981) and have an optimal temperature between 25 and 30 °C (Coesel & Wandenaar, 1990). These organisms' population maxima in the Northern Hemisphere are usually associated with the summer and fall seasons (June-August or September-October) (Canter & Lund, 1966; Lund, 1971; Coesel & Kooijman-van-Blokland, 1991). In the tropics and subtropics, however, the occurrence of population maxima is associated with atelomixis, a singular characteristic of warm monomictic lakes in which thermal stability is noticeably high (Barbosa & Padisák, 2002; Tavera & Martínez-Almeida, 2005). Atelomixis occurs during the night and early morning, allowing the identification of total mixing during the entire dry period or partial mixing of the epilimnion during the rainy period (Barbosa & Padisák, 2002).
3. The association of desmids with underwater light conditions is underexplored: small desmids are adapted to low light intensities and eutrophic lakes, whereas large desmids dominate in oligotrophic lakes, where organic matter is scarce and light is more available (Coesel, 1982). The eutrophication process may decrease the abundance of desmids, and the structural diversity of desmids may also decrease as the richness of the aquatic vegetation decreases (Coesel, 1978).

The adaptive survival strategies of desmids are characterized by K tendencies (the tendency to act as S-strategists). Desmids are strong competitors in the epilimnion in the presence of

biomass stability during the thermal stratification period (Reynolds, 1984). Desmids were formerly included by Reynolds *et al*. (2002) in the **N** and **P** functional groups, both proposed for epilimnetic species living in low-latitude or temperate lakes during the summer. These groups show a strong reliance on physical mixing and, consequently, require a continuous or semi-continuous mixing layer. These groups are representative of shallow lakes whose mean depth is 2-3 m or slightly deeper. The groups are also representative of the epilimnion of stratified lakes, where mixing occurs. Padisák *et al*. (2009) suggested that group **N** should be restricted to temperate environments and that all low- latitude species should be transferred to group **Na** according to the Souza *et al*. (2008) criteria. This group would include all desmid species with small isodiametric cells such as those of *Cosmarium*, *Staurodesmus* and *Staurastrum*. It would also include filamentous individual organisms (e.g., *Teilingia* and *Spondylosium*) that are sensitive to mixing and typical of oligo-mesotrophic lakes subjected to atelomixis. Species of functional group **P** have very similar requirements to those of group **N.** However, they are able to live in more eutrophic waters and tolerate low light availability.

The present study aimed to describe the effects of different disturbance events and abiotic factors on desmid biomass in two warm monomictic lakes: Dom Helvécio (large and deep) and Carioca (small and shallow).

METHODS

Study area

Rio Doce State Park is located approximately in the middle of the Rio Doce Lake District, southeast Brazil (19°29′S, 42°28′W) (Meis & Tundisi, 1997). Among the lakes in Rio Doce State Park are Lake Carioca, which is mesotrophic, shallow and round-shaped, with a surface area of 0.14 km^2 and a maximum depth of 11.8 m, and Lake Dom Helvécio, which is oligotrophic and dendritic in shape, with a surface area of 5.27 km^2 and a maximum depth of 39.2 m

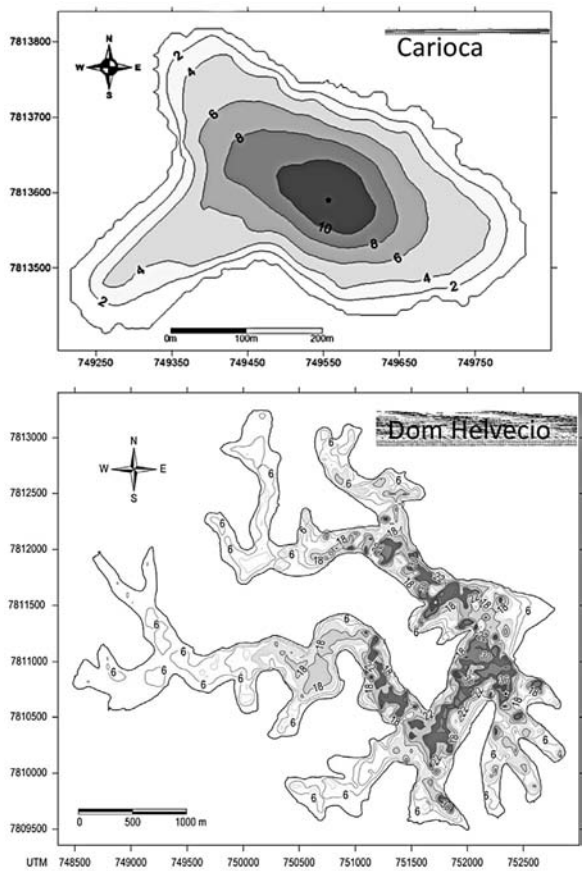

Figure 1. Bathymetry of Lakes Carioca and Dom Helvécio, southeast Brazil, with depth isolines (Bezerra-Neto & Pinto-Coelho 2008; Bezerra-Neto *et al.* 2010). *Batimetría de los lagos Carioca y Dom Helvécio, sureste de Brasil.*

(Bezerra-Neto & Pinto-Coelho, 2008). Both lakes are warm monomictic, are stratified between September and April and are basically isothermal from May through August (Henry & Barbosa, 1989) (Fig. 1).

Sampling

Monthly samples were collected over a five-year period (January 2002-December 2006) in the limnetic region of the two lakes. The percentage of incident light was estimated from Secchi disk measurements (Cole, 1994). Vertical profiles of water temperature, pH and electrical conductivity were obtained with a multiparameter probe (Horiba® sensor 220 model U 22). The

stability of stratification was calculated according to Idso (1973). The mixing zone (Z_{mix}) was identified from the temperature profiles, and the Z_{eu} : Z_{mix} ratio was used as a light availability index (Jensen *et al*., 1994).

Nutrients and phytoplankton samples were collected monthly with a van Dorn bottle from the subsurface (100 % incident light), the Secchi disk depth (15 % incident light), three times the Secchi disk depth (1 % incident light) and the aphotic zone. Total phosphorus (TP), soluble reactive phosphorus (SRP → PO_4^{-3}), total nitrogen (TN) and dissolved inorganic nitrogen (DIN → NO_3^-, NO_2^-, NH_4^+) were measured according to Golterman *et al*. (1978) and Mackereth *et al*. (1978), respectively. The DIN:SRP molar ratio was used to evaluate possible nitrogen or phosphorus limitation, where: DIN:SRP < 13 indicates limiting nitrogen, DIN:SRP >50 indicates limiting phosphorus, and 13 < DIN:SRP < 50 indicates that neither of these nutrients is limiting (Morris & Lewis, 1988; Kosten *et al.*, 2009).

For specific and infra-specific identification of the taxa, classical taxonomic literature was consulted (Teiling, 1967; Croasdale, *et al*., 1983; Prescott, 1975; Prescott *et al*., 1972, 1975, 1977, 1981, 1982).

Desmids were quantitatively estimated according to Utermöhl (1958). The sedimentation time determinations followed Lund *et al*. (1958). The biomass and the surface:volume ratio (S:V) were obtained in terms of geometric shapes (Hillebrand *et al*., 1999), assuming that the unit of fresh weight, expressed in terms of mass, was 1 mm^3 L^{-1} = 1 mg L^{-1} (Wetzel & Likens, 2000).

Desmid species were classified according to the following criteria:

1. Frequency of occurrence (%):

   rare ($F \leq 10$ %),
   common (10 % < $F \leq 50$ %) or
   very common ($F > 50$ %).

2. Habit or life form: simple (*Cosmarium*), filamentous (intact filaments of *Spondylosium* and *Teilingia*), complex (*Staurastrum*), intermediate (*Staurodesmus*) or cylindrical (*Closterium* and *Pleurotaenium*).

3. MLD, Maximum Linear Dimension (adapted from Sieburth *et al*., 1978): class 1 (<10 μ m, ultraplankton), class 2 (11-20 μ m, nanoplankton), class 3 (21-50 μ m, microplankton) or class 4 (>50 μ m, net plankton).

4. Functional groups (*sensu* Reynolds *et al*., 2002; Padisák *et al*., 2009).

A multivariate descriptive analysis of the five-year abiotic and biotic variables was performed with canonical correspondence analysis (CCA). A Monte Carlo test was used, and the variability of the data was explained in terms of the canonic coefficient (Ter-Braak, 1986). The Pearson correlation coefficient (r) resulting from the relationship between the ordination values was used, as well as the individual variables for the ordination (McCune & Mefford, 1997).

For a descriptive multivariate analysis of the variables at a monthly scale, the thermal stability, Z_{mix}, Secchi disc depth and light attenuation coefficient were selected from a principal component analysis (PCA) (Barbosa *et al.*, 2012). The biomass was integrated arithmetically ($mm^3 \cdot m^{-2}$) including monthly values at each depth. The arithmetic integration of the water column was performed in terms of the sum of the area of each stratum (depth), composed of two axes: the depth of the water column and the other variable to be integrated (biomass). The programs used were FITOPAC (Shepherd, 1996), for the transformation of data, and PC-ORD for Windows, version 5.0 (McCune & Mefford, 1997).

The average depth of desmid occurrence (Pm_i), i.e., the depth at which the mean biomass (mg L^{-1}) i is located in the water column, was calculated with the Dini *et al*. (1993) formula, used to describe the daytime vertical migration (DVM) of zooplankton. In this study, this formula was used to assess the difference in the maximum biomass between the stratification and mixing periods. Different months were compared with a two-tailed t test. These analyses were performed with Statistica software, version 5.1 (Statsoft).

RESULTS

During stratification, significant differences in desmid biomass were observed between depths in Lake Dom Helvécio (one-way Anova, $p = 0.001$, $n = 60$). The maximum value of desmid biomass (4.97 mg L^{-1}) was measured during stratification in January 2002, at 10 % light incidence. During the mixing period, maximum values (3.99 mg L^{-1}) were measured in May 2002 at 1 % light incidence (Figs. 2a-b). In both lakes, the three most representative

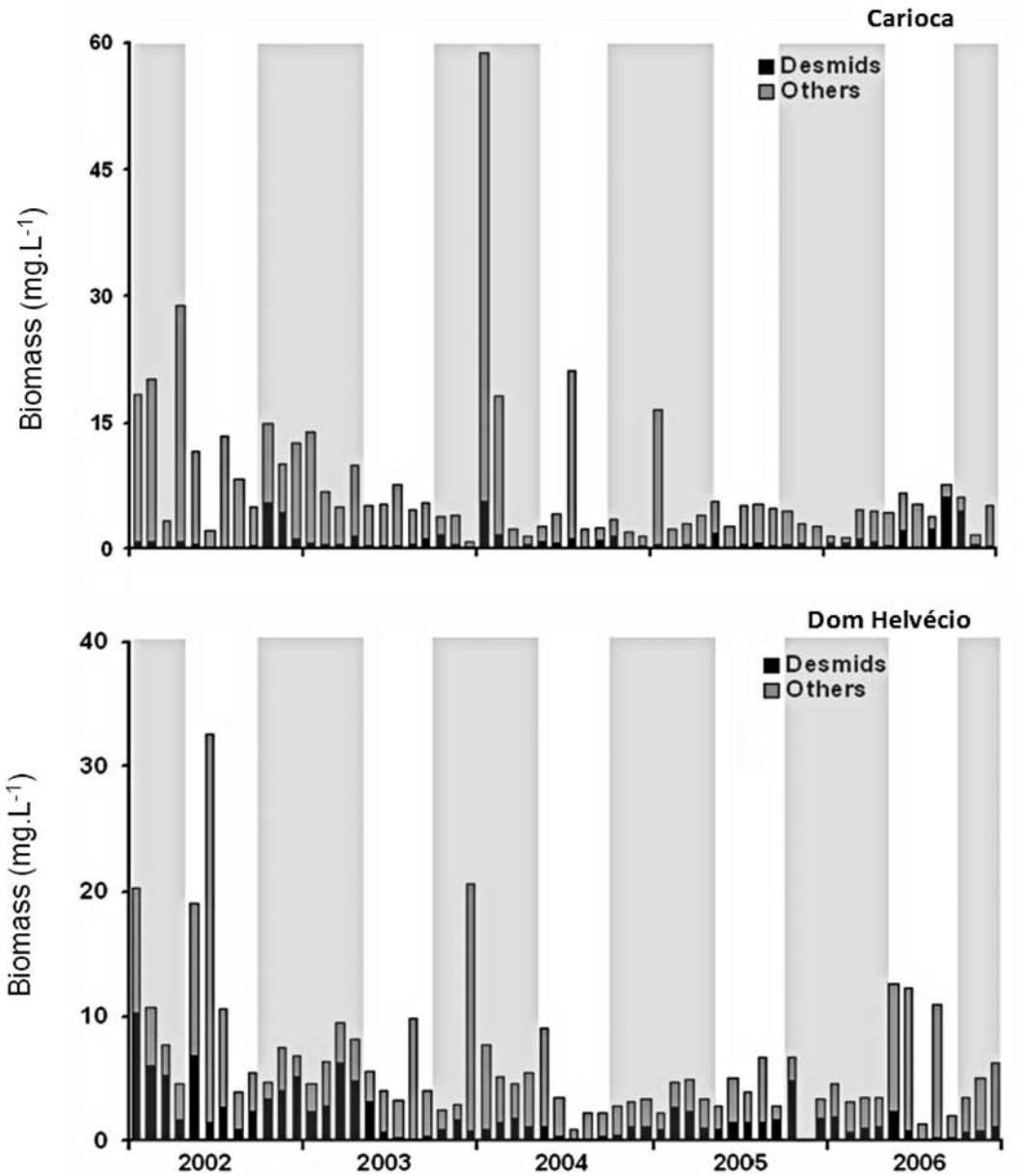

Figure 2. Biomass of desmids and other algal classes (mg L^{-1}) at Lakes Carioca and Dom Helvécio during 2002-2006. Clear spaces: non-sampling period. Grey stripes correspond to stratification periods. *Biomasa (mg L^{-1}) de las desmidiáceas y otras clases de algas en los lagos Carioca y Dom Helvécio durante el período 2002-2006. Espacios en blanco: periodo sin muestreo. Columnas grises corresponden a los períodos de estratificación.*

genera (maximum 90 % of total desmid biomass) were *Cosmarium*, *Staurastrum* and *Staurodesmus* (Fig. 3). *C. asphaerosporum* var. *strigosum* was the dominant species (> 50 %) along the entire temporal scale.

During stratification at Lake Carioca, coexistence of *C. asphaerosporum* var. *strigosum* (40 %), *S. panduriforme* (13 %), *T. granulata* (10 %), *C. bioculatum* (7 %) and *S. iotanum* var. *iotanum* (5 %) was detected at depths up to 1 %

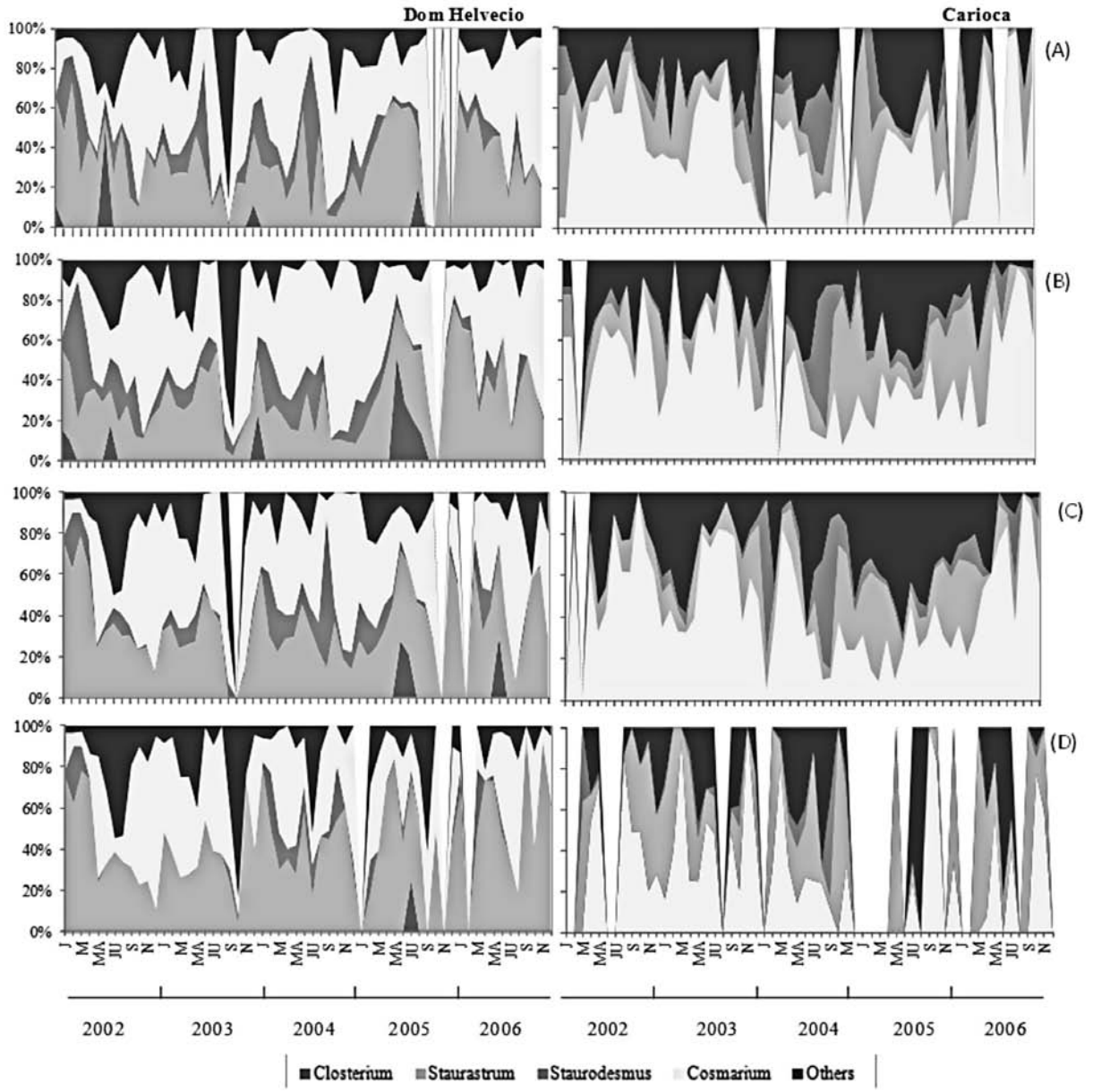

Figure 3. Relative abundance (%) of the major desmid genera at four depths in Lakes Carioca and Dom Helvécio during 2002-2006. Sub-surface (A), 10 % light incidence (B), 1 % light incidence (C) and aphotic zone (D). Clear spaces: non-sampling period. *Abundancia relativa (%) de los principales géneros de desmidiáceas a lo largo de las cuatro profundidades de muestreo en los lagos Carioca y Dom Helvécio durante el período 2002-2006. Sub-superficie (A), 10 % de incidencia de luz (B), 1 % de incidencia de luz (C) y zona afótica (D). Espacios en blanco: período sin muestreo.*

light incidence (3-6 m), corresponding to 80 % of the desmids' total biomass (Table 1).

The maximum biomass of *C. asphaerosporum* var. *strigosum* (3.37 mg L^{-1}) was identified in September 2006 at the 1 % light incidence depth. The molar dissolved ratio was relatively high (DIN:SRP = 102), and the maximum value recorded for NO_3^- was 46 µg L^{-1}. In January 2004, *C. bioculatum* (1.15 mg L^{-1}), *T. granulata* (2.16 mg L^{-1}) and *S. panduriforme* (0.86 mg L^{-1})

Table 1. Classification of desmids of Lakes Carioca and Dom Helvécio (MLD: Size structure, FG: Functional Group,*: occurrence only at Lake Dom Helvécio, **: species with biomass (mg L^{-1}) below 1 %). *Clasificación de las desmidiáceas de los lagos Carioca y Dom Helvécio: (MLD: Máxima Dimensión Linear, FG: Grupo Funcional, *: ocurrencia restringida al lago Dom Helvécio, **: especies con biomasa (mg L^{-1}) menor a 1 %).*

			Carioca		Dom Helvécio	
Species	**Abreviation**	**Form**	**MLD**	**FG**	**MLD**	**FG**
Closterium aciculare West	Clac	Cylindrical	4	**	4	P
C. moliniferum Ehr. *ex* Ralfs	Clmol	Cylindrical	*	**	4	**
Cosmarium asphaerosporum Nordst. var. *strigosum* Nordst.	Cosasp	Simple	1	Na	1	**
C. bioculatum Bréb.	Cosbio	Simple	1	**	1	**
C. contractum Kirchn. var. *contractum*	Coscont	Simple	2	**	2	Na
C. moniliforme (Turp.) Ralfs	Cosmon	Simple	1	**	1	**
Cosmarium regnelii Wille	Cosregn	Simple	1	**	1	**
Pleurotaenium trabecula (Ehr.) *ex* Näg.	Pleurotr	Cylindrical	*	**	4	**
Spondylosium panduriforme (*Heim*.) *Teil*.	Sponpan	Simple	1	Na	1	**
Staurastrum erostellum West & West	Staeros	Complex	-	-	2	**
S. forficulatum Lund.	Stafor	Complex	3	**	3	**
S. gemelliparum Nordst.	Stagemel	Complex	2	**	2	**
S. gracile Ralfs	Stagrac	Complex	-	-	3	**
S. hirsutum (Ehr.) Ralfs	Stahirs	Complex	*	**	2	**
S. iotanum Wolle var. *iotanum*	StaiotG	Complex	2	**	2	**
S. iotanum Wolle Morphotype 1	StaiotG	Complex	2	**	2	**
S. laeve Ralfs	Stalae	Complex	2	**	2	**
S. laeve Morphotype 1	StalaeP	Complex	2	**	2	**
S. leptocladum Nordst. var. *cornutum* West & West	Stalepto	Complex	*	**	4	**
S. muticum (Bréb.) *ex* Ralfs var. *muticum*	Stamut	Complex	4	**	4	**
S. rotula Nordst.	Starot	Complex	2	Na	2	Na
S. smithii (G.M. Smith) Teil.	Stasmi	Complex	*	**	1	Na
Staurastrum sp.	Stasp	Complex	3	**	3	**
S. subcruciantum Cook & Wills	Stsubc	Complex	2	**	-	**
S. subunguiferum Fritsch & Rich	Stasubun	Complex	2	**	2	**
S. taylorii Grönbl.	Statayl	Complex	1	**	1	Na
S. tetracerum (Kütz.) Ralfs *ex* Ralfs var. *tetracerum* f. *tetracerum*	Sttetet	Complex	1	**	1	**
S. tetracerum var. *tetracerum* (Kütz.) Ralfs *ex* Ralfs var. *tortum* (Teil.) Borge	Statetor	Complex	1	**	1	**
S. trifidum Nordst. var. *glabrum* Lag.	Statrif	Complex	2	**	**	**
S. wolleanum Butl.	Stawol	Intermediate	-	**	4	**
S. convergens (Ehr. *ex* Ralfs) Teil.	Stocon	Intermediate	3	**	3	**
S. crassus (West) Flor.	Stocra	Intermediate	2	Na	2	Na
S. cuspidatus (Bréb.) Teil.	Stocusp	Complex	-	-	2	**
S. dejectus (Bréb.) Teil.	Stodej	Intermediate	3	**	3	**
S. incus (Bréb.) Teil. var. *ralfsii* (W. West) Teil.	StoinR	Intermediate	1	**	1	**
S. jaculiferus (West & West) Teill.	Stojacu	Intermediate	*	**	3	**
Teilingia granulata (Roy & Biss.) Bourr.	Teilgra	Simple	1	Na	1	Na
Teilingia granulata (Roy & Biss.) Bourr. Morfotipo 1	TeilgraG	Simple	1	Na	1	**
Xanthidium conccinum Arch.	Xconc	Complex	**	**	1	**
Xanthidium sp.	Xsp	Complex	**	**	2	**

maximum values were identified at the 1 % light incidence depth. The Z_{eu} : Z_{mix} = 1.3 ratio indicated a clear epilimnion (Fig. 4).

During the mixing period, however, *C. asphaerosporum* var. *strigosum* (0.9-3.4 mg L^{-1}) maximum values were measured at the Z_{eu} from June to September 2006. During this period, the $N\text{-}NO_3^-$ concentration at Z_{eu} varied between 2.6 and 20.1 mg L^{-1}, and $N\text{-}NH_4^+$ varied from 115.8 to 77.2 μg L^{-1}. Their molar ratio (DIN:SRP) was always > 50 if Z_{mix} reached the lake bottom.

The analysis of the intermediate and complex desmid forms (*Staurastrum* and *Staurodesmus*) with MLD < 20 μ m showed that the maximum values were recorded between the surface and the 1 % light incidence depth during the stratification period, corresponding to the epilimnetic and metalimnetic layers. *S. crassus* (maximum 0.67 mg L^{-1}), *Staurastrum* sp. (maximum 0.45 mg L^{-1}), *S. incus* var. *ralfsii* (maximum 0.43 mg L^{-1}), *S. iotanum* var. *iotanum* (maximum 0.35 mg L^{-1}) and *S. laeve* (maximum 0.29 mg L^{-1}) were the most common species during the stratification period.

Rarely occurring species (complex forms) with MLD > 20 μm were best documented below the 10 % light incidence depth. Among these species were *S. rotula* (1.34 mg L^{-1}, May 2005), *S. dejectus* (0.89 mg L^{-1}, February 2004), *S. trifidum* (0.48 mg L^{-1}, September 2006), *S. cuspidatus* (0.36 mg L^{-1}, August 2006) and *S. gemelliparum* (0.25 mg L^{-1}, December 2002). However, in contrast to the observations at Lake Dom Helvécio, morphologically complex forms (MLD > 20 μm) showed maximum biomass values very similar to those of the rarely occurring forms.

At Lake Dom Helvécio, the co-occurrence of *C. contractum* (22 %), *S. taylorii* (17 %), *C. asphaerosporum* var. *strigosum* (15 %), *S. crassus* (13 %), *T. granulata* (6 %) and *S. laeve* (5 %) was recorded during stratification at the 1 % light incidence depth (approximately 9 m).

A maximum of *C. asphaerosporum* var. *strigosum* (1.09 mg L^{-1}) was detected in May 2002 at the 1 % light incidence depth, with Z_{mix} located at a depth of 10 m and Z_{eu} at a depth of 9 m (Z_{eu} : Z_{mix} = 0.9). In November and December 2003, maximum values for *C. contractum* (1.39-2.47 mg L^{-1}) were found between the surface and the 1 % light incidence depth. During these months, the Z_{eu} : Z_{mix} ratio was 0.95, and the concentrations of both $P\text{-}PO_4^{-3}$ (2.2-3.5 μg L^{-1}) and $N\text{-}NO_3^-$ (3.6-12 μg L^{-1}) were comparatively low. Maxima for *S. panduriforme* (0.33 mg L^{-1}) and *T. granulata* (0.54 mg

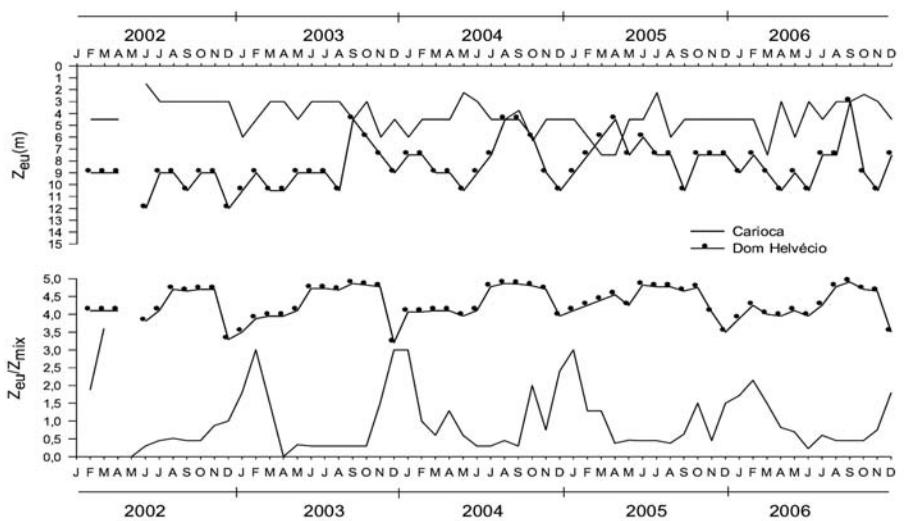

Figure 4. Monthly variation in the euphotic zone and the euphotic zone:mixing zone ratio in Lakes Carioca and Dom Helvécio during the study period (2002-2006). *Variación mensual de la zona eufótica y de la relación zona eufótica:zona de mezcla en los lagos Carioca y Dom Helvécio durante el período de estudio (2002-2006).*

L^{-1}) were observed between the surface and the 1 % light incidence depth (March-May 2003).

The analysis of the intermediate and complex forms (*Staurastrum* and *Staurodesmus*) with an MLD up to 20 μm showed that the maximum values occurred during the stratification period at Z_{mix} (Z_{mix} = 12 m). Among the commonly occurring species, *S. crassus* (maximum 2.5 mg L^{-1}, February-March 2002), *S. taylorii* (maximum 2.25 mg L^{-1}, January-February 2002), *S. laeve* (maximum 0.35 mg L^{-1}, May 2002) and *S. tetracerum* var. *tetracerum* f. *tetracerum* (maximum 0.64 mg L^{-1} at Z_{aph}, October 2005) were noteworthy. The commonly occurring species with MLD > 20 μm also had their greatest occurrence between the surface and the 1 % light incidence depth, occasionally during the mixing period (June-September) and frequently during stratification (October-February). Among the commonly occurring species, *S. wolleanum* (0.37 mg L^{-1}, January 2006), *S. rotula* (0.35 mg L^{-1}, December 2002), *S. muticum* (0.1 mg L^{-1}, January 2005) and *S. gemelliparum* (0.03 mg L^{-1}, January 2002) were noteworthy. Rarely occurring species were also identified, including *S. leptocladum* (0.13 mg L^{-1}, October 2002) and *S. dejectus* (0.02 mg L^{-1}, April and July 2003).

The common planktonic species included *C. aciculare* and *C. moniliferum*. The maximum values of *C. aciculare* were observed in July 2002 (1.01 mg L^{-1}) and June-September 2005 (0.08-0.30 mg L^{-1}), always at the lake subsurface. This species was also present during the stratification period (0.2-0.4 mg L^{-1}).

Effects of thermal stability and underwater light climate on desmid biomass

The average depth of biomass (mg L^{-1}) for functional group **Na** (Fig. 5) indicated that typical desmid epilimnetic communities occurred at both Carioca (MLD < 20 μm) and Dom Helvécio (MLD > 20 μm) Lakes. A comparison between the data for the stratification period and the data for the mixing period for both lakes noted no significant differences ($p > 0.05$). The only differences occurred in the quantitative decrease in species biomass (≤ 80 %) during the mixing period.

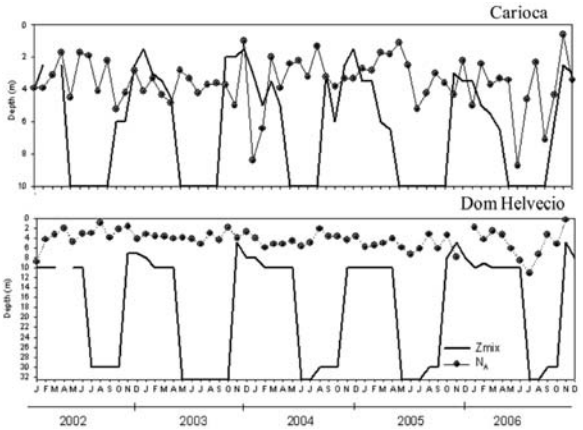

Figure 5. Annual variation in the mean depth of the biomass (mg L^{-1}) of functional group **Na** at Lakes Carioca (A) and Dom Helvécio (B) during 2002-2006. Continuous lines: mixing layer. *Variación anual de la profundidad promedio del grupo funcional **Na** en los lagos Carioca (A) y Dom Helvécio (B) durante el período 2002-2006. Línea continua: zona de mezcla.*

A Canonical Correspondence Analysis performed with 39 species and 6 abiotic factors resulted in eigenvalues of $\lambda = 0.13$ and $\lambda = 0.036$ for axes 1 and 2, respectively. The species-environment correlations for axes 1 (0.89) and 2 (0.685) were high, indicating a strong correlation between the distribution of the desmid species and the environmental variables used for ordination (Table 2). A Monte Carlo permutation test showed that axes 1 and 2 were both statistically significant, as simultaneously identified by CCA ($p < 0.01$), indicating an absence of random events and the presence of relationships between environmental variables and desmid species (Fig. 6). The sampling units for Lake Dom Helvécio formed a group to the right of axis 1, and the units for Lake Carioca formed a group to the left of this axis. The canonical coefficients for axis 1 and axis 2 showed that thermal stability and the Secchi disk depth were the most important environmental variables for the ordination.

The canonical coefficients indicated that thermal stability ($r = -0.63$), the mixing layer ($r = -0.59$) and the light attenuation coefficient ($r = 0.35$) were the most important factors associated with the axis 1 ordination. Axis 2 presented higher canonical coefficients for the Secchi disk depth ($r = -0.77$), thermal stability ($r = 0.54$), electrical conductivity ($r = -0.46$),

Table 2. Canonical coefficient and intra-set correlations from a Canonical Correspondence Analysis (CCA) between environmental variables and species characteristic of Lakes Dom Helvécio and Carioca. *Coeficiente Canónico y correlación intra-set del Análisis de Correspondencia Canónica (ACC) entre las variables ambientales y las especies descriptivas de los lagos Dom Helvécio y Carioca.*

Variable		Canonic coefficient Axis 1	Axis 2
Electrical conductivity	EC	−0.14	−0.46
$N-NH_4$	NH4	0.09	0.3
CO_2	CO2	−0.03	0.17
Secchi disk	DS	0.09	−0.77
Light attenuation coefficient	K0	0.35	−0.19
Thermal stability	S	−0.63	0.54

$N\text{-}NH_4^+$ ($r = 0.3$) and the light attenuation coefficient ($r = -0.19$) (Table 2).

A tendency toward the formation of species groups that were representative of the stratification and mixing periods was detected at Lake Dom Helvécio, but no clear-cut separation of such groups occurred at Lake Carioca. On the negative side of axis 1, the sampling units for Lake Dom Helvécio were grouped in association with the following species: *C. aciculare* ($r = -0.91$), *C. contractum* ($r = -0.56$), *S. laeve* ($r = -0.11$) and *S. smithii* ($r = -0.16$). This

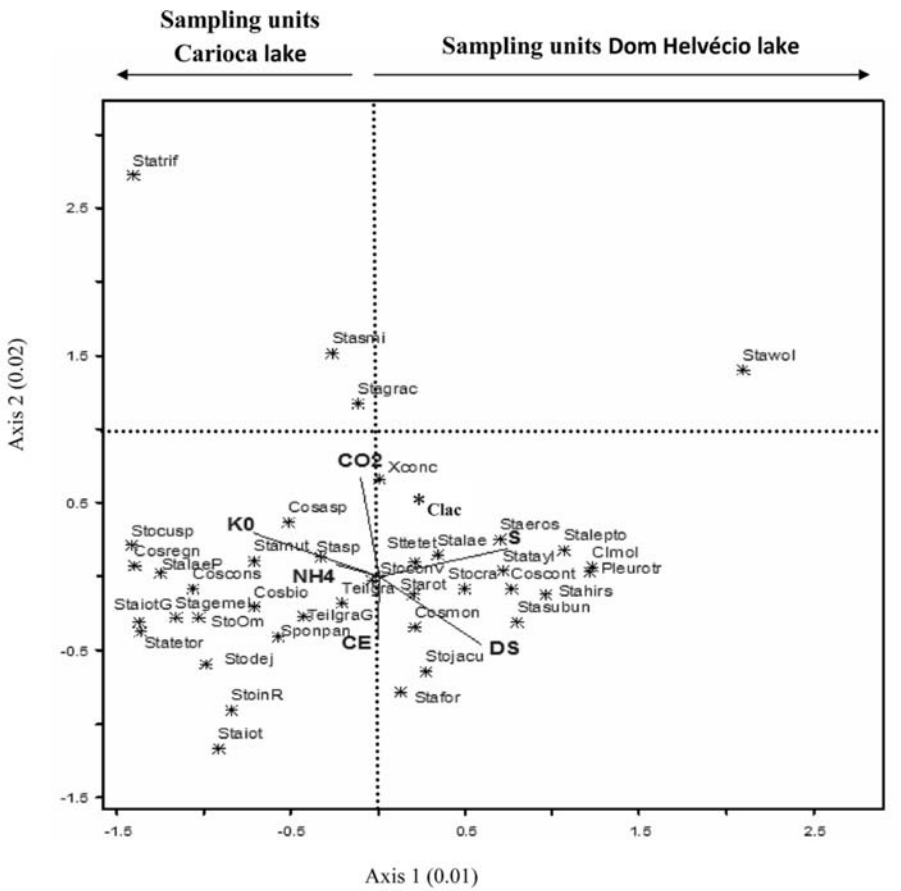

Figure 6. CCA ordination diagram of the sampling units in relation to the desmid species (mg L^{-1}) at Lakes DomHelvécio and Carioca and the principal abiotic variables during 2002-2006. (Abbreviations of species in Table 1). *Ordenación del diagrama del ACC de las unidades muestrales en relación a las especies de desmidiáceas (mg L^{-1}) en los lagos Dom Helvécio y Carioca y las principales variables abióticas durante el período 2002-2006 (Abreviaciones de las especies en Tabla 1).*

species group showed a close relationship with thermal stability ($r = -0.68$). The other group combined the species *P. trabecula* ($r = -0.93$), *S. hirsutum* ($r = -0.85$), *S. rotula* ($r = -0.71$), all associated with Z_{mix} ($r = -0.61$) and water transparency ($r = -0.57$).

On the positive side of axis 1, species characteristic of Lake Carioca were grouped together based on the high light attenuation coefficient ($r = 0.42$) and N-NH_4^+ ($r = 0.33$) concentration. The highest correlations were found for *S. iotanum* var. *iotanum* ($r = 0.77$), *S. laeve* ($r = 1.17$) and *S. incus* var. *ralfsii* ($r = 0.98$). In the central portion of the ordination, a group was observed corresponding to the common species association between the two lakes and the months.

DISCUSSION

The long stratification period (202 days) associated with high temperatures (>25 °C) and high thermal stability, acidic water (pH < 7), low light availability, high availability of nutrients and structural changes at a lower Z_{mix} (Lake Carioca: 1.5-6.5 m) or larger scale (Lake Dom Helvécio: 5-12 m) significantly affected the biomass of desmids, as indicated by the CCA results. Previous studies have shown that in certain tropical lakes, the largest desmid populations occur during the period of thermal stratification (summer). Furthermore, the growth of these algae has been detected in the epilimnion of tropical lakes (Barbosa & Padisák, 2002; Tavera & Martínez-Almeida, 2005; Souza *et al*., 2008).

The adaptation of desmids to turbulent environments (Brook, 1981) requires an optimum temperature between 25 and 30 °C (Coesel & Wandenaar, 1990) and the ability to tolerate nutritional deficits (Reynolds *et al.*, 2002), conditions observed in the epilimnion of lakes in general. Yinxin & Minjuan (2005) reported that *Cosmarium*, *Staurastrum*, *Staurodesmus* and *Closterium* (> 70 % of the density and biomass) were dominant at six stations during three seasons (spring, summer and autumn) but not during the winter. Desmid populations associated with increased surface temperature have also been previously identified in tropical lakes (Clarke, 2008), particularly if the increase in temperature is associated with thermal stratification.

A greater biomass of desmids was found in the epilimnion of the lakes, and the physical stability of the lakes had a substantial influence on the long-term maintenance of this biomass. However, the biomass values decreased drastically (≥ 70 %) during the mixing period. Consequently, the desmid community would show a low adaptive flexibility and persistence in unstable pelagic lakes (i.e., during the mixing period), thus reinforcing the hypothesis that desmids exhibit high ecological resilience in the face of significant fluctuations between the stratification and mixing periods. Despite their preference or affinity for the thermal stratification period, *C. asphaerosporum* var. *strigosum*, *S. taylorii* and *C. aciculare* also occurred during the mixing period.

Most of the desmids collected (MLD < 20) belonged to functional group **Na**. Species of functional group **P**, which show requirements very similar to those of group **N**, were also found in both lakes and were represented by *C. aciculare*, which attained maximum values at Lake Dom Helvécio during the mixing period. *Closterium* species were very well adapted to the higher levels of organic matter and the lower levels of light intensity in the monomictic lakes (Brook & Williamson, 1988). These species also gained a strong adaptive benefit from their physiological affinity to the mixing conditions (Pollingher, 1986) in the monomictic lakes.

The Z_{eu} : Z_{mix} ratio identified a well-lighted epilimnion in Lakes Carioca ($0.9 < Z_{eu}/z_{mix} < 3.6$) and Dom Helvécio ($0.9 < Z_{eu}/Z_{mix} < 1.8$). This result suggested that desmid communities are most likely not light limited. Lower transparency (0.5-2.5 m) and greater light attenuation (0.85-3.4 m) were observed in Lake Carioca due to its high content of pigmented organic matter (Bezerra-Neto *et al*., 2006). Eutrophic swamps and wetlands tend to be dominated by small desmids with short generation times (e. g., *Cosmarium granatum* Brébisson, *Cosmarium subgranatum* Nordstedt and *Cosmarium subtumidum* Nordstedt), whereas oligotrophic lakes are dominated by large desmids (e. g., *Micraste-*

rias, *Tetmemorus* and *Pleurotaenium*) (Coesel, 1982), situations similar to those observed in Lakes Carioca and Dom Helvécio, respectively. Studies of the effect of light on phytoplankton morphology in wetlands have shown that small organisms occur in light-limited environments, whereas larger forms occur if light is relatively abundant (O'Farrel *et al*., 2007).

At Lake Carioca, the maximum biomass was associated with the nanoplanktonic fraction (MLD < 20 μm). In Lake Dom Helvécio, the nano- and microplanktonic (> 20 μm) species coexisted. Brook (1981) reported that desmid species tend to behave as K-strategists, with high volumes and low intrinsic growth rates, low rates of population increase (Coesel & Kooijman-van-Blokland, 1991) and high light demands (Reynolds, 1984). However, the dominant desmids in Lakes Carioca and Dom Helvécio have a lower MLD (≤ 20 μm), a small volume ($< 10^3$) and greater dispersion. These characteristics would allow rapid colonization and nutrient absorption. In addition to these factors, a low sinking rate would also be potentially favored by the morphological features cited above. These features would facilitate the establishment of forms with the ability to achieve dominance under stressful conditions (i.e., low light and nutrient availability) (Reynolds, 2006).

Planktonic desmids are commonly associated with oligo-mesotrophic waters (Nygaard, 1949; Coesel, 1975, 2001). However, the relationship between the trophic level of a lake and the species richness, diversity and biomass of planktonic desmids is highly debatable. In addition to their association with oligotrophic lakes, the great majority of desmid species (except for the cosmopolitan species) are considered oligosaprobic (Coesel, 1983), i.e., not tolerant to organic pollution. Although planktonic desmids are commonly associated with oligo-mesotrophic waters, their trophic relationships and level are still very uncertain (Coesel, 1975, 2001).

