On the non-malacostracan crustaceans (Crustacea: Branchiopoda, Copepoda, Ostracoda) from the inland waters of Fthiotida (Greece)

FEDERICO MARRONE1,*, MARCO ARCULEO1, CHRISTOS GEORGIADIS2,3, FABIO STOCH4

1 University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), via Archirafi 18, 90123 Palermo (Italy)
2 Department of Biology, College of Science, United Arab Emirates University, P.O. Box 15551, Al Ain, (United Arab Emirates)
3 Section of Zoology – Marine Biology, Department of Biology, National and Kapodistrian University of Athens, 15784 Zografou (Greece)
4 Evolutionary Biology & Ecology, Université Libre de Bruxelles, Avenue F.D. Roosevelt 50, 1050 Brussels (Belgium)

*corresponding author: federico.marrone@unipa.it

Keywords: Arctodiaptomus alpinus, Cross-taxon congruence, Diaptomus cf. serbicus, Leptestheria dahalacensis, Temporary ponds, Wlassicsia pannonica.

SUMMARY
In the frame of the activities of the LIFE11 NAT/GR/1014 ForOpenForests, some water bodies occurring in the "Ethnikos Drymos Oitis” (GR2440004) and “Oros Kallidromo” (GR2440006) (Sterea Ellada) were investigated with the aim of providing a first census of the composition and diversity of their crustacean fauna. Overall, the sampling of 15 water bodies (7 of them listed as “Mediterranean temporary ponds” sensu EU “Habitats Directive”) led to the finding of 13 branchiopod, 11 copepod, and 7 ostracod taxa, including 4 species new for mainland Greece, i.e. the copepods Arctodiaptomus alpinus (Imhoff, 1885) and Diaptomus cf. serbicus, and the branchiopods Leptestheria dahalacensis (Rüppel, 1837) and Wlassicsia pannonica Daday, 1904. The comparative analysis of the observed species assemblages and richness suggests that the protection of those ponds identified as “priority habitats” according to the “Habitats Directive” is effective for the specialized and peculiar crustacean biota of these ecosystems, but it is not sufficient in order to preserve efficiently the whole diversity of temporary pond-dwelling crustaceans occurring in the study area. Therefore, the implementation of synergistic conservation measures dedicated to both “priority” and “non-priority” habitats is desirable.
INTRODUCTION

Non-malacostracan, free-living crustaceans are among the most abundant and diverse invertebrate taxa inhabiting temporary ponds (e.g., Williams 2005). Thanks to the production of drought-resistant resting stages, these crustaceans can survive through the dry phases periodically experienced by these unstable ecosystems (Incagnone et al. 2015).

Due to their unusual aspect, high rate of endemism, and keystone ecosystem role, the so-called ‘large branchiopods’ (Branchiopoda: Anostraca, Notostraca, Spinicaudata) and the calanoid copepods (Copepoda: Calanoida) are considered “flagship taxa” of the animal biota inhabiting Mediterranean temporary ponds (e.g., Sahuquillo and Miracle 2013, Alfonso et al. 2016, Marrone et al. 2016). Notwithstanding the paramount importance of crustaceans in pond ecosystems, to date only few data are available about the non-malacostracan crustaceans inhabiting the inland waters of Greece (see Abatzopoulos et al. 1999, Marrone 2006, Marrone et al. 2019, and references therein); such lack of data is particularly regrettable since, due to its geographical location and complex physiography, Greece is expected to host an extraordinarily rich and diverse crustacean fauna (e.g., Griffiths et al. 2004, Blondel et al. 2010, Marrone et al. 2017).

Within the frame of the activities of the LIFE11 NAT/GR/1014 ‘ForOpenForests’ program, we investigated the crustacean fauna of the temporary water bodies occurring in the Natura 2000 sites "Ethnikos Drymos Oitis" (GR2440004) and “Oros Kallidromo” (GR2440006), located in the eastern Aegean (Attiko-Voitia) Greek ecoregional unit, as defined by Zogaris et al. (2009). Moreover, a comparison between the crustacean assemblages inhabiting the habitat of Community Interest 3170* (Mediterranean temporary ponds, listed in Annex 1 of the Habitats Directive 92/43/EEC) and those observed in other temporary and permanent water bodies occurring in the study area was carried out.

