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). *Eubranchipus (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. *fide* 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 Eeast 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;

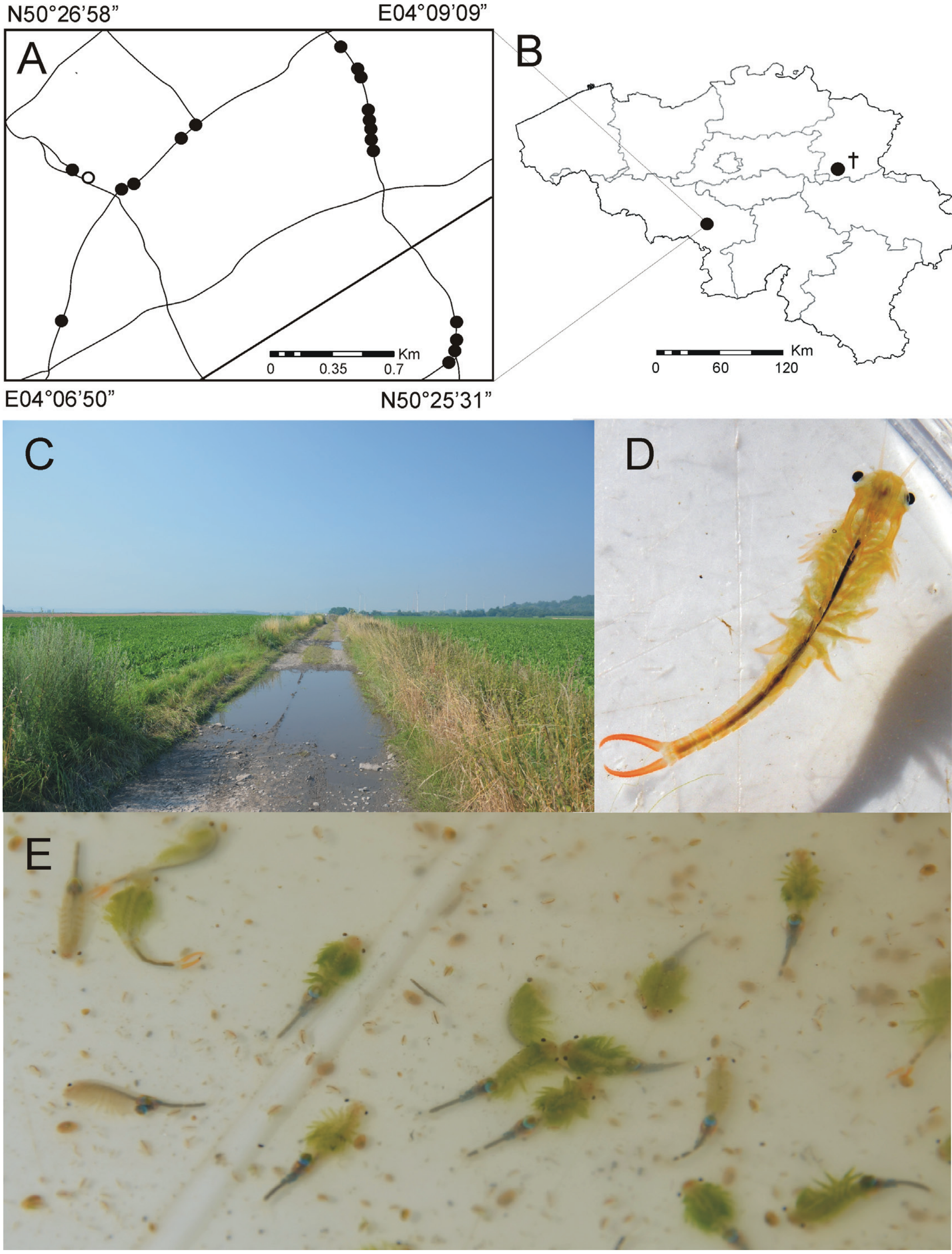

Fig. 1. – Distribution of the discovered *Branchipus schaefferi* populations in Hainaut (A) with thin and thick black lines representing unsealed and sealed roads, respectively. Filled symbols represent wheel track populations, the empty symbol corresponds to a population in a farmland pond. (B) Overview 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 µS 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. cancriformis* (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; µS cm^{-1}), water temperature (T; °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 (± 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.

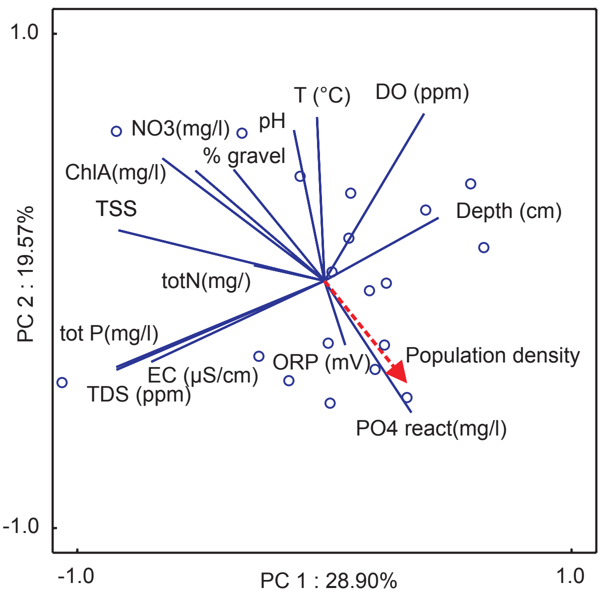

Fig. 2. – PCA triplot showing associations between environmental variables (vectors), site scores (circles) and the supplementary variable Population density (arrow). Environmental variables were centered and standardised prior to analysis. Population densities were transformed to ranks.

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

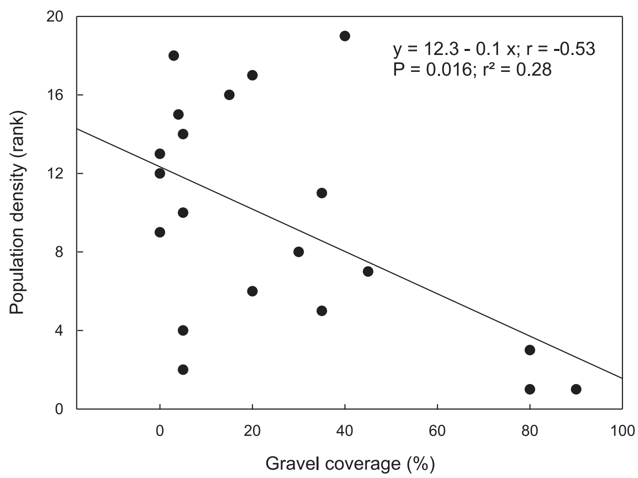

Fig. 3. – Scatterplot showing the significant negative relationship between the percentage of surface area of each habitat covered with gravel and the density of fairy shrimp found in the active populations during sampling.

TABLE 1

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

Variable	**Average ± st. dev.**	**Range**
Surface (m²)	32.4 ± 75.0	0.2 - 314
Depth (cm)	7.8 ± 3.8	2 - 15
Conductivity (µS/cm)	573.4 ± 274.6	133 - 1335
Dissolved oxygen (ppm)	38.6 ± 1.47	6.20 - 1.35
pH	8.10 ± 0.30	7.69 - 8.57
Temperature (°C)	32.1 ± 2.3	27.73 - 35.7
Total Dissolved Solids (ppm)	286.9 ± 137.2	67 - 668
TSS	222.8 ± 205.0	34.0 - 873.7
Chl A (mg/l)	0.011 ± 0.007	0.002 - 0.03
Total N (mg/l)	4.92 ± 3.45	0 - 10.4
Nitrate (mg/l)	4.51 ± 2.49	0 - 9
Total P (mg/l)	2.01 ± 0.76	0.68 - 3.62
Reactive phosphate (mg/l)	1.06 ± 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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REFERENCES

Al-Sayed H & Zainal K (2005). The occurence of anostracans - fairy shrimps *Branchipus schaefferi* in vernal pools of Bahrain. Journal of Arid Environments, 61:447-460.

Beladjal L, Peiren N, Vandekerckhove TTM & Mertens J (2003). Different life histories of the co-occuring fairy shrimps *Branchipus schaefferi* and *Streptocephalus torvicornis* (Anostraca). Journal of Crustacean Biology, 23(2):300-307.

Beladjal L, Weekers PHH & Mertens J (2007). Life cycle and genetic differences of two *Branchipus schaefferi* populations from Morocco (Anostraca: Crustacea). Animal Biology, 57(4):409-421.

Belk D (1998). Global status and trends in ephemeral pool invertebrate conservation: implications for Californian fairy shrimp. In: Witham, CW, Bauder ET, Belk D, Ferren WRJr & Ornduff, R (eds), Ecology, conservation, and management of vernal pool ecosystems, California native plant society, Sacramento: 147-150.

Belk D & Brtek J (1995). Checklist of the Anostraca. Hydrobiologia, 298(1-3):315-353.

Boven L, Vanschoenwinkel B, De Roeck ER, Hulsmans A & Brendonck L (2008). Diversity and distribution of large branchiopods in Kiskunsag (Hungary) in relation to local habitat and spatial factors: implications for their conservation. Marine and Freshwater Research, 59(10):940-950.

Brtek J & Thiéry A (1995). The geographic distribution of the European branchiopods (Anostraca, Notostraca, Spinicaudata, Laevicaudata). Hydrobiologia, 298(1-3):263-280.

Brendonck L (1989a). A review of the phyllopods (Crustacea: Anostraca, Notostraca, Conchostraca) of the Belgian fauna. In: Wouters K & Baert L (eds) Invertébrés de Belgique. Institut Royal des Sciences naturelles de Belgique, Bruxelles: 129-135.

Brendonck L (1989b). *Leptestheria dahalacensis* (Rüppel, 1837), a Conchostracan new for the Belgian fauna. *Bulletin de l'institut royal des sciences naturelles de Belgique, Biologie*, 59:59-62.

Brendonck L (1996). Diapause, quiescence, hatching requirements: What we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). In: International Symposium on Diapause in the Crustacea: Sep 12-17 1996; St Petersburg, Russia: Kluwer Academic Publishers: 85-97.

Brendonck L, Rogers DC, Olesen J, Weeks S, Hoeh WR (2008). Global diversity of large branchiopods (Crustacea: Branchiopoda) in freshwater. Hydrobiologia, 595:167-176.

Defaye D, Rabet N & Thiéry A (1998). Atlas et bibliographie des crustacés branchiopodes (Anostraca, Notostraca, Spinicaudata) de France. Vol. 32.

Eder E, Hödl W & Gottwald R (1997). Distribution and phenology of large branchiopods in Austria. Hydrobiologia, 359:13 - 22.

Engelmann M & Hahn T (2004) Vorkommen von *Lepidurus apus, Triops cancriformis, Eubranchipus* (*Siphonophanes*) *grubii, Tanymastix stagnali*s und *Branchipus schaefferi* in Deutschland und Österreich (Crustacea: Notostraca und Anostraca). Faun. Abh. 25: 3–67.

Hilt S, Gross EM, Hupfer M, Morscheid H, Mählmann J, Melzer A, Poltz J, Sandrock S, Scharf E-M, Schneider S & van de weyer K (2006). Restoration of submerged vegetation in shallow eutrophic lakes – A guideline and state of the art in Germany. *Limnologica - Ecology and Management of Inland Waters*, 36(3):155-171.

Hössler J, Maier G & Tessenow U (1995). Some notes on the ecology of a German *Branchipus schaefferi* population (Crustacea: Anostraca). Hydrobiologia, 298:105-112.

Jeppesen E, Christoffersen K, Landkildehus F, Lauridsen T, Amsinck SL, Riget F & Sondergaard M. (2001) Fish and crustaceans in northeast Greenland lakes with special emphasis on interactions between Arctic charr (*Salvelinus alpinus*), *Lepidurus arcticus* and benthic chydorids. Hydrobiologia, 442, 329-337.

Lahr J (1998). An ecological assessment of the hazard of eight insecticides used in Desert Locust control, to invertebrates in temporary ponds in the Sahel. Aquatic Ecology 32:153-162.

Legendre P & Legendre L (1998) Numerical Ecology: Elsevier.

Loneux M & ThiÉry A (1998) Révision des grands Branchiopodes conservés au Musée de Zoologie de l'Université de Liège: Intérêt des collections muséologiques. *Les Naturalistes belges*, 79(2):33-47.

Loneux M & Walravens E (1998). Observation recente de Chirocephalus diaphanus (Prevost in Jurin, 1820) en Belgique: appel aux naturalistes. Les Naturalistes belges, 79:9-14.

Maier G, Hössler J & Tessenow U (1998). Succession of physical and chemical conditions and of crustacean communities in some small, manmade water bodies. International Review of Hydrobiology, 83(5-6):405-418.

Marrone F & Mura G (2006). Updated status of Anostraca, Notostraca and Spinicaudata (Crustacea Branchiopoda) in Sicily (Italy): review and new records. Naturalista Siciliano 30:3-19.

Mura G (1999). Current status of the Anostraca of Italy. Hydrobiologia, 405:57-65.

Mura G (2001). Life history strategy of *Chirocephalus ruffoi* (Crustacea, Anostraca) in Mediterranean temporary mountain pools. Hydrobiologia, 462:145-156.

Nhiwatiwa T, Brendonck L, Waterkeyn A, Vanschoenwinkel B (2011). The importance of landscape and habitat properties in explaining instantaneous and long-term distributions of large branchiopods in subtropical temporary pans. Freshwater Biology, 56(10):1992-2008.

Paulssen L (2000). De kieuwpootkreeft *Chirocephalus diaphanus* (Crustacea: Branchiopoda) ontdekt in Limburg. Natuurhistorisch Maandblad, 89:226-229.

Petkovski S (1997). On the presence of the genus *Branchipus* Schaeffer, 1766 (Crustacea : Anostraca) in Macedonia. Hydrobiologia, 359:37-44.

Petrov B, Cvetkovic DM (1997). Community structure of branchiopods (Anostraca, Notostraca and Conchostraca) in the Banat province in Yugoslavia. Hydrobiologia, 359:23-28.

Petrov B, Petrov I (1997). The status of Anostraca, Notostraca and Conchostraca (Crustacea : Branchiopoda) in Yugoslavia. Hydrobiologia, 359:29-35.

Rogers DC: Aquatic macroinvertebrate occurrences and population trends in constructed and natural vernal pools in Folsom, California. In: C.W. Witham ETB, D. Belk, W.R. Ferren, Jr., and R. Ornduff (eds), Ecology, conservation, and management of vernal pool ecosystems. California Native Plant Society, Sacramento: 224-235.

Soesbergen M (2008). Oerkreeft in karrenspoor. In: *Passie voor kleine beestjes*. Edited by Kleukers R, Berg M, van Strien W. Leiden: EIS Nederland;.

Spencer M, Blaustein L, Schwartz SS & Cohen JE (1999). Species richness and the proportion of predatory animal species in temporary freshwater pools: relationships with habitat size and permanence. Ecology Letters, 2(3):157-166.

Thiéry A (1987): Les crustacés branchiopodes Anostraca Notostraca & Conchostraca des milieux limniques temporaires (dayas) au Maroc. Taxonomie, biogéographie, écologie. Doctoral thesis, Université Aix-Marseille III, Marseille.

Thiéry A, Brtek J & Gasc C (1995). Cyst morphology of European branchiopods (Crustacea: Anostraca, Notostraca, Spinicaudata, Laevicaudata). Bulletin du Museum National d'Histoire Naturelle, Section A Zoologie Biologie et Ecologie Animales, 17(1-2):107-139.

Vanschoenwinkel B, Gielen S, Vandewaerde H, Seaman M & Brendonck L (2008A). Relative importance of different dispersal vectors for small aquatic invertebrates in a rock pool metacommunity. Ecography, 31:567-577.

Vanschoenwinkel B, Waterkeyn A, Vandecaetsbeek T, Pineau O, Grillas P & Brendonck L (2008B): Dispersal of freshwater invertebrates by large terrestrial mammals: a case study with wild boar (*Sus scrofa*) in Mediterranean wetlands. Freshwater Biology, 53:2264-2273.

Vanschoenwinkel B, Mergeay J, Pinceel T, Waterkeyn A, Vandewaerde H, Seaman M, Brendonck L (2011). Long distance dispersal of zooplankton endemic to isolated mountaintops - an example of an ecological process operating on an evolutionary time scale. PLoS ONE, 6(11). http://dx.doi.org/10.1371/journal.pone.0026730

Waterkeyn A, Grillas P, De Roeck ERM, Boven L & Brendonck L (2009). Assemblage structure and dynamics of large branchiopods in Mediterranean temporary wetlands: patterns and processes. Freshwater Biology, 54(6):1256-1270.

Waterkeyn A, Vanschoenwinkel B, Elsen S, Anton-Pardo M, Grillas P & Brendonck L (2010). Unintentional dispersal of aquatic invertebrates via footwear and motor vehicles in a Mediterranean wetland area. Aquatic Conservation: Marine and Freshwater Ecosystems, 20:580-587.

Williams DD (2006). The biology of temporary waters. Oxford University Press, Oxford, UK.

Willems T & de Leander L (2006). *Triops* herontdekt. Bertram, 4(3):11-15.

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