The presence of *P. trabecula* and *C. aciculare* at Lake Dom Helvécio indicates a low to medium level of pollution (Coesel, 1983; Negearnpat & Peerapornpisal, 2007). However, the floristic composition of the desmids in Lake Carioca included both species typical of eutrophic lakes and species associated with oligotrophic lakes. This result indicated that, although the lake is located in an area in which the environment is protected, the changes occurring in the lake are typical of shallow ponds. Eutrophication processes are associated with decreases in the richness of desmids (Coesel, 1978). This association results from the increase in organic pollution, which decreases the structural diversity of the environment in conjunction with the decreased richness of the aquatic vegetation (Coesel *et al*., 1978). A decrease in diversity in Lake Donghu (China) was identified between 1960 and 1990 and related to increases in phosphorus and nitrogen and a decrease in water transparency (Yinxin & Minjuan, 2005). A relatively low richness of desmids may be favored by several factors intrinsic to Lake Carioca, including shading by the terrestrial vegetation, a lower richness of macrophytes and low water transparency, (Felisberto & Rodrigues, 2005).

In summary, the physical stability of the lakes is the principal driver favoring the dominance of desmids in tropical warm monomictic lakes, although stress due to the limitation of inorganic nutrients (Lake Dom Helvécio) and the limited amount of light (Lake Carioca) also determines the composition and abundance of functional groups and adaptive strategies.

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REFERENCES

BARBOSA, F. A. R. & J. PADISÁK. 2002. The forgotten lake stratification pattern: atelomixis, and its ecological importance. *Verhandlungen der*

Internationalen Vereinigung für Theoretische und Angewandte Limnologie, 28: 1385–1395.

BARBOSA, L. G., F. A. R. BARBOSA & C. E. de M. BICUDO. 2012. Inter-annual chemical stratification in Brazilian natural lakes: meromixis and hypolimnetic memory. *Acta Limnologica Brasiliensia*, 24(2) 127–139.

BEZERRA-NETO, J. F., L. S. BRIGUENTI & R. M. PINTO-COELHO. 2010. A new morphometric study of Carioca Lake, Parque Estadual do Rio Doce (PERD), Minas Gerais State, Brazil. *Acta Scientiarum. Biological Sciences*, 32: 49–54.

BEZERRA-NETO, J. F. & R. M. PINTO-COELHO. 2008. Morphometric study of Lake Dom Helvécio, Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil: a re-evaluation. *Acta Limnologica Brasiliensia*, 20: 161–167.

BEZERRA-NETO, J. F., P. M. BARBOSA & F. A. R. BARBOSA. 2006. Temporal variation of attenuation of the visible radiation in four lakes of Parque Estadual do Rio Doce (PERD), Minas Gerais, Brasil. *Acta Limnologica Brasiliensia*, 18: 39–46.

BROOK, A. J. 1981. *The Biology of Desmids*. Oxford: Blackwell Scientific Publications. 275 pp. (Botanical Monographs, 16).

BROOK, A. J. & D. B. WILLIAMSON.1988. The survival of desmids on the drying mud of a small lake. In: *Algae and the aquatic environment*. F. E. Round (ed.): 185–196. Biopress. Bristol.

CANTER, H. M. & J. W. G. LUND. 1966. The periodicity of planktonic desmids in Windermere, England. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 16: 163–172.

CLARKE, E., O. M. OLARINMOYE, F. G. OWODEINDE, A. O. ADEBOYEJO, A. JIMOH, S. L. AKINTOLA, & O. J. ADERINOLA, 2008. The dynamics of *Desmidacean* populations in Ologe lagoon, lagos, Nigeria. *Journal of Cell and Animal Biology*, 2: 021–030.

COESEL, P. F. M. 1975. The relevance of desmids in the biological typology and evaluation of fresh waters. *Aquatic Ecology*, 9: 93–101.

COESEL, P. F. M. 1978. Environmental changes in the Oisterwijk pool area, as evident from the composition of the desmid flora. *Aquatic Ecology*, 12: 52–53.

COESEL, P. F. M. 1982. Structural characteristics and adaptations of desmid communities. *Journal of Ecology*, 70: 163–177.

COESEL, P. F. M. 1983. The significance of desmids as indicators of the trophic status of freshwaters. *Schweizerische Zeitschrift für Hydrologie*, 45: 388–393.

COESEL, P. F. M. 1986. Structure and dynamics of desmid communities in hydrosere vegetation in a mesotrophic quivering bog. *Beihefte zum Nova Hedwigia*, 56: 119–143.

COESEL, P. F. M. 1993. Poor physiological adaptation to alkaline culture conditions in *Closterium acutum* var. *variabile*, a planktonic desmid from eutrophic waters. *European Journal of Phycology*, 28: 53–57.

COESEL, P. F. M. 2001. A method for quantifying conservation value in lentic freshwater habitats using desmids as indicator organisms. *Biodiversity and Conservation*, 10: 177–187.

COESEL, P. F. M. & H. KOOIJMAN-VAN-BLOKLAND. 1991. Seasonality of planktonic desmid species in Lake Maarsseveen (The Netherlands) related to experimentally determined growth rates in a temperature-light gradient. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 24: 763–767.

COESEL, P. F. M. & H. KOOIJMAN-VAN-BLOKLAND. 1994. Distribution and seasonality of desmids in the Maarsseveen Lakes area. *Netherlands Journal of Aquatic Ecology*, 28: 19–24.

COESEL, P. F. M., R. KWAKKESTEIN & A. VERSCHOOR. 1978. Oligotrophication and eutrophication tendencies in some Dutch moorland pools, as reflected in their desmid flora. *Hydrobiologia*, 61: 21–31.

COESEL, P. F. M. & K. WANDENAAR. 1990. Growth responses of planktonic desmids in a light-temperature gradient. *Freshwater Biology*, 23: 551–560.

COLE, G. A. 1994. *Textbook of Limnology*. Waveland Press. Illinois. 412 pp.

CROASDALE, H. T., C. E. M. BICUDO & G. W. PRESCOTT. 1983. A Synopsis of North American Desmids. University Nebraska Press. Lincoln. 2(5): 470 pp.

DINI, M. L., P. A. SORANNO, M. SCHEUERELL & S. R. CARPENTER. 1993. Effects of predators and food supply on diel vertical migration of *Daphnia*. In: *The trophic cascades in lakes*. S. R. Carpenter & J. F. Kitchell (eds.): 153–171. Cambridge University Press. Cambridge.

FELISBERTO, S. A. & L. RODRIGUES. 2005. Influência do gradiente longitudinal (riobarragem) na similaridade das comunidades de desmídias

perifíticas. *Revista Brasileira de Botânica*, 28: 241–254.

GERRATH, J. F. 1993. The biology of desmids: a decade of progress. In: *Progress in Phycological Research*. F. E. Round, D. J. Chapman (eds.): 79–193. Biopress Ltd. Bristol.

GOLTERMAN, H. L., R. S. CLYMO & M. A. M. OHMSTAD. 1978. *Methods for physical and chemical analysis of freshwaters*. Blackwell Scientific Publications. Oxford. 214 pp.

HARRIS, G. P. 1986. *Phytoplankton ecology: structure, function and fluctuation.* Chapman and Hall. London. 384 pp.

HENRY, R. & F. A. R. BARBOSA.1989. Thermal structure, heat content and stability of two lakes in the National Rio Doce Valley Lakes (Minas Gerais, Brazil). *Hydrobiologia*, 181: 189–199.

HILLEBRAND, H., C. D. DÜRSELEN, D. KIRSCHTEL, D. POLLINGHER & T. ZOHARY. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 35: 403–424.

HUSZAR, V. L. M., L. H. S. SILVA, P. DOMINGOS, M. MARINHO & S. MELO. 1998. Phytoplankton species composition is more sensitive than OECD criteria to the trophic status of three Brasilian tropical lakes. *Hydrobiologia*, 369–370: 59–71.

IDSO, S. 1973. On the concept of lake stability. *Limnology and Oceanography*, 18: 681–683.

JENSEN, P., E. JEPPESEN, K. OLRIK & P. KRISTENSEN. 1994. Impact of nutrients and physical factors on the shift from Cyanobacterial to Chlorophyte dominance in shallow Danish lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 51: 1692–1699.

KOROLEFF, F. 1976. Determination of ammonia. In: *Methods of seawater analysis*. K. Grasshoff (ed.): 126–133. Verlag Chemie. Weinheim.

KOSTEN, S., V. L. M. HUSZAR, N. MAZZEO, M. SCHEFFER, L. S. L. STERNBERG, & E. JEPPESEN. 2009. Lake and watershed characteristics rather than climate influence nutrient limitation in shallow lakes. *Ecological Applications*, 19: 1791–1804.

LUND, J. W. G. 1971. The seasonal periodicity of three planktonic desmids in Windermere. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 19: 3–25.

LUND, J. W. G., C. KIPLING & E. D. LECREN. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, 11: 143–170.

MACKERETH, F. J. H., J. HERON & J. F. TALLING. 1978. *Water Analysis. Scientific Publication (n^o 36)*. Freshwater Biological Association. Ambleside. 120 pp.

McCUNE, B. & M. J. MEFFORD. 1997. PC-ORD. *Multivariate analysis of ecological data, Version 3.0*. MjM Software Design. Oregon.

MEIS, M. R. M. & J. G. TUNDISI. 1997. Geomorphological and limnological process as a basis for lake tipology. The middle Rio Doce lake system. In: *Limnological Studies in the Rio Doce Valley Lakes*. J. G. Tundisi & Y. Saijo (eds.): 25–50. Brazilian Academy of Sciences, São Carlos, 25–50.

MORRIS, D. P. & W. M. LEWIS. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. *Freshwater Biology*, 20: 315–327.

MOSS, B. 1972. The influence of environmental factors on the distribution of freshwater algae: an experimental study, 1: introduction and the influence of calcium concentration. *Journal of Ecology*, 60: 917–932.

MOSS, B. 1973. The influence of environmental factors on the distribution of freshwater algae: an experimental study, 2: the role of pH and the carbon dioxide-bicarbonate system. *Journal of Ecology*, 61: 157–177.

NASELLI-FLORES, L., J. PADISÁK, M.T. DOKULIL & I. CHORUS. 2003. Equilibrium/steady-state concept in phytoplankton ecology. *Hydrobiologia*, 502: 395–403.

NGEARNPAT, N. & Y. PEERAPOMPISAL. 2007. Application of desmid diversity in assessing the water quality of 12 freshwater resources in Thailand. *Journal of Applied Phycology*, 19: 667–674.

NYGAARD, G. 1949. Hydrobiological studies on some Danish ponds and lakes, 2: the quotient hypothesis and some new or little known phytoplankton organisms. *Kongelige Danske Videnskabernes Selskab*, 7: 1–293.

O'FARRELL, I., P. TEZANOS-PINTO & I. IZAGUIRRE. 2007. Phytoplankton morphological response to the underwater light conditions in a vegetated wetland. *Hydrobiologia*, 578: 65–77.

PADISÁK, J., L. O. CROSSETTI & L. NASELLI-FLORES. 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. *Hydrobiologia*, 621: 1–19.

POLLINGHER, U. 1986. Phytoplankton periodicity in a subtropical lake (Lake Kinneret, Israel). *Hydrobiologia*, 138: 127–138.

PRESCOTT, G. W. 1975. *Algae of the western great lakes area*. Wm. C. Brown Company Publishers. 6th edition. 977 pp.

PRESCOTT, G. W., H. T. CROASDALE & W. C. VINYARD 1972. *A Synopsis of North American Desmids*. University Nebraska Press. Lincoln. 1(2): 84 pp.

PRESCOTT, G. W., H. T. CROASDALE & W. C. VINYARD. 1975. *A Synopsis of North American Desmids*. University Nebraska Press. Lincoln. 2(1): 267 pp.

PRESCOTT, G. W., H. T. CROASDALE & W. C. VINYARD. 1977. *A Synopsis of North American Desmids*. University Nebraska Press. Lincoln. 2(2): 413 pp.

PRESCOTT, G. W., H. T. CROASDALE, W. C. VINYARD & C. E. M. BICUDO. 1981. *A Synopsis of North American Desmids*. University Nebraska Press. Lincoln. 2(3): 720 pp.

PRESCOTT, G. W., W. C. VINYARD & C. E. M. BICUDO. 1982. *A Synopsis of North American Desmids.* University Nebraska Press. Lincoln. 2(3): 700 pp.

REYNOLDS, C. S. 1984. *The ecology of freshwater phytoplankton*. Cambridge University Press. Cambridge. 365 pp.

REYNOLDS, C. S. 2006. *Ecology of phytoplankton*. Cambridge University Press. Cambridge. 535 pp.

REYNOLDS, C. S., V. HUZSAR, C. KRUK, L. NASELLI-FLORES & S. MELO. 2002. Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research*, 24(5): 417–428.

SHEPHERD, G. J. 1996. *FITOPAC 1: manual de usuário*. Universidade Estadual de Campinas, Campinas. 95 pp.

SIEBURTH, J. McN., V. SMETACEK & J. LENZ. Pelagic ecosystem structure: heterotrophic compartments of plankton and their relationship to plankton size fractions. *Limnology and Oceanography*, 23: 1256–1263.

SOUZA, M. B. G., C. BARROS, F. A. R. BARBOSA, E. HAJNAL & J. PADISÁK. 2008. Role of atelomixis in replacement of phytoplankton assemblages in Dom Helvécio Lake, Southeast Brazil. *Hydrobiologia*, 607(1): 211–224.

TAVERA, R. & V. MARTÍNEZ-ALMEIDA. 2005. A hydrobiological study to interpret the presence of desmids in Lake Zirahuén, Mexico. *Limnologica*, 35: 61–69.

TEILING, E. 1967. *The desmid genus Staurodesmus*. *A taxonomic study.* Arkiv für Botanik, 6(11): 467–629.

TER-BRAAK, C. J. F. 1986. Canonical Correspondence Analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, 67: 1167–1179.

UTERMÖHL, H. 1958. Zur Vervolkomnung der quantitative Phytoplankton-methodik. *Mitteilungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 9: 1–38.

WETZEL, R. G. & G. E. LIKENS. 2000. *Limnological analyses*. Springer-Verlag NewYork Inc. New York. 391 pp.

WOELKERLING, W. J. & S. B. GOUGH. 1976. Wisconsin desmids, 3: desmid community composition and distribution in relation to lake type and water chemistry. *Hydrobiologia*, 51: 3–32.

YINXIN, W. & Y. MINJUAN. 2005. New desmids material from Donghu Lake, Wuhan, China. *Chinese Journal of Oceanology and Limnology*, 23(2): 210–217.

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Comportamiento térmico en ríos mediterráneos andinos de la zona centro-sur de Chile

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ABSTRACT

Thermal behavior of Mediterranean Andean streams in South-Central Chile

Despite of the importance of temperature in the fluvial ecosystems, the knowledge of its spatial and temporal variability in Andean rivers is limited, thus it is necessary to clarify the relative importance of site-specific factors in controlling the temperature of rivers. The aim of this research was to characterize the thermal regime of five Andean streams through an altitudinal gradient. The results show that there is a spatial and temporal variability in water temperature with a relatively rapid rise in temperature in mid-December, being stable in January and February, and a rapid decrease at the beginning of March and April. The thermal heterogeneity recorded in the high Andean zone of the Biobio River Basin makes it clear that geomorphology characteristics of each site are important in regulating water temperature, associated mainly with the altitude and shade; this latter mainly generated by high mountains and in some cases by the timberline. It is known that thermal variability, mainly the maximum and minimum mean temperatures; causing severe stress on stenotherm organisms. Therefore, a deeper knowledge of the river temperature is essential for the management and future protection of the Andean freshwater ecosystems to mitigate the impacts associated to the global warming.

Key words: Local factors, altitudinal pattern, thermal regime, Andean streams.

RESUMEN

Comportamiento térmico en ríos mediterráneos andinos de la zona centro-sur de Chile

A pesar de la importancia que tiene la temperatura en los sistemas fluviales, el conocimiento de su variabilidad espacio-temporal en ríos de Chile es limitado. Tales estudios son necesarios para aclarar la importancia relativa de los factores locales a la hora de controlar la temperatura de los ríos. La presente investigación tuvo por objetivo caracterizar el régimen térmico de cinco ríos andinos de la región del Biobío a través de un gradiente altitudinal. Los resultados muestran que existe una variabilidad espacio-temporal en la temperatura del agua con un incremento relativamente rápido de la temperatura a mediados de diciembre, manteniéndose en enero y febrero, para disminuir rápidamente a principio de marzo y abril. La heterogeneidad térmica encontrada en la zona andina de la cuenca del río Biobío deja de manifiesto que las características geomorfológicas de cada sitio son relevantes en la regulación de la temperatura del agua, asociada principalmente a la altitud y sombra, esta última generada principalmente por las altas montañas y en algunos casos por la vegetación arbórea.

Se prevé que cambios principalmente en las temperaturas medias máximas y mínimas podrían ocasionar un severo estrés en organismos estenotermos. Por lo tanto, un conocimiento detallado de la temperatura en ríos andinos podría proveer de información necesaria para direccionar la mitigación de los impactos asociados al calentamiento global.

Palabras clave: *Factores locales, patrón altitudinal, régimen térmico, ríos andinos.*

INTRODUCCIÓN

La temperatura es una de las variables del hábitat físico más importantes en los ecosistemas fluviales, debido a que afecta la respuesta ecológica funcional y estructural de los organismos acuáticos (Vannote & Sweeney, 1980; Hawkins *et al.*, 1997; Jacobsen *et al.*, 1997), así como las reacciones fisicoquímicas que ocurren en dichos sistemas (Berner & Berner, 1996; Webb, 1996; Erickson & Stefan, 2000). No obstante, el conocimiento ligado a la variabilidad térmica natural de los ríos es limitado, dado el nivel de alteración que presentan estos sistemas (Malmqvist & Rundle, 2002). Además de esto, se proyecta que la temperatura superficial del planeta seguirá aumentando como resultado del calentamiento global (3-5 °C durante los próximos años; IPCC, 2007), lo cual condicionaría no solo los regímenes térmicos estacionales, sino también la respuesta ecológica de los sistemas fluviales, principalmente la distribución de las especies dulceacuícolas (Heino, 2002; Caissie, 2006; Brown *et al.*, 2007).

En condiciones naturales, el factor climático es el principal modelador de la temperatura en ríos, sobre todo aquellos parámetros que presentan una variabilidad interanual (p. ej. estacionalidad del sol, temperatura del aire, viento y humedad relativa; Malcolm *et al.*, 2004). Sin embargo, también existen factores locales (p. ej. vegetación de ribera, aguas subterráneas) que contribuyen a la heterogeneidad térmica, los cuales han sido identificados por Alexander & Caissie (2003), Johnson (2004), Royer & Minshall (1997) y Poff & Ward (1990). Estos factores raramente influyen de forma independiente en la temperatura, por lo que su importancia es un desafío, debido a que pueden variar en forma diaria, estacional, así como espacialmente. Estudios desarrollados durante las últimas décadas han permitido constatar que la radiación solar de onda corta y la temperatura del aire son los reguladores primarios de la temperatura en los sistemas fluviales (Sinokrot & Stefan, 1994; Webb & Zhang, 1997; Evans *et al.*, 1998). Alexander & Caissie (2003) demuestran que el intercambio de calor entre la atmósfera y la superficie del agua explica gran parte de las fluctuaciones térmicas diarias en ríos someros. Sin embargo, la naturaleza del intercambio energético puede variar significativamente de acuerdo con las características geomorfológicas del río (p. ej. rápidos, rápidos-someros, pozas; Webb *et al.*, 2008).

En Chile, la diversidad geológica y continental conforma una realidad territorial latitudinal-altitudinal de tal magnitud, que permite diferenciar térmicamente una variedad de ecosistemas acuáticos. Sin embargo, los estudios del régimen térmico son muy limitados, conociéndose solo los de Link *et al.* (2012), Monsalve *et al.* (2012) y Link *et al.* (2009). Esto genera una escasez de información sobre la dinámica y heterogeneidad térmica de los ríos en condiciones naturales. En este contexto, los ríos alto-andinos se presentan como unidades ecológicas favorables para estudiar los patrones térmicos, ya que a menudo se encuentran escasamente alterados y presentan un gradiente térmico que depende marcadamente de las condiciones ambientales locales y la fuente que los alimenta (p. ej. glaciar, subterránea y/o termal). Ambos factores son importantes para moderar las temperaturas (Poole & Berman, 2001; Caissie, 2006), en particular las altas temperaturas estivales, que aumentarían como consecuencia del calentamiento global (Falvey & Garreaud, 2009), siendo limitante para la distribución y supervivencia futura de ciertas especies acuáticas (p. ej. Plecoptera; Palma & Figueroa, 2008) de las zonas andinas. De acuerdo con lo planteado anteriormente, el objetivo del presente trabajo es caracterizar el régimen térmico de cinco ríos alto-andinos de la región del Biobío a través de un gradiente altitudinal, para identificar los factores locales que afectan la temperatura de los ríos.

MATERIAL Y MÉTODOS

Área de estudio

La zona de estudio se localizó entre los 37.74-38.08°S; 71.39-71.14°W y consideró cinco ríos alto-andinos de la cuenca del río Biobío: Lomín, Chaquilvín, Quepuca, Pangue y Queuco (Fig. 1). Todos los sitios presentan características climáticas típicas de ríos mediterráneos (Gasith & Resh, 1999). Se describe el régimen hidrológico de

todos los sitios como pluvio-nival, con pico de máximo caudal en invierno y primavera. El sedimento de la zona de estudio está constituido principalmente por un conglomerado de roca ígnea con intrusiones sedimentarias, asociado fundamentalmente a las características fluvio-glacio-volcánicas de la zona (Mardones *et al.*, 1992). El sustrato acuático consiste en una mezcla de cantos rodados, piedras, grava y arena, mientras que la vegetación está constituida por una mezcla entre bosque nativo perenne (p. ej. *Drymis winteri*), caducifolio (*Nothophagus spp.*), matorral y estepa andina (Hajek, 1991; Dallman, 1998). La figura 1 muestra el uso de suelo de cada una de las microcuencas estudiadas. Los tramos de río fueron clasificados de acuerdo con sus diferencias de altitud: ríos de mayor altura (> 800 msnm) con mezcla de matorral y estepa alto-andina; de altitud media (500-800 msnm) con bosque achaparrado y renoval nativo, y de baja altitud (< 500 msnm) con mezcla de bosque y renoval nativo. El renoval nativo corresponde al bosque nativo secundario originado por semillas y/o reproducción vegetativa después de una perturbación antrópica o natural. Cada sitio de estudio fue caracterizado incluyendo

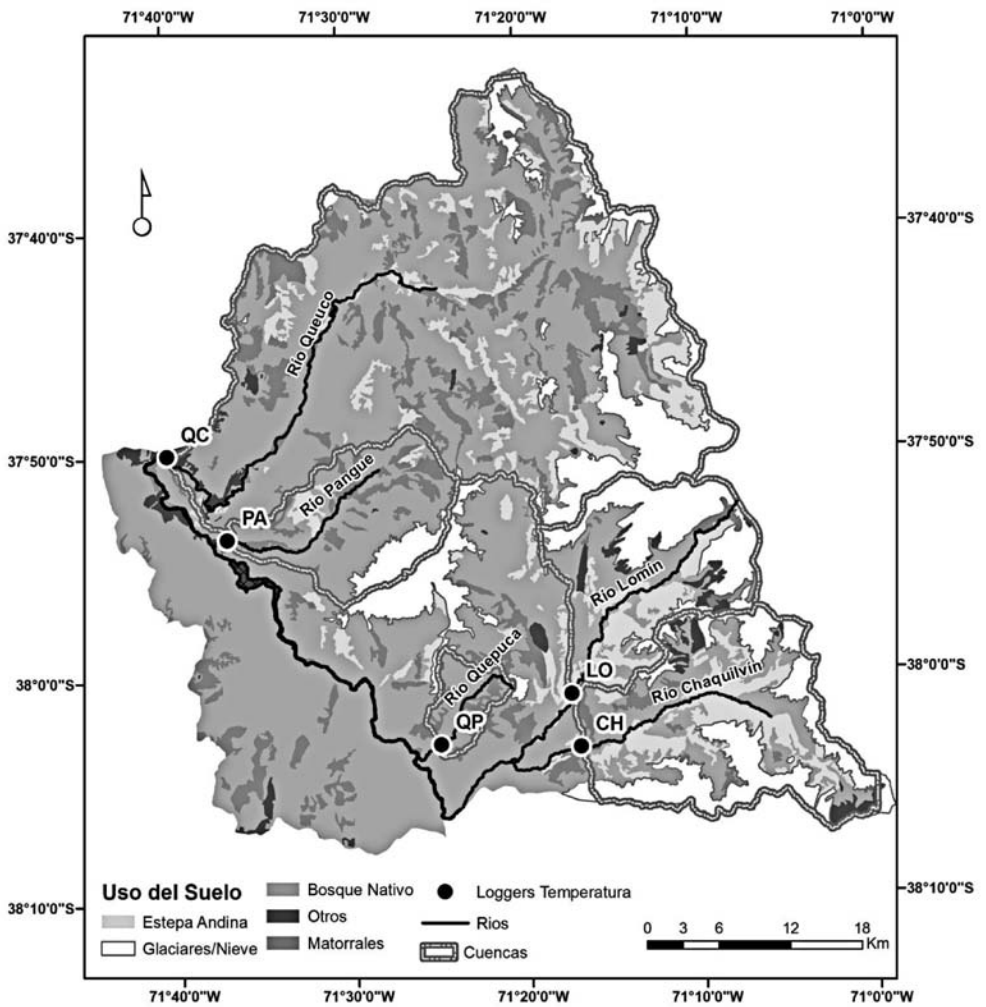

Figura 1. Localización de los sitios de estudio en el sector andino de la cuenca del río Biobío. *Location of study sites in the Andean zone of the Biobio River basin.*

Tabla 1. Caracterización física de los cinco ríos andinos estudiados desde julio de 2010 a junio de 2011. LO = Lomín; CH = Chaquilvín; QP = Quepuca; PA = Pangue y QC = Queuco. *Physical characterization of the five Andean rivers studied during July 2010 and June 2011. LO = Lomín; CH = Chaquilvín; QP = Quepuca; PA = Pangue y QC= Queuco.*

Sitio	LO	CH	QP	PA	QC
Localización	38°00′57.24″S 71°17′24.02″W	38°03′21.5″S 71°16′52.29″W	38°03′8.05″S 71°24′57.22″W	37°53′45.04″S 71°36′51.79″W	37°49′56.9″S 71°40′12.54″W
Altitud (msnm)	936	882	750	475	370
Área de la cuenca (km^2)	214.52	298.92	35.32	156.03	983.61
Aspecto	E	NE	NE	NO	E
Ancho máximo del río (m)	8-12	18-25	5-7	7-13	45-65
Profundidad (m)	0.20-0.40	0.21-0.50	0.15-0.37	0.22-0.45	0.20-0.51
Orden	2	3	2	3	3
Distancia desde el origen (km)	25.919	19.737	10.827	17.827	71.858

datos geográficos, además de características físicas del río (Tabla 1).

Recolección de datos

La temperatura del agua y del aire fue medida continuamente utilizando registradores de datos marca HOBO, modelo UA-001-08 (−20 ° a 70 °C), con una precisión de ± 0.5 °C. Estos fueron programados mediante el software HOBOware para registrar y almacenar la temperatura a intervalos de 15 min; además se calibraron durante 24 horas previamente a su instalación en cada sitio de estudio, siguiendo la metodología basada en Haidekker & Hering, 2008. Los registradores se introdujeron en tubos de PVC (diámetro = 35 mm; longitud = 15 cm) a fin de evitar que recibieran radiación directa que alterara la temperatura del sensor. El área de drenaje, la distancia desde el origen y el orden del río de cada sitio de muestreo fueron obtenidos de las bases de datos cartográficas del IGM (Instituto Geográfico Militar; 1:50 000), mientras que la ubicación geográfica y la altitud (msnm) se determinaron utilizando un GPS Garmin Xtrex. El ancho y la profundidad media se estimaron in situ en cada lugar de muestreo. La velocidad de la corriente se midió con un velocímetro digital Flow Probe, modelo FP111.

Análisis estadístico

La evaluación de heterogeneidad térmica se basó en los registros efectuados en los cinco ríos desde julio de 2010 a junio de 2011, a excepción del río Queuco, donde la recogida de datos comenzó en agosto 2010. Para cada sitio de estudio se determinaron cuatro variables térmicas: (i) temperatura media diaria, (ii) temperatura máxima diaria, (iii) temperatura mínima diaria, y (iv) rango térmico diario, el cual fue calculado como la diferencia entre la temperatura máxima y mínima diaria (λ media diaria). Además, se estimó la tasa media de calentamiento y de enfriamiento en primavera y otoño (°C $día^{-1}$) (Uehlinger *et al.*, 2003), ambas obtenidas de la regresión lineal de la temperatura media diaria en el período de tiempo 21 septiembre-21 diciembre y 21 marzo-21 junio, respectivamente. Paralelamente, se determinó la cantidad de horas que cada sitio recibe de radiación solar directa, a partir de la relación entre el ángulo cenital y el efecto de sombra generado por las altas montañas, siguiendo la metodología propuesta por Tung *et al.* (2006).

Se utilizó el análisis de varianza de un factor (ANOVA) con test de Tuckey *post hoc* para establecer diferencias en las variables térmicas (temperatura media diaria y rango térmico) y la estacionalidad entre los sitios y épocas de estudio. Las correlaciones y modelos de regresión se consideraron significativos cuando $p < 0.05$. Como una medida de asociación entre la temperatura del aire y la del agua, se estimó la correlación de Pearson (r) para cada sitio de estudio. Finalmente, se determinaron los grados-día para el período julio 2010-junio 2011 (Arscott *et al.*, 2001). Este parámetro fue calculado a escala mensual y anual a partir de la suma de las temperaturas me-

dias diarias por encima de 0 °C, estableciendo la influencia de la energía térmica disponible en los organismos acuáticos.

RESULTADOS

La temperatura media diaria del aire presentó un patrón sinusoidal con una media diaria máxima, ocurrida el 23 de enero, que fluctuó entre 21.52 °C (río Quepuca) y 26.47 °C (río Queuco), y una mínima, el 22 de julio, que fluctuó entre −6.32 °C (río Chaquilvín) y 0.88 °C (río Queuco) (Fig. 2). La variabilidad media anual de la temperatura del aire entre los sitios con mayor y menor altitud fluctuó entre 9.36 y 12.49 °C, respectivamente. La comparación en esta microescala temporal (julio de 2010 a junio de 2011) reveló que a altitudes bajas las temperaturas son más cálidas, mientras que a altitudes media-altas los patrones térmicos del aire son más fríos y similares entre sí.

La temperatura del agua muestra un patrón sinusoidal similar al registrado en la temperatura del aire (véanse Fig. 2 y Tabla 2). En gene-

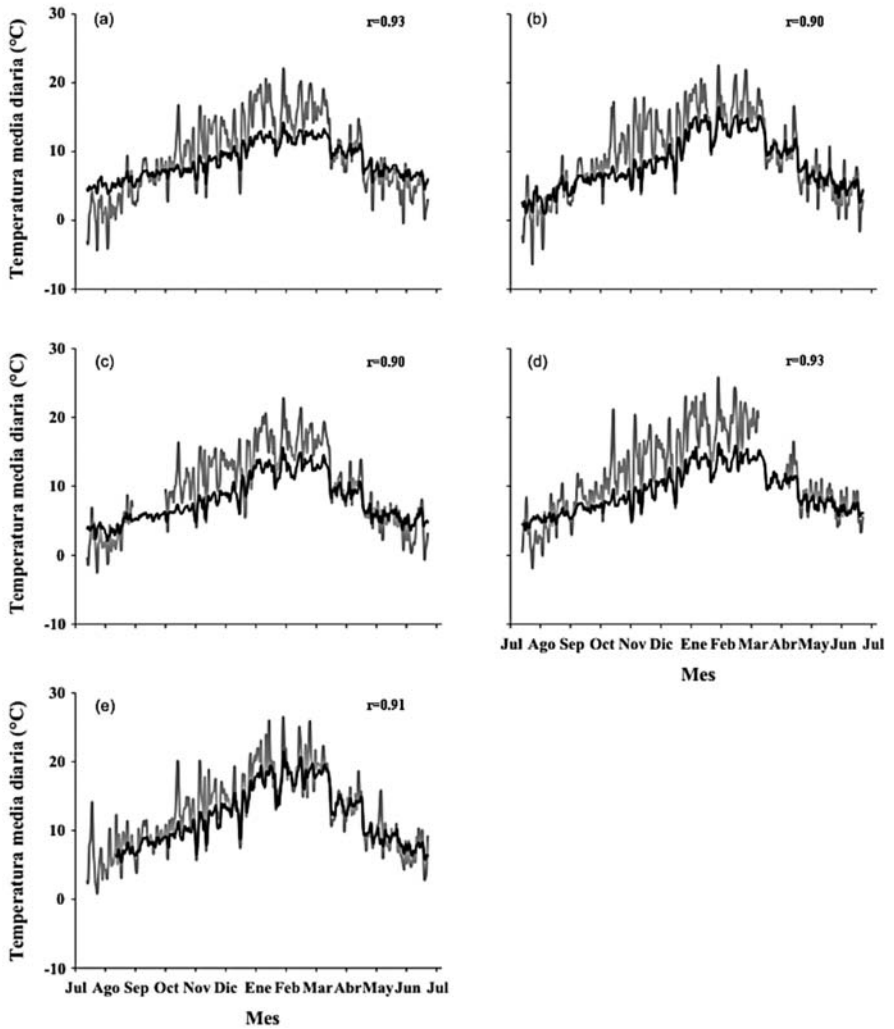

Figura 2. Temperatura media diaria del aire (línea clara) y agua (línea oscura) durante un ciclo anual de los ríos (a) Lomín, (b) Chaquilvín, (c) Quepuca, (d) Pangue y (e) Queuco. *Daily mean air (light line) and water (dark line) temperature during an annual cycle in the (a) Lomín, (b) Chaquilvín, (c) Quepuca, (d) Pangue, (e) Queuco streams.*

Tabla 2. Características térmicas del agua (°C) y horas de radiación directa (% RD) reportadas durante el ciclo anual julio de 2010 a junio de 2011, en ríos mediterráneos alto-andinos del centro-sur de Chile. *Thermal characteristics of water (°C) and hours of direct radiation (% DR) reported during an annual cycle, from July 20120 to June 2011, in Andean Mediterranean rivers in South-Central Chile.*

Río	Altitud	$T_{media\ anual}$	$T_{media\ mensual}$	$T_{media\ diaria}$	$\lambda_{media\ diaria}$	$T_{máx}$	Mes	$T_{mín}$	Mes	Grados-día	% RD
Lomín	936	8.07	5.01-12.16	3.78-14.04	0.51-7.08	16.90	Enero	2.41	Agosto	2976	40.02
Chaquilvín	882	7.53	2.82-13.65	0.97-16.32	0.73-9.63	19.28	Enero	0.12	Julio	2822	46.63
Quepuca	750	7.96	3.96-12.95	2.14-15.56	0.51-8.49	19.28	Enero	1.00	Agosto	2716	42.85
Pangue	475	8.85	4.93-13.88	3.52-15.91	0.40-8.60	20.04	Enero	2.73	Julio	3234	45.78
Queuco	370	11.87	5.43-21.49	6.84-18.20	0.20 -6.78	24.54	Enero	1.87	Julio	3763	40.30

ral, los registros térmicos de los cinco ríos alto-andinos estuvieron caracterizados por un incremento relativamente rápido de la temperatura a mediados de diciembre, manteniéndose en enero y febrero, para disminuir rápidamente a principio de marzo y abril. Llama la atención que el río Pangue (475 msnm) presentó un patrón térmico similar a los ríos de mayor altitud, Lomín (936 msnm) y Chaquilvín (882 msnm), y distinto al del río Queuco (370 msnm), de menor altitud. Esto podría deberse a que el río Pangue tiene afluentes importantes de origen glaciar, mientras que el Queuco se nutre de la escorrentía superficial y aportes subterráneos. Las temperaturas fueron máximas en enero para todos los sitios de estudio, mientras que las mínimas fueron en julio (Chaquilvín, Pangue y Queuco) y agosto (Lomín y Quepuca). En cuanto al rango térmico, este tuvo su mayor amplitud en el río Chaquilvín, con valores entre 0.73-9.63 °C, mientras que el río Queuco presentó la menor amplitud, con valores entre 0.20-6.78 °C. En todos los sitios de estudio se evidenció que la temperatura alcanzó los valores más altos durante la época estival, cuando los caudales son mínimos, lo cual queda reflejado en las mediciones de profundidad y ancho de la llanura de inundación, presentando una mayor variabilidad en el río Queuco y menor en el río Quepuca (véase Tabla 1). En este sentido, la variabilidad en la temperatura media diaria del agua puede ser estimada a partir de la temperatura media diaria del aire (n = 311-360; $p < 0.001$) y las horas de radiación directa en cada sitio de estudio. A este respecto se observó que la temperatura media anual aumentó de 7.53 a 11.87 °C a medida que disminuía la altitud (de 936 a 370 msnm). Estas diferencias en variabilidad espacio-temporal de la temperatura media diaria también se reflejaron en los patrones de grados-día mensual y anual (Figs. 3a y 3b). La variación de grados-día anual registró valores en-

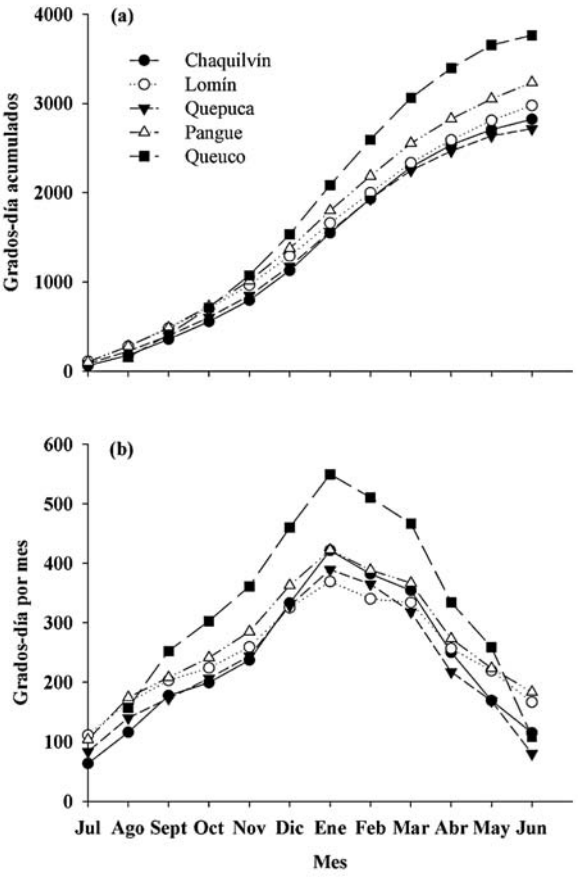

Figura 3. Grados-día acumulados durante el ciclo anual de estudio de julio de 2010 a junio de 2011, (a) grados-día acumulados y (b) grados-día mensuales para cada uno de los ríos andinos. *Cumulative degree-days during the annual cycle of the study period, from July 2010 to June 2011, (a) cumulative and (b) monthly average degree-days for each of the Andean streams.*

tre 2716 y 3763, alcanzando valores más altos a medida que disminuía la altitud. La mayor variabilidad en los patrones de grados-día está en función de las temperaturas de verano (Fig. 3b). Sin embargo, las temperaturas de invierno son importantes para los grados-día en cada sitio, siendo reconocidas por el aumento relativamente lineal entre julio y septiembre.

Los efectos de la estacionalidad sobre la temperatura media diaria y rango medio diario fueron significativos en todos los sitios de estudio ($p < 0.001$). Estas interacciones se condicen con el aumento de la temperatura del aire y una disminución en la profundidad y ancho de la llanura de inundación en época estival. Mientras que la tasa de calentamiento de los ríos fluctuó en primavera desde 0.0624 (río Queuco) a 0.0395 °C día^{-1} (río Chaquilvín), y la tasa de enfriamiento de otoño registró valores entre −0.0525 (río Lomín) y −0.0902 °C día^{-1} (río Queuco). En ambos casos las tasas de cambio incrementaron significativamente (modelo de regresión lineal, $p < 0.001$) con la distancia al origen.

Finalmente, la heterogeneidad térmica de los ríos a mayor altitud respecto al río ubicado a menor altitud se muestra en la figura 4. La diferencia altitudinal en la temperatura media diaria fluctúa entre 0.50-7.65 °C, con una media de 3.15 °C (Queuco-Lomín), y 1.46-5.74 °C, con una media de 3.42 °C (Queuco-Chaquilvín). En época estival (21 diciembre-21 marzo) se presenta la mayor heterogeneidad térmica con variaciones entre 2.54-7.65 °C, con una media de 5.63 °C (Queuco-Lomín, y 2.59-5.74 °C, con una media de 4.39 °C (Queuco-Chaquilvín). En ambos casos la relación fue positiva, debido a que los ríos de altitudes mayores siempre presentaron temperaturas más frías que los tramos más bajos.

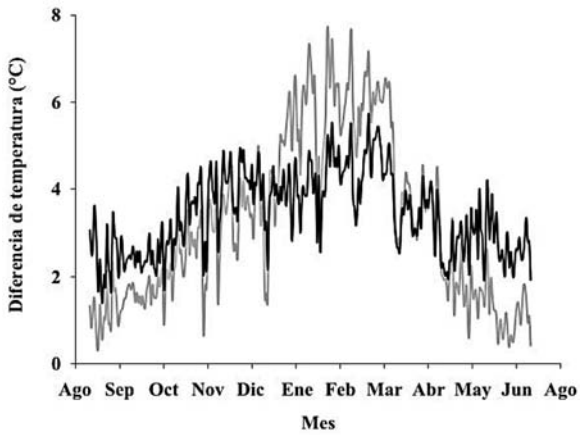

Figura 4. Discontinuidad térmica a través del gradiente altitudinal. Diferencias entre los ríos Queuco-Lomín (línea oscura) y Queco-Chaquilvín (línea clara) durante el período agosto de 2010 a junio de 2011. *Thermal discontinuity through altitudinal gradient. Differences among Queuco-Lomín (dark line), and Queco-Chaquilvín (light line) streams from August 2010 to June 2011.*

DISCUSIÓN

La variabilidad espacio-temporal de la temperatura es compleja y expresa la influencia local y regional (Uehlinger *et al.*, 2003). En este contexto, la resolución de los registradores de datos (15 min) reveló claras diferencias espacio-temporales en la temperatura del agua de los cinco ríos estudiados. La variabilidad inter-sitio en la temperatura media diaria del agua fue más evidente durante la época estival. Esto se relaciona con los resultados obtenidos por Webb & Zhang (1997) y Evans *et al.* (1998) en ríos del Hemisferio Norte, y en Chile Link *et al.* (2012) para los ríos Vergara e Itata, quienes lo atribuyen a la radiación neta dominada por la entrada de energía solar durante los meses de verano. Esto fue observado en el río Quepuca, que presentó bajas temperaturas, lo cual estaría asociado al desarrollo de una amplia densidad arbórea, abrupta topografía del canal y una baja relación ancho-profundidad. Dichos factores se asocian a la reducción en la cantidad de radiación solar directa y al área disponible para el intercambio de energía desde la atmósfera al río (Hawkins *et al.*, 1997; Webb & Zhang, 1997; Arscott *et al.*, 2001; Poole & Berman, 2001; Malcolm *et al.*, 2004). A pesar de que presenta aproximadamente un 43 % de horas con radiación solar directa, la secuencia de sombra generada por el dosel arbóreo y las altas montañas serían los condicionantes a la hora de absorber o reflejar la radiación de onda corta.

Contrariamente, los patrones térmicos encontrados en los ríos Lomín y Chaquilvín responden más a la dinámica meteorológica temporal local, debido a que los sitios se encuentran localiza-

dos en una zona de valle con escasa secuencia de sombra (matorral y estepa alto-andina), pero con un área disponible mayor para el intercambio de energía (véase en Tabla 1 ancho y profundidad del río), lo cual lleva a que estos sistemas tengan una capacidad térmica relativamente baja. Esta condición concuerda con lo reportado por Isaak & Hubert (2001), donde el régimen térmico de estos sistemas está más ligado a la radiación solar y a la temperatura del aire. Si bien ambos sistemas se encuentran a una altitud similar, la amplitud térmica entre los sitios es diferente; una situación similar se registró con las temperaturas mínimas y máximas. Estas diferencias inter-sitio se atribuyen a que la relación ancho-profundidad, orientación del río y las horas de radiación directa son distintas, así, Chaquilvín presenta una orientación noreste con un área mayor para intercambio recibiendo en promedio anual 46.6 % de radicación directa, que no solo facilita la cantidad e intensidad de luz que recibe el río durante el día, sino también la capacidad de absorción de energía (Johnson & Jones, 2000).

Nuestros resultados concuerdan con la relación altitud-temperatura para los hábitats alto-andinos de zonas templadas (Nagy & Grabherr, 2009). Estas diferencias en los patrones térmicos pueden atribuirse primariamente a la altitud y secundariamente a la combinación de distintos factores que se interrelacionan entre sí para influenciar el régimen térmico de estos sistemas fluviales. Así, en el río Queuco, la topografía del canal, la relación ancho-profundidad, la vegetación de ribera, el tipo de sustrato (mayor porcentaje de roca sedimentaria), un mayor aporte de temperatura desde ríos tributarios y el volumen de agua, condicionarían dichos patrones. Mientras que la similitud de los patrones térmicos entre el río Pangue y los ríos de mayor altitud, se asocia a que en su cabecera recibe un aporte importante de alimentación glacial, que estaría determinando la heterogeneidad térmica, debido a la inclusión de temperaturas bajas, en algunos casos cercanas a 0 °C. Sin embargo, se requieren más estudios para estimar la influencia glacial sobre los ríos mediterráneos alto-andinos.

Las fluctuaciones térmicas de invierno no presentaron una variación diaria significativa, pero a mediados de marzo y abril se produce un rápido decaimiento de los patrones térmicos en todos los sitios estudiados; esta disminución se asocia a la intensidad de las primeras lluvias otoñales, que determinaron la naturaleza de la respuesta hidrológica. La respuesta de la temperatura a las condiciones climáticas ha sido reportada como condicionante en la dinámica térmica (Brown *et al.*, 2006). La heterogeneidad en la temperatura del agua entre los sitios de estudio también se ve reflejada en las tasas de cambio y los grados día acumulados durante un ciclo anual; ambos patrones se asocian a la capacidad de *buffer* de cada río individualmente.

En este trabajo hemos relacionado las diferencias térmicas entre ríos con factores locales como altitud, insolación y presencia de vegetación. A partir de estos datos será necesario *a posteriori* estimar un balance térmico de cada uno de los ríos considerando datos de caudal e intercambio con el hiporreos; este último puede ser importante como regulador de la temperatura del agua (Evans *et al.*, 1998), y como refugio térmico para los organismos (Acuña & Tockner, 2009; Wood *et al.*, 2010). Además, los patrones térmicos encontrados podrían ser fuertemente alterados como consecuencia del calentamiento global (Caissie, 2006). En el caso particular de Chile, los registros de temperatura en la zona andina muestran un incremento en la temperatura media diaria del aire de aproximadamente +0.25 °C/década entre 1979-2006 (Conama, 2006; Falvey & Garreaud, 2009). Por lo que un conocimiento detallado de la dinámica de la temperatura de los ríos es esencial para la gestión, protección y conservación futura de los sistemas acuáticos, así como para direccionar la mitigación de los impactos asociados al calentamiento global.

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BIBLIOGRAFÍA

ACUÑA, V. & K. TOCKNER. 2009. Surface-subsurface water exchange rates along alluvial river reaches control the thermal patterns in an Alpine river network. *Freshwater Biology*, 54: 306–320.

ALEXANDER, M. D. & D. CAISSIE. 2003. Variability and Comparison of Hyporheic Water Temperatures and Seepage Fluxes in a Small Atlantic Salmon Stream1. *Ground Water*, 41: 72–82.

ARSCOTT, D. B., K. TOCKNER & J. V. WARD. 2001. Thermal heterogeneity along a braided floodplain river (Tagliamento River, northeastern Italy). *Canadian Journal of Fisheries and Aquatic Sciences*, 58 (12): 2359–2373.

BERNER, E. K. & R. A. BERNER. 1996. *Global environment: Water, air and geochemical cycles*. Prentice Hall, Old Tappan, New Jersey.

BROWN, L. E., D. M. HANNAH & A. M. MILNER. 2006. Hydroclimatological influences on water column and streambed thermal dynamics in an alpine river system. *Journal of Hydrology*, 325: 1–20.

BROWN, L. E., D. M. HANNAH & A. M. MILNER. 2007. Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. *Global Change Biology*, 13: 958–966.

CAISSIE, D. 2006. The thermal regime of rivers: a review. *Freshwater Biology*, 51: 1389–1406.

CONAMA. 2006. *Estudio de la variabilidad climática en Chile para el siglo XXI*. Departamento de Geofísica, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Santiago, Chile.

DALLMAN, P. R. 1998. *Plant life in the world's mediterranean climates*. University of California Press, Los Angeles, USA.

ERICKSON, T. R. & H. G. STEFAN. 2000. Linear air-water temperature correlations for streams during open water periods. *Journal of Hydrologic Engineering*, 5 (3): 317–321.

EVANS, E. C., G. R. MCGREGOR & G. E. PETTS. 1998. River energy budgets with special reference to river bed processes. *Hydrological Processes*, 12: 575–595.

FALVEY, M. & R. D. GARREAUD. 2009. Regional cooling in a warming world: Recent temperature trends in the southeast Pacific and along the west coast of subtropical South America (1979-2006). *Journal of Geophysical Research*, 114 (D4): 7–14.

GASITH, A. & V. H. RESH. 1999. Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. *Annual Review of Ecology and Systematics*, 30: 51–81.

HAIDEKKER, A. & D. HERING. 2008. Relationship between benthic insects (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera) and temperature in small and medium-sized streams in Germany: A multivariate study. *Aquatic Ecology*, 42 (3): 463–481.

HAJEK, E. R. 1991. El medio ambiente en Chile. In: *La situación ambiental en América Latina*. E. R. HAJEK (ed.): 237–294. CIEDLA, Buenos Aires.

HAWKINS, C. P., J. N. HOGUE, L. M. DECKER & J. W. FEMINELLA. 1997. Channel Morphology, Water Temperature, and Assemblage Structure of Stream Insects. *Journal of the North American Benthological Society*, 16: 728–749.

HEINO, J. 2002. Concordance of species richness patterns among multiple freshwater taxa: a regional perspective. *Biodiversity and Conservation*, 11: 137–147.

IPCC. 2007. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.

ISAAK, D. J. & W. A. HUBERT. 2001. A hypothesis about factors that affect maximum summer stream temperatures across montane landscapes. *Journal of the American Water Resources Association*, 37: 351–366.

JACOBSEN, D., R. SCHULTZ & A. ENCALADA. 1997. Structure and diversity of stream invertebrate assemblages the influence of temperature with altitude and latitude. *Freshwater Biology*, 38: 247–261.

JOHNSON, S. L. 2004. Factors influencing stream temperatures in small streams: substrate effects and a shading experiment. *Canadian Journal of Fisheries and Aquatic Sciences*, 61: 913–923.

JOHNSON, S. L. & J. I. JONES. 2000. Stream temperature responses to forest harvest and debris flows in western Cascades Oregon. *Canadian*

Journal of Fisheries and Aquatic Sciences, 57: 30–39.

LINK, O., A. HUERTA, A. STEHR, A. MONSALVE, C. MEIER & M. AGUAYO. 2012. The solar-to-stream power ratio: A dimensionless number explaining diel fluctuations of temperature in mesoscale rivers. *River Research and Applications*: n/a-n/a.

LINK, O., A. MONSALVE, A. STEHR, A. GARCÍA & R. URRUTIA. 2009. Régimen Térmico del Río Itata. In: *La cuenca hidrográfica del río Itata, aportes científicos para su gestión sustentable*. O. Parra, J. C. Castilla, H. Romero, R. Quiñones & A. Camaño (eds.): 44–57. Editorial Universidad de Concepción, Concepción, Chile.

MALCOLM, I. A., D. M. HANNAH, M. J. DONAGHY, C. SOULSBY & A. F. YOUNGSON. 2004. The influence of riparian woodland on the spatial and temporal variability of stream water temperatures in an upland salmon stream. *Hydrology and Earth System Sciences*, 8: 449–459.

MALMQVIST, B. & S. RUNDLE. 2002. Threats to the running water ecosystems of the world. *Environmental Conservation*, 19: 134–153.

MARDONES, M., E. JAQUE & D. ZAIO. 1992. *Geomorfología de la hoya hidrográfica del río Bio-bío*. Instituto Geográfico Militar, Santiago, Chile.

MONSALVE, A., O. LINK & A. STEHR. 2012. Régimen térmico de ríos: desarrollo, verificación y aplicación de un modelo numérico. *Tecnología y Ciencias del Agua*, 3: 41–56.

NAGY, L. & G. GRABHERR. 2009. *The Biology of Alpine Habitats*. Oxford University Press, New York.

PALMA, A. & R. FIGUEROA. 2008. Latitudinal diversity of plecoptera (Insecta) on local and global scales. *Illiesia*, 4: 81–90.

POFF, N. L. & J. V. WARD. 1990. Physical habitat templates of lotic systems-recovery in the context of historical pattern of spatiotemporal heterogeneity. *Environmental Management*, 14: 629–645.

POOLE, G. C. & C. H. BERMAN. 2001. An ecological perspective on in-stream temperature: Natural heat dynamics and mechanisms of human-caused thermal degradation. *Environmental Management*, 27: 787–802.

ROYER, T. V. & G. W. MINSHALL. 1997. Temperature patterns in small streams following wildfire. *Archiv für Hydrobiologie*, 140: 237–242.

SINOKROT, B. A. & H. G. STEFAN. 1994. Stream water-temperature sensitivy to weather and bed parameters. *Journal of Hydraulic Engineering-Asce*, 120: 722–736.

TUNG, C. P., T. Y. LEE & Y. C. YANG. 2006. Modelling climate-change impacts on stream temperature of Formosan landlocked salmon habitat. *Hydrological Processes*, 20: 1629–1649.

UEHLINGER, U., F. MALARD & J. V. WARD. 2003. Thermal patterns in the surface waters of a glacial river corridor (Val Roseg, Switzerland). *Freshwater Biology*, 48: 284–300.

VANNOTE, R. L. & B. W. SWEENEY. 1980. Geographic Analysis of Thermal Equilibria: A Conceptual Model for Evaluating the Effect of Natural and Modified Thermal Regimes on Aquatic Insect Communities. *The American Naturalist*, 115: 667–695.

WEBB, B. W. 1996. Trends in stream and river temperature. *Hydrological Processes*, 10: 205–226.

WEBB, B. W., D. M. HANNAH, R. D. MOORE, L. E. BROWN & F. NOBILIS. 2008. Recent advances in stream and river temperature research. *Hydrological Processes*, 22: 902–918.

WEBB, B. W. & Y. ZHANG. 1997. Spatial and seasonal variability in the components of the river heat budget. *Hydrological Processes*, 11: 79–101.

WOOD, P. J., A. J. BOULTON, S. LITTLE & R. STUBBINGTON. 2010. Is the hyporheic zone a refugium for aquatic macroinvertebrates during severe low flow conditions? *Fundamental and Applied Limnology/Archiv fur Hydrobiologie*, 176: 377–390.

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Freshwater food web studies: a plea for multiple tracer approach

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ABSTRACT

Freshwater food web studies: a plea for multiple tracer approach

Food webs are complex systems of interactions between ecosystem species. Beyond the direct analysis of stomach contents, stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) have been used widely to evaluate these trophic relationships and calculate the relative contribution of food sources to a consumer's diet using mixing models. However, there are still some constraints on the use of these traditional tracers that limit their output. Here, we briefly comment on the potential of using multiple tracers (i.e., stable isotopes of C, N and H; trace metals), and applying recent numerical approaches (i.e. Bayesian mixing models) to advance the understanding of complex aquatic food webs. Stable isotopes of hydrogen (δ^{2}H), normally used to examine large-scale migration patterns of terrestrial animals, have been recently proposed as a complementary trophic tracer in aquatic ecosystems. The principle for this application is the large isotopic difference in δ^{2}H among food items that can be found in some aquatic systems. Other potential trophic indicators are such substances that accumulate through diet (e.g., trace metals). These substances are traditionally studied from bioaccumulation or toxicological perspectives, but there are indications that encourage their use for tracing food web interactions. Bayesian mixing models, which are able to incorporate several sources of variability and multiple food sources in the model, can help to solve puzzling results. In summary, we suggest that the simultaneous use of multiple tracers will provide more reliable results than any of them in isolation. The challenge is to develop methods to combine them enhancing their strengths and minimizing uncertainty.

Key words: Stable isotopes, food webs, deuterium, trace metals, Bayesian mixing models.

RESUMEN

Estudio de redes tróficas acuáticas: una llamada al uso de múltiples marcadores

Las redes tróficas son sistemas complejos de interacciones entre las especies del ecosistema. Más allá del análisis directo de los contenidos estomacales, los isótopos estables del carbono ($\delta^{13}C$) y nitrógeno ($\delta^{15}N$) se han utilizado con frecuencia para evaluar las conexiones tróficas y calcular la contribución relativa de cada alimento en la dieta de los consumidores mediante modelos de mezcla. Sin embargo, todavía hay algunas restricciones en el uso de estos marcadores tradicionales que limitan su rendimiento. En este artículo comentamos brevemente la posibilidad de utilizar varios marcadores (i.e., los isótopos estables del C, N y H; metales traza), y aplicar nuevos métodos numéricos (i.e., modelos de mezcla bayesianos) para avanzar en la comprensión de las complejas redes tróficas acuáticas. Los isótopos estables del hidrógeno ($\delta^{2}H$), que normalmente se usan para examinar patrones de migración de los animales terrestres a grandes escalas, se han propuesto recientemente como un trazador trófico complementario en los ecosistemas acuáticos. Su aplicación se basa en la gran variabilidad de $\delta^{2}H$ entre las fuentes de alimento que se puede encontrar en algunos sistemas acuáticos. Otros posibles indicadores tróficos son tales sustancias que se acumulan a través de la dieta (por ejemplo, metales traza). Estas sustancias han sido tradicionalmente estudiadas desde perspectivas de bioacumulación o toxicológicas, pero hay indicios que estimulan su aplicación en el estudio de las interacciones tróficas. Los modelos bayesianos de mezcla, que son capaces de incorporar varias fuentes de variabilidad y múltiples fuentes de alimentos, pueden ayudar a resolver casos ambiguos. En resumen, sugerimos que el uso simultáneo de varios marcadores proporcionará resultados más fiables que cualquiera de ellos de forma individual. El reto está en desarrollar métodos para combinarlos aprovechando sus fortalezas y minimizando las incertidumbres.

Palabras clave: *Isótopos estables, redes tróficas, deuterio, metales traza, modelos bayesianos de mezcla.*

INTRODUCTION

Ecosystems are organized communities of producers, consumers, and decomposers along with an abiotic environment that influences species growth, reproduction, and dispersal (Covich, 2001). The early approach to trophic structure was a simple linear food chain where species aggregate into discrete trophic levels (plants-herbivores-carnivores) with declining numbers of individuals at higher levels (Elton, 1927). Soon, this simplistic view of ecosystem trophic structure was revised. Lindeman (1942) introduced the tropho-dynamics viewpoint in an article of exceptional significance for ecology. This paradigm included some basic principles, such as that energy transfer efficiency between trophic levels should limit the size and length of the food chain; and that increase in biomass at higher trophic levels can only be sustained if biomass turnover at lower levels is higher. The latter, for instance, occurs in marine pelagic food webs, in which short-living algal cells (i.e., phytoplankton) support a large biomass of longer-living zooplankton, and the biomass of heterotrophs exceeds that of autotrophs (Odum, 1971; Gasol *et al.*, 1997). Currently, the food chain view (Fig. 1a) has been replaced by the more realistic food web concept (Fig. 1b), which due to its complexity is a challenging research topic.

Food webs are networks of trophic interactions within an ecosystem, characterized by the number of species involved and the nature, number and intensity of their connections. The static view of trophic structure of ecosystems has been substituted by a concept that sees complexity and dynamism as an intrinsic property of food webs (Pimm, 1984; Polis & Strong, 1996). However, how variable trophic links are over space and time, how we can measure these complex interactions are questions difficult to approach.

Progress in the understanding of this complexity and dynamism requires development of new theoretical and operative frameworks, and the eventual merging of them. Here, we discuss pros and cons of the use of some new tracers (i.e., δ^2H, trace metals) and numerical approaches (i.e., Bayesian mixing models) that might be helpful to advance the understanding of the food web structure in aquatic ecosystems.

STABLE ISOTOPE ECOLOGY

Stable isotopes have been used widely in ecological research during the last decades (West *et al.*,

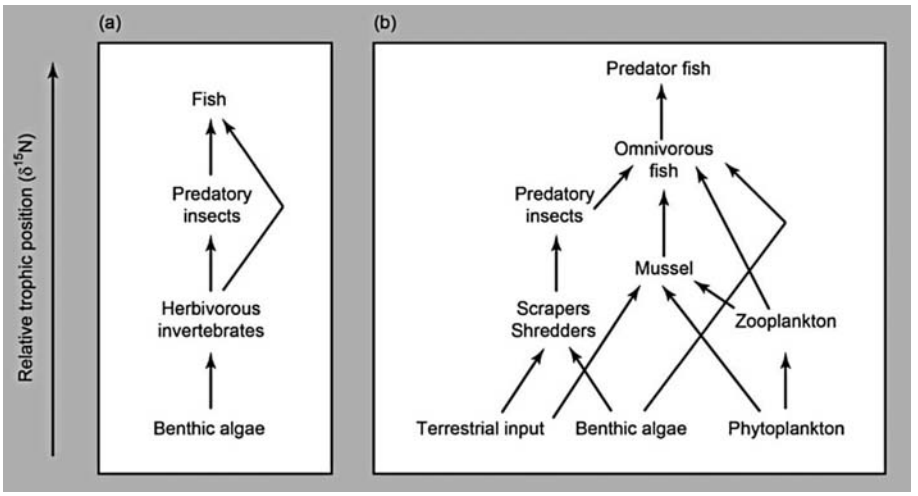

Figure 1. Rather than ordinary food chains (a), trophic relationships between aquatic organisms constitute complex food webs (b), which are difficult to disentangle without a set of different tracers. *Más allá de simples cadenas tróficas (a), las relaciones tróficas entre los organismos acuáticos forman complejas redes tróficas (b), las cuales son difíciles de desenmarañar sin un conjunto diverso de trazadores.*

2006). Particularly, after the development of continuous flow methods (CF-IRMS) of isotopic analyses, which are faster and cheaper techniques, stable isotope analyses have expanded to a widespread use in biology. Stable isotopes are forms of a given element that differ in atomic mass since they have the same number of protons, but different number of neutrons. This mass difference generates a variation in abundance of the heavier to the lighter isotope in organism tissues due to the different reaction and transport rates for molecules. Stable isotope measurements are generally expressed as the relative isotope-ratio difference or isotope delta (δ) values. They are usually reported in parts per thousand (‰) deviations from an international standard, as follows:

$$\delta X = [(R_A/R_{std}) - 1] \quad (1)$$

where R_A and R_{std} are the isotope ratio of the heavier and lighter isotope of the element X (e.g., $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{2}H/^{1}H$) in the sample and the international standard (e.g., PDB, AIR, and VSMOW, respectively).

In ecological studies, stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been applied to gain insight into food web structures (e.g., Peterson & Fry, 1987; Cabana & Rasmussen, 1994). Basically, the isotope ratio of a consumer reflects those of its diet with some trophic isotopic discrimination. The discrimination factor can vary according to species and tissues but, usually, average values from meta-analysis studies are applied in the calculations. Isotopic differences provide insight for investigating the relative use of food sources, contributions of different habitats to the entire food web, general degree of omnivory and other relevant aspects of the food web structure (France, 1995; Vander Zanden *et al.*, 1999). Stable isotopes provide some advantages to assess feeding relationships compared to traditional approaches (e.g., stomach content studies). For instance, they can trace the animal diet over different time periods, compared to the snapshot that using stomach content represents. Isotope turnover rates of each tissue vary and may provide information of animal diet over different time intervals (Karasov & Martínez del Rio, 2007). Tissues with fast turnover rates will achieve earlier the isotopic equilibrium and will reflect diet changes at shorter temporal scales.

BAYESIAN MIXING MODELS

Mixing models evaluate the relative contribution of different food items to consumer's diet (Phillips & Gregg, 2003), and their estimation can be refined incorporating differences in food stoichiometry (Phillips & Koch, 2002). Among several existing models, the one-isotope, two-source model (Boecklen *et al.*, 2011) is extensively used. It is based on the following assumptions:

$$\delta_T = f_A(\delta_A + \Delta_A) + f_B(\delta_B + \Delta_B) \quad (2)$$

$$1 = f_A + f_B \quad (3)$$

where δ_T, δ_A, and δ_B are the isotope value in the consumer's tissue, source A and source B, respectively; f_A and f_B are the fractional contribution for each source; and Δ is the diet-tissue trophic discrimination.

Trophic discrimination factors (e.g., $\Delta^{15}N$ and $\Delta^{13}C$) must be assigned a priori to each dietary food component to build mixing models. There are some aspects that require attention before applying these models. For example, consumers may lie outside the mixing polygon delimited by all the potential sources because some key end-member source is lacking or there are large differences in stoichiometry among food sources.

Recent stable-isotope mixing models (e.g., SIAR and MixSIR; Moore & Semmens, 2008; Parnell *et al.*, 2010) implement the Bayesian approach that may enable more accurate estimates to track trophic links in complex food webs than traditional approaches. The advantages of these models to previous approaches are the possibility to incorporate the variation in diet-tissue trophic discriminations (McCutchan *et al.*, 2003; Caut *et al.*, 2009) and prior information. Diet-tissue trophic discrimination can vary among consumers depending on sampled tissue (Pinnegar

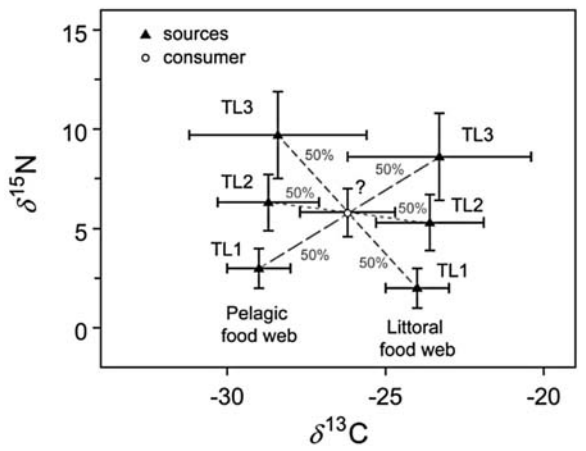

Figure 2. Hypothetical case illustrating the limitation of using two isotopic tracers in complex food webs. Three equally feasible solutions for the diet of a consumer can be estimated based on the δ^{13}C and δ^{15}N values of its potential food. There are three combinations of two 50 % food items (illustrated by different grey broken lines) from distinct trophic levels (TL): (i) TL1 (pelagic) and TL3 (littoral); (ii) TL3 (pelagic) and TL1 (littoral); and (iii) TL2 (pelagic) and TL2 (littoral). Uncertainties of the isotopic values were estimated as standard deviations (SDs) from 1,000 bootstrap iterations of the mixing model. New values of model parameters were drawn from normal distributions described by the estimated means and SDs for each iteration. No trophic discrimination was assumed. *Caso hipotético que ilustra la limitación del uso de dos trazadores en redes tróficas complejas. Se dan tres soluciones igualmente plausibles para la dieta de un consumidor estimadas a partir de los valores de δ^{13}C y δ^{15}N de su comida potencial. Hay tres combinaciones de dos fuentes de comida con una contribución del 50 % (ilustradas por distintas líneas segmentadas grises) procedentes de distintos niveles tróficos (TL): (i) TL1 (pelágica) y TL3 (litoral); (ii) TL3 (pelágica) y TL1 (litoral); y (iii) TL2 (pelágica) y TL2 (litoral). La variabilidad de los valores isotópicos se estimó como desviaciones estándar (DS) de 1000 iteraciones de remuestreo del modelo de mezcla. Nuevos valores de los parámetros del modelo se extrajeron de distribuciones normales determinadas por las medias y DS para cada iteración. Se asumió que no hay discriminación trófica.*

& Polunin, 1999), protein composition and proportion in the diet (Kelly & Martínez del Rio, 2010; Robbins *et al.*, 2010), food nitrogen content (Adams & Sterner, 2000), an animal growth and ingestion rates (Gaye-Siessegger *et al.*, 2004; Martínez del Rio *et al.*, 2009). The uncertainty of model estimates increases when consumers potentially feed on many sources (Phillips & Gregg, 2003). This uncertainty may be reduced using stomach content analysis to disregard some food sources (Catalan *et al.*, 2004), or introducing it in the Bayesian mixing models as prior information. In any case, stomach content is always useful to examine unexpected associations between food and consumers. However, it is necessary to keep in mind that stable isotopes and stomach contents are techniques that can refer to different time scales.

When using only two isotope tracers (e.g., δ^{13}C and δ^{15}N), consumers that potentially feed on totally different food sources can lie in the same isotopic niche (Fig. 2). Despite the advances of Bayesian mixing models, there is no way to disentangle the correct food composition. Figure 2 illustrates such case with a hypothetic example: the isotope values of a unique consumer in the δ^{13}C-δ^{15}N space could be feasibly interpreted by three completely different diet compositions within a complex food web. In addition to this complex case, limited power for discerning source contributions also occurs when there is little isotopic differentiation among sources or high variation in the diet-tissue trophic discrimination (Phillips & Gregg, 2003; Moore & Semmens, 2008; Bond & Diamond, 2011). In complex food webs, additional constraints might be useful to simplify the mixing models; for instance, gut contents could be used to discard certain sources and, in cases where the isotopic values of some sources are not statistically different, these sources can be pooled to reduce their number. However, increasing the number of tracers, which increase the degrees of freedom for the estimation of trophic links, may be more powerful for solving misleading cases.

STABLE ISOTOPES OF HYDROGEN

Stable hydrogen isotope ratios (δ^{2}H) have been used for tracking large-scale terrestrial migration movements and wildlife provenance. This approach is based on the well-known spatial isotope landscapes (or isoscapes) and the strong correlation between δ^{2}H values in precipitation and those in tissues from a given location (Hobson *et al.*, 2012). Hydrogen isotopes have also been used to distinguish between allochthonous and autochthonous sources (leaf litter versus primary producers) in a consumer diet (Doucett *et al.*, 2007). Aside from these applications, the

large δ^2H differences among the basal carbon sources of the food web in aquatic systems indicate that there is potential for distinguishing food web pathways. This means high δ^2H variability among food web components and, thus, increased interaction resolution if used in combination with the traditional stable isotopes.

The use of δ^2H as a dietary tracer in aquatic ecosystems is promising. However, previously, the mechanisms and processes that determine the variation of H isotopes in aquatic systems must be understood sufficiently in order to build a theoretical δ^2H framework for food web studies. Recent applications of δ^2H as an aquatic food web tracer assume a trophic compounding effect of water rather than trophic isotope discrimination (Solomon *et al.*, 2009; Soto *et al.*, 2011b), as an explanation to the trophic δ^2H patterns found by Birchall *et al.* (2005). This apparent trophic discrimination is caused by the H isotopic exchange with water *in vivo* during protein synthesis (Soto *et al.*, 2013). Controlled experiments have shown that the water contribution to tissue H varies with the type of organism. Therefore, in contrast with C and N, H in the consumer's tissues is derived both from diet and environmental water. The contribution of ambient water to tissue H at each trophic step through the food web determines the ^{2}H enrichment in consumers compared to their diet. Furthermore, there are other mechanisms of variation for H isotopes in organisms; for instance, the effect of the metabolic water derived from the metabolism of lipids. Ideally, in a given location, researchers should be able to determine diet δ^2H values for a consumer knowing the stable isotopic composition of the environmental water and that of metabolic water from ingested food components using mass-balance models.

Analytically, there are other factors that do not make trivial the δ^2H application for trophic purposes. (i) The isotopic exchangeability of H in organic samples with ambient vapour adds uncontrolled uncertainty to δ^2H measurements. This can make δ^2H results not comparable among laboratories unless they use some method to correct for the exchangeable H, such as the Comparative Equilibration method (Wassenaar & Hobson, 2000, 2003). (ii) Variation in the lipid content of tissues induces uncertainty because lipids are highly depleted in ^{2}H compared to the protein of the same animal tissue (Hobson *et al.*, 1999; Soto *et al.*, 2013). In addition, lipids do not have exchangeable H with ambient water vapour in the laboratory (Wassenaar & Hobson, 2000), in contrast with the calibrated standards used with the Comparative Equilibration method. Thus, lipids should be removed from samples before δ^2H measurements to avoid uncertainties in the evaluation of trophic relationships (Jardine *et al.*, 2009).

TRACE METAL BIOACCUMULATION

Trace metal bioaccumulation in aquatic organisms has been studied widely during last decades, being a major concern due to the impacts in human health (Luoma & Rainbow, 2008). However, trace metals have been seldom used to trace dietary sources (Stewart *et al.*, 2004) compared with their potential. Trace metals are ubiquitous elements whose environment concentrations depend on the natural background and contamination spills due to industrial production and agricultural treatments. Trace metal bioaccumulation in aquatic organisms results from exposure to medium and diet and involves complex mechanisms such as assimilation, storage, metabolism, and elimination of contaminants. Comparative studies considering several food web components for distinct contaminants are rare but hold a great potential for environmental biomonitoring and food web considerations (Soto *et al.*, 2011a). Food web components showed differences in trace metal concentrations among aquatic species with the same apparent trace metal exposure (Soto *et al.*, 2011a) and the difference in biomonitoring capacity is also the basis for the proposal that trace metals can complement stable isotopes in unveiling aquatic food web structure.

Concentrations of chemical elements that bioaccumulate in an organism should correlate to some degree to concentrations in its diet. Similarly to stable isotopes and according to trace metal bioaccumulation models, the trace metal concentrations in aquatic species are

affected by physiological characteristics of the species (e.g., growth, elimination rates, ingestion rates), which usually are related to animal size (Trudel & Rasmussen, 1997; Trudel *et al.*, 2000). Therefore, the relative differences of trace metal bioaccumulation among organisms can provide insights into species trophic relationships, and also the species metabolic requirements. Trace metals may become particularly useful when i) studying species with similar rates of key physiological processes (e.g., ingestion, trace metal elimination), and ii) the variation of trace metal concentration in food sources is high.

Combining trace metals and stable isotopes for evaluating trophic links appears promising. Cabana & Rasmussen (1994) showed that Hg bioaccumulation was related to the trophic position (δ^{15}N) and that the knowledge of the degree of omnivory could predict realistically the Hg biomagnification. Biomagnification of trace metals along food chains occurs because uptake from the diet is higher than elimination. On the contrary, As concentrations are biodiminished along food chains (Chen & Folt, 2000). The same explanation applies to δ^{15}N in which organisms retain ^{15}N preferentially over ^{14}N. There are cases in which trace metal discriminate better among trophic levels than stable isotopes. An example is shown in figure 3, corresponding to the macroinvertebrate food web of Flix reservoir. Predators and consumers (collector/scrapers) can scarcely be differentiated using C and N stable isotopes.

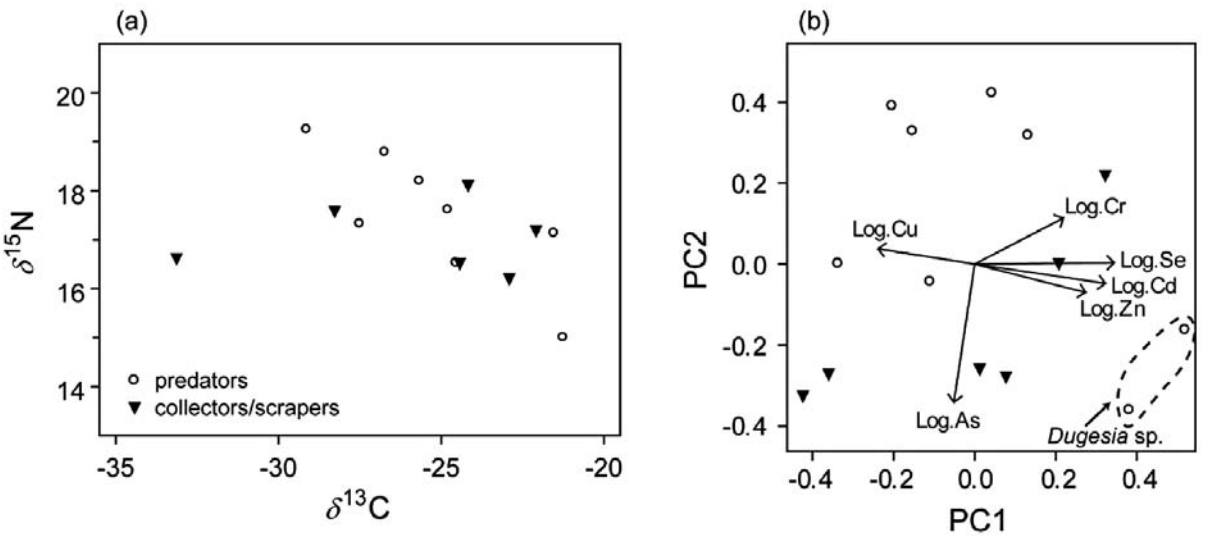

Figure 3. A case study example showing a situation in which trace metals discriminate better among trophic groups than stable isotopes. Data correspond to macroinvertebrates from the Flix reservoir (Ebro River, Spain) sampled in 2006. (a) The δ^{13}C and δ^{15}N values (Soto *et al*., 2011b). (b) Principal component biplot of trace metal concentrations (trace metal data from Soto *et al*., 2011a). The principal components (PC1 and PC2, respectively) explained 50 % and 17 % of the variation, respectively. Predators are clearly discriminated from consumers by the biodiminished arsenic (As) concentrations. Only the peculiar case of Turbellaria (*Dugesia* sp.) does not follow the pattern. Predator taxa include: *Dugesia* sp. (Turbellaria), Hydrophilidae adults (Coleoptera, Insecta), Coenagrionidae (Odonata, Insecta), and *Naucoris* sp. (Naucoridae, Heteroptera, Insecta); and collectors/scrapers include: *Physa* sp. (Gastropoda, Mollusca), *Cloëon* sp. (Baetidae, Ephemeroptera, Insecta), and Hydrophilidae larvae (Coleoptera, Insecta). *Un caso de estudio que ilustra una situación en que los metales traza discriminan mejor entre grupos tróficos que los isótopos estables. Los datos corresponden a macroinvertebrados del embalse de Flix (río Ebro, España) muestreados durante el 2006. (a) Valores de δ^{13}C y δ^{15}N (Soto et al., 2011b). (b) Biplot de un análisis de componentes principales de las concentraciones de metales traza (datos de metales traza de Soto et al., 2011a). Las componentes principales (PC1 y PC2) explican un 50 % y un 17 % de la variación, respectivamente. Los depredadores se discriminan claramente de los consumidores por el efecto de biodismiución del arsénico (As). Sólo el caso peculiar de los Turbelarios (i.e., Dugesia, sp.) no sigue la pauta. Los depredadores incluyen: Dugesia sp. (Turbellaria), adultos de Hydrophilidae (Coleoptera, Insecta), Coenagrionidae (Odonata, Insecta), y Naucoris sp. (Naucoridae, Heteroptera, Insecta); y los consumidores: Physa sp. (Gastropoda, Mollusca), Cloëon sp. (Baetidae, Ephemeroptera, Insecta), y larvas de Hydrophilidae (Coleoptera, Insecta).*

However, the trace metal signatures clearly differentiate between the two trophic groups. More research regarding the consistency of the multivariate trace metal patterns should be undertaken.

There are already some studies that point towards a use of multiple tracers. Soto *et al.*, (2011b) found that Hg and As concentrations in fish from a reservoir were positively and negatively correlated, respectively, with trophic indicators based on C and N stable isotopes. The combination of trace metals and stable isotopes (δ^{13}C and δ^{15}N) were also useful to show a potential trophic meaning of δ^{2}H values which was unclear only using δ^{13}C and δ^{15}N (Soto *et al.*, 2011b). A case study from San Francisco Bay, organisms feeding on bivalves had much higher selenium concentrations than those species that fed on crustaceans because of the lower loss rate constant of selenium in bivalves (Stewart *et al.*, 2004). Organochlorine contaminants have also been occasionally used in feeding ecology, in cases where the interpretation of trophic position with only stable isotopes as indicators could be imprecise (Fisk *et al.*, 2002).

In summary, we suggest that increasing the number of tracers by combining trace metals (or other bioaccumulated contaminants) and stable isotopes can be a powerful technique in systems where the isotope values of consumers result insufficient to separate feeding modes.

FINAL REMARKS

The use of δ^{13}C and δ^{15}N is helpful for revealing the food web structure of aquatic ecosystems, but usually insufficient for disentangling complex food webs. It cannot be expected that only two tracers solve the myriad of possible interactions that occur in food webs. Complementary trophic tracers of considerable potential are other stable isotopes (i.e., δ^{2}H) and substances that bioaccumulate through food webs (i.e., trace metals, organic pollutants).

For a confident use of δ^{2}H in aquatic food web studies, the potential confounding effect of seasonal and spatial hydrogen isotopic variation of environmental water should be taken into account along with other mechanisms that drive the hydrogen isotopic variability.

Differential trace metal accumulation patterns occur among food web organisms. This diversity is the basis to suggest trace metal use for studying food webs. Any increase in understanding the bioaccumulation process will serve also the application of trace metals as food web tracers. The combined use of both techniques (stable isotopes and trace metals) can be highly complementary, particularly, when values in the potential food sources show little variability for one of them.

In addition to using more tracers, the numerical estimations can also be improved. Bayesian mixing models are a powerful tool to obtain reliable results because can take into account sources of variability in the data interpretation. These models can be performed at both population and individual levels to trace the links of complex food webs. Quantitative estimation of trophic food webs may have applications that range from theoretical (e.g., food web stability) to applied investigations (e.g., invasive species diet).

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REFERENCES

ADAMS, T. S. & R. W. STERNER. 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnology and Oceanography,* 45: 601–607.

BIRCHALL, J., T. C. O'CONNELL, T. H. E. HEATON & R. E. M. HEDGES. 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. *Journal of Animal Ecology,* 74: 877–881.

BOECKLEN, W. J., C. T. YARNES, B. A. COOK & A. C. JAMES. 2011. On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, 42: 411–440.

BOND, A. L. & A. W. DIAMOND. 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, 21: 1017–1023.

CABANA, G. & J. B. RASMUSSEN. 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature*, 372 (6503): 255–257.

CATALAN, J., M. VENTURA, I. VIVES & J. O. GRIMALT. 2004. The roles of food and water in the bioaccumulation of organochlorine compounds in high mountain lake fish. *Environmental Science and Technology*, 38: 4269–4275.

CAUT, S., E. ANGULO & F. COURCHAMP. 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46: 443–453.

CHEN, C. Y. & C. L. FOLT. 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. *Environmental Science and Technology*, 34: 3878–3884.

COVICH, A. P. 2001. Energy Flow and Ecosystems. In: *Encyclopedia of Biodiversity*. A. L. Simon (ed.): 509–523. Elsevier, New York, USA.

DOUCETT, R. R., J. C. MARKS, D. W. BLINN, M. CARON & B. A. HUNGATE. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology*, 88: 1587–1592.

ELTON, C. S. 1927. *Animal ecology*. Macmillan. New York. USA.

FISK, A. T., S. A. TITTLEMIER, J. L. PRANSCHKE & R. J. NORSTROM. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland Sharks. *Ecology*, 83 (8): 2162–2172.

FRANCE, R. L. 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography*, 40: 1310–1313.

GASOL, J. M., P. A. DEL GIORGIO & C. M. DUARTE. 1997. Biomass distribution in marine planktonic communities. *Limnology and Oceanography*, 42: 1353–1363.

GAYE-SIESSEGGER, J., U. FOCKEN, S. MUETZEL, H. ABEL & K. BECKER. 2004. Feeding level and individual metabolic rate affect δ^{13}C and δ^{15}N values in carp: implications for food web studies. *Oecologia*, 138: 175–183.

HOBSON, K. A., L. ATWELL & L. I. WASSENAAR. 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Sciences*, 96: 8003–8006.

HOBSON, K. A., D. X. SOTO, D. R. PAULSON, L. I. WASSENAAR & J. H. MATTHEWS. 2012. A dragonfly (δ^2H) isoscape for North America: a new tool for determining natal origins of migratory aquatic emergent insects. *Methods in Ecology and Evolution*, 3: 766–772.

JARDINE, T. D., K. A. KIDD & R. A. CUNJAK. 2009. An evaluation of deuterium as a food source tracer in temperate streams of eastern Canada. *Journal of the North American Benthological Society*, 28: 885–893.

KARASOV, W. H. & C. MARTÍNEZ DEL RIO. 2007. *Physiological ecology: How animals process energy, nutrients, and toxins*. Princeton University Press. Princeton.

KELLY, L. J. & C. MARTÍNEZ DEL RIO. 2010. The fate of carbon in growing fish: an experimental study of isotopic routing. *Physiological and Biochemical Zoology*, 83: 473–480.

LINDEMAN, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology*, 23: 399–417.

LUOMA, S. N. & P. S. RAINBOW. 2008. *Metal contamination in aquatic environments: science and lateral management*. Cambridge University Press. New York.

MARTÍNEZ DEL RIO, C., N. WOLF, S. A. CARLETON & L. Z. GANNES. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, 84: 91–111.

MCCUTCHAN, J. H., JR., W. M. LEWIS, JR., C. KENDALL & C. C. MCGRATH. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102: 378–390.

MOORE, J. W. & B. X. SEMMENS. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters*, 11: 470–480.

ODUM, E. P. 1971. *Fundamentals of ecology*. Saunders. Philadelphia.

PARNELL, A. C., R. INGER, S. BEARHOP & A. L. JACKSON. 2010. Source partitioning using stable isotopes: Coping with too much variation. *PLoS ONE*, 5: e9672.

PETERSON, B. J. & B. FRY. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18: 293–320.

PHILLIPS, D. & P. KOCH. 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, 130: 114–125.

PHILLIPS, D. L. & J. W. GREGG. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, 136: 261–269.

PIMM, S. L. 1984. The complexity and stability of ecosystems. *Nature*, 307 (5949): 321–326.

PINNEGAR, J. K. & N. V. C. POLUNIN. 1999. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. *Functional Ecology*, 13: 225–231.

POLIS, G. A. & D. R. STRONG. 1996. Food web complexity and community dynamics. *The American Naturalist*, 147: 813–846.

ROBBINS, C. T., L. A. FELICETTI & S. T. FLORIN. 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. *Oecologia*, 162: 571–579.

SOLOMON, C. T., J. J. COLE, R. R. DOUCETT, M. L. PACE, N. D. PRESTON, L. E. SMITH & B. C. WEIDEL. 2009. The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia*, 161: 313–324.

SOTO, D. X., R. ROIG, E. GACIA & J. CATALAN. 2011a. Differential accumulation of mercury and other trace metals in the food web components of a reservoir impacted by a chlor-alkali plant (Flix, Ebro River, Spain): Implications for biomonitoring. *Environmental Pollution*, 159: 1481–1489.

SOTO, D. X., L. I. WASSENAAR, K. A. HOBSON & J. CATALAN. 2011b. Effects of size and diet on stable hydrogen isotope values (δD) in fish: implications for tracing origins of individuals and their food sources. *Canadian Journal of Fisheries and Aquatic Sciences*, 68: 2011–2019.

SOTO, D. X., L. I. WASSENAAR & K. A. HOBSON. 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Functional Ecology*, 27: 535–543.

STEWART, A. R., S. N. LUOMA, C. E. SCHLEKAT, M. A. DOBLIN & K. A. HIEB. 2004. Food web pathway determines how selenium affects aquatic ecosystems: A San Francisco Bay case study. *Environmental Science and Technology*, 38: 4519–4526.

TRUDEL, M. & J. B. RASMUSSEN. 1997. Modeling the elimination of mercury by fish. *Environmental Science and Technology*, 31: 1716–1722.

TRUDEL, M., A. TREMBLAY, R. SCHETAGNE & J. B. RASMUSSEN. 2000. Estimating food consumption rates of fish using a mercury mass balance model. *Canadian Journal of Fisheries and Aquatic Sciences*, 57: 414–428.

VANDER ZANDEN, M. J., J. M. CASSELMAN & J. B. RASMUSSEN. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, 401 (6752): 464–467.

WASSENAAR, L. I. & K. A. HOBSON. 2000. Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. *Environmental Science and Technology*, 34: 2354–2360.

WASSENAAR, L. I. & K. A. HOBSON. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies*, 39: 211–217.

WEST, J. B., G. J. BOWEN, T. E. CERLING & J. R. EHLERINGER. 2006. Stable isotopes as one of nature's ecological recorders. *Trends in Ecology & Evolution*, 21: 408–414.

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Filtration rates of the non-native Chinese mystery snail (*Bellamya chinensis*) and potential impacts on microbial communities

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ABSTRACT

Filtration rates of the non-native Chinese mystery snail (*Bellamya chinensis*) and potential impacts on microbial communities

Invasive species in the phylum Mollusca, including gastropods and bivalves, have caused substantial impacts in freshwater ecosystems. The Chinese mystery snail, *Bellamya chinensis*, is a large viviparid snail native to Southeastern Asia and widely introduced throughout United States and parts of Canada and Europe. *B. chinensis* is a facultative filter-feeding detritivore that can both graze epiphytic diatoms using its radula and filter-feed its breathing water. Despite mounting concern associated with the expanding range and increasing abundance of *B. chinensis* in many parts of its invaded range, the potential ecological impacts of this non-native species remain largely unknown. Here, we used a series of laboratory experiments to assess filtration rates of *B. chinensis* and quantify its effects on microbial communities. According to both microcosm (24-hour, 4-L suspension) and mesocosm (5-day, 90-L suspension) experimental trials, *B. chinensis* exhibited an average filtration rate of 106-113 mL snail^{-1}h^{-1} (1.45 mL mg DW^{-1}h^{-1}) and an individual maximum of 471 mL snail^{-1}h^{-1} (6.15 mL mg DW^{-1}h^{-1}). These values are comparable to reported filtration rates for high-profile invasive, freshwater bivalves. Relationships between snail size and filtration rate relationship suggests that *B. chinensis* display an ontogenetic shift in feeding behavior from primarily radular grazing to increased filter-feeding at threshold size of approximately 44 mm shell height. Our experiments also revealed that high snail densities can result in small, significant shifts in bacterial community composition. These results suggest that *B. chinensis* may influence microbial communities either directly by using bacteria as a food source or indirectly by producing sufficiently large quantities of fecal and pseudo-fecal material to affect bacterial activity and growth. The overall ecological effects and importance of *B. chinensis* filtration behavior remain unclear, but our experimental results suggest that these impacts may be large and should be further investigated to better understand its potential role in coupling benthic and pelagic food webs in lake ecosystems.

Key words: Invasive species, chlorophyll-*a*, *Dreissena polymorpha*, *Corbicula fluminea*, *Potamopyrgus antipodarum*, *Pomacea canaliculata.*

RESUMEN

Tasas de filtración de la especie introducida* Bellamya chinensis *y su potencial impacto en las comunidades microbianas

Las especies invasoras del phyllum Mollusca, incluyendo los gasterópodos y bivalvos, han causado impactos importantes en los ecosistemas dulceacuícolas. Bellamya chinensis, *es un vivíparo de gran tamaño, nativo del sureste de Asia y ampliamente introducido a lo largo de los Estados Unidos y parte de Canadá y Europa.* B. chinensis *es una especie detritivora- filtradora facultativa, que puede tanto ramonear diatomeas epifitas usando su rádula como filtrar el agua que respiran. A pesar de la creciente preocupación asociada al incremento en la abundancia y rango de distribución de* B. chinensis *en las regiones ya colonizadas, el potencial impacto ecológico de esta especie introducida permanece ampliamente desconocido. En este estudio, usamos series de experimentos de laboratorio para evaluar las tasas de filtración de* B. chinensis *y cuantificar su efecto en las comunidades microbianas. De acuerdo con los experimentos realizados tanto en los microcosmos (24-hour, 4-L suspensión) como en los mesocosmos (5-day, 90-L suspensión),* B. chinensis *mostró una tasa promedio de filtración de 106-113 mL caracol^{-1}h^{-1} (1.45 mL mg peso seco^{-1}h^{-1}) y un máximo por individuo de 471 mL caracol^{-1}h^{-1} (6.15 mL mg peso seco^{-1}h^{-1}). Estos valores son comparables a otros reportados para especies de bivalvos dulceacuícolas altamente invasivas. La relación entre el tamaño de los caracoles y las tasas de filtración sugieren que* B. chinensis *muestra un cambio ontogénico*

en la manera de alimentarse, de ramoneo a una mayor alimentación por filtración, a partir de un umbral de tamaño de la concha de aproximadamente 44 mm de altura. Nuestros experimentos también revelan que altas densidades de caracoles generan pequeños cambios pero significativos en las comunidades microbianas. Estos resultados sugieren que B. chinensis *afectaría las comunidades microbianas de forma directa usando las bacterias como fuente de alimentación o indirectamente al producir una cantidad de materia fecal o seudo-fecal, suficiente para afectar la actividad y crecimiento bacteriano. El impacto ecológico global y el comportamiento como filtrador de* B. chinensis *aún no son claros, pero nuestros resultados experimentales sugieren que estos impactos pueden ser importantes y se deben investigar mejor para entender más su papel potencial en el acoplamiento de las redes tróficas bentónicas y pelágicas en los sistemas lacustres.*

Palabras clave: *Especies invasoras, clorofila*-a, Dreissena polymorpha, Corbicula fluminea, Potamopyrgus antipodarum, Pomacea canaliculata.

INTRODUCTION

Invasive species in the phylum Mollusca, including gastropods and bivalves, have caused substantial impacts in freshwater ecosystems, including alteration of water biogeochemistry, extirpation of native species, and modification of entire food webs (Strayer, 1999; Sousa *et al*. 2009). Notable examples range from the widespread invasion of zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) throughout North America (Strayer, 2009; Higgins & Vander Zanden, 2010) to the numerous introductions of Asian clam (*Corbicula fluminea*: Hakenkamp *et al*., 2001; Vaughn & Spooner, 2006), golden mussel (*Limnoperna fortunei*: Ricciardi, 1998; Boltovskoy *et al*., 2009), New Zealand mudsnail (*Potamopyrgus antipodarum*: Hall *et al*., 2003; Kerans *et al*., 2005) and golden apple snail (*Pomacea canaliculata*: Carlsson *et al*., 2004; Rawlings *et al*. 2007) in recent decades. In one such example, the invasion of golden apple snails in Southeast Asia drove wetlands from a macrophyte-dominated, clear water state to a turbid, phytoplankton-dominated state with concomitant shifts in the biological communities (Carlsson, *et al*. 2004). Although the ecosystem consequences of the aforementioned invaders have garnered significant scientific attention, we know very little of the ecology and potential ecological impacts of numerous other non-native mollusks (Strayer, 2012).

The Chinese mystery snail, *Bellamya* [= *Cipangopaludina*] *chinensis* (Gray, 1834), is a viviparid snail native to southeastern Asia and first documented in the United States over a century ago in Chinese markets of San Francisco (Wood, 1892). *Bellamya chinensis* (hereafter "*Bellamya*") is the second largest snail in North America (approaching 70 mm maximum shell height), and its thick outer shell and a hard operculum flap covering the aperture (shell opening) provide a high degree of protection from predators and unfavorable environmental conditions. *Bellamya* was likely introduced to the United States multiple times through the aquarium trade and water gardening industry or for culinary purposes (Mackie, 1999), and it is now widely distributed in lakes and slow-moving rivers across North America. Recent evidence also suggests that *Bellamya* is highly resistant to desiccation leading to the potential for overland transport via boats (Havel, 2011). *Bellamya*'s invaded range includes at least 37 states in the United States, several provinces of southern Canada (Jokinen, 1982; Bury *et al*., 2007), and parts of Europe (Soes *et al*., 2011). During the 1960s, *Bellamya* were reportedly removed from the Laurentian Great Lakes by the metric ton (Mills *et al.*, 1993).

Despite mounting concern associated with the expanding range and increasing abundance of *Bellamya* in many parts of its invaded range (Bury *et al*., 2007; Karatayev *et al*., 2009; Solomon *et al*., 2010; Chaine *et al*., 2012), the

potential ecological impacts of this non-native species remain speculative. Johnson *et* al. (2009) found that the presence of *Bellamya* caused substantial declines in the growth and abundance of two native snails in an experimental setting, reduced algal biomass, and increased the N:P ratio in the water column. Similarly, Clark (2009) found that *Bellamya* caused decreased growth rates in a native *Physa* snail. Results from a field survey by Solomon *et al.* (2010) revealed no difference in snail assemblage structure associated with *Bellamya* presence or abundance at the scale of an entire lake, although some native snail species tended not to occur at sites where *Bellamya* was abundant. With the exception of these studies, little else is known about the potential ecological effects of this species, including its degree of reliance on benthic vs. pelagic resources and/or its effects on microbial communities. However, these impacts may be substantial because *Bellamya* is unique in that it is a facultative filter-feeding detritivore that switches between grazing benthic and epiphytic diatoms using its radula and filter-feeding the water column (Plinski *et al*., 1978; Dillon, 2000). Indeed, the popularity of using *Bellamya* to clarify water in the pet aquarium and water garden trades attests to their efficiency as filter-feeders.

Bacteria are critical in freshwater ecosystems because they cycle essential nutrients and remove harmful toxins or chemicals from the water (Newton *et al*., 2011). To date, little is known about impacts of species invasions on microbial communities (Ehrenfeld, 2010) and how these changes may alter nutrient cycles in invaded ecosystems (van der Putten *et al*., 2007). *Bellamya* may interact either directly with microbial populations by utilizing bacteria as a food source or indirectly by influencing the composition, activity, and growth of microbial communities via production of large quantities of fecal and pseudo-fecal material. Previous studies of invasive zebra mussel have shown direct effects on the abundance of major groups of microbes (e.g., Frischer *et al*., 2000; Lavrentyev *et al*., 2000; Lohner *et al*., 2007) and a significant increase in the abundance of sediment-associated bacteria (Higgins *et al*., 2010).

In the present study, we provide the first quantification of filtration rates by *Bellayma* and investigate its potential effects on the diversity and composition of freshwater microbial communities. Given the vast diversity of microbial cells in the environment, the characterization of microbial community structure has been a challenge. Here, we used a ribosomal DNA fingerprinting technique (specifically, automated rRNA intergenic spacer analysis) to examine shifts in the whole bacterial community (including both rare and abundant taxa) in response to increasing densities of *Bellamya* in an experimental setting.

METHODS

Experimental design

We evaluated the filter feeding capacity of *Bellamya* using an indoor microcosm experiment during February 2009 in the School of Aquatic and Fishery Science's hatchery at the University of Washington. Glass microcosms (4-L volume) were filled with water directly from Lake Washington and experienced ambient photo-period (L:D = 12:12 hours) and water temperatures (16.8 °C ±0.3 °C). Natural seston was kept suspended using an aeration (bubbling) device. To each of 72 microcosms we added either none (control, $n = 18$) or one snail (collected from the wild) from each of three size ranges: 30-40 mm ($n = 18$), 40-50 mm ($n = 18$), 50-60 mm ($n = 18$); measured according to shell height. At time zero (initial) and 24 hours (final) of the experiment we siphoned water samples from two inches below the surface of the water in the center of the microcosm for chlorophyll-*a* analysis. Ash-free dry weight (DW) of each snail was determined.

We assessed both filtration rates and the effects of *Bellamya* on microbial community composition using an indoor mesocosm experiment during August 2009. Mesocosms (90-L volume, 0.18 m^2 area) were covered with 5 kg of coarse gravel (0.5-2.0 cm in diameter, 3 cm depth), filled with water directly from Lake Washington, and experienced ambient photo-period (L:D = 14:10 hours) and water temperatures (21.5 °C ±0.5 °C).

Natural seston was kept suspended using an aeration (bubbling) device. Four pairs of ceramic tiles were placed on the bottom surface of each mesocosm in each cardinal direction (total of 8 tiles per mesocosm). After a five-day pre-experiment period, we added 0 (control), 2 (low) or 4 (high) snails to each of 6 mesocosms (replicates), representing a total of 18 mesocosms. These snail densities (11 and 22 ind m^{-2}, respectively) are comparable to those reported in surveys of Wisconsin, Washington State and European lakes, which ranged from 1 to 38 ind m^{-2} (Olden *et al*., 2009; Solomon *et al*., 2010; Sousa *et al*., 2009). Snails measured 45-50 mm in shell height and were collected from the wild one-day prior to the start of the experiment. On day zero (initial) and day five (final) of the experiment we (1) collected water samples from two inches below the surface of the water in the center of the mesocosm for chlorophyll-*a*and microbial assemblage analysis, and (2) collected periphyton by scraping the top of one tile in each of the cardinal directions (total of four tiles per time period).

Chlorophyll-*a* concentration and filtration rate

For both the microcosm and mesocosm experiments, chlorophyll *a* (mg m^{-3}) was measured by filtering a 500 mL aliquot of water through a 47 mm Whatman GF/C filter paper and using a Turner Designs spectrophotometer following standardized protocols (http://lter.limnology.wisc.edu/research/protocols). A large number of studies have shown that filtration rates can be reliability measured using the clearance method and that consumption models based on natural seston produce more accurate predictions compared to data based on algal monocultures (reviewed in Riisgård, 2001). Filtration rates were estimated following Coughlan (1969) and many other studies by measuring the decline in chlorophyll-*a* over the experimental period:

$$FR = \frac{M}{n} \times \frac{(\ln C_{\text{initial}} - \ln C_{\text{final}}) - \left(\ln \bar{C}'_{\text{initial}} - \ln \bar{C}'_{\text{final}}\right)}{t}$$

where *FR* is the filtration rate (volume cleared per snail per hour: mL $\text{snail}^{-1}\text{h}^{-1}$), *M* is the volume of the water suspension (mL), *n* is the number of snails, C_{initial} and C_{final} are the initial and final concentrations in the snail treatment, $\bar{C}'_{\text{initial}}$ and $\bar{C}'_{\text{final}}$ are the mean initial and final concentrations in control treatments, and *t* is elapsed time (hours). In this formulation, the mean change in control concentrations (without snails) was subtracted from the change in experimental concentrations (with snails). Reductions of chlorophyll-*a* in controls would likely be attributable to zooplankton grazing and/or pigment degradation. Both experiments involved relatively long incubation times (24 and 120 hours), therefore small amounts of pseudofecal material were generated by the snails and could have been re-suspended in the water column. Thus, some particulate material may have been re-filtered, and therefore the measured filtration rates presented should be considered conservative estimates compared to a situation where pseudofeces have been removed during the course of the experiment.

Bacterial community composition

Bacterial community composition was assessed using automated ribosomal intergenic spacer analysis (ARISA: Fisher & Triplett, 1999). ARISA generates fingerprints of the microbial community based on the length heterogeneity in the intergenic spacer region between the 16S and 23S rRNA genes, which varies among organisms. Although ARISA has similar limitations as other PCR-based fingerprinting approaches (Fisher & Triplett, 1999), it has been shown to give a robust, high-resolution view of bacterial assemblages in aquatic ecosystems (Brown *et al*., 2005; Yannarell & Triplett, 2005) and can represent species-level taxonomic resolution (98-99 % sequence similarity; Brown *et al*., 2005). A sample of 500 mL of water was filtered in duplicate onto a 0.22 μ m filter (Supor-200, Pall Gelman, East Hills, NY), followed with 3 mL preservation buffer (10 mM Tris pH 8.0, 100 mM EDTA, 0.5 M NaCl) and frozen at −80° until analysis. DNA was extracted from replicate filters using the Qiagen DNEasy Blood and Tissue Mini-kit (Qiagen, Valencia, CA). The 16S-23S

intergenic region was amplified using the polymerase chain reaction (PCR) from the total extracted DNA using 6-FAM-labelled universal 1406-F primer (5′-TGYACACACCGCCCGT-3′) and bacterial specific primer 23S-R (5′-GGGTT BCCCCATTCRG-3′) (Fisher & Triplett, 1999; Yannarell *et al*., 2003). For each sample, four independent PCRs were performed, pooled, ethanol precipitated to remove unincorporated primers and run on a MegaBace 96 capillary sequencer along with ROX labeled size standards (50-1500 bp ladder, BioVentures, Inc). This sequencer is routinely used for fragment analysis and can resolve differences of 2 bp in fragments in the 300-400 bp range and differences of 10 bp for larger fragments (1000-1500 bp).

Fragment lengths were sized using DAX software (http://www.dax.nl/dax.htm) and a signal to noise cutoff was used to verify presence of peaks. Operational taxonomic units (OTUs) were generated by binning ARISA fragments into successively larger length bins based on their size and eliminating fragments that were <150 and >1300 bp (Brown *et al*., 2005). Although OTUs are arbitrarily numbered groups of taxa and are not traceable to phylogenetic or functional groups, the ARISA approach provides a rapid and affordable way to assess changes in taxonomic composition of bacterial communities. We used both peak area and maximum peak area to estimate relative abundance of OTUs in our samples (Yannarell & Triplett, 2005), as well as examined OTU presence-absence.

Data analysis

Results from the microcosm experiments were used to assess the relationship between snail size (shell height, mm) and filtration rate (mL $snail^{-1}h^{-1}$). Initial data exploration revealed that a linear model was not appropriate for the entire range of values and that a non-linear function was also insufficient. This prompted the comparison of three models: linear regression, quadratic regression and piecewise regression. Piecewise or segmented regression recognizes that different linear relationships may occur over different ranges of the independent variable (in this case, snail size). Breakpoints are values on the x-axis where a change in the slope of the different linear relationships can be defined; these breaks may or may not be known before the analysis (Toms & Lesperance, 2003). Although this method is flexible enough to include segments (e.g., linear, polynomial) and breakpoints (e.g., sharp, smooth) of different forms, we estimated one sharp breakpoint and two linear, continuous functions.

Results from the mesocosm experiment were analyzed using univariate and multivariate statistics to assess the effects of *Bellamya* on algal biomass (water column and substrate) and to quantify impacts on microbial (OTU) community composition. Student's t-tests tested for differences in chlorophyll-*a* between initial and final conditions for the low ($n = 2$) and high ($n = 4$) snail density treatments (after controlling for changes in the control treatment).

Multivariate analyses of microbial community composition included Mantel's tests, analysis of multivariate similarity (ANOSIM) and non-metric multidimensional scaling (NMDS). We used Sørensen's similarity index (OTU presence-absence) and Bray-Curtis similarity index (OTU abundance according to area and peak height assignments) to summarize compositional similarity between treatment-replicate samples (3 treatment levels × 6 replicates = 18 observations). Sørensen index was used because it represents the complement to the Bray–Curtis index when using presence–absence data (Legendre & Legendre, 1998), thus allowing for a direct comparison of the multivariate results based on OTU presence-absence and abundance (peak height, maximum area). Similarity matrices were calculated separately for each for treatment (control, low, high) to allow for a comparison between the initial and final conditions.

Deep sequencing of microbial assemblages using 454 sequencing allows for the detection of extremely low abundance taxa. These extremely low abundance or unique taxa may influence the results from multivariate analyses and make data interpretation less clear (Rudi *et al*., 2007; Gobet *et al*., 2010). To assess how the occurrence of rare taxa might affect the results, Mantel tests were performed to assess the relationships between

dissimilarity matrices of the entire OTU dataset and two reduced datasets where OTUs that occurred in < 2 % or < 5 % of the samples were removed. Gastropods can often hosts their own unique microbial assemblages, therefore OTUs that were only present in the *Bellamya* treatments (i.e. were unique to snails) were removed from all datasets. We found statistically significant pairwise correlations between all datasets (Mantel R = 0.82-0.99, all $P < 0.001$), indicating that the choice of OTU occurrence vs. abundance and the effect of rare OTUs did not heavily influence the results from the multivariate analysis. Consequently, we used the full dataset (not excluding "rare" OTU) containing OTU presence-absence.

We assessed the effects of *Bellamya* on bacterial community composition using the complementarily approaches of multivariate ordination (NMDS) and analyses of variance (ANOSIM). NMDS is an ordination method that preserves the rank ordered distances between observations in ordination space; here it was used to visualize differences in microbial composition between the initial and final time periods in each of the snail treatments (control, low, high). NMDS is an iterative approach that rearranges observations in ordination space to minimize a measure of disagreement (referred to as stress) between the compositional dissimilarities and the distance between points in the ordination diagram (Kruskal, 1964). We used a distance matrix based on Sørensen's similarity index to ordinate the observations (replicates) in 2 dimensions with 100 random starts and tested the significance of the stress value with a Monte Carlo randomization test.

We used ANOSIM (Clarke, 1993), a nonparametric multivariate procedure analogous to analysis of variance, to test for differences in microbial community composition between the initial and final time periods in each of the snail treatments (control, low, high). ANOSIM tests predefined groups against random groups in ordination space by comparing the average of all rank similarities among observations within groups to the average of rank similarities among observations between groups (expressed as a R_{ANOSIM} statistic). We conducted 999 random permutations to assess the statistical significance of R. All analyses were conducted in R version 2.15 (R Development Core Team 2012).

RESULTS

Filtration rates

The mean filtration rate in the 24-hour microcosm experiment (where *Bellamya* were examined individually) was 106 mL snail^{-1}h^{-1} (SD = 145) or 1.45 mL mg DW^{-1}h^{-1} (SD = 2.30); snails ranged from 30 to 60 mm in size. The individual maximum filtration rate was 471 mL snail^{-1}h^{-1} (6.15 mL mg DW^{-1}h^{-1}). The mean filtration rate, based on the 5-day mesocosm experiment where *Bellamya* were examined in groups of 2 or 4 individuals, was 113 mL snail^{-1}h^{-1} (SD = 158). Estimated filtration rates were over two times greater in the high- versus low-density treatment (159 vs. 66 mL snail^{-1}h^{-1}), suggesting that individual snails were filtering at a faster rate when in the presence of more conspecifics. Notably, we found very similar mean filtration rates for snails in the 45-50 mm size range based on the 24-hour

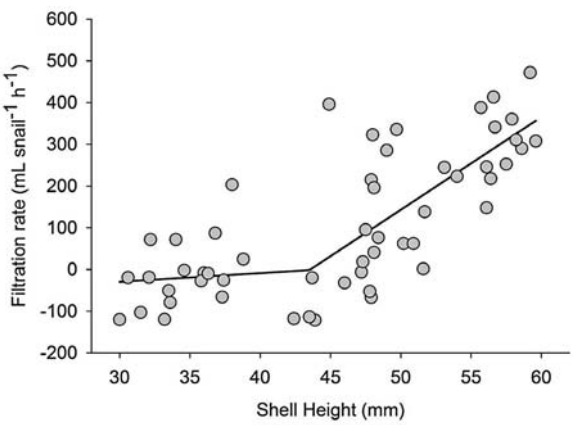

Figure 1. *Bellamya* filtration rates (mL mussel^{-1}h^{-1}) as a function of snail shell height (mm) based on the microcosm experiment. Snails were held in isolation for 24 hours in a 4-L of water containing natural seston. Negative values reflect greater declines in chlorophyll-*a* concentrations in the control compared to the treatment microcosms. *Tasas de filtración de* Bellamya *(mL mejillón^{-1}h^{-1}) como función de la altura de la concha (mm) basadas en los experimentos en microcosmos. Los caracoles fueron aislados durante 24 horas en 4 litros de agua con seston natural. Valores negativos reflejan mayor reducción en la concentración de la clorofila-*a*, en el control que en los microcosmos tratados.*

microcosm experiment (110 ± 145 mL snail^{-1}h^{-1}) and 5-day mesocosm experiments (113 ± 158 mL snail^{-1}h^{-1}) despite differences in initial chlorophyll-*a* concentrations and water temperatures.

Bellamya filtration rates increased with snail size (Fig. 1). Although statistical support for a linear (adj. $R^2 = 0.52$, $F_{1,52} = 56.7$, $p < 0.001$) and quadratic relationship (adj. $R^2 = 0.55$, $F_{2,51} = 34.2$, $p < 0.001$) was evident, the strongest model was represented by the piecewise regression (adj. $R^2 = 0.56$, $F_{3,50} = 34.2$, $p < 0.001$). Based on the piecewise model there was strong support for an estimated breakpoint of 43.5 ± 4.1 mm (mean ± 1 SE) ($t = 10.51$, $p < 0.001$); snails less than the threshold size of 43.5 mm showed no evidence of a relationship with filtration rate (slope = 1.6, $t = 0.20$, $p = 0.984$), whereas snails exceeding the threshold size demonstrated a strong positive relationship with filtration rate (slope = 356.4, $t = 9.60$, $p < 0.001$).

Water column and benthic algal biomass (chlorophyll-*a*)

Water column chlorophyll-*a* decreased in the presence of *Bellamya* during the mesocosm experiments, with a 54 % decrease in the low-density treatment ($t = -1.64$, $p = 0.162$) and a 155 % decrease in the high-density treatment ($t = -2.92$, $p = 0.033$) (Fig. 2A). Reductions in chlorophyll-*a* were three times greater in the high- compared to the low-density treatments, despite having only twice the number of snails (11 vs. 22 snails m^{-2}). By contrast, benthic chlorophyll-*a* showed variable responses to the *Bellamya* treatments (Fig. 2B). The low-density treatment resulted in a 64 % reduction in chlorophyll-*a* ($t = -2.14$, $p = 0.085$), whereas the high-density treatment resulted in a 40 % increase in chlorophyll-*a* ($t = -3.01$, $p = 0.030$).

Bacterial community composition

Bacterial community composition changed significantly over the course of the mesocosm experiment in the high-density treatment ($R_{ANOSIM} = 0.132$, $p = 0.049$) but showed little change in both the low-density treatment ($R_{ANOSIM} = 0.147$, $p = 0.111$) as well as the control ($R_{ANOSIM} = 0.075$, $p = 0.176$). NMDS ordination plots showed that differences in community composition were generally modest, but distinction between initial and final time peri-

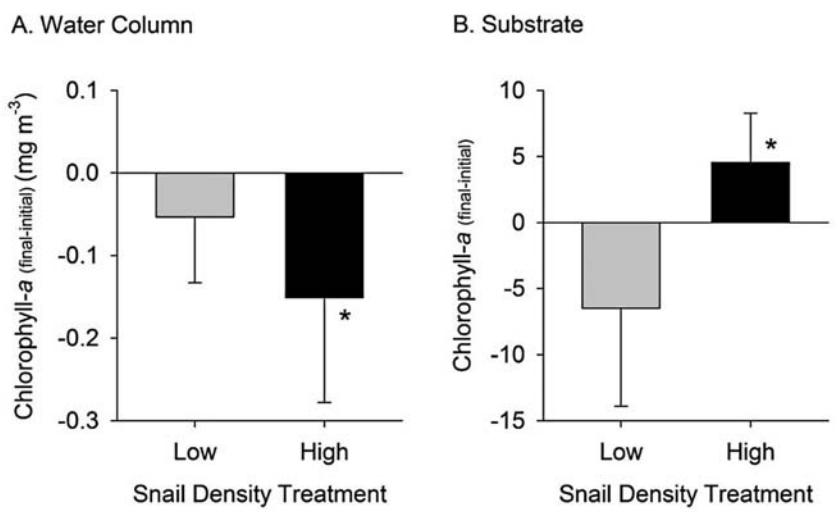

Figure 2. Change in water column (A) and benthic (B) chlorophyll *a* (mg m^{-3}) over the course of the mesocosm experiment for low (grey bar) and high (black bar) snail density treatments. * indicates that mean is statistically different from zero based on a Student's t-test and $p < 0.05$. *Cambios en la concentración de clorofila a (mg m^{-3}) en la columna de agua (A) y en el bentos (B) durante el experimento en el mesocosmos, para tratamientos con bajas (barra gris) y altas (barra negra) densidades de caracoles. * significa que la media es estadísticamente diferente de cero basándose en el t-test Student y* $p < 0.05$.

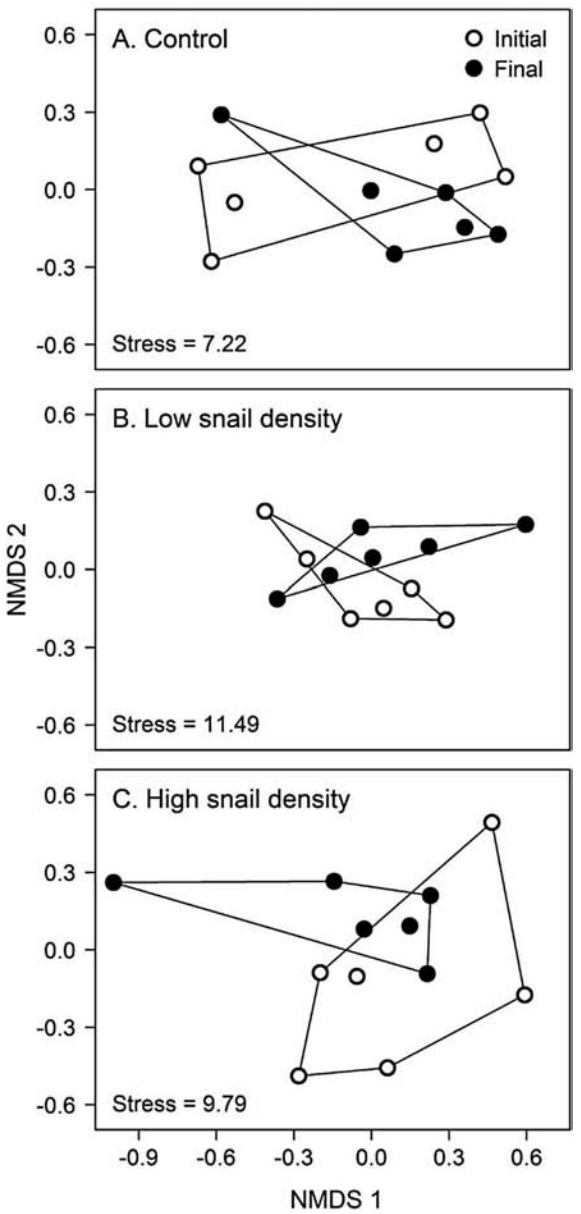

Figure 3. NMDS ordinations summarizing patterns in microbial community composition in (A) control, (B) low and (C) high *Bellamya* density treatments plots. *Patrones de ordenación NMDS, de la composición de la comunidad microbiana en los tratamientos con las diferentes densidades de* Bellamya*; control (A), baja (B) y alta (C).*

ods was greater in the presence of *Bellamya* (Fig. 3B, C) compared to the control (Fig. 3A). In addition, high-density snail treatments had reduced differences in community composition among replicates (Fig. 3C), as demonstrated by a higher average rank similarities in the final time period (86 %) compared to the initial time period (61 %). Snail density did not have a significant impact on total bacterial abundance over the course of the experiment (results not shown).

DISCUSSION

Chinese mystery snail, *Bellamya chinensis*, is a versatile consumer that switches between grazing microalgae using its radula and filter-feeding its breathing water (Plinski *et al*., 1977; Dillon, 2000). Our results showed that *Bellamya* demonstrated high rates of water filtration, caused significant declines in pelagic algal biomass under experimental settings and impacted bacterial community composition.

According to both microcosm (24-hour) and mesocosm (5-day) experimental trials, *Bellamya* exhibited an average filtration rate of 106-113 mL $snail^{-1}h^{-1}$ (1.45 mL mg $DW^{-1}h^{-1}$) and an individual maximum of 471 mL $snail^{-1}h^{-1}$ (6.15 mL mg $DW^{-1}h^{-1}$). A number of difficulties arise when attempting to compare the filtration rates of *Bellamya* to those estimated for non-native bivalves in past studies. Important factors such as concentration of suspension, the nature of the suspension used, and the degree to which "refiltration" occurs within chambers differ from one experiment to the next. Despite these challenges, a qualitative comparison suggests that that *Bellamya* filtration rates on natural seston are reasonably comparable to reported values for other invasive, freshwater and marine bivalves, including zebra mussel (*Dreissena polymorpha*), quagga mussel (*Dreissena bugensis*), Asian clam (*Corbicula fluminea*), golden mussel (*Limnoperna fortunei*) blue mussel (*Mytilus edulis*) (Table 1). Consequently, in certain environments, we expect that *Bellamya* filtering-feeding behavior may shift primary production from pelagic to benthic zones.

Across the wide size spectrum of *Bellamya* examined in our study we found that filtration rates were positively, and non-linearly, related to snail shell height. *Bellamya* filtration rates were explained by a quadratic relationship with snail size, although we found slightly stronger

statistical support for a marked increase at a threshold size of 43.5 mm; below that size estimated filtration rates were near zero (and had no relationship with size), but above that size filtration rates increased with size. This provides the first evidence that *Bellamya* may display an ontogenetic shift in feeding behavior from primarily radular grazing to filter-feeding with increasing size (age). Our results also suggest that *Bellamya* may increase their filter-feeding behavior in the presence of higher densities of conspecifics. Two lines of evidence support this statement: (1) estimated filtration rates were over two times greater in the high- versus low-density treatment (159 vs. 66 mL snail^{-1}h^{-1}) and (2) greater proportional decrease in water column chlorophyll-*a* in high vs. low density treatment were observed. This shift from grazing benthic microalgae to filter-feeding at higher densities may explain the opposing increase (high-density treatment) vs. decrease (low-density treatment) in benthic chlorophyll-*a*. This is likely caused, in part, by greater total snail excretion leading to elevated N:P in the water column (Johnson *et al.*, 2009) that promotes periphyton production.

Knowledge of how invasive species may affect bacterial diversity, abundance, and associated processes are important to understand potential impacts on ecosystem function. Previous studies have focused almost exclusively on invasive bivalves, specifically zebra and quagga mussel and have revealed shifts in benthic bacterial communities associated with invasions (Findlay *et al*., 1998; Frischer *et al*., 2000; Lavrentyev *et al*., 2000; Viergutz *et al*., 2007). For example, Lohner *et al*. (2007) observed an

Table 1. Comparison of feeding rates of *Bellamya chinensis* found in this study with those of other freshwater and marine invasive bivalves (adult estimates). *Comparación de las tasas de filtración de* Bellamya chinensis *encontradas en este estudio con las de otras especies invasoras de bivalvos dulceacuícolas y marinas (estimaciones en adultos).*

Species	Filtration Rate (mL ind^{-1}h^{-1})	Filtration Rate (mL mg DW^{-1}h^{-1})	Reference
Bellamya chinensis	106-113	1.5	this study
Dreissena polymorpha		16.4	Kondratev (1963)
		3.2	Micheev (1966)
		16.5	Kryger and Riisgård (1988)
	78-170	1.6-3.5	Reeders et al. (1989)
		4.3	Reeders and bij de Vaate (1990)
		1.9	Aldridge et al. (1995)
		16.2	Fanslow et al. (1995)
	375	9.1	Sprung (1995)
	40	4.1	Berg et al. (1996)
	125	2.1	
	114-133		Roditi et al. (1996)
	22-80	6.1-13.5	Lei et al. (1996)
	125-223	4.6-9.1	Horgan and Mills (1997)
	110-225	3.1-6.9	Diggins (2001)
	60-170	3.8-10.7	
Dreissena bugensis	40-200	2.2-10.8	Diggins (2001)
	120-310	2.7-7.0	
Corbicula fluminea	347	20.5	Buttner and Heidinger (1981)
	567	1.9	Way et al. (1990)
	490	2.2	Silverman et al. (1997)
Limnoperna fortunei	19-133	11.9-24.5	Rückert et al. (2004)
	125-350	9.9-29.5	Sylvester et al. (2005)
	100-214	1.5-3.1	Cataldo et al. (2012)
Mytilus edulis	17-2767	1.1-11.3	Winter (1973)
		1.9	Bayne and Scullard (1977)
	281-799		Clausen and Riisgård (1996)

increase in bacterial density, activity, metabolic diversity and structure in zebra mussel clusters relative to bare sediment. Here we observed a shift in bacterial community composition and decreased community variability in the water column under high *Bellamya* filtration rates, but no change in total abundance.

Bellamya may interact directly with microbial populations by utilizing bacteria as a food source, resulting in a community shift when bacteria respond with different growth rates. Alternatively, *Bellamya*-bacterial interactions may be indirect, mediated by the production of large quantities of fecal and pseudo-fecal material that differentially affect the composition, activity, and growth of bacterial taxa. Recent observations made for zebra mussel in the Hudson River, New York, suggest that there is increased total bacterial biomass and heterotrophic activity associated with zebra mussel colonies compared with surface sediments (Findlay *et al*., 1998; Strayer, 1999). Invasive bivalves can directly affect the abundance of major groups of microbes through feeding and altering resource partitioning, but whether those affects are biased towards particular bacterial functional groups remains understudied. Frischer *et al*. (2000) reported that zebra mussels are likely to promote the enrichment of particular bacteria groups, including Gammaproteobacteria and Betaproteobacteria and negatively impact Deltaproteobacteria and Flavobacteria. Unfortunately, ARISA does not allow for the identification of particular bacterial taxa or groups. However, the reduced differences in bacterial community composition among replicates in the high-density *Bellamya* treatments suggest that filtering differentially impacted bacterial taxa.

Changes in microbial abundance can have cascading ecosystem effects. Our laboratory experiments revealed that *Bellayma* are capable of high filtration rates, thus adding to the small but growing body of research suggesting that *Bellamya* may play an important role in freshwater ecosystems. Although we know very little about the feeding and nutrition of *Bellamya*, limited studies from over three decades ago suggest that *Bellamya* feeds primarily on periphyton. Stomach content analysis indicated that diatoms were the largest portion of *Bellamya* diet in the Ottawa River, and that radular feeding showed no selectivity among periphyton taxa (Stanczykowska *et al*., 1972; Plinski *et al*., 1978; Jokinen, 1982). More recent research examining carbon stable isotope ratios of *Bellamya* collected from one Wisconsin lake suggests heavy reliance on benthic resources (Solomon *et al*., 2010), although this has not been rigorously tested. Given the filter-feeding behavior of Bellamya (this study) and its effects on N:P that define ecological stochiometry and control algal growth (Johnson *et al*., 2009), additional experimental and field efforts are needed to understand the biology and ecology of *Bellamya chinesis*. In conclusion, even though *Bellamya* are only facultative filter-feeders (compared to exclusive filter-feeders such as mussels), they have the potential to serve an important, yet unexplored role in coupling benthic and pelagic food webs in lake ecosystems (Vadeboncoeur *et al*., 2002; Wagner *et al*. 2012); this topic requires more investigation.

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REFERENCES

ALDRIDGE, D. W., B. S. PAYNE & A. C. MILLER. 1995. Oxygen consumption, nitrogenous excretion, and filtration rates of *Dreissena polymorpha*

at acclimation temperatures between 20 and 32 °C. *Canadian Journal of Fisheries and Aquatic Sciences,* 52: 1761–1767.

BAYNE, B. L. & C. SCULLARD. 1977. Rates of nitrogen excretion by species of *Mytilus* (Bivalvia: Mollusca). *Journal of the Marine Biological Association of the United Kingdom,* 57: 355–369.

BERG, D. J., S. W. FISHER & P. F. LANDRUM. 1996. Clearance and processing of algal particles by zebra mussels (*Dreissena polymorpha*). *Journal of Great Lakes Research,* 22: 779–788.

BOLTOVSKOY, D., A. KARATAYEV, L. BURLAKOVA, D. CATALDO, V. KARATAYEV, F. SYLVESTER & A. MARINELARENA. 2009. Significant ecosystem-wide effects of the swiftly spreading invasive freshwater bivalve *Limnoperna fortunei*. *Hydrobiologia,* 636: 271–284.

BROWN, M. V., M. S. SCHWALBACH, I. HEWSON & J. A. FUHRMAN. 2005. Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to a time series. *Environmental Microbiology,* 7: 1466–1479.

BURY, J. A., B. E. SIETMAN & B. N. KARNS. 2007. Distribution of the non-native viviparid snails, *Bellamya chinensis* and *Viviparus georgianus,* in Minnesota and the first record of *Bellamya japonica* from Wisconsin. *Journal of Freshwater Ecology,* 22: 697–703.

BUTTNER, J. & R. HEIDINGER. 1981. Rate of filtration in the Asiatic clam, *Corbicula fluminea*. *Transactions of the Illinois State Academy of Science,* 74: 13–17.

CARLSSON, N. O. L., C. BRONMARK & L. A. HANSSON. 2004. Invading herbivory: The golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology,* 85: 1575–1580.

CATALDO, D., I. O' FARRELL, E. PAOLUCCI, F. SYLVESTER & D. BOLTOVSKOY. 2012. Impact of the invasive golden mussel (*Limnoperna fortunei*) on phytoplankton and nutrient cycling. *Aquatic Invasions,* 7: 91–100.

CHAINE, N. M. & 12 OTHER AUTHORS. 2012. Population estimate of Chinese mystery snail (*Bellamya chinensis*) in a Nebraska reservoir. *Bio Invasions Records,* 1: 283–287.

CLARK, G. T. 2009. *Distribution, growth, and competitive impacts of the exotic Chinese mystery snail (Bellamya chinesis) in the James River, southwest Missouri*. MS Thesis, Missouri State University, Missouri, U.S.

CLARKE, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology,* 18: 117–143.

CLAUSEN, I. & H. U. RIISGARD. 1996. Growth, filtration and respiration in the mussel *Mytilus edulis*: No evidence for physiological regulation of the filter-pump to nutritional needs. *Marine Ecology Progress Series,* 141: 37–45.

COUGHLAN, J. 1969. The estimation of filtering rates from the clearance of suspensions. *Marine Biology,* 2: 356–358.

DIGGINS, T. P. 2001. A seasonal comparison of suspended sediment filtration by quagga (*Dreissena bugensis*) and zebra (*D. polymorpha*) mussels. *Journal of Great Lakes Research,* 27: 457–466.

DILLON, R. T. 2000. *The Ecology of Freshwater Molluscs,* Cambridge, Cambridge University Press.

EHRENFELD, J. G. 2010. Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics,* 41: 59–80.

FANSLOW, D. L., T. F. NALEPA & G. A. LANG. 1995. Filtration rates of the zebra mussel (*Dreissena polymorpha*) on natural seston from Saginaw Bay, Lake Huron. *Journal of Great Lakes Research,* 21: 489–500.

FISHER, M. M. & E. W. TRIPLETT. 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Applied and Environmental Microbiology,* 65: 4630–4636.

FRISCHER, M. E., S. A. NIERZWICKI-BAUER, R. H. PARSONS, K. VATHANODORN & K. R. WAITKUS. 2000. Interactions between zebra mussels (*Dreissena polymorpha*) and microbial communities. *Canadian Journal of Fisheries and Aquatic Sciences,* 57: 591–599.

GOBET, A., C. QUINCE & A. RAMETTE. 2010. Multivariate cutoff level analysis (MultiCoLA) of large community data sets. *Nucleic Acids Research,* 38: e155.

HAKENKAMP, C. C., S. G. RIBBLETT, M. A. PALMER, C. M. SWAN, J. W. REID & M. R. GOODISON. 2001. The impact of an introduced bivalve (*Corbicula fluminea*) on the benthos of a sandy stream. *Freshwater Biology,* 46: 491–501.

HALL, R., J. L. TANK & M. F. DYBDAHL. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. *Frontiers in Ecology and the Environment,* 1: 407–411.

HAVEL, J. E. 2011. Survival of the exotic Chinese mystery snail (*Cipangopaludina chinensis malleata*) during air exposure and implications for overland dispersal by boats. *Hydrobiologia,* 668: 195–202.

HIGGINS, S. N., M. J. VANDER ZANDEN, L. N. JOPPA & Y. VADEBONCOEUR. 2011. The effect of dreissenid invasions on chlorophyll and the chlorophyll: total phosphorus ratio in north-temperate lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* 68: 319–329.

HORGAN, M. J. & E. L. MILLS. 1997. Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): implications for freshwater lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* 54: 249–255.

JOHNSON, P. T. J., J. D. OLDEN, C. T. SOLOMON & M. J. VANDER ZANDEN. 2009. Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia,* 159: 161–170.

JOKINEN, E. H. 1982. *Cipangopaludina chinensis* (Gastropoda, Viviparidae) in North America; review and update. *Nautilus,* 96: 89–95.

KARATAYEV, A. Y., L. E. BURLAKOVA, V. A. KARATAYEV & D. K. PADILLA. 2009. Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia,* 619: 181–194.

KERANS, B. L., M. E. DYBDAHL, M. M. GANGLOFF & J. E. JANNOT. 2005. *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the Greater Yellowstone Ecosystem. *Journal of the North American Benthological Society,* 24: 123–138.

KONDRATEV, G. P. 1963. O nekotorye osobennostjah filtracii u presnovodnyh molljuskov. *Nauchn. Dokl. Vyssh. Shk. Biol. Nauki.,* 1: 13–16.

KRUSKAL, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. *Psychometrika,* 29: 115–129.

KRYGER, J. & H. U. RIISGÅRD. 1988. Filtration rate capacities of 6 species of European freshwater bivalves. *Oecologia,* 77: 34–38.

LAVRENTYEV, P. J., W. S. GARDNER & L. Y. YANG. 2000. Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface. *Aquatic Microbial Ecology,* 21: 187–194.

LEGENDRE, P. & L. LEGENDRE 1998. *Numerical Ecology,* Amsterdam, The Netherlands, Elsevier Scientific.

LEI, J., B. S. PAYNE & S. Y. WANG. 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha*. *Canadian Journal of Fisheries and Aquatic Sciences,* 53: 29–37.

LOHNER, R. N., SIGLER, V., MAYER, C. M. & C. BALOGH. 2007. A comparison of the benthic bacterial communities within and surrounding Dreissena clusters in lakes. *Microbial Ecology,* 54: 469–477.

MACKIE, G. L. 1999. Introduction of molluscs through the import for live food. *In:* CLAUDI, R. & J. LEACH. (eds.) *Non-indigenous freshwater organisms: vectors biology and impacts.* Boca Raton: Lewis Publishers.

MICHEEV, V. P. 1966. O skorosti fil tracii vody Drejssenoj. *TrI Inst. Biol. Vodokhran Akad. Nauk. SSSR,* 12: 134–138.

MILLS, E. L., J. H. LEACH, J. T. CARLTON & C. L. SECOR. 1993. Exotic species in the Great Lakes-a history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research,* 19: 1–54.

NEWTON, R. J., S. E. JONES, A. EILER, K. D. MCMAHON & S. BERTILSSON. 2011. A guide to the natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews,* 75: 14–49.

OLDEN, J. D., E. R. LARSON & M. C. MIMS. 2009. Home-field advantage: native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*). *Aquatic Ecology,* 43: 1073–1084.

PLINSKI, M., W. LAWACZ, A. M. STANCZYKOWSKA & E. MAGNIN. 1978. Etude quantitative et qualitative de la nourriture des *Vivaparus malleatus* (Reeve) (Gastropoda, Prosobranchia) dans deux lacs de la region de Montreal. *Canadian Journal of Zoology,* 56: 272–279.

RAWLINGS, T. A., K. A. HAYES, R. H. COWIE & T. M. COLLINS. 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evolutionary Biology,* 7: 97.

REEDERS, H. H. & A. BIJ DE VAATE. 1990. Zebra mussels (*Dreissena polymorpha*): a new perspective for water quality management. *Hydrobiologia,* 200/201: 437–450.

REEDERS, H. H., A. B. DEVAATE & F. J. SLIM. 1989. The filtration rate of *Dreissena polymorpha* (Bivalvia) in 3 Dutch Lakes with reference to biological water quality management. *Freshwater Biology,* 22: 133–141.

RICCIARDI, A. 1998. Global range expansion of the Asian mussel *Limnoperna fortunei* (Mytilidae): Another fouling threat to freshwater systems. *Biofouling,* 13: 97–106.

RIISGÅRD, H. U. 2001. On measurement of filtration rates in bivalves - the stony road to reliable data: review and interpretation. *Marine Ecology Progress Series,* 211: 275–291.

RODITI, H. A., N. F. CARACO, J. J. COLE & D. L. STRAYER. 1996. Filtration of Hudson River water by the zebra mussel (*Dreissena polymorpha*). *Estuaries,* 19: 824–832.

RÜCKERT, G., M.C., S. C. & M. E. ROLLA. 2004. Alimentação de *Limnoperna fortunei* (Dunker 1857): taxas de filtração com ênfase ao uso de Cyanobacteria. *Acta Scientiarum, Biological Sciences,* 26: 421–429.

RUDI, K., M. ZIMONJA, P. TROSVIK & T. NAES. 2007. Use of multivariate statistics for 16S rRNA gene analysis of microbial communities. *International Journal of Food Microbiology,* 120: 95–99.

SILVERMAN, H., S. J. NICHOLS, J. S. CHERRY, E. ACHBERGER, J. W. LYNN & T. H. DIETZ. 1997. Clearance of laboratory-cultured bacteria by freshwater bivalves: differences between lentic and lotic unionids. *Canadian Journal of Zoology,* 75: 1857–1866.

SOES, D. M., G. D. MAJOOR & S. M. A. KEULEN. 2011. *Bellamya chinensis* (Gray, 1834) (Gastropoda: Viviparidae), a new alien snail species for the European fauna. *Aquatic Invasions,* 6: 97–102.

SOLOMON, C. T., J. D. OLDEN, P. T. J. JOHNSON, R. T. DILLON & M. J. VANDER ZANDEN. 2010. Distribution and community-level effects of the Chinese mystery snail (*Bellamya chinensis*) in northern Wisconsin lakes. *Biological Invasions,* 12: 1591–1605.

SOUSA, R., J. L. GUTIERREZ & D. C. ALDRIDGE. 2009. Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions,* 11: 2367–2385.

SPRUNG, M. 1995. Physiological energetics of the zebra mussel *Dreissena polymorpha* in Lakes. 2. Food uptake and gross growth efficiency. *Hydrobiologia,* 304: 133–146.

STANCZYKOWSKA, A. M., M. M. PLINSKI & E. MAGNIN. 1972. Study on 3 populations of *Viviparus malleatus* (Reeve) (Gastropoda, Prosobranchia) of region of Montreal. 2. Qualitative and quantitative study on nutrition. *Canadian Journal of Zoology,* 50: 1617–1624.

STRAYER, D. L. 1999. Effects of alien species on freshwater mollusks in North America. *Journal of the North American Benthological Society,* 18: 74–98.

STRAYER, D. L. 2012. Eight questions about invasions and ecosystem functioning. *Ecology Letters,* 15: 1199–1210.

SYLVESTER, F., J. DORADO, D. BOLTOVSKOY, A. JUAREZ & D. CATALDO. 2005. Filtration rates of the invasive pest bivalve *Limnoperna fortunei* as a function of size and temperature. *Hydrobiologia,* 534: 71–80.

TOMS, J. D. & M. L. LESPERANCE. 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology,* 84: 2034–2041.

VADEBONCOEUR, Y., M. J. VANDER ZANDEN & D. M. LODGE. 2002. Putting the lake back together: re-integrating benthic pathways into lake food web models. *BioScience,* 52: 44–54.

VAN DER PUTTEN, W. H., J. N. KLIRONOMOS & D. A. WARDLE. 2007. Microbial ecology of biological invasions. *ISME Journal,* 1: 28–37.

VAUGHN, C. C. & D. E. SPOONER. 2006. Scale-dependent associations between native freshwater mussels and invasive Corbicula. *Hydrobiologia,* 568: 331–339.

VIERGUTZ, C., M. KATHOL, H. NORF, H. ARNDT & M. WEITERE. 2007. Control of microbial communities by the macrofauna: a sensitive interaction in the context of extreme summer temperatures? *Oecologia,* 151: 115–124.

WAGNER, A., S. VOLKMAN & P. M. A. DETTINTINGER-KLEMM. 2012. Benthic–pelagic coupling in lake ecosystems: the key role of chironomid pupae as prey of pelagic fish. *Ecosphere,* 3(2): 14. http://dx.doi.org/10.1890/ES11-00181.1.

WAY, C. M., D. J. HORNBACH, C. A. MILLER-WAY, B. S. PAYNE & A. C. MILLER. 1990. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia, Corbiculidae). *Canadian Journal of Zoology,* 68: 115–120.

WINTER, J. E. 1973. The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by continuous automatic recording apparatus. *Marine Biology,* 22: 317–328.

WOOD, W. M. 1892. *Paludina japonica* Mart. for sale in the San Francisco Chinese markets. *Nautilus,* 5: 114–115.

YANNARELL, A. C., A. D. KENT, G. H. LAUSTER, T. K. KRATZ & E. W. TRIPLETT. 2003. Temporal patterns in bacterial communities in three temperate lakes of different trophic status. *Microbial Ecology,* 46: 391–405.

YANNARELL, A. C. & E. W. TRIPLETT. 2005. Geographic and environmental sources of variation in lake bacterial community composition. *Applied and Environmental Microbiology,* 71: 227–239.

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The trophic ecology of the red swamp crayfish (*Procambarus clarkii*) in Mediterranean aquatic ecosystems: a stable isotope study

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ABSTRACT

The trophic ecology of the red swamp crayfish (*Procambarus clarkii*) in Mediterranean aquatic ecosystems: a stable isotope study

The red swamp crayfish (*Procambarus clarkii*) is an invasive species in most of its current distribution range. As an omnivorous species that feeds on items of many trophic levels and is eaten by many others, it occupies a key trophic position within the invaded food webs. This trophic position, in combination with its active physiology, makes *P. clarkii* a suitable organism for ecotoxicological studies and, more specifically, a bioindicator of heavy metal pollution. These characteristics also make *P. clarkii* a likely vector of contaminants toward higher trophic levels. In this study, we (i) describe aquatic food webs in three contrasting Mediterranean wetlands in the lower Guadalquivir River Basin, southwestern Spain, each populated by invasive *P. clarkii* but having a different heavy metal concentration, (ii) assess the trophic role of crayfish and temporal trends in its diet using stable isotope analysis (δ^{13}C and δ^{15}N), and (iii) assess the relationship of crayfish isotopic signatures to the content of heavy metals (Cu, Zn, Pb, Cd, As) bioaccumulated in crayfish body tissues. We detected significant between-site differences in carbon and nitrogen isotopic signatures but found significant between-date differences only for nitrogen signatures. Between-site changes in carbon and nitrogen isotopes were due primarily to variations in the relative contribution of autochthonous *vs.* allochthonous primary producers and shifts in crayfish abundance through time, respectively. Isotopic food web models were used to distinguish between systems driven by a detritus-based energy pathway and systems supported by detritus and primary producers. The trophic positions estimated for crayfish and other invertebrates at each site were low, suggesting the prevalence of omnivory and the occurrence of a trophic continuum rather than discrete levels. Isotopically, crayfish occupy a predator position in the observed food webs, which is consistent with the predominance of animal food sources in the species' diet. No significant changes were found between crayfish ontogenetic stages using isotopic ratios. The site with the highest concentration of heavy metals showed the highest δ^{13}C and δ^{15}N values, and a significant correlation was found between five heavy metal elements (As, Cd, Zn. Cu, Pb) measured in crayfish and their nitrogen isotope signatures ($r = 0.72$, $p < 0.0001$), thus reinforcing its contamination biomarker role.

Key words: Food webs, δ^{13}C, δ^{15}N, crayfish, stable isotopes, Mediterranean wetlands, heavy metals.

RESUMEN

Ecología trófica del cangrejo rojo (Procambarus clarkii) en ecosistemas acuáticos mediterráneos: un estudio sobre isótopos estables

*El cangrejo rojo (*Procambarus clarkii*) es una especie invasora en la mayor parte de su área de distribución actual. Ocupa un estatus trófico clave dentro de las redes tróficas invadidas al ser una especie omnívora que se alimenta de muchos recursos tróficos, además de ser presa de otros consumidores. Tal posición de especie clave, junto con su activa fisiología, hace que sea un vector potencial de contaminantes a posiciones superiores en las redes tróficas, y por lo tanto, un excelente bioindicador de contaminación por metales pesados que ha sido muy utilizado en estudios ecotoxicológicos. En este estudio describimos las redes tróficas de tres humedales mediterráneos de la cuenca del Bajo Guadalquivir (SO, España), que poseen densas poblaciones de* P. clarkii *y que muestran diferentes grados de afección por contaminación de metales pesados, usando isótopos estables (δ^{13}C and δ^{15}N). Además, se explora la relación que existe entre las señales isotópicas y las concentraciones de metales pesados (Cu, Zn, Pb, Cd, As) bioacumuladas en sus tejidos. Se detectaron diferencias significativas en las señales isotópicas de carbono y nitrógeno entre las diferentes localidades, mientras que las diferencias temporales sólo fueron reflejadas por la señal del nitrógeno. Los cambios medios que se dan en las señales del carbono y nitrógeno en cada localidad,*

son debidas a la contribución relativa de los productores primarios autóctonos respecto a la de los alóctonos y cambios en la abundancia de cangrejos a lo largo del tiempo, respectivamente. La descripción de las redes tróficas realizada a través de los isótopos estables distingue entre los sistemas con flujos energéticos basados en detritus, y los basados en productores primarios junto con detritus. Las posiciones tróficas estimadas para los cangrejos e invertebrados en cada localidad fueron bajas, sugiriendo que existe un predominio de la omnivoría y la existencia de un "continuo trófico" mas que la existencia de niveles discretos. Isotópicamente, los cangrejos ocupan una posición de depredador en las redes tróficas que concuerda con el predominio de recursos de origen animal en su dieta. No se encontraron cambios ontogenéticos en la dieta de los distintos estadíos de cangrejo según las señales isotópicas. La localidad con una mayor contaminación de metales pesados mostró una relación directamente proporcional significativa con las señales isotópicas de $\delta^{13}C$ *y* $\delta^{15}N$ *(*r *= 0.72,* p *<0.0001), reforzando así su papel como biomarcador.*

Palabras clave: *Redes tróficas,* $\delta^{13}C$*,* $\delta^{15}N$*, cangrejo, isótopos estables, humedales mediterráneos, metales pesados.*

INTRODUCTION

Among the several alien species of freshwater crayfish that have been introduced in Spain during recent decades, the red swamp crayfish (*Procambarus clarkii* Girard) has been the most successful and therefore the most environmentally threatening of them all (Gutiérrez-Yurrita, *et al*., 1999). *P. clarkii* was introduced in 1973 at two aquaculture installations in Sevilla and Badajoz, southwestern Spain (Habsburgo-Lorena, 1983) and spread throughout the Mediterranean region and central Europe during the subsequent three decades (Alonso *et al*., 2000). Its broad ecological tolerance, rapid growth, high fecundity and resistance to diseases explain such achievement (Montes *et al*., 1993; Gherardi & Barbaresi, 2000).

The establishment of red swamp crayfish populations in Mediterranean wetlands and rivers in southwestern Spain was so successful because of the similar environmental conditions with *P. clarkii*'s homeland aquatic systems (the southeastern areas of the USA) (Geiger *et al*., 2005). The species' establishment in Spain was also greatly promoted by intentional and repeated translocations by humans for economic or recreational purposes (Montes *et al*., 1993). The presence of this alien species has significant impacts on the structure and functioning of the invaded aquatic ecosystems. An example of such an impact is the physical alteration of the habitat structure, primarily through shredding of macrophytes during feeding and indirectly through bioturbation of sediments (Geiger *et al*., 2005). These mechanisms are considered to be responsible for the change observed in many invaded ecosystems from a natural, macrophyte-dominated, transparent water equilibrium state to a turbid, eutrophic, phytoplankton-dominated equilibrium state (Anastácio & Marques 1995; Angeler *et al*., 2001; Rodríguez *et al*., 2003). Because *P. clarkii* is an opportunistic, omnivorous feeder with high assimilation efficiencies that rapidly develops dense populations (Gutiérrez-Yurrita *et al*., 1999), it is expected to have strong effects on aquatic food webs and to impact trophic levels both below (Ilhéu & Bernardo, 1993, 1995; Gutiérrez-Yurrita *et al*., 1998) and above its own (Geiger *et al*., 2005; Tablado *et al*., 2010). Despite *P. clarkii*'s major role in structuring aquatic food webs, information regarding the species' diet in natural habitats is scarce (Feminella & Resh, 1986, 1989; D'Abramo & Robinson, 1989; Ilhéu & Bernardo, 1993, 1995; Gutiérrez-Yurrita *et al*., 1998; Alcorlo *et al*., 2004). Studies of food webs have traditionally been based on stomach content analyses for the placement of species at their proper trophic position (Hobson & Welch, 1992). This approach, however, often relies on data gathered on a casual rather than on a regular basis making trophic models particularly susceptible to both the temporal and the spatial scales used (Paine, 1988). In recent years, the biological interpretation of changes in the relative abundance of naturally

occurring stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) has provided an alternative method for characterising food web structures and dynamics (Peterson *et al*., 1985; Peterson & Fry, 1987; Lajtha & Michener, 1994; Hershey & Peterson, 1996; Gannes *et al*., 1998; Inger & Bearhop 2008; Martínez del Río *et al*., 2009). This approach is based on the fact that stable-isotope ratios of nitrogen and carbon in the tissues of consumers reflect those in their prey in a predictable manner (DeNiro & Epstein, 1978, 1981).

In addition, the polytrophic character of crayfish has important consequences for ecosystem management because of the role that these animals play in the transport of xenobiotic substances throughout the food web. *P. clarkii* can ingest and eventually store large amounts of heavy metals in its tissues (Maranhao *et al*., 1995) and is able to adjust, using complex physiological mechanisms, to survive in polluted environments (Naqvi & Flagge, 1990; Naqvi and Howel, 1993; Allison *et al*., 2000). *P. clarkii* has therefore been widely used as a biomarker (Depledge & Fossi, 1994; Anderson *et al*., 1997a, 1997b; Schilderman *et al*., 1999; Schlenk, 1999; Antón *et al*., 2000; Alcorlo *et al*., 2006; Martín-Díaz *et al*., 2006; Vioque-Fernández *et al*., 2007; Vioque-Fernández *et al*., 2009; Faria *et al*., 2010; Suárez-Serrano *et al*., 2010) to efficiently trace the relocation of heavy metals from non-biological (primarily sediments) to biological compartments (food webs) in the ecosystem.

The estimation of the amount of pollutants that *P. clarkii* mobilises and stores as biomass is ecologically relevant because this species is now the major prey item for many endangered and legally protected vertebrate species in the Lower Guadalquivir Basin (Adrian & Delibes, 1987; Palomares & Delibes, 1991; Senra & Alés, 1992; Correia, 2001; Tablado *et al*., 2010). Pollution effects can potentially travel far from the source area through the trophic relationships that link crayfish with wide-ranging predator species (e.g., herons, spoonbills, storks, otters).

The general aim of the present study is to describe the trophic position of *P. clarkii* and its role as a heavy metal bioindicator in three Mediterranean wetlands exposed to different land-uses (one in a natural protected area, one surrounded by agricultural land and one affected by a toxic mine spill). Our specific aims are as follows: 1) to describe the food webs using carbon and nitrogen stable isotopes, with emphasis on temporal and habitat variations in the diet of *P. clarkii*; 2) to determine whether *P. clarkii* occupies the same trophic position in each of the three environments; 3) to investigate whether isotopic signatures describe changes in diet in relation to crayfish ontogeny; and 4) to analyse the relationship between nitrogen isotopic signatures and heavy metal bioaccumulation in *P. clarkii* tissues.

Our hypothesis is that the isotopic descriptions of the assembled food webs in three types of aquatic ecosystem will reflect the differing structures of the food web patterns developed. According to this hypothesis, 1) the isotopic signals of crayfish will reveal distinct positions in each system depending on the unequal contribution of the exploited food resources and the ontogenetic shifts in diet, and 2) the nitrogen isotopic signature of *P. clarkii* will be correlated with the levels of heavy metal contamination because of the dissimilar communities developed at each site.

METHODS

Study area

The study was conducted in three tributaries of the Lower Guadalquivir River (SW Spain) close to the northern boundary of Doñana National Park (Fig. 1). The three sampling sites are subject to differing land-use and anthropogenic pressures and have contrasting morphological features in terms of the nature and grain size of stream bed sediments and of the channel shape (Montes *et al.*, 1998).

Site 1 (*Brazo del Este*, UTM: 29SQB633117) is located in a section of a stream that collects water runoff with a high content of pesticides and fertilisers from the surrounding rice fields (Cano & Ocete, 1997; Vioque-Fernández *et al.*, 2009). The agricultural activity in this area has resulted in the discharge of pesticides with lead (Pb), copper (Cu) and arsenic (As) (http://npic.

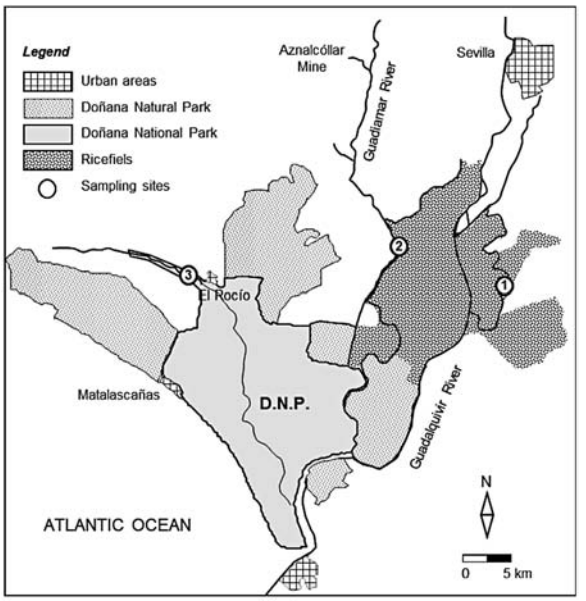

Figure 1. Schematic map of the studied area, sampling stations and mine location. 1.-Brazo del Este, 2.-Puente de los Vaqueros, 3.-Charco de la Boca. *Mapa de la zona de estudio, donde se muestran las estaciones de muestreo y la mina. 1.-Brazo del Este, 2.-Puente de los Vaqueros, 3.-Charco de la Boca.*

orst.edu/ingred/aifact.html) into the surrounding aquatic ecosystems for many years (Vioque–Fernández *et al.*, 2009). The riverbanks are covered by well-developed cattail (*Typha* sp.) stands. The sediment is muddy and rich in clay and silt and has a thick, organic-rich, black layer on top.

Site 2 (*Puente de los Vaqueros*, UTM: 29SQB495168) is located in the lower watercourse of the Guadiamar Basin (Fig. 1). Although the Guadiamar River was formerly one of the main tributaries of both the Guadalquivir River and its associated marshland, it is nowadays dramatically transformed in a multitude of channels that distribute and collect irrigation water to and from the surrounding rice fields. In April 1998, the entire basin was severely affected by a toxic spill from the Aznalcóllar mine that released 5 Hm^3 of acid water and metal-rich sludge (As-0.6 %; Pb-1.2 %; Zn-0.8 % dry weight, and other metals) (Meharg *et al.*, 1999). In addition to mining activity, the stream also receives sewage dumping from the nearby urban areas (Prat *et al.*, 1999). In the sampling area, the emergent vegetation on the banks has been reduced to small patches of common reed (*Phragmites australis*) and cattail (*Typha* sp.). The riverbed sediments are very rich in silt, which causes the water to be turbid most of the time.

Site 3 (*Charco de la Boca*, UTM: 29SQB 227121) is located in a wetland within the boundaries of Doñana National Park (Fig. 1). The entire area is covered by pines (*Pinus pinea*) growing on sandy soils. The littoral zone of the wetland is rich in silt and clay and is occupied by belts of *Phragmites australis* and cattail *Typha* sp., which provide shelter and food to many waterfowl and other bird species (Montes *et al.*, 1998).

Field data collection

Samples were collected during periods when crayfish were very abundant and their populations included individuals of varying sizes and developmental stages: autumn (November 2000-date 1 and 2001-date 3; the end of the growing season for crayfish), and mid-Spring (April 2001-date 2; which is the growing season). Three replicate samples for each of the categories used in the food web (i.e., fish, crayfish, invertebrates -zooplankton and zoobenthos-, aquatic flora, detritus and sediments) were taken on each date. Limnological descriptors [depth (cm), turbidity (cm, Secchi disk depth), water temperature (°C) and dissolved oxygen (mg l^{-1} and %, WTW-oxymeter), conductivity (μS cm^{-1}, WTW LF96-conductivimeter), and pH (Merck- indicators paper)] were measured *in situ*. Sediment samples were obtained with a core-sampler (54 mm inner diameter, 50 cm height). The upper layer (5 cm deep), where most of the detritus is stored, was sliced out and kept in a polyethylene bag hermetically closed. This fraction containing the organic matter in the sediment will be referred to hereafter as detritus. The so-called phytoplanktonic fraction, a mixture of true algae plus particulate organic matter (0.45-100 μm), was collected on a pre-ashed (500 °C for 4 h) glass fibre filter (Whatman, CF/C) and stored in a hermetic polyethylene bag kept in darkness. Helophyte samples were obtained from emergent plant species (*Phragmites australis* in sites 2 and 3, and *Typha* in all sites). Samples of submerged

vegetation were restricted to the terrestrial grass *Cynodon dactylon* in sites 2 and 3. Healthy green leaves and stems were handpicked from various stands and put in hermetic polyethylene bags. A hand net (250 μm mesh size) was used for collecting invertebrates. Samples were obtained by filtering water along 50 m long transects for five to ten minutes. Material collected in this way was placed in polyethylene bottles (500 ml). Fish, crayfish and shrimp were collected at each site using 10 shrimp traps (‘*nasa camaronera’*). The traps were checked after 24 h. When captures were too small (e.g., $n < 10$ individuals), the traps were left in the field for a second day. Captured animals were individually placed in hermetic plastic bags.

All sediment and biological samples were kept refrigerated (4 °C) until freezing on the same day of collection. The samples remained frozen until they were prepared for stable isotope analysis. Crayfish selected for the analysis of heavy metal contents (ten individuals from each site) were measured *in situ* for body length (distance from the edge of the rostrum to the telson, expressed in cm) and mass (using a portable Scaltec balance and expressed in g with an accuracy of 0.1 g). An additional sample of zooplankton and benthos was taken and fixed (4 % neutralised formaldehyde) for further taxonomic identification.

Stable isotope analysis

For the isotopic analysis of sediments, 30-50 mg of each frozen sample was thawed, placed in a 250 ml Erlenmeyer flask, and suspended in 100 ml of 0.2 N HCl for 24 h at room temperature to remove carbonates. The sediments were then rinsed thoroughly with deionised water and dried at 60 °C until no further water loss occurred (Cifuentes *et al.*, 1988; Bernasconi *et al*., 1997). The samples were then homogenised using a mortar and pestle and stored dry in 15 ml vials until analysed.

Filters of phytoplankton samples were dried at 60 °C until no water loss was recorded and stored dry in vials until analysed (Gearing *et al*., 1984). Macrophyte and helophyte samples were thawed, epiphytic material was removed from leaves and stems by gentle wiping and washed with deionised water, and all of these materials were dried at 60 °C (LaZerte & Szalados, 1982; Boon & Bunn, 1994). The dried periphyton material was homogenised using a mortar and pestle and stored dry in 15 ml vials until analysed (France, 1999). The aquatic plant tissues were milled using an analytical mill (model IKA-A10).

The dominant planktonic and benthic taxa were sorted by hand from the thawed samples. In several cases, insects and microcrustaceans had to be pooled by taxonomic groups (i.e., Order) to obtain sufficient material for isotopic analysis. Ostracods (crustaceans with a calcareous carapace) were suspended in a solution of 0.2 N HCl for 24 h at room temperature to remove carbonates. The samples were then rinsed thoroughly with deionised water, dried at 60 °C until no water loss was recorded, homogenised with a mortar and pestle and stored dry in 15 ml vials until analysed (Gearing *et al*., 1984).

The abdominal muscles of crayfish (France, 1996a) and muscle samples above the lateral line of fish (Kwak & Zedler, 1997) were dissected after thawing of the samples. The samples were rinsed thoroughly with deionised water, dried, powdered, and stored at room temperature (20 to 25 °C) until isotope analysis.

Stable carbon and nitrogen analyses were performed on 1 mg subsamples of homogenised materials by loading them into tin cups and combusting at 1800 °C in a Carbo Erba 1108-CHNS elemental analyser. The resultant CO_2 and N_2 gases were analysed using a Micromass Isochrom continuous-flow isotope ratio mass spectrometer (CFIRMS) with every 9 unknowns separated by two or three laboratory standards (NBS22, sucrose, atropine, benzoic).

Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = \left[(R_{\text{sample}} / R_{\text{standard}}) - 1 \right] \times 1000$$

were X is the isotope ^{15}N or ^{13}C, and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The R_{standard} for ^{15}N is that for atmospheric N_2 (air) and the

$R_{standard}$ for ^{13}C is that for Pee Bee Belemmnite (PDB) limestone formation. Based on numerous measurements of organic and inorganic standards by the lab that performed the analyses, the precision of these measurements was estimated to be ±0.1 and ±0.2 ‰ for carbon and nitrogen, respectively.

Isotopic models

The trophic position of food web items at each site was estimated assuming a constant enrichment value of 3.4 ‰ in the nitrogen stable isotope, in accordance with previous studies performed in other aquatic ecosystems (Minagawa & Wada, 1984; DeNiro & Epstein, 1981; Whitledge & Rabeni, 1997; Ponsard & Arditi, 2001). Accordingly, the trophic level was computed by the following equation:

$$TL = 1 + \frac{(D_p - D_b)}{3.4}$$

where TL is the trophic level of a consumer and D_p is the $\delta^{15}N$ value of the whole body tissues of consumers in the case of insects and microcrustaceans or the $\delta^{15}N$ value of the consumer's muscle tissue in the case of fish and crayfish (‰). D_b is the baseline $\delta^{15}N$ value; *i.e.*, the isotopic value of the main food sources of the consumer. Detritus was assumed to be the main food source for all of the consumers in the food web except the microcrustaceans, for which phytoplankton were assumed to be the main food source.

The above approach is a simplistic way to summarise the various enrichment factors that might occur between different food web items. Further studies are required to identify more precisely the $\delta^{15}N$ enrichment factors between consecutive trophic levels. Alternatively, the use of an average enrichment factor of 3.4 ‰, as actually found in several aquatic food webs, appears to provide a good estimate of the trophic interactions that we are trying to elucidate.

We used a concentration–weighted linear mixing model of $\delta^{15}N$ and $\delta^{13}C$ values (Phillips & Koch 2002; http://www.epa.gov.wed/pages/models.htm) to estimate the proportion of the crayfish diet allocated to each of the major food items (invertebrates, vegetative material or detritus) as determined in previous conventional crayfish dietary studies that analysed stomach contents and were performed at the same sites (Gutiérrez Yurrita *et al*. 1998, Alcorlo *et al.*, 2004) and in other studies (Brown, 1990; Whitledge & Rabeni, 1997; Evans-White *et al.*, 2001). This model assumes that for each element, the contribution of a source is proportional to the contributed mass times the elemental concentration in that source. Isotopic values for food sources must be adjusted by the appropriate fractionation values to account for trophic fractionation (Phillips & Koch, 2002). In this study, we used 3.4 and 0.8 ‰ fractionation factors for nitrogen and carbon isotopes, respectively (Ponsard & Arditi, 2001). The elementary compositions of C and N of the food web items determined were analysed with an elemental analyser (Carbo Erba 1108-CHNS) during the isotopic determinations.

Such models have both mathematical and biological limitations (Ben-David & Shell, 2001; Phillips, 2001; Phillips & Gregg, 2001). An overestimation of the contribution to the diet may occur if one source has a signal similar to that of the sink, and exact solutions are not possible unless all of the end members are included. The ranges of source contributions can be determined. The breadth of these ranges depends on the geometry of the mixing space (e.g., mixing triangles) and the similarity of the source and mixture isotopic signatures. When the mixture lies near the periphery of the convex polygon connecting the sources, the sources on that side of the mixing diagram predominate and the ranges of the possible contributions from each source are well constrained. We followed the suggestion of Ben-David & Shell (2001) and the procedure of Jones & Waldron (2003), and used these models as a heuristic tool to investigate the patterns of our data.

Heavy metal analysis

Heavy metal analyses of arsenic (As), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) were performed for the crayfish sampled in November 2001. Because *P. clarkii* is exploited

for commercial use in the study area and the legal limits for human consumption are expressed in fresh weight, we expressed the heavy metal concentrations as $\mu g \cdot g^{-1}$ fresh weight.

The entire body of each crayfish was powdered and homogenised, and a 2-g subsample was digested in 10 ml of nitric acid (65 %) for at least 30 min. A posterior microwave digestion was performed using a CEM Microwave Digestion System 2100 and following the protocol App. Note BI-7 "EPA 3052". The samples were then cooled, filtered (Whatman fiber glass filters, 47 mm diameter), brought to a volume of 100 ml with deionised water, and each heavy metal item was analysed following the appropriate procedure. As and Pb were measured using Electrothermal Atomic Absorption Spectrometry with a graphite furnace. Cd, Zn and Cu were measured using Inductively Coupled Plasma Emission Spectroscopy (EPA 6010 B).

Statistical analyses

The $\delta^{13}C$ and $\delta^{15}N$ values estimated in each biological compartment of the food web were compared simultaneously between sites, dates and their interaction (date × site), using a multi-way analysis of variance (MANOVA) with the Wilks' lambda statistic. Certain samples in which only one of the δ values was available were excluded from the analysis. When post-hoc comparisons were required, we used Tukey's Honestly Significant Difference test (HSD). The normality of distributions was assessed by the inspection of normal plots. Departures from homogeneity of variances were assessed by Levene's test. Log-transformation of dependent variable was performed when necessary after exploring the correction of the heteroscedasticity of the transformed data.

A two-sample *t* test for a two-tailed hypothesis was performed to assess the variation in mean $\delta^{15}N$ values resulting from ontogenetic changes between young and adult crayfish.

Multiple correlation and regression analyses were performed to determine whether crayfish nitrogen stable isotope abundances were influenced by the heavy metal contents measured at all sites pooled together and at each site separately.

RESULTS

Limnological parameters and community composition

The three aquatic ecosystems sampled are characterised by their shallowness and a slightly saline character (Table 1). Sites 1 and 2 have conductivity and pH values that are higher than those of site 3. This finding is most likely due to the nature of the underlying geological materials, which are sandier and more acid in the area of site 3.

The community composition in the studied sites is shown in APPENDIX 1 (www.limnetica.net/internet). Crustaceans, particularly cla-

Table 1. Ranges of variation of physico-chemical parameters of water at the three sampling sites. *Rangos de variación de los parámetros físico-químicos del agua en las tres estaciones de muestro.*

Sampling site	Z	Secchi	T	Conductivity	pH	O_2	O_2
	(cm)	(cm)	(°C)	($\mu S \cdot cm^{-1}$)		($mg \cdot l^{-1}$)	(%)
Site 1							
mean	40.58	10.50	19.10	3296.20	8.02	5.84	90.60
range	24.5-57	6-15	7.3-26.5	2600-4711	7.5-8.5	1.9-10.31	78-103.2
Site 2							
mean	41.00	9.53	20.05	1859.88	7.91	10.05	119.25
range	24-67	7-12	11.2-28.9	1173-3334	7-8.5	6.08-17.6	58.5-184
Site 3							
mean	24.25	11.00	15.08	584.25	6.91	5.16	56.40
range	7-37.5	7-15	11-16.9	425-730	6.5-8	3.8-7.48	39-73

docerans and copepods, and aquatic bugs (Insecta: Heteroptera) were the most species-rich animal groups. For certain rare taxa (ostracods and mayflies) it was not possible to obtain sufficient biomass for the stable isotope analyses, and these taxa were therefore excluded from the statistical analysis. Rotifers and ciliates were also excluded because of their low sampled biomass.

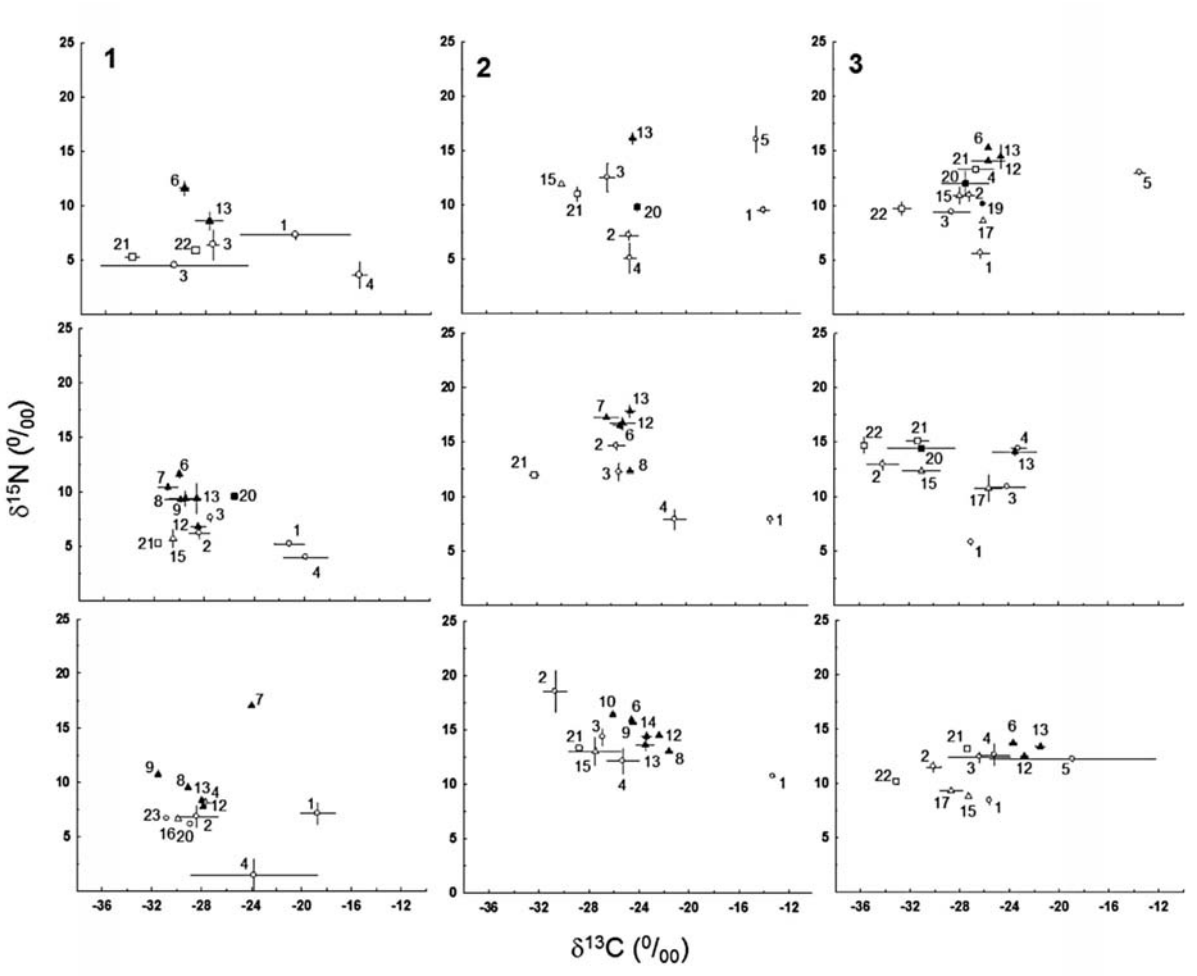

Figure 2. Stable isotope ratios for carbon (δ^{13}C, ‰, mean ± S.D.) and nitrogen (δ^{15}N, ‰, mean ± S.D.) for food web components at each of the sampling sites (upper: November 2000, middle: April 2001, lower: November 2001). Trophic items are as follows: 1.-detritus, 2.-phytoplankton, 3.-helophytes, 4.-periphyton, 5.-submersed macrophytes, 6.-*Gambusia holbrooki*, 7.-*Anguilla anguilla*, 8.-*Liza ramada*, 9.-*Cyprinus carpio*, 10.-*Barbus sclateri*, 11.-crayfish (< 3 cm), 12.-crayfish (< 7 cm), 13.-crayfish (> 7 cm), 14.-*Palaemon serratus* (shrimp), 15.-heteroptera, 16.-odonata, 17.-coleoptera, 18.-ephemeroptera, 19.-gastropoda, 20.-diptera, 21.-copepoda, 22.-cladocera, 23.-microcrustaceans (copepoda and cladocera). Trophic categories are symbolised as: ○ primary producers, ● invertebrate herbivores (ephemeroptera and gastropoda), △ invertivores (heteroptera and odonata), omnivores (fish, shrimp and crayfish), ▲ detritivores (diptera), filter feeders (copepoda and cladocera). *Razones isotópicas de carbono ($\delta^{13}C$, ‰, media ± D.E.) y nitrógeno ($\delta^{15}N$, ‰, media ± D.E.) para los componentes de la red trófica de las tres estaciones (arriba: noviembre 2000, en el medio: abril 2001, debajo: noviembre 2001). Los recursos tróficos son los siguientes: 1.-detritus, 2.-fitoplancton, 3.-helófitos, 4.-perifiton, 5.-macrófitos sumergidos, 6.*-Gambusia holbrooki, *7.*-Anguilla anguilla, *8.*-Liza ramada, *9.*-Cyprinus carpio, *10.*-Barbus sclateri, *11.*-cangrejos (< 3 cm), 12.-cangrejos (< 7 cm), 13.-cangrejos (> 7 cm), 14.*-Palaemon serratus *(camarones), 15.-heterópteros, 16.-odonatos, 17.-coleópteros, 18.-efemerópteros, 19.-gasterópodos, 20.-dípteros, 21.-copépodos, 22.-cladóceros, 23.-microcrustáceos (copépodos + cladóceros). Categorías tróficas simbolizadas como: ○ productores primarios, ● invertebrados herbívoros (efemerópteros y gasterópodos), △ invertívoros (heterópteros y odonatos), omnívoros (peces, camarones y cangrejos), ▲ detritívoros (dípteros), fitradores (copépodos y cladóceros).*

Food web structure revealed through the use of stable isotopes

MANOVA showed no significant interaction (date x site) (λ Wilks' = 0.97, Rao's $R_{(8,594)}$ = 1.03, p = 0.4) but did show significant differences between dates (λ Wilks' = 0.64, Rao's $R_{(4,594)}$ = 36.13, p < 0.0001) and sites (λ Wilks' = 0.96, Rao's $R_{(4,594)}$ = 2.67, p = 0.03) for δ^{13}C and δ^{15}N values, suggesting that the space and time factors have independent effects on the isotope values. Indeed, significant differences on δ^{15}N ($F_{(2,298)}$ = 3.72, p = 0.02) were observed only for dates 2 and 3 (Tukey's HSD test; p = 0.025 and p = 0.018, respectively), but no significant differences were observed for δ^{13}C. The δ^{15}N values for site 2 increased through time, as follows: November 2000 < April 2001 < November 2001. In regard to carbon isotopes, no significant differences in δ^{13}C were observed, except for the last date on site 3 (Tukey's HSD test, p = 0.026).

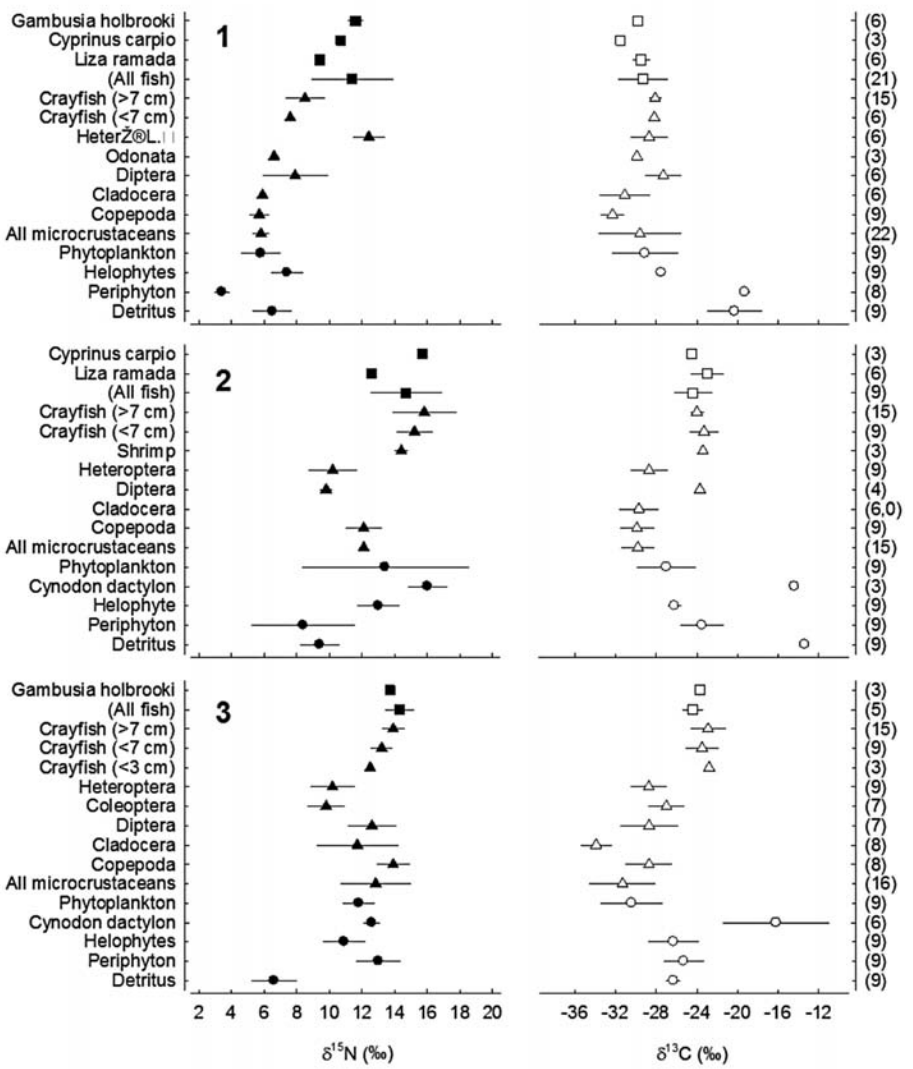

Figure 3. Stable nitrogen and carbon isotope concentration in food web components of the three sites during the sampling period. Sample sizes are given in parentheses following the names of species. Circle (●): primary producers; triangle (▲) : invertebrates; square (■): vertebrates. *Concentración de isótopos estables de carbono y nitrógeno de los componentes de las redes tróficas de las tres estaciones durante el periodo de muestreo. Círculos: productores primarios, triángulos: invertebrados, cuadrados: vertebrados.*

The average whole system content of $\delta^{13}C$ and $\delta^{15}N$ was highest for site 2 (which was affected by the heavy metals from the toxic spill), intermediate for site 3, and lowest for site 1 ($\delta^{13}C$: $F_{(2,298)} = 15.48$, $p < 0.0001$; $\delta^{15}N$: $F_{(2,298)} = 58$, $p < 0.0001$) (Fig. 2).

Detritus and periphyton drove the food webs in the habitats with soft and muddy bottom, *i.e.*, sites 1 and 2, whereas the trophic dynamics in site 3 (which had a sandy bottom) were driven primarily by detritus and submersed vegetation (Fig. 2). The higher trophic positions in all of the systems were occupied by fish and crayfish, with crustaceans and insects at intermediate positions.

Natural abundance of $\delta^{13}C$ and $\delta^{15}N$

The range values of $\delta^{13}C$ for the three sites differed mainly in their basal components. For site 1, the lowest and highest mean values corresponded to copepods and periphyton, respectively (-34.3 ± 1.2; -19.3 ± 0.5 ‰, APPENDIX 2, www.limnetica.net/internet). Similarly, copepods at site 2 had the lowest $\delta^{13}C$ value (-29.9 ± 1.7 ‰) while detritus had the highest value (-13.4 ± 0.4 ‰) (APPENDIX 3, www.limnetica.net/internet). For Site 3, the lowest value was found for cladocerans (-33.9 ± 1.4 ‰) and the highest value was found for the submersed grass *Cynodon dactylon* (-16.2 ± 5.2 ‰) (APPENDIX 4, www.limnetica.net/internet).

The $\delta^{15}N$ values for the three systems fluctuated in a variable manner. For sites 1 and 2, the lowest $\delta^{15}N$ values were found for periphyton (3.4 ± 0.5 ‰ and 8.4 ± 3.2 ‰respectively) and the highest values were found for eels, *Anguilla anguilla* (13.7 ± 3.6 ‰ and 17.2 ± 0.2 ‰, respectively) (APPENDIX 2 and 3). Finally, $\delta^{15}N$ values in site 3 fluctuate between 6.61 ± 1.42 ‰ for detritus to 14.3 ± 0.9 ‰ for the all-fish sample (APPENDIX 4).

The trophic positions were very close together, and very short trophic distances were found between species. The lowest crayfish values were found for site 1 (APPENDIX 2-4). For a better understanding of these variations in the $\delta^{13}C$ and $\delta^{15}N$ ratios, we constructed an isotope scaling for the different compartments analysed for each system over the entire sampling period (Fig. 3). Adult crayfish always appeared in higher trophic positions than juveniles. No significant differences were found between the $\delta^{15}N$ values of juvenile and adult crayfish, except for site 3 ($t_{2(0.05)} = -2.68$, $p = 0.016$) (APPENDIX 2-4; Figs. 3 and 4).

Trophic role of red swamp crayfish: applying a mixed model

We estimated the resources most frequently used by crayfish using two sources: stable isotopes and traditional diet studies that were performed in the same geographic area (Gutiérrez-Yurrita *et al*., 1998; Alcorlo *et al*., 2004). The results of the application of the concentration-weighted mixing model using the equations proposed by Phillips and Koch (2002) were inconsistent with the isotopic data for site 1 but fit well with the isotopic data from the other sampling sites (Table 2).

Nitrogen stable isotope ratios and heavy metals

The highest concentrations of heavy metals in crayfish were found for site 2, except for As

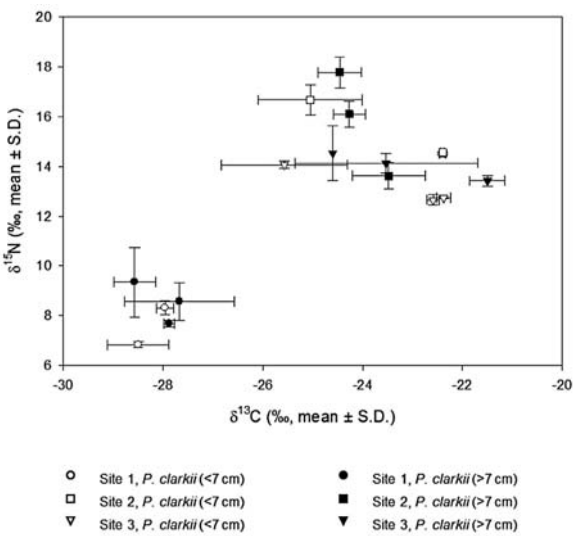

Figure 4. Stable nitrogen and carbon isotope concentration for juvenile and adult crayfish at the three sites (site 1, n = 9, 15; site 2, n = 9, 15; site 3, n = 6, 15). *Concentración de isótopos estables de carbono y nitrógeno de los cangrejos juveniles y adultos de las tres estaciones (Estación 1, n = 9, 15; estación 2, n = 9, 15; estación 3, n = 6, 15).*

which was higher at site 1 (Fig. 5). The Cu and Pb concentrations were well above the legal limits for heavy metal content in crustacean foodstuffs included in the Spanish law and EC Regulation No. 1881/2006 (20 ppm and 0.3 ppm for Cu and Pb, respectively). Arsenic (As) concentrations were high at each of the sites (0.56–0.98, 0.36–2.04 and 0.79–1.02 μg g^{-1} or ppm for sites 1, 2, and 3, respectively) when compared with the provisional tolerable daily intake suggested by the European Food Safety Authority (2 μg kg^{-1} body weight) (EFSA, 2009).

The contribution of heavy metals to the $\delta^{15}N$ signatures in crayfish was estimated through a multiple regression analysis of the five heavy metals analysed over the $\delta^{15}N$ signatures of crayfish (pooled individuals of the three sites) as the dependent variable and explained 51 % of the total variation of crayfish $\delta^{15}N$ ($r = 0.72$, $r^2 = 0.51$, $F_{(5,34)} = 7.35$, $p < 0.0001$). Further multiple regression analyses were performed for each site. Only site 3 showed significant results, with 93 % of the variance in $\delta^{15}N$ explained by the heavy metal contents of crayfish and with Cu ($r = -0.79$, $p < 0.05$) and Cd ($r = -0.78$, $p < 0.05$) as the elements that accounted for most of this relationship.

DISCUSSION

In this study, the spatial variability (between-site differences) was significant for both $\delta^{13}C$ and $\delta^{15}N$ isotope signatures, whereas the temporal variability (between-date differences) was significant only for average $\delta^{15}N$ signatures. The pattern of variation of $\delta^{13}C$ values was related to the variability of the basal food sources (mainly

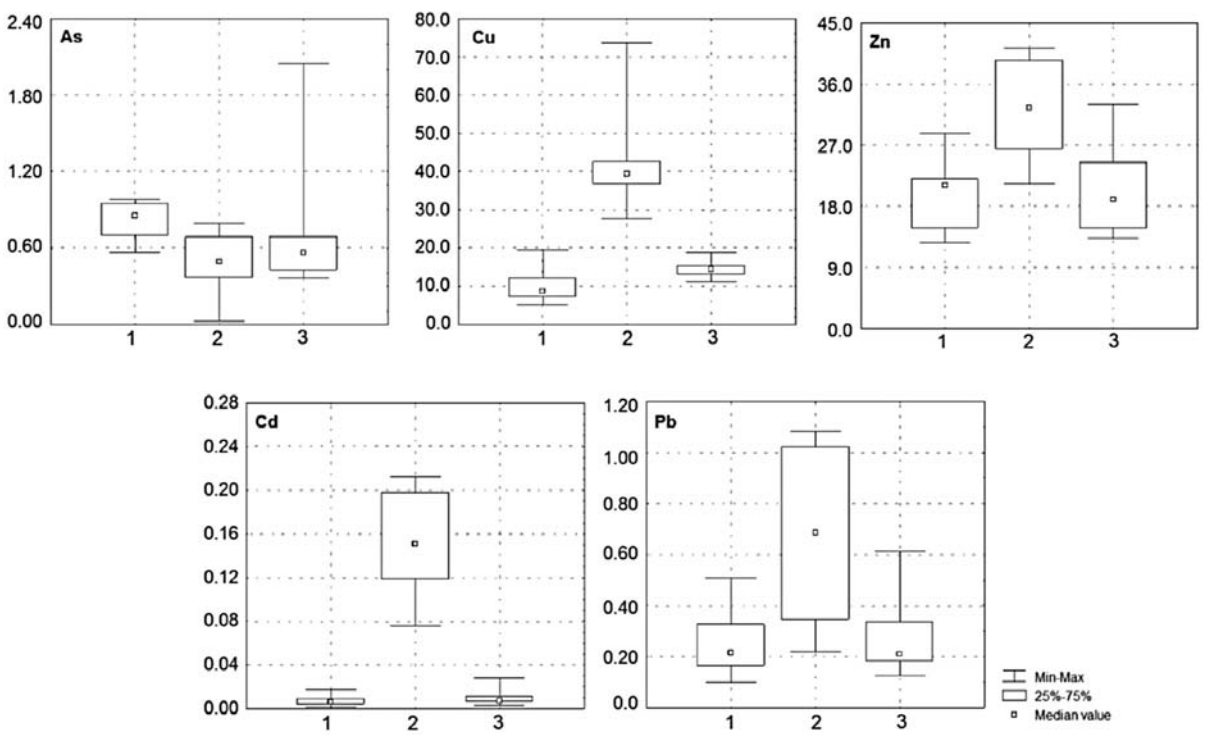

Figure 5. Patterns of variability of five heavy metals analysed in whole body crayfish from the three studied ecosystems, expressed as μg · g^{-1} fresh weight. Sample sizes of each crayfish sample are given in parentheses for each site. Site 1 (n = 10); site 2 (n = 20); site 3 (n = 10). *Patrones de variabilidad de los metales pesados analizados en los cuerpos enteros de cangrejos en los tres ecosistemas estudiados expresados en μg · g^{-1} peso fresco. El tamaño muestral de los cangrejos analizados se da entre paréntesis para cada estación. Estación (n = 10), estación 2 (n = 20); estación 3 (n = 10).*

detritus and primary producers) at each site. The amount of detritus for each sampling site varied depending on the underlying lithology and organic matter inputs from biological communities within the area or nearby (autochthonous or allochthonous inputs, respectively). The δ^{13}C values for detritus from sites 1 and 2 (the muddy sites) indicated that the sites were both enriched in carbon and had very similar ranges but differed markedly from the value for sandy site 3. These values are similar to those found in highly productive freshwater ecosystems that store large amounts of detritus, such as Laguna Madre of Texas (−16 ‰) (Fry & Sherr, 1984). The δ^{13}C value for site 3 was lower and more similar to the average values of terrestrial organic matter (−26 ‰) and estuaries (−25.8 ‰) in the Delaware estuary, Philadelphia (Cifuentes *et al.,* 1988) or of littoral areas (−29.9 ‰ in Wards Creek, Virginian Atlantic Coast) (Garman & Macko, 1998).

Helophytes and macrophytes showed smaller δ^{13}C values compared with detritus. This effect can be used to estimate the relative importance of these primary producers to herbivores. Allochthonous (*Cynodon dactylon*) and autochthonous (phytoplankton, periphyton, helophytes) food sources can be easily identified according to their δ^{13}C signatures. Because *C. dactylon* is a gramineous plant with a C-4 photosynthetic pathway, it is expected to have higher δ^{13}C values (LaZerte & Szalados, 1982). This species, which is associated with areas of groundwater discharge, is the only terrestrial plant that remains green throughout the dry season and is an important food source for numerous waterfowl species that live in the area (González-Bernáldez, 1997). Our isotopic food web models (Figs. 2 and 3, Table 2) are consistent with systems driven by a detritus-based energy pathway (sites 1 and 2) and with a system based on both primary producers and detritus (site 3).

The mean δ^{15}N values for detritus fell within the range observed for other aquatic ecosystems at many sites (3.93–15.70 ‰ in Lake Lugano, Switzerland [Bernasconi *et al.*, 1997]; 5.5–18.7 ‰ in the Delaware estuary [Cifuentes *et al.*, 1988]), suggesting that nitrate is not limiting to primary producers. At each site, the primary producers and consumers showed changes in their δ^{15}N signatures that are consistent with species replacement within the communities that inhabit these ecosystems (see APPENDIX 1). Whereas the δ^{15}N values of species varied significantly between sites, the isotopic signatures of consumers fit well within the ranges of potential food sources at each site. Invertebrates displayed a broad range of trophic strategies including suspension feeders (*e.g.*, copepods or cladocerans), detritivores or 'collectors' (*sensu* (Merrit and Cummins 1996)) (*e.g.*, dipterans and ostracods), herbivores or 'macrophyte piercers' (Merritt and Cummins, op. cit.) (*e.g.*, mayflies), carnivores (*e.g.*, many heteroptera, coleoptera and odonata), and omnivores (*e.g.*, fish and crayfish). Detritivores and grazers appear to be primarily omnivorous, as indicated by their high δ^{15}N values, which are similar to those of predators. At all sites, the variation in δ^{15}N within trophic levels was low, and trophic levels were not easy to discriminate. The high variation and overlap observed in isotopic signatures (Figs. 2 and 3) showed a group effect for different trophic

Table 2. Contribution of food items to crayfish of the three study sites determined by the concentration–weighted linear mixing model. *Contribución de los distintos recursos alimenticios de los tres casos de estudio al cangrejo calculados según un modelo lineal de mezcla ponderado.*

Site	Detritus	Primary producers	Invertebrates	Food items included in the model
1	0.515	–0.259	0.744	detritus, periphyton, shrimps, heteroptera, odonata and diptera
2	0.637	0.020	0.343	detritus, periphyton, submersed macrophytes, shrimps, heteroptera and diptera
3	0.737	0.246	0.017	detritus, periphyton, submersed macrophytes, copepoda, cladocera, shrimps, heteroptera and diptera

species in the same taxonomic category, which suggests that animals act as opportunistic feeders that focus on food sources according to their availability, as reported in other field studies (Zah *et al*., 2001).

The gradual enrichment in $\delta^{15}N$ values between species in our simplified model suggested that the concept of trophic levels with discrete jumps in trophic position along a food chain is inappropriate. Co-existing species appeared to feed along a continuum of trophic levels, as has been observed in other systems (Hobson *et al.*, 1994; France & Peters, 1997; Vander Zanden *et al.*, 1999).

The omnivore trophic role of crayfish is particularly relevant in these ecosystems. Gut content studies and stable isotopes analyses have revealed detritus as a major component of the diet of many crayfish species (Creed, 1994; Whitledge & Rabeni, 1997; Gutiérrez-Yurrita *et al*., 1998; Alcorlo *et al.*, 2004). Our concentration-weighed mixing isotopic model estimated that the proportions of detritus in crayfish tissues were 63 % and 73 % for sites 2 and 3, respectively. The fact that crayfish from site 1 did not fall in the mixing triangle (Table 2) suggests that the chosen fractionation factors should be corrected because of the sensitivity of this type of model to the discrimination factors used, as suggested by Bond & Antony (2011), or that there are no substantial differences in isotopic composition among sources, as suggested by Phillips & Gregg (2001). There was also a small contribution from other invertebrates, as revealed by their $\delta^{15}N$ signatures, which fall within the expected enrichment range of 3.4 ‰ (APPENDIX 2-4). The estimated contribution of primary producers to the crayfish $\delta^{15}N$ signal seems to be very small. The $\delta^{13}C$ signatures of *P. clarkii* revealed that the animals used primary producer food sources in each of the three sites, both autochthonous sources (detritus and the primary producers of the system [phytoplankton, periphyton and helophytes]) and the allochthonous sources (terrestrial submersed vegetation) (Table 2).

In regard to the changes in diet corresponding to the ontogeny of crayfish, it has long been recognised that juveniles of many species feed predominantly on invertebrate prey, whereas adults shift their food requirements to vegetation and detritus (Goddard, 1988; France, 1996b). The stable isotope analysis in the present study did not identify differences in nutritional pathways between crayfish of different sizes (Fig. 4). The ontogenetic shift in diet detected in gut analyses may indicate that lower volumes of invertebrates are able to satisfy the energetic requirements for adult growth (Parkyn *et al.*, 2001). Gut content analyses identify crayfish as omnivores that process detritus and vegetation and prey upon other invertebrates, such as chironomids, water bugs, cladocerans, snails and mayflies (Alcorlo *et al.*, 2004). On the other hand, isotopic signatures indicated that crayfish tend toward a predator position (Figs. 2 and 3). These findings suggest that, despite the use of several food sources, crayfish feed predominantly on animal prey and that these prey are more important in terms of assimilation and incorporation into crayfish biomass. Similar results have been found in studies of other crayfish species, such as *Paranephrops planifrons* in New Zealand streams (Parkyn *et al*., 2001; Hollows *et al*. 2002) and *Orconectes nais* and *O. neglectus* in a tallgrass prairie stream in Kansas (Evans-White *et al.*, 2001).

We observed a consistent relationship between the concentrations of heavy metals (As, Cd, Zn, Cu, Pb) bioaccumulated in crayfish tissues and their nitrogen isotope signatures. This relationship, however, became weak when it was estimated site by site. The metals that have the greatest influence on $\delta^{15}N$ are Cu and Cd, whose accumulation has been found to be dose (i.e., Cd)- and time (i.e., Cu)-dependent in other studies performed in the area (Alcorlo *et al*., 2006). The bioaccumulation of different metals by crayfish depends on their environmental concentration and the nature and/or possible role of these metals as essential elements for crayfish metabolism. Cu is an essential metal whose levels are regulated and controlled within certain limits because it forms part of active enzyme centres and respiratory pigments (Rainbow, 1995), whereas Cd is a non-essential metal

(Rainbow, 1997) and tend to be detoxified by metallothionein proteins located in the digestive glands of *P. clarkii* (Del Ramo *et al*., 1989). These results are related to the different metal assimilation routes used by crayfish, *i.e.*, direct uptake from water or indirect uptake from food.

Given that crayfish behave as polytrophic species (omnivorous) in the systems where they occur establishing a high number of trophic interactions with other biological compartments of the systems; its trophic role has important consequences for the management of the ecosystems where crayfish is present. For example, crayfish can efficiently transport xenobiotic substances throughout the trophic web in polluted areas, such as the one described in this study (González *et al.*, 1985; Hernández *et al*., 1992). Because crayfish constitute an important food source for vertebrate predators, such as fish (*Anguilla anguilla*), birds (e.g., *Ciconia ciconia* in Correia, 2001) and mammals (e.g., *Lutra lutra* in Delibes & Adrián, 1987; Palomares & Delibes, 1991; Beja, 1996) we strongly recommend a long-term monitoring programme to keep track of the transfer of pollutants to higher order food web levels mediated by crayfish.

Finally, we conclude that our isotopic food web models are consistent with systems driven by a detritus-based energy pathway (sites 1 and 2) and with a system based on both primary producers and detritus. The trophic positions estimated for crayfish and other invertebrates at each site were low, suggesting the prevalence of omnivory and the occurrence of a trophic continuum rather than discrete levels. Isotopically, crayfish occupied a predator position in the food webs, consistently with a predominance of animal sources in their diet. However, no significant changes between crayfish ontogenetic stages were found using isotopic ratios in this study. The site with the highest concentration of heavy metals showed $\delta^{13}C$ and $\delta^{15}N$ ratios, and we found a significant correlation between five heavy metal elements (As, Cd, Zn. Cu, Pb) measured in crayfish and their nitrogen isotope signatures ($r = 0.72$, $p < 0.0001$). These findings support the role of *P. clarkii* as a pollution biomarker.

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REFERENCES

ADRIAN, M. I. & M. DELIBES. 1987. Food habits of the otter (*Lutra lutra*) in two habitats of the Doñana National Park, SW Spain. *Journal of Zoology*, 212: 399–406.

ALCORLO, P., W. GEIGER & M. OTERO. 2004. Feeding preferences and food selection of the red swamp crayfish, *Procambarus clarkii*, in habitats differing in food item diversity. *Crustaceana*, 44: 435–453.

ALCORLO, P., M. OTERO, M. CREHUET. A. BALTANÁS & C. MONTES. 2006. The use of the red swamp crayfish (*Procambarus clarkii*, Girard) as indicator of the bioavailability of heavy metals in environmental monitoring in the River Guadiamar (SW, Spain). *Science of the Total Environment*, 366: 380–390.

ALLISON, G., L. J. B. LAURENSON, G. PISTONE, F. STAGNITTI & P. L. JONES. 2000. Effects of

dietary copper on the Australian Freshwater Crayfish *Cherax destructor*. *Ecotoxicology and Environmental Safety*, 46: 117–123.

ALONSO, F., C. TEMIÑO & J. DIÉGUEZ-URIBEONDO. 2000. La situation de l'écrevisse à pieds blancs, *Austropotamobius pallipes* (Lereboullet, 1858), en Espagne: répartition et législation. *Bulletin FranÇais De La Pêche Et De La Pisciculture*, 356: 31–54.

ANASTÁCIO, P. M. & J. C. MARQUES. 1995 Population Biology and production of the red swamp crayfish *Procambarus clarkii* (Girard) in the lower Mondego river Valley, Portugal, *Journal of Crustacean Biology*, 15: 156–168.

ANDERSON, M. B., P. REDDY, J. E. PRESLAN, M. FINGERMAN, J. BOLLINGER, L. JOLIBOIS, G. MAHESHWARUDU & WJ. GEORGES. 1997a. Metal accumulation in crayfish, *Procambarus clarkii*, exposed to a petroleum-contaminated Bayou in Louisiana. *Ecotoxicology and Environmental Safety*, 37: 267–272.

ANDERSON, M. B., J. E. PRESLAN, L. JOLIBOIS, J. BOLLINGER & W. J. GEORGES. 1997b. Bioaccumulation of lead nitrate in Red Swamp Crayfish (*Procambarus clarkii*). *Journal of Hazardous Materials*, 154: 15–29.

ANGELER, D., S. SÁNCHEZ-CARRILLO, G. GARCÍA & M. A. COBELAS. 2001. The influence of *Procambarus clarkii* (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. *Hydrobiologia*, 464: 89–98.

ANTÓN, A., T. SERRANO, E. ANGULO, G. FERRERO, A. RALLO. 2000. The use of two species of crayfish as environmental quality sentinels: the relationship between heavy metal content, cell and tissue biomarkers and physico-chemical characteristics of the environment. *Science of the Total Environment*, 247: 239–251.

BEJA, P. R. 1996. An analysis of otter *Lutra lutra* predation on introduced american crayfish *Procambarus clarkii* in Iberian streams. *Journal of Applied Ecology*, 33: 1156–70.

BEN-DAVID, M. & D. M. SCHELL. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. *Oecologia*, 127, 180–184.

BERNASCONI, S. M., A. BARBIERI & M. SIMONA. 1997. Carbon and nitrogen isotope variations in sedimenting organic matter in Lake Lugano. *Limnology and Oceanography*, 42: 1755–1765.

BOND, A. L. & W. D. ANTONY. 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecological Applications*, 21(4): 1017–1023.

BOON P. I. & S. E. BUNN. 1994. Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. *Aquatic Botany*, 48: 99–108.

BROWN, P. B. 1990 Review of crayfish nutrition. *Crustacean Nutrition Newsletter*, 6: 68–69.

CANO, E. & M. E. OCETE 1997. Population biology of red swamp crayfish, *Procambarus clarkii* (Girard, 1852) in the Guadalquivir river marshes. Spain. *Crustaceana*, 70: 553–561.

CIFUENTES, L. A., J. H. SHARP & M. L. FOGEL. 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. *Journal of the North American Benthological Society*, 33: 1102–1115.

CORREIA, A. M. 2001. Seasonal and interspecific evaluation of presation by mammals and birds on the introduced red swamp crayfish *Procambarus clarkii* (Crustacea, Cambaridae) in a freshwater marsh (Portugal). *Journal of Zoology, London*, 255: 533–41.

CREED, R. P. 1994. Direct and indirect effects of crayfish grazing in a stream community. *Ecology*, 75: 2091–2103.

D'ABRAMO L. R. & E. H. ROBINSON. 1989. Nutrition of crayfish. *Aquatic Science*, 1: 711–728.

DEL RAMO, J., A. PASTOR, A. TORREBLANCA, J. MEDINA & J. DÍAZ-MAYANS. 1989. Cadmium-Blinding proteins in midgut gland of freshwater crayfish *Procambarus clarkii*. *Bulletin of Environmental Contamination and Toxicology*, 42: 241–246.

DELIBES, M. & I. ADRIÁN 1987. Effects of crayfish introduction on otter *Lutra lutra* in the Doñana National Park, SW Spain. *Biological Conservation*, 42: 153–159.

DENIRO, M. J. & S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica Et Cosmochimica Acta*, 42: 495–506.

DENIRO, M. J. & S. EPSTEIN. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica Et Cosmochimica Acta*, 45: 341–351.

EFSA-Panel on Contaminants in the Food Chain (CONTAM). 2009. Scientific Opinion on Arsenic in Food. *EFSA Journal*; 7(10): 1351. [199 pp.].

doi:10.2903/j.efsa.2009.1351. Available online: www.efsa.europa.eu.

EVANS-WHITE, M., W. K. DODDS, L. J. GRAY & K. M. FRITZ. 2001 A comparison of the trophic ecology of the crayfishes (*Orconectes nais* (Faxon) and *Orconectes neglectus* (Faxon)) and the central stoneroller minnow (*Campostoma anomalum* (Rafinesque)): omnivory in a tallgrass prairie stream. *Hydrobiologia*, 462: 131–144.

FARIA, M., D. HUERTAS, D. X. SOTO, J. O GRIMALT, J. CATALAN, M. C. RIVA & C. BARATA, 2010. Contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Dreissena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). *Chemosphere*; 78: 232–240.

FEMINELLA, J. W. & V. H. RESH. 1986. Effects of crayfish grazing on mosquito habitat at Coyote Hills Marsh. In: Proceedings and Papers of the Fifty-Fourth Annual Conference of the California Mosquito and Vector Control Association. *The 54th Annual Conference of the California Mosquito and Vector Control Association*, University of California, USA: 101–103.

FEMINELLA, J. W. & V. H. RESH. 1989. Submerged macrophytes and grazing crayfish: an experimental study of herbivory in California freshwater marsh. *Holartic Ecology*, 12: 1–8.

FRANCE, R. L. 1996a. Ontogenetic shift in crayfish δ^{13}C as a measure of land-water ecotonal coupling. *Oecologia*, 107: 239–242.

FRANCE, R. L. 1996b. Benthic-pelagic uncoupling of carbon flow. *Trends in Ecology and Evolution*, 11: 471.

FRANCE, R. L. 1999. Relationships between DOC concentration and epilithon stable isotopes in boreal lakes. *Freshwater Biology*, 41: 101–105.

FRANCE, R. L. & R. H. PETERS. 1997. Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. *Canadian Journal of Fisheries and Aquatic Sciences*, 54: 1255–1258.

FRY, B. & E. B. SHERR. 1984. δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science*, 27: 13–47.

GANNES L. Z., C. M. RÍO & P. KOCH. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry Physiology*, 119: 725–737.

GARMAN, G. C. & S. A. MACKO. 1998. Contribution of marine-derived organic matter to an Atlantic coast, freshwater, tidal stream by anadromous clupeid fishes. *Journal of The North American Benthological Society*, 17: 277–285.

GEARING J. N., P. J. GEARING, D. T. RUDNICK, A. G. REQUEJO & M. J. HUTCHINS. 1984. Isotopic variability of organic carbon in a phytoplankton-based temperate estuary. *Geochimica Et Cosmochimica Acta*, 48: 1089–1098.

GEIGER, W., P. ALCORLO, A. BALTANÁS & C. MONTES. 2005. Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions*, 7: 49–73.

GHERARDI, F. & S. BARBARESI. 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv fur Hydrobiologie*, 150: 153–168.

GODDARD, J. S. 1988. Food and feeding. In: *Freshwater Crayfish Biology, Management and Exploitation*. Holdich, D.M. & R.S. Lowery (eds): 145–166. Croom-Helm Timber Press, London.

GONZÁLEZ-BERNÁLDEZ, F. 1997. Gramíneas pratenses de Madrid, Consejería de Medio Ambiente y Desarrollo Regional de la Comunidad Autónoma de Madrid, 287 pp.

GONZÁLEZ, J., L. M. HERNÁNDEZ, A. HERNÁN & G. BALUJA. 1985. Multivariate analysis of water contamination by heavy metals at Doñana National Park. *Bulletin of Environmental Contamination & Toxicology*, 35: 266–271.

GUTIÉRREZ-YURRITA, P. J., G. SANCHO, M. A. BRAVO, A. BALTANÁS & C. MONTES. 1998. Diet of the red swamp crayfish *Procambarus clarkii* in natural ecosystems of the Doñana National Park temporary fresh-water marsh (Spain). *Journal of Crustacean Biology*, 14: 120–127.

GUTIÉRREZ-YURRITA, P. J., J. M. MARTÍNEZ, M. A. BRAVO-UTRERA, C. MONTES, M. ILHÉU & J. M. BERNARDO. 1999. The status of crayfish populations in Spain and Portugal. In: *Crayfish in Europe as alien species.* F. Gherardi & D.M. Holdich (eds): 161–192. Balkema AA, Rotterdam.

HABSBURGO-LORENA, A. S. 1983. Socioeconomic aspects of the crawfish industry in Spain. *Freshwater Crayfish*, 5: 552–554.

HERNÁNDEZ, L. M., M. A. FERNÁNDEZ & M. J. GONZÁLEZ. 1992. Organochlorine pollutants in water, soils and earthworms in the Guadalquivir

river, Spain. *Bulletin of Environmental Contamination & Toxicology*, 49: 192–198.

HERSHEY, A. E. & B. J. PETERSON. 1996. Stream food webs. In: *Methods in stream ecology* F.R. Hauer & G.A. Lamberti (eds): 511–529. Academic Press, San Diego, California.

HOBSON, K. A. & H. E. WELCH. 1992. Determination of trophic relationships within a high Artic marine food web using δ^{13}C and δ^{15}N analysis. *Marine Ecology Progress Series*, 84: 9–18.

HOBSON, K. A., J. F. PIATT & J. PITOCCHELLI. 1994. Using stable isotopes to determine seabirds trophic relationships. *Journal of Animal Ecology*, 63: 786–798.

HOLLOWS, J. W., C. R. TOWNSEND & K. J. COLLIER. 2002. Diet of crayfish *Paranephrops zealandicus* in bush and pasture streams: insights from stable isotopes and stomach analysis. *New Zealand Journal of Marine and Freshwater Research*, 36: 129–142.

ILHÉU, M. & J. M. BERNARDO. 1993. Experimental evaluation of food preference of red swamp crayfish, *Procambarus clarkii*: Vegetal versus animal. *Freshwater Crayfish*, 9: 359–364.

ILHÉU, M. & J. M. BERNARDO. 1995. Trophic ecology of red swamp crayfish *Procambarus clarkii* (Girard)-Preferences and digestibility of plant foods. *Freshwater Crayfish*, 10: 132–139.

INGER, R. & S. BEARHOP. 2008. Applications of stable isotope analysis to avian ecology. *Ibis*, 150: 447–461.

JONES, J. I. & S. WALDRON. 2003. Combined stable isotope and gut contents analysis of food webs in plant-dominated, shallow lakes. *Freshwater Biology*, 48: 1396–1407.

KWAK, T. J. & J. B. ZEDLER. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia*, 110: 262–277.

LAJTHA, K. & R. H. MICHENER. 1994. Introduction. In: *Stable isotopes in ecology and environmental science* K. Lajtha & R.H. Michener (eds.). Blackwell Scientific Publications, London, U.K.

LAZERTE, B. D. & J. E. SZALADOS. 1982. Stable carbon isotope ratio of submerged freshwater macrophytes. *Limnology and Oceanography*, 27: 413–418.

MARANHÃO, P., J. C. MARQUES & V. MADEIRA. 1995. Copper concentrations in soft tissues of the red swamp crayfish *Procambarus clarkii* (Girard, 1852), after exposure to a range of dissolved copper concentrations. *Freshwater Crayfish*, 10: 282–286.

MARTÍNEZ DEL RIO, C., N. WOLF, S. A. CARLETON, & L. Z. GANNES. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews of the Cambridge Philosophical Society*, 84: 91–111.

MARTÍN-DÍAZ, M. L., S. R. TUBERTY, C. MCKENNEY, J. BLASCO, C. SARASQUETE & T. A. DEL VALLS. 2006. The use of bioaccumulation, biomarkers and histopathology diseases in *Procambarus clarkii* to establish bioavailability of Cd and Zn after a mining spill. *Environmental Monitoring and Assessment*, 116 (1–3): 169–184.

MEHARG, A. A., D. OSBORN, D. J. PAIN, A. SÁNCHEZ & M. A. NAVESO. 1999. Contamination of Doñana food-chains after the Aznalcóllar mine disaster. *Environmental Pollution*, 105: 387–390.

MERRIT, R. W. & K. W. CUMMINS. 1996. An Introduction to the Aquatic Insects of North America: 3rd Edition. Kendall/Hunt publishing company.

MINAGAWA, M. & E. WADA. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. *Geochimica Et Cosmochimica Acta*, 48: 1135–1140.

MONTES, C., M. A. BRAVO-UTRERA, A. BALTANÁS, C. DUARTE & P. J. GUTIÉRREZ-YURRITA. 1993. *Bases ecológicas para la gestión del cangrejo rojo de las marismas del Parque Nacional de Doñana, España.* Madrid, España: ICONA.

MONTES, C., F. BORJA, M. A. BRAVO & J. M. MOREIRA. 1998. *Reconocimiento biofísico de espacios naturales protegidos. Doñana: una aproximación ecosistémica*. Consejería de Medio Ambiente, Junta de Andalucía, España. 311 pp.

NAQVI, S. M. & C. T. FLAGGE. 1990. Chronic effects of arsenic on American red crayfish, *Procambarus clarkii*, exposed to Monosodium Methanearsonate (MSMA) Herbicide. *Bulletin of Environmental Contamination & Toxicology*, 45: 101–106.

NAQVI, S. M. & R. D. HOWELL. 1993. Toxicity of cadmium and lead to juvenile red swamp crayfish, *Procambarus clarkii*, and effects on fecundity of adults. *Bulletin of Environmental Contamination & Toxicology*, 51: 303–308.

NATIONAL PESTICIDE INFORMATION CENTER. http://npic.orst.edu/ingred/aifact.html (last visit 17th july 2012).

PAINE, R. T. 1988. Food webs: road maps of interactions or grist for theoretical deveplopment? *Ecology*, 69, 1648–1654.

PALOMARES F. & M. DELIBES 1991. Alimentación del meloncillo *Herpestes ichneumon* y de la gineta *Genetta genetta* en la Reserva Biológica de Doñana, S.O. de la Peninsula Ibérica. *Doñana Acta Vertebrata*, 18: 5–20.

PARKYN, S. M., K. J. COLLIER & B. J. HICKS. 2001. New Zealand stream crayfish: functional omnivores but trophic predators? *Freshwater Biology*, 46: 641–652.

PETERSON, B. J., R. W. HOWARTH & R. H. GARRITT. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science*, 227: 1361–1363.

PETERSON, B. J. & B. FRY. 1987. Stable isotopes in ecosystem studies. *Annual Reviews of Ecology and Systematics*, 18: 293–320.

PHILLIPS, D. L. 2001. Mixing models in analyses of diet using multiple isotopes: a critique. *Oecologia*, 127: 166–170.

PHILLIPS, D. L. & J. W. GREGG. 2001. Uncertainty in source partitioning using stable isotopes. *Oecologia*, 127: 171–179.

PHILLIPS, D. L. & P. L. KOCH. 2002. Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, 130: 114–125.

PONSARD, S. & R. ARDITI. 2001. Detecting omnivory with δ^{15}N . *Trends in Ecology and Evolution*, 16: 20–21.

PRAT, N., J. TOJA, C. SOLÀ, M. D. BURGOS, M. PLANS & M. RIERADEVALL. 1999. Effect of dumping and cleaning activities on the aquatic ecosystems of the Guadiamar River following a toxic flood. *The Science of the Total Environment*, 242: 231–248.

RAINBOW, P. S. 1995. Physiology, Physicochemistry and metal uptake: a crustacean perspective. *Marine Pollution Bulletin*, 31: 55–59.

RAINBOW, P. S. 1997. Ecophysiology of trace metal uptake in Crustaceans. *Estuarine Coastal and Shelf Science*, 44: 169–175.

RODRÍGUEZ, C. F., E. BECARÉS & M. FERNÁNDEZ-ALÁEZ. 2003. Shift from clear to turbid phase in lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (*Procambarus clarkii*) . *Hydrobiologia*, 506–509: 421–426.

SCHILDERMAN, P. A. E. L., E. J. C. MOONEN, L. M. MAAS, I. WELLE & J. C. S. KLEINJANS. 1999. Use of crayfish in biomonitoring studies of environmental pollution of the River Meuse. *Ecotoxicology and Environmetal Safety*, 44: 241–252.

SCHLENK, D. 1999. Necessity of defining biomarkers for use in Ecological Risk Assessment. *Marine Pollution Bulletin*, 39(1–12): 48–53.

SENRA, A. & E. E. ALÉS. 1992. The decline of white stork *Ciconia ciconia* population of western Andalusia between 1976 and 1988: causes and proposals for conservation. *Biological Conservation*, 61: 51–57.

SUÁREZ-SERRANO, A., C. ALCARAZ, C. IBÁÑEZ, R. TROBAJO & C. BARATA. 2010. *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology and Environmental Safety*, 73: 280–286.

TABLADO, Z., J. L. TELLA, J. A. SÁNCHEZ-ZAPATA & F. HIRALDO. 2010. The paradox of the long-term positive effects of a North American crayfish on a European Community of predators. *Conservation Biology*, 24: 1230–1238.

VANDER ZANDEN, M. J., J. M. CASSELMAN & J. B. RASMUSSEN. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, 401: 464–469.

VIOQUE-FERNÁNDEZ, A., E. ALVES DE ALMEIDA, J. BALLESTEROS, T. GARCÍA-BARRERA, J. L. GÓMEZ-ARIZA & J. LÓPEZ-BAREA. 2007. Doñana National Park survey using crayfish (*Procambarus clarkii*) as bioindicator: esterase inhibition and pollutant levels. *Toxicology Letters*, 168: 260–268.

VIOQUE-FERNÁNDEZ, A., E. ALVES DE ALMEIDA & J. LÓPEZ-BAREA. 2009. Assessment of Doñana National Park contamination in *Procambarus clarkii*: Integration of conventional biomarkers and proteomic approaches. *The Science of the Total Environment*, 407(5): 1784–1797.

WHITLEDGE, G. W. & C. F. RABENI. 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 54: 2555–2563.

ZAH, R., P. BURGHERR, S. M. BERNASCONI & U. UEHLINGER. 2001. Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream. *Freshwater Biology*, 46: 871–882.

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Spectrophotometric methods for the determination of photosynthetic pigments in stratified lakes: a critical analysis based on comparisons with HPLC determinations in a model lake

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ABSTRACT

Spectrophotometric methods for the determination of photosynthetic pigments in stratified lakes: a critical analysis based on comparisons with HPLC determinations in a model lake

High-performance liquid chromatography (HPLC) is an accurate method for photosynthetic pigment analysis; however, spectrophotometric equations are also frequently used for pigment quantification in aquatic systems. Here, we present a critical analysis of the most-used spectrophotometric equations by comparing the results obtained using these equations with unambiguous HPLC determinations. The study was performed in Lake La Cruz (central Spain). In this meromictic lake with strong thermal stratification, photosynthetic populations occur in different, vertically stratified layers. Eukaryotic algae and picocyanobacteria are mostly located in oxic layers, whereas purple sulphur bacteria grow at the oxic-anoxic interface and below, and green sulphur bacteria occur primarily in deeper anoxic layers. This broad diversity of photosynthetic microorganisms involves a complex mixture of photosynthetic pigments that often exhibit overlapping absorption spectra. We tested spectrophotometric equations using samples that represented the entire range of spatial and temporal variability of the lake. For chlorophyll-*a*, the best correlations with all tested equations were observed for oxic layers. Regardless of where the sample was obtained, the best fit for chlorophyll-*a* was produced by the equation of Overmann & Tilzer, which is specifically designed to handle mixtures of chlorophyll-*a* and bacteriochlorophyll-*d* from green sulphur bacteria. Trichromatic equations for determining chlorophyll-*b* and -*c* exhibited strong interferences in anoxic waters, whereas in the upper layers of the lake, concentrations of these pigments were usually below the detection limit, restricting the use of these equations. The equations of Takahashi & Ichimura for bacterial pigments slightly overestimated both bacteriochlorophyll-*a* and -*d* by approximately 10 % and underestimated bacteriochlorophyll-*c* by nearly 23 %, although for bacteriochlorophyll-*d*, the correlation was better than those obtained using the dichromatic equations of Parkin & Brock and Overmann & Tilzer, respectively. Total carotenoid abundance can be assessed with the equation designed for this purpose by Strickland & Parsons (1972); however, the accuracy of the results differs with depth and is strongly biased by the presence of the bacterial carotenoid okenone. On the other hand, dual-wavelength carotenoid-to-chlorophyll-*a* ratios (430/665 and 480/665) only produced acceptable results in the epilimnion, with the occurrence of the bacterial carotenoid okenone in the microaerobic and anoxic layers again producing significant interference. Additionally, the wide variation with depth of the carotenoid composition undermined the validity of these dual-wavelength ratios. In conclusion, our findings indicate that a complete and unambiguous study of photosynthetic pigments in highly stratified lakes with overlapping populations of phototrophic microorganisms requires the use of HPLC techniques. Anyway, our results demonstrate that chlorophyll-*a* and bacteriochlorophyll-*a* and -*d* can be directly measured in oxic and anoxic layers, respectively, using spectrophotometric methods with an error lower than 10 %. However, according to our results, chlorophyll-*b* and -*c* and bacteriochlorophyll-*c* cannot be accurately estimated by spectrophotometric methods in stratified lakes.

Key words: Stratified lakes, photosynthetic pigments, chlorophylls, bacteriochlorophylls, spectrophotometry, high performance liquid chromatography (HPLC), phytoplankton, photosynthetic bacteria.

RESUMEN

Determinación de pigmentos fotosintéticos por métodos espectrofotométricos en lagos estratificados: un análisis crítico basado en la comparación con determinaciones por HPLC en un lago modelo

*La cromatografía líquida de alta eficacia (HPLC) es un método preciso para el análisis de pigmentos fotosintéticos, sin embargo las ecuaciones espectrofotométricas todavía se usan frecuentemente en sistemas acuáticos. En este trabajo mostramos un análisis crítico de algunas de estas ecuaciones comparando los resultados obtenidos con las determinaciones más precisas realizadas por HPLC. El estudio se realizó tomando como modelo la Laguna de La Cruz (centro de España). En este lago meromíctico, que además presenta una fuerte estratificación térmica estival, las poblaciones de microorganismos fotosintéticos se sitúan verticalmente estratificadas en diferentes capas. Mientras que las algas eucariotas y las picocianobacterias se localizan en las capas óxicas, las bacterias púrpuras del azufre crecen en la interfase óxico-anóxica y por debajo de esta y las bacterias verdes del azufre se localizan en capas anóxicas más profundas. Esta amplia diversidad de microorganismos fotosintéticos implica una compleja mezcla de pigmentos fotosintéticos en los extractos, los cuales a menudo muestran espectros de absorción que se superponen. Hemos ensayado las ecuaciones espectrofotométricas con muestras que abarcan toda la variabilidad espacial y temporal del lago. Para la clorofila-*a *encontramos buenas correlaciones en las capas óxicas para la mayoría de las ecuaciones ensayadas. Sin embargo, la ecuación que mejor funciona en general para la determinación de la concentración de clorofila-a, independientemente del origen de la muestra, es la dada por Overmann & Tilzer, que está diseñada específicamente para mezclas de clorofila-*a *con bacterioclorofila-*d *de bacterias verdes del azufre. Las ecuaciones tricromáticas para la determinación de clorofila-*b *y* c *presentaron fuertes interferencias en aguas anóxicas, mientras que en las capas superiores del lago las concentraciones se encontraron normalmente por debajo del límite de detección, lo que restringía la posibilidad de uso de estas ecuaciones. Las ecuaciones de Takahashi & Ichimura (1970) para pigmentos bacterianos sobreestimaban tanto la concentración de bacterioclorofila-*a *como la* d*, aproximadamente en un 10 %, y subestimaban la concentración de bacterioclorofila-*c *casi en un 23 %; si bien para la determinación de bacterioclorofila-*d *la correlación fue mejor que la obtenida por las ecuaciones dicromáticas de Parkin & Brock y de Overmann & Tilzer. La abundancia de carotenos totales puede ser estimada por la ecuación de Strickland & Parsons (1972) diseñada para este propósito, sin embargo la precisión en los resultados difiere con la profundidad y se ve tremendamente sesgada por la presencia del caroteno bacteriano okenona. Por otro lado, las relaciones caroteno/clorofila-*a *basada en medidas a doble longitud de onda (430/665 y 480/665) solo mostraban resultados aceptables en el epilimnion, ya que en otras capas la aparición de carotenos bacterianos como la okenona produce interferencias significativas. Además, la gran variación en profundidad de la composición de carotenos socava la validez de estas relaciones fundamentadas en medidas a doble longitud de onda. En conclusión, nuestros resultados indican que el estudio de los pigmentos fotosintéticos completo y sin ambigüedades en lagos fuertemente estratificados y con solapamiento de sus poblaciones de organismos fototróficos requiere del uso de HPLC. Sin embargo, nuestros resultados también demuestran que las concentraciones de clorofila-*a *y bacterioclorofila-*a *y* d *pueden ser directamente medidas, tanto en profundidades óxicas como anóxicas, usando ecuaciones espectrofotométricas con un error inferior a un 10 %. Por el contrario, de acuerdo con nuestros resultados ni la concentración de clorofila-*b *y* c*, ni la de bacterioclorofila-*c *pueden ser estimadas con precisión por métodos espectrofotométricos en lagos estratificados.*

Palabras clave: *Lagos estratificados, pigmentos fotosintéticos, clorofilas, bacterioclorofilas, espectrofotometría, cromatografía líquida de alta eficacia (HPLC), fitoplancton, bacterias fotosintéticas.*

INTRODUCTION

Since the early days of research in limnology, the correct quantification of photosynthetic pigments has been a topic of great interest. For many years, spectrophotometric methods were the only methods available for pigment quantification and were an important concern in limnology. Despite the more recent appearance of chromatographic techniques (High Performance Liquid Chromatography-HPLC) for quantifying pigments, it seems advisable to continue using spectroscopic techniques and use chromatographic techniques to calibrate previously obtained results. Questions related to the best methods for quantifying pigments have been raised for a long time, primarily in seawater and in non-stratified systems (Murray *et al*., 1986; Salinas, 1988; Pinckney *et al*., 1994; Latasa *et al*., 1996; Mantoura *et al*., 1997; Dos Santos *et al*., 2003; Ritchie, 2008; Jodłowska & Latała, 2011). However, there is a lack of information for highly stratified systems with a complex mixture of photosynthetic organisms in the vertical profile and marked seasonal changes in those organisms' relative abundances.

Conventionally, limnological studies use chlorophyll-*a* concentration, the analysis of which is faster than cell counting procedures, to estimate phytoplankton biomass and/or primary productivity. Chlorophyll-*a* concentration is also a common and reliable indicator of water quality and trophic status used in management routines. The analytical differentiation of photosynthetic pigments is valuable for elucidating the composition of phytoplankton communities because some pigments are chemotaxonomic biomarkers (Wright & Jeffrey, 2006). In this context, chlorophyll-*a* is a universal marker of phytoplankton abundance, but chlorophyll-*b* and -*c* can be good indicators of the abundance of specific algal groups such as chlorophytes or bacillariophytes/chrysophytes, respectively. Accordingly, chlorophyll *b*/*a* and *c*/*a* ratios have been suggested as proxies of the relative dominance of these algal groups (MacLulich, 1986). In anoxic waters such as the anoxic hypolimnion of stratified lakes and reservoirs or the monimolimnion of meromictic lakes, it is also possible to assess the biomass and structure of the photosynthetic bacterial community through the quantification of the different bacteriochlorophylls. Furthermore, the total or relative concentration of carotenoids has long been used to investigate the physiological condition of phytoplankton populations, which may vary depending on light conditions (Jodłowska & Latała, 2011) or nutrient availability (Shimura & Fujita, 1975). In this context, the carotenoid-to-chlorophyll-*a* ratio has been proposed as a measure of the accumulation of fatty acids in freshwater microalgae, which co-occurs with nitrogen starvation or light stress (Solovchenko *et al.*, 2009). These simple relationships among pigments can thus be considered as tentative proxies of the physiological status of algal populations, with results that are more practical than those of more complex and expensive approaches.

The spectrophotometric methods for quantifying photosynthetic pigments in samples containing pigments from different sources are based on conventional visible absorbance measurements of organic extracts. In this case,

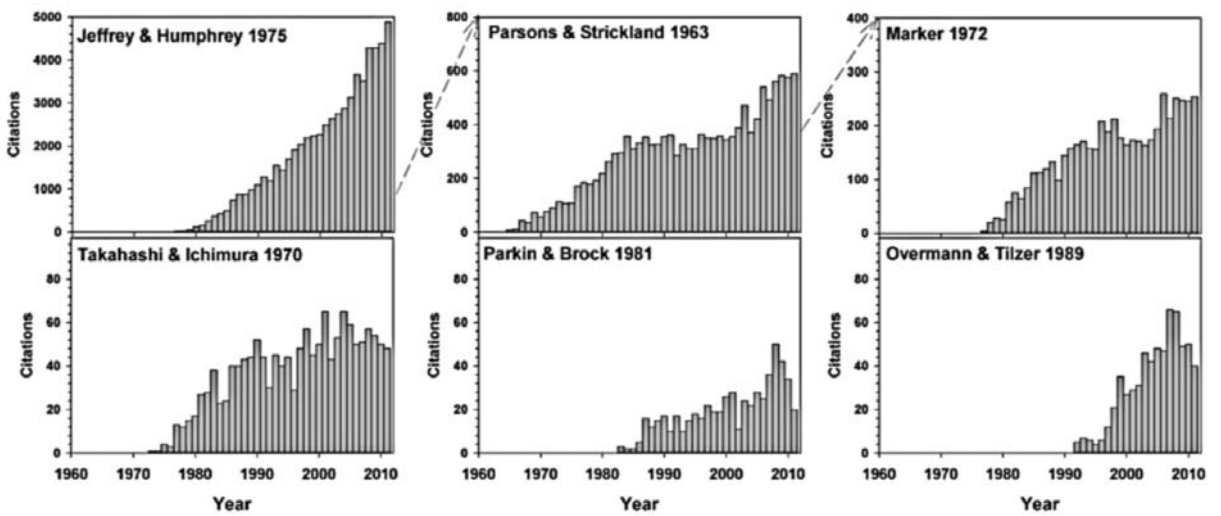

Figure 1. Summary of citations, by year of publication, of the most-used equations in the spectrophotometric determination of the photosynthetic pigments that were tested in our study (source: ISI -Thomson Reuters, Web of Knowledge). The equations proposed by Parsons & Strickland (1963) are most commonly cited as Strickland & Parsons (1972) from their Seawater Analysis Manual, and consequently, the number of citations in ISI-WOK under represents their usage. Note the differences in scale for the number of citations. *Informe de citas, por año de publicación, de los métodos de cálculo más utilizados en la determinación espectrofotométrica de pigmentos fotosintéticos y analizados en este estudio (fuente: ISI-Thomson Reuters, Web of Knowledge). Las ecuaciones de Parsons & Strickland (1963) se citan normalmente refiriendo el manual de análisis de aguas marinas de Strickland & Parsons (1972), por lo que los datos de la cita del artículo de 1963 obtenido del ISI-WOK estarían subestimando el uso real de estas ecuaciones. Nótese las diferentes escalas para el número de citaciones.*

pigments can be extracted from cells using different organic solvents such as acetone, methanol, ethanol, diethyl ether, dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF). Acetone is probably the most-used solvent (Ritchie, 2006), as it produces sharper absorption peaks compared to other solvents. On the other hand, it is more volatile and flammable than other solvents. Acetone is also considered to be less efficient at extracting pigments compared to other solvents (Ritchie, 2006); however, pigment recovery can be improved in different ways, such as by using sonication (Schagerl & Künzl, 2007).

The methods used for pigment quantification vary in accuracy and cost-effectiveness. Quantification by HPLC allows the discrimination of chlorophyll, its derivatives and carotenoids (Mantoura & Llewellyn, 1983). HPLC further improves the pigment detection limit in comparison to other methods, but also requires the use of expensive instruments and the need for certain technical capabilities for data processing. By contrast, the direct spectrophotometric quantification of samples containing a mixture of pigments is more affordable and widely used today (Fig. 1). A standardised HPLC protocol for photosynthetic pigment determination has already been compiled in the 21st edition of the APHA Standard Methods (2005). However, the management agencies responsible for controlling water quality, such as the US EPA in its Method 447.0 (Arar, 1997), still recommend spectrophotometric methods, for instance, that of Jeffrey & Humphrey (1975). The direct spectrophotometric quantification of a mixture of pigments requires the use of equations that utilise molar

Table 1. The most-used equations for the spectrophotometric determination of the photosynthetic pigments that were tested in this study. (v: acetone volume added for extraction, V: Total water volume filtered for pigment extraction). (TC: Total number of citations, ACI: Average Citations per Item, SPU: Specified plant pigments units). *Ecuaciones más usadas para la determinación espectrofotométrica de los pigmentos fotosintéticos testados en este estudio. (v: Volumen de acetona añadido para la extracción, V: Volumen total de agua filtrado para la extracción). (TC: Número total de citas, ACI: Media de citas por item, SPU: Unidades de pigmentos específicos).*

Monochromatic				TC	ACI
Marker 1972	Chl *a*	(μg l^{-1})	13.14*DO665*(v/V)	5359	33.08
Dichromatic (Chl *a*-Bchl *d*)					
Parkin & Brock 1981	chl *a*	(μg l^{-1})	11.9*DO663*((DO663/DO654*0.93)-0.33*(v/V)	593	21.18
	Bchl *d*	(μg l^{-1})	10.2*D654((DO663/DO654*(-0.99)+1.55)*(v/V)		
Overmann & Tilzer 1989	chl *a*	(μg l^{-1})	(1.315*DO663-0.643*DO651+0.005)*v*10^3/(V*d*84)	684	23.59
	Bchl *d* + *e*	(μg l^{-1})	(1.315*DO651-0.643*DO663+0.005)*v*10^3/(V*d*98)		
Trichromatic					
Strickland & Parsons 1972	Chl *a*	(μg l^{-1})	11.6*DO665-1.3*DO645-0.14*DO630*(v/V)	13441	34.91
	Chl *b*	(μg l^{-1})	20.7*DO645-4.34*DO665-4.42*DO630*(v/V)		
	Chl *c*	(μg l^{-1})	55*DO630-4.64*DO665-16.3*DO645*(v/V)		
Jeffrey & Humphrey 1975	Chl *a*	(μg l^{-1})	11.85*DO664-1.54*DO647-0.08*DO630*(v/V)	66647	29.53
	Chl *b*	(μg l^{-1})	-5.47*DO664+21.03*DO647-2.66*DO630*(v/V)		
	Chl *c*	(μg l^{-1})	-1.67*DO664-7.60*DO647+24.52*DO630*(v/V)		
Bacterioclorophylls					
Takahashi & Ichimura 1970	Bchl *a*	(μg l^{-1})	25.2*DO772*(v/V)	1489	21.58
	Bchl *c*	(μg l^{-1})	10.8*DO662*(v/V)		
	Bchl *d*	(μg l^{-1})	10.2*DO654*(v/V)		
	Bchl *e*	(μg l^{-1})	10.2*DO652*(v/V)		
Carotenoids					
Strickland & Parsons 1972	Carotenoids	(μSPU l^{-1})	4*DO480*(v/V)	—	—
Index 430/480	Car/chl-*a* ratio	—	DO430/DO665	—	—
Index 480/665	Car/chl-*a* ratio	—	DO480/DO665	—	—

extinction coefficients for each specific pigment (Table 1). These equations may use only one extinction coefficient (i.e., monochromatic) or include several coefficients to minimise the interference produced by overlapping absorption spectra (i.e., trichromatic). Spectrophotometric analyses may yield divergent results depending on which equation is used (Mantoura *et al*., 1997).

Stratified lakes and reservoirs presenting oxic-anoxic interfaces support plankton communities whose composition can notably differ in its content of photosynthetic pigments (Steenbergen & Korthals 1982). Furthermore, the spatial distributions of different groups of these diverse phototrophs usually overlap within the water column (Miracle *et al*., 1992, Camacho *et al*., 2001). In deeper water, anoxia and the characteristics of the light that penetrates to greater depths in these lakes allow the development of photosynthetic bacterial populations (Takahashi & Ichimura, 1970), which coexist with settled senescent epilimnetic phytoplankton and unicellular picocyanobacteria, forming deep chlorophyll maxima. All of these factors increase the pigment complexity of samples, thus intensifying the previously addressed impreciseness of spectrophotometric measures (Stal *et al*., 1984).

The strength and suitability of some spectrophotometric algorithms in stratified lakes are considered in this study by challenging the results obtained with spectrophotometric equations with reliable quantifications performed on chromatographically isolated pigments. The study was conducted with samples from meromictic Lake La Cruz, situated in central Spain, which was selected as a model lake because it presents the most possible combinations of overlapping microorganisms within its different vertical gradients. In addition to its permanent monimolimnion, this lake annually develops a strong thermal stratification and a relatively complex distribution of different photosynthetic microorganisms along its vertical profile. From the results of this analysis, we provide recommendations to be considered before undertaking limnological studies in this type of multi-layered

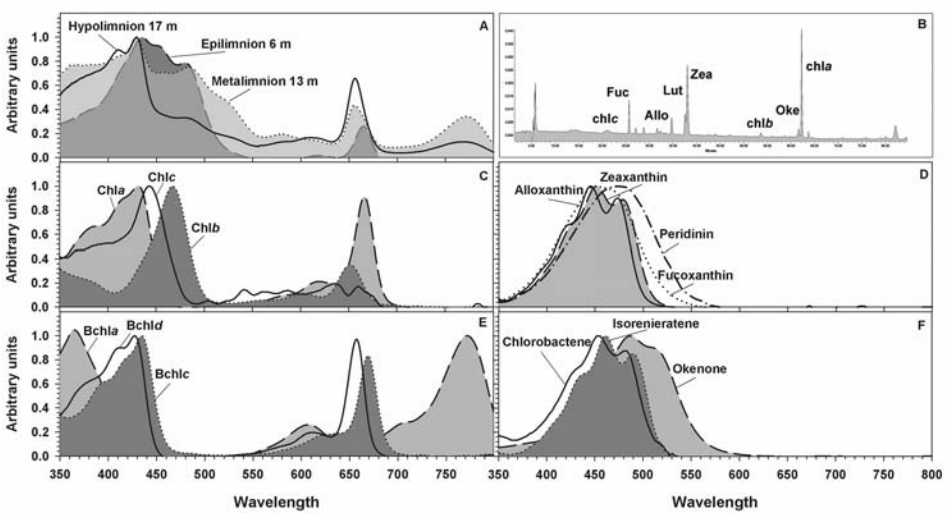

Figure 2. A) Spectrophotometric absorption spectra of the pigment extracts from samples collected from different depths (epilimnion, metalimnion and hypolimnion) of Lake La Cruz in June 2006. B) HPLC profile of a sample from the metalimnetic waters of Lake La Cruz collected in June 2006. C and D) Individual absorption spectra of the most abundant photosynthetic pigments in oxic waters of Lake La Cruz. E and F) Individual absorption spectra of the most abundant photosynthetic pigments in anoxic waters of Lake La Cruz. (All individual data spectra from HPLC profiles, samples collected June 2006). *A) Espectros de absorción espectrofotométricos de los pigmentos extraídos a diferentes profundidades (epilimnion, metalimnion e hipolimnion) en la Laguna de La Cruz, junio 2006. B) Perfiles por cromatografía líquida (HPLC) de una muestra de aguas metalimnéticas de la Laguna de La Cruz, junio 2006. C y D) Espectros de absorción individuales de los pigmentos fotosintéticos más abundantes en las aguas óxicas de la Laguna de La Cruz. E y F) Espectros de absorción individuales de los pigmentos fotosintéticos más abundantes en las aguas anóxicas de la Laguna de La Cruz. (Todos los datos de los espectros individuales provienen de los perfiles de HPLC, en muestras de junio de 2006).*

and complex freshwater environment, attempting to establish the particular trade-offs that can occur when using these equations. In this sense, our study aims to facilitate the selection of simple spectrophotometric methods in stratified water columns when the application of more expensive procedures such as HPLC is not suitable, primarily by considering that spectrophotometric methods are the most common means of obtaining certain key limnological data.

METHODS

Study site

Samples for pigment analysis were collected from Lake La Cruz, a meromictic karstic lake situated in a doline in Cañada del Hoyo (Cuenca, central Spain) (UTM 30 X596163 Y4427009, datum WGS84) with a surface area of 10,100 m^2 and a maximum depth of 21 meters. This lake exhibits a strong thermal stratification that develops each year between April and October (Camacho *et al*. 2003) and a permanently anoxic monimolimnion (Vicente & Miracle, 1988; Rodrigo *et al*., 2000, 2001). The water samples were obtained following the procedures described in Miracle *et al.* (1992). The samples used for the study correspond to 12 limnological surveys conducted between December 2005 and January 2007 and include a total of 235 samples obtained throughout the vertical profile, 144 and 91 from oxic and anoxic layers, respectively (Fig. 2).

Pigment extraction procedure

For pigment extraction, the seston was recovered by filtering water from each depth layer through GF/F filters (Whatman 47 mm). After that, the filters were placed on corning tubes containing 5 ml of 90 % acetone (modified from Strickland & Parsons 1972). To facilitate the extraction, samples were sonicated several times. Samples were later stored at −20 °C in the dark for at least 8 hours. After this, the tubes were centrifuged (3000× g for 10 min) and the pellet discarded. The supernatant was then filtered onto PTFE 0.2 μm filters (VWR) and processed within the following 3 hours. The spectrophotometric and HPLC determinations were made in parallel with two aliquots from the same extract.

Pigment determination by VIS-spectrophotometry

Spectrophotometric measurements were performed with 2 ml of filtered extract in 1 cm quartz cuvettes using a Beckman DU-7 UV-Visible spectrophotometer. The scan spectra from 350 to 900 nm, with a resolution of 0.5 nm, were digitally recorded and processed. The concentrations of different photosynthetic pigments were obtained using the algorithms compiled in Table 1.

Pigment determination via high-performance liquid chromatography (HPLC)

To analyse pigment composition using HPLC, a 150 μl aliquot of each extract was injected into a Waters HPLC system with a Waters 996 photodiode Array Detector. To improve the separation of pigments, before injection, the aliquots were mixed with a volume of ammonium acetate (ion pairing agent) to reach a final concentration of 0.1 mM. The system was equipped with two columns (Spherisorb S5 ODS2) working in series and running for 95 min at 35 °C in a methanol/ammonium acetate/acetone gradient modified from Pinckney *et al*. 1996 to improve separation (see gradient in Table 2). Eluted pigments were detected by a photodiode array detector at a range of absorbance of 380–800 nm. The peak identities were determined by comparing the retention times and spectra with either pure standards purchased from DHI® or chromatograms acquired from pure algal or sulphur bacterial cultures. The pigment concentrations were obtained from the curves made with the standards by integrating the area under the cross-section at the wavelength of maximum absorption of each pigment. In all cases, linear correlations were measured with the intercept forced to zero. For some algae carotenoids and all bacterial pigments, concentrations were calculated us-

Table 2. Solvent gradient applied for HPLC measurements (after Pinckney *et al*., 1996). *Gradientes de los diferentes solventes aplicados en las medidas por HPLC (según Pinckney* et al.*, 1996).*

Time (min)	Flow (ml min^{-1})	% Methanol	% Ammonium acetate (0.1 M)	% Acetone
0	0.8	80	20	0
5	0.8	80	10	10
45	1.25	80	5	15
50	1.5	80	0	20
65	0.8	80	0	20
67	0.8	80	20	0
95	0.8	80	20	0

ing specific extinction coefficients (Foppen, 1971 & Jeffrey *et al*., 1997).

Data analyses

For each studied pigment, the Pearson correlation coefficient was used to evaluate the agreement between measures performed by spectrophotometry and HPLC. Student t-tests were performed to determine whether the Pearson correlation coefficients were statistically significant, establishing a confidence value of $p \leq 0.05$. The slopes of the correlations indicated whether the equations underestimated or overestimated pigment concen-

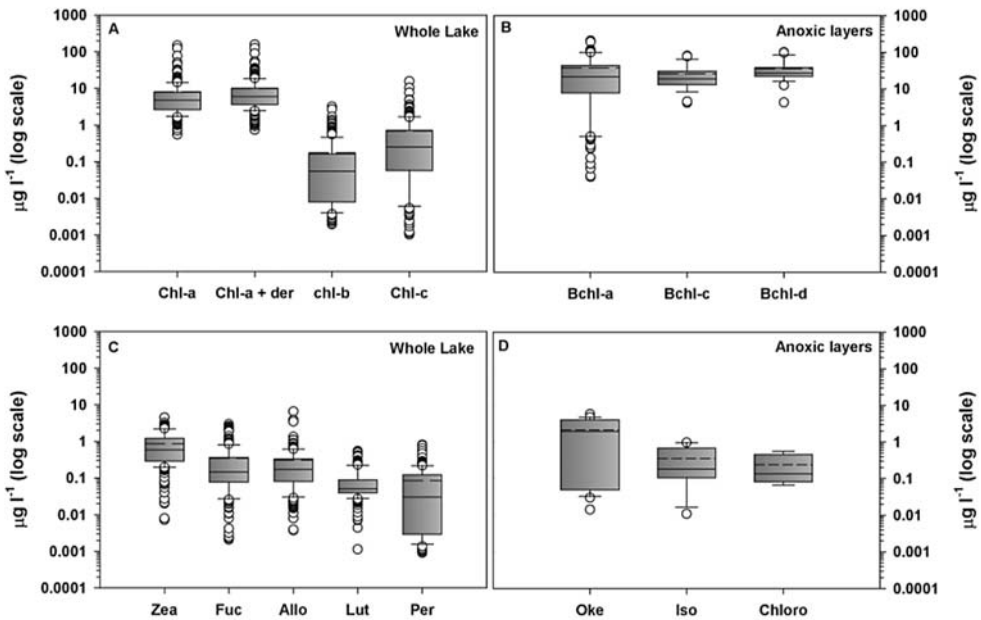

Figure 3. Box-plot distribution (log of concentration, µg l^{-1})) of the most abundant photosynthetic pigments found in Lake La Cruz. The statistical parameters of the raw data set used in this study are shown. A) Chlorophyll-*a*, plus derivatives, *b* and *c* in all of the samples. B) Bacteriochlorophyll-*a*, *c* and *d* in the samples from anoxic waters. C) Primary algae or cyanobacterial carotenoids in all of the samples (Zea: Zeaxanthin, Fuc: Fucoxanthin, Alo: Alloxanthin, Lut: Lutein, Per: Peridinin). D) Primary bacterial carotenoids in samples from anoxic waters (Oke: Okenone, Iso: Isorenieratene, Chloro: Chlorobactene). *Diagrama de cajas de la distribución (logaritmo de la concentración (µg l^{-1})) de los pigmentos fotosintéticos más abundantes encontrados en la laguna de La Cruz. Se muestran los datos estadísticos en bruto usados en el presente estudio. A) Clorofila-*a *y derivados,* b *y* c *en todas las muestras B) Bacterioclorofilas-*a, c *y* d *en muestras de aguas anóxicas. C) Principales carotenos de algas y cianobacterias en todas las muestras (Zea: Zeaxantina, Fuc: Fucoxantina, Alo: Alloxantina, Lut: Luteina, Per: Peridinina). D) Principales carotenos bacterianos en muestras de aguas anóxicas (Oke: Okenona, Iso: Isorrenierateno, Chloro: Clorobacteno).*

trations. All of the data correlations and statistical analyses were performed using University of Valencia-licensed SPSS 17.0 software.

RESULTS

During the studied period, the concentrations of all of the analysed photosynthetic pigments showed high variability (Fig. 3). In the entire water column, the concentration of chlorophyll-*a* varied from 0.5 to 85 $\mu g \cdot l^{-1}$ and its derivatives (primarily chlorophyllide and pheophytin) represented between 3 and 60 % of the total chlorophyll-*a* concentration, with a high degree of variability within the vertical profile and over time. We observed pigment concentrations below the detection limit of the spectrophotometric method (i.e., 0.5 $\mu g \cdot l^{-1}$) in 89 % of the samples for chlorophyll-*b* and in 61 % for chlorophyll-*c*. The highest measured concentrations of chlorophyll-*b* and -*c* were 3.2 and 15.7 $\mu g \cdot l^{-1}$, respectively. In the anoxic layers, bacteriochlorophylls also varied widely, with an average of 20 $\mu g \cdot l^{-1}$ and maximum values of approximately 200 $\mu g \cdot l^{-1}$ in a narrow layer of the lake just around the deep chlorophyll maximum (DCM). Figure 3C presents the most abundant taxon-specific carotenoids observed in the lake. More than 15 different carotenoids were recovered in the chromatographic analysis for Lake La Cruz. The more relevant carotenoids detected from chlorophytes were zeaxanthin, lutein, violaxanthin, and antheroxanthin. Other relevant carotenoids were zeaxanthin and myxoxantophyll in cyanobacteria, alloxanthin from cryptophytes and fucoxanthin and diatoxanthin from bacillariophytes. Other ubiquitous carotenoids, such as β-carotene, were also regularly observed in samples. Among bacterial carotenoids, okenone was the most abundant; this carotenoid is characteristic of some purple sulphur bacteria (PSB), with *Lamprocystis purpurea* being the dominant species in Lake La Cruz. Also observed in the samples were the signature carotenoids of green sulphur bacteria (GSB), isorenieratene and chlorobactene.

The relationships between the chlorophyll-*a* concentrations measured using HPLC and the estimates made with different spectrophotometric equations are shown in figure 4 and Table 3. The HPLC data are expressed as either the total amount of native chlorophyll-*a* (Fig. 4, plots on left column) or the sum of the native form and derivatives (plots on right column), which were primarily chlorophyllide-*a* and pheophytin-*a*, although small amounts of pheophorbides were also present and taken into account. There were important differences in the responses of the equations studied in relation to the depth of the lake, which implies an important component of spatial variability in the water column that yields different accuracies depending on whether samples are from oxic or anoxic water.

The correlations between the spectrophotometric determinations of chlorophyll-*a* (native plus derivate forms) and the HPLC measurements (Table 3), made for all data in the vertical profile (oxic and anoxic samples), showed that the overall monochromatic and trichromatic equations (Marker, 1972; Strickland & Parsons 1972; Jeffrey & Humphrey 1975), although significantly correlated, had low Pearson values and high errors (approximately 25 %) with slopes that strongly underestimate the concentration of chlorophyll-*a* (with respect to HPLC) when all samples were jointly considered. The spectrophotometric equations with a correction for bacteriochlorophyll (Parkin & Brock, 1981 and Overmann & Tilzer, 1989) showed better correlations (higher Pearson values). However, the equation of Parkin & Brock (1981) had a similar % error than the monochromatic and trichromatic equations but, in this case, a high overestimation of chlorophyll-*a* concentration (slope 1.235). Overall, the equation whose predictions best fit all of the data was that given by Overmann & Tilzer (1989), showing a low error (1.9 %) and no considerable deviation of the estimated chlorophyll-*a* concentration (slope 1.019).

For oxic samples, all equations overestimated the spectrophotometric chlorophyll-*a* concentration with respect to the native chlorophyll-*a* values (Figs. 4 A, C, E, G, I) measured by HPLC. However, when derivative forms are also considered, as all equations do, monochromatic and trichromatic equations (Marker, 1972;

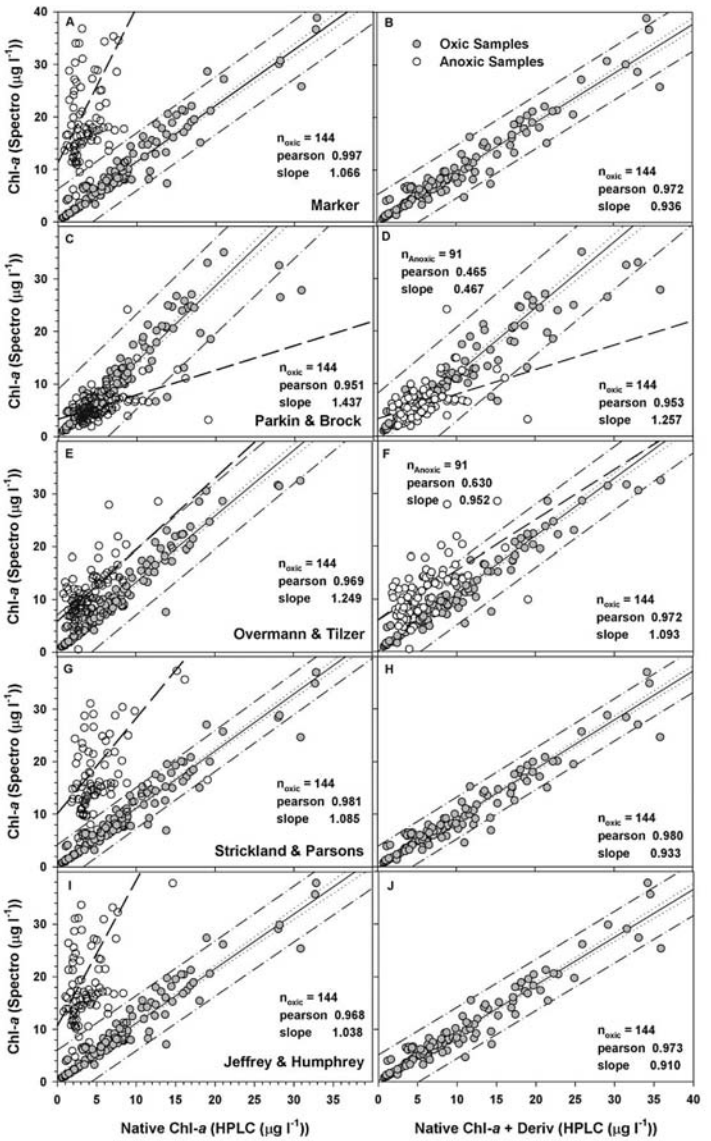

Figure 4. Relationship between the chlorophyll-*a* concentrations (µg l^{-1}) obtained using different spectrophotometric equations and through HPLC. In the left column (A, C, E, G, and I), correlations are shown between the concentrations obtained by the spectrophotometric equations and the concentrations of native chlorophyll-*a* determined by HPLC (including allomer and epimer) for both oxic and anoxic samples. The right column (B, D, F, H, and J) plots the correlations between the concentrations obtained by spectrophotometric equations and the total chlorophyll-*a* concentrations determined by HPLC (native plus derivate pigments, chlorophyllide-*a*, pheophytin-*a* and pheophorbide-*a*). Anoxic samples have been excluded for equations that do not include a correction for bacteriochlorophylls and have been maintained only for the equations of Parkin & Brock (1981) and Overmann & Tilzer (1989). (Solid line: correlation of oxic samples; dotted line: confidence intervals; dash-dot line: prediction intervals; dash line: correlation of anoxic samples). *Relación entre las concentraciones de clorofila-*a *(µg l^{-1}) obtenidas mediante las diferentes ecuaciones espectrofotométricas estudiadas respecto a las obtenidas por HPLC. En la columna de la izquierda (A, C, E, G, e I) se muestran las correlaciones entre las concentraciones estimadas por las ecuaciones espectrofotométricas y la concentración de clorofila-a nativa medida por HPLC (sumando su alomero y epimero). En la columna derecha (B, D, F, H, y J) se muestran las correlaciones entre las concentraciones estimadas por ecuaciones espectrofotométricas y la concentración total de clorofila-a medida por HPLC (sumando a la nativa los pigmentos derivados como clorofilida-*a, *feofitina-*a *y feoforbido-*a*). Las muestras anóxicas han sido excluidas para las ecuaciones sin corrección para bacterioclorofilas y mantenidas solo para Parkin & Brock (1981) y Overmann & Tilzer (1989) (Línea continua: Correlación de las muestras óxicas. Línea de puntos: intervalos de confianza. Línea de rayas y puntos: intervalo de predicción. Línea de rayas: correlación de las muestras de anoxia).*

Table 3. Summary of the statistical data (Pearson's correlation coefficient, slope and % error) on the correlations between the different spectrophotometric equations tested and the HPLC results for all samples as well as for oxic and anoxic layers separately. The equations resulting in the lowest disagreement (lowest % error) with the HPLC measurements are marked in bold and are thus the most recommendable for the respective case. The % error indicates the difference between the values calculated with the spectrophotometric formulae and the HPLC measurements, where negative errors represent an underestimation of values by the spectrophotometric determinations compared to the HPLC values, whereas positive errors indicate overestimation. Correlations refer to all forms of each pigment (e.g., chlorophyll-*a* plus derivative concentrations for chlorophyll-*a*). *Resumen de los datos estadísticos (coeficiente de correlación de Pearson, pendiente y % de error) de las correlaciones entre las diferentes ecuaciones espectrofotométricas probadas y las determinaciones por HPLC para todas las muestras, asi como para las muestras óxicas y anóxicas por separado. Se muestran en negrita, para la aplicación de distintas ecuaciones, aquellas que presentan una menor discrepancia con la determinación por HPLC (% error), siendo en consecuencia las más recomendables para el caso particular. El % de error toma valores negativos cuando se produce una subestimación por parte de las determinaciones espectrofotométricas en comparación con las medidas por HPLC, mientras que toma valores positivos cuando se da una sobreestimación. Las correlaciones se refieren a todas las formas de cada* pigmento (p.ej. *concentraciones de clorofila*-a y *sus derivados en el caso de la clorofila*-a).

			Marker 1972			Jeffrey & Humphrey 1975			Strickland & Parsons 1972			Parkin & Brock 1981			Overmann & Tilzer 1989			Takahashi & Ichimura 1970		
Layer	*n*	Pigment	pearson	slope	% error	pearson	slope	% error	pearson	slope	% error	pearson	slope	% error	pearson	slope	% error	pearson	slope	% error
Total	235	chl a	0.462	0.736	−26.4	0.486	0.724	−27.6	0.516	0.754	−24.6	0.946	1.235	23.5	0.924	1.019	**1.9**			
	235	chl b				−0.217	−18.4	−1935.4	−0.214	−12.9	−1391.0									
	235	chl c				0.081	0.019	−98.1	0.081	0.043	−95.7									
	118	Carotenoids							0.243	1.492	49.2									
Oxic	144	chl a	0.972	0.936	−6.4	0.973	0.910	−9.0	0.980	0.933	−6.7	0.954	1.257	25.7	0.972	0.944	**–5.6**			
	144	chl b				0.324	0.875	**–12.5**	0.377	0.632	−36.8									
	144	chl c				0.655	0.362	−63.8	0.689	0.834	**–16.6**									
	93	Carotenoids							0.919	1.620	62.0									
Anoxic	91	chl a	0.447	2.963	196.3	0.455	2.772	177.2	0.459	2.557	155.7	0.465	0.467	−53.3	0.630	0.952	**–4.8**			
	91	chl b				—	—	—	—	—	—									
	91	chl c				—	—	—-	—	—	—									
	91	bchl a																0.987	1.090	**9.0**
	25	bchl d										0.985	1.124	12.4	0.949	0.440	−56.0	0.983	1.102	**10.2**
	25	bchl c																0.979	0.774	**–22.6**
	25	Carotenoids							0.970	3.224	222.4									

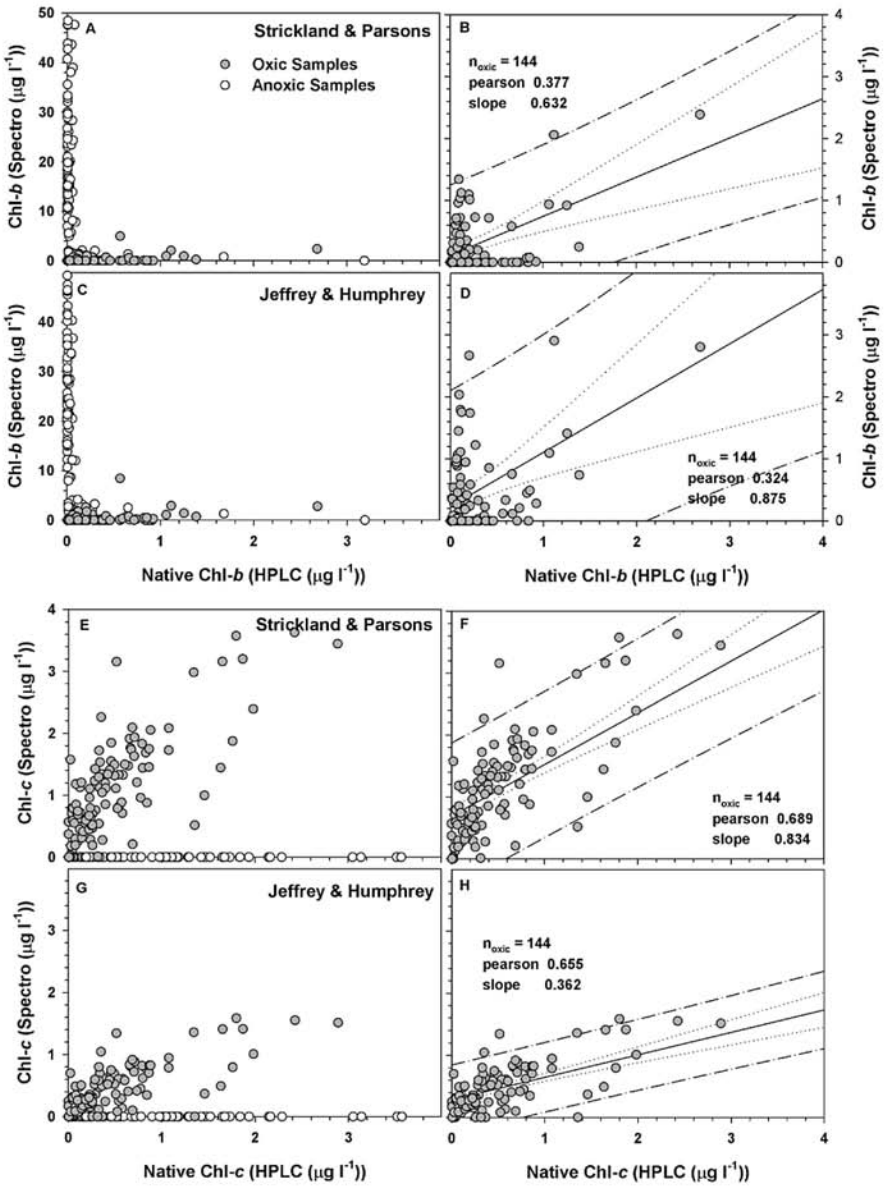

Figure 5. A to D) Relationships between the chlorophyll-*b* concentrations (μg l^{-1}) obtained using the spectrophotometric equations proposed by Strickland & Parsons (A and B) and by Jeffrey & Humphrey (C and D) and the concentrations measured using HPLC. E-H) Relationships between the chlorophyll-*c* concentrations (μg l^{-1}) obtained using the equations of Strickland & Parsons (E and F) and Jeffrey & Humphrey (G and H) and the concentrations measured using HPLC. Note the change of scale in chlorophyll-*b* plots A-B compared to C-D. (Solid line: correlation of oxic samples; dotted line: confidence intervals; dash-dot line: prediction intervals). No correlation was found when all samples (oxic and anoxic) were included (left plots). The right plots include only oxic samples. *A-D) Relación entre las concentraciones (μg l^{-1}) de clorofila-*b *obtenidas mediante las ecuaciones espectrofotométricas de Strickland & Parsons (A y B) y Jeffrey & Humphrey (C y D) respecto a las obtenidas por HPLC. E-H) Relación entre las concentraciones (μg l^{-1}) de clorofila-c obtenidas mediante las ecuaciones espectrofotométricas de Strickland & Parsons (E y F) y Jeffrey & Humphrey (G y H) respecto a las obtenidas por HPLC. Nótese el cambio de escala para las figuras de la clorofila-b, A-B, respecto a las C-D, (Línea continua: Correlación de las muestras óxicas. Línea de puntos: intervalos de confianza. Línea de rayas y puntos: intervalo de predicción). Cuando se incluyeron todas las muestras (óxicas y anóxicas, gráficas de la izquierda) no se encontró ninguna correlación, en las gráficas de la derecha se incluyen solo las muestras óxicas.*

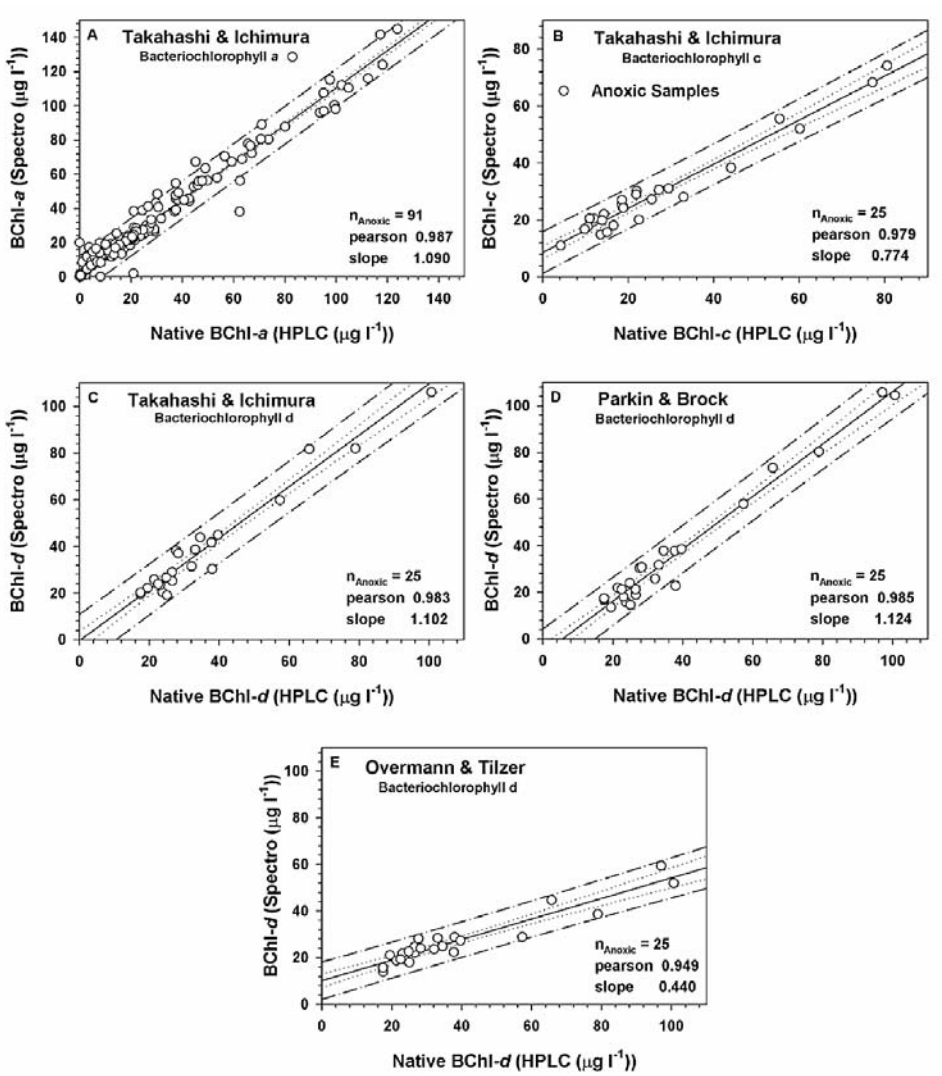

Figure 6. Plots include only the anoxic samples. A) Relationship between the bacteriochlorophyll-*a* concentrations (μg l^{-1}) obtained by Takahashi & Ichimura's spectrophotometric equation and those obtained by HPLC. B) Relationship between the bacteriochlorophyll-*c* concentrations (μg l^{-1}) obtained by Takahashi & Ichimura's spectrophotometric equation and those obtained by HPLC. C-D-E) Relationship between the bacteriochlorophyll-*d* concentrations (μg l^{-1}) obtained using the spectrophotometric equations of Takahashi & Ichimura (C) Parkin & Brock (D) and Overmann & Tilzer (E) and those obtained using HPLC. (Solid line: correlation of anoxic samples; dotted line: confidence intervals; dash-dot line: prediction intervals). *Incluye solo muestras anóxicas. A) Relación entre las concentraciones de bacterioclorofila-*a *(μg l^{-1}) obtenidas mediante la ecuación espectrofotométrica de Takahashi & Ichimura respecto a las obtenidas por HPLC. B) Relación entre las concentraciones de bacterioclorofila-*c *(μg l^{-1}) obtenidas mediante la ecuación espectrofotométrica de Takahashi & Ichimura respecto a las obtenidas por HPLC. C-D-E) Relación entre las concentraciones de bacterioclorofila-*d *(μg l^{-1}) obtenida mediante las ecuaciones espectrofotométricas de Takahashi & Ichimura (C) Parkin & Brock (D) y Overmann & Tilzer (E) respecto a las obtenidas por HPLC. (Línea continua: Correlación de las muestras anóxicas. Línea de puntos: intervalos de confianza; línea de rayas y puntos: intervalo de predicción).*

Strickland & Parsons 1972; Jeffrey & Humphrey 1975) underestimated chlorophyll-*a* (plus derivatives) concentrations (Figs. 4 B, H, J). Because spectrophotometric equations include the determination of all forms (native plus derivatives), Table 3, which synthesises the results of the application of the different equations, was made by considering the correlations of spec-

trophotometric determinations with the HPLC concentrations of native plus derivate forms of chlorophyll-*a*. Concerning the equations that correct chlorophyll-*a* concentration for the presence of bacteriochlorophyll-*d* (*d* + *e*), that of Parkin & Brock (1981) exhibited a high error (25.7 %), overestimating chlorophyll-*a*. Although for oxic samples all but Parkin & Brock's equation for chlorophyll-*a* produced relatively low errors (under 10 %), the best values were again provided by the Overmann & Tilzer (1989) equation, with an error of 5.6 %, whereas the most-used Jeffrey & Humphrey (1975) equation produced an error of 9.0 %.

Concerning the anoxic samples, no significant correlation was found for the monochromatic and trichromatic equations that did not include a bacteriochlorophyll-*d* correction for chlorophyll-*a* concentration (dashed line in Figs. 4 A, C, E, G, I), which resulted in large errors (Table 3). Those equations are thus unsuitable for determining chlorophyll-*a* in anoxic waters presenting green photosynthetic bacteria. For the equations with a bacteriochlorophyll-*d* correction (Figs. 4 D, F), that of Parkin & Brock (1981) exhibited a low Pearson correlation (r = 0.465), low slope (0.467), and high error (53 %), with a strong underestimation with respect to HPLC values. Again, Overmann & Tilzer's (1989) equation showed the best correlation (r = 0.630), with a good slope (0.952) and low error (4.8 %).

Figure 5 presents the relationship between the two analytical methods, but in this case for the measurement of chlorophyll-*b* and -*c*, for which only the trichromatic equations of Strickland & Parsons (1972) and Jeffrey & Humphrey (1975) had previously been proposed. As with chlorophyll-*a*, the trichromatic equations were inadequate in predicting the concentrations of these pigments in the anoxic layers, where chlorophyll-*b* and -*c* likely represent settling algae. When all samples were jointly considered (Fig. 5, plots on left, Table 3), there was again no correlation between the spectrophotometric and

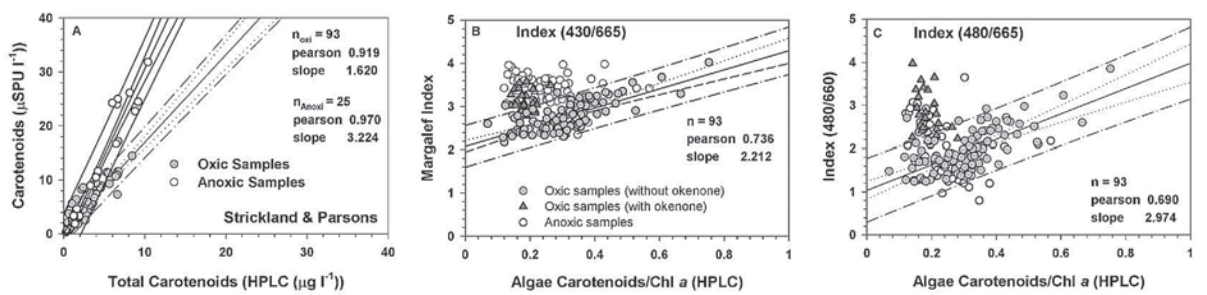

Figure 7. Relationships between the different carotenoid indices obtained by spectrophotometric formulae (relative or absorbance units) and the carotenoid concentrations (µg l^{-1}) measured by HPLC. A) Correlation between the Strickland & Parsons (1972) estimation of carotenoid abundance and the total carotenoid concentration (µg l^{-1}) measured by HPLC for oxic and anoxic samples. B) Correlation, for oxic samples not presenting okenone, between the absorbance 430/665 index and the ratio of total HPLC-measured algal carotenoid concentration to total (native plus derivatives) chlorophyll-a concentration. C) Correlation, for oxic samples not presenting okenone, between the absorbance index 480/665 and the ratio of total HPLC-measured algal carotenoid concentration to total chlorophyll-a concentration (solid lines in plot A correspond to either the oxic and anoxic samples, whereas in plots B and C they correspond only to oxic samples not presenting okenone. Dotted line: confidence intervals; dash-dot line: prediction intervals). *Relación entre los diferentes índices de carotenos obtenidos por espectrofotometría (unidades relativas o de absorbancia) y las concentraciones de carotenos (µg l^{-1}) medidas por HPLC. A) Correlación entre la estimación de la abundancia de carotenos estimada mediante la expresión de Strickland & Parsons (1972) y la concentración total de carotenos (µg l^{-1}) medida por HPLC para las muestras óxicas y anóxicas. B) Correlación, para las muestras óxicas que no presentan okenona, entre el índice de absorbancia 430/665 y el ratio entre las concentraciones de carotenos algales respecto a las concentraciones de clorofila-a total (nativa más derivados) medidas por HPLC. C) Correlación, para las muestras óxicas que no presentan okenona, entre el índice de absorbancia 480/665 y el ratio entre las concentraciones de carotenos algales respecto a las concentraciones de clorofila-a total medidas por HPLC. (Línea continua: en el gráfico A corresponde a las muestras óxicas y, por separado, a las muestras anóxicas, mientras que en los gráficos B y C corresponde solo a las muestras óxicas que no presentan okenona, Línea de puntos: intervalos de confianza; línea de rayas y puntos: intervalo de predicción).*

HPLC measures. Even when considering only the oxic samples (Fig. 5, plots on the right, and Table 3), the accuracy of the spectrophotometric determinations was very low, as the concentrations of these pigments in the lake were also low.

Concerning bacteriochlorophylls, (Fig. 6), Takahashi & Ichimura (1970) provided an equation for the calculation of bacteriochlorophyll-*a* that fits well with HPLC determinations, showing a high correlation coefficient. This correlation is facilitated by the higher number of samples for the analysis compared to the lower number for other bacteriochlorophylls. The equations of Takahasi & Ichimura (1970), which are the most extensively used to estimate bacterial pigments, slightly overestimated both bacteriochlorophyll−*a* and bacteriochlorophyll−*d* by approximately 10 %, whereas bacteriochlorophyll−*c* was underestimated by approximately 23 %. A lower accuracy was observed for the calculation of bacteriochlorophyll−*d* by means of the Parkin & Brock (1981) equation, which overestimated concentrations by 12 %. By contrast, the equation of Overmann & Tilzer (1989) greatly underestimated bacteriochlorophyll−*d* concentrations by more than 50 %.

The comparison between carotenoid quantifications performed on non-purified extracts using the Strickland & Parsons (1972) equations and through HPLC determinations are shown in figure 7. Both direct quantifications and absorbance ratios were also sensitive to the samples' origin. Although a statistically significant correlation was observed when comparing the total carotenoids measured with the equation of Strickland & Parsons (1972) with the measures obtained by HPLC, the different slopes of 1.62 for oxic layers and 3.22 for anoxic layers indicate that the response of Strickland & Parsons' equations is different when bacterial carotenoids are involved. Thus, these equations are not suitable to be used indistinctly for oxic and anoxic samples. On the other hand, the two dual-wavelength absorbance ratios (i.e., 430/665 and 480/665) (Margalef, 1983) exhibited moderately good correlations for only the epilimnetic samples, whereas the metalimnetic and hypolimnetic samples (that include the bacterial carotenoid okenone) did not show any significant relationships with the carotenoid/chlorophyll-*a* ratios estimated from the HPLC measures.

DISCUSSION

Although HPLC analyses provide higher precision and improve pigment characterisation compared to routine spectrophotometric methods (Wright & Jeffrey, 2006), the former are also more expensive and time-consuming. Thus, for many applications, direct spectrophotometric measures may offer a high enough level of precision to obtain optimum results. Our examination of cited works in the Thomson Reuters (formerly ISI) Web of Knowledge reveals that at present, spectrophotometric equations are extensively used (Fig. 1). The number of citations is continuously increasing for some of these equations, such as those proposed by Jeffrey & Humphrey (1975) and Strickland & Parsons (1972) in the mid-1970s. This preference for spectrophotometric methods is evident because routine spectrophotometric procedures avoid cost and time compared to HPLC, but the inherent inaccuracies of the method require caution when applying the different formulae and when interpreting the results, particularly when the study involves stratified water columns. Several studies, including ours, demonstrate that the validity of some equations depends on the origin of the samples and that the presence or absence of certain pigment forms may cause the accuracy of the determination to vary. For example, the absorption characteristics of chlorophyll-*a* derivatives introduce some bias into the quantification of native chlorophyll-*a* (Salinas, 1988; Rowan 1989; Jacobsen & Rai, 1990; Pinckney *et al.*, 1994; Ritchie 2008). To explore this issue, we compared the linear correlations of quantifications obtained by both considering and not considering these chlorophyll-*a* derivatives. When chlorophyll-*a* derivatives were excluded from the HPLC addition of pigment forms (Fig. 4, left plots), all equations overestimated the native chlorophyll-*a* concentrations due to

the presence of these degradation derivatives, although the overestimates were generally less than 10 %. However, upon the consideration of derivative forms in the HPLC measurements, all equations other than dichromatic ones (those of Parkin & Brock, 1981, and Overmann & Tilzer, 1989) underestimated the HPLC measurements. It has previously been suggested that the use of spectrophotometric methods can be acceptable if degraded forms represent less than 5 % of total chlorophyll-*a* (Jodłowska & Latała, 2011). The error of our estimates was commonly less than 10 % (Fig. 4), which could be explained by the presence of these derivatives, except for the case of the Parkin & Brock's equation that consistently and greatly overestimates chlorophyll-*a* concentrations.

There are also spectrophotometric protocols, not tested in this study, that allow the correction of chlorophyll-*a* concentrations with respect to their degraded compounds through a previous acidification step (Lorenzen, 1967). By means of this acidification, native chlorophyll-*a* is transformed into phaeophytin-*a*. Chlorophyllide-*a* is the result of the loss of the phytol chain and is a common degraded form of chlorophyll-*a* whose occurrence has been associated with cell senescence due to the enzymatic activity of chlorophyllases (Louda *et al*., 1998, 2002) and/or disruption produced by predation (Barlow *et al*., 1988). In Lake La Cruz, high concentrations of this pigment are usually associated with the metalimnetic deep chlorophyll maximum, which invalidates the use of such methods for the studied lake, as the presence of high quantities of chlorophyllide-*a* in the extracts detracts from the validity of the results even after acidification (Plante-Cuny *et al.*, 1993). For that reason, we did not evaluate such methods.

In highly stratified lakes, additional interference in the quantification of chlorophyll-*a* is generated by spectral overlap with bacteriochlorophylls. These pigments occur primarily in the anoxic and microaerobic layers, where photosynthetic sulphur bacteria thrive. This would explain why neither the monochromatic (Marker, 1972) nor the trichromatic (Strickland & Parsons, 1972; Jeffrey & Humphrey, 1975) equations that we tested produced good estimates of chlorophyll-*a* for the anoxic layers of the lake. In the early 1980s, Parkin & Brock (1981) proposed a dichromatic equation to estimate chlorophyll-*a* concentrations in the presence of bacteriochlorophyll-*d*, which is the main source of interference for chlorophyll-*a* concentration estimations. Since then, this equation has been widely used in these types of lakes. The effectiveness of this equation was later challenged by Overmann & Tilzer (1989), who proposed an alternative algorithm. In our study, the equation of Parkin & Brock (1981) did not correctly approximate chlorophyll-*a* concentrations when bacteriochlorophyll-*d* was present, and we do not recommend its use in such cases. The equation of Overmann & Tilzer (1989), however, offered the best results in measuring chlorophyll-*a* concentrations regardless of the sample's origin (oxic or anoxic water), and it was the only equation that produced good estimations for anoxic samples. In contrast, for the oxic samples, no equations except that of Parkin & Brock (1981) deviated notably from the linear distribution, thus indicating a high measurement accuracy of these equations compared to the results of HPLC and making any of these equations suitable for the determination of chlorophyll-*a* in oxic waters.

Trichromatic equations are widely used in spectrophotometric procedures for the determination of chlorophyll-*b* and -*c* (Jeffrey *et al.*, 1997), which provide chemotaxonomic information concerning phytoplankton community composition. The two most-used equations were defined by Parsons & Strickland (1963) (usually reported by Strickland & Parsons, 1972 and later almost identically assumed by SCOR-UNESCO, 1966) and by Jeffrey & Humphrey (1975). The equation of Jeffrey & Humphrey (1975) is used even more often, most likely because it is the equation recommended by the US EPA. However, a critical aspect of these trichromatic equations is that they are not accurate in estimating pigments at low concentrations. Unicellular picocyanobacteria and photosynthetic bacteria dominate the phytoplankton community of the meromictic Lake La Cruz (Rodrigo *et al*., 2001; Camacho *et al*., 2003). By contrast, algal groups

containing chlorophyll-*b* (i.e., chlorophytes) or chlorophyll-*c* (i.e., bacillariophytes and chrysophytes) regularly exhibit lower abundances in the studied lake, which implies that the possible use of these equations is complicated by these pigment concentrations being near or below the equations' resolution limit. This appears to particularly affect the estimation of chlorophyll-*b*, which in our case produced the worst statistical results, contrasting with Ritchie's (2008) observations of a lower accuracy of these equations for the calculation of chlorophyll-*c* compared to chlorophyll-*b*. Moreover, in lake layers where green photosynthetic bacteria occur, such as the metalimnion and hypolimnion of Lake La Cruz, bacteriochlorophylls are likely producing this bias because of the spectra overlap, which can be observed in figure 2. In our case, chlorophyll-*b* estimation is more sensitive to this bias compared to chlorophyll-*c*.

To assess bacteriochlorophyll (*a*, *c* and *d*) concentrations, we applied the equations of Takahashi & Ichimura (1970), which are the most commonly utilised in freshwater ecosystems. We also assessed the dichromatic equations of Parkin & Brock (1981) and Overmann & Tilzer (1989), which were designed for the calculation of chlorophyll-*a* concentration when bacteriochlorophyll-*d* is present and also allow for the estimation of the concentrations of bacteriochlorophyll-*d* and -*e*. Bacteriochlorophyll-*e* was not present in samples from Lake La Cruz, as confirmed by the HPLC analysis; under these circumstances, we assume that the equation of Overmann & Tilzer (1989) could be used to measure the concentration of bacteriochlorophyll-*d*. During the mixing period, photosynthetic sulphur bacteria in Lake La Cruz might incidentally occur in oxic layers due to the lack of control of buoyancy and the formation of aggregates that can remain for a long time in the mixolimnion; however, such bacteria naturally develop in the anoxic monimolimnion and hypolimnion when sufficient light reaches these sulphide-containing depths (Casamayor *et al.*, 2011). It is for this reason that correlations were only explored for the anoxic layers of the lake (Fig. 6). In contrast with chlorophyll-*a*, the derivatives of these bacterial pigments are present only in very low amounts, making any correction unnecessary. Bacteriochlorophyll-*a* is found in purple sulphur bacteria (PSB). In Lake La Cruz, *Lamprocystis purpurea* dominates among other PSB species (Casamayor *et al.*, 2011). The correlations obtained for this pigment between the equations of Takahashi & Ichimura (1970) and the HPLC determinations are in our case statistically satisfactory, indicating an underestimation of the bacteriochlorophyll-*a* concentration by only 8 %. Concerning bacteriochlorophyll-*c* and -*d*, which occur in green sulphur bacteria (GSB), among which *Chlorobium clathratiforme* is abundant in Lake La Cruz, the results obtained with the equations of Takahashi & Ichimura (1970) show a better fit for bacteriochlorophyll-*d*. In this case, bacteriochlorophyll-*c* was here underestimated by approximately 23 %. Concerning the other equations, that of Parkin & Brock (1981) produced a good correlation for bacteriochlorophyll-*d*, whereas the equation of Overmann & Tilzer (1989) appears to notably underestimate the concentration of this pigment. For this last result, it should be noted that Overmann & Tilzer's equation was designed to assess mixtures of bacteriochlorophyll-*d* and -*e*, which is not our case.

There are several analytical approaches to assess the carotenoid-to-chlorophyll ratios in phytoplankton. These include simple ratios of absorbance among specific wavelengths (e.g., 480/665 nm or 430/665 nm) that assess the carotenoid-to-chlorophyll-*a* relationship (Margalef, 1983), as well as quantitative methods such as HPLC determination. The former are rarely reported in the scientific literature; however, they are still formally used in management and technical studies. One of the first attempts to spectrophotometrically quantify carotenoid concentrations was made by Richards & Thomson (1952), but this produced inadequate results when diatoms and/or chrysophytes occurred in samples. Strickland & Parsons (1972) proposed an equation that partially solved this problem; however, it assumed that the relationship among the concentrations of different carotenoids re-

mains somewhat constant throughout the water column, which is not true for highly stratified lakes such as Lake La Cruz. Although the wavelengths used in these equations are within the ranges of maximum absorption of all carotenoids, a notable variation in the relative composition of carotenoids with depth, such as would be expected for highly stratified lakes, can introduce an important bias. In any case, carotenoid-to-chlorophyll-*a* ratios can be suitable for qualitative analyses and represent readily obtainable optical signatures that can be used as proxies for some phytoplankton community properties. However, our results indicate that only epilimnetic waters can be accurately analysed in this way due to the interference of some bacterial carotenoids such as okenone in microaerophilic and anoxic waters.

In summary, our results demonstrate that in highly stratified aquatic systems, the concentrations of only some algal and bacterial photosynthetic pigments can be estimated with an appropriate accuracy using spectrophotometric algorithms. Furthermore, the use of only one of these types of equations is not always recommended for the entire water column, but the change from one equation to another depends on the mixture and relative abundance of the different photosynthetic microorganisms. In any case, for chlorophyll-*a* determinations, we found the equation by Overmann & Tilzer (1989) to perform the best under all circumstances; thus, we recommend its use for stratified water columns showing vertical stratification of photosynthetic microorganisms. Concerning bacteriochlorophylls, the formulae by Takahahi & Ichimura perform well in anoxic waters, where phototrophic bacteria thrive. Similar considerations for other pigments, such as chlorophyll-*b* and –*c*, and carotenoid concentrations or ratios can be made by reviewing the information synthesised in Table 3. This table summarises the strength of each equation when applied to a stratified system such as our model lake and could serve as a reference for the selection of the most appropriate equation when spectrophotometric methods are used for photosynthetic pigment determinations.

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REFERENCES

APHA–AWWA–WEF. 2005. *Standard methods for the examination of water and watershed. 21th edition*. American public health association. Washington DC, USA. 1200 pp.

ARAR, E. J. 1997. Determination of chlorophylls *a* and *b* and identification of other pigments of interest in marine and freshwater algae using high performance liquid chromatography with visible wavelength detection. Method 447.0, U.S. Environmental Protection Agency.

BARLOW, R. G., P. H. BURKILL & R. F. C. MANTOURA. 1988. Grazing and degradation of algal pigments by marine protozoan *Oxyrrhis marina*. *Journal of Experimental Marine*, 119: 119–129.

CAMACHO, A., J. PEREZ, A. CHICOTE, M. FLORIN, M. M. SQUIRES, C. LEHMANN & R. BACHOFEN. 2001. Microbial microstratification, inorganic carbon photoassimilation and dark carbon fixation at the chemocline of the meromictic Lake Cadagno (Switzerland) and its relevance to the food web. *Aquatic Sciences*, 63: 91–106.

CAMACHO, A., A. PICAZO, M. R. MIRACLE & E. VICENTE. 2003. Spatial distribution and temporal dynamics of picocyanobacteria in a meromictic karstic lake. *Archiv für Hydrobiologie Supplementband*, 148: 171–184.

CASAMAYOR, E. O., M. LLIROS, A. PICAZO, A. BARBERÁN, C. M. BORREGO & A. CAMACHO. 2011. Contribution of deep dark fixation processes to overall CO_2 incorporation and large vertical changes of microbial populations in stratified karstic lakes. *Aquatic Sciences*, 74: 61–75.

DOS SANTOS, A. C. A., M. C. CALIJURI, E. M. MORAES, M. A. T. ADORNO, P. B. FALCO, D. P. CARVALHO, G. L. B. D. P., DEBERDT, G. L. B. & BENASSI, S. F. 2003. Comparison of three methods for chlorophyll determination: spec-

trophotometry and fluorimetry in samples containing pigment mixtures and spectrophotometry in samples with separate pigments through High Performance Liquid Chromatography. *Acta Limnologica Brasiliensia*, 15: 7–18.

FOPPEN, F. H. 1971. Tables for identification of carotenoid pigments. *Chromatographic Reviews*, 14: 133–298.

JACOBSEN, T. R. & H. RAI. 1990. Comparison of spectrophotometric, fluorometric and High Performance Liquid Chromatography Methods for Determination of chlorophyll a in aquatic samples: Effects of solvent and extraction procedures. *Internationale Revue gesamten Hydrobiologie*, 75: 207–217.

JEFFREY, S. W. & G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen*, 167: 191–194.

JEFFREY, S. W., MANTOURA, R. F. C. & S.W. WRIGHT. 1997. *Phytoplankton pigments in oceanography: guidelines to modern methods*. UNESCO, Paris, 661 pp.

JODŁOWSKA, S. & A. LATAŁA. 2011. The comparison of spectrophotometric method and high performance liquid chromatography in photosynthetic pigments analysis. *Online Journal of Biological Sciences*, 11: 63–69.

LATASA, M., R. R. BIDIGARE, M. E. ONDRUSEK, AND M. C. KENNICUTT II. 1996. HPLC analysis of algal pigments: a comparison exercise among laboratories and recommendations for improved analytical performance. Marine Chemistry, 51: 315–324.

LORENZEN, C. J. 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnology & Oceanography*, 12: 343–346.

LOUDA, J. W., J. L. LEI LIU, M. N. WINFREE & E. W. BAKER. 1998. Chlorophyll-a degradation during cellular senescence and death. *Organic Geochemistry*, 29: 1233–1251.

LOUDA, J. W., LIU, L., & E. W. BAKER. 2002. Senescence and deathrelated alteration of chlorophylls and carotenoids in marine phytoplankton. *Organic Geochemistry*, 33: 1635–1653.

MACLULICH, J. H. 1986. Experimental evaluation of methods for sampling and assaying intertidal epilithic microalgae. *Marine Ecology Progress Series*, 34: 275–280.

MANTOURA, R. F. C., & C. A. LLEWELLYN. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Analytica Chimica Acta*, 151: 297–314.

MANTOURA, R. F. C., S. W. JEFFREY, C. A. LLEWELLYN, H. CLAUSTRE, & C. E. MORALES. 1997. Comparison between spectrophotometric, fluorometric and HPLC methods for chlorophyll analysis. In *Phytoplankton pigments in oceanography: guidelines to modern methods*. S. W. Jeffrey *et al*. (eds.). 361–380. UNESCO, Paris.

MARGALEF, R. 1983. *Limnologia*. Ediciones Omega, S.A., Barcelona, Spain.

MARKER, A. F. H. 1972. The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. *Freshwater Biology*, 2: 361–385.

MIRACLE, M. R., E. VICENTE & C. PEDRÓS-ALIÓ. 1992. Biological studies of Spanish meromictic and stratified karstic lakes. *Limnetica*, 8: 59–77.

MURRAY, A. P., C. F. GIBBS, A. R. LONGMORE & D. J. FLETT. 1986. Determination of chlorophyll in marine waters: intercomparison of a rapid HPLC method with full HPLC, spectrophotometric and fluorometric methods. *Marine Chemistry*, 19: 211–227.

OVERMANN, J. R. & M. M. TILZER. 1989. Control of primary productivity and the significance of photosynthetic bacteria in a meromictic kettle lake. Mittlerer Buchensee, West-Germany. *Aquatic Sciences*, 51: 261–278.

PARKIN, T. & T. BROCK. 1981. Photosynthetic bacterial production and carbon mineralization in a meromictic lake. *Archiv für Hydrobiologie*, 91: 366–382.

PARSONS, T. R. & J. D. H. STRICKLAND. 1963. Discussion of spectrophotometric determination of marine-plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. *Journal of Marine Research*, 21: 155–163.

PINCKNEY, J., R. PAPA & R. ZINGMARK. 1994. Comparison of high-performance liquid chromatographic, spectrophotometric, and fluorometric methods for determining chlorophyll a concentrations in estuarine sediments. *Journal of Microbiological Methods*, 19: 59–66.

PINCKNEY, J. L., D. F. MILLIE, K. E. HOWE, H. W. PAERL & J. P. HURLEY.1996. Flow scintillation counting of 14C-labeled microalgal photosynthetic pigments. *Journal of Plankton Research*, 18: 1867–1880.

PLANTE-CUNY, M. R., C. BARRANGUET, D. BONIN & C. GRENZ. 1993. Does chlorophyllide a reduce reliability of chlorophyll a measurements in marine coastal sediments? *Aquatic Sciences*, 55: 19–30.

RICHARDS, F. A. & T. G. THOMPSON. 1952. The estimation and characterization of plankton populations by pigment analysis. II. A spectrophotometric method for the estimation of plankton pigments. *Journal of Marine Research*, 11: 156–172.

RITCHIE, R. J. 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. *Photosynthesis Research*, 89: 27–41.

RITCHIE, R. J. 2008. Universal chlorophyll equations for estimating chlorophylls *a*, *b*, *c*, and *d* and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica*, 46: 115–126.

RODRIGO, M. A., E. VICENTE & M. R. MIRACLE. 2000. The role of light and concentration gradients in the vertical stratification and seasonal development of phototrophic bacteria in a meromictic lake. *Archiv für Hydrobiologie*, 148: 533–548.

RODRIGO, M. A., M. R. MIRACLE & E. VICENTE. 2001. The meromictic Lake La Cruz (Central Spain). Patterns of stratification. *Aquatic Sciences*, 63: 406–416.

ROWAN, K. 1989. *Photosynthetic Pigments of Algae.* Cambridge University Press.

SALINAS, J. T. 1988. A critical comparison of methods for the determination of phytoplankton chlorophyll. Ph.D. Thesis. Oregon State University, U.S.A.

SCHAGERL, M., & G. KÜNZL. 2007. Chlorophyll *a* extraction from freshwater algae *a* reevaluation. *Biologia*, 62: 270–275.

SCOR-UNESCO. 1966. *Determination of photosynthetic pigments in seawater. Monographs on Oceanographic Methodology*, UNESCO, Paris, vol. 1, p.11–18.

SHIMURA, S. & Y. FUJITA. 1975. Changes in the activity of fucoxanthin-excited photosynthesis in the marine diatom *Phaeodactylum tricornutum* grown under different culture conditions. *Marine Biology*, 33: 185–194.

SOLOVCHENKO, A. E., I. KHOZIN-GOLDBERG, Z. COHEN & M. N. MERZLYAK. 2009. Carotenoid-to-chlorophyll ratio as a proxy for assay of total fatty acids and arachidonic acid content in the green microalga *Parietochloris incise*. *Journal of Applied Phycology*, 21: 361–366.

STAL, L. J., H. VAN GEMERDEN & W. E. KRUMBEIN. 1984. The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. *Journal of Microbiological Methods*, 2: 295–306.

STEENBERGEN, C. L. M. & H. J. KORTHALS. 1982. Distribution of phototrophic microorganisms in the anaerobic and microaerophilic strata of Lake Vechten (The Netherlands). Pigment analysis and the role of primary production. *Limnology & Oceanography*, 27: 883–895.

STRICKLAND, J. D. H. & T. R. PARSONS. 1972. *A Practical Handbook of Seawater Analysis*. 2nd ed., Bull. Fish. Res. Bd. Can. No. 167, 310 pp.

TAKAHASHI, M. & S. ICHIMURA. 1970. Photosynthetic properties and growth of photosynthetic sulfur bacteria in lakes. *Limnology & Oceanography*, 15: 929–944.

VICENTE, E. & M. R. MIRACLE. 1988. Physicochemical and microbial stratification in a meromictic karstic lake of Spain. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 23: 522–529.

WRIGHT, S. W. & S. W. JEFFREY. 2006. *Pigment markers for phytoplankton production. In:* The handbook of environmental chemistry Volume 2. Marine organic matter: Biomarkers, isotopes and DNA. J. K. Volkman (ed.): 71–104. Springer Verlag, Basel.

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Limnetica publica artículos originales de investigación sobre la ecología de las aguas continentales. El ámbito de publicación de *Limnetica* incluye la ecología de ríos, lagos, embalses, lagunas costeras, zonas húmedas, biogeoquímica, paleolimnología, desarrollo de metodologías, taxonomía, biogeografía y todos los aspectos de la ecología acuática continental teórica y aplicada como gestión y conservación, evaluación de impactos, ecotoxicología y contaminación. Por este motivo *Limnetica* aceptará para su publicación artículos científicos presentando avances del estado del conocimiento, de desarrollo tecnológico así como los que resulten de aplicaciones prácticas novedosas en las especialidades de interés de la revista.

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Las Tablas constituyen una de las partes más costosas en tiempo y presupuesto por lo que se ruega se preparen procurando ocupar el mínimo espacio posible. Las tablas pueden tener la anchura de una columna (8 cm) o dos columnas (16 cm) y su longitud no puede exceder de 25 cm. Se incluirán al final del manuscrito y tendrán numeración arábiga. En el texto siempre se citarán de forma completa (p.e. Según se puede ver en la Tabla 6... o, Los datos (Tabla 6) indican que..., etc.) y nunca en forma abreviada –Tab. 6 o tab. 6. Las leyendas de las tablas se presentarán en castellano e inglés y se incluirán en el mismo apartado que el texto de las figuras. No deberán usarse líneas verticales y los encabezamientos de las columnas deberán ser breves. Se prestará particular atención en no publicar tablas que dupliquen información que ya está en forma de figuras.

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SEAR, D. A. 2010. Integrating science and practice fro the suitable management of in-channel salmonid habitat. In: *Salmonid Fisheries: Freshwater habitat management.* P.S. Kemp (ed.): 81-119. Wiley-Blackwell, Chichester. UK.

CASTRO, M., J. MARTÍN-VIDE & S. ALONSO. 2005. El clima de España: pasado, presente y escenarios de clima para el siglo XXI. In: *Evaluación preliminar de los impactos en España por efecto del Cambio Climático.* J. M. Moreno Rodríguez (ed.): 113-146. Ministerio de Medio Ambiente, Madrid, Spain.

- Congresos:

GEORGE, D. G. 2006. Using airborne remote sensing to study the mixing characteristics of lakes and reservoirs. Proceedings of the 10th European Workshop on Physical Processes in Natural Waters. June 26-28, 2006. Granada, Spain: 2001-207.

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MORENO-OSTOS, E. 2004. *Spatial dynamics of phytoplankton in El Gergal reservoir (Seville, Spain).* Ph.D. Thesis. University of Granada, Spain.

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En el manuscrito se listarán únicamente los trabajos citados en el texto; en éste, las referencias se harán en minúsculas (Kalff, 2002; Dolz & Velasco, 1991; Rueda *et al*., 2006). En ningún caso se aceptarán como referencias trabajos no publicados (p.e. en preparación) o aún no aceptados (p.e. enviado). Sí se podrán incluir citas de trabajos aceptados para su publicación (en prensa). Se recuerda la conveniencia de reducir al máximo las referencias bibliográficas de difícil consulta como informes, resúmenes a congresos, etc.

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Figure calls must be made in complete, lower case form when in the text (e.g., Location of sampling sites is shown in figure 1), in abbreviated, upper case when going in a parenthesis and not directly related to the text [e.g., Samples were taken monthly at five sites along the river (Fig. 1)].

Units must be expressed preferably following the International System (I.S.), with abbreviated symbols when preceded by numeric expressions. Values combining two units must be expressed with the corresponding arithmetic sign, like m/s, mol/m^3, ind/l, but when there are more than two units exponentials must be used, like in mgC m^{-2} h^{-1}, µmol m^{-2} s^{-1}.

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