Figure 1. Geographical location of the sampled sites (Sterea Ellada, Greece). 3170* sites are reported in green, “non-3170*” sites are reported in yellow.

MATERIALS AND METHODS

Samples were collected from 13 temporary and two permanent water bodies in the study area on May and November 2017 (see Fig. 1, Table 1, Appendix 1); the geographical coordinates and elevation of each site were recorded using a hand-held GPS apparatus, and an alphanumeric code was attributed to each of them (Table 1). The locality names and any other information originating from the Greek language follow the transliteration rules as set by the ELOT 743/ISO 843 system, to properly standardize the wording. This should be an integral part of all such reports, as it streamlines the proper databasing of specimens and avoids data-mining misinterpretations based on misspellings (see above example of Ethnikos Drymos Oitis, which could be erroneously inscribed as Ethnikos Drimos Itis).

Seven out of the 15 sampled water bodies could be ascribed to the priority habitat type “Mediterranean temporary ponds (3170*)”; these are freshwater temporary ponds located between 989 and 1914 m a.s.l. The ponds on Oiti are characterized by a somewhat regular alternation between their wet and dry
phases, while those on Kallidromo are rather irregular, with the wet phase not being in sync between them (Delipetrou et al. 2015a). The main typical flora species in the water bodies of Oiti are annuals such as *Lythrum thymifolia* L., *Limosella aquatic* L., *Ranunculus lateriflorus* DC, *Myosurus minimus* L., and *Veronica oetae* L. -A. Gustavsson 1978, with the latter being a local endemic and registered as a priority species in Annex II of the EU92/43 Directive (Karetsos et al. 2018, Delipetrou et al. 2015b). For the Kallidromo ponds, typical species are *Verbena supina* L., *Heliotropium supinum* L., *Mentha pulegium* L., and *Cyperus fuscus* L. The vegetation shows temporal and spatial variation, depending mainly on water depth and inundation period (Delipetrou et al. 2015a, Karetsos 2002). As for the fauna, Legakis (2015) recorded larval forms of insects (e.g., Odonata, Trichoptera, Ephemeroptera, Heteroptera), predatory adult beetles (Coleoptera; probably Hydrophilidae and Gyrinidae), as well as Platyhelminthes and Annelida. Six other water bodies, located along an elevational range comprised between 1 and 1536 m a.s.l., were characterized by a temporary hydroperiod, but did not host the phytosociological associations typical of habitat 3170*.

Water temperature (°C) and electrical conductivity (µS/cm) were measured using a hand-held Hanna Instruments HI9835 multiprobe. Three arbitrary qualitative classes were used to estimate water turbidity (from 1: crystal-clear water, to 3: extremely turbid water). Macrophyte cover was estimated visually observing the pond surface; the percentage of macrophyte cover was used as a proxy for the structural complexity of aquatic vegetation and hence, habitat complexity, varying from 0 (low complexity, macrophytes absent) to 3 (high complexity, 100% macrophyte cover).

A 200µm mesh-sized hand net was used to sample crustaceans along shorelines and through submerged vegetation; a 125µm mesh-sized towing net was used to collect samples in the open waters. Collected samples were fixed *in situ* in 96% ethanol.

Table 1. List of the sampled sites. Geographical coordinates are in decimal degrees, WGS84 datum.

| Sites                          | Mountain body | ID Code | Latitude   | Longitude   | Elevation (m a.s.l.) | Hydroperiod | Habitat 3170* |
|-------------------------------|---------------|---------|------------|-------------|----------------------|-------------|---------------|
| Limni Nevropolis              | Kallidromo    | GR161   | 38.751484  | 22.492133   | 989                  | Temporary   | YES           |
| Limni Louka                   | Oiti          | GR163   | 38.843127  | 22.323721   | 1159                 | Temporary   | YES           |
| Limni Greveno                 | Oiti          | GR167   | 38.823732  | 22.282342   | 1893                 | Temporary   | YES           |
| Limni Alikaina                | Oiti          | GR168   | 38.808661  | 22.258798   | 1914                 | Temporary   | YES           |
| Limni Livadies                | Oiti          | GR169   | 38.821925  | 22.271584   | 1813                 | Temporary   | YES           |
| Mourouza                      | Kallidromo    | GR173   | 38.751262  | 22.504197   | 1075                 | Temporary   | YES           |
| Mourouzos                     | Kallidromo    | GR174   | 38.750576  | 22.505173   | 1075                 | Temporary   | YES           |
| Pond 1 Anopia                 | Kallidromo    | GR160   | 38.765111  | 22.512111   | 906                  | Temporary   | NO            |
| Nevropoli pool                | Kallidromo    | GR162   | 38.751625  | 22.494335   | 989                  | Temporary   | NO            |
| Anthili rice fields           | -             | GR164   | 38.821856  | 22.491015   | 5                    | Temporary   | NO            |
| Pond 1 Katavothra             | Oiti          | GR165   | 38.763363  | 22.301297   | 1536                 | Temporary   | NO            |
| Pond at the mouth of Spercheios river | -          | GR170   | 38.865764  | 22.526366   | 1                    | Temporary   | NO            |
| Pond close to the village of Kastriotissa | Oiti      | GR172   | 38.761030  | 22.22049    | 1400                 | Temporary   | NO            |
| Man-made pond on the path to Livadies | Oiti      | GR166   | 38.820980  | 22.274155   | 1808                 | Permanent   | NO            |
| Reservoir at the mouth of the Spercheios river | -          | GR171   | 38.865176  | 22.526493   | 1                    | Permanent   | NO            |
The *ex situ* re-hydration of dry sediment, known as “Sars’ method” (van Damme and Dumont 2010) was used as a complement to the study of the crustacean samples collected during the inundated phase of temporary water bodies. Accordingly, sediment samples were collected from some of the sampled water bodies (i.e., GR164, GR168, GR173, GR174, see Table 1) and cultured in laboratory following the methods described by Marrone et al. (2019).

Crustacean specimens collected in the field or coming from laboratory cultures were sorted and identified according to Cottarelli and Mura (1983), Alonso (1996), Flössner (2000) and Korn et al. (2006) (Branchiopoda), Dussart (1967, 1969) and Kiefer (1978) (Copepoda), and Meisch (2000) (Ostracoda); identifications and nomenclature were updated following the most recent taxonomic literature. Undissected crustacean specimens were stored in 95% ethanol at -20°C; dissected soft parts were mounted in glycerol in sealed microscope slides.

Because of the intricate taxonomy of the anostracan genus *Chirocephalus* and, in particular, of the species included in the *C. diaphanus* group, five specimens of *C. diaphanus sensu lato* from sites GR161, GR163, GR167, GR169 and GR174 (see Table 1) were studied by amplifying and sequencing a fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I (COI); comparative sequences of *C. diaphanus* s.l. were downloaded from GenBank and included in the analysis (see Marrone et al. 2019, for details on the laboratory protocols and data analysis). Likewise, a fragment of the mitochondrial 12S gene was amplified for performing molecular identification of the specimens of the natostracan genus *Triops* raised from the sediment collected in the Anthili rice fields (see Tziortzis et al. 2014 for details on the laboratory protocols and data analysis). The new sequences were deposited in GenBank with the accession numbers MK748499-MK748503 (*Chirocephalus* COI sequences) and MK736275 (*Triops* 12S sequence). For each dataset, best-fitting evolutionary models were selected under the Akaike Information Criterion (AIC) with MrModeltest 2.2 (Nylander 2004). Bayesian inference (BI) and maximum likelihood (ML) analyses were performed as implemented by MrBayes 3.2 (Ronquist et al. 2012) and PhyML v.3 (Guindon and Gascuel 2003), respectively.

Data obtained from the field surveys were stored as a binary (presence/absence) species composition data matrix due to the difficulty to compare species abundances obtained using different collecting methods. Sites GR168 and GR170 were excluded from the analysis since no crustaceans were collected there, nor obtained from culturing the sediment samples. Site GR162 was also not included, since it hosts a subset of the fauna occurring in the neighbouring site GR161, which is likely the source area for a part, or the totality, of the fauna recorded in GR162 (cf. Table 2). Each sample was attributed to its habitat type defined as (i) Mediterranean temporary ponds (habitat 3170*) and (ii) non-3170 natural ponds; two water bodies were classified as rice fields (“Anthili rice fields”, code GR164) and brackish water permanent ponds (reservoir located at the mouth of the Spercheios river, code GR171), respectively. All statistical analyses were performed using R software 3.3.2 (R Development Core Team 2016). A pairwise dissimilarity matrix based on the Sorensen's index of dissimilarity was computed using the R package ‘vegan’ (Oksanen et al. 2017). The significance of attributing sites on the basis of their dissimilarities to the two main different habitat types defined above (3170* and non-3170* ponds) was assessed by means of Analysis of Similarities (ANOSIM) using the ‘vegan’ R package. Ordination of sites was performed by non-Metric Multidimensional Scaling (nMDS) using the ‘vegan’ R package. Species were superimposed on the nMDS ordination plain using their Spearman’s correlation coefficients with axes.
Table 2. Checklist and distribution of the collected non-malacostracan crustaceans.

| Taxa                        | Acronym | Sites of occurrence (3170* only) | Sites of occurrence (non-3170*) |
|-----------------------------|---------|----------------------------------|--------------------------------|
| **BRANCHIOPODA**            |         |                                  |                                |
| Anostraca                   |         |                                  |                                |
| Chirocephalidae             |         |                                  |                                |
| Chirocephalus diaphanus     | Cdia    | GR161, GR163, GR167, GR169, GR173, GR174 | GR162                          |
| **Notostraca**              |         |                                  |                                |
| Triops cancriformis         | Tcan    |                                  | GR164                          |
| **Spinicaudata**            |         |                                  |                                |
| Leptestheriidae             |         |                                  |                                |
| Leptestheria dahalacensis   | Ldah    |                                  | GR164                          |
| **Anomopoda**               |         |                                  |                                |
| **Moinidae**                |         |                                  |                                |
| Moina brachiata             | Mbra    | GR161, GR169, GR173, GR174       | GR162, GR164, GR165            |
| Moina macrocopa             | Mmac    |                                  |                                |
| **Daphniidae**              |         |                                  |                                |
| Ceriodaphnia reticulata     | Cret    | GR169                            | GR166                          |
| Sinocephalus vetulus        | Svet    | GR161                            |                                |
| Daphnia (Ctenodaphnia)      | Daphnii | Datk                            | GR173, GR174                   |
| Daphnia (Ctenodaphnia) cheveuxi Richard, 1896 | Dche | GR173, GR174 | |
| **Chydoridae**              |         |                                  |                                |
| Chydorus sphaericus         | Csph    | GR161                            | GR160, GR165, GR166, GR172     |
| Coronatella rectangula G.O. Sars, 1862 | Cret | GR161                            | GR166                          |
| **Macrothrichidae**         |         |                                  |                                |
| **COPEPODA**                |         |                                  |                                |
| Calanoida                   |         |                                  |                                |
| Diaptomidae                 |         |                                  |                                |
| Arctodiaptomus alpinus      | Aalp    | GR161, GR173, GR174              | GR165, GR166                   |
| Arctodiaptomus pectinicornis| Apec    |                                  |                                |
| Diaptomus cf. serbicus      | Dser    | GR173, GR174                     |                                |
| Pseudodiaptomidae           |         |                                  |                                |
| Calaniopoda aquaedulcis     | Caqu    |                                  |                                |
| **Cyclopoidea**             |         |                                  |                                |
| Cyclopidae                  |         |                                  |                                |
| Eucyclops serrulatus        | Eser    | GR161                            | GR166, GR171                   |
| Metacyclops minutus         | Mnin    | GR161, GR169, GR173, GR174       | GR162                          |
| Cyclops ankyrae Mann        | Cank    | GR163                            | GR165                          |
| Diacyclops lubbecki         | Dlub    | GR160                            |                                |
| Microcyclops rubellus       | Mrub    |                                  | GR160                          |
| Acanthocyclus einei Mirabdullayev & Defaye, 2004 | Aein | GR169 | GR160, GR165, GR166, GR171, GR172 |
| **Harpacticoida**           |         |                                  |                                |
| Canthocamptidae             |         |                                  |                                |
| **OSTRACODA**               |         |                                  |                                |
| Podocopa                    |         |                                  |                                |
| Eucypris virens             | Evir    | GR161                            |                                |
| Heterocypris incongruens   | Hinc    | GR161, GR167, GR169, GR173, GR174 | GR164                          |
| Potamocypris unicaudata     | Puni    | GR161                            |                                |
| Tonnacypris lutaria         | Tlut    | GR167, GR173, GR174              |                                |
| Cypria ophtalmica           | Coph    |                                  | GR166                          |
| **Ilyocyprididae**          |         |                                  |                                |
| Ilyocypris decipiens       | Idec    | GR161                            |                                |
| **Canodinae**               |         |                                  |                                |
| Cyclocypris ovum            | Covu    | GR160, GR165, GR166, GR172       |                                |
RESULTS

Details on the sampling sites and dates, and on the environmental parameters registered for each sampling, are reported in Appendix 1.

Overall, based on the study of both field-collected and laboratory-reared samples, 13 branchiopod, 11 copepod and 7 ostracod taxa were observed in the 15 sampled water bodies (Table 2). Oddly, no crustaceans were collected in Limni Alykaina (GR168) and in the temporary salty pond at the mouth of the Spercheios river (GR170), nor they were obtained through the culturing of the sediment.

All the collected specimens were identified at the species level; however, the diaptomid copepods belonging to the genus Diaptomus collected in November 2017 in Mourouza (GR173) and Mourouzos (GR174) displayed a peculiar combination of morphocharacters that deserves a more detailed analysis. Accordingly, this taxon is here provisionally reported as Diaptomus cf. serbicus.

The most frequently occurring crustacean species within the study area were the anostracan Chirocephalus diaphanus s.l., the ostracod Heterocypris incongruens, the
cyclopoid *Acanthocyclops einslei* and the cladoceran *Moina brachiata*, recorded in six sites each. Conversely, in the frame of the present survey, several crustacean species were observed in single water bodies, thus showing a very restricted distribution in the study area.

The mtDNA sequences of the analysed *Chirocephalus diaphanus* s.l. specimens place them in a well-characterized clade that is phylogenetically close to the other Balkan populations for which molecular data are available (Fig. 2). The analysis of the mtDNA sequence of the notostracan specimen raised from sediment collected in the Anthili rice fields assigned it to a widespread lineage of *Triops cancrriformis* sensu stricto (Fig. 3).

The crustacean assemblage occurring in the Mediterranean temporary ponds (3170*) was significantly different from those occurring in the other temporary ponds and in the few sampled permanent water bodies (ANOSIM analysis, Global R=0.853, P<0.01). This is clearly displayed in the nMDS plot, which showed four separate clusters (Fig. 4a) corresponding to the four different habitat types examined. The anostracan *Chirocephalus diaphanus* s.l., together with the cladocerans *Daphnia atkinsoni* and *D. chevreuxi*, the calanoids *Arctodiaptomus alpinus* and *Diaptomus* cf. *serbicust*, the cyclopoid *Metacyclops minutus*, and the ostracods *Heterocypris incongruens* and *Tonnacypris lutaria* were preferentially found in the...
Mediterranean temporary ponds (Fig. 4b); *Triops cancriformis*, together with the spinicaudatan *Leptestheria dahalacensis* and the cladocerans *Moina macrocopa* and *Wlassicsia pannonica*, characterized the rice fields. The other species were found preferentially in other types of temporary and permanent ponds (see Fig. 4b), while the pseudodiaptomid calanoid *Calanipeda aquaedulcis* characterized the brackish water pond.

**DISCUSSION**

Notes on the collected species

1. Branchiopoda

Overall, three large branchiopod species were collected in the frame of the present survey (Table 2). *Triops cancriformis* and *Leptestheria dahalacensis*, both collected in the Anthili rice fields, are widespread Palearctic taxa, often linked with rice fields throughout the western Palearctic. In Greece, the presence of *Triops cancriformis* was already reported for rice fields in the Axios delta (Kazantzidis and Goutner 2005), and some specimens collected in Athens are conserved in the collections of the Natural History Museum “La Specola” of the University of Florence, Italy (Innocenti 2009). The spinicaudatan *Leptestheria dahalacensis* is new for the fauna of the country and, to our knowledge, this represents the first record of the whole order Spinicaudata for Greece. Both taxa are possibly allochthonous in the study area, and the resting eggs of these species could have been unwarily introduced in the Anthili rice fields, along with rice seeds or seedlings. However, lacking detailed information on the checklist and distribution of Greek large branchiopods (see the discussion in Abatzopoulos et al. 1999), their native status cannot at present be excluded.

The molecular analysis of six *Chirocephalus diaphanus* specimens from the study sites showed the existence of a well-characterised clade nested within the “easternmost clade” of *C. diaphanus* s.l., which should, in fact, be possibly ascribed to *C. carinatus* Dayad, 1910 or *Chirocephalus romanicus* Stoicescu, 1992 (see discussion in Marrone et al. 2019) (Fig. 2). Interestingly, *C. diaphanus* s.l. was observed in all the surveyed 3170* ponds (with the obvious exception of Limni Alikaina, GR168), whilst proved to be absent in all the other surveyed water bodies with the single exception of GR162, i.e. a shallow temporary pool located a few metres apart from Limni Nevropolis. *Chirocephalus diaphanus* s.l. is a rare taxon in Greece, where
it has been reported for Makedonia (Daday 1910), Voioitia (Pesta 1921), Zakynthos (Stephanides 1948), and Kriti (Marrone et al. 2019).

Among the ten collected anomopod species, the macrothrichid *Wlassicsia pannonica* is a new record for the fauna of Greece. It was collected in the Anthili rice fields (GR164), where it was possibly introduced along with the large branchiopods *Triops cancriformis* and *Leptestheria dahalacensis*. The other recorded anomopod species are widespread Palearctic taxa already known to occur in the country.

2. **Copepoda**

The collected harpacticoid and cyclopoid copepod species are rather widespread in the Mediterranean area; conversely, all the collected diaptomid species are of biogeographical interest. In particular, *Arctodiaptomus alpinus* is a Palearctic species, typical of high-elevation ponds (Błędzki and Rybak 2016, Marrone et al. 2017) whose presence in mainland Greece was to date unknown (see discussion in Marrone et al. 2019). *Arctodiaptomus pectinicornis* is an eastern European species reported in Greece only in two lakes close to the North Macedonia border (Kiefer 1978, Zarfdjian and Economidis 1989). To date, no information on the phenology and distribution of *Diaptomus* cf. *serbicicus* collected in November 2017 in Limni Mourouza (GR173) and Limni Mourouzos (GR174) are available. This taxon is morphologically close to *Diaptomus serbicicus*, a species reported from the Balkans to Italy (Kiefer 1978, Marrone et al. 2017), which in Greece is reported to occur only in the island of Kerkyra (Stephanides 1948, Zarfdjian and Economidis 1989).

3. **Ostracoda**

Among the collected ostracods, the cypridid *Potamocypris unicaudata* is new for the fauna of Greece. *Heterocypris incongruens* and *Tonnacypris lutaria*, two species typical of temporary ponds, dominated the ostracod assemblages of the 3170* sites only; conversely, the more euryecious *Cyclocypris ovum* was found only in the “non-3170*” ponds.

Notes on the assemblages

The collected crustaceans allowed us to determine four different assemblages, each one linked to a different habitat type (Figs. 3 and 4d). It is not surprising that the faunas occurring in the Anthili rice fields (GR164) and in the fish-inhabited brackish-water reservoir located at the mouth of the Spercheios River (GR171) are well-characterized and distinct from one another, and from those inhabiting the studied temporary ponds. Conversely, the sharp segregation of the crustacean assemblages occurring in the 3170* temporary ponds from those occurring in neighbouring, “non-3170*” temporary water bodies, was not obvious. Such a segregation has to be ascribed to the dominant presence of taxa linked to strictly temporary water bodies in the “3170* ponds” (i.e. *Chirocephalus diaphanus* s.l., *Daphnia atkinsoni*, *D. chevreuxi*, *Arctodiaptomus alpinus*, *Diaptomus* cf. *serbicicus*, *Metacyclops minutus*, *Tonnacypris lutaria*), which are replaced by more euryecious taxa in the “non-3170*” sampled ponds (e.g., *Chydorus sphaericus*, *Arctodiaptomus pectinicornis*, *Acanthocyclops einslei*, *Cyclocypris ovum*). Among the 3170* ponds, Limni Nevropolis showed a mixed fauna, including both temporary pond-dwellers (e.g., *Chirocephalus diaphanus* s.l.) and more euryecious taxa (e.g., *Simocephalus vetulus*, *Chydorus sphaericus*). This is due to the astatic nature of Limni Nevropolis, which is fed both by rainfall surface run-off and by a spring.

CONCLUSIONS

The data collected so far from the two Sites of Community Interest (SIC) present in the Fthiotida area suggest the occurrence of
different crustacean assemblages in “3170*” vs. “non-3170*” temporary ponds. This finding stresses the peculiarity of the biota of Mediterranean temporary ponds as defined by the EU “Habitats Directive” and, at the same time, highlights that an integrative analysis of different biological communities, using datasets from different taxonomic groups, is desirable to ensure effective choices for the long-term conservation of the whole biological diversity of a given area (Bagella et al. 2011, Guareschi et al. 2015). Accordingly, precautionary management efforts should be carried out in order to preserve these important water bodies. The current focus on the priority habitat “Mediterranean Temporary Ponds” grants an “umbrella protection effect” to their peculiar crustacean assemblages, whereas those species recorded only (or mostly) in the other temporary ponds should be the object of different, albeit less stringent, management measures. As highlighted by the high incidence of species recorded from single sites, the currently available data probably do not allow getting an exhaustive picture of the diversity of the crustacean fauna in the study area, and thus, there is a time-sensitive need for other surveys for a deeper understanding of these fragile ecosystems.

ACKNOWLEDGEMENTS

P. Delipetrou (National and Kapodistrian University, Athens) and I. Dimitriadis (National and Kapodistrian University, Athens) are warmly acknowledged for the support they provided in the planning and carrying out of the sampling activities as well as Anna N. Chapman (National and Kapodistrian University, Athens) for helping out during the sampling and curation of the specimens. V. Pieri (Piacenza, Italy) kindly identified the ostracods.

REFERENCES

Abatzopoulos, T.J., Brendonck, L. & Sorgeloos, P. (1999) First record of Branchinella spinosa (Milne-Edwards) (Crustacea: Branchiopoda: Anostraca) from Greece. International Journal of Salt Lake Research, 8, 351-360. DOI: 10.1007/BF02442120

Alfonso, G., Beccarisi, L., Pieri, V., Frassanito, A. & Belmonte, G. (2016) Using crustaceans to identify different pond types. A case study from the Alta Murgia National Park, Apulia (Southeastern Italy). Hydrobiologia, 782, 53-69. DOI: 10.1007/s10750-016-2669-y

Alonso, M., 1996. Crustacea, Branchiopoda. In: (M.A. Ramos et al. eds.) Fauna Iberica, vol. 7. Museo Nacional de Ciencias Naturales. CSIC, Madrid.

Bagella, S., Gascón, S., Caria, M.C., Sala, J. & Boix, D. (2011) Cross-taxon congruence in Mediterranean temporary wetlands: vascular plants, crustaceans, and coleopterans. Community Ecology, 12, 40-50. DOI: 10.1556/ComEc.12.2011.1.6

Bledzki, L.A. & Rybak, J.I. (2016). Freshwater crustacean zooplankton of Europe. Springer Nature, Switzerland. DOI: 10.1007/978-3-319-29871-9

Blondel, J., Aronson, J., Bodiou, J.Y. & Boeuf, G. (2010) The Mediterranean Region: biodiversity in space and time. 2nd edition. Oxford University Press, 392 pp.

Cottarelli, V. & Mura, G. (1983) Anostraci, Notostraci, Conocostraci. Guide per il riconoscimento delle specie animali delle acque interne italiane. Consiglio Nazionale delle Ricerche, 18, 73 pp.

Daday, E. (1910) Quelques phyllopodes anostracés nouveaux. Appendice a la monographie systématique des Phyllopodes Anostracés. Annales des sciences naturelles. Zoologie, 12, 241-264.

Delipetrou, P., Dimitriadis, I., Zikos, A., Sarika, M. & Georgiou, K. (2015a) Base study and interannual variation of temporary pond (3170*) communities in Mt. Oiti and Mt. Kallidromo. Part A: Flora and Vegetation. Deliverable A.3.1.a for the project LIFE11 NAT/GR/2014 -
ForOpenForests. National and Kapodistrian University of Athens - HSPN, 67 p.

Delipetrou, P., Dimitriadis, I., Koutsovoulou, K., Thanos, C. & Georgiou, K. (2015b) Population dynamics of Veronica oetaea. Deliverable A.4.1. for the project LIFE11 NAT/GR/2014 - ForOpenForests. National and Kapodistrian University of Athens - HSPN, 20 p.

Dussart, B. (1967) Les copépodes des eaux continentales d’Europe Occidentale. I. Calanoïdes et Harpacticoides. Boubée et Cie, Paris.

Dussart, B. (1967) Les copépodes des eaux continentales d’Europe Occidentale. II. Cyclopoïdes et biologie. Boubée et Cie, Paris.

Flössner, D. (2000) Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys Publisher, Leiden, 428 pp.

Griffiths, H.I., Kryštufek, B. & Reed, J.M. (2004) Balkan Biodiversity. Pattern and Process in the European Hotspot. Kluwer Academic Publisher.

Guareschi, S., Abellán, P., Laini, A., Green, J.C., Sanchez-Zapata, J.A., Velasco, J. & Millán, A. (2015) Cross-taxon congruence in wetlands: assessing the value of waterbirds as surrogates of macroinvertebrate biodiversity in Mediterranean Ramsar sites. Ecological Indicators, 49, 204-215. DOI: 10.1016/j.ecolind.2014.10.012

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology, 52, 696-704. DOI: 10.1080/10635150390235520

Incagnone, G., Marrone, F., Barone, R., Robba, L. & Naselli-Flores, L. (2015) How do freshwater organisms cross the “dry ocean”? A review on passive dispersal and colonization processes with a special focus on temporary ponds. Hydrobiologia, 750, 103-123. DOI: 10.1007/s10750-014-2110-3

Innocenti, G. (2009) Collections of the Natural History Museum, Zoological section “La Specola” of the University of Florence XXVII. Crustacea, Classes Branchiopoda, Ostracoda and Maxillopoda, Subclasses Branchiura and Copepoda. Atti della Società Toscana di Scienze Naturali - Memorie serie B, 116, 51-59.

Legakis, A. (2015) Base study and interannual variation of temporary pond (3170*) communities in Mt. Oiti and Mt. Kallidromo. Part A: Flora and Vegetation. Deliverable A.3.1.a for the project LIFE11 NAT/GR/2014 - ForOpenForests. National and Kapodistrian University of Athens - HSPN, 13 pp.

Karetsos, G.K. (2002) Study of the flora and vegetation of Mount Oiti. Ph.D. Thesis, University of Patras, Department of Biology, Section of Plant Biology, Laboratory of Plant Ecology, Patras, 311 pp.

Karetsos, G., Solomou, A.D., Trigas, P. & Tsagari, K. (2018) The vascular flora of Mt. Oiti National Park and the surrounding area in Greece. Journal of Forest Science, 64(10), 435-454. DOI: 10.17221/65/2018-JFS

Kazantzidis, S. & Goutner, V. (2005) The diet of nestlings of three Ardeidