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Rediscovery of *Branchipus schaefferi* (Branchiopoda: Anostraca) in Belgium - notes on habitat requirements and conservation management

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ABSTRACT. Fairy shrimps (Crustacea, Anostraca) are specialized inhabitants of inland water bodies that periodically dry or freeze over. Here we report the first observation since 1997 of a member of this basal crustacean order in Belgium and the first sighting of the species *Branchipus schaefferi* Fischer, 1834 since 1930. Nineteen populations were found in a restricted area located 55 km SE of Brussels in the Province of Hainaut. Based on a field survey, we discuss the habitat characteristics of these populations. We discuss also the distribution and habitat requirements of the species based on literature and formulate a number of guidelines for the conservation of this species as well as other large branchiopods in densely settled areas with intensive agriculture such as Belgium. Finally, we formulate a number of likely explanations for the lack of recent observations of these organisms in Western Europe and in Belgium.

KEY WORDS: fairy shrimp, temporal pools, wheel tracks, conservation management

INTRODUCTION

Fairy shrimps and brine shrimps together make up the order Anostraca. With the closely related clam shrimps (Spinicaudata, Laevicaudata and Cyclestherida) and tadpole shrimps (Notostraca) they are often referred to as large branchiopods. Together with a number of extinct orders they form the class Branchiopoda. Due to their size, large branchiopods are sensitive to fish predation. Therefore, they typically occur in aquatic habitats that cannot sustain significant fish populations (but see JEPPESEN et al., 2001), either because they are highly saline, periodically dry or regularly freeze solid. Brine shrimps (*Artemia* and *Parartemia* spp.) and some members of the fairy shrimp genera *Branchinella*, *Branchinectella*, *Branchinecta* and *Phallocryptus* are typical for salt pans in different parts of the world. Most fairy shrimp, on the other hand, generally inhabit temporary freshwater habitats, ranging from large wetlands,

vernal ponds and marshes to rock pools, wheel tracks, small puddles and flooded rice fields. Although the class Branchiopoda is an old group with a near global distribution (BRENDONCK et al., 2008), Belgian records of fairy shrimp and other large branchiopod populations are extremely scant. Museum collections in Brussels and Liège were inventoried by BRENDONCK (1989a) and LONEUX & THIÉRY (1998), respectively, revealing a historic species richness of seven. These include three fairy shrimp species: *Chirocephalus diaphanus* Prevost, 1803; *Eubranchipus* (*Siphonophanes*) *grubii* Dybowski, 1860 and *Branchipus schaefferi* Fischer, 1834; two clam shrimp species: *Limnadia lenticularis* Linnaeus, 1761 and *Leptestheria dahalacensis* (Rüppel, 1837) and two tadpole shrimp species: *Lepidurus apus* Linnaeus, 1758 and *Triops cancriformis* Bosc, 1801.

The fairy shrimp *C. diaphanus* is a widespread species that is found in most of Western Europe

and North Africa with a range extending northward into Great Britain and eastward to the Black Sea. In Belgium, *C. diaphanus* was found in Halen in 1903 (Brendonck, 1989A) and in Sint-Truiden in 1930 (LONEUX & THIÉRY, 1998) and was last seen in Hamois in 1998 (LONEUX & WALRAVENS, 1998). *Eubbranchipus (Siphonophanes) grubii* is a Central and Eastern European coldwater species and may have been observed in Belgium in 1970 in Geel (K. WOUTERS, pers. comm. *vide* BRENDONCK, 1998), but its presence was never confirmed. Currently, the nearest populations are located in The Netherlands (North Brabant, Gelderland, Overijssel and Limburg) (BRENDONCK, 1989A; SOESBERGEN, 2008), Germany (Rhineland-Palatinate, Nordrhein-Westfalen) (MAIER, 1998; ENGELMANN & HAHN, 2004) and France (Alsace) (DEFAYE et al., 1998). *Branchipus schaefferi* is a eurytherm species that is widespread in Europe and around the Mediterranean basin with additional records from Northern Africa and Asia (BRTEK & THIÉRY, 1995; AL-SAYED & ZAINAL, 2005) and was encountered only once in Belgium (Sint-Truiden, 1930) (LONEUX & THIÉRY, 1998). The closest currently-known populations are located in Western Germany (Rhineland-Palatinate, Nordrhein-Westfalen) (MAIER, 1998; ENGELMANN & HAHN, 2004) and North West (Ault) and North East France (Alsace region) (DEFAYE et al., 1998). The clam shrimp *L. lenticularis* is a Holarctic species most abundant in northern temperate climates. In Belgium, it was reported from a marsh in Genk in 1946 and Zolder-Zonhoven in 1959 (BRENDONCK, 1989A). *Leptestheria dahalacensis* (Rüppel, 1837) was encountered in Belgium in 1989 in a recently-inundated fishpond near Brussels, presumably having been introduced inadvertently with mud from temporary ponds in carp nurseries in Eastern Europe (BRENDONCK et al., 1989B). The tadpole shrimp *L. apus* is widespread in Europe and has been reported in Belgium from Balen or Halen (date unknown), but nothing else is known about the population (BRENDONCK, 1998). The second tadpole shrimp species, *T. cancriformis*, is also widespread in Europe and historically known from Halen (in 1892 and 1903), Sint

Truiden (in 1917, 1929 and 1930), Ferrières (in 1905 and 1906) and Leuven (date unknown) (BRENDONCK, 1998; LONEUX & THIÉRY, 1998). The only confirmed currently-persisting population in Belgium is a *T. cancriformis* population discovered in 2006 on a military domain in Brasschaat in the province of Antwerp (WILLEMS & DE LEANDER, 2006). Despite the relatively intensive monitoring of many aquatic habitats, fairy shrimps have not been observed in Belgium since 1998 and clam shrimps have not been seen since 1989.

In this paper we report the rediscovery of the order Anostraca in Belgium represented by at least 19 populations of *B. schaefferi*, which has not been observed since 1930. We discuss the autecology of the species based on published literature and the biotic and abiotic characteristics of the habitats in which it was found. Based on this we formulate guidelines for more effective detection, monitoring and conservation of this species and other large branchiopods in intensively-developed regions such as Belgium.

MATERIALS AND METHODS

Notes on the ecology and distribution of the species

Branchipus schaefferi (Fig. 1) is a eurythermic species that can be found from late spring until fall in temperate regions (HÖSSLER et al., 1995; EDER et al., 1997) and in Southern France (DEFAYE et al., 1998; WATERKEYN et al., 2009) or throughout the year in warmer regions around the Mediterranean basin such as Morocco (BELK & BRTEK, 1995; BRTEK & THIÉRY, 1995; THIÉRY, 1987; MARRONE & MURA, 2006). It is most frequently found in small shallow ponds, puddles or wheel tracks with turbid water and scarce vegetation (HÖSSLER et al., 1995; PETROV & PETROV, 1997; DEFAYE et al., 1998; BOVEN et al., 2008). The species can also be found in other habitat types, such as flooded rice fields (PETKOVSKI, 1997; MURA, 2001) and mountain habitats (EDER et al., 1997; DEFAYE et al., 1998;

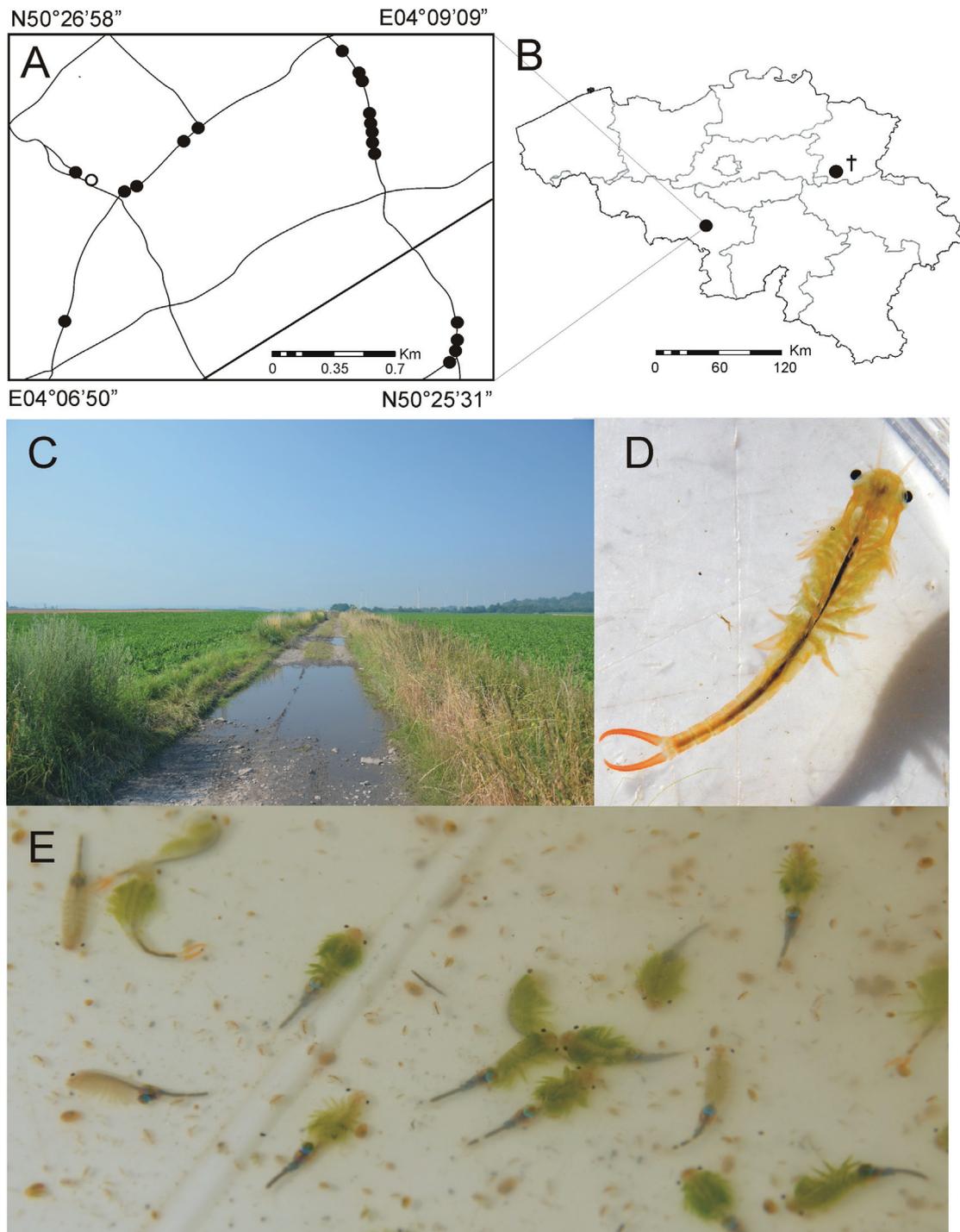


Fig. 1. – Distribution of the discovered *Branchipus schaefferi* populations in Hainaut (A) with thin and thick black lines representing unsealed and sealed roads, respectively. Filled symbols represent wheel track populations, the empty symbol corresponds to a population in a farmland pond. (B) Overview of the only two records of this species in Belgium, indicating the newly discovered populations near Binche (filled symbol) and the historic locality near Sint-Truiden (†). The middle panel shows a typical *B. schaefferi* wheel track habitat near Binche (Picture: B. Vanschoenwinkel) (C) and a close up of an adult male showing the antennal structure characteristic of the species (Picture: A. Waterkeyn) (D). The lower panel shows an isolate of a wheel track zooplankton community including many fairy shrimps. Females can be discerned based on the presence of a blue brood pouch (Picture: B. Vanschoenwinkel) (E).

MURA, 1999; THIÉRY, 1987). Exceptionally, it can occur in permanent waters, as is the case in Germany (HÖSSLER et al., 1995). It is considered a rather tolerant species, since it can survive in ponds with short inundations, high turbidities (THIÉRY, 1987), high conductivities (up to 4500 $\mu\text{S cm}^{-1}$) (WATERKEYN et al., 2010), high temperatures (MARRONE & MURA, 2006), eutrophication due to cattle manure (THIÉRY, 1987) and high altitudes (up to 2600 m a.s.l.) (THIÉRY, 1987).

Dormant eggs of *Branchipus schaefferi* hatch within 1 to 6 days after inundation, while maturation takes 7 to 30 days, depending on the temperature and food conditions (WATERKEYN et al., 2009 and references therein). They can survive for up to 2.5 months (HÖSSLER et al., 1995; BELADJAL et al., 2003) and grow up to 20–25 mm (THIÉRY, 1991; HÖSSLER et al., 1995; PETKOVSKI, 1997; DEFAYE et al., 1998; AL-SAYED & ZAINAL, 2005). The females have a brightly-turquoise colored brood sac and can produce up to 242 dormant eggs per day (maximum reached 21 to 27 days after hatching) (BELADJAL et al., 2007). Eggs are typically angular and wrinkled and more or less spherical ranging from 195 to 290 μm in size (THIÉRY et al., 1995). Different life stages of *B. schaefferi* can co-occur, probably due to several hatching peaks triggered by additional rainfall (HÖSSLER et al., 1995; PETROV & PETROV, 1997; AL-SAYED & ZAINAL, 2005). *Branchipus schaefferi* often co-exists with one or several other large branchiopods, such as the notostracan *T. cancrivormis* (most often reported), spinicaudatans (*Imnadia yeyetta*, *I. banatica*, *L. dahalacensis* or *L. saetosa*), or other anostracans (*Tanymastix stagnalis*, *Branchinecta ferox*, *Streptocephalus torvicornis*, *C. diaphanus*, *C. carnuntanus*, *C. brevipapis*) (PETROV & CVETKOVIC, 1997; PETKOVSKI, 1997; DEFAYE et al., 1998; MAIER et al., 1999; MARRONE & MURA, 2006; BOVEN et al., 2008; WATERKEYN et al., 2009).

Study site and sampling protocol

After a first unconfirmed sighting of a large branchiopod-like crustacean in the area in 2002 (Dupriez, pers. com.), populations of the species were discovered in a wheel track North of Binche (province of Hainaut; Belgium) on 23 July 2012 by Pascal Dupriez during a survey for natterjack toads (*Bufo calamita*). The observation was reported as a potential sighting to the KU Leuven nationwide large branchiopod survey (<http://bio.kuleuven.be/de/dea/branchiopodhunters/index.php>). Thirty other potential habitats in that area were surveyed on 25, 26 and 27 July 2012. These included the two types of temporary ponds present in the area: wheel tracks as well as a couple of pools situated in the corner of crop fields. Several wheel tracks were dry so the presence of fairy shrimp in these could not be determined in the field. In order to reliably define habitats and populations we used conservative criteria. A set of tracks that showed obvious signs of connections or that very likely could connect during floods was considered as a single habitat potentially housing a single population. Proximate tracks were only considered as separate habitats if they were independent depressions separated by a clear topographic barrier. In total, water quality variables were measured in 22 habitats (19 of which contained *B. schaefferi*). Measurements were taken between 11.00 and 15.00 under sunny conditions and included conductivity (EC; $\mu\text{S cm}^{-1}$), water temperature (T; $^{\circ}\text{C}$), pH, oxygen concentration (DO; ppm), total dissolved solids (TDS; ppm), and total suspended solids (TSS; ppm) using a HI9828 Multiparameter Meter (Hanna instruments, Ann Arbor, MI, USA). Chlorophyll-a concentration (ChlA; mg L^{-1}) and turbidity were determined using a hand held AquaFluor fluorometer (Turner Designs, Sunnyville, CA, USA). Nutrient concentrations (mg/L) were quantified spectrophotometrically using a Hach DR2400 spectrophotometer (Hach company, Loveland, CO, USA) by means of the following methods: total N (persulphate digestion method), total P (acid persulphate digestion method using PhosVer® 3), reactive phosphate (PhosVer® 3 method) and Nitrate (chromotropic

acid method). The bottom of many wheel track habitats was partly or almost entirely covered with gravel. The gravel coverage ($\pm 10\%$) was estimated and included as an additional predictor variable in the analyses. Habitat size was assessed by measuring length, width, max depth and volume calculated using the formula for the volume of a half ellipsoid. In case pools consisted of two superficially connected tracks, the volume was calculated as two separate half ellipsoids. An aquarium net (mesh 0.5 mm) was initially used to qualitatively check for the presence of *B. schaefferi*. In order to quantify density of fairy shrimp (number of individuals per L), quantitative zooplankton samples were taken by scooping a total of 12 L of water using a 0.5 L beaker and filtering this over a 64 μm zooplankton net. If fairy shrimp densities were very high less water was filtered. Samples were stored in 90% non-denatured ethanol. Total population sizes were obtained by multiplying densities with calculated water volumes.

Analyses

The relationship between measured environmental variables and fairy shrimp density was analysed using multiple linear regression. Due to large variation in fairy shrimp densities including several outliers, analyses were performed using density ranks rather than the untransformed data. This transformation helped to meet the linear regression assumption of homoscedasticity. In order to reduce the set of predictor variables, only variables with a clear trend of association (Spearman correlation coefficient > 0.20 or < -0.20) with the response variable were included in a multiple regression model. Both stepwise forward and backward selection procedures were used in order to remove non significant terms from the model and select a final consensus model taking into account both adjusted r^2 and Akaike's information criterion as decisive factors. First order interactions were also considered. Associations between fairy shrimp densities and measured environmental variables were visualised using principal

component analysis (PCA) triplot. This plot shows the relative positions of different habitats along the two dominant axes of environmental variation (PC1 and PC2) while simultaneously showing the associations between habitats and environmental variables (shown as vectors) and associations between environmental variables. Environmental variables were centered and standardised prior to analyses. In order to obtain an objective representation of the environmental variation and its relationship with the response variable of interest (fairy shrimp density), the latter was plotted as a supplementary variable that does not affect the ordination (LEGENDRE & LEGENDRE, 1998). All analyses were performed in Statistica 10 (Statsoft 2011, Tulsa, OK, USA).

RESULTS

B. schaefferi was found in a total of 18 wheel tracks present in a local network of unsealed roads in a rural area covering a total area of approximately 7 km² North of Binche in the Province of Hainaut. These roads were separated from surrounding crop fields (mainly potato and wheat) by an elevated ridge of approximately 1.5 m wide and 30 cm high, preventing excessive runoff from the fields into the tracks. Additionally, the species was found in a single temporary pond in the corner of a field of sugar beets in the same area. Most habitats housed large populations (Average: 4315; Range: 1 - 44000) of adults of both sexes. The environmental characteristics of the different *B. schaefferi* habitats are provided in Table 1. In general, habitats were relatively shallow, turbid and lacked aquatic vegetation. Water temperatures up to 35°C were recorded. Macroinvertebrate species richness was relatively low in all habitats. Besides *B. schaefferi*, the only branchiopod crustacean present was *Moina branchiata*. Other inhabitants included at least one ostracod species, water bugs (Corixidae), mayfly larvae (Baetidae), and the larvae of several dipterans (Chironomidae, Culicidae, *Eristalis* sp.). Principal components analysis was used to visualize associations between densities of *B. schaefferi* and environmental variables. The

first two principal components captured 48.47 % of total variation. The triplot suggests a positive association between fairy shrimp population density and reactive phosphate, and negative associations with pH, gravel coverage, nitrate and chlorophyll *a* (Fig. 2). However, gravel coverage was the only significant variable that was retained in the constructed regression models associated with lower fairy shrimp densities (Fig. 3). Associations with other environmental variables were not significant.

DISCUSSION

The current study shows that *B. schaefferi* is still present in Belgium after not being reported for almost 72 years. This was the first sighting of fairy shrimp in Belgium since *C. diaphanus* was last detected in Hamois in 1998 (LONEUX & WALRAVENS, 1998). Observation of *B. schaefferi* in summer during a warm period and after heavy rains is consistent with the known phenology of this heat-tolerant eurythermic species (DEFAYE et al., 1998). Most of the remaining populations

of *B. schaefferi* in Western and Eastern Europe are known from wheel tracks (BOVEN et al., 2008; DEFAYE et al., 1998), probably since these are the most commonly-remaining temporary aquatic habitat types in anthropogenically-modified landscapes. The species is well adapted to time stress, displaying traits such as a short life cycle and high fecundity (HÖSSLER et al., 1995; PETROV & PETROV, 1997; DEFAYE et al., 1998). Therefore it is well adapted for living in these short-lived temporary aquatic systems, which often hold water for only a couple of weeks.

In general, population densities in most of the studied habitats were high (Average: 2.18 ± 4.1 ind./L; Range: 0.01-18) suggesting that these populations are well established. Although nutrient concentrations were moderate to high, chlorophyll *a* concentrations were low indicating that this could be top-down controlled by the grazing zooplankton community. Freshwater zooplankton communities, and fairy shrimp in particular, can be sensitive to pesticides or to oxygen stress as a result of eutrophication (LAHR, 1998; ROGERS, 1998). As such, the fact that populations were doing well despite the presence of intensive agriculture in the immediate vicinity could illustrate that the buffer zones (elevated ridge covered with grasses, herbs and small shrubs) that are present between

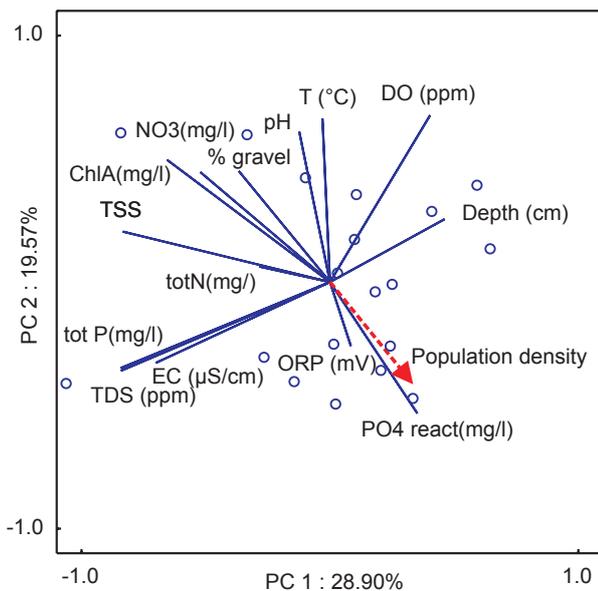


Fig. 2. – PCA triplot showing associations between environmental variables (vectors), site scores (circles) and the supplementary variable Population density (arrow). Environmental variables were centered and standardised prior to analysis. Population densities were transformed to ranks.

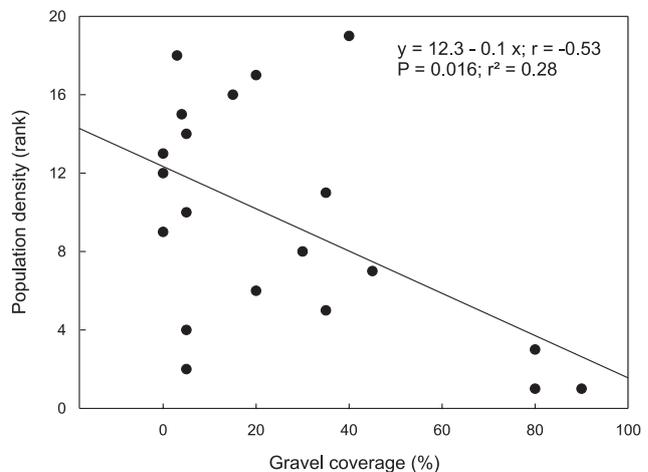


Fig. 3. – Scatterplot showing the significant negative relationship between the percentage of surface area of each habitat covered with gravel and the density of fairy shrimp found in the active populations during sampling.

TABLE 1

Environmental variables measured in the ponds containing *B. schaefferi* (n = 19).

Variable	Average \pm st. dev.	Range
Surface (m ²)	32.4 \pm 75.0	0.2 - 314
Depth (cm)	7.8 \pm 3.8	2 - 15
Conductivity (μ S/cm)	573.4 \pm 274.6	133 - 1335
Dissolved oxygen (ppm)	38.6 \pm 1.47	6.20 - 1.35
pH	8.10 \pm 0.30	7.69 - 8.57
Temperature ($^{\circ}$ C)	32.1 \pm 2.3	27.73 - 35.7
Total Dissolved Solids (ppm)	286.9 \pm 137.2	67 - 668
TSS	222.8 \pm 205.0	34.0 - 873.7
Chl A (mg/l)	0.011 \pm 0.007	0.002 - 0.03
Total N (mg/l)	4.92 \pm 3.45	0 - 10.4
Nitrate (mg/l)	4.51 \pm 2.49	0 - 9
Total P (mg/l)	2.01 \pm 0.76	0.68 - 3.62
Reactive phosphate (mg/l)	1.06 \pm 0.78	0.22 - 2.7

the wheel tracks and the surrounding fields are effective. However, the presence of a large population in the corner of a sugar beet field fed by surface runoff suggests that the species may, in fact, be quite resistant, as was also suggested by THIÉRY (1987). The species, however, remains vulnerable to habitat destruction. In many areas wheel tracks are filled with gravel in order to facilitate passing of traffic. Here, it was shown that in the studied area fairy shrimp population densities were much lower in habitats with ample gravel coverage. This effect can have different origins. Gravel application can reduce the depth and potential length of inundations (hydroperiod) of the habitat or alter water chemistry and make them less suitable for fairy shrimp. However, we found no indications for associations between gravel coverage and water levels or any of the measured environmental variables (Spearman R; all $P > 0.10$). As such, the effect might be of a purely physical nature. Gravel application can, for instance, cover the dormant egg bank and shield fairy shrimp resting eggs from receiving hatching cues, such as light, impeding successful recruitment. Filling of roadside ditches presumably also led to the disappearance of the last known

Belgian population of *C. diaphanus* in Hamois (LONEUX, pers. com.; VANSCHOENWINKEL, pers. obs.). Consequently, it is advisable to refrain from adding additional gravel, debris or other sediments to existing wheel tracks if these populations are to be preserved. In some cases, however, this may be unavoidable. When wheel tracks become too deep to allow passage of vehicles we propose that they should not be filled up completely allowing for the presence of about 10 cm of standing water after rains, as was the situation observed for the fairy shrimp populations in this study. Ideally, the top layer surface sediment (± 4 cm) should be temporarily removed prior to graveling and replaced on top of the gravel to ensure that the resting egg bank will not be covered and fairy shrimp may continue to hatch during future inundations. Restricting access of vehicles altogether is probably not recommended as the disturbance provided by passing vehicles is necessary to maintain these wheel tracks. Additionally, previous research has shown that walkers and motor vehicles can be important dispersal vectors for large branchiopod crustaceans (WATERKEYN et al., 2010). Regular exchange of eggs between populations may ensure healthy metapopulation dynamics

with recolonization rates compensating for occasional extinctions. The spatial organization of the populations in this study located along an unsealed road network could be illustrative of these processes but this will have to be confirmed using genetic analyses.

Relict populations or products of a recent introduction?

Although it remains to be confirmed using genetic analyses, it is plausible that the discovered populations represent relicts, rather than a recent establishment of the species. First of all, the presence of the species is consistent with the species' distribution and its historic presence in Belgium (LONEUX & THIÉRY, 1998). Currently known populations are present in Northern France (Alsace) and Western Germany (Rhineland-palatinate) at about 200 to 300 km from the Belgian locality (LONEUX & THIÉRY, 1998). Secondly, the occurrence of a substantial number of populations, usually consisting of numerous individuals, suggests that the populations are likely to have been in the area for at least several decades. Finally, the fact that the populations were found in an old agricultural area with unsealed roads that are probably more than 100 years old, makes continuous and prolonged persistence of the species in the area a likely scenario. An upcoming phylogeographic study across the species' range (including specimens from this study) documenting the phylogenetic relationships among the remaining European lineages, will likely provide more conclusive evidence concerning the origin of the Belgian populations.

A hidden existence

The current study illustrates that populations of fairy shrimp can remain undetected, although individuals are relatively large (1 - 4 cm) and conspicuous and often characterized by bright coloration, and even in relatively well-studied and monitored regions, such as Belgium. The

reasons for this are manifold. First of all, fairy shrimp and other large branchiopods are typically only present during specific seasons, hatching from the dormant egg bank after specific hatching cues (BRENDONCK, 1996). If such cues do not present themselves, it is common that years will go by without active populations developing in the field (WATERKEYN et al., 2009). This is possible since they produce long-lived resistant, dormant eggs. For instance, the most common species in Western Europe, *C. diaphanus* and *E. (S.) grubii*, are usually only present during the colder winter months and the beginning of spring (DEFAYE et al., 1998), at a time when there is typically no monitoring. Secondly, even when eggs hatch and adults develop, they can easily remain un-noted as fairy shrimps often inhabit small and inconspicuous systems, such as wheel tracks and puddles in meadows and cropland where few people wander. These habitats are also often considered of low conservation interest and are therefore not monitored. Thirdly, water in wheel tracks is often turbid obscuring potential inhabitants. Finally, active populations in the water column often only persist for several weeks as a result of their short life span and the gradual increase of predation (by e.g. beetles, dragonflies, notonectids) throughout the inundation (SPENCER et al., 1999).

Towards effective conservation

Large branchiopods are threatened in many parts of the world and notably in Western Europe. The main reason for this is the loss of temporary aquatic habitats as a result of intensive agriculture and urbanisation, and the few remaining habitats are often degraded (BELK, 1998). Although 29 fairy shrimp species are red listed by IUCN, and several species are included in local red lists (e.g. in the Alsace), at the moment there is no legal basis for protection of large branchiopods in Belgium. Before a species can be red listed it must be shown that sufficient effort has been put into localizing and monitoring populations. Given the hidden existence of the members of this group, they are typically overlooked in

standard biodiversity inventory surveys. We therefore recommend that sampling campaigns should be strategically planned and undertaken at specific moments when the chances of finding large branchiopods are highest. For instance, early spring (February, March) and preferably three to four weeks after prolific rains, is an excellent time to find relatively slow-growing cold water species such as *C. diaphanus*, *S. grubii* and *L. apus*. On the other hand, a summer drought followed by heavy rainfall presents ideal conditions for hatching and development of warm water species, such as *B. schaefferi* and *T. cancriformis*, which can be detected from about 10 days - 3 weeks after inundation.

Due to the frequent disturbance typical of ephemeral habitats, local populations may regularly go extinct. Therefore, in order to persist regionally, dispersal and recolonization from nearby populations (metapopulation dynamics) are likely to be important. Promising localities to find branchiopods therefore include areas where temporary water bodies are abundant and have historically been abundant. Although the local dispersal potential of large branchiopods is quite high (VANSCHOENWINKEL et al., 2008A,B), successful long distance dispersal (several km) events are rare (VANSCHOENWINKEL et al., 2011). Therefore, recently-formed temporary water bodies, such as bomb craters and human-made temporary ponds, may be suitable in terms of their environmental conditions, but may not be colonized, even when large branchiopods are present in the region. Nevertheless, occasionally isolated relict populations have been detected (PAULSEN, 2000). Finally, over longer time scales, temporary pond systems typically accumulate sediments and disappear. Therefore, physical disturbances that counteract this process can be beneficial. Large mammals often maintain temporary water bodies by wallowing in them covering their skin with mud (VANSCHOENWINKEL et al., 2008B). These turbid, unattractive systems often hold a large diversity of branchiopod crustaceans (NHIWATIWA et al., 2011). In recent times, many large branchiopod populations have been found in habitats that

are frequently disturbed by humans, such as wheel tracks (e.g. BOVEN et al., 2008). The last remaining *Triops* population in Belgium persists in a muddy track used by tanks and other military vehicles (WILLEMS & DE LEANDER, 2006). Similarly, military domains in Eastern Europe are known for their diversity of large branchiopods (MAIER et al., 1998). The presence of natural habitat that was historically set apart, unsealed roads with puddles and wheel tracks and regular physical disturbance by vehicles, makes military domains particularly suitable areas that may be acting as refuges for temporary pond fauna, such as large branchiopods.

While public incentive to conserve a rare group of crustaceans may be limited, it is important to realize that temporary ponds not only house a unique crustacean fauna, but are also of vital importance for other endangered species of plants and animals (WILLIAMS, 2006). These include macrophytes, dragonflies and amphibians specifically linked with temporary waters. Substantial efforts and financial support have been directed at protecting certain endangered amphibians that use temporary ponds for breeding, such as the natterjack toad (*Bufo calamita*) and the fire bellied toad (*Bombina bombina*). Temporary pond restoration and construction projects performed for these 'flagship' species (e.g. EU life project *Bombina*) are likely to be beneficial for other typical temporary pond organisms too. For instance, different rare macrophytes were shown to re-emerge from old seed banks during pond restoration projects (HILT et al., 2006). Due to the prolonged viability of their dormant eggs (BRENDONCK, 1996), it is not unlikely that large branchiopods may emerge from old egg banks present in the sediment. Consequently, a habitat-oriented conservation strategy protecting the few remaining high quality temporary ponds and increasing temporary pond densities in the landscape is likely to be most beneficial as a large number of organism groups, including large branchiopods, will benefit from them. In a landscape dominated by agriculture, the use of vegetation buffer zones and ridges is likely to

be beneficial preventing runoff of nutrients and pesticides (DECLERCK et al., 2006), even though some species such as *B. schaefferi* may be quite tolerant. Finally, we would also encourage the re-evaluation of marginal aquatic systems such as wheel tracks as they may contain unique biota, such as *B. schaefferi*.

CONCLUSIONS

This paper reports the rediscovery and the first biotope description of *B. schaefferi* in Belgium and analyses the link between habitat characteristics and population densities. For the studied wheel track populations it was shown that extensive gravel application was associated with lower fairy shrimp population densities, suggesting that this practice should be avoided if populations are to be preserved. In terms of conservation management, we conclude that a habitat-oriented approach preserving natural processes of desiccation and disturbance is likely to be most effective for the conservation of fairy shrimp as well as other typical temporary pond organisms.

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Diet composition of the Mediterranean horse mackerel, *Trachurus mediterraneus* (STEINDACHNER, 1868) (Osteichthyes: Carangidae), from the Aegean Sea

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ABSTRACT. Data on feeding habits in aquatic ecosystems are of great importance in determining the role that a certain fish species plays in its habitat and in related ecosystems. In this study, the diet composition of Mediterranean horse mackerel, *Trachurus mediterraneus* was investigated for 728 specimens from the central Aegean Sea to compare our data with those from other regions of the Mediterranean Sea and throughout all four seasons. Catches from five major groups were identified. Crustaceans (particularly copepods) proved to be the most important food item considering the index of relative importance (IRI). At least 58 different copepod species were identified. Abundant copepod species that occur in every season were *Corycaeus* sp., *Oncaea media*, *Oncaea* spp., *Isias clavipes*, *Euterpina acutifrons* and *Oithona nana*. *Isias clavipes* occurred only in spring, with all of them appearing in the diet with a %IRI >10. Although found in a relatively smaller quantity, teleost larvae ranked as the second most important prey in the overall diet, with increasing quantities in fish larger than 18.0 cm.

KEYWORDS: Mediterranean horse mackerel, *Trachurus mediterraneus*, diet composition, prey, food, Aegean Sea

INTRODUCTION

Mediterranean horse mackerel, *Trachurus mediterraneus* (STEINDACHNER, 1868) is a common semipelagic carangid fish distributed across the entire Mediterranean Sea and the eastern Atlantic coasts (SMITH-VANIZ 1986). Despite its abundance and commercial value, few data are available on its feeding habits. BEN-SALEM (1988) conducted a study on the stomach contents of *T. trachurus* and *T. mediterraneus* in the eastern Atlantic and the Mediterranean but only provided frequency of occurrence of food items because of a low number of samples. Quantitative analysis of main prey in *T. mediterraneus* was made by KYRTATOS (1998) based on specimens from the central Aegean Sea between 1979 and 1980. SEVER & BAYHAN (SAHINOGLU) (1999) presented preliminary results from analysing the main food

of this species in Izmir Bay. Diet composition and feeding intensity of Mediterranean horse mackerel in the eastern Adriatic were also examined by ŠANTIĆ et al. (2004).

Data of feeding studies in aquatic ecosystems are of great importance in determining the role that a certain species plays in its habitat and related ecosystems. Although food composition of the species has been comprehensively studied in various regions of the Mediterranean Sea, there are no studies in which groups of food items have been compared for different seasons and in relation to the length categories of the target species. It is the purpose of our study to identify the most important food groups of the horse mackerel and their seasonal variation.

We believe that the results of the present study can be applied to stock management of the

investigated Mediterranean region for both the target species and other species with which it competes for food in the same habitat.

MATERIAL AND METHODS

Samples were obtained from commercial fishermen, who generally use purse seine and gill nets in Izmir and Candarli Bays, Turkish Aegean Sea, which covered significant fishing grounds in the central part of the sea in 2008. A total of 728 *T. mediterraneus* were collected all year round, with total lengths ranging from 9.7 to 25.3 cm (175 in June and August; 192 in September, October and November; 180 January and February; 181 in March, April and May). Fish were dissected soon after capture and stomachs removed and stored in formalin (4 %) until contents were analysed. Stomach contents were homogenized in petri dishes and then examined using a SZX7 Olympus stereo microscope with 0.8-5.6 x (zoom) and 10 x resolution. Except for Copepoda, for which identification was made at the species or generic level, prey items were identified to the lowest possible taxonomic level. Once counted, the individuals of the same prey species were weighed together (wet weight to the nearest ± 0.0001 g), after excess moisture was removed by blotting prey items on tissue paper.

The following indices were used to quantify the importance of different prey items in the diets of *T. mediterraneus*: (i) percentage frequency of occurrence (%F) for each prey group computed separately = number of stomachs in which a food item was found divided by the total number of non-empty stomachs, multiplied by 100 (ii) percentage numerical abundance (%Cn) = number of each prey item in all non-empty stomachs, divided by the total number of food items in all stomachs, multiplied by 100 and (iii) percentage gravimetric composition (%Cw) = wet weight of each prey item, divided by the total weight of stomach contents, multiplied by 100 (HYSLOP 1980). Main food items were identified using the index of relative importance (IRI) by PINKAS et al (1971). This index has been expressed as: $IRI = \%F \times (\%Cn + \%Cw)$

where % Cn and % F are, respectively, numerical abundance and the frequency of occurrence, and % Cw is the volumetric percentage of the prey type. In addition, %IRI was calculated, being the proportion of IRI of each prey type in relation to the total IRI value. $\%IRI = (IRI / \sum IRI) \times 100$. The vacuity index (VI) was used for feeding intensity. The percentage of empty stomachs to the total number of examined stomachs was expressed as the vacuity index (VI). Vacuity index (%VI): $\%VI = 100 \times (\text{number of empty stomachs} / \text{number of examined stomachs})$ (BERG 1979).

RESULTS

Of a total of 728 stomachs of the Mediterranean horse mackerel, 106 were empty (14.56 %). VI was low during autumn (12.77 %) and spring (13.33 %) but somewhat higher values were observed in winter (15.56 %) and summer (16.67 %). Stomach contents of *T. mediterraneus* included prey from five major taxonomical groups (Polychaeta, Crustacea, Mollusca, Chaetognatha, Osteichthyes). Frequency of occurrence, numerical abundance, gravimetric composition and index of relative importance of different prey species found in the stomachs are presented in Table 1. Given numerical occurrence, there was a clear dominance of crustaceans comprising 92.65 % of the diet in total. Fish larvae (47.23 %) and crustaceans (46.19 %) were present in similar proportions regarding gravimetric composition, both of which formed the major weight percentage of prey. According to the %IRI, crustaceans were the most important prey group while other taxa, i.e. molluscs and teleost fishes were less important in the diet. Of crustaceans, copepods contributed most importantly to the diet (%IRI=78.38), followed by larvae of decapod crustaceans (%IRI=7.63), and the non-crustacean bivalves (%IRI=5.85) and fish larvae (%IRI=5.75). Copepods were the primary food in all seasons, despite quantities ranging between 55.48 and 88.03 of %IRI (Table 2). The majority of the copepods were identified to species or generic level and at least 58 different prey taxa were determined (Table 3). Abundant

TABLE 1

Diet composition of *Trachurus mediterraneus* (%F: frequency of occurrence, %Cn: percentage numerical composition, %Cw: percentage gravimetric composition, IRI: index of relative importance and %IRI: percentage index of relative importance).

Prey groups	%F	%Cn	%Cw	IRI	%IRI
Polychaeta					
Errantia	0.96	0.06	0.09	0.14	<0.01
Crustacea					
Ostracoda	2.57	0.16	0.24	1.04	0.01
Copepoda	75.24	79.49	27.29	8033.56	78.38
Cladocera	16.08	1.10	0.97	33.19	0.32
Mysidacea	19.94	1.55	2.38	78.39	0.77
Euphausiacea	14.47	0.97	3.18	59.99	0.59
Isopoda	0.96	0.06	0.023	0.07	<0.01
Amphipoda	1.29	0.07	0.28	0.46	<0.01
Natantia	0.96	0.06	0.43	0.47	<0.01
Brachyura	7.40	0.72	1.96	19.82	0.19
Stomatopoda	2.25	0.14	0.29	0.97	0.01
Decapod larvae (unidentified.)	44.69	8.34	9.16	782.03	7.63
Mollusca					
Gastropoda (Mesogastropoda)	19.94	1.75	0.76	49.89	0.49
Bivalvia (Filibranchiata)	61.09	4.32	5.50	599.96	5.85
Cephalopoda	1.93	0.20	0.16	0.60	0.01
Chaetognatha					
<i>Sagitta</i> sp.	0.64	0.05	0.13	0.16	<0.01
Osteichthyes					
Eggs and larvae of teleosts	12.22	0.97	47.23	588.90	5.75

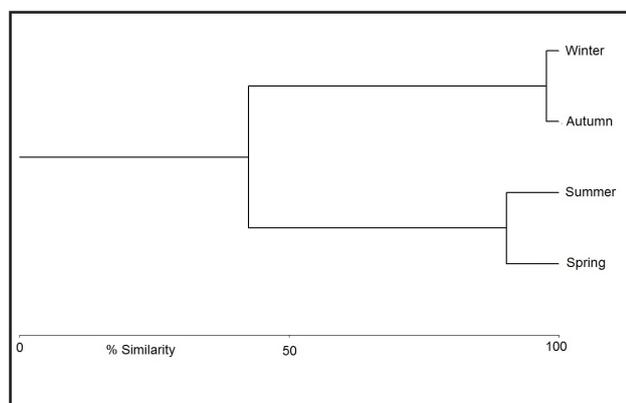


Fig. 1. – Dendrogram of the cluster analysis showing diet similarity (%W) in relation to seasons, using Bray-Curtis index.

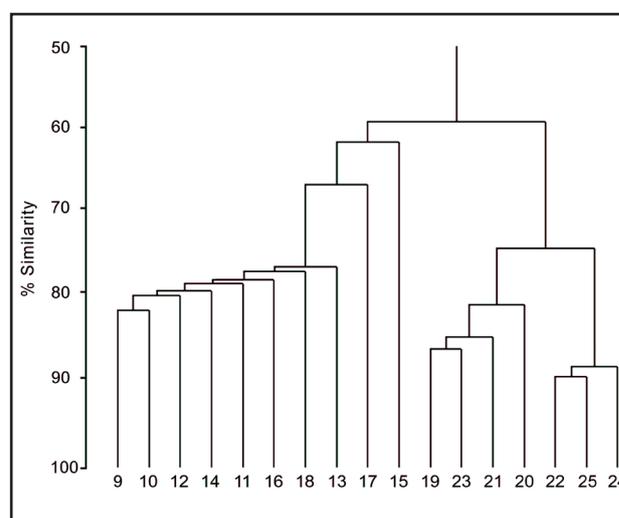


Fig. 2. – Dendrogram of the cluster analysis showing diet similarity (%W) in relation to fish size, using Bray-Curtis index.

TABLE 2

Percentage index of relative importance (%IRI) of species and prey groups by seasons for *T. mediterraneus*.

Prey groups	January and February	March, April and May	June and August	September, October and November
N	180	181	175	192
Mean TL (cm)	18.4	17.8	18.6	18.0
Std. Dev.	1.09	1.33	1.49	1.57
Prey groups				
Polychaeta				
Errantia		<0.01	<0.01	<0.01
Crustacea				
Ostracoda	0.01	0.01	0.01	0.02
Copepoda	56.64	55.48	78.79	88.03
Cladocera	0.01	0.05	0.75	0.83
Mysidacea	5.92	0.95	0.52	0.71
Euphausiacea	<0.01	<0.01		<0.01
Isopoda	0.72	0.03	0.04	0.21
Amphipoda	0.01	<0.01	<0.01	0.01
Natantia		0.01	<0.01	0.01
Brachyura	0.15	0.09	0.30	0.17
Stomatopoda			0.02	0.04
Decapod larvae (unident.)	27.75	22.86	0.15	0.15
Mollusca				
Gastropoda (Mesogastropoda)	0.02	5.63	<0.01	0.01
Bivalvia (Filibranchiata)	8.78	6.61	1.20	9.81
Cephalopoda		0.05		<0.01
Chaetognatha				
<i>Sagitta</i> sp.		0.01		
Osteichthyes				
Eggs and larvae of teleosts	<0.01	8.23	18.23	<0.01

copepod species that occurred in every season were *Corycaeus* sp., *Oncaea media*, *Oncaea* spp., *Isias clavipes*, *Euterpina acutifrons* and *Oithona nana*. *Isias clavipes* occurred only in spring, and all of them appeared in the diet with a %IRI>10. A clear peak of decapod crustacean larvae was observed during winter and spring, which sharply decreased to %IRI<1 in all other seasons. Mollusca was the second most important category except in summer. Teleost larvae were present in the diet throughout the year but the most remarkable quantity was in summer. A comparison of seasonal %IRI and %W values of

major taxonomical groups based on Bray-Curtis index revealed that winter and autumn were 98.98 % and 97.69 % similar whereas the other seasons had an 88.91 % and 90.26 % diet similarity (Table 2, Fig. 1). Following examination of diet composition in relation to fish size, two groups were formed based on weight percentage of food items (Fig. 2). Gravimetric composition of prey ranged from 0.51 to 1.34 g (mean = 1.06 g) for fish lengths of up to 18.0 cm, which increased to a mean prey weight of 2.53 g (1.81–3.51 g) for fishes larger than 18.0 cm, primarily due to a diet shift to teleost larvae.

TABLE 3
Percentage index of relative importance (%IRI) of copepoda species by seasons for *T. mediterraneus*.

Copepoda Species	Winter	Spring	Summer	Autumn	Copepoda Species	Winter	Spring	Summer	Autumn
<i>Acartia clausi</i>	4.012	3.925	-	3.533	<i>Copilia</i> sp.	0.014	0.311	-	-
<i>Acartia latisetosa</i>	-	-	-	0.064	<i>Corycaeus flaccus</i>	0.123	-	-	-
<i>Acartia</i> sp.	0.014	0.011	-	-	<i>Corycaeus limbatus</i>	0.014	-	1.166	-
<i>Calanus</i> sp.	0.123	-	0.014	-	<i>Corycaeus typicus</i>	9.419	3.102	1.020	0.467
<i>Calocalanus pavo</i>	0.014	0.011	0.014	-	<i>Corycaeus brehmi</i>	0.014	0.644	0.170	-
<i>Candacia aethiopica</i>	0.014	-	-	-	<i>Corycaeus giesbrechti</i>	0.341	0.102	-	-
<i>Candacia armata</i>	0.874	0.045	-	-	<i>Corycaeus ovalis</i>	0.014	-	-	-
<i>Candacia simplex</i>	0.055	-	0.014	-	<i>Corycaeus clausi</i>	1.582	-	0.028	-
<i>Candacia</i> sp.	4.955	0.045	0.014	0.016	<i>Corycaeus</i> sp.	16.041	1.265	2.823	0.467
<i>Centropages krøyeri</i>	-	-	0.397	0.398	<i>Corycaeus latus</i>	-	-	0.170	-
<i>Centropages typicus</i>	0.218	8.721	1.133	0.143	<i>Corycella rostrata</i>	9.219	6.301	1.813	0.170
<i>Centropages</i> sp.	-	-	-	0.016	<i>Lubbockia squillimana</i>	0.014	-	-	-
<i>Clausocalanus arcuicornis</i>	0.123	0.011	-	-	<i>Oncaea media</i>	15.084	15.408	27.588	28.502
<i>Clausocalanus furcatus</i>	0.014	0.119	0.057	-	<i>Oncaea mediterranea</i>	4.618	0.311	4.836	0.398
<i>Clausocalanus</i> sp.	0.218	1.187	-	0.085	<i>Oncaea</i> spp.	12.788	8.908	26.280	12.577
<i>Copepod nauplius</i>	-	-	-	0.021	<i>Oncaea venusta</i>	0.123	-	0.944	-
<i>Euchaeta marina</i>	0.014	-	-	-	<i>Oncaea conifera</i>	-	-	-	0.016
<i>Euterpina acutifrons</i>	1.147	11.031	8.867	26.202	<i>Oithona nana</i>	-	0.102	0.014	14.345
<i>Ischnocalanus plumulosus</i>	-	-	0.057	-	<i>Oithona plumifera</i>	-	0.011	0.057	0.016
<i>Isias clavipes</i>	0.014	15.263	0.605	0.064	<i>Oithona</i> sp.	-	2.374	0.014	-
<i>Labidocera wollastoni</i>	-	2.786	-	-	<i>Sapphirina</i> sp.	-	-	0.170	-
<i>Nannocalanus minor</i>	3.494	0.204	0.510	-	<i>Cyclopoida</i> (unident.)	0.014	0.011	-	-
<i>Paracalanus nanus</i>	-	0.119	-	-	<i>Clytemnestra scutellata</i>	0.007	-	-	-
<i>Paracalanus parvus</i>	0.014	0.119	-	0.551	<i>Clytemnestra rostrata</i>	-	-	0.038	-
<i>Paracalanus</i> sp.	0.055	-	-	0.016	<i>Clytemnestra</i> sp.	-	0.011	-	-
<i>Pleuromamma abdominalis</i>	-	-	-	-	<i>Microsetella norvegica</i>	0.027	0.057	0.113	1.604
<i>Pleuromamma</i> sp.	0.014	-	-	-	<i>Microsetella rosea</i>	0.007	-	-	-
<i>Temora stylifera</i>	9.419	8.338	5.301	1.766	<i>Microsetella</i> sp.	0.014	-	0.057	-
<i>Calanoida</i> (unident.)	9.724	9.079	8.265	7.524	<i>Harpacticoida</i> (unident.)	0.014	0.011	3.498	1.041

DISCUSSION

Results from our study clearly reveal that *T. mediterraneus* is a zooplanktophagous species, concurring with earlier findings (FISCHER et al 1987, DEUDERO 2001, ŠANTIĆ et al 2004). A wide range of prey items was found in stomachs but several of them were rarely consumed. Crustaceans clearly dominate the diet in terms of numerical occurrence (92.65 %) but they shared the great portion of the diet together with teleost larvae, as far as gravimetric occurrence is concerned. The difference observed between %Cn and %Cw is due to the fact that Mediterranean horse mackerel frequently fed on planktonic crustaceans, whose total weights were low in comparison with the less frequently ingested teleost larvae. Similar results were obtained in the Adriatic Sea (ŠANTIĆ et al 2004) for *T. mediterraneus*, as well as for the other species of the genus, *T. trachurus* (JARDAS et al 2004). Of the crustaceans, copepods were the most important prey group (78.38) followed by decapod larvae (7.63), while others (Amphipoda, Euphausiacea, Mysidacea etc.) had %IRI < 1. It is striking to note that BEN-SALEM (1988) did not record any copepod species in stomachs of Mediterranean horse mackerel from the Aegean Sea. However, in view of the low number of full stomachs (n=43) found by BEN-SALEM (1988), relevant evidence does not seem comparable. Copepods are known to be abundant all year round in the Aegean Sea (MORAITOU-APOSTOLOPOULOU 1972, STERGIU et al 1997), and they are also of great importance in the diet of several pelagic and semi-pelagic fishes such as *Sardina pilchardus* (SEVER et al. 2005) and *Scomber japonicus* (SEVER et al. 2006). No copepods were observed in the diet of Adriatic Sea specimens of *T. mediterraneus* either, while euphausiids consisted of more than 50 % of the total IRI (ŠANTIĆ et al. 2004). This prey shift between different localities could be a good indication for the ability of *T. mediterraneus* to modify its diet in response to available food items. A sudden change in the feeding habits of the Mediterranean horse mackerel was also reported along the southern Spanish coast due to

the availability of new and abundant food items (FERNANDEZ-JOVER et al. 2007).

In general, the percentage of empty stomachs of *T. mediterraneus* was relatively low when compared to results of previous studies. The VI ranged between 12.77 % and 16.67 % according to season, which is much lower than the overall ratio of 50.50 % observed by Šantić et al. (2004) in the Adriatic Sea. Percentage of empty stomachs was also high in the Gulf of Lyon (32.50 %) while percentages closer to our study were observed at the Tunisian coast (16.46 %) and Aegean Sea (14.50 %) (BEN-SALEM 1988). This difference may be attributed to the favorable environmental conditions in the Aegean Sea supporting feeding intensity of *T. mediterraneus* throughout the year probably with abundant food supplies. There was no prominent variation in seasonal diet in our study. Crustaceans were the primary prey all year round, with lowest %IRI in spring (79.48) and highest in winter (91.20). Molluscs, especially Bivalvia ranked as the second most important category, excluding the summer season when the dominant copepod prey were followed by teleost larvae. In contrast, KYRTATOS (1998) indicated differences in the seasonal feeding habits of the horse mackerel, with a clear decrease of teleost larvae from spring to winter and an increase of copepods, mysids and other crustaceans during the corresponding interval. Little diet variation was observed in Adriatic Sea specimens of *T. mediterraneus* among seasons (ŠANTIĆ et al. 2004). Mean prey weight in stomachs increased with fish size as expected. Food preferences and quantity change with growth and the ability of large mackerels to capture larger prey increases (ŠANTIĆ et al. 2005). In our study, teleost larvae became importance in the diet of Mediterranean horse mackerel with lengths greater than 18 cm, although this prey shift was not very strong even in fish of the maximum length examined in the study. Also other researchers found that larvae and postlarvae of teleosts are important for the diet of *Trachurus* spp. especially for individuals larger than 20 cm (ŠANTIĆ et al. 2004, FERNANDEZ-JOVER et al. 2007). Although total lengths of up to 60 cm have been reported for

the horse mackerel in other studies (for example, FISCHER et al. 1987), it appears unlikely to observe *T. mediterraneus* specimens larger than 40 cm in the Adriatic Sea (ŠANTIĆ et al. 2005).

Nowadays, trophic levels are used for the development of ecosystem-origin fishery management strategies. Depending on the trophic controls that are formed upwards from the bottom of the food chain and downwards from the top of the food chain, the long-term changes of fish stocks through the years and intervals between the low and high stock regimes could be defined (DASKALOV 2002, DASKALOV et al. 2007). As a consequence, the findings obtained from this research will contribute to the ecosystem-origin fishery management that will be conducted in the Aegean Sea.

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Seasonal Changes in the thermal tolerances of *Odontophrynus occidentalis* (BERG, 1896) (Anura: Cycloramphidae)

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ABSTRACT. We studied the thermal tolerances of *Odontophrynus occidentalis* during the dry and wet seasons of the Monte Desert in San Juan Province, Argentina. This toad had differences in CT_{max} between dry and wet seasons, with CT_{max} values being greater in the wet season (Austral summer). Operative temperature, body temperature, and environmental maximal temperature were related to CT_{max} suggesting seasonal acclimatization in *O. occidentalis*. Additionally, the CT_{max} recorded for *O. occidentalis* was 36.1 °C, and the maximum ambient temperature measured during the toads' activity time was 37 °C. The CT_{min} of *O. occidentalis* was 4.1 °C while the minimum environmental temperature recorded was 7.2 °C. The wide tolerance range observed and the relationship between tolerance limits and the environmental extremes indicate that seasonal acclimatization is an effective mechanism by which toads can raise their thermal tolerance. This is a highly relevant adaptation allowing them to survive in the challenging conditions of the Monte Desert.

KEY WORDS: Amphibia; Argentina; Monte Desert; Seasonal Acclimatization; Thermal Tolerance

INTRODUCTION

All organisms possess some capacity to modify their behavioral and physiological characteristics in response to environmental temperature (ANGILLETTA, 2009). The thermal extremes minimum and maximum (critical temperatures) are defined as “the thermal point at which locomotory activity becomes disorganized and an animal loses its ability to escape from conditions that will promptly lead to its death” (COWLES & BOGERT, 1944). The critical thermal maximum (CT_{max}) and critical thermal minimum (CT_{min}) are widely used to define the range of thermal tolerances of animals within their environments (DOUGHTY, 1994; HUEY & STEVENSON, 1979; STEBBINS & COHEN, 1995). In amphibians, thermal tolerances are influenced by hydration (CLAUSSEN, 1969; POUGH & WILSON, 1970),

nutritional status (CUPP, 1980), photoperiod (HUTCHISON, 1961; HUTCHISON & KOSH, 1964; HUTCHISON & FERRANCE, 1970), melatonin (ERSKINE & HUTCHISON, 1982), fatigue (BURKE & POUGH, 1976) and relative humidity (SANABRIA et al., 2012). Also, daily and seasonal fluctuations in environmental temperatures affect variation in the thermal tolerances of many species of ectotherms (ROME et al., 2002). Finally, life-history parameters and reproductive activity also influence temperature tolerances. For example, male toads (*Rhinella arenarum*) have higher CT_{max} in the reproductive season when they are active during the day than in the post-reproductive season when they are active only at night (SANABRIA & QUIROGA, 2011a). This variation in thermal tolerances, being associated with local climatic variation (daily or seasonal) and the organism's natural history, is an

important form of physiological acclimatization (IUPS THERMAL COMMISSION, 2003).

Physiological strategies such as acclimatization enable animals to compensate for, and thus avoid, the consequences of thermal extremes, which can be lethal or cause permanent damage to cells, membranes, and proteins (BRATTSTROM & LAWRENCE, 1962). It is possible that animals do not tolerate temperatures above 50 °C (KAY, 1998) through biochemical restrictions imposed by the denaturation of proteins, as the upper limit that proteins can tolerate before denaturation is between 45–50 °C. All the mechanisms above are associated with critical temperature extremes (HUEY & STEVENSON, 1979; HILL et al., 2006) and influence the survival of ectotherms during brief periods of exposure to potentially lethal temperatures (BRATTSTROM & LAWRENCE, 1962).

The thermal tolerances and acclimatization abilities of South American amphibians – especially those inhabiting deserts – have rarely been studied. We investigated the thermal tolerances of a population of the toad *Odontophrynus occidentalis* in the Monte Desert of San Juan, Argentina. We compared the thermal tolerances (CT_{\max} and CT_{\min}) of *O. occidentalis* between the dry and wet season. In addition, we tested the relationship that CT_{\max} and CT_{\min} have with operative temperatures (T_e) and several abiotic characteristics of the environment. We hypothesized that thermal tolerances of *O. occidentalis* toads change between dry and wet season, and that the thermal tolerance parameters (CT_{\max} and CT_{\min}) are related to abiotic parameters because the toads of the desert experience an acclimatization process during the year.

MATERIAL AND METHODS

Study site

The Quebrada de Las Flores is localized 55 km east of the city of San Juan, Caucete Department, San Juan Province, Argentina (31.70079 °S, 68.09506 °W, elevation: 800 m). The small

saline stream (7710 mS cm⁻¹) that runs through this canyon fluctuates dramatically in rates of daily and seasonal flow. The dominant plants in the area are *Deuterocohnia longipetala*, *Larrea cuneifolia* and *L. divaricata*, *Prosopis* sp., *Typha dominguensis* and *Baccharis salicifolia*. Quebrada de Las Flores lies within the Monte phytogeographic province, a region characterized by an arid climate with a mean annual temperature of 17.3 °C, a mean maximum temperature of 25.7 °C, a mean minimum temperature of 10.4 °C, and a mean annual precipitation of 89 mm falling primarily in summer (CABRERA, 1976).

Measuring of Field Body Temperatures and Environmental Parameters

We compared the thermal biology of toads during the wet and dry seasons from November 2008 to October 2009. The wet season has heavy rains from December to March and the dry season lasts from April to November. We collected individuals haphazardly using the technique of visual encounter (HEYER et al., 2001).

We measured the body temperatures (T_b) of surface-active adult toads ($N = 35$) at the time of capture with a digital thermometer (± 0.1 °C; TES 1312, TES Electrical Electronic Corp., Taipei, Taiwan) by inserting probes (28 AWG type-K catheter probes, TES TP-K01) ~1 cm into the cloaca of each toad. T_b s were measured from August to November (dry season) and December to March (wet season). Thereafter, we measured toad body sizes (snout–vent length, SVL) to the nearest mm with a transparent ruler.

Environmental data, including the maximum and minimum temperature (T_{\max} , T_{\min}), effective heliophany (number of hours of light in the field), and relative humidity (RH) for the study period were obtained from the Instituto Nacional de Técnicas Agropecuarias (INTA), Pocito, San Juan.

Estimating Critical Thermal Maximum and Minimum

Immediately after capture, toads were transported to the laboratory to measure their

thermal tolerances; the measurements were made on 25 toads (9 for the dry season and 16 for the wet season). Critical thermal maximum (CT_{max}) was determined using the methods described by HUTCHINSON (1961). Individual toads were placed in a glass container (15 x 30 cm) with 300 ml of water at ambient temperature (25 ± 2 °C). A catheter probe (TES TP-K01) was inserted ~1 cm into the cloaca to measure T_b . The catheter probe was fixed into the cloaca with a drop of instant glue (La Gotita, Poxipol®, Buenos Aires, Argentina). The toads were kept in captivity for 2 d during which the probes detached on their own. An electric heating mantle raised the toad's temperature at a rate of approximately 1 °C min^{-1} . The righting reflex was checked at regular intervals of two minutes after the toads showed a voluntary escape behavior when being turned on their backs. When the righting reflex was lost, CT_{max} was recorded.

After the measurement of the critical temperature (CT_{max}), the toads were placed in individual terrariums (40x20x30) with free access to water and food at ambient temperature. After 72 h, we measured the CT_{min} . To determine CT_{min} , we followed the same procedure described above for CT_{max} except that we placed the glass container inside a larger plastic container containing water and ice, which reduced the toad's temperature at a rate of approximately 1 °C min^{-1} . The T_b at which the righting reflex was lost was recorded as the animal's CT_{min} .

All measurements were taken at night, since the natural activity period of this toad species is nocturnal.

Construction and Calibration of Plaster Operative Temperature Models

Four plaster models were constructed using methods described by Tracy et al. (2007). We sacrificed two adult male *O. occidentalis* (mean SVL = 52 ± 0.5 mm), by injecting an overdose of anesthesia (2.5 ml of 2% xylocaine and 2% lidocaine HCl, AstraZeneca Labs, Bs. As., Argentina) into their lymphatic sacs. Toads were

fixed in their water-conservation posture with 10% formaldehyde (formalin) for 3 d. Thereafter, the toads were placed in acetic silicone (Dow Corning, Bs. As., Argentina) to create a flexible matrix. This matrix was filled with a preparation of plaster (30% plaster of Paris and 70% water, by volume) to obtain replicas of the specimens. To prevent dehydration, a water source was connected to the models by inserting a silicone tube (outer and inner diameters 4 mm and 2 mm, respectively) into the left side of the toad model until the core was reached and the tube was connected to a 1-L plastic bottle filled with distilled water. Capillary action kept the models hydrated. In addition, we inserted a thermistor from a data logger (Hobo H8, Onset Computer Corporation, USA) into the models to record their temperature every 5 min.

The four models were calibrated by comparing them to one male toad (SVL=53 mm). This toad was anesthetized with 0.5 ml of 2% xylocaine and 2% lidocaine HCl (AstraZeneca Labs, Buenos Aires, Argentina). The calibration was performed under field conditions T_e models remained in the field for 3 d mo^{-1} . T_e (sensu: BAKKEN, 1992) was recorded every 5 min. We only used T_e measurements that were recorded during the daily activity periods of the toads.

Data Analyses

We calculated the mean and standard error for all thermal data, and analyzed relationships using non-parametric Spearman correlations. We applied the Mann–Whitney U test to evaluate differences between the dry and wet seasons and to evaluate the differences in thermal parameters between the species studied. We used non parametric statistical analysis, because the data were not normally distributed and there was no homogeneity in the variance. The PAST version 9.4 (HAMMER, 2001) statistical packet was used for statistical analysis.

RESULTS

Calibrations of Plaster Operative Temperature Models

We obtained significant regression coefficients ($r^2 = 0.98$, $P = 0.00001$) for the comparisons of model and toad temperatures for the calibration. The slight difference in temperature between the model and the T_b of the toad used for calibration (0.4 ± 0.2 °C) was not statistically significant ($U = 191.5$, $P > 0.81$).

Operative Temperatures and Environmental Parameters

The mean of T_e was measured as 18.31 ± 0.4 °C (range: 14.5–21.3 °C). The mean environmental thermal maximum (T_{max}) reached 30.5 ± 0.8 °C (range: 14.4–37.0 °C) and the mean environmental thermal minimum (T_{min}) 15.6 ± 0.8 °C (range: 7.2–21.0 °C). The effective heliophany had a mean of 10.6 ± 0.3 h (range: 6.8–12.4 h) and the mean relative humidity was $55.9 \pm 0.73\%$ (range: 47–64%).

Relationship Between Thermal Tolerances and Environmental Parameters

The mean tolerance range of *O. occidentalis* was 30.17 ± 0.26 °C (wet season) and 30 ± 0.56 °C (Dry season), not showing any significant

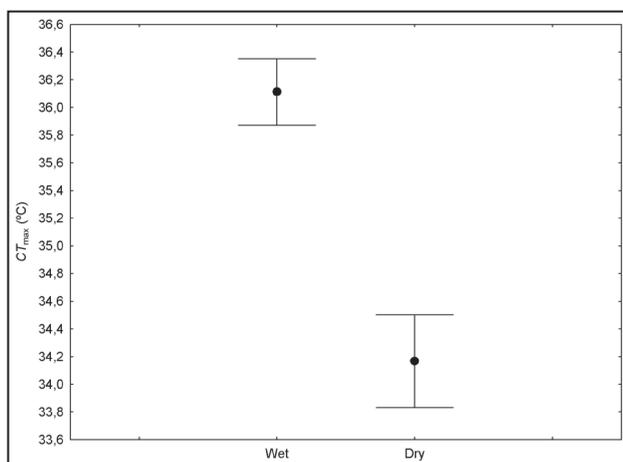


Fig. 1. – Variation of critical thermal maximum between wet and dry season, for *Odontophrynus occidentalis* from the Monte Desert of Argentina.

differences between seasons ($U = 40.5$, $P > 0.1$). CT_{max} was significantly higher in the wet (36.1 ± 0.2 °C) than in the dry seasons (34.1 ± 0.3 °C) ($N = 25$, $U = 10.5$, $P < 0.00004$; Fig. 1). CT_{max} was significantly correlated to T_b ($r = 0.45$, $P < 0.02$; Fig. 2) as well as to T_e ($r = 0.53$, $P < 0.006$; Fig. 2), T_{max} ($r = 0.44$, $P < 0.02$; Fig. 3) and T_{min} ($r = 0.45$, $P < 0.02$; Fig. 3). No relationship was found with the other variables such as SVL ($P > 0.3$) or effective heliophany ($P > 0.3$) and RH ($P > 0.2$).

The CT_{min} of *O. occidentalis* was significantly higher in the wet (4.9 ± 0.1 °C) than in the dry seasons (4.1 ± 0.4 °C) ($N = 35$, $U = 54$, $P < 0.002$; Fig. 4). Also CT_{min} was significantly correlated with T_b ($r = 0.41$, $P < 0.01$) but no significant relationship was found between CT_{min} and SVL ($P > 0.1$), T_{min} ($P > 0.15$), T_e ($P > 0.12$), T_{max} ($P < 0.3$), effective heliophany ($P > 0.9$), or RH ($P > 0.2$).

DISCUSSION

Odontophrynus occidentalis showed a different CT_{max} in the dry compared to the wet seasons, and the CT_{max} values were greater in the wet season (Austral summer). In addition, T_e , T_b , T_{max} , and T_{min} were significantly related to CT_{max} . This suggests that the acclimatization process (HUTCHISON & MANESS, 1979) could be mainly

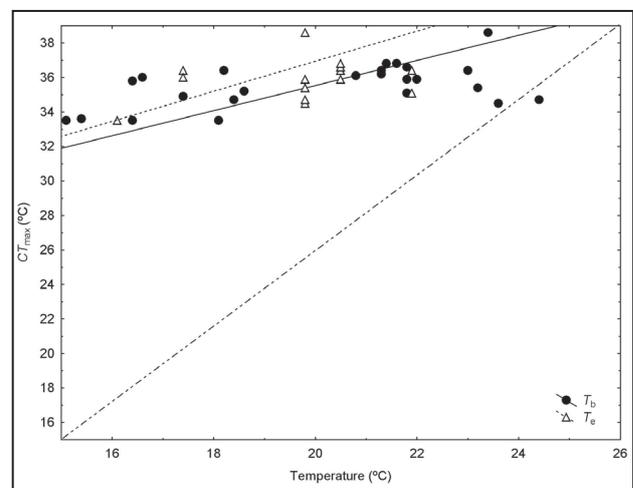


Fig. 2. – Relationship between the critical thermal maximum and body (white dots) and operative (black dots) temperatures for *Odontophrynus occidentalis* from the Monte Desert of Argentina.

regulated by environmental conditions. The four variables (T_e , T_b , T_{max} , and T_{min}) have an important role in the acclimatization of the toads. Thermal tolerances (CT_{min} and CT_{max}) vary with changes in local weather conditions where amphibians develop (BRATTSTROM, 1968; HUTCHISON, 1961; HUTCHISON & KOSH, 1964; HUTCHISON & FERRANCE, 1970; ROME et al., 2002) and with the physiological condition of the animals (BURKE & POUGH, 1976; CLAUSSEN, 1969; CUPP, 1980; ERSKINE & HUTCHISON, 1982). It is likely that the changes in temperature between the dry and wet seasons are the environmental cues stimulating seasonal thermal acclimatization in *O. occidentalis*. *Rhinella arenarum* at the same latitude exhibits an acclimatization process, where the climatic parameters are a principal modulator (SANABRIA et al., 2012). In contrast, changes in photoperiod have been suggested to be the principal acclimatization cue for other ectotherms such as salamanders, turtles, and the frog *Lithobates pipiens* (HUTCHISON & KOSH, 1964; HUTCHISON, 1961; HUTCHISON & FERRANCE, 1970). Photoperiod affects the acclimation of some amphibians, especially anurans with diurnal activity that gain heat energy via basking (BRATTSTROM, 1968; DUELLMAN & TRUEB, 1986). Apparently, in adults of *O. occidentalis*, which are nocturnal, photoperiod is probably not significant for acclimatization,

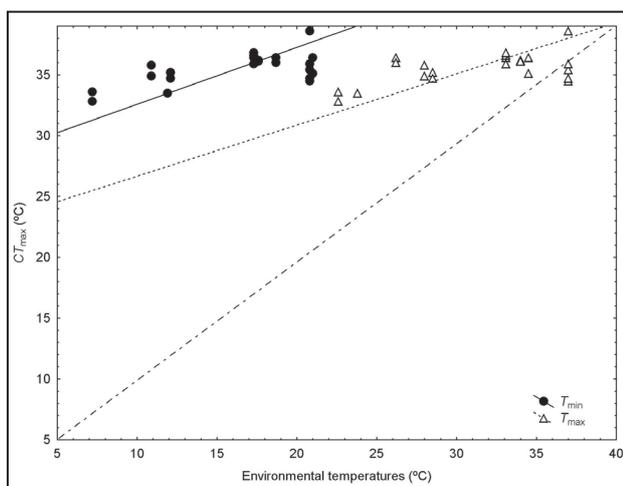


Fig. 3. – Relationship between the critical thermal maximum and environmental thermal maximum (black dots) and minimum (white dots) for *Odontophrynus occidentalis* from the Monte Desert of Argentina.

because there was no association between effective heliophany and thermal tolerances. However, the tadpoles of *Odontophrynus occidentalis* have a long larval period prior to metamorphosis with diurnal activity. This tadpole shows changes in the thermal parameters in relation to the photoperiod. Apparently, in this condition, the photoperiod is a modulator of acclimatization and confers more tolerance to environmental temperature with an increase in hours of light (SANABRIA & QUIROGA, 2011b).

The critical thermal minimum has not been widely investigated in amphibians, although there have been a few anecdotal studies (DUELLMAN & TRUEB, 1986). We found the CT_{min} of *O. occidentalis* to be significantly different between wet and dry seasons. Our data furthermore allows us to calculate the thermal tolerance range of the species in the dry and wet seasons. *O. occidentalis* showed no significant differences in mean values of thermal ranges during the wet and dry seasons. Ecologically, the thermal tolerance range is considered as the safety margin of a species, allowing it to escape extreme temperatures (ANGILLETTA, 2009; HUEY & STEVENSON, 1979). Species that have wide thermal tolerance ranges are generally associated with environments that have strong seasonal fluctuations in temperature (NAVAS et al., 2008), such as deserts (WARNER, 2004).

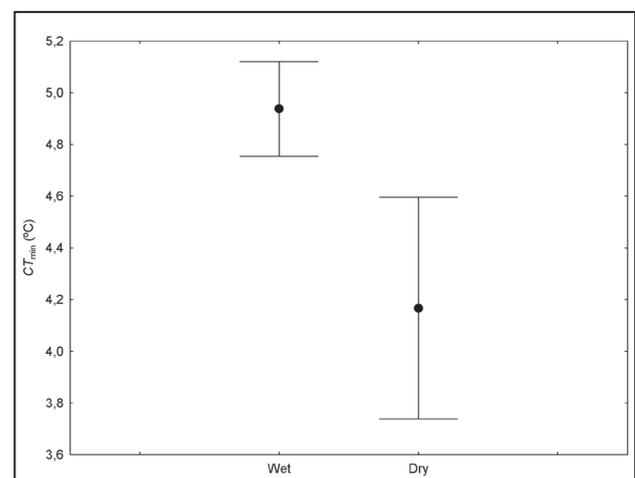


Fig. 4. – Variation of the critical thermal minimum between the wet and dry season for *Odontophrynus occidentalis* from the Monte Desert of Argentina.

Studies of desert-dwelling anuran larvae, where high temperatures are common, have proven that these taxa possess high thermal tolerances (BROWN, 1969; CUPP, 1980; HOPPE, 1978; ULTSCHetal., 1999; WHITFORD, 2002). In contrast, tropical frogs living in aseasonal environments do not have to undergo acclimatization because temperatures are relatively constant throughout the year (DUELLMAN & TRUEB, 1986; NAVAS, 1997). Having wide thermal tolerance ranges allows a species to quickly acclimatize to extreme temperatures and thus avoid the generation of heat shock proteins and the energetic costs of their production (ANGILLETTA, 2009; HILL et al., 2006).

The toads in this study were found to have extreme temperature tolerances close to daily minimum (T_{\min}) and maximum (T_{\max}) environmental temperatures. This indicates that the species studied here became adapted to extreme daily temperature fluctuations. The wide thermal range detected and the relationship with the environmental parameters (acclimatization) is an efficient mechanism by which toads can raise thermal tolerance (BRATTSTROM, 1963). However, further studies are required to understand the relationship between the thermal extremes of amphibians of the desert and the environmental parameters that influence the thermal physiology of these species. Additional studies are needed to understand the relationship between the thermal tolerances of this desert amphibian and the environmental parameters that influence its thermal physiology.

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Soil mite communities (Acari: Gamasina) from different ecosystem types from Romania

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ABSTRACT. The study aimed to characterize the distribution of soil mite populations (Acari: Gamasina) from several terrestrial ecosystems, some of them little studied in Romania as well as in Europe. We studied the Gamasina communities in seven ecosystem types from the Doftana Valley, Prahova County: *Luzulo-Fagetum* beech forest; Medio-European limestone beech forest of the *Cephalanthero-Fagion*; Pannonic woods with *Quercus petraea* and *Carpinus betulus*; Galio-carpinetum oak – hornbeam forests; Alpine rivers and their ligneous vegetation with *Myricaria germanica*; alluvial shrub, characteristic for a hilly-mountain area, with *Salix purpurea*; adjacent area to an inland cliff ecosystem. A total of 240 soil samples, 63 species and 475 individuals were analysed. The samples were taken from May 2006 till September 2010. The highest numerical abundance and species diversity was found in the area located nearby to an inland cliff, which is an early succession stage ecosystem. Considering the dominance and constancy indices quantified for mites from all investigated areas, the highest percent was recorded by the recedent-subrecedent and accessory-accidental species. Based on similarity index two different characteristic groups of gamasid populations were delimited: one for deciduous forests and one for shrubs. DCA ordination successfully handled the variation in soil mite species communities taking account of the soil type.

KEY WORDS: similarity, population, mite, dominance, constancy.

INTRODUCTION

Gamasina mites are very dynamic, free living organisms. Being mostly predators, they are permanently in search of food and favourable habitats for their development. As predators, they do not change the soil structure or plant productivity directly, but have an indirect effect on ecosystem functions by influencing the populations of other organisms (KOEHLER, 1997, 1999; GULVIK, 2007; SALMANE & BRUMELIS, 2008).

Although the response of the soil mite communities to specific environmental conditions characterizing various ecosystem

types (e.g., forest, grassland, arable field, sand dunes, etc.), quantified in terms of changes in population parameters (abundance, species diversity, distribution, dominance, frequency), has been examined, little information is available for Gamasina populations from shrub and cliff ecosystems (KOEHLER, 1997, 1999; MINOR & CIANCIOLO, 2007; GWIAZDOWICZ, 2007; BEDANO & RUF, 2007, 2010; SALMANE & BRUMELIS, 2010).

In Romania, some preliminary data from the studied area have been reported previously (VASILIU OROMULU et al., 2008; PAUCĂ-COMĂNESCU et al., 2009; MANU, 2008, 2009, 2010, 2011). Other research regarding the

Gamasina fauna from hilly forests has been carried out in Moldavia (CĂLUGĂR, 2001-2003, 2004-2006). All these studies provided fragmented information concerning soil mite communities from different types of ecosystems. The main aim of this paper is to present the distribution of soil mite populations (Acari: Gamasina) from several terrestrial ecosystems, some of them little studied in Romania, as well as in Europe (as in the case of shrubs and cliffs) and to highlight the similarities or dissimilarities between them.

MATERIALS AND METHODS

The research was done in seven ecosystem types from the Doftana Valley, Prahova County, Romania, during 2006-2010. According to the Natura 2000 classification, the following ecosystems types were investigated: 9110 *Luzulo-Fagetum* beech forest (BF); 9150

Medio-European limestone beech forest of the *Cephalanthero-Fagion* (BC); 91G0 Pannonic woods with *Quercus petraea* and *Carpinus betulus* (QC); 9170 galio-carpinetum oak – hornbeam forests (GCO); 3230 Alpine rivers and their ligneous vegetation with *Myricaria germanica* (AR); alluvial shrub, characteristic for a hilly-mountain area, with *Salix purpurea* (R 4418) (AS); adjacent area to an inland cliff ecosystem in early succession stage (more than 20% are pioneer species, as: *Cytisus nigricans*, *Rosa canina* and *Hippophaë rhamnoides*) (IC) (DONIȚĂ et al., 2005). All areas belong to the Romanian Subcarpathians (Fig. 1). A brief description of the studied ecosystems, based on Paucă- Comănescu et al., 2008 and Onete et al., 2011, is presented in Table 1.

In total, 240 soil core samples were collected with a MacFadyen corer (5 cm diameter), to 10 cm depth. The distance between ecosystems

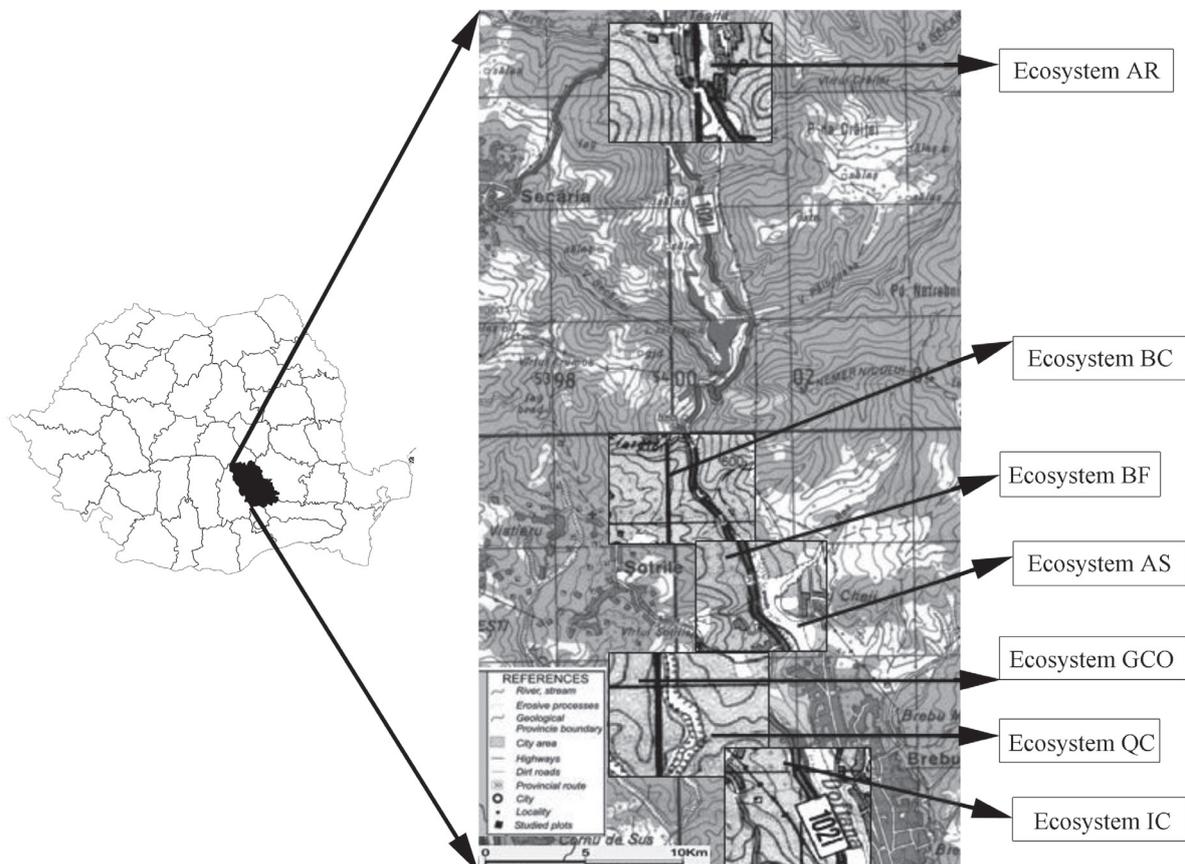


Fig. 1. – Location of the studied ecosystems in the Doftana Valley, Prahova County, Romania.

Table 1

Description of the investigated ecosystems from the Doftana Valley.

Ecosystem	BF	BC
Geographical coordinates	N: 45°13'39.67"; E: 25°43'48.61"	N: 45°11'27.09"; E: 25°44'50.24"
Altitude	600 m	490 m
Slope	30°	40°
Type of soil	Acid brown, brown iron-alluvial podzols, oligo and oligomesobasic, with moderate and low humidity, middle to deep clayey-sandy, with small-middle edaphic volume.	The humid – calcareous soil is not uniform in structure, varying from calcicolous, clayey till argillaceous or with mull humus. It has a good airflow and drainage.
Conservative value	Moderate	Moderate
Ecosystem	QC	GCO
Geographical coordinates	N: 45°09'49.46; E: 25°45'06.62"	N: 45°09'45.62; E: 25°44'52.60"
Altitude	500 m	490 m
Slope	25°	flat
Type of soil	Alluvial, with the upper 25 cm moderate in humus, middle trophicity; water is permanently available.	Brown eumesobasic, rich in humus, sandy-clay texture, well structured on humus layer, good drainage, low acidity on superior level.
Conservative value	Moderate	Moderate
Ecosystem	AR	AS
Geographical coordinates	N: 45°16'56.38"; E: 25°45'35.08"	N 45° 20'40,1" E 25°74'51,3
Altitude	663 m	485 m
Slope	flat	flat
Type of soil	Sandy-clay, with an increased humidity.	Sandy-clay, with an increased humidity.
Conservative value	High: Emerald protection	High: Emerald protection
Ecosystem	IC	
Geographical coordinates	N: 45° 12' 31,1"; E: 25° 44' 23,5"	
Altitude	537 m	
Slope	50° – 70°	
Type of soil	Three classes: clayey till argillaceous on the moderate and strongly inclined peaks, which are seriously affected by erosion; brown eumesobasic till pseudogleic, which have a mineral component formed at the soil surface, connected to a thin humified organic matter layer; typically alluvial soil.	
Conservative value	Moderate	

varied from 1 km to 25 km. Samples were taken randomly. The extraction was performed with a modified Berlese-Tullgren extractor, in ethanol and the mite samples were clarified in lactic acid. All mites were identified to species level.

The statistical analysis was conducted with the aid of the BioDiversity Pro 2.0 software, designed and developed by Neil McAleece and provided by The Natural History Museum, London. The Jaccard similarity index (q) was calculated using the formula: $q = c / (a + b - c)$, where a = number of species from ecosystem A; b = number of species from ecosystem B; c = number of species shared between ecosystems A and B.

Using statistical information, dominance was calculated, according to Engelmann's dominance classification (ENGELMANN, 1978).

The dominance was calculated using the formula:

$D = 100 * n / N$, where:

n - number of individuals of one species from one sample;

N - total number of individuals of all species from one sample.

The dominance classes for the identified mites were:

eudominants = over 10% (D5); dominants = 5.1-10% (D4); subdominants = 2.1 – 5% (D3); recedents = 1.1- 2% (D2) and subrecedents \leq 1.1% (D1).

The constancy was determined following the methodology of Skorupski et al. (2009) and obtained as:

$C = 100 * pA / P$, where:

pA = number of samples with species A;

P = total number of samples.

The mite species were classified in four constancy classes: euconstants = 75.1-100% (C4), constants = 50.1-75% (C3), accessory = 25.1-50% (C2) and accidental = 1-25% (C1).

We used detrended correspondence analysis

(DCA) to investigate spatial variation of soil mite community structure. DCA is an indirect eigenvector ordination technique based upon reciprocal averaging, which corrects for the 'arch effect' observed in correspondence analysis, and displays effectively primary gradients within communities. Pearson product-moment correlations were used to determine if DCA axes scores were correlated with any of the environmental variables (GAUSCH, 1982; LEGENDRE & LEGENDRE, 2000). The following types of soils, as variables, were investigated (M (mixt) - SC (sandy clay) - BE (brown eumezobasic) – HC (humid calcaros) – A (alluvial) – AB (acid brown). The scales used are different to facilitate interpretation, and a selection of species and sites is shown to facilitate visualization. Species codes are given in Table 4.

We also used canonical correspondence analysis (CCA) to investigate associations between soil mite communities and environmental variables: habitat type (Ht), altitude (Alt), slope (Spl) and soil type (St) (TER BRAAK & VERDONSCROT, 1995; LEGENDRE & LEGENDRE, 2000). CCA is a multivariate direct ordination technique, which extracts synthetic environmental gradients that maximize niche separation within communities, thereby facilitating the interpretation of how species abundances relate to environmental variables. Non-transformed species abundance data and all four environmental variables for each sample were included in the analysis.

CCA and DCA were carried out using the vegan package in R version 1.8.1 (R DEVELOPMENT CORE TEAM, 2003; OKSANEN et al., 2011).

RESULTS

A total of 475 Gamasina mites were counted, belonging to 63 species. 45.03% of the species were only found in a single ecosystem 4 (Table 2).

The graph analysing cumulative abundance (%) plotted against species rank revealed differences

Table 2

Gamasina species identified in the forest ecosystems from the Doftana Valley (ecosystems BF, BC, QC, GCO).

Species	BF		BC		QC		GCO	
	D%	C%	D%	C%	D%	C%	D%	C%
<i>Alloparasitus oblongus</i> Halbert, 1915	-	-	-	-	1.22	10	-	-
<i>Amblyseius</i> sp.	-	-	1.61	10	-	-	-	-
<i>Arctoseius cetratus</i> Sellnicki, 1940	9.09	20	-	-	-	-	-	-
<i>Asca bicornis</i> Canestrini & Fanzago, 1887	-	-	-	-	1.22	10	-	-
<i>Cheroseius</i> sp.	3.03	10	-	-	-	-	-	-
<i>Cheroseius viduus</i> C.L.Koch, 1839	-	-	-	-	-	-	7.94	30
<i>Dendrolaelaps willmanni</i> Hirschmann, 1963	-	-	-	-	2.44	10	-	-
<i>Epicrius mollis</i> Kramer, 1876	1.52	10	-	-	-	-	-	-
<i>Eviphis ostrinus</i> C.L.Koch, 1836	-	-	-	-	-	-	1.59	10
<i>Geholaspis mandibularis</i> Berlese, 1904	-	-	-	-	1.22	10	-	-
<i>Holoparasitus calcaratus</i> C.L.Koch, 1839	-	-	-	-	1.22	10	-	-
<i>Hypoaspis aculeifer</i> Canestrini, 1883	15.15	40	8.06	30	-	-	1.59	10
<i>Hypoaspis brevipilis</i> Hirschmann, 1969	-	-	-	-	2.44	20	-	-
<i>Leptogamasus parvulus</i> Berlese, 1903	-	-	3.22	20	6.10	30	6.35	30
<i>Leptogamasus variabilis</i> Juvara-Balș, 1981	-	-	1.61	10	-	-	-	-
<i>Lysigamasus conus</i> Karg, 1971	3.03	10	-	-	-	-	-	-
<i>Lysigamasus lapponicus</i> Tragardh, 1910	3.03	10	9.67	40	2.44	20	15.87	50
<i>Lysigamasus misellus</i> Berlese, 1903	-	-	-	-	-	-	1.59	10
<i>Lysigamasus neoruncatellus</i> Schweizer, 1961	-	-	-	-	3.66	30	-	-
<i>Lysigamasus truncus</i> Schweizer, 1961	-	-	1.61	10	1.22	10	-	-
<i>Macrocheles carinatus</i> C.L.Koch, 1839	-	-	-	-	3.66	10	-	-
<i>Macrocheles decolotaus</i> C.L. Koch, 1893	-	-	-	-	1.22	10	-	-
<i>Macrocheles montanus</i> Willmann, 1951	3.03	10	-	-	3.66	20	1.59	10
<i>Olopachys suecicus</i> Sellnick, 1950	-	-	1.61	10	6.10	50	1.59	10
<i>Olopachys vysockajae</i> Koroleva, 1976	-	-	-	-	1.22	10	-	-
<i>Pachylaelaps furcifer</i> Oudemans, 1903	-	-	-	-	-	-	6.35	30
<i>Pachyseius humeralis</i> Berlese, 1910	3.03	10	-	-	-	-	-	-
<i>Pergamasus crassipes</i> Linne, 1758	-	-	1.61	10	-	-	-	-
<i>Pergamasus longicornis</i> Berlese, 1906	-	-	-	-	-	-	1.59	10
<i>Pergamasus quisquiliarum</i> R & C. Canestrini, 1882	-	-	-	-	1.22	10	9.52	40
<i>Prozercon fimbriatus</i> C.L.Koch, 1839	33.33	50	14.52	50	7.32	20	11.11	40
<i>Prozercon rafalski</i> Blaszak, 1971	-	-	-	-	3.66	30	-	-
<i>Prozercon</i> sp.	-	-	3.22	20	-	-	-	-
<i>Prozercon traegardhi</i> Halbert, 1923	15.15	10	11.29	30	14.63	60	3.17	20
<i>Pseudolaelaps doderoi</i> Berlese, 1910	-	-	11.29	30	-	-	-	-
<i>Rhodacarellus perspicuus</i> Halaskova, 1958	-	-	-	-	-	-	1.59	10
<i>Veigaia exigua</i> Berlese, 1917	6.06	10	4.84	20	-	-	-	-
<i>Veigaia nemorensis</i> C.L. Koch, 1839	3.03	10	22.58	60	23.17	80	23.81	70
<i>Vulgarogamasus zschokkei</i> Schweizer, 1922	-	-	-	-	-	-	3.17	10
<i>Zercon fageticola</i> Halaskova, 1969	3.03	10	-	-	3.66	30	-	-
<i>Zercon peltatus</i> C.L. Koch, 1836	-	-	-	-	7.32	40	1.59	10

between mite communities from the investigated ecosystems. The highest values were recorded for mite communities from ecosystem IC, while the lowest were from ecosystem BF. In the other ecosystems species had smaller abundance decreasing from QC to AR to GCO to BC to AS (Fig. 2).

Taking account of the dominance index quantified for these invertebrates in forests, the highest numbers of eudominant and dominant species were recorded in ecosystem BC (28.57% and 14.28%, respectively of the total number of identified soil mites) and the lowest in ecosystem QC (9.09% and 18.18%, respectively). These species were: *Lysigamasus lapponicus* (Tragardh, 1910), *Pseudolaelaps doderoi* (Berlese, 1910), *Veigaia nemorensis* (C.L.Koch, 1839), *Cheroseius viduus* (C.L.Koch, 1839), *Hypoaspis aculeifer* (Canestrini, 1883), *Prozercon fimbriatus* (C.L.Koch, 1839), *Prozercon traegardhi* (Halbert, 1923), *Zercon peltatus* (C.L.Koch, 1836). The most numerous subrecedent species were found in ecosystem GCO (45.08%). In ecosystem BF no recedent or subrecedent species were recorded. Analysis of the constancy index revealed *Veigaia nemorensis* as a constant species in ecosystems BC and GCO, and as a euconstant species in ecosystem

QC. The majority of the identified mites were classified as accessory and accidental species (75% - 16.67%) (Table 2).

The highest numbers of eudominant and dominant species were recorded in ecosystem AR, shrub with *Myricaria germanica* (16.67% and 25%, respectively) and the lowest in ecosystem IC, adjacent area of the cliff (2.94% and 1.47%, respectively). In the shrub ecosystem, these species were: *Asca bicornis* (Canestrini and Fanzago, 1887), *Hypoaspis aculeifer*, *Pachyseius humeralis* (Berlese, 1910), *Lysigamasus conus* (Karg, 1971), *Lysigamasus lapponicus*, *Macrocheles carinatus* (C.L. Koch, 1839) and in adjacent area to the cliff: *Geholaspis mandibularis* (Berlese, 1904), *Prozercon traegardhi* (Halbert, 1923), *Veigaia nemorensis*, *Veigaia planicola* (Berlese, 1905). 78.18% of the total number of species from shrub with *Salix purpurea* (ecosystem AS) were recedents and subrecedents. *Veigaia nemorensis*, *Veigaia planicola* and *Prozercon traegardhi* were identified as euconstant species, *Leptogamasus obesus* (Holtzmann, 1969) as constant, all of them found in ecosystem IC. The shrub ecosystems (AR and AS) were characterized only by accessory (8.33% in ecosystem AR; 33.33% in ecosystem AS) and accidental species

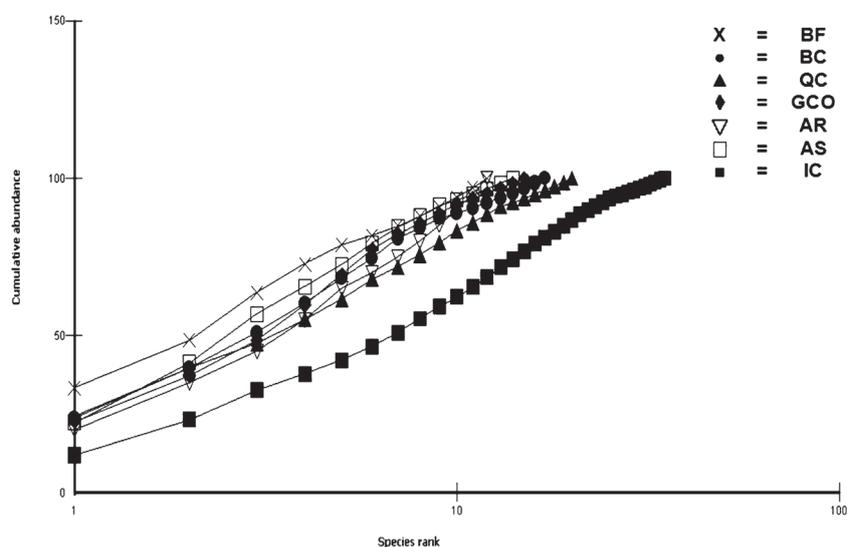


Fig. 2. – Cumulative abundance (%) of the identified Gamasina species from the investigated ecosystems.

Table 3

Gamasina species identified in the shrub and adjacent area to the cliff ecosystems from the Doftana Valley (ecosystems AR, AS, IC).

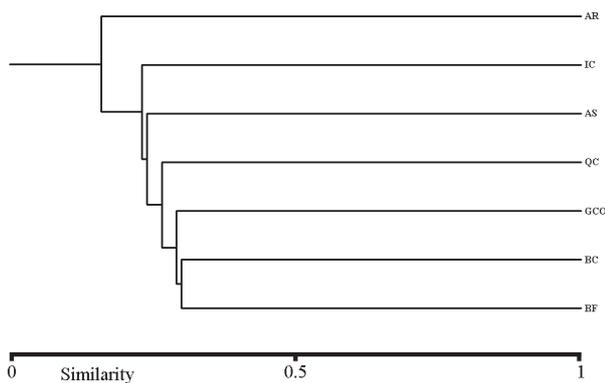
Species	AR		AS		IC	
	D%	C%	D%	C%	D%	C%
<i>Arctoseius resinae</i> Karg, 1969	-	-	-	-	0.63	10
<i>Asca bicornis</i> Canestrini & Fanzago, 1887	20	30	-	-	0.63	10
<i>Eviphis ostrinus</i> C.L.Koch, 1836	-	-	3.45	20	1.89	30
<i>Geholaspis longisetosus</i> Balogh, 1958	-	-	-	-	1.89	20
<i>Geholaspis mandibularis</i> Berlese, 1904	-	-	18.97	50	5.03	50
<i>Hypoaspis aculeifer</i> Canestrini, 1883	10	10	15.52	50	1.26	20
<i>Hypoaspis milles</i> Berlese, 1892	-	-	3.45	10	-	-
<i>Hypoaspis</i> sp.	10	20	-	-	-	-
<i>Leptogamasus obesus</i> Holtzmann, 1969	-	-	-	-	4.40	70
<i>Leptogamasus</i> sp.	-	-	5.17	20	-	-
<i>Lysigamasus conus</i> Karg, 1971	15	20	-	-	-	-
<i>Lysigamasus neoruncatellus</i> Schweizer, 1961	5	10	-	-	0.63	10
<i>Lysigamasus</i> sp.	-	-	1.72	10	0.63	10
<i>Lysigamaus lapponicus</i> Tragardh, 1910	-	-	6.90	20	0.63	10
<i>Macrocheles carinatus</i> C.L.Koch, 1839	-	-	8.62	30	-	-
<i>Macrocheles matrius</i> Hull, 1925	-	-	-	-	2.52	30
<i>Macrocheles montanus</i> Willmann, 1951	-	-	1.72	10	1.26	20
<i>Macrocheles</i> sp.	-	-	-	-	3.14	30
<i>Olopachys suecicus</i> Sellnick, 1950	-	-	-	-	1.26	20
<i>Olopachys vysotskajae</i> Koroleva, 1976	-	-	-	-	1.26	20
<i>Pachylaelaps dubius</i> Hirschmann & Krauss, 1965	-	-	-	-	0.63	10
<i>Pachylaelaps furcifer</i> Oudemans, 1903	5	10	1.72	10	4.40	50
<i>Pachylaelaps pectinifer</i> R & C. Canestrini, 1882	-	-	-	-	0.63	10
<i>Pachyseius humeralis</i> Berlese, 1910	10	20	-	-	3.14	20
<i>Parasitellus</i> sp.	-	-	-	-	0.63	10
<i>Pergamasus barbarus</i> Berlese, 1904	-	-	1.72	10	1.89	30
<i>Pergamasus longicornis</i> Berlese, 1906	-	-	-	-	2.52	30
<i>Pergamasus quisquiliarum</i> R & C. Canestrini, 1882	5	10	-	-	-	-
<i>Poecilochirus carabi</i> G. & R. Canestrini, 1882	-	-	-	-	0.63	10
<i>Prozercon carsticus</i> Halaskova, 1963	-	-	-	-	4.40	50
<i>Prozercon</i> sp.	5	10	-	-	-	-
<i>Prozercon traegardhi</i> Halbert, 1923	-	-	-	-	9.43	80
<i>Pseudolaelaps doderoi</i> Berlese, 1910	5	10	-	-	2.52	40
<i>Rhodacarellus perspicuus</i> Halaskova, 1958	5	10	-	-	-	-
<i>Rhodacarus denticulatus</i> Berlese, 1921	5	10	-	-	-	-
<i>Veigaia exigua</i> Berlese, 1917	-	-	6.90	30	3.14	40
<i>Veigaia nemorensis</i> C.L. Koch, 1839	-	-	22.41	50	11.95	100
<i>Veigaia planicola</i> Berlese, 1892	-	-	-	-	11.32	80
<i>Veigaia propinqua</i> Willmann, 1936	-	-	-	-	1.89	10
<i>Vulgarogamasus kraepelini</i> Berlese, 1905	-	-	-	-	1.89	20
<i>Zercon foveolatus</i> Halaskova, 1969	-	-	-	-	4.40	50
<i>Zercon hungaricus</i> Sellnick, 1958	-	-	-	-	3.14	10
<i>Zercon peltadoides</i> Halaskova, 1970	-	-	-	-	3.77	40
<i>Zercon triangularis</i> C.L.Koch, 1836	-	-	1.72	10	-	-

Table 4

Pearson product-moment correlations between DCA axes site scores and environmental variable.

	Habitat type	Altitude	Slope	Soil type
DCA1	-0.422**	0.188	0.05	-0.450**
DCA2	-0.394**	-0.255*	0.081	-0.382**

* p < 0.5, ** p < 0.01



(91.67% in ecosystem AR; 66.66% in ecosystem AS) (Table 3).

The highest values of the Jaccard similarity index (q), which considers the species abundance from each investigated ecosystem, were recorded between mite populations from ecosystems BC and BF (q= 0.30), GCO-BC (q= 0,29) and GCO-QC (q= 0.26). In addition, the pairs of ecosystems AS-IC (q=0.23), AS-BF (q= 0.238) and AS-GCO (q= 0.24) had close values of the similarity index (Fig. 3).

Fig. 3. – Dendrogram depicting similarities between the Gamasina communities between the seven investigated ecosystem types.

The first axis of the DCA ordination accounted for 26.6% of the variance in soil mite community

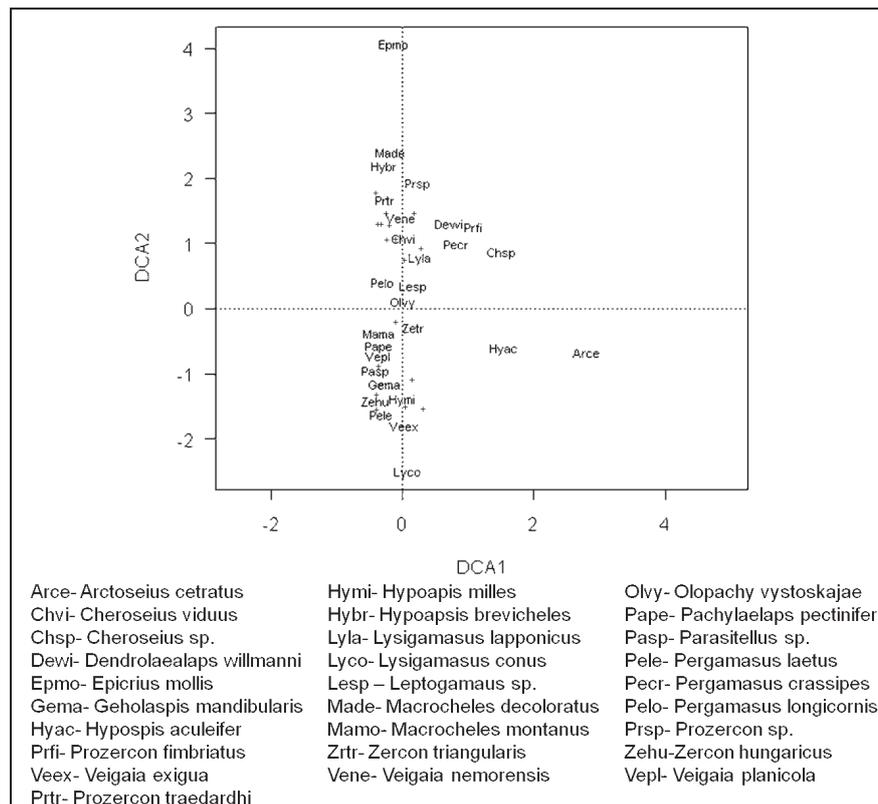


Fig. 4. – DCA ordination of soil mite species on the first two axes.

data (eigenvalue = 0.20) with a gradient length of 1.5 standard deviation units (Fig. 4). Site scores along axis one of the DCA ordination diagram clustered along the type of soil gradient. The second axis explained 14.42% of the variance (eigenvalue = 0.14) with a gradient length of 1.8 standard deviation units and represented an altitude gradient (high to low) (Fig. 5). DCA axis 1 was negatively correlated with soil type and habitat type whereas DCA axis 2 was most correlated with altitude (Table 4).

DISCUSSION

The number of Gamasina species (63) obtained in the investigated ecosystems is in concordance with the findings from other temperate ecosystems, where this parameter varies from 13 to 98 (KOEHLER, 1997, 1999; SKORUPSKI, 2001; MORAZA, 2006; GWIAZDOWICZ, 2007; GULVIK, 2007; SKORUPSKI et al., 2009; SALAMANE & BRUMELIS, 2010). In general, dominant species were common eurytopic detriticol species, with

a wide ecological range. The highest values of abundance and frequency of occurrence were recorded in leaf litter and soil detritus from deciduous temperate forests. The fact that these species are dominant in all investigated ecosystems means that they do not have a certain preferred habitat, being capable of adapting to any environmental conditions (GWIAZDOWICZ, 2007).

Despite the poor vegetation and soil (mineral component formed at soil surface, connected to a thin humified organic matter layer), species diversity was the highest in ecosystem IC. In the early ecological succession, the mite's abundance and diversity can be very high and their trophical specialization is not so strict. Gamasina are able to exploit spatially and temporarily restricted habitats. High dispersal capability is characteristic of many species; dispersal occurs by wind drift, phoresy on insects or from adjacent species-rich ecotones, and the recolonization of various source and sink patches is an important part of the life strategy of many species (KOEHLER, 1999; MINOR & CIANCIOLO, 2007).

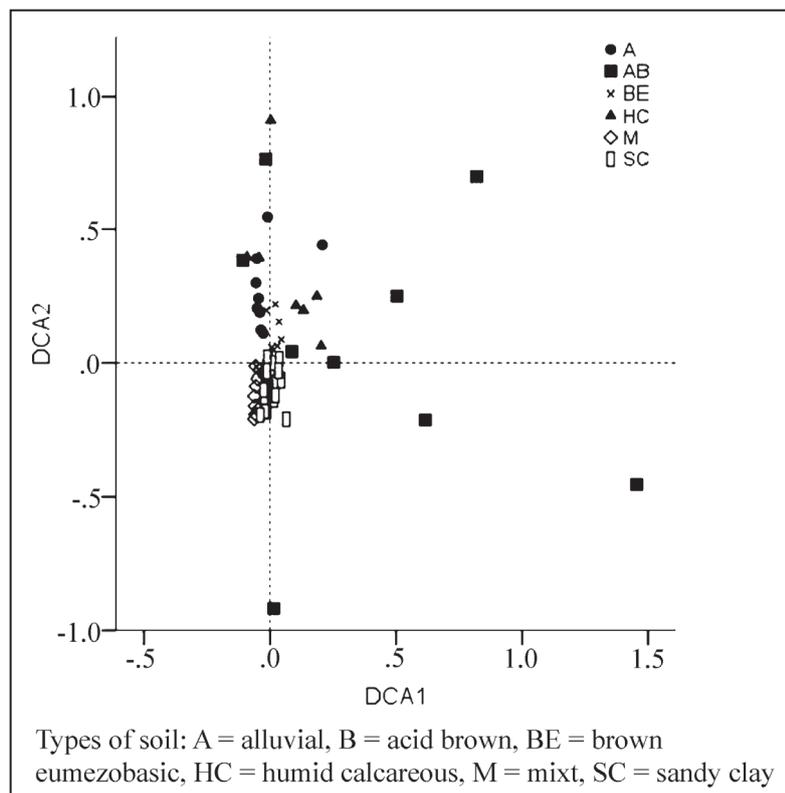


Fig. 5. – DCA ordination of surveyed sites coded by soil type.

Differences were found in dominance and constancy indices for individual mite species in each investigated ecosystem. The high percent of recedent - subrecedent species (ecosystem GCO) and of accessory - accidental species (ecosystems BC, QC, AR and AS) showed that the unfavourable environmental conditions (type of vegetation, slope, sandy soils without organic matter and the dryness) influence the stability of the studied populations. Being predators, they are very mobile species, always migrating to favourable habitats in search for food.

Veigaia nemorensis (C.L.Koch, 1839) was identified as a euconstant-constant species in six out of the seven studied ecosystems. This is likely due to its wide niche breadth, being a well-recognised ubiquitous species (SALMANE, 2001; SALAMANE & BRUMELIS, 2010).

The dendrogram showed that the deciduous forests (ecosystems BF, BC, QC, GCO) have more common species in comparison with the other ecosystems, highlighted by the high value of the similarity index. A common characteristic was the presence of humus in all forests, which represents a proper habitat for Gamasina species, providing them a proper trophic source (springtails, nematodes, enchytreides and other mites). The mite populations from shrub ecosystems (AR, AS) and adjacent area of the cliff (IC) formed a distinct group, based on characteristic species composition (especially in ecosystem IC). This phenomenon was reflected in dissimilarity between Gamasina mite populations from ecosystems BC-AR, AR-QC, AR-AS and AS-IC. These differences could be explained through both large geographic distance between ecosystem AR and the other ecosystems (about 15 km) and the characteristic environmental conditions (type of soil, vegetation).

The DCA showed that the species *Epicrius mollis*, *Geholapsis mandibularis*, *Hypospis aculeifer*, *Veigaia exigua*, *Veigaia nemorensis*, *Macrocheles montanus* *Prozercon fimbriatus* and *Prozercon traegardhi* are highly correlated with different types of soils. As these mites are

predators, the soil of the investigated habitats represents the trophic reservoir for them. Acid soils allow proper development of invertebrate populations (oribatids, springtails, nematodes, etc), which are the favoured food of gamasids (KOEHLER, 1997, 1999; GULVIK, 2007).

The soil type from deciduous forest, characterized by increased soil humidity and presence of organic matter (decayed wood), is proper habitat for predatory species, e.g. *Veigaia nemorensis* and *Hypospis aculeifer* and also for the hygromesophilous omnivorous species *Zercon fageticola* and *Zercon peltatus* (MASAN & FENDA, 2004; SABELIS & LESNA, 2010; GWIAZDOWICZ ET AL., 2011).

In ecosystems GCO and AR two small species were identified. Due to their small body dimensions (0.25-0.45 mm), *Asca bicornis* and *Rhodacarellus perspicuus* adapt easily to rough conditions from shrub ecosystems (dryness, sandy soils low in organic matter), as they are able to migrate deep in the soil, till 20 cm (KOEHLER, 1997; BERNT et al., 2003, GULVIK, 2007; MANU, 2008).

The high number of accessory and accidental species from ecosystems AS and IC indicates that these habitats are not preferred by mites. Being very mobile predatory invertebrates, they are able to migrate from adjacent ecosystems, and such migration may explain why these species were not directly correlated with the ecosystem type.

CONCLUSIONS

Each studied ecosystem was characterized by the dominant species, which are characteristically for temperate ecosystems. The ecosystem nearby the inland cliff, which is in early succession stage, was characterised by the highest numerical abundance and species diversity. *Veigaia nemorensis* was identified in six ecosystems, having the widest ecological plasticity.

Considering the dominance and constancy indices, the highest values were recorded for recedent - subrecedent and accessory - accidental species in all ecosystems. This phenomenon showed that these soil mite communities cannot be considered as stable populations, since they are continuously migrating in search for food.

Analysing the distribution of the Gamasina fauna we observed different composition structure, depending on vegetation (habitat type), soil conditions and geographical position. The similarity analysis between mite populations showed two distinct groups: species from deciduous forests and those from shrubs. The multivariate analysis showed us that the soil mite communities are strongly influenced by the environmental variables, especially by the type of soil.

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Autecology of the extant ostracod fauna of Lake Ohrid and adjacent waters - a key to paleoenvironmental reconstruction

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ABSTRACT. Understanding the ecology of bioindicators such as ostracods is essential in order to reconstruct past environmental and climate change from analysis of fossil assemblages preserved in lake sediment cores. Knowledge of the ecology of ancient Lake Ohrid's ostracod fauna is very limited and open to debate. In advance of the Ohrid ICDP-Drilling project, which has potential to generate high-resolution long-term paleoenvironmental data of global importance in paleoclimate research, we sampled Lake Ohrid and a wide range of habitat types in its surroundings to assess 1) the composition of ostracod assemblages in lakes, springs, streams, and short-lived seasonal water bodies, 2) the geographical distribution of ostracods, and 3) the ecological characteristics of individual ostracod species. In total, 40 species were collected alive, and seven species were preserved as valves and empty carapaces. Of the 40 ostracod species, twelve were endemic to Lake Ohrid. The most common genus in the lake was *Candona*, represented by 13 living species, followed by *Paralimnocythere*, represented by five living species. The most frequent species was *Cypria obliqua*. Species with distinct distributions included *Heterocypris incongruens*, *Candonopsis kingsleii*, and *Cypria lacustris*. The most common species in shallow, flooded areas was *H. incongruens*, and the most prominent species in ditches was *C. kingsleii*. *C. lacustris* was widely distributed in channels, springs, lakes, and rivers. Statistical analyses were performed on a "Lake Ohrid" dataset, comprising the subset of samples from Lake Ohrid alone, and an "entire" dataset comprising all samples collected. The unweighted pair group mean average (UPGMA) clustering was mainly controlled by species-specific depth preferences. Canonical Correspondence Analysis (CCA) with forward selection identified water depth, water temperature, and pH as variables that best explained the ostracod distribution in Lake Ohrid. The lack of significance of conductivity and dissolved oxygen in CCA of Ohrid data highlight the uniformity across the lake of the well-mixed waters. In the entire area, CCA revealed that ostracod distribution was best explained by water depth, salinity, conductivity, pH, and dissolved oxygen. Salinity was probably selected by CCA due to the presence of *Eucypris virens* and *Bradleystrandesia reticulata* in short-lived seasonal water bodies. Water depth is an important, although indirect, influence on ostracod species distribution, which is probably associated with other factors such as sediment texture and food supply. Some species appeared to be indicators for multiple environmental variables, such as lake level and water temperature.

KEY WORDS: freshwater Ostracoda, endemism, ancient lakes, multivariate analysis, training set

INTRODUCTION

Ostracods (Arthropoda) are bivalved crustaceans with adults being typically 0.5 to 3.0mm long. They are environmentally and geographically diverse, and are often abundant in almost all aquatic and in some terrestrial habitats (DE DECKKER & FORESTER, 1988; HOLMES, 2001; SMITH & DELORME, 2009). In common with diatoms, cladocerans, and thecamoebians,

for example, ostracods are one of the few aquatic organisms that can be recovered as fossils in statistically relevant numbers. They are strong tools for paleoecological interpretations because of their excellent shell preservation, their small size, diversity, and sensitivity to a wide range of environmental variables (DELORME, 1969). Traditionally, most ostracod specialists have focused more on taxonomic problems than on defining ecological response. For robust

paleoenvironmental interpretation of preserved fossil ostracod records, a sound knowledge of ostracod ecology is vital (DE DECKKER & FORESTER, 1988). Important environmental variables determining the presence and distribution of ostracod species can be divided into habitat characteristics and the nature of the host water. The former includes the size, energy level, permanence of the water body, water depth, sediment texture, presence and types of aquatic plants, availability of food, and predation. The latter comprises nutrient status, pH, dissolved oxygen content, salinity, temperature, and ionic composition of the host water (HOLMES, 2001). Besides their potential as a tool for paleoecological reconstruction, they can also be used as indicators of ecosystem health. For example, KÜLKÖYLÜOĞLU (2004) suggested that ostracods are excellent water quality indicators. Similarly, PIERI et al. (2012) investigated the impact of wastewater discharge on ostracod density, richness, and community composition, highlighting their value as water quality indicators. Transfer functions are a common approach to reconstruct past environments. REED et al. (2008) applied a diatom-inferred total phosphorus transfer function to demonstrate that Lake Uluabat (Turkey) has been eutrophic since prior to the 19th century. MEZQUITA et al. (2005) found that ostracods from the eastern Iberian Peninsula have potential both for reconstructing past water temperatures and water chemistry (solute concentration and composition). However, transfer functions are not infallible. Sometimes, due to the nature of variation in the calibration dataset, ecologically insignificant variables may appear to exert a significant influence on species distribution. To minimize this risk, care must be taken to ensure that both the training set and the variation in the environmental variable of interest are sufficiently large (TELFORD & BIRKS, 2011a, b).

In Lake Ohrid, the pioneering investigation of the ostracod fauna in 1934 (KLIE, 1934) was soon followed by a series of others (HOLMES, 1937; KLIE, 1939a, b, 1942; PETKOVSKI, 1960a, b, c; MIKULIĆ, 1961; PETKOVSKI, 1969a, b; MIKULIĆ & PLJAKIĆ, 1970). These studies

focused on acquisition of the species inventory and taxonomy. Except for some information on species sampling depth, knowledge on the autecology of the predominantly endemic ostracods from Lake Ohrid and its surroundings is lacking (BELMECHERI et al., 2009; AUFGEBAUER et al., 2012). This study aims to define baseline ecological information to contribute to future studies on the long sequence obtained during the ICDP-Drilling in Lake Ohrid in 2013. In parallel, it aims to generate information on the indicator value of individual ostracod species for water quality assessment, which could be used to tackle the “creeping biodiversity crisis” in this World Heritage Site (KOSTOSKI et al., 2010). To achieve this goal, we collected surface sediments and water samples from different habitat types, e.g., Lakes Ohrid, Prespa, Dojran, and Ioannina as well as short-lived seasonal water bodies, springs, and streams, and compiled a detailed species inventory. To attempt to define species-specific environmental preferences, optimum values and tolerance ranges were estimated using the transfer function approach (SMOL, 2002). Furthermore, the geographical distribution of the ostracod species in the region was determined.

MATERIALS AND METHODS

Study area

Situated in the southern Balkans (Fig. 1), the study area is located in a highly diverse biogeographic region, characterized by a large number of ancient lakes including Lakes Ohrid, Prespa, Dojran, and Ioannina (GRIFFITHS et al., 2002b; FROGLEY & PREECE, 2004; REED et al., 2004; ALBRECHT et al., 2009) which are a focus for the current study. The region is relatively arid, with fewer lakes than, for example, northwestern Europe. Lakes Ohrid and Prespa are both deep and relatively low in nutrients, whereas Lakes Dojran and Ioannina are shallow and currently hypereutrophic. The oligotrophic, karstic Lake Ohrid lies on the border between Macedonia and Albania at 695m a.s.l. It has a surface area of 358km² and a maximum depth of 288.7m. Lake

Ohrid is directly connected with Lake Prespa via underground karstic channels (SALEMAA, 1994; ALBRECHT & WILKE, 2008). Both lakes are Quaternary graben-shaped lakes formed by a combination of post-Pliocene uplift and gradual subsidence (ALIAJ et al., 2001). Lake Prespa, with a surface area of 254km², is situated on the Macedonian-Albanian-Greek border at an altitude of 849m. Its maximum water depth is 48m, having undergone a ca. 6m reduction in lake level since the 1950s due to increasing water abstraction for irrigation. The lake may also be suffering from anthropogenic eutrophication (LÖFFLER et al., 1998; MATZINGER et al., 2006). Lake Dojran (146-148m a.s.l.) lies between the borders of Macedonia and Greece in a karstified basin formed by Tertiary volcanic and tectonic activity. The maximum surface area is approximately 40km², the maximum depth

was 10.4m in the 1930s but by 1995 had fallen by 4.8m due to water abstraction (GRIFFITHS et al., 2002a). The southernmost study site is Lake Ioannina (also known as Lake Pamvotis), located in northwest Greece at 480m a.s.l. in a karst polje, a depression, formed during the Late Pliocene to Early Pleistocene. The catchment consists mainly of Mesozoic and early Cenozoic limestone bedrock overlain by thick deposits of Late Pliocene and Quaternary lake sediments. The lake level of Lake Ioannina also dropped markedly during the first half of the twentieth century as a result of agricultural drainage. The modern lake is a remnant of the ancient lake, with a surface area of ca. 23km² and a maximum water depth of 10m (FROGLEY et al., 2001; LENG et al., 2010).

The watersheds of Lakes Prespa and Ohrid are estimated to be 1391km² and 1002km², respectively. Because of the irregular and strongly developed surface terrain morphology partly underlain by deep karst it is difficult to define the exact watershed and catchment areas. Numerous karst springs occur within Lake Ohrid and at the lakeshore (POPOVSKA & BONACCI, 2007). At Sveti Naum, on the southeastern shore of Lake Ohrid, eight springs form a small pond, which collects the spring waters before draining into Lake Ohrid (JORDANOSKA et al., 2010). The Tushemisht spring zone, west of Sveti Naum, in Albania, consists of 80 individual springs. Other springs are the Biljana, at the Hydrobiological Institute in the City of Ohrid, and the Bej wells which are located northeast of Lake Ohrid (POPOVSKA & BONACCI, 2007). The Kališta springs are located at the northwestern shore of the lake, the Šum springs north of Lake Ohrid, and the Korita springs between Lake Ohrid and Lake Prespa (Fig. 1).

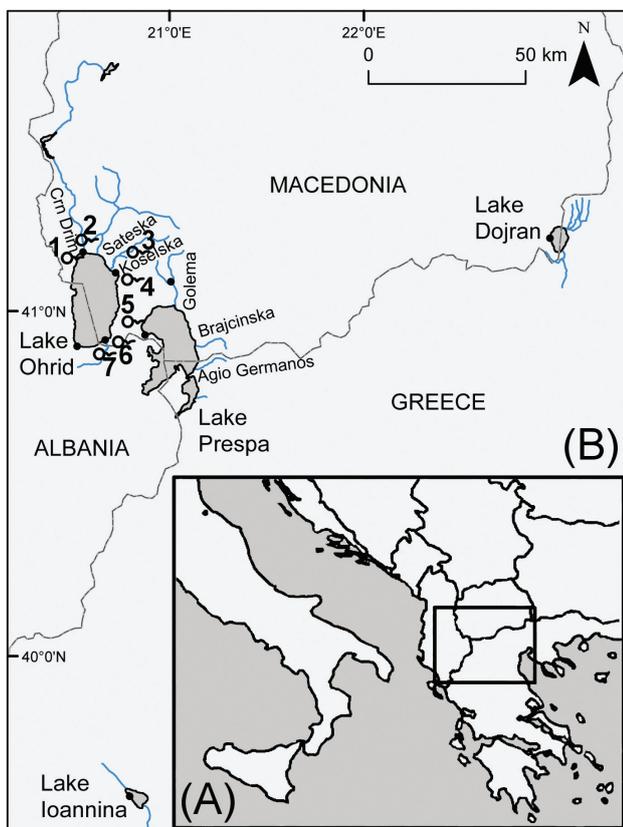


Fig. 1. – Map showing the location of the study area (marked with a square) in Macedonia, Albania, and Greece (A) and basic hydrological features (B). Numbers indicate major sampled springs (1: Kališta springs, 2: Šum springs, 3: Bej wells, 4: Biljana springs, 5: Korita springs, 6: Sveti Naum, 7: Tushemisht springs).

Lakes Ohrid and Prespa are influenced by a continental climate. For the period 1961-1990 the average annual air temperature was 11.1°C in the City of Ohrid. The minimum air temperature was -5.7°C and the maximum 31.5°C. In Resen, 10km north of Lake Prespa, average annual air temperature of 9.5°C was registered. The

minimum air temperature was -8.7°C and the maximum air temperature 30.5°C (POPOVSKA & BONACCI, 2007). Maximum precipitation occurs in December and March, whereas the late summer is dry. In summer, the winds are predominantly southwestern (SALEMAA, 1994). Lake Dojran has a Mediterranean climate with an average annual precipitation of 623.4mm (average for the period 1961-1990) (POPOVSKA & BONACCI, 2008) in Nov Dojran, west of the lake. The summers are long and hot, and the winters are mild. Highest average long-term monthly temperatures occur in July and August (19.1°C and 18.7°C) and lowest temperatures in January and February (0.6°C and 1.9°C) (POPOVSKA & BONACCI, 2008). Lake Ioannina is influenced by a semi-humid Mediterranean climate. The winters are cold and wet with a mean temperature in January of 4.9°C . Summers are hot and dry, with a mean temperature in July of 24.9°C . The mean annual temperature is 14.4°C , and the annual precipitation is approximately 1100-1200mm (TZEDAKIS, 2000; ROMERO et al., 2002).

Field and laboratory analysis

A total of 335 surface sediment samples were collected during four field campaigns in March/April 2009, August/September 2009, February 2010, and June 2010 in Lake Ohrid (Macedonia, Albania) and its surrounding areas including Lakes Prespa (Macedonia, Albania, Greece) and Ioannina (Greece) as well as from springs, rivers, flooded areas, ditches, and channels. Samples taken in September 2009 from Lake Dojran (Macedonia, Greece) were provided by Thomas Wilke and his team, Justus-Liebig-Universität Gießen, Germany. Deeper parts of Lake Ohrid (from 5 to 280m water depths) were sampled with a gravity multicorer, consisting of three cores with a diameter of 11cm each, and short core tops (uppermost $2\text{cm} \cong 190\text{cm}^3$) were used for ostracod analysis. In shallower waters and in places where sediments were stony, it was impossible to use the multicorer. There, samples were retrieved with either an exhaustor, spoon net, UWITEC-gravity corer, Ekman grab

(uppermost 2cm), dredge or with a sediment suction device. This device is composed of a manual membrane pump with a flexible hose of 5m length to which a metal pipe is attached allowing for sampling between rocks and stones. All samples were washed through a $125\mu\text{m}$ mesh sieve immediately after collection and were preserved in 95-99% ethanol. *In situ*, dissolved oxygen, pH, conductivity, salinity, and water temperature were measured with a handheld multi-sensor (Multi 3500i Hand-Held Meter, WTW) and water transparency with a Secchi disc. A WinLab Data Line Photometer (Windaus) was used to analyze nutrients and major ions including sulfate, phosphate, ammonium, nitrite, and nitrate. Water samples were collected for the additional measurement of major and trace elements. Lake waters from deeper sites ($>5\text{m}$) were obtained either with a Niskin Type water sampler, or bottom water trapped above core tops during collection of short cores was used. Substrate was described in the field and classified into detritus, sapropel, artificial, rocks ($>200\text{mm}$), stones (200-63mm), gravel (63-2mm), sand (2-0.063mm), and silt/clay ($<0.063\text{mm}$). Water chemistry was analyzed at the Technische Universität Braunschweig, Germany, using Ion Chromatography with a 761 Compact IC Metrohm and Inductively Coupled Plasma Optical Emission with an ICP-OES Jobin Yvon JY 50 P, and at the Freie Universität Berlin, Germany, using a DIONEX DX-500 Ion Chromatograph, ICP-OES Perkin Elmer Optima 2100DV, and Technicon DOC-Auto-Analyzer II. Surface sediment samples were hand-sorted under a dissecting microscope with a magnification up to 50x. The preparation of soft parts used for identification of species was carried out using traditional embedding in Hydro-MatrixTM (Micro Tech Lab). Scanning Electron Microscope (SEM) pictures of ostracod valves were taken at the Zoological Museum of the Universität Hamburg with a Scanning electron microscope LEO 1525 (Carl Zeiss Inc.), and at the Universität Köln with a CamScan-CS-44 (CamScan). Digital images were taken with a light microscope (DM5000B; camera type Leica DFC320), and single pictures were stacked

with the software package CombineZ. Ostracod identification was based on taxonomic work by KLIE (1934, 1939a, b, 1942), PETKOVSKI (1960a, b, c), MIKULIĆ (1961), PETKOVSKI (1969a, b), MEISCH (2000), and PETKOVSKI et al. (2002).

Data analysis

Quantitative data analysis was based on adults of living ostracods. Indeterminate juveniles, empty carapaces and valves were omitted because they may have been transported by drift, particularly in shallow depths and on rocky substrates, and thus do not necessarily present the true species assemblage (PARK et al., 2003). Analyses are based on relative abundances, because this is more robust. In total 74 samples did not contain ostracods and were therefore excluded from analysis; the dataset therefore comprises 211 samples. Secchi depth was also excluded from statistical analyses as the Secchi disk was in most cases visible all the way down to the bottom of the aquatic habitat (<20m) and Secchi depth therefore corresponds to water depth. Pearson correlation, cluster analysis, and ordinations were performed with two different datasets to determine whether ostracod species in Lake Ohrid were affected by other environmental variables than those found over the full sample area. The entire dataset consisted of all sampled habitats, whereas the Lake-Ohrid dataset included only the samples from Lake Ohrid.

The unweighted pair group mean average (UPGMA) with Jaccard's coefficient was used to show a possible clustering relationship among ostracods; it was performed with the program MVSP (MultiVariate Statistical Package), version 3.21. The program Past, version 2.12 (HAMMER et al., 2001) was used for SIMPER (Similarity percentage) to assess ostracod species that accounted for the greatest observed differences in ostracod composition between various habitats, and for Pearson correlations, to assess relationships between the environmental variables. Distribution maps of ostracod species were created in ESRI Arc Map 9.3. Grapher version 9.1.536 was used to prepare pie charts

illustrating the link between grain size and ostracod distribution.

Detrended Correspondence Analysis (DCA) (HILL & GAUCH, 1980), with detrending by segments and non-linear rescaling, was used to decide whether unimodal (gradient length >2 SD (standard deviation units)) or linear (gradient length <2 SD) based numerical techniques should be used (SMOL, 2002). Because DCA revealed gradient lengths of 4.71 (first axis) and 3.77 SD (second axis) for the entire dataset, as well as 4.82 (first axis) and 4.38 SD (second axis) for the Lake-Ohrid dataset, we used Canonical Correspondence Analysis (CCA) (TER BRAAK, 1986) to explain the relationships between ostracod species and their environment. For CCA analysis, rare species were down-weighted, ostracod data were log-transformed, and environmental variables were added by manual forward selection using the Monte Carlo permutation test with 999 permutations. All ordinations were performed using Canoco version 4.5 (TER BRAAK & SMILAUER, 2002), and ordination diagrams were made with CanoDraw 4.0. Weighted averaging (WA) regression with inverse deshrinking was used to calculate the ecological tolerance range and the optima of the most common ostracod species. This was performed with the program C2 version 1.7.2 (JUGGINS, 2007) for significant environmental variables only ($P < 0.05$). The 16 rare species, present in ≤ 3 waterbodies each, were excluded from UPGMA clustering, DCA, CCA, and WA to avoid distortion.

RESULTS

Habitat preferences

A total of 47 ostracod species was found, belonging to 18 genera (Table 1, Figs 1S-3S, supplementary material). Forty species were collected alive, and seven species were identified using valves or empty carapaces only. The soft parts of the ostracods were used for preliminary species identification and a detailed description

TABLE 1

Taxonomic ranking of ostracod species (species marked with asterisks (*) were identified using valves or empty carapaces). Abbreviations of each taxon (used in subsequent tables and figures) are indicated after their respective names.

Class Ostracoda Latreille, 1806	
Order Podocopida Sars, 1866	
Suborder Podocopina Sars, 1866	
Infraorder Cypridocopina Jones, 1901	
Superfamily Cypridoidea Baird, 1845	
Family Candonidae Kaufmann, 1900	
Subfamily Candoninae Kaufmann, 1900	
Genus <i>Candona</i> s. str. Baird, 1845	
<i>Candona bimucronata</i> Klie, 1937	Cbimu
* <i>Candona expansa</i> Mikulić, 1961	
<i>Candona goricensis</i> Mikulić, 1961	Cgori
<i>Candona hadzistei</i> Petkovski et al., 2002	Chadz
<i>Candona hartmanni</i> Petkovski, 1969	Chart
* <i>Candona holmesi</i> Petkovski, 1960	
<i>Candona litoralis</i> Mikulić, 1961	Clito
<i>Candona margaritana</i> Mikulić, 1961	Cmarga
* <i>Candona marginata</i> Klie, 1942	
<i>Candona marginatoides</i> Petkovski, 1960	Cmargi
<i>Candona media</i> Klie, 1939	Cmedi
<i>Candona ohrida</i> Holmes, 1937	Cohri
<i>Candona ovalis</i> Mikulić, 1961	Coval
<i>Candona trapeziformis</i> Klie, 1939	Ctrap
* <i>Candona triangulata</i> Klie, 1939	
<i>Candona vidua</i> Klie, 1941	Cvidu
Genus <i>Fabaeformiscandona</i> Krstić, 1972	
<i>Fabaeformiscandona krstici</i> Petkovski, 1969	Fkrst
Genus <i>Pseudocandona</i> Kaufmann, 1900	
<i>Pseudocandona compressa</i> (Koch, 1838)	Pcomp
Genus <i>Candonopsis</i> Vávra, 1891	
<i>Candonopsis kingsleii</i> (Brady & Robertson, 1870)	Cking
Subfamily Cyclocypridinae Kaufmann, 1900	
Genus <i>Cypria</i> Zenker, 1854	
<i>Cypria lacustris</i> Sars, 1890	Cypla
<i>Cypria obliqua</i> Klie, 1939	Cyobl
* <i>Cypria ophthalmica</i> (Jurine, 1820)	
Genus <i>Cyclocypris</i> Brady & Norman, 1889	
<i>Cyclocypris ovum</i> Jurine, 1820	Covum
Family Ilyocyprididae Kaufmann, 1900	
Subfamily Ilyocypridinae Kaufmann, 1900	
Genus <i>Ilyocypris</i> Brady & Norman, 1889	
<i>Ilyocypris bradyi</i> Sars, 1890	Ibrad
Family Cyprididae Baird, 1845	
Subfamily Eucypridinae Bronshtein, 1947	
Genus <i>Eucypris</i> Vávra, 1891	
<i>Eucypris virens</i> (Jurine, 1820)	Evire

<i>Eucypris</i> sp.	Esp
Genus <i>Prionocypris</i> Brady & Norman, 1896	
<i>Prionocypris zenkeri</i> (Chyzer & Toth, 1858)	Pzenk
Subfamily Cypricerinae McKenzie, 1971	
Genus <i>Bradleystrandesia</i> Broodbakker, 1983	
<i>Bradleystrandesia reticulata</i> (Zaddach, 1844)	Breti
Subfamily Herpetocypridinae Kaufmann, 1900	
Genus <i>Herpetocypris</i> Brady & Norman, 1889	
<i>Herpetocypris</i> sp.	Hsp
<i>Herpetocypris</i> sp. 2	Hsp2
Genus <i>Psychrodromus</i> Danielopol & McKenzie, 1977	
<i>Psychrodromus fontinalis</i> (Wolf, 1920)	Pfont
<i>Psychrodromus olivaceus</i> (Brady & Norman, 1889)	Poliv
<i>Psychrodromus</i> sp.	Psp
Subfamily Cyprinotinae Bronshtein, 1947	
Genus <i>Heterocypris</i> Claus, 1892	
<i>Heterocypris incongruens</i> (Ramdohr, 1808)	Hinco
<i>Heterocypris reptans</i> (Baird, 1835)	Hrept
* <i>Heterocypris salina</i> (Brady, 1868)	
Subfamily Dolerocypridinae Triebel, 1961	
Genus <i>Dolerocypris</i> Kaufmann, 1900	
<i>Dolerocypris sinensis</i> (Sars, 1903)	Dsine
Superfamily Cytheroidea Baird, 1850	
Family Leptocytheridae Hanai, 1957	
Subfamily Leptocytherinae Hanai, 1957	
Genus <i>Amnicythere</i> Devoto, 1965	
<i>Amnicythere karamani</i> (Klie, 1939)	Akara
Family Limnocytheridae Klie, 1938	
Subfamily Limnocytherinae Klie, 1938	
Genus <i>Paralimnocythere</i> Carbonnel, 1965	
<i>Paralimnocythere alata</i> (Klie, 1939)	Palat
<i>Paralimnocythere georgevitschi</i> (Petkovski, 1960)	Pgeor
<i>Paralimnocythere karamani</i> (Petkovski, 1960)	Pkara
<i>Paralimnocythere ochridense</i> (Klie, 1934)	Pochr
<i>Paralimnocythere slavei</i> Petkovski, 1969	Pslav
* <i>Paralimnocythere umbonata</i> (Klie, 1939)	
Subfamily Timiriaseviinae Mandelstam, 1960	
Genus <i>Kovalevskiella</i> Klein, 1963	
<i>Kovalevskiella</i> sp.	Ksp
Family Cytherideidae Sars, 1925	
Genus <i>Cytherissa</i> Sars, 1925	
<i>Cytherissa lacustris</i> (Sars, 1863)	Cytla
Infraorder Darwinulocopina Sohn, 1988	
Superfamily Darwinuloidea Brady & Norman, 1889	
Family Darwinulidae Brady & Norman, 1889	
Genus <i>Darwinula</i> Brady & Robertson, 1885	
<i>Darwinula stevensoni</i> (Brady & Robertson, 1870)	Dstev

and documentation of the Ohrid ostracod soft part morphology is planned for the near future (Lorenschat et al., in preparation). In certain cases, it is possible to determine ostracods to the species level on valve morphology alone. For example, the valves of *Amnicythere karamani* (Klie, 1939) are, in contrast to other *Amnicythere* species in the western Balkan Peninsula, covered with coarse pits (PETKOVSKI & KEYSER, 1992), while the valves of the species of *Paralimnocythere* have a specific number and typical array of prominent ridges, tubercles, and ala (lateral projections) (MEISCH, 2000). Distinctive features of *Candona* species are the valve size and the valve outlines, as described in the literature, e.g., MIKULIĆ (1961).

Thirty-two out of the 40 living ostracod species occurred in Lake Ohrid, and twelve were discovered exclusively in the lake (Fig. 4S, supplementary material). Furthermore, valves and empty carapaces of *Candona expansa* Mikulić, 1961, *Candona holmesi* Petkovski, 1960, *Candona marginata* Klie, 1942, *Candona triangulata* Klie, 1939, and *Cypria ophthalmica* (Jurine, 1820) were found in Lake Ohrid. Empty carapaces of *Paralimnocythere umbonata* (Klie, 1939) occurred in one sample from the springs of Sveti Naum and in Lake Ohrid. In the littoral zone of Lake Dojran, living specimens of *Cypria lacustris* Sars, 1890 and *Herpetocypris* sp. as well as valves and empty carapaces of *Heterocypris salina* (Brady, 1868) were collected. The littoral of Lake Prespa was inhabited by *Candona marginatoides* Petkovski, 1960, *Cycloocypris ovum* Jurine, 1820, *Cypria lacustris*, *Darwinula stevensoni* (Brady & Robertson, 1870), *A. karamani*, *Paralimnocythere ochridense* (Klie, 1934), and *Bradleystrandesia reticulata* (Zaddach, 1844). *Candona media* Klie, 1939 and *D. stevensoni* were the only species that were recovered alive in the littoral of Lake Ioannina. The most common genus in the study area was *Candona*, represented by a total of 13 living species, followed by *Paralimnocythere* with five living species. The most common species was *Cypria obliqua* Klie, 1939 which was found in 120 samples (36%) (Fig. 4S, supplementary

material). Other common species were *C. media* (in 76 samples), *Cypria lacustris* (in 70 samples), and *A. karamani* (in 60 samples). Furthermore, most of the sampled habitats were inhabited by a large number of juvenile ostracods of the genera *Candona* and *Cycloocypris* that were indeterminable to species level. For instance, in a sample taken with the suction device at 0.5m water depth on the eastern shore of the lake, a total of 1432 ostracod specimens was found, of which 1358 were juvenile Candonids. The 74 samples devoid of ostracods were mainly those of rivers and streams with a strong water current and a substrate mainly consisting of rocks, stones, and gravel. Samples from the littoral of Lake Ohrid, where coarse material (rocks, stones, and gravel) predominated, and wave action was strong, were also devoid of ostracods. The deepest sample from Lake Ohrid that did not contain ostracods was taken in 20m water depth at the eastern shore (coarse material and steeply sloping shore). All the other sampled localities from deeper waters in Lake Ohrid were inhabited by ostracods.

Relative abundance species data from the entire dataset, consisting of 211 surface sediment samples, clustered into six main groups (Fig. 5S, supplementary material). Group 1 contained only the species *Heterocypris incongruens* (Ramdohr, 1808). This was the only species found in the surrounding areas of Lake Ohrid, not in the lake itself; and it always occurred in very shallow water (maximum sampling depth 0.3m). The only species in Cluster Group 2 was *Paralimnocythere karamani* (Petkovski, 1960). It was only discovered at three localities in Lake Ohrid and in Sveti Naum, in shallow waters from 0.2m down to 54m depth and at low water temperatures (<11.5°C). Five species (*Ilyocypris bradyi* Sars, 1890, *Cytherissa lacustris* (Sars, 1863), *B. reticulata*, *Eucypris virens* (Jurine, 1820), and *Candona hartmanni* Petkovski, 1969) were found in Cluster Group 3. All species in this group inhabited water depths from 0.5m down to 40m, and waters with temperatures between 17°C and 23°C. Group 3 can be divided into four subgroups (a, b, c, and d) (Fig. 5S, supplementary material). The most common species was *C.*

hartmanni (subgroup d) with six occurrences in Lake Ohrid and two occurrences in springs (Sveti Naum and Bej wells). *B. reticulata* and *E. virens* (subgroup c) were species associated to waters with a very high conductivity. *Candona litoralis* Mikulić, 1961 (cluster group 4) was the only species in Lake Ohrid restricted to the shallower littoral (maximum sampling depth 5m). *Candona hadzistei* Petkovski et al., 2002, *Fabaeformiscandona krstici* Petkovski, 1969, *C. marginatoides*, *C. media*, *Candona ovalis* Mikulić, 1961, *Candona vidua* Klie, 1941, *C. obliqua*, *A. karamani*, and *Candona trapeziformis* Klie, 1939 clustered in Group 5. *C. trapeziformis* inhabited Lake Ohrid down to 163m depth and all the other species occurred down to 280m water depth. Cluster Group 6 included *C. ovum*, *Cypria lacustris*, *P. ochridense*, *Paralimnocythere slavei* Petkovski, 1969, *Candona ohrida* Holmes, 1937, *Candonopsis kingsleii* (Brady & Robertson, 1870), and *D. stevensoni*. They colonized different water depths but were more common at lower depths (1.7-19.5m). Furthermore, all species were found in waters with a maximum temperature of 26°C.

For the Lake Ohrid dataset, consisting of 160 surface sediment samples, the UPGMA dendrogram revealed three major clustering groups (Fig. 6S, supplementary material). Group 1 included *C. litoralis* and *C. hartmanni*. These species were restricted to 5m and 10m water depth, respectively. Cluster Groups 2 and 3 of the Lake-Ohrid dataset were similar to Cluster Groups 5 and 6 from the entire dataset.

Ostracods occurred in almost all aquatic habitats. Species-specific environmental preferences were suggested by the SIMPER output (Table 1S, supplementary material). All lakes, except for Lake Ohrid, were considered as an individual group because samples were taken only in the littoral zone, so that these do not reflect the complete ostracod fauna of the lakes (e.g., species that inhabit only deeper parts of the lakes were not found). SIMPER indicated that the maximum average dissimilarity between the habitats was 99.96% (flooded areas and

rivers), and the minimum 38.05% (channels and lake habitats). SIMPER results also define characteristic indicator species for specific habitats: *H. incongruens* for flooded areas, *C. kingsleii* for ditches, *C. obliqua* for Lake Ohrid, and *Cypria lacustris* for channels, springs, lakes, and rivers.

Candona margaritana Mikulić, 1961, *Kovalevskiella* sp., and *P. karamani* were restricted to sediments with a minimum of 50% sand content (Fig. 7S, supplementary material). Six species (*Candona bimucronata* Klie, 1937, *C. litoralis*, *C. ohrida*, *C. kingsleii*, *D. stevensoni*, and *P. slavei*) were found on substratum that consisted of at least 50% of coarse sediment (rocks, stones, and gravel), and 16 species preferred mainly silty sediment.

Response to limnological variables

Correlations between the variables (Table 2S and Table 3S, supplementary material) were similar in both datasets, with few significant correlations. The only noticeable difference was the high correlation between NO_2 and NH_4 (0.84) in the entire dataset compared to 0.47 in Lake Ohrid dataset.

The first CCA focused on the entire dataset and the first two axes of the DCA explained 20.9% of the variability in species data. The sum of all canonical eigenvalues was 0.91. The first two axes of the CCA explained 6.5% (Table 2) of the variance in the species data. Water depth, salinity, conductivity, pH, and dissolved oxygen were identified by forward selection as variables that best explained the variation in ostracod species data (Table 3). The first synthetic gradient was positively correlated with salinity and conductivity and negatively with water depth, pH, and dissolved oxygen (Fig. 2). Furthermore, dissolved oxygen and pH were negatively correlated with the second axis, and conductivity, salinity, and water depth were positively correlated with the second axis. All species located with negative axis 2 scores, were found in water depths down to 280m, except for

TABLE 2

Summary of results from CCA performed on the entire dataset.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.34	0.14	0.11	0.08	7.27
Species-environment correlation	0.73	0.55	0.46	0.47	
Cumulative percentage variance of species data	4.70	6.50	8.00	9.10	
of species-environment relation	37.30	52.00	63.80	72.50	
Sum of all eigenvalues					7.27
Sum of all canonical eigenvalues					0.91

C. trapeziformis which occurred in a maximum water depth of 163m. *B. reticulata* and *E. virens* are located in the upper right quadrant of the biplot and were sampled in habitats with high conductivity (Fig. 2). In the majority of cases, the most common ostracod species are grouped in the center of the ordination biplot, for example, *Cypria lacustris*, underlining their apparently broad tolerance ranges.

The second CCA focused on the Lake Ohrid dataset only. In the initial DCA, the first and second axes together explained 23.2% of the variance in species data; the sum of all canonical

TABLE 3

Results of forward selection of explanatory variables (P -values ($P < 0.05$) and total percent variance explained by each environmental variable) for the entire dataset.

Variable	P -value	% total variance explained
Water depth	0.001	22
Salinity	0.003	12
Conductivity	0.007	8
pH	0.003	11
Dissolved oxygen	0.050	5

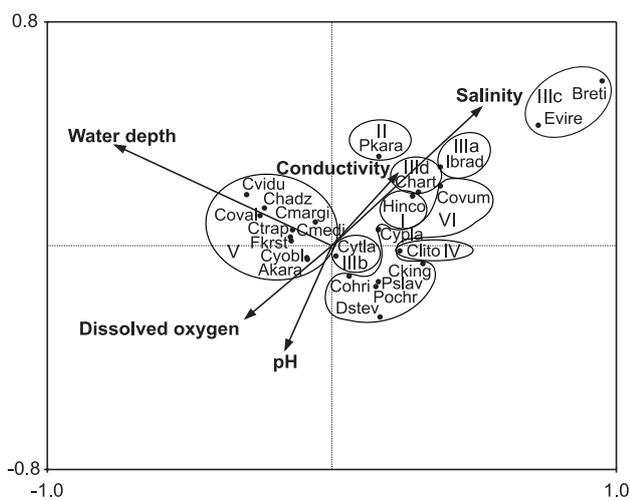


Fig. 2. – Canonical Correspondence Analysis (CCA) biplot of ostracod species and forward selected environmental variables from 211 surface sediment samples taken in Lake Ohrid and its surroundings. Species codes as in Table 1 and roman numerals and characters indicate species assemblages.

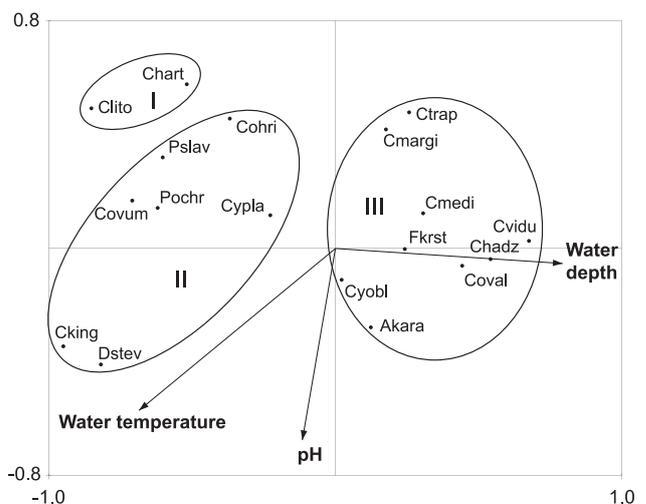


Fig. 3. – Canonical Correspondence Analysis (CCA) biplot of ostracod species and forward selected environmental variables from 160 surface sediment samples taken in Lake Ohrid. Species codes as in Table 1 and roman numerals indicate species assemblages.

TABLE 4

Summary of results from CCA performed on the Lake-Ohrid dataset.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.25	0.09	0.07	0.05	4.14
Species-environment correlation	0.73	0.56	0.48	0.50	
Cumulative percentage variance of species data	6.10	8.40	10.00	11.30	
of species-environment relation	40.50	55.50	66.40	75.00	
Sum of all eigenvalues					4.14
Sum of all canonical eigenvalues					0.63

eigenvalues was 0.63. The first two axes of the CCA together explained 8.4% of the variance in the species data (Table 4). Water depth, water temperature, and pH were identified by forward selection as variables that best explain the species distribution (Table 5). The first axis was positively correlated with water depth and negatively with water temperature, and the second axis was strongly negatively correlated with pH (Fig. 3). Species related to deeper waters were located on the upper right side of the biplot. *C. obliqua* was the most common species in Lake Ohrid, and is located in the centre of the biplot.

As indicated by SIMPER, some species occurred in specific habitats and others in a wide range of different environments. Whereas the latter species have a wide tolerance range for particular environmental variables, the former species have a narrow distribution range and are less tolerant. All estimated optima, tolerance ranges, and standard errors for the significant variables and for all species are listed in Table 4S, supplementary material. Nine ostracod species

occurred in Lake Ohrid down to 280m water depth: *C. hadzistei*, *F. krstici*, *C. marginatoides*, *C. media*, *C. ovalis*, *C. vidua*, *C. obliqua*, *Cypria lacustris*, and *A. karamani*. Eight out of these nine species have a high tolerance for water depth (72.3-97.3m); only *Cypria lacustris* showed a lower tolerance range (49.9m) and optimum (19.5m) for depth. *B. reticulata*, *C. hartmanni*, *C. kingsleii*, *C. litoralis*, *E. virens*, and *H. incongruens* occurred in all sampled habitats down to 10 m water depth and all of them have relatively low tolerance ranges (<3.0m) and optimum values (<1.7m). *C. trapeziformis* was the species that always occurred at low water temperatures (< 9.9°C). This species showed the lowest optimum (8.2°C) and a low tolerance range (0.7°C). *H. incongruens* was the species that thrived at highest temperatures (maximum: 33.8°C); it has a high optimum (32.8°C), and a high tolerance range (16.1°C).

DISCUSSION

Understanding the autecology of single ostracod species is important for interpreting past climate and environmental changes. This study provides the first information about the autecology of ostracod species mostly endemic to Lake Ohrid and its surroundings. A total of 40 living ostracod species were found in 335 surface sediment samples from Lake Ohrid and its surroundings of which 32 occurred in the lake itself and twelve species were exclusively found in Lake Ohrid. We collected seven

TABLE 5

Results of forward selection of explanatory variables (*P*-values (*P*<0.05) and total percent variance explained by each environmental variable) for the Lake-Ohrid dataset.

Variable	<i>P</i> -value	% total variance explained
Water depth	0.001	17
Water temperature	0.001	9
pH	0.017	5

ostracod species in Lake Prespa and, except for *B. reticulata*, all other species were also found by PETKOVSKI (1960b). The two living species, *Herpetocypris* sp. and *Cypria lacustris* sampled in the littoral of Lake Dojran, were not found by GRIFFITHS et al. (2002a). In the littoral of Lake Ioannina, we only found two living species, *C. media* and *D. stevensoni*; only the latter had been recorded there before (FROGLEY et al., 2001).

Cluster analysis and CCA were used to identify the relationship between environmental variables and ostracod distribution. In the UPGMA dendrogram rare species were usually clustered in small groups as is the case in Groups 1-4 of the entire dataset (Fig. 5S, supplementary material), and in Group 1 of the Ohrid dataset (Fig. 6S, supplementary material). Clustering of both datasets was mainly affected by water depth and forward selection of CCA identified water depth, salinity, conductivity, pH, and dissolved oxygen as variables that determine the ostracod distribution in the entire study area. Salinity was probably forward selected because of the presence of species such as *E. virens* and *B. reticulata*, mostly found in waters with the highest salinity (0.1‰). The biplot (Fig. 2) indicated the same ostracod assemblages that were visible in the UPGMA cluster (Fig. 5S, supplementary material). For the Lake Ohrid dataset, water depth, water temperature, and pH were identified as variables that best explained the species distribution within the lake. Probably due to the uniformity of conductivity and dissolved oxygen within the lake, these two variables were not identified as major environmental variables determining ostracod distribution. They did, however, play a major role in determining the distribution of ostracods in the Lake Ohrid surroundings. Strikingly, the “Ohrid-biplot” (Fig. 3) revealed the same groups as the UPGMA cluster (Fig. 6S, supplementary material). Cluster analysis as well as CCA for both datasets revealed water depth as the variable with the strongest apparent influence on ostracod species distribution; while some species that occurred in Lake Ohrid at all depths down to 280m had a relatively broad tolerance to depth, those species

that only colonized waters down to 10m depth (*B. reticulata*, *C. hartmanni*, *C. kingsleii*, *C. litoralis*, *E. virens*, and *H. incongruens*) had low tolerances, thus representing excellent indicator species. The results of our CCA analyses agree with several other studies (e.g., BUNBURY & GAJEWSKI, 2005) confirming that water depth is an influential factor affecting ostracod species distribution. As a note of caution, NEALE (1964) has previously argued that the direct influence of water depth *per se* is probably modest. Its apparent influence probably results from a combination of other environmental variables that are closely linked to water depth, such as water temperature, light intensity and hence photosynthesis; these in turn affect both food supply and shelter (NEALE, 1964). It is difficult to identify which variable is linked to water depth in Lake Ohrid, and the Pearson correlations provide no clear evidence. In the entire dataset (Table 2S, supplementary material) water depth showed no correlation and therefore no relationship to any environmental variable. In the Lake Ohrid dataset (Table 3S, supplementary material) water depth is strongly positively correlated to silt (0.83) but otherwise all the other variables are not associated to water depth. This might indicate that the distribution of ostracod species in Lake Ohrid is determined in part by the availability of silt, linked, for example, to food availability. Another factor, which was not observed by us in the field but which may control ostracod distribution is wave energy (MISCHKE et al., 2010) and concomitant sediment disturbance and stability of the environment. Water depth, however, is an important variable in paleoenvironmental reconstructions (MISCHKE et al., 2010) because it provides information about hydrological changes of a lake system that may have been caused by climate change; on the assumption that these relationships are maintained over time, we have a potential tool to reconstruct fluctuations in water depth.

Water temperature also plays an important role in controlling ostracod distribution (e.g., NEALE, 1964; HORNE, 2007; VAN DER MEEREN et al., 2010). It influences occurrence, growth rate, size, and life span of individual species

(HOLMES, 2001). Here, *C. trapeziformis*, *C. vidua* and *P. karamani* are indicator species for low water temperatures. These species were found in waters with a maximum temperature of 14.8°C, and their temperature tolerances were very narrow (0.7-1.5°C) (Fig. 4).

Another factor known to affect ostracod distribution is nutrient status; in spite of the inclusion of hypereutrophic lakes in the dataset, nutrient variables were not identified as significant in forward selection. The low and rather stable nutrient status in oligotrophic Lake Ohrid and in all sampled spring habitats may be responsible for this insensitivity.

The clear distinction between Lake Ohrid and water bodies in its surroundings, as indicated by SIMPER (Table 1S, supplementary material), is not surprising because of the high level of endemism. But this distinction underlines that paleoreconstruction will have to rely mainly on modern ecological distribution within the lake itself. More detailed studies of seasonality and niche requirements of key taxa are required, and

a more extensive regional training set would be of little value for improving reconstructions.

CONCLUSIONS

A comprehensive investigation of ostracod distribution in a variety of aquatic habitat types (lakes, springs, rivers, and channels) in the southeastern Balkan Peninsula retrieved a total of 47 ostracod species (40 living species and seven species detected by valves and empty carapaces) belonging to 18 genera and seven families (Candonidae, Ilyocyprididae, Cyprididae, Leptocytheridae, Limnocytheridae, Cytherideidae, and Darwinulidae). *C. obliqua*, *Cypria lacustris*, *C. media*, and *A. karamani* were the most common species. Short-lived seasonal waterbodies, channels, ditches, rivers, and springs were characterized by widespread species such as *H. incongruens*, *Heterocypris reptans* (Baird, 1835), *C. kingsleii*, and *Psychrodromus fontinalis* (Wolf, 1920). The ostracod composition of Lake Ohrid is highly distinct from its surroundings, with *C. obliqua* being the most abundant species. Species-specific distribution of ostracods is influenced by a complex interconnection of physical-chemical water characteristics. From canonical correspondence analyses, water depth, salinity, conductivity, pH, and dissolved oxygen were the main determining factors for ostracod distribution in the entire study area. Ostracods in Lake Ohrid were mainly controlled by water depth, water temperature, and pH. Some species appeared to be strong indicator species for important environmental variables: *C. trapeziformis*, *C. vidua*, and *P. karamani* for cold waters (<14.8°C, tolerance <1.5°C) and *B. reticulata*, *C. hartmanni*, *C. kingsleii*, *C. littoralis*, *E. virens*, and *H. incongruens* for shallow water depth. Because of the now established autecology of the ostracod species from Lake Ohrid it is possible to use fossil ostracod assemblages from sediment cores as paleoenvironmental indicators, although a more intensive monitoring programme, including wave energy quantification, is necessary to further strengthen understanding.

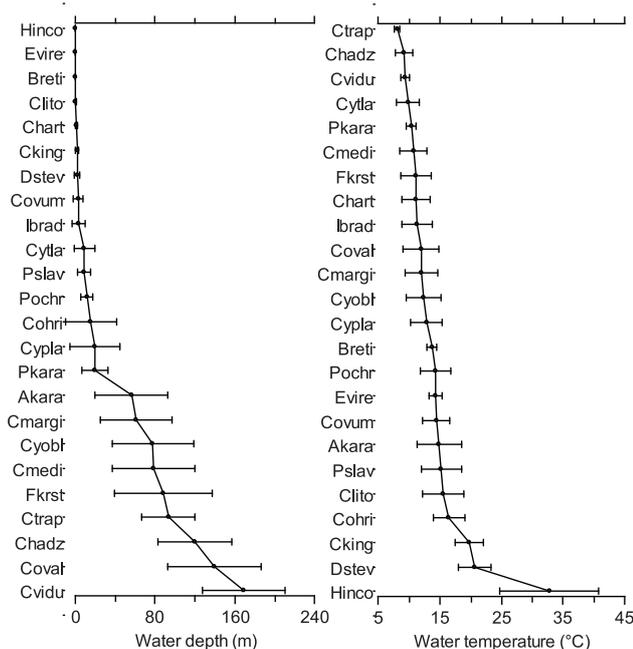


Fig. 4.—Ostracod species optima and tolerance ranges for water depth and water temperature estimated by weighted averaging (WA) regression.

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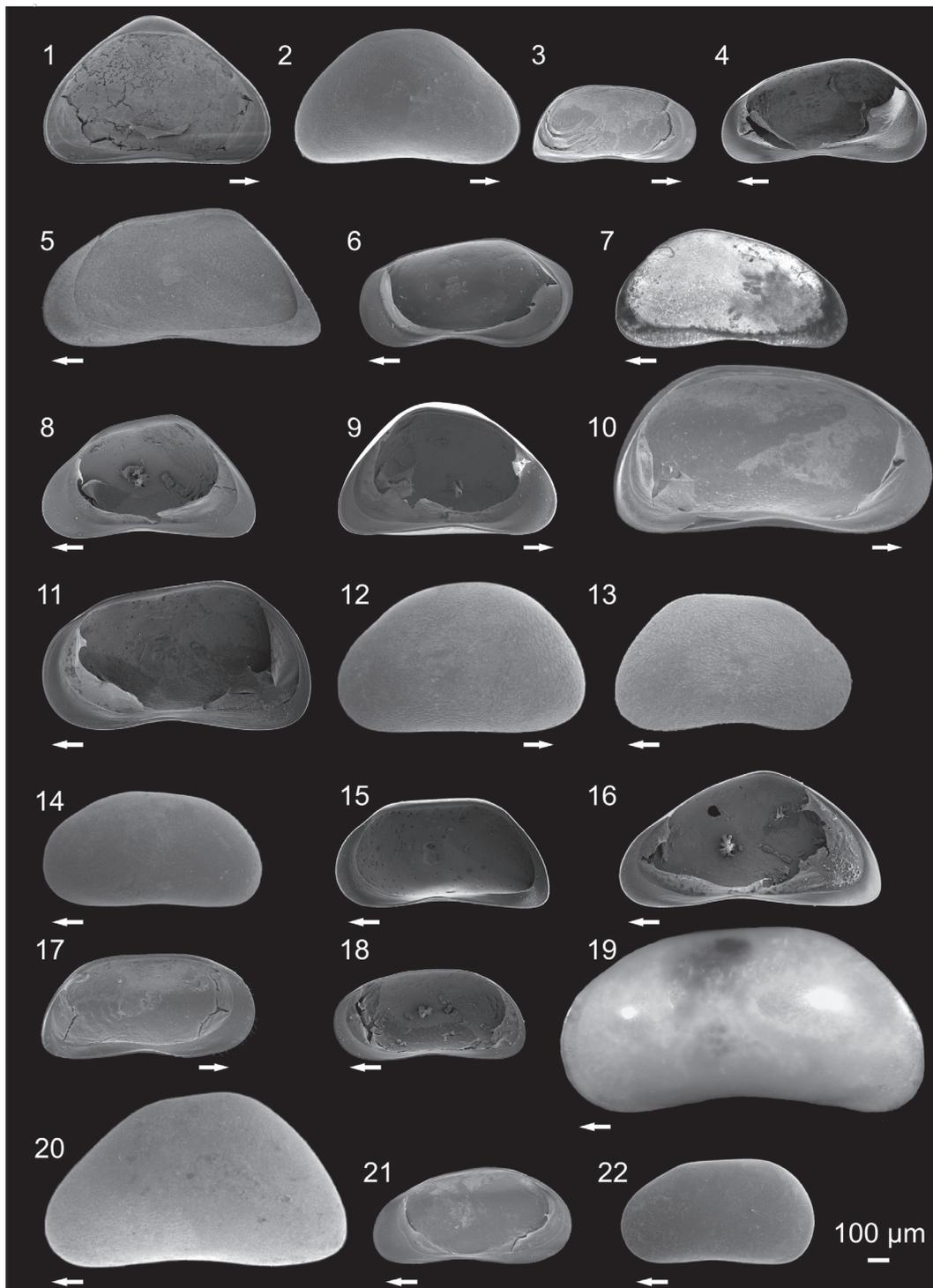


Fig. 1S. – *Candona goricensis* (1) Left valve (LV), internal view, female; *Candona goricensis* (2) Right valve (RV), external view, female; *Candona hadzistei* (3) LV, internal view, male; *Candona hartmanni* (4) RV, internal view, female; *Candona holmesi* (5) RV, internal view, female; *Fabaeformiscandona krstici* (6) RV, internal view, male; *Candona litoralis* (7) LV, external view, male; *Candona margaritana* (8) RV, internal view, female; *Candona margaritana* (9) LV, internal view, female; *Candona marginatoides* (10) LV, internal view, female; *Candona media* (11) RV, internal view, female; *Candona ohrida* (12) RV, external view, male; *Candona ohrida* (13) LV, external view, male; *Candona ovalis* (14) LV, external view, female; *Candona trapeziformis* (15) RV, internal view, female; *Candona triangulata* (16) RV, internal view, female; *Candona vidua* (17) LV, internal view, female; *Candona marginata* (18) RV, internal view, female; *Candona bimucronata* (19) LV, external view, female; *Candona expansa* (20) LV, external view, female; *Candonopsis kingsleii* (21) RV, internal view, male; *Pseudocandona compressa* (22) LV, external view, female. Arrows point to anterior.

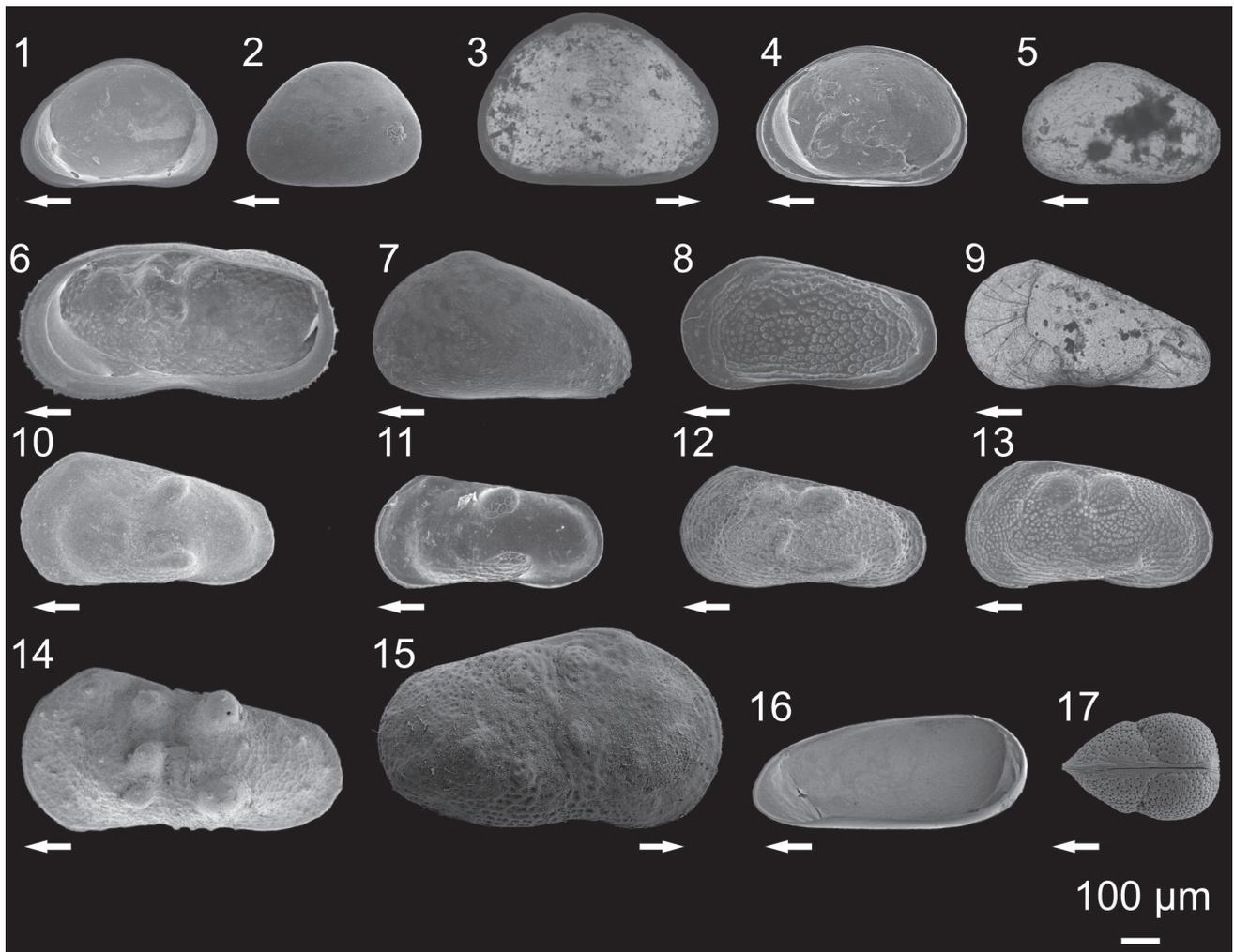


Fig. 2S. – *Cypria lacustris* (1) Right valve (RV), internal view, female; *Cypria obliqua* (2) Left valve (LV), external view, female; *Cypria ophtalmica* (3) LV, internal view, isolated valve; *Cyclocypris ovum* (4) RV, internal view, female; *Heterocypris salina* (5) LV, external view, isolated valve; *Ilyocypris bradyi* (6) RV, internal view, female; *Prionocypris zenkeri* (7) LV, external view, juvenile; *Amnicythere karamani* (8) LV, external view, female; *Paralimnocythere alata* (9) LV, external view, female; *Paralimnocythere georgevitschi* (10) LV, external view, female; *Paralimnocythere karamani* (11) LV, external view, female; *Paralimnocythere ochridense* (12) LV, external view, female; *Paralimnocythere slavei* (13) LV, external view, female; *Paralimnocythere umbonata* (14) LV, external view, female; *Cytherissa lacustris* (15) RV, external view, female; *Darwinula stevensoni* (16) RV, internal view, female; *Kovalevskiella* sp. (17) dorsal view, female. Arrows point to anterior.

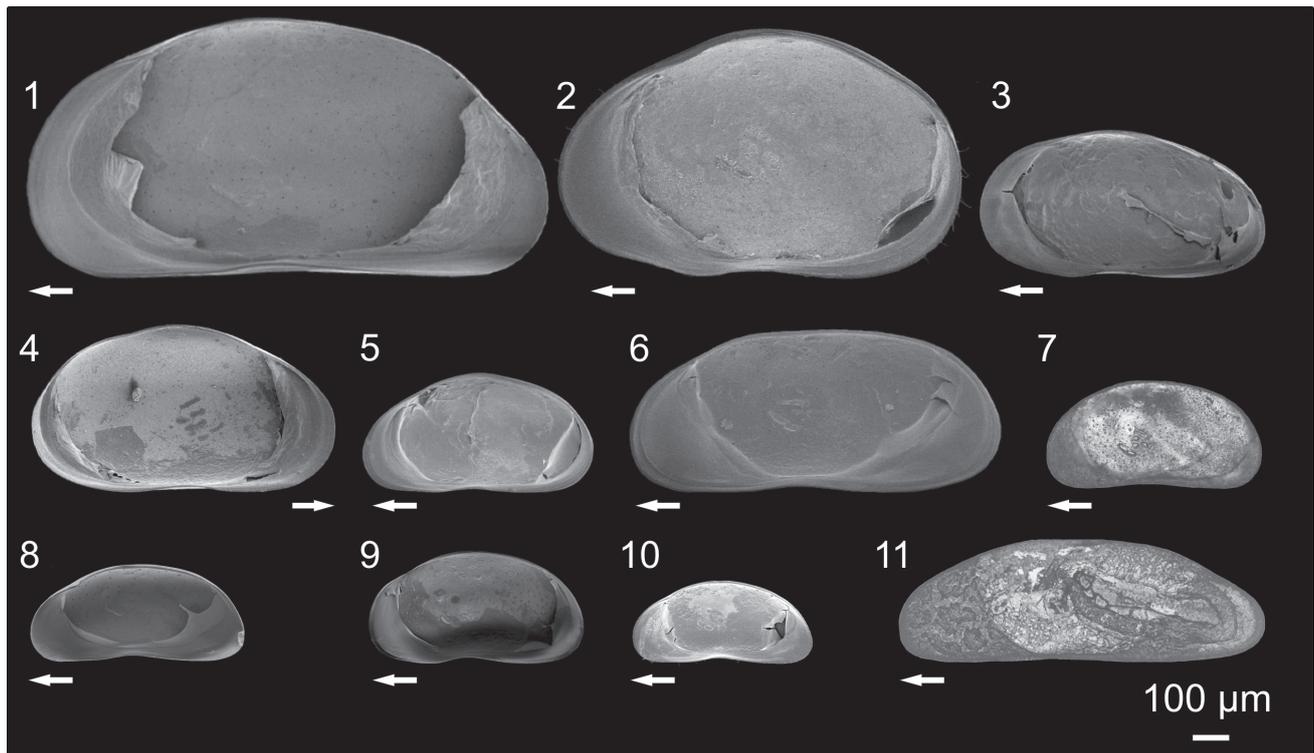


Fig. 3S. – *Eucypris* sp. (1) Right valve (RV), internal view, female; *Eucypris virens* (2) RV, internal view, female; *Bradleystrandesia reticulata* (3) RV, internal view, female; *Heterocypris incongruens* (4) Left valve (LV), internal view, female; *Heterocypris reptans* (5) RV, internal view, female; *Herpetocypris* sp. (6) RV, internal view, female; *Herpetocypris* sp. 2 (7) LV, external view, female; *Psychrodromus fontinalis* (8) RV, internal view, female; *Psychrodromus olivaceus* (9) RV, internal view, female; *Psychrodromus* sp. (10) RV, internal view, female; *Dolerocypris sinensis* (11) LV, external view, female. Arrows point to anterior.

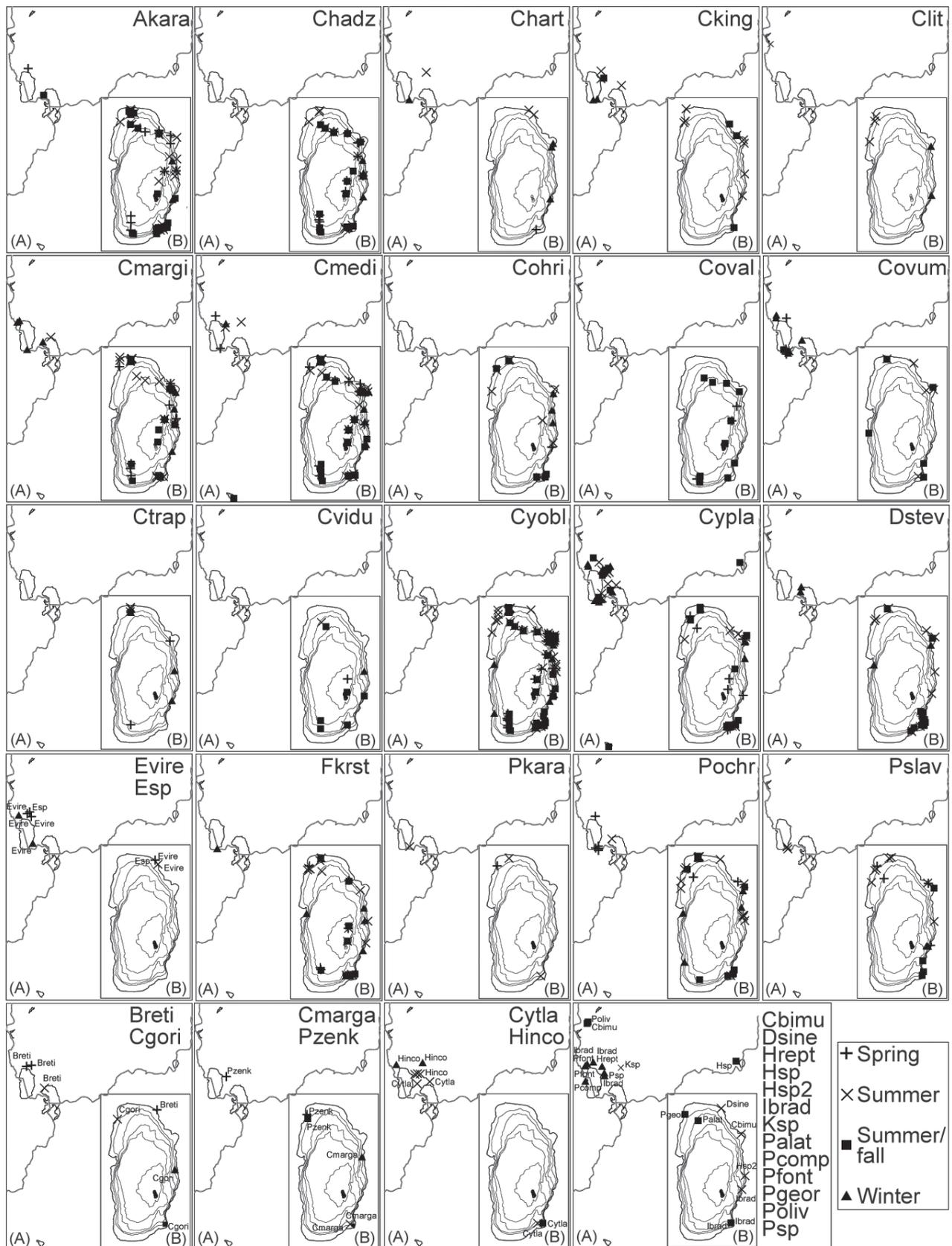


Fig. 4S. – Distribution maps of the 40 living ostracod species in the study area (A) and in Lake Ohrid (B). Species codes as in Table 1.

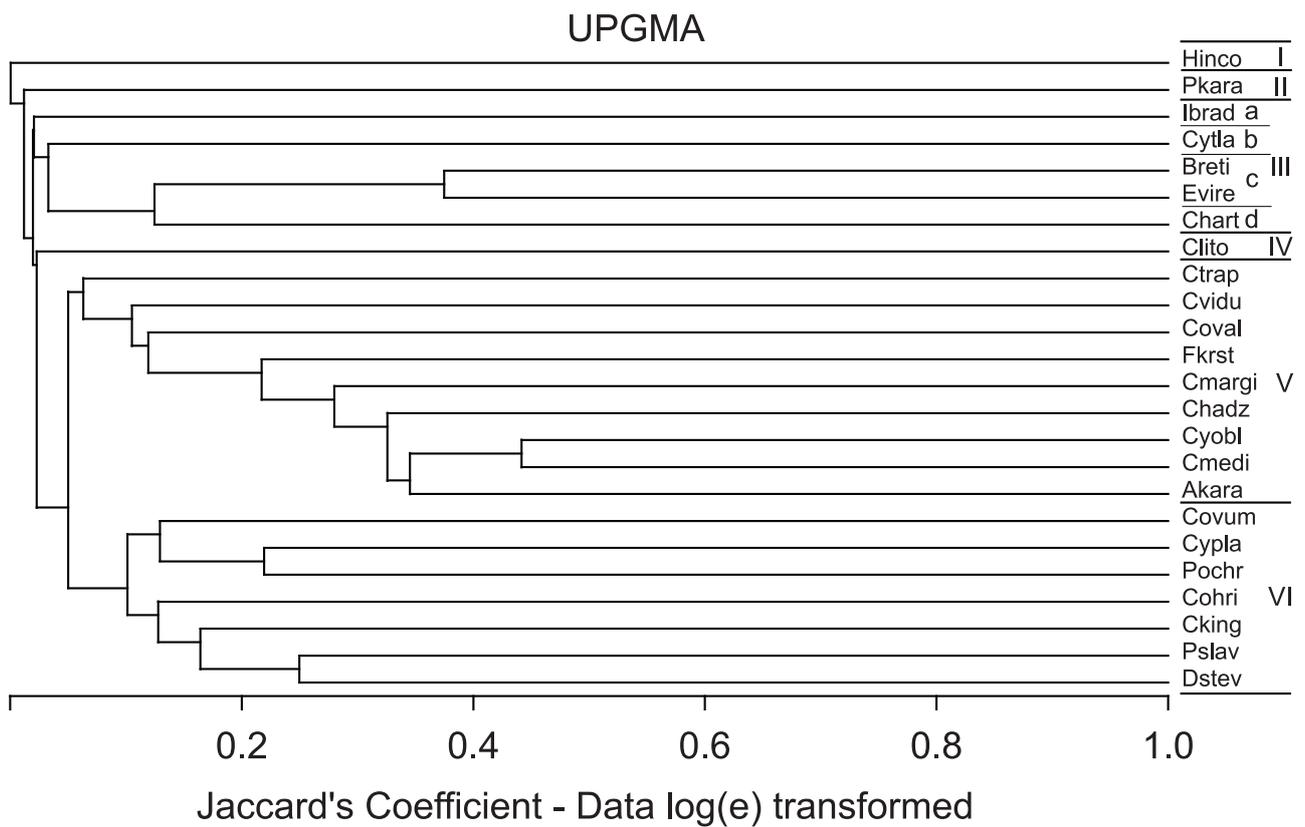


Fig. 5S. – Dendrogram for the unweighted pair group mean average (UPGMA) for the entire dataset. Roman numerals and characters after species codes (as in Table 1) indicate the clustering groups.

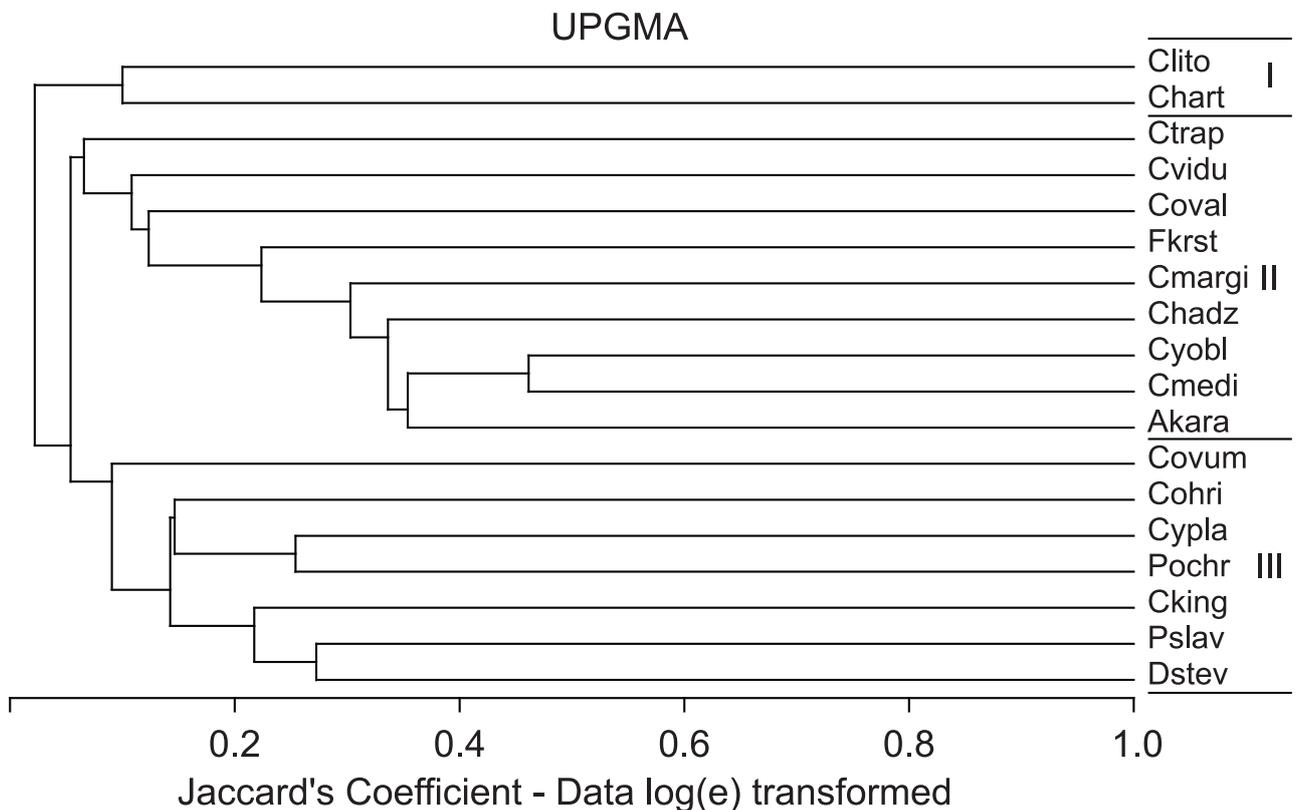


Fig. 6S. – Dendrogram for the unweighted pair group mean average (UPGMA) for the Lake-Ohrid dataset. Roman numerals after species codes (as in Table 1) indicate the clustering groups.

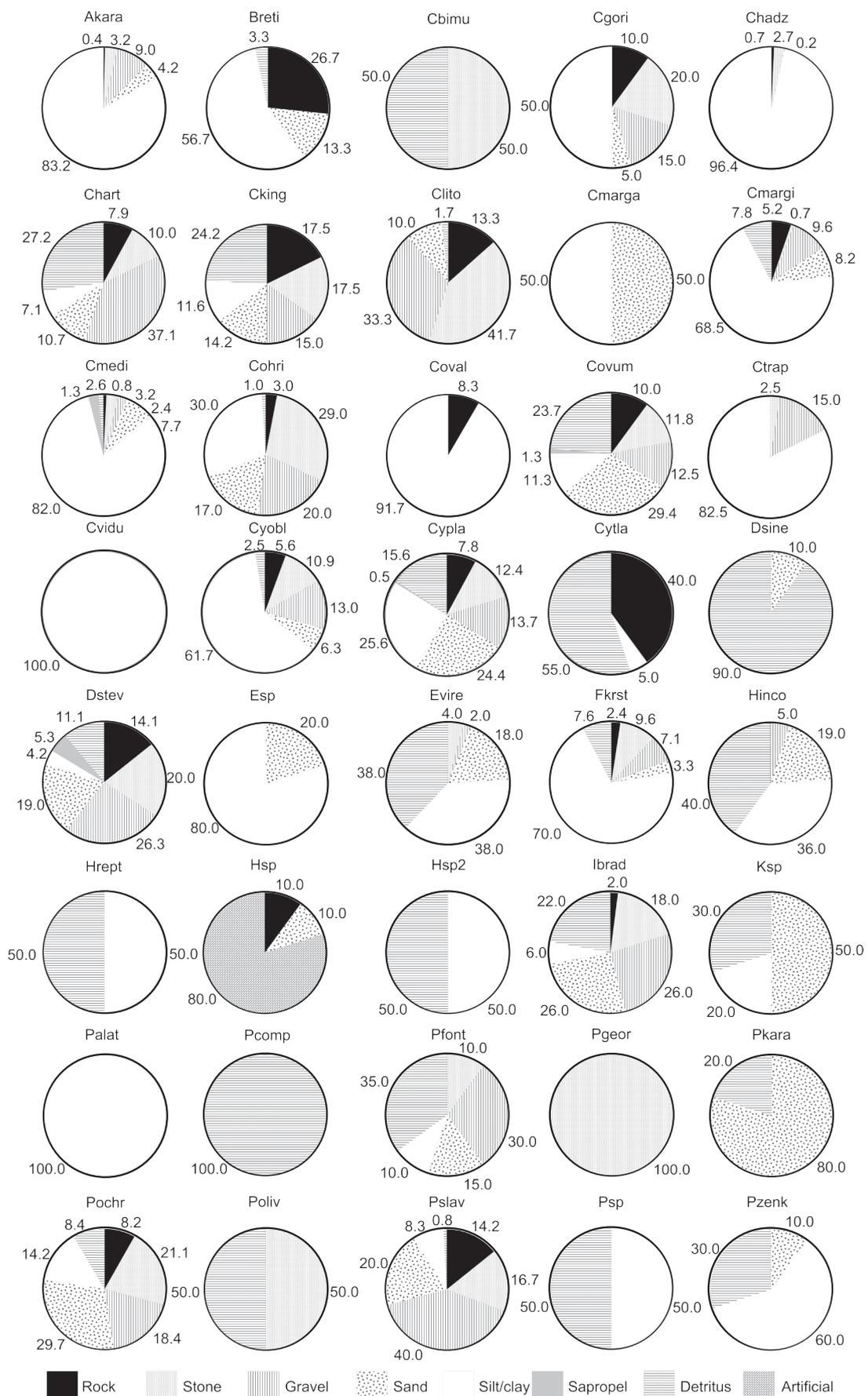


Fig. 7S. – Pie diagram showing the average sediment composition at the sampling locations for the 40 living ostracod species. Species codes as in Table 1.

TABLE 1S

Summary of the similarity percentage (SIMPER) analysis for the first five main ostracod species contributing to the average dissimilarity between habitats (Diss. contr.: Dissimilarity contribution, Cum. diss.: Cumulative dissimilarity, and Av. diss.: Average dissimilarity).

Ohrhid vs. channel	Diss. contr. (%)	Cum. diss. (%)	Ohrhid vs. ditches	Diss. contr. (%)	Cum. diss. (%)	Ohrhid vs. lakes	Diss. contr. (%)	Cum. diss. (%)
Cypla	43.85	49.56	Cyobl	21.54	23.06	Cypla	28.76	32.48
Cyobl	21.54	73.91	Cypla	21.18	45.73	Cyobl	21.54	56.80
Akara	3.38	77.73	Cking	13.88	60.59	Dstev	9.78	67.85
Cmargi	2.69	80.78	Breti	10.13	71.44	Akara	6.30	74.97
Pochr	2.68	83.80	Cmargi	6.61	78.52	Cmedi	4.78	80.36
Av. diss.: 88.48%			Av. diss.: 93.40%			Av. diss.: 88.55%		
Ohrhid vs. flooded areas	Diss. contr. (%)	Cum. diss. (%)	Ohrhid vs. rivers	Diss. contr. (%)	Cum. diss. (%)	Ohrhid vs. springs	Diss. contr. (%)	Cum. diss. (%)
Hinco	25.00	25.47	Cyobl	21.54	22.72	Cyobl	21.54	23.23
Cyobl	21.54	47.42	Cypla	16.76	40.40	Cypla	20.26	45.08
Ibrad	9.75	57.35	Cking	14.30	55.48	Covum	10.59	56.51
Pochr	6.70	64.18	Cmedi	8.88	64.84	Pochr	5.69	62.65
Evire	6.33	70.63	Covum	7.91	73.18	Pslav	5.44	68.51
Av. diss.: 98.15%			Av. diss.: 94.81%			Av. diss.: 92.73%		
Channels vs. ditches	Diss. contr. (%)	Cum. diss. (%)	Channels vs. lakes	Diss. contr. (%)	Cum. diss. (%)	Channels vs. flooded areas	Diss. contr. (%)	Cum. diss. (%)
Cypla	29.48	49.49	Cypla	18.71	49.17	Cypla	49.60	49.80
Cking	13.58	72.28	Dstev	9.04	72.91	Hinco	25.00	74.90
Breti	10.06	89.17	Akara	4.00	83.42	Ibrad	9.71	84.65
Cmargi	4.78	97.19	Cmedi	3.33	92.18	Evire	6.19	90.86
Cypla	0.78	98.50	Covum	1.32	85.64	Pochr	4.67	95.55
Av. diss.: 59.57%			Av. diss.: 38.05%			Av. diss.: 99.60%		
Channels vs. rivers	Diss. contr. (%)	Cum. diss. (%)	Channels vs. springs	Diss. contr. (%)	Cum. diss. (%)	Ditches vs. lakes	Diss. contr. (%)	Cum. diss. (%)
Cypla	35.54	49.60	Cypla	29.88	49.46	Cypla	25.54	34.55
Cking	13.90	69.00	Covum	10.08	66.15	Cking	13.58	52.91
Cmedi	7.53	79.51	Pslav	4.29	73.25	Breti	10.06	66.53

Clito	7.14	89.48	Pochr	3.61	79.22	Dstev	9.04	78.75
Covum	7.14	99.45	Chart	2.78	83.82	Cmargi	4.94	85.43
Av. diss.: 71.66%			Av. diss.: 60.40%			Av. diss.: 73.92%		
Ditches vs. flooded areas	Diss. contr. (%)	Cum. diss. (%)	Ditches vs. rivers	Diss. contr. (%)	Cum. diss. (%)	Ditches vs. springs	Diss. contr. (%)	Cum. diss. (%)
Hinco	25.00	25.35	Cypla	22.99	28.56	Cypla	23.35	28.62
Cypla	20.31	45.94	Cking	19.72	53.06	Cking	13.94	45.71
Cking	13.58	59.70	Breti	10.06	65.56	Covum	10.11	58.10
Breti	10.11	69.95	Cmedi	7.58	74.97	Breti	10.08	70.45
Ibrad	9.77	79.86	Covum	7.34	84.08	Cmargi	4.79	76.32
Av. diss.: 98.63%			Av. diss.: 80.50%			Av. diss.: 81.58%		
Lakes vs. flooded areas	Diss. contr. (%)	Cum. diss. (%)	Lakes vs. rivers	Diss. contr. (%)	Cum. diss. (%)	Lakes vs. springs	Diss. contr. (%)	Cum. diss. (%)
Cypla	31.05	31.34	Cypla	27.59	34.16	Cypla	23.23	33.27
Hinco	25.00	56.58	Cking	13.90	51.37	Dstev	10.60	48.46
Ibrad	9.71	66.38	Cmedi	9.76	63.45	Covum	10.35	63.28
Dstev	9.04	75.50	Dstev	9.04	74.63	Pslav	4.29	69.41
Evire	6.19	81.75	Covum	8.08	84.64	Pochr	4.23	75.48
Av. diss.: 99.07%			Av. diss.: 80.77%			Av. diss.: 69.82%		
Flooded areas vs. rivers	Diss. contr. (%)	Cum. diss. (%)	Flooded areas vs. springs	Diss. contr. (%)	Cum. diss. (%)	Rivers vs. springs	Diss. contr. (%)	Cum. diss. (%)
Hinco	25.00	25.01	Hinco	25.00	25.29	Cypla	22.80	26.87
Cypla	14.29	39.30	Cypla	19.87	45.40	Cking	14.57	44.04
Cking	13.90	53.21	Covum	10.08	55.60	Covum	14.34	60.95
Ibrad	9.71	62.93	Ibrad	9.72	65.43	Cmedi	7.53	69.82
Cmedi	7.56	70.49	Pochr	7.43	72.94	Clito	7.14	78.24
Av. diss.: 99.96%			Av. diss.: 98.84%			Av. diss.: 84.86%		

TABLE 2S

Pearson correlation between pairs of environmental variables and habitat types for the entire dataset. Variables with high correlations ($\pm/0.7-1.0$) are bold and underlined and correlations between 0.6-0.7 are bold.

	Salinity	Depth	DO	Temp	pH	SO ₄	PO ₄	NH ₄	NO ₂	NO ₃	Cond	Ca	Fe	Mg	K	Na	Mn	Sr	Cl	DOC	Ohrtd	Channel	Ditch	Lake	Flooded	River	Sprng	Rocks	Stones	Gravel	Sand	Silt	Sapropel	Detritus					
Salinity	1.00																																						
Depth	-0.18	1.00																																					
DO	-0.10	0.08	1.00																																				
Temp	0.03	-0.23	0.35	1.00																																			
pH	0.09	0.17	0.51	0.54	1.00																																		
SO ₄	-0.16	0.32	0.37	0.06	0.32	1.00																																	
PO ₄	0.18	0.00	0.07	-0.02	0.00	-0.02	1.00																																
NH ₄	-0.11	0.18	0.05	-0.06	-0.01	0.23	0.06	1.00																															
NO ₂	-0.04	-0.02	-0.09	-0.09	-0.13	0.00	0.10	0.84	1.00																														
NO ₃	-0.07	0.16	0.08	-0.07	0.07	0.17	0.00	0.05	0.03	1.00																													
Cond	0.27	0.03	0.33	0.34	0.29	0.11	0.05	0.02	-0.05	0.07	1.00																												
Ca	-0.05	0.12	0.26	-0.06	0.22	0.41	-0.05	0.04	-0.03	0.09	0.04	1.00																											
Fe	-0.09	0.04	0.24	-0.08	0.18	0.26	-0.04	-0.08	-0.04	-0.07	0.00	0.73	1.00																										
Mg	-0.10	0.12	0.28	-0.05	0.22	0.42	-0.05	0.05	-0.03	-0.09	0.03	0.98	0.71	1.00																									
K	-0.10	0.10	0.28	-0.03	0.22	0.42	-0.05	0.07	-0.03	-0.09	0.03	0.96	0.68	0.98	1.00																								
Na	-0.09	0.12	0.27	-0.04	0.21	0.42	-0.05	0.07	-0.03	-0.09	0.03	0.95	0.66	0.98	0.99	1.00																							
Mn	-0.07	0.09	0.18	-0.09	0.16	0.20	-0.03	-0.07	-0.03	-0.06	0.01	0.65	0.92	0.60	0.56	0.51	1.00																						
Sr	-0.09	0.12	0.27	-0.05	0.22	0.42	-0.05	0.05	-0.03	-0.09	0.03	0.98	0.72	1.00	0.98	0.98	0.61	1.00																					
Cl	-0.10	0.37	0.31	-0.06	0.19	0.45	0.09	0.15	-0.02	0.00	0.08	0.24	0.17	0.24	0.24	0.25	0.13	0.24	1.00																				
DOC	-0.07	0.11	0.20	-0.02	0.18	0.30	-0.04	0.07	-0.02	-0.07	0.04	0.74	0.25	0.77	0.73	0.72	0.23	0.76	0.18	1.00																			
Ohrtd	-0.46	0.37	0.30	0.14	0.32	0.32	-0.09	0.01	-0.11	0.01	-0.21	0.21	0.18	0.24	0.24	0.23	0.15	0.23	0.21	0.18	1.00																		
Channel	-0.05	-0.11	-0.06	-0.10	-0.09	-0.08	0.03	0.33	0.40	0.04	-0.04	-0.08	-0.05	-0.08	-0.07	-0.07	-0.05	-0.08	-0.09	-0.06	-0.30	1.00																	
Ditch	-0.04	-0.10	-0.12	-0.06	-0.28	-0.12	-0.02	-0.06	-0.02	-0.04	0.12	-0.07	-0.05	-0.07	-0.07	-0.07	-0.04	-0.07	-0.08	-0.05	-0.27	-0.03	1.00																
Lake	-0.04	-0.10	-0.12	0.05	0.00	0.04	-0.01	0.03	-0.01	-0.04	-0.01	-0.07	-0.05	-0.07	-0.07	-0.07	-0.04	-0.07	-0.03	-0.05	-0.27	-0.03	-0.02	1.00															
Flooded	0.22	-0.13	-0.08	-0.04	-0.26	-0.15	-0.02	-0.08	-0.03	-0.05	0.07	-0.09	-0.06	-0.09	-0.09	-0.09	-0.05	-0.09	-0.10	-0.07	-0.35	-0.03	-0.03	-0.03	1.00														
River	-0.05	-0.12	-0.20	-0.04	-0.07	-0.14	-0.01	0.01	0.02	0.05	0.12	-0.08	-0.06	-0.08	-0.08	-0.08	-0.05	-0.08	-0.09	-0.06	-0.32	-0.03	-0.03	-0.04	1.00														
Springs	0.62	-0.21	-0.11	-0.09	-0.06	-0.20	0.15	-0.13	-0.05	0.00	0.14	-0.08	-0.10	-0.12	-0.13	-0.11	-0.09	-0.12	-0.08	-0.09	-0.58	-0.06	-0.05	-0.07	-0.06	1.00													
Rocks	0.18	-0.18	-0.07	0.19	0.00	-0.12	-0.03	-0.09	-0.02	-0.05	0.02	-0.12	-0.09	-0.13	-0.12	-0.12	-0.07	-0.13	-0.14	-0.10	-0.13	-0.05	0.12	0.04	-0.06	0.03	0.15	1.00											
Stones	0.08	-0.25	-0.04	0.13	-0.04	0.03	-0.05	-0.12	-0.04	-0.07	-0.03	-0.18	-0.13	-0.18	-0.17	-0.17	-0.11	-0.18	-0.16	-0.14	0.03	-0.07	-0.06	0.06	-0.08	0.03	0.15	1.00											
Gravel	0.00	-0.26	-0.09	0.10	-0.15	-0.19	-0.05	-0.15	-0.04	-0.09	-0.03	-0.20	-0.14	-0.20	-0.20	-0.20	-0.12	-0.20	-0.23	-0.16	0.00	-0.01	-0.03	-0.04	0.03	0.03	0.00	0.28	1.00										
Sand	0.22	-0.29	-0.12	-0.03	-0.14	-0.16	-0.05	-0.11	-0.01	0.02	0.08	-0.13	-0.15	-0.18	-0.18	-0.17	-0.12	-0.17	-0.15	-0.13	-0.43	0.01	0.04	0.12	0.16	0.20	0.32	-0.05	0.02	0.19	1.00								
Silt	-0.15	0.77	0.02	-0.28	-0.04	0.20	0.05	0.19	0.07	0.24	0.09	-0.06	-0.10	-0.07	-0.08	-0.07	0.00	-0.06	0.26	-0.02	0.13	0.07	0.01	-0.09	0.04	-0.09	-0.16	-0.18	-0.25	-0.26	-0.18	1.00							
Sapropel	-0.02	-0.05	-0.08	0.11	0.04	0.04	0.00	0.05	0.01	-0.02	0.06	-0.04	-0.03	-0.04	-0.04	-0.04	-0.04	-0.04	0.07	-0.03	-0.14	-0.01	-0.01	0.43	-0.02	-0.02	0.02	-0.02	-0.03	-0.02	-0.02	-0.05	1.00						
Detritus	0.26	-0.23	-0.18	-0.10	-0.11	-0.24	-0.03	-0.02	0.06	-0.06	0.01	-0.14	-0.11	-0.15	-0.15	-0.15	-0.09	-0.15	-0.17	-0.11	-0.40	0.14	0.06	-0.01	0.24	-0.02	0.33	0.02	-0.07	-0.08	0.10	-0.11	-0.02	1.00					

TABLE 3S
 Pearson correlation between pairs of environmental variables for the Lake-Ohrid dataset. Variables with high correlations ($\pm 0.7-1.0$) are bold and underlined and correlations between 0.6-0.7 are bold.

	Depth	Salinity	DO	Temp	pH	SO ₄	PO ₄	NH ₄	NO ₂	NO ₃	Cond	Ca	Fe	Mg	K	Na	Mn	Sr	Cl	DOC	Rocks	Stones	Gravel	Sand	Silt	Detritus
Depth	1.00																									
Salinity	-0.06	1.00																								
DO	-0.04	-0.13	1.00																							
Temp	-0.36	0.09	0.29	1.00																						
pH	0.08	0.04	0.53	0.53	1.00																					
SO ₄	0.24	-0.07	0.31	-0.04	0.27	1.00																				
PO ₄	0.05	-0.01	0.14	-0.02	0.02	-0.06	1.00																			
NH ₄	0.35	-0.05	0.22	-0.04	0.14	0.44	-0.04	1.00																		
NO ₂	0.19	-0.04	0.09	0.12	0.15	0.44	0.01	0.47	1.00																	
NO ₃	0.18	-0.02	0.09	-0.12	0.04	0.21	0.02	0.00	0.01	1.00																
Cond	0.21	-0.03	0.58	0.24	0.54	0.37	0.05	0.29	0.25	0.11	1.00															
Ca	0.05	0.04	0.24	-0.12	0.21	0.38	-0.05	0.10	-0.04	-0.10	0.15	1.00														
Fe	-0.02	-0.03	0.23	-0.12	0.17	0.23	-0.04	-0.14	-0.13	-0.08	0.07	0.75	1.00													
Mg	0.04	-0.04	0.25	-0.10	0.21	0.38	-0.05	0.10	-0.04	-0.10	0.15	0.99	0.70	1.00												
K	0.02	-0.04	0.26	-0.08	0.20	0.39	-0.05	0.13	-0.01	-0.10	0.15	0.97	0.67	0.98	1.00											
Na	0.04	-0.04	0.25	-0.09	0.18	0.39	-0.05	0.14	0.00	-0.10	0.15	0.95	0.65	0.98	0.99	1.00										
Mn	0.04	-0.02	0.17	-0.13	0.16	0.17	-0.03	-0.14	-0.13	-0.07	0.07	0.66	0.92	0.59	0.55	0.50	1.00									
Sr	0.05	-0.04	0.25	-0.11	0.21	0.38	-0.05	0.11	-0.03	-0.10	0.15	0.99	0.71	1.00	0.98	0.98	0.60	1.00								
Cl	0.34	-0.05	0.29	-0.14	0.15	0.43	0.22	0.29	0.09	-0.08	0.23	0.20	0.14	0.21	0.21	0.21	0.11	0.21	1.00							
DOC	0.06	-0.03	0.18	-0.05	0.17	0.26	-0.04	0.14	-0.01	-0.08	0.14	0.73	0.23	0.76	0.71	0.71	0.21	0.75	0.15	1.00						
Rocks	-0.19	-0.02	0.01	0.26	0.07	-0.07	-0.02	-0.11	0.08	-0.04	0.08	-0.13	-0.09	-0.13	-0.13	-0.13	-0.08	-0.13	-0.14	-0.10	1.00					
Stones	-0.31	-0.03	0.10	0.17	-0.11	-0.04	-0.04	-0.20	0.11	-0.07	-0.01	-0.21	-0.15	-0.21	-0.21	-0.20	-0.12	-0.21	-0.23	-0.16	0.24	1.00				
Gravel	-0.31	-0.04	0.14	0.17	-0.26	-0.22	-0.04	-0.24	-0.05	-0.09	0.00	-0.23	-0.16	-0.23	-0.23	-0.23	-0.14	-0.23	-0.25	-0.18	0.05	0.27	1.00			
Sand	-0.28	-0.03	0.08	0.09	-0.20	-0.11	-0.04	-0.15	0.02	-0.06	0.03	-0.20	-0.14	-0.20	-0.20	-0.20	-0.12	-0.20	-0.22	-0.15	-0.02	0.11	0.34	1.00		
Silt	0.83	-0.02	0.05	-0.35	-0.03	0.20	0.15	0.26	0.10	0.28	0.20	-0.08	-0.14	-0.10	-0.12	-0.10	-0.02	-0.10	0.29	-0.04	-0.16	-0.26	-0.16	1.00		
Detritus	-0.15	0.36	-0.09	-0.01	0.00	-0.14	-0.02	-0.05	-0.09	-0.03	0.04	-0.10	-0.07	-0.10	-0.10	-0.10	-0.06	-0.10	-0.11	-0.08	-0.02	0.01	-0.05	0.06	-0.10	1.00

TABLE 4S

Estimated optima, tolerance ranges, and standard errors (SE) for significant variables determined with weighted averaging (WA) model. Abbreviations for species see Table 1.

Species	Water depth (m)			Water temperature (°C)			Salinity (‰)			pH			DO (mg l ⁻¹)			Conductivity (µS cm ⁻¹)								
	Opt	SE _{Opt}	Tol	SE _{Opt}	SE _{Tol}	Tol	SE _{Opt}	SE _{Tol}	Tol	SE _{Opt}	SE _{Tol}	Tol	SE _{Opt}	SE _{Tol}	Tol	SE _{Opt}	SE _{Tol}	Tol						
code																								
Akara	56.7	73	14.7	12.2	14.9	7.1	1.6	0.5	0.00	0.04	0.00	0.00	8.29	0.46	0.08	0.06	9.3	1.6	0.4	0.4	232.9	12.5	2.2	3.9
Brefi	0.5	0.1	0.1	13.1	13.7	1.7	1.3	1.4	0.10	0.04	0.00	0.00	9.16	0.43	0.00	0.00	6.2	0.6	0.4	0.7	957.8	245.9	185.6	99.1
Chadz	119.9	74.2	14	5.6	9.2	2.7	0.4	0.6	0.00	0.04	0.00	0.00	8.09	0.37	0.07	0.06	9.2	1.5	0.3	0.2	237.3	5.8	1.1	0.7
Chart	1.3	2.4	0.8	5.3	11.2	4.4	2.2	1.6	0.03	0.05	0.02	0.01	8.87	0.28	0.14	0.10	5.7	5.2	3.1	1.6	311.3	103.5	56.3	35.2
Cking	1.7	3	0.9	1.1	19.8	4.5	1.6	1	0.01	0.04	0.01	0.01	8.56	0.22	0.07	0.07	7.4	2.2	0.9	1.1	411.7	333.8	136.2	158.8
Clito	0.5	1	0.2	5.1	15.5	6.7	3.8	2.7	0.00	0.04	0.00	0.00	8.82	0.31	0.19	0.13	1.4	5.2	3.1	1.7	233.7	0.6	0.4	21
Cmargi	61.1	72.3	13.2	10.9	12.1	5.3	1.4	0.8	0.00	0.02	0.00	0.01	8.28	0.44	0.11	0.04	8.3	2.2	0.5	0.4	245	34.5	9.8	16.4
Cmedi	78.3	83.2	14	7.5	10.8	4.5	0.9	1	0.00	0.01	0.00	0.01	8.24	0.50	0.07	0.06	8.8	2.7	0.6	0.6	244.2	32.6	5.6	11.1
Cohri	15.8	51.6	16.5	28.3	16.5	5.1	1.6	1.4	0.00	0.04	0.00	0.00	8.54	0.32	0.14	0.07	8.9	1.6	0.6	0.6	230.7	6.7	2.7	2.4
Coval	139.6	94.7	29.2	16	12	5.9	2	2.1	0.00	0.04	0.00	0.00	8.10	0.36	0.13	0.07	8.2	1.4	0.4	0.2	236.7	9.7	3.1	2.9
Covum	3.3	8.5	1.5	3.9	14.4	4.5	1	0.7	0.05	0.05	0.01	0.00	8.20	0.62	0.18	0.11	5.8	2.7	1	0.5	343.3	122.1	32.6	35.8
Ctrap	93.6	53.4	24.3	17.5	8.2	0.7	0.4	0.8	0.00	0.04	0.00	0.00	8.00	0.57	0.24	0.23	9.9	0.5	0.3	0.6	232.3	8.1	6.4	11.5
Cvidu	168.7	83.1	25.2	20.3	9.5	1.1	0.4	0.3	0.00	0.04	0.00	0.00	8.04	0.24	0.06	0.09	7.7	1.7	0.7	0.5	241.3	7.8	2.8	1.9
Cyobl	77.9	81.6	8.9	6.7	12.4	5.7	0.5	0.4	0.00	0.01	0.00	0.01	8.37	0.53	0.05	0.04	8.8	1.9	0.2	0.2	234	18.4	2	7
Cypla	19.5	49.9	7.1	16.2	12.9	5.1	0.7	0.4	0.01	0.04	0.00	0.00	8.25	0.52	0.07	0.06	7.7	3.2	0.5	0.5	269	43.8	6.2	4
Cytla	9	20.1	10.5	6.9	9.9	3.7	2	2.2	0.00	0.04	0.00	0.00	8.52	0.48	0.31	0.15	9.3	1.9	1	0.6	257.4	24.3	14	11.5
Dstev	2.3	3.8	0.9	0.9	20.6	5.3	1.4	1.4	0.00	0.04	0.00	0.00	8.62	0.26	0.06	0.08	7.9	3.1	0.8	0.7	239.1	35.3	10.2	11.6
Evire	0.3	0.2	0.1	8.9	14.4	2	1	0.8	0.10	0.06	0.04	0.02	9.13	0.36	0.25	0.16	6.5	1.3	0.9	0.9	604.2	207.5	132.1	81.3
Fkrst	88.5	97.3	27.9	14.2	11.2	4.9	1.7	1	0.00	0.04	0.00	0.00	8.28	0.53	0.18	0.09	8.1	2.4	0.6	0.5	238.3	10.7	3.1	2.8
Hinco	0.1	0.1	0	13.5	32.8	16.1	10.6	5.2	0.10	0.07	0.04	0.01	8.12	0.14	0.09	0.15	8.5	0.3	0.2	0.9	458	140	83.2	42.8
Ibrad	3.6	12.3	8.2	7.1	11.3	5	2.9	2.2	0.09	0.04	0.03	0.02	7.94	0.18	0.19	0.18	4.8	2.2	1.7	1.1	337.8	52.5	38.2	21.7
Pkara	19.9	26.7	14.8	9.4	10.4	1.5	0.9	0.9	0.05	0.07	0.04	0.01	8.54	1.13	0.58	0.42	7.4	2.7	1.5	1.1	264.5	67.3	34.7	24
Pochr	11.5	12.9	2.8	1.4	14.3	5	0.9	0.6	0.01	0.04	0.01	0.01	8.64	0.74	0.19	0.13	8.2	2.3	0.6	0.4	252.4	39.4	10.5	5.9
Pslav	9.2	11.6	3.5	2.9	15.3	6.6	2.4	1.3	0.00	0.04	0.00	0.00	8.29	0.37	0.14	0.07	8.4	1.8	0.8	0.4	260.3	45	20	13.3

Visual discrimination of shapes in the ant *Myrmica rubra* (Hymenoptera, Formicidae)

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ABSTRACT. Using collective differential operant conditioning, it could be shown that workers of the species *Myrmica rubra* distinguish different filled shapes of similar size (e.g. a black square from a black circle, a black triangle from a black circle, and a black triangle from a black square). They are unable to discriminate hollow shapes (e.g. a rectangle from an ellipse, or a lozenge from an ellipse) of similar size. When presented with hollow shapes, *M. rubra* workers rely more on the length of the perimeter than on the actual shape. A hollow lozenge and rectangle of identical height and width but with a different perimeter can be partly distinguished; hollow shapes of different height and width but identical perimeter are not at all discriminated. The visual perception ability in *M. rubra* is weaker than in *M. ruginodis* but superior to that in *M. sabuleti*; this assessment is consistent with the eye morphology and the navigation system of the three species.

KEY WORDS: differential conditioning, filled and hollow shapes, operant conditioning, visual cues.

INTRODUCTION

Ants are known to essentially use odors (principally pheromones) for communication, brood care, recruitment of congeners, food collection and nest relocation. However, they also use visual perception to perform tasks, such as foraging, returning to the nest after finding either a new food source or a new nest site and returning to the foraging area after having removed a dead nestmate. The fact that ants use their visual perception to forage has been shown for numerous species, e.g. *Gigantiops destructor* (BEUGNON et al., 2005), *Cataglyphis bicolor* (COLLETT et al., 1992), *Leptothorax albipennis* (PRATT et al., 2001), *Formica rufa* (NICHOLSON et al., 1999), *Tapinoma sessile* and *Camponotus pennsylvanicus* (KLOTZ & REID, 1992), *Myrmica sabuleti* (CAMMAERTS & RACHIDI, 2009), *Myrmica ruginodis* (CAMMAERTS et al., 2011). In general, foraging ants orient themselves using visual cues according to the snapshot and sketch map models (PASSERA & ARON, 2005).

When examining an ant species' navigation system, it is essential to also analyze that species'

ability to see and discriminate shapes, forms, dimension, orientation, coloration and other parameters.

Studies on the visual perception of ants have generally concerned species with large eyes and good vision, e.g. *Formica rufa* (VOWLES, 1965; VOSS, 1967).

Previously, we examined the visual perception (CAMMAERTS, 2004a, 2007a, b, 2008) and the orientation system (CAMMAERTS & LAMBERT, 2009; CAMMAERTS & RACHIDI, 2009) of an ant with medium-sized eyes, *Myrmica sabuleti*, as well as the visual perception (CAMMAERTS, 2012a) and orientation system (CAMMAERTS et al., 2012) of an ant with somewhat larger eyes: *M. ruginodis*. The workers of *M. sabuleti* essentially use odors to find their way, while workers of *M. ruginodis* rely primarily on visual cues as long as vision is possible (references here above). For these two species, their visual perception and navigation system are in agreement with one another and are also in accordance with their usual habitat, eye morphology and subtended angle of vision (RACHIDI et al., 2008;

CAMMAERTS, 2004a, 2011). Workers of *Myrmica rubra* have eyes of intermediate size compared with *M. sabuleti* and *M. ruginodis* (RACHIDI et al., 2008) as well as a subtended angle of vision of intermediate value (CAMMAERTS, 2011).

In this study, we investigated how the visual perception of these ants could be characterized. Answering this question will provide further insight into the comparative study of the eye morphology, the subtended angle of vision and the visual perception of three closely related species, probably revealing accordance between these morphological and physiological studies. The travelling system of *M. rubra* has been elucidated (CAMMAERTS, 2012b): this species uses both its olfaction and its vision to explore the environment. Such a system differs from – and is, in fact, intermediate between – those of *M. sabuleti* (use of odors) and *M. ruginodis* (use of visual cues) (references here above). Is the navigation system of *M. rubra* in agreement with its visual perception and is its visual perception in accordance with its preferred habitat as is the case for the two other *Myrmica* species? Answering this question requires a detailed analysis of the visual perception of workers of *M. rubra*. Finally, such a study of this species' visual perception will consolidate a series of ecological, morphological, physiological and ethological studies on three closely related species, possibly helping to understand niche differentiation between these three related species.

MATERIAL AND METHODS

Collection and maintenance of ants

The experiments were performed on six experimental colonies derived from large colonies collected in the Aise valley (Ardenne, Belgium) on open grassland. These colonies were demographically identical: each contained a queen, about 500 workers and brood. They were maintained in the laboratory in artificial nests made of one to three glass tubes half-filled with water, a cotton-plug separating the ants from the water (Fig. 1A). The glass tubes were deposited

in trays (43 cm x 28 cm x 7 cm), the sides of which were covered with talc. The trays served as foraging areas wherein food was provided and the ants were trained, as well as tested on the floor of the trays (Fig. 1A, B).

Temperature was maintained at $20^{\circ} \pm 2^{\circ}$ C. Humidity was approximately 80% and remained constant over the course of the experiment. Light intensity was held constant at 600 lux when maintaining the ants (e.g. providing food, renewing nesting tubes) and during the training and testing periods. Otherwise, the light intensity was adjusted to about 120 lux using a dimmer.

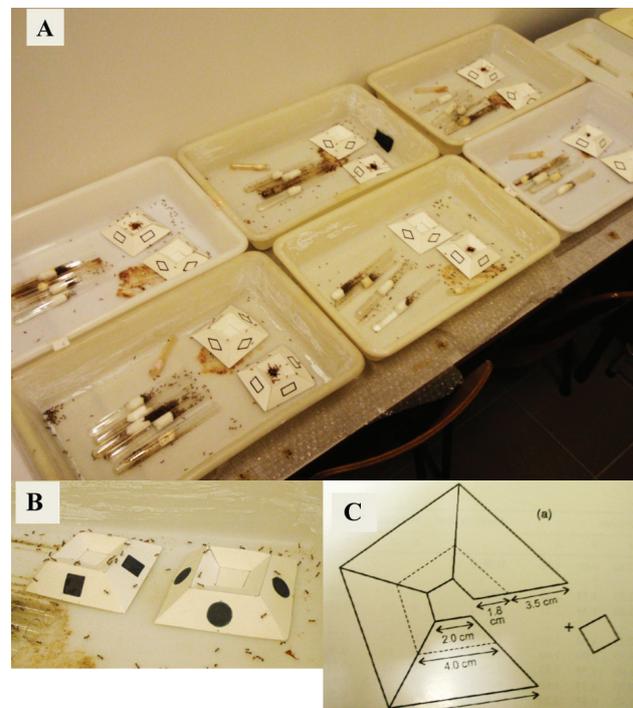


Fig. 1. – **A:** experimental design; training of six experimental colonies to a hollow black rectangle and lozenge of similar perimeter, the rectangle being the ‘rewarded’ cue. **B:** testing the ants in presence of a filled black square (previously ‘rewarded’) and circle of same area; the ants discriminated between the two shapes. **C:** experimental device: a kind of truncated pyramid with a reverse pyramid at its top, constructed from one piece of paper Steinbach® ((a)), closed with a piece of same paper (+). The angle between the base and the edge of a face equaled $52^{\circ} 5'$. To study the ants' visual perception, cues were drawn on the center of each face of the device and two pyramidal devices, each one with a given kind of cue, were presented to the ants.

Sugared water was permanently offered in a small glass tube plugged with cotton, and chopped cockroach was served twice a week on a glass-slide. No meat food was given during experiments since it served as a reward during training (Fig. 1A).

Experimental device

The experimental device was made of very strong white paper (Steinbach®), which was cut to a precise form and dimension (Fig. 1C). Paper was folded to form a pyramid at the base and folded again inward at the top into an inverted pyramid. A strip of Sellotape paper® was attached on the interior to hold the device together. The inner inverted pyramid was closed by taping a square piece of the same kind of paper to its base. A cue was drawn with a black water proof marker in the center of each of the four outer surfaces of a device.

During an experiment, the six colonies were trained prior to testing (see below) with a pair of experimental devices each provided with one kind of cue (Fig. 1A, B). All colonies had their own devices for training and for testing. In other words, one complete experiment required 12 devices for training (= 6 devices with one kind of cue + 6 devices with another kind of cue), and another 12 devices for testing (= 6 with the first kind of cue + 6 with the second kind of cue).

Experimental protocol

For performing an entire experiment, the ants of the six colonies were trained for six days, then tested a first time, thereafter again trained during three days and finally tested a second time.

Ant training (Fig. 1A)

A pair of pyramidal devices was placed in each colony's tray, each device with a different cue drawn on the four sides. A piece of dead cockroach was placed in the inner square room of one of the two devices. The cue associated with food (= the "rewarded" cue) was considered as the correct cue, i.e. the one the ants should

choose during the tests. In the course of each 6-day and 3-day training periods, the pair of pyramidal devices was turned and relocated 6 and 3 times, respectively, but never periodically, and the food was then renewed. This procedure prevented the ants from depositing a recruiting trail (CAMMAERTS, 1978) and from acquiring spatial and temporal learning (CAMMAERTS, 2004b). Moreover, due to the relocations, each cue was exposed to the ants in an identical way.

Ant testing (Fig. 1B)

All training devices were removed from the colonies and replaced with testing devices. During the tests, no food was provided. The ants present on each pyramidal device were counted fifteen times (during fifteen minutes) and the mean values of these counts were calculated for each of the two kinds of cues, first for each colony and second for all the colonies i.e. the total mean values (Table 1). The six mean values obtained for the six colonies for one kind of cue were compared to the corresponding six mean values obtained for the other kind of cue using the non parametric Wilcoxon signed rank test (SIEGEL & CASTELLAN, 1989) This statistical test was separately used for each experimental test, each providing the two series of values to be compared. As a checking statistical analysis, the total mean values were also compared using the non-parametric 2 x 2 table contingency χ^2 test (SIEGEL & CASTELLAN, 1989), two values of the table being the experimentally obtained ones, and two values being those expected if ants did not discriminate the two presented cues.

Each experiment used the foragers of six large colonies, included 48 cues, and lasted 6 + 1 + 3 + 1 = 11 days. Eventual bias and imperfection relative to the drawing of the cues and the presentation of the pyramidal devices to the ants probably cancel out each other.

Presented cues (Fig. 2)

To study the ants' discrimination of equally-sized filled shapes, a black square (c = 1.4 cm), a black circle (d = 1.58 cm) and a black triangle (b = 2.13 cm, h = 1.8 cm) were used.

TABLE 1

Mean numbers of ants responding, during two tests, to previously rewarded cues *versus* unrewarded. The cues (Fig. 2) were presented on the four faces of two pyramidal devices (Fig. 1) and the ants (of six colonies) that were present on these devices were counted fifteen times before establishing the mean values for each colony (column 2) and for all the colonies (column 2 on the right), for each kind of cue. The latter values are also schematically presented as black ('correct') and gray ('wrong') lines. Column 3: results of non-parametric Wilcoxon tests, with N, T and P according to the nomenclature of SIEGEL & CATELLAN, 1989.

rewarded and unrewarded cues	test 1	mean n ^{ets} of ants in front of the rewarded and the unrewarded cue								statistics		
	test 2	for:	each colony						all colonies	N	T	P
control experiment 1) two identical cues		12.3	4.7	10.8	5.8	2.5	2.0	—	6.37	6	-15	0.219
		12.1	4.2	11.2	9.7	2.8	2.3	—	7.06			
filled shapes 2) a filled black square and circle		17.1	7.8	8.4	10.9	2.9	4.7	—	8.62	6	21	0.016
		11.8	4.1	2.8	4.3	1.8	2.4	—	2.89			
		17.1	8.9	8.9	12.5	7.1	4.5	—	9.82	6	21	0.016
		14.3	3.5	4.5	3.3	2.3	2.1	—	5.01			
3) a filled black triangle and circle		29.2	15.1	26.9	18.7	8.9	3.5	—	17.04	6	21	0.016
		11.0	3.5	15.1	3.3	0.5	0.4	—	5.70			
		15.5	19.3	10.7	16.1	3.2	2.7	—	11.27	6	21	0.016
		13.5	10.9	6.0	4.3	0.6	0.8	—	6.01			
4) a filled black triangle and square		30.4	11.0	24.4	13.5	1.8	2.9	—	14.00	6	21	0.016
		27.0	7.7	16.4	10.3	0.7	0.8	—	10.47			
		11.5	12.5	10.2	8.4	7.0	5.6	—	9.20	6	21	0.016
		6.1	4.7	4.8	1.1	1.7	1.5	—	3.31			
		28.9	10.5	30.0	10.1	6.9	6.1	—	15.42	6	21	0.016
		11.6	2.4	19.5	4.3	1.8	2.3	—	6.98			
hollow forms # perimeter 5) a hollow black rectangle and ellipse of same height and width		31.9	8.5	14.3	8.3	1.9	4.8	—	9.95	6	21	0.016
		14.7	6.6	11.0	4.1	0.8	3.2	—	7.73			
		21.8	4.7	8.9	9.3	0.1	1.9	—	7.78	6	12	0.422
		17.4	6.6	10.3	5.4	1.1	1.1	—	6.81			
6) a hollow black lozenge and ellipse of same height and width		23.3	12.9	14.1	8.4	0.0	3.1	—	10.29	5	-12	0.156
		24.1	12.7	15.8	8.0	1.0	3.1	—	10.79			
		21.0	23.1	15.1	6.1	1.9	4.5	—	11.94	6	-15	0.219
		30.6	16.9	18.1	9.5	2.3	4.4	—	13.63			
7) a hollow black lozenge and rectangle of same height and width		25.7	17.0	9.7	7.3	3.1	4.1	—	11.16	6	18	0.078
		26.9	11.8	8.7	6.9	1.6	3.3	—	9.86			
		29.7	19.5	22.4	10.0	3.9	11.5	—	16.15	6	21	0.016
		28.9	18.3	7.9	9.9	1.9	2.5	—	11.56			
hollow forms ≈ perimeter 8) before training, a hollow black rectangle and lozenge of ≈ perimeter		10.6	28.8	12.2	5.6	0.9	6.8	—	10.82	6	18	0.078
		11.0	28.0	8.8	5.4	0.8	6.0	—	10.00			
9) after training, a hollow black rectangle and lozenge of same perimeter		5.5	18.0	11.7	0.9	1.3	0.7	—	6.37	5	9	0.406
		5.7	15.3	8.6	1.7	2.0	0.7	—	5.66			
		7.5	12.5	8.9	4.6	2.4	1.0	—	6.16	6	14	0.281
		7.7	17.0	9.1	3.5	3.2	0.8	—	6.90			

To study the ants' discrimination of hollow shapes, three hollow shapes of the same height and width were used [a hollow rectangle ($L = 3.2$ cm, $l = 1.6$ cm), a hollow ellipse ($D = 3.2$ cm, $d = 1.6$ cm), a hollow lozenge ($D = 3.2$ cm, $d = 1.6$ cm)], in addition to two hollow shapes of the same perimeter [a hollow rectangle ($L = 2.24$ cm, $l = 1.12$ cm), a hollow lozenge ($D = 3$ cm, $d = 1.5$ cm)]. All hollow shapes were made of black lines 1 mm thick.

RESULTS

Control experiment

For checking our experimental method, two pyramidal devices provided with exactly the same cues [two filled black circles ($d = 0.8$ cm) horizontally set at 0.8 cm of distance from one another, in the center of each side of the device] were presented to the ants, a piece of dead cockroach being offered in one of the two devices. After training, during the subsequent control experiment, the ants visited similarly the two pyramidal devices. Statistical tests showed that ants were not differently numerous on the two devices (Table 1, line 1). The experimental method was thus unbiased.

Discrimination of filled shapes

After having been trained to a given filled shape in the presence of another different unrewarded filled shape, statistically more foragers came during the test experiments onto the device provided with the 'rewarded' cue (Table 1, lines 2, 3, 4). It can thus be concluded that workers of *M. rubra* could discriminate – e.g. a filled black square and a filled black circle even when these shapes had exactly the same area (Table 1, line 2; Fig. 1 B); - a filled black triangle and a filled black circle of equal area (Table 1, line 3); - a filled black circle and a filled black square of equal area (Table 1, line 4; a supplementary test having been made previously to the two usual ones, after 4 training days).

Discrimination of hollow shapes of equal height and width but of different perimeter

After training to a hollow black rectangle (as the rewarded cue) and a hollow black ellipse (unrewarded), the number of ants at the rectangle in the test experiments was statistically slightly greater than those at the ellipse (Table 1, line 5). After training to a hollow black lozenge (rewarded cue) and a hollow black ellipse (unrewarded), the ants were found in statistically equal numbers at

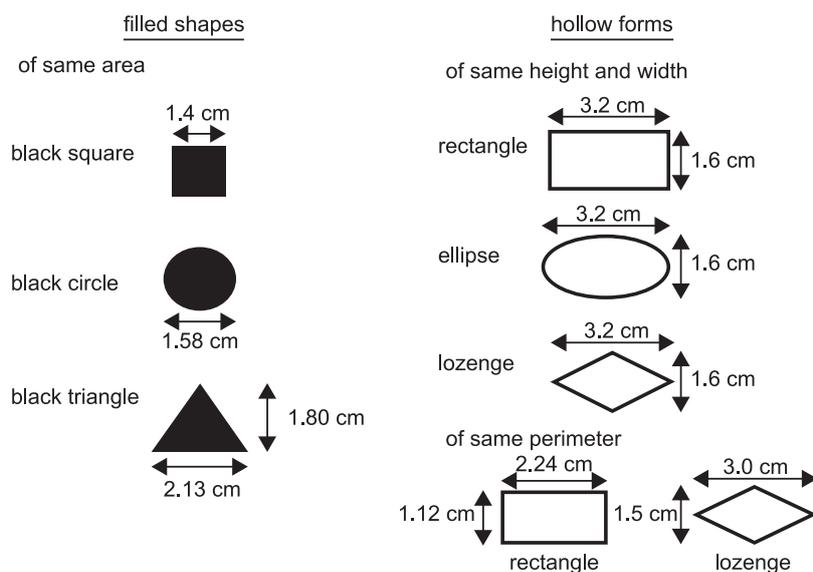


Fig. 2. – Cues presented to the ants: filled black shapes of equal area (square, circle, triangle), hollow black forms of equal height and width (rectangle, ellipse, lozenge) and hollow black forms of equal perimeter (rectangle, lozenge).

both shapes in the test experiments (Table 1, line 6). Workers of *Myrmica rubra* are thus poorly able to distinguish between hollow shapes, i.e. between hollow rectangle and ellipse, or between hollow lozenge and ellipse.

The three hollow shapes had the same height (1.6 cm) and width (3.2 cm) but somewhat different perimeters. The perimeter of the rectangle was 9.60 cm, that of the ellipse 7.54 cm, and that of the lozenge 7.16 cm. Ants were thus poorly able to discriminate between hollow shapes when the difference in the perimeter was small. In the next experiments, the ants were presented with hollow shapes having clear differences in perimeter, i.e. the lozenge and rectangle.

After training to a hollow lozenge ('rewarded'; perimeter of 7.16 cm) and a hollow rectangle of same height and width but with a larger perimeter (unrewarded; perimeter of 9.60 cm), more ants were counted, in the test experiments, at the device provided with the hollow lozenge. This observation was statistically significant on the basis of the Wilcoxon test (ants of five colonies out of six for test 1 and ants of all colonies for test 2 successfully discriminating the shapes) though not significant on the basis of the χ^2 test (Table 1, line 7). Workers of *M. rubra* thus perceived a slight difference between the two presented hollow forms or required more time to better distinguish the difference. It can be hypothesized that although these workers have difficulties in discriminating the hollow shapes, they are able to achieve this by relying on the total length of the shape's perimeter. This hypothesis was submitted to a further experiment.

Discrimination of hollow shapes of similar perimeter

Firstly, after a delay period of one month, the ants were tested prior to training in front of a hollow black rectangle and a hollow black lozenge of similar perimeter. The tested ants showed no preference for one or the other of these two forms (Table 1, line 8). They showed

no natural preference for a given form and had lost their previous conditioning.

Secondly, after training to the following shapes (rewarded hollow black rectangle: L = 2.24 cm, l = 1.16 cm, perimeter = 6.80 cm; non-rewarded hollow black lozenge: D = 3 cm, d = 1.5 cm, perimeter = 4 x 1.677 cm = 6.71 cm) (note that in the previous tests, the lozenge shape was associated with the reward), workers came in equal numbers to the rectangle and the lozenge in the test experiments (Table 1, line 9). The Wilcoxon test and the χ^2 one are in agreement for such a verdict. The two presented hollow shapes were thus similarly perceived by the ants. Thus workers of *Myrmica rubra* rely more on the length of the perimeters of hollow forms than on their actual shapes to sufficiently distinguish these hollow forms.

DISCUSSION

Main findings and remarks

Using collective operant conditioning, it could be showed that workers of *M. rubra* distinguish filled shapes from one another but fail to do so for hollow shapes, then relying more on the total perimeter than on actual shape.

The difficulty in discriminating between hollow shapes exhibited by these workers may be due to the fact that several ant species may see with a slight convexity, therefore seeing rectangles and lozenges as similar to ellipses. These ants may utilize characteristics of cues other than the exact shape, such as dimension, area, perimeter, angularity, convexity, line-thickness, contrast, and brightness. This could account for the conflicting findings of certain authors regarding the visual perception ability of some insects, e.g. bees (HORRIDGE, 1999, HEMPEL & GIURFA, 2003).

Comments on the methodology

In the course of the present work, the position of each cue – the 'rewarded' as well as the

‘unrewarded’ – was always on the same place of the experimental device, i.e. in the center of one side. Each experimental device was rotated and relocated several times during training while the ants’ artificial nests were never moved or otherwise disturbed during the course of an experiment. The number of cues (48 each time), the number of tested ants (the foragers of six colonies), and number of times the ants were counted (15 x 6 vs. 15 x 6 each time) were held constant to ensure consistency in the results. Finally, as an added precaution, in experiment 9, the ants coming to the rectangle were rewarded, although previously in experiment 7 the lozenge was the ‘rewarded’ cue.

Integration of the present results with other findings on the same species and with those on two other species of *Myrmica*

The visual perception ability of workers of *M. rubra* is of higher quality than that of workers of *M. sabuleti*, which fails to discriminate filled shapes (CAMMAERTS, 2008), but of lower quality compared to individuals of *M. ruginodis*, which are able to discriminate between hollow shapes as well as other cues (CAMMAERTS, 2012a).

It may be presumed that workers of *M. rubra* can perceive the dimension, inclination, orientation and number of elements of a cue in a similar way to workers of *M. sabuleti* (CAMMAERTS 2004a, 2008). The fact that foragers of *M. rubra* poorly discriminate hollow shapes does not imperil their use of visual cues for travel. They simply use what they can see, even if imperfectly and also use any encountered odorous elements (CAMMAERTS, 2012b).

In summary, the perceptive abilities of the three species can be characterized as follows.

M. sabuleti has comparatively small eyes (with a mean of 109 ommatidia, RACHIDI et al., 2008), a subtended angle of vision equaling 5° 12’ (CAMMAERTS, 2004a), a low-performance visual perception (CAMMAERTS, 2008), and the workers rely primarily on odors to travel (CAMMAERTS

& RACHIDI, 2009). The species nests in open fields with small odorous plants (CAMMAERTS & CAMMAERTS, personal observations). *M. ruginodis* has comparatively large eyes (with a mean of 149 ommatidia, RACHIDI et al., 2008), a subtended angle of vision equaling 3° 10’ (CAMMAERTS, 2011), a high-performance visual perception (CAMMAERTS, 2012a), and the workers essentially use visual cues located above them to travel (CAMMAERTS et al., 2012). This species nests near clearings under branches where the sky is partly visible (CAMMAERTS & CAMMAERTS, personal observations). *M. rubra* has eyes of intermediate size (with a mean of 129 ommatidia, RACHIDI et al., 2008), a subtended angle of vision equaling 3° 50’ (CAMMAERTS, 2011) and a visual perception of middle quality (present work). This species nests in open grass lands (CAMMAERTS & CAMMAERTS, personal observations). The orientation system of foragers of *M. rubra* has been investigated: these ants use their vision and their olfaction as best as they can, according to the circumstances (CAMMAERTS, 2012b).

The three closely related *Myrmica* species have thus been examined at ecological, morphological, physiological and ethological points; the results of these different studies are in agreement with one another and in accordance with each species’ usual habitat.

Comments about other works on the subject

The topic of insect vision has been broadly studied (WEHNER, 1981), and in particular for species of Odonata, Diptera and especially bees (Hymenoptera). Ants have not been commonly investigated in this respect. The species, which have been investigated so far, are ants having good vision and large eyes, *Formica* spp. (VOSS, 1967) and *Cataglyphis* spp. (PASTERGUE-RUIZ & BEUGNON, 1995). The present work examines the visual perception of an ant species having eyes of intermediate size.

While the visual perception system of ants has been neglected to an extent, their orientation

system has received considerable attention. In general, ants memorize and use encountered visual cues according to a snapshot model (CARTWRIGHT & COLLETT, 1983; NAREDA et al., 2007) and a sketch map model (BEUGNON et al., 1996).

We argue that to efficiently investigate the orientation system of an ant species, one should know beforehand about their visual perception abilities. Do the ants concentrate on cues located in front of them or above them? Do they have color vision? Do they perceive their environment with perspective? Are they sensitive to dimension and orientation of a cue? We conducted such research on *M. sabuleti* before attempting to analyze the species' navigation system (CAMMAERTS, 2004a, 2007a, b, 2008; CAMMAERTS & RACHIDI, 2009). We also studied the visual perception and the travelling system of *M. ruginodis* (CAMMAERTS, 2012a; CAMMAERTS et al., 2012) and finally, we examined the traveling system of *M. rubra* (CAMMAERTS, 2012b) and, at last, this latter species' visual perception (present work which thus fills the last gap).

Visual perception is nearly always studied via visual conditioning or learning, which is in itself also a complex physiological ability (AVERGUÈS et al., 2011). The procedure of visual conditioning is time-consuming (CAMMAERTS et al., 2011) and difficult since it brings together two complex physiological abilities. However, it is the only practical method available. The results are generally surprising. Ants, as well as other insects, often appear to have a better sense of vision than previously expected - e.g. *M. ruginodis* (CAMMAERTS, 2012a). Ants can learn (i.e. can be conditioned to) a large number of cues (CAMMAERTS, 2004a, 2008). They have a rather long visual memory (e.g. *M. sabuleti*, CAMMAERTS et al., 2011) and sometimes a surprisingly long one (e.g. *M. ruginodis*, CAMMAERTS & NEMEGHAIRE, 2012). The visual memory of workers of *M. rubra* has also been assessed and is rather long lasting (CAMMAERTS, 2012c).

The ability of insects to discriminate shapes (such as squares, triangles, lozenges) has been

disputed by some researchers (HORRIDGE, 2006 and references therein). It must be freely admitted that workers of *M. sabuleti* cannot discriminate filled or hollow shapes except when these shapes are presented with concavity (CAMMAERTS, 2008). However, foragers of *M. ruginodis* can perfectly discriminate filled (black or white) and hollow shapes (CAMMAERTS, 2012a) and foragers of *M. rubra* can distinguish filled black shapes from one another but not hollow shapes, especially when the perimeters of the shapes are very similar (present work). The ability to truly distinguish shapes from one another may depend on characteristics of the shapes, such as dimension (adapted to the animals' size), contrast (black, grey), filling (filled, hollow or transparent) and shape (triangle, square, hexagon, dodecagon or circle). Additional factors influencing insect visual performance are the animal's subtended angle of vision ($3^{\circ} 30'$ might be a limit for a good discrimination), the morphology and physiology of the eyes (i.e., the shape of the eye, the number of ommatidia, the performance of each ommatidium, the existence of several kinds of ommatidia), the performance of the neuronal structures that handle visual perception (i.e., the histological organization of the optic lobes, etc.). Since the three species we have studied (*M. sabuleti*, *M. ruginodis*, *M. rubra*) are closely related and since we conducted the experiments using cues of similar dimension, contrast and shape, we tentatively argue that the observed differences in their visual abilities are a consequence of differences in eye morphology, number of ommatidia (RACHIDI et al., 2008) and subtended angle of vision (CAMMAERTS, 2011).

A thorough study of an animal's visual perception should include ethological experiments, as well as a complementary physiological analysis at the neuronal level, as has been done for olfactory perception in bees (GIURFA, 2001; GUERRIERI et al., 2005; DEISIG et al., 2006).

In conclusion, our contribution to the ethological study of animal visual perception consists in establishing what three species of ants with middle-sized eyes can discriminate

(CAMMAERTS, 2004a, 2008, 2012a, present work), the proximate reasons for their ability or inability to discriminate (i.e., eye morphology: RACHIDI et al., 2008; subtended angle of vision: CAMMAERTS, 2004a; CAMMAERTS, 2011), what their visual and olfactory learning characteristics are (CAMMAERTS et al., 2011; CAMMAERTS & NEMEGHAIRE, 2012; CAMMAERTS, 2012c), how they negotiate their way (CAMMAERTS & RACHIDI, 2009; CAMMAERTS et al., 2012; CAMMAERTS, 2012b) and why they nest in such usual natural biotopes (CAMMAERTS & CAMMAERTS, personal observation).

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Kinematic analysis of swimming ontogeny in seabass (*Dicentrarchus labrax*)

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ABSTRACT. Swimming has been investigated in multiple species, but few studies consider the establishment of swimming through ontogeny. This study describes the establishment of cyclical swimming in *Dicentrarchus labrax*, a marine fish from cold, temperate waters. The data were compared with results from previous studies on two subtropical freshwater catfish species (*Clarias gariepinus* and *Corydoras aeneus*). The three species have different modes of locomotion each during their adult stage (anguilliform, subacarangiform and carangiform). The swimming of *Dicentrarchus labrax* was recorded with a high-speed video camera (500 fps) from 0 to 288 hours and from 960 to 2496 hours post-hatching. Three indices, i.e. coefficient of determination (r^2), coefficient of variation (CV), and Strouhal number (St), were used to investigate the establishment and efficiency of swimming. Important differences in the timing of swimming establishment were observed between the seabass and the two catfish species. The two catfish species display a sine-shaped swimming mode immediately or soon after hatching, and the efficiency of movement substantially improves during the first days of life. For seabass, however, establishment of swimming is slower during the same developmental period. These differences may be related to a faster developmental rate in the catfishes that allows them to swim rapidly in an intermediate regime flow and to develop the required morphology to establish efficient movements earlier.

KEY WORDS: swimming, ontogeny, body-caudal locomotion, Strouhal number, larvae

INTRODUCTION

In most fish, swimming corresponds to the propagation of a wave of increasing amplitude from head to tail (GRAY, 1933), which is commonly named the body/caudal fin locomotion (BCF) (BREder, 1926; LINDSEY, 1978; VIDELER, 1993). Depending primarily on the parameter of size, undulatory movements generate thrust in a range of flow regimes, by exploiting viscous or inertial forces or both (VIDELER, 1993). The ratio between the viscous and inertial forces can be assessed by the Reynolds number (Re). At hatching, larvae are only a few millimeters long and the corresponding flow regime is dominated by viscous forces (Re values below 100) (MCHENRY & LAUDER, 2005; VIDELER, 2011). This means that larvae must use large body wave

amplitudes along their whole body to enable them to exploit the viscous force optimally (WEBB & WEIHS, 1986). This set of movements corresponds to the anguilliform swimming mode (MÜLLER & VAN LEEUWEN, 2004; MAUGUIT et al., 2010a,b). Experiments showed that an increase in the viscosity of the medium induced an increase in the amplitude of the movement in the anterior part of the body (HORNER & JAYNE, 2008; DANOS, 2012). During growth, fish experience a gradual change from a viscous to an intermediate regime ($Re > 300$) and then from the intermediate to an inertial flow regime ($Re > 1000$) (MÜLLER & VIDELER, 1996; OSSE & VAN DEN BOOGAART, 2000; MCHENRY & LAUDER, 2005). Simultaneously, skeletal, muscular and nervous systems develop while new structures (i.e. fins) are recruited, and other structures

(i.e. yolk-sacs) are lost. These changes are important in setting physical limits to locomotor performance (WEBB, 1984), and are expected to affect the ontogeny of swimming (FUIMAN & WEBB, 1988). However, the developmental process driving the swimming capacity has received little attention. The kinematics of swimming in fish larvae has been studied in zebrafish *Danio rerio* (Hamilton, 1822) (FUIMAN & WEBB, 1988; MÜLLER & VAN LEEUWEN, 2004; FONTAINE et al., 2008; MÜLLER et al., 2008), the common carp *Cyprinus carpio* (L., 1758) (OSSE, 1990; OSSE & VAN DEN BOOGAART, 2000), the European plaice *Pleuronectes platessa* (L., 1758) (BATTY, 1981) and the Atlantic herring *Clupea harengus* (L., 1758) (BATTY, 1984). However, these studies only focused on a few larval stages (from 2 to 7) and did not provide a global view of the developmental cycle.

More recently, MAUGUIT et al. (2010a,b) more thoroughly examined the development of swimming by studying 21 and 23 larval stages in *Clarias gariepinus* (Burchell, 1822) and *Corydoras aeneus* (Gill, 1858), respectively. Both studies used a new kind of analysis that allows a better understanding of the changes in swimming during fish growth. This method is based on the work of VIDELER (1993). In theory, during one undulatory movement, each body part follows a pure sine-like trajectory when two conditions are observed: (1) the swimming speed is constant during the execution of one swimming movement and (2) the lateral amplitude of each body part is small (VIDELER, 1993). In practice, neither condition is met. First, the thrust oscillates during one tail-beat as the tail beats back and forth. Consequently, the swimming speed also fluctuates (MÜLLER & VAN LEEUWEN, 2004). Second, the body wave amplitude increases from the pivot point to the tail (VIDELER, 1981). The pivot point, located just behind the head, characterizes the position on the body where the smallest amplitude of lateral movement is usually observed. Consequently, the propulsive wave deviates moderately from a pure sine-like trajectory at this pivot point and more markedly at the tail, where the amplitude is maximal.

Variations in swimming speed during one tail-beat are stronger in the viscous flow regime of small, slow swimmers than in the inertial flow regime of large, fast swimmers (MÜLLER & VAN LEEUWEN, 2004). In a viscous flow regime, large amplitudes of each body part are required to better exploit forces of the medium while in an inertial flow regime, smaller amplitudes allow better penetration in the water (BATTY, 1981).

Thus, we predict larvae may be unable to perform sine-shaped movements at hatching. During ontogeny, as body length and absolute swimming speed increase and morphological developments occur, we expect larvae to progressively improve their swimming ability towards sinusoidal movements.

Our aim is to use the method of MAUGUIT et al. (2010a) to describe the ontogeny of the swimming in *Dicentrarchus labrax* (seabass) (L. 1758) from hatching to 104 days post-hatching. The data will then facilitate more comprehensive comparisons with the two species studied by MAUGUIT et al. (2010a,b): *Clarias gariepinus* and *Corydoras aeneus*. The two catfish species live in subtropical regions in freshwater. Both stay generally in quiet waters near the bottom (TEUGELS, 1986; BURGESS, 1992). At adult stage, the two species differ in their swimming mode, one using the anguilliform mode (*C. gariepinus*) and the other the carangiform mode (*C. aeneus*) (MAUGUIT et al., 2010a,b). *Dicentrarchus labrax* generally favours turbulent environments in cold and temperate seawaters. Larvae hatch in the open sea and, once juveniles, drift towards the coast and estuaries (PICKETT & PAWSON, 1994). At adult stage, seabass use a subcarangiform swimming mode (PALOMARES, 1991; HERSKIN & STEFFENSEN, 1998). The differences between habitats and way of life should affect the growth and the rate of morphological development of the species and in this way the establishment of swimming behaviour. Being a marine fish living in cold/temperate water, the seabass should have slower growth and rate of morphological development than the two catfish species. We expect that the establishment of swimming in *D.*

labrax will take more time than in *C. gariepinus* and in *C. aeneus*.

MATERIALS AND METHODS

In this study the larval period was determined as the period between hatching and the ossification of the caudal fin. Once the caudal fin was ossified, fish were considered as juveniles. Larvae of *D. labrax* were bred at Aquanord hatchery (Gravelines, France) in a community tank filled with running seawater at a temperature of 15°C. Larvae were kept in the darkness until 216 hours post-hatching (hPH). Then, light was turned on with a photoperiod of 12h and larvae were fed with brine shrimps three times a day (*Artemia salina* L., 1758). Juveniles (whose caudal fin is ossified) were also bred in a community tank at a temperature of 20°C. Fish were sampled at different stages: 0, 4, 8, 12, 16, 20, 24, 32, 40, 48, 60, 72, 96, 120, 144, 168, 192, 216, 240, 264 and 288 hPH for larvae and 960, 1896 and 2496 hPH (respectively 40, 79 and 104 days post-hatching) for juveniles. For each stage, 15 individuals were sampled and were separated into three batches of five fish. In each batch, swimming was recorded at 500 frames s⁻¹ with a high-speed digital video camera (RedLake MotionPro 2000; RedLake, San Diego, California, USA) from a dorsal view. Swimming sequence was recorded only once per batch to avoid studying the same individual twice. A total of three independent replicates was collected for each stage. Note that a sequence suitable for further analysis was defined when fish performed one complete undulatory movement in a straight direction and at a constant speed. For each swimming movement, the first three tail strokes were not recorded to avoid inclusion of acceleration movements.

Three tanks of variable dimensions were used: 7.5 cm × 2.5 cm, 14.5 × 8 cm, 123 × 8.5 cm respectively for larvae (3 to 5.6 mm TL), small juveniles (<20 mm TL) and large juveniles (>20 mm TL). The water level was always five times the fish height. Fish were free to move in all directions and at variable swimming speeds. The

camera was mounted on a Stereozoom Microscope (Leica M10; Leica, Wetzlar, Germany) for larvae and equipped with a lens (Linus Mevis 25 mm, 1.6: 16; Linus; Munich, Germany) for juveniles. The two smallest tanks were lit from the top and the bottom (light reflected by a mirror) with two 240W Volpi 6000-1 cold light sources (Volpi, Schlieren, Switzerland) and optical fibers. The largest tank was lit from the top with two 1000W spotlights (IFF Q1250).

The analyses of sequences of the swimming movements were carried out with Midas software (RedLake, version 2.1.1). For larvae and juveniles, a total of 63 and nine sequences respectively were analyzed in an earth-bound frame of reference. The fish midline was divided into seven equal segments by selecting eight landmarks between the snout and the tail tip, *i.e.* at 0, 0.14, 0.29, 0.43, 0.57, 0.71, 0.86 and 1 TL (respectively LM1 to LM8, Fig. 1). A

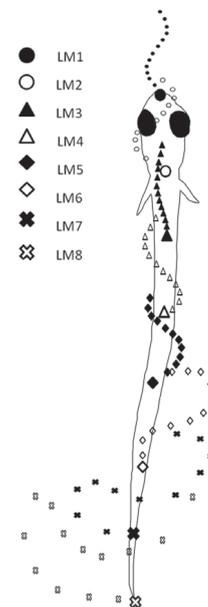


Fig. 1. – Drawing of a swimming movement executed by a seabass larva (*Dicentrarchus labrax*). The eight paths made up of different markers represent the trajectory of each landmark (LM1-LM8) during the execution of a complete undulatory swimming movement. The larger symbol of each type indicates the placement of the landmarks on the fish body.

preliminary study determined the useful number of landmarks. The use of eight landmarks allowed placing the second one near the pivot point (where the amplitude of the movement is the weakest along the body). The x-axis was defined as the direction of fish displacement and the y-axis was perpendicular to the x-axis. A transformation was done to move the first point of the trajectory of each landmark to the x-axis origin ($x = 0$). The aim of this transformation was to use the same referential for analyzing the motions of each landmark independently of its position on the fish.

For each sequence, direct and indirect parameters were selected to describe the swimming movement. The direct parameters were total fish length (TL; mm), relative swimming speed (U ; TLs^{-1}), tail-beat frequency (f ; s^{-1}), the wavelength of the propulsive wave (λ) and the maximal amplitude movement of each landmark (A_{1-8} ; %TL).

The indirect parameters were the three indices used by MAUGUIT et al. (2010b). The first index made it possible to assess the sinusoidal trajectories of the swimming movement. From a distribution of the observed values of x , and knowing the movement amplitude (A_{1-8}) and its wavelength, a theoretical sinusoid could be calculated for the various landmarks at each developmental stage. The sinusoidal function linking y_{th} (theoretical y) and x is $y_{th} = A \sin(\omega x + \varphi)$, where A is the amplitude (in mm), ω the pulsation (in radian mm^{-1}), and φ the initial phase (in radians). The amplitude (A) and the pulsation (ω) were determined from the observed coordinates: $A = y_{max}$ and $\omega = 2\pi T_x^{-1}$, where y_{max} is the maximum amplitude observed during one tail-beat and T_x is the period of motion. For each landmark, the y_{th} values were calculated by varying φ between 0 and 2π by increments of 0.01 radian (this yields 628 theoretical sinusoids) to obtain the best fit between the observed and theoretical curves. The φ value of the theoretical sinusoid having the best fit (the highest r^2) with the observed motion was then selected. These sinusoids correspond to the theoretical ideal

trajectories that each landmark should follow during execution of a complete undulatory movement (i.e. to the situation that should be observed at the adult stage). The paths observed for the various landmarks throughout ontogeny were compared with these theoretical sinusoids by determining the coefficient of determination (r^2) between the two curves. The coefficients of determination of LM1-LM8 (r^2_{1-8}) provided an objective index of the similarity between this motion and the adult sinusoid motion for each landmark placed on a fish. The r^2_{mean} , the mean of the r^2_1 to r^2_6 , provided a global index of the body movement profile at a specific developmental stage. Swimming movements were considered to be sinusoidal when r^2_{mean} was ≥ 0.95 . Landmarks 7 and 8 were not taken into account for the r^2_{mean} . Landmarks 7 and 8 were on the caudal region. This region is not yet developed in larvae (no ray-finned caudal fin and no intrinsic muscles), which therefore are unable to fully control their tail-tip movements. However the caudal fin follows a better sinusoidal path once developed (in juveniles); the caudal fin has intrinsic muscles that are able to alter the movement (LAUDER, 1989, 2000). In consequence, the caudal region does not behave the same way as the rest of the body. For this reason the corresponding r^2 were not taken into account for the r^2_{mean} .

The coefficient of variation of r^2 (CV), the second index, was calculated with the r^2_1 - r^2_6 values to gauge whether, at a given developmental stage, the movement appeared to have the same level of organization in different parts of the body.

The third index used was the Strouhal number:

$$[\text{Formula 1}] St = \frac{f \times A_8}{U^{-1}}$$

where f is the tail-beat frequency, A_8 the maximum amplitude of the caudal fin and U the swimming speed. The St value describes how fast the tail is flapping relative to how fast the fish is swimming. The value of this index is dependent on the Re values (BORAZJANI & SOTIROPOULOS, 2008, 2009). For adult fish whose corresponding

Re values are very high, the swimming efficiency is optimal when the St values range from 0.25 to 0.45 (BORAZJANI & SOTIROPOULOS, 2008, 2009; LAUDER & TYTELL, 2006; TAYLOR et al., 2003). But for fish whose corresponding Re values are only 300, the optimal St values go down to 1.3 (BORAZJANI & SOTIROPOULOS, 2008, 2009).

Other parameters about fish morphology and flow regime were also calculated: yolk-sac volume (V_v) and Reynolds number (Re). The shape of the yolk-sac was assumed to be a prolate spheroid (BAGARINAO, 1986), and the volume was calculated as follows:

$$[\text{Formula 2}] V_v = \left(\frac{\pi}{6}\right) \times L_v H^2$$

where L_v and H are respectively the length and the height of the yolk-sac. This parameter was measured on photos using Vistamatrix version 1.34 (SkillCrest LLC, Tucson, Arizona, USA).

The effects of size, swimming speed and yolk-sac volume (independent variables) on r^2_{mean} , CV, St and the amplitudes of LM1 to LM8, were investigated by multiple polynomial regressions of the third degree (the use of a higher degree did not improve the accuracy of the analysis). The best-fit model was identified in a stepwise forward-selection manner and was selected based on the lowest p -value. Only significant values were included in the models (Student's t test, $p < 0.05$) and presented in the results. In graphs showing effects of size and swimming speed on r^2_{mean} , CV and St values, data are presented in a surface plot where the size is on the x axis, the swimming speed is on the y axis, and the values of the dependent variable are provided by a color code. The surface that fitted best to the points of observation was determined by the weighted least squares method (McLAIN, 1974). Nonparametric correlation (Spearman) was also determined between Reynolds and Strouhal numbers.

RESULTS

Three movements from three different fish were

analyzed for each stage. The sample is similar to the ones of the two studies of MAUGUIT et al. (2010a,b) (72 sequences for *D. labrax*, 93 for *C. gariepinus* and 73 for *C. aeneus*).

Swimming ontogeny in the larval period (from hatching to 288 hPH)

Larvae measured 3.3 ± 0.2 mm ($n = 3$) at hatching and were observed until they grew to 5.5 ± 0.1 mm (= 288 hPH; $n = 3$). The yolk-sac (initially 0.4 ± 0.01 mm³, $n = 3$) was totally resorbed between 96 and 120 hPH. Pectoral fins appeared at a size of 4.93 ± 0.21 mm (96 hPH; $n = 3$). Independent of age, size and morphology, the swimming bouts could be split into two groups swimming at different speeds. According to the terminology used in BLAXTER (1969), the first group used a steady swimming speed (1.31 to 4.88 TLs⁻¹) ($n = 50$) while the second performed a burst of swimming (12.73 to 27.62 TLs⁻¹) ($n = 13$). None of the larvae swam at the intermediate speed, between 4.9 and 12.73 TLs⁻¹. Tail-beat frequencies were 20.47 ± 3.69 Hz for the steady swimming group and 49.07 ± 5.39 Hz for the burst of swimming group. In burst of swimming larvae, the six fastest bouts (24 to 27.62 TLs⁻¹) were in an intermediate regime ($Re > 300$). All the other recorded swimming bouts were in a viscous flow regime ($Re \leq 300$).

Swimming movements were observed at hatching. These movements were not completely sinusoidal ($r^2_{\text{mean}} = 0.77 \pm 0.17$), but r^2_{mean} values continuously increased with growth (Fig. 2A; $F_{[4,58]} = 24.170$, $p < 0.001$). At the size of 5.2 mm, all larvae were able to perform sinusoidal movements ($r^2_{\text{mean}} \geq 0.95$) while swimming speed ranged from 1.9 to 23.23 TLs⁻¹ (Fig. 2A). Moreover, independent of size, there was a strong correlation between a sinusoidal movement and swimming speed (Figure 2A; $F_{[4,58]} = 24.170$, $p < 0.001$): all larvae with a burst of swimming (>12 TLs⁻¹) performed sinusoidal movements independent of size (Fig. 2A). During ontogeny, the sinusoidal swimming motion was established first in the middle part of the body near the pivot point (LM3-LM4). The same acquisition was

then observed along almost all the rest of the body (LM1-LM2; LM5). After larvae reached a size of 5.2 mm, all body landmarks (LM1 to LM6) exhibited sinusoidal paths (Fig. 3A). This variation in the time of acquisition of sinusoidal movement for different parts of the body induced high values of CV in larvae (ca 12%) (Fig. 2B).

Independent of size, fish swimming at least at 12 TLs^{-1} (= burst of swimming) show CV around 2% (Fig. 2B; $F_{[3,59]} = 7.6278, p < 0.001$), with all the body parts (LM1-LM6) exhibiting sinusoidal trajectories. Although the r^2_7 and the r^2_8 values increased regularly with growth and with speed ($F_{4,58} = 13.209, p < 0.001$ for r^2_7 and $F_{4,58} =$

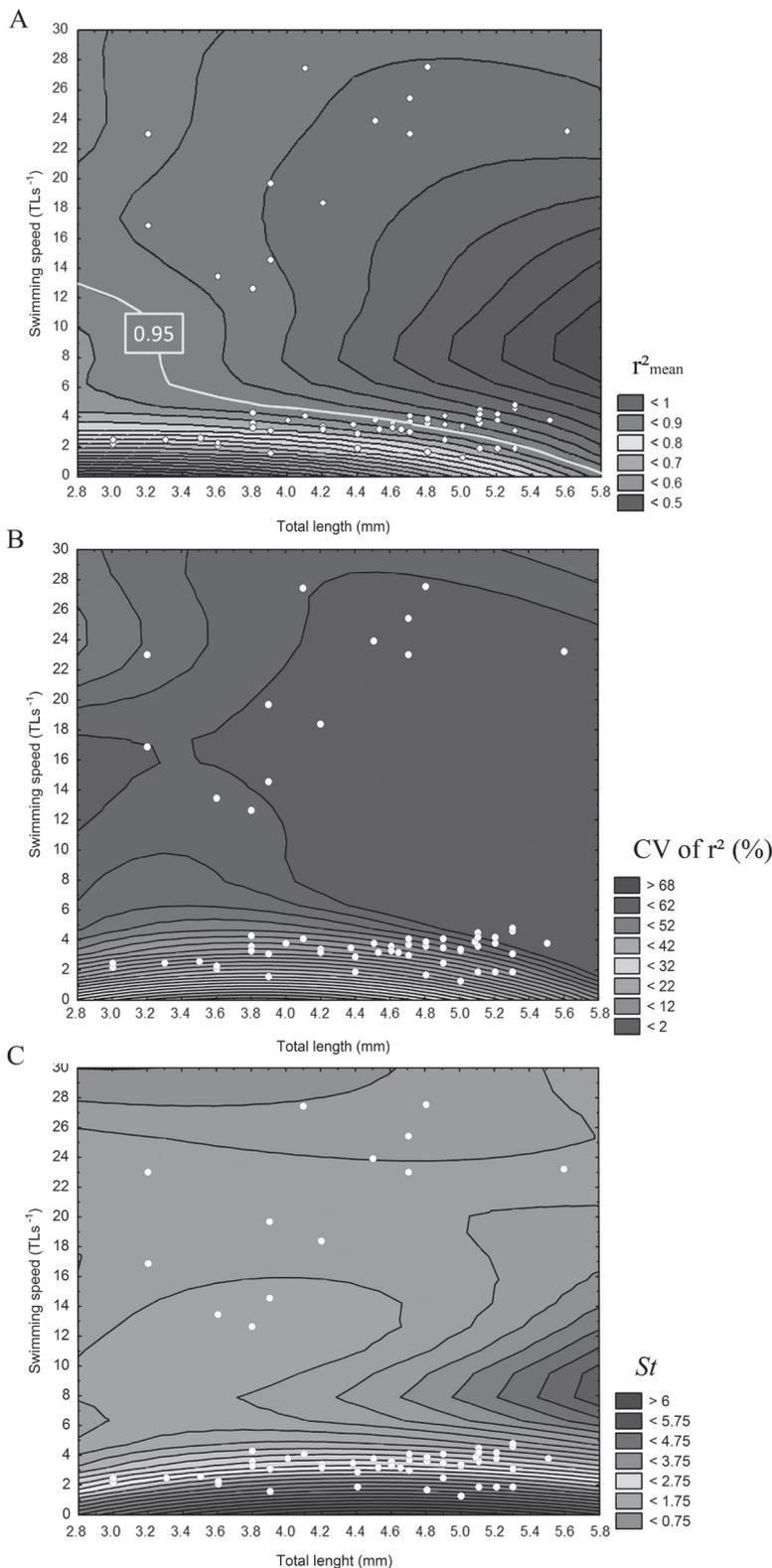


Fig. 2. – Contour plot representing the changes in (A) mean coefficient of determination (r^2_{mean}), (B) coefficient of variation of r^2 (CV of r^2) and (C) Strouhal number (St) as a function of the total fish length (TL) and relative swimming speed (U) of larvae of seabass (*Dicentrarchus labrax*). The index values are given by the colour code. The white circles represent observations made during the present study. Each circle corresponds to one fish. These data were used to fit the isoclines by means of the weighted least square distance method. In A the line represents the threshold value of 0.95. White circles located to the left of this line correspond to fish that did not execute fully sinusoidal swimming movements, whereas those to the right of this line correspond to fish with sinusoidal swimming movements.

11.060, $p < 0.001$ for r^2), sinusoidal movements were rarely observed at the level of the tail.

At hatching, larvae swam with high Strouhal numbers (> 2), and this index did not change significantly at the end of the larval period studied (Fig. 2C; $F_{[3,59]} = 27.527$, $p < 0.001$). During the period studied, larvae did not grow very much and so Re values did not differ substantially:

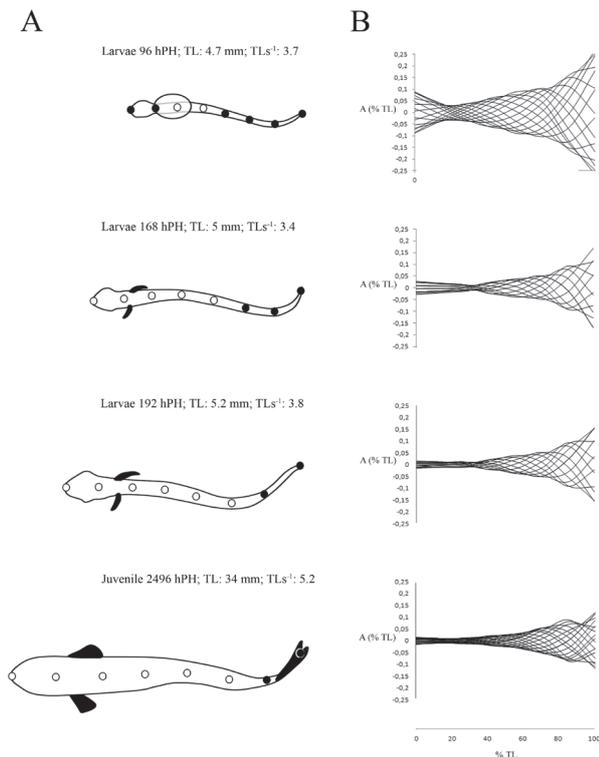


Fig. 3. – **A.** Representation of four stages: three larval and one juvenile. The eight landmarks used in the analysis of the swimming movement are indicated on the midline of each fish. White landmarks indicate that all fish from the size indicated reached at least a value of 0.95 for the corresponding r^2 . **B.** Midline kinematics of seabass (*Dicentrarchus labrax*) larvae and juveniles during cyclic swimming at different stages of ontogeny. The superimposed midlines (time step 4 ms) of one tail-beat cycle show the amplitude envelope of the body wave. Each amplitude envelope corresponds to the fish represented by the black shape. The amplitude is expressed as a proportion of total fish length (TL), the x axis representing the total fish length in percentage.

from 20 ± 3.5 ($n = 3$) at hatching to 94 ± 17 ($n = 3$) at 288 hPH (only for steady swimming). It is well known that St values are strongly correlated with Re values (BORAZJANI & SOTIROPOULOS, 2008), so no relation between size of larvae and St values was discovered. However, a strong correspondence was observed with swimming speed: the values of Re increased considerably for larvae with bursts of swimming ($Re = 323 \pm 160$; $n = 13$), inducing a decrease in St number values (1.12 ± 0.28) (Fig. 2C; $F_{[3,59]} = 27.527$, $p < 0.001$).

During growth, the relative amplitudes of the different body parts (LM1-6) decreased in parallel to a better sine-shaped swimming mode (Fig. 3B; $F_{[2,60]} = 51.144$ (LM1); 38.647 (LM2); 54.265 (LM3); 51.700 (LM4); 33.702 (LM5); 40.640 (LM6); $p < 0.001$). VIDELER (1993) and BREDER (1926) defined a subcarangiform mode as a swimming movement where the amplitude increases significantly in the posterior part of the body and where between 0.5 and 1 wavelength of propulsive wave (λ) is observed in the fish body. Despite the decrease in the amplitude movement of the LM1-6 during growth, all larvae swam with more than one λ on the body. So, the transition from an anguilliform to a subcarangiform mode was not yet complete. Except at the caudal tip (LM8), the amplitude of movement increased with swimming speed ($F_{[2,60]} = 51.144$ (LM1); 38.647 (LM2); 54.265 (LM3); 51.700 (LM4); 33.702 (LM5); 40.640 (LM6); 29.592 (LM7); $p < 0.001$).

The impact of yolk-sac resorption was also studied on the three indices and lateral displacements of the body during swimming movements (A_1 to A_8), but no significant relationship was found.

Swimming mode in juvenile stages (960-2496 hPH)

The size of the juveniles ranged from 14.3 to 34.2 mm, and all had a fully ossified caudal fin. Their swimming speed ranged from 2.32 to 23.4 TLs⁻¹ with a tail-beat frequency of 14.8 ± 10.72 Hz. Juveniles between 14 and 19 mm swam in the intermediate flow regime with Re values

from 509 to 848. Larger juveniles (25 to 34 mm) evolved in the inertial flow regime with Re values from 5242 to 13301.

The r^2_{mean} was always higher than 0.95 (0.98 ± 0.01), and the CV was low ($1.84 \pm 0.94\%$). Although the caudal fin followed better sinusoidal paths than in larvae, the threshold value of 0.95 was not always reached. The Strouhal numbers ranged from 0.48 to 1.58, with the lowest values being found for juveniles swimming in an inertial regime. Juveniles performed swimming movements with an important increase of the amplitude in the posterior part of the body and with a propulsive wave between 0.5 and 1 wavelength. Therefore, the transition to a subcarangiform mode occurred between the larval and juvenile stages.

DISCUSSION

All *Dicentrarchus labrax* larvae start their life swimming with an anguilliform motion that is functional in all types of hydrodynamic flow regimes (GRAY & HANCOCK, 1955, LIGHTHILL, 1969, WEBB & WEIHS, 1986). In addition, the anguilliform mode is used by all fish larvae, no matter what mode they eventually use as adults (OSSE & VAN DEN BOOGAART, 2000; MÜLLER & VAN LEEUWEN, 2004; MAUGUIT et al., 2010a,b). During growth, swimming movements become more sinusoidal, made up of movements having less relative amplitude. The St values do not change with size in larvae but are substantially reduced in juveniles that pass into the intermediate regime flow and have a subcarangiform swimming mode. However, it appears that swimming speed has a strong influence on all parameters studied, regardless of size and morphological development.

Previous studies on the ontogeny of swimming abilities in different fish species concerned only a few larval stages (BATTY, 1981, 1984; FUIMAN & WEBB, 1988, OSSE, 1990, OSSE & VAN DEN BOOGAART, 2000; FONTAINE et al., 2008; MÜLLER et al., 2008), restricting the accurate comparisons

between taxa. The study on zebrafish by MÜLLER et al. (2004) dealt with several larvae stages but none of the different indices used in our studies were used, so comparisons are difficult to make. For this reason, we can only compare our data to the data for *Clarias gariepinus* and *Corydoras aeneus* (MAUGUIT et al., 2010a,b) for which many developmental stages were also studied.

However, the biology of these three species differs in several points that can influence the establishment of swimming behaviour. *Dicentrarchus labrax* is a marine fish that lives in cold/temperate water. As a consequence, larval seabass were reared in seawater at 15°C. Being subtropical catfish species, larvae of *C. gariepinus* and *C. aeneus* were reared in freshwater at 28 and 25°C, respectively (MAUGUIT et al., 2010a,b). The time of hatching after fertilization differs between species; 24h only for *C. gariepinus*, 72h for *C. aeneus* (MAUGUIT et al., 2010a,b) and 96h for *D. labrax*. The temperature and the salinity are both ecological factors that act directly through receptors to increase or decrease growth (BOEUF et al., 2001), while the time between fertilization and hatching can have an effect on the morphological stage of larvae at hatching. The differences between these parameters can, probably, largely explain the differences observed in ontogeny of swimming development in the three species studied.

Figure 4 provides a panel contrasting the main modifications in swimming abilities between the three species that live in two different environments and have different modes of swimming in the adult stage.

At hatching, only *C. aeneus* has pectoral fins and is the only species to perform sinusoidal swimming (MAUGUIT et al., 2010b). *Clarias gariepinus* larvae hatch the earliest after fertilization and are unable to perform any swimming at this stage (MAUGUIT et al., 2010a). *Dicentrarchus labrax* larvae spend the longest time in the egg phase, but live in colder water with higher salinity, which should decrease their developmental, growth rate. At hatching, *D.*

labrax cannot perform sinusoidal swimming ($r^2 = 0.77 \pm 0.17$) (Fig. 4). In various species it has been observed that oscillations in the anterior part of the body during swimming movements in larvae decrease once the pectorals fins appear (BATTY, 1981, OSSE, 1990, THORSEN et al., 2004). The present study confirms this statement. In *D. labrax*, pectoral fins appear at 4.93 ± 0.21 mm and almost all the body (LM1-LM5) follows a sinusoidal path from a size of 5 mm (Fig. 3A).

A common characteristic in the ontogeny of swimming in the three species is a progressive decrease in the amplitude of movement in the anterior part of the body. The amplitudes of LM1 to LM4 for catfishes and of LM1 to LM5 for the seabass decrease by 50% in the first days after hatching (Fig. 4). This amplitude reduction was

earlier thought to explain the change in the *St* number (MAUGUIT et al. 2010a,b). However, this assumption is not verified in *D. labrax* (Fig. 4). The *St* number has mostly been studied in fish moving in an inertial regime (TAYLOR et al., 2003, TYTELL, 2004; LAUDER & TYTELL, 2006). More recently, studies by BORAZJANI & SOTIROPOULOS (2008, 2009) showed the *St* number is inversely related to *Re* values: an *Re* of 300 (intermediate regime) corresponds to an optimal *St* number of 1.3, and an *Re* of 4000 (inertial regime) corresponds to an optimal *St* of 0.6. This correlation between *Re* and *St* is also observed in our results (Fig. 5). The fact that changes in *St* number are first of all related to changes in *Re* number could explain why catfish species reach a low *St* number faster than seabass (Fig. 4). Because catfish species

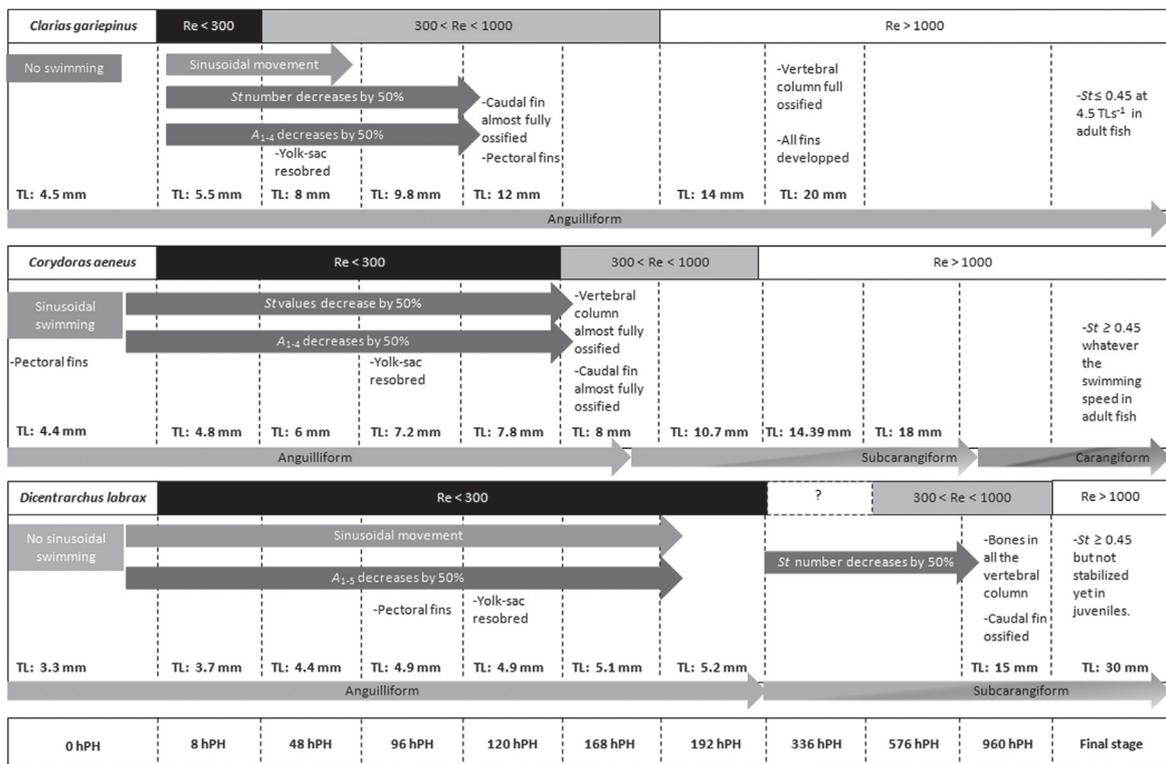


Fig. 4. – Major steps in the swimming ontogeny in *Clarias gariepinus*, in *Corydoras aeneus* and in *Dicentrarchus labrax*. The data on the catfishes are obtained from the studies of MAUGUIT et al; (2010a,b). The acquisition of sinusoidal swimming, the evolution of *St* values and the changes in swimming mode are expressed in relation to age, size, regime flow and morphological changes in the three species. For *D. labrax*, the transition between the viscous and the intermediate regime is lacking. For each species, all morphological changes cited were observed in the fish in the corresponding studies on ontogeny of swimming and not from the literature. The gradual transition between different swimming modes is indicated by bicolour arrows.

grow faster, they are able to earlier reach high Re values and the intermediate regime at a cruise speed. Having slower growth, seabass larvae are restricted longer to a small size and to a viscous flow. In this flow, *D. labrax* larvae need to swim at high frequency (22.85 ± 2.09 Hz; $n = 63$), which decreases their efficiency. Consequently, the major decrease in St does not occur until the first juvenile stage studied (ca 15 mm TL: 960 hPH), corresponding to swimming in an intermediate regime at a cruising speed (Fig. 4). In this regime flow, the total drag decreases, the tail-beat frequency is slower (14.8 ± 10.72 Hz; $n = 9$) and the swimming efficiency improves ($St = 0.095 \pm 0.04$; $n = 9$). Additionally to the change in regime flow, the ossification of caudal fin rays corresponds to an important decrease in tail-beat frequency and in St number in all the species (Fig.4). The ossification of the vertebral column and caudal fin increases the stiffness of the body and allows better force transmission (BATTY, 1981; LONG et al., 1994; MCHENRY et al., 1995; HALE, 1999). For *C. aeneus* and *D. labrax*, this ossification also occurs before the transition in swimming mode, demonstrating that a rigid axis

is required for a subcarangiform and carangiform mode.

The swimming speed appears to strongly affect the values of r^2_{mean} , CV and St in *D. labrax*. All larvae swimming at high speed are able to perform a sinusoidal swimming motion ($r^2_{\text{mean}} \geq 0.95$) with a constant motion along the length of the body (CV = 2.45 ± 1.99 ; $n = 13$). According to the study by BUDICK & O'MALLEY (2000) on the fast swimming speed in *Danio rerio*, the ability in larvae to execute very fast movements could be due to a good synchronization of motoneurons firing along the body. This improved synchronization could partially explain the good coordination along the body during fast swimming movements. Larvae also show decreasing St number with increasing swimming speed, which is related to the way the Re number varies. At high speed, Re number increases considerably for swimming larvae. Consequently, total drag decreases and efficiency increases (St number decreases).

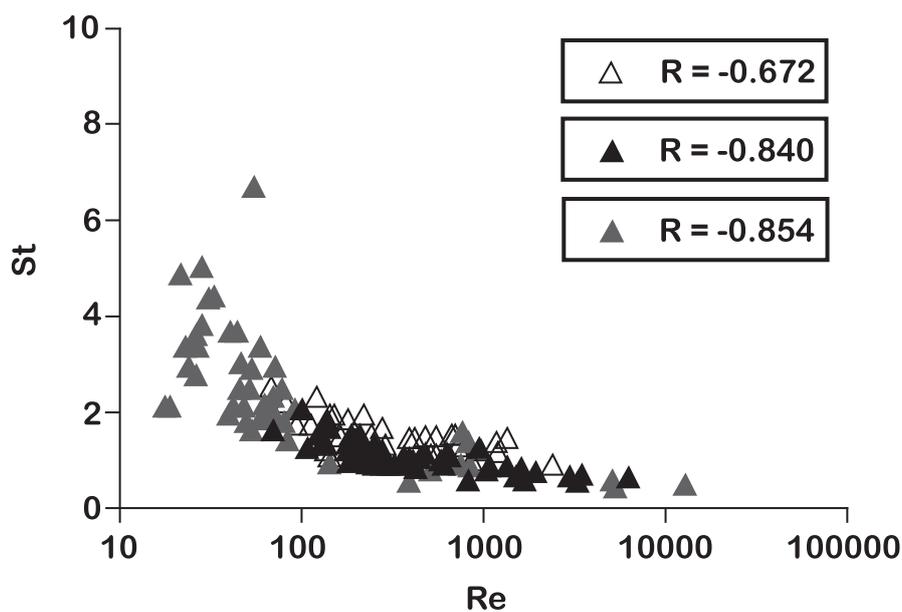


Fig. 5. – Correlation between St and Re values in *Clarias gariepinus* (in white), *Corydoras aeneus* (in dark grey) and *Dicentrarchus labrax* (in light grey). The x axis is presented in \log_{10} . The values of the correlation (R) are indicated.

CONCLUSION

The gradual establishment of adult swimming movement is not the same between the three species studied, but varies according to morphological development and growth rate. However it does follow the same pattern: 1) they transition from a viscous to an intermediate and then to an inertial flow regime; 2) they begin with an anguilliform swimming mode; 3) for the three species the decrease in tail-beat frequency and in St correspond to an increase in Re and are correlated to ossification of caudal fin rays.

Being a marine fish, *D. labrax* growth rate is lower than that of the two catfishes. Therefore sea bass larvae stay in low Re values longer and have consequently high St values during their larval period. However the viscous flow regime does not impede the establishment of a sinusoidal movement since stabilization of r^2_{mean} and CV occur in this regime.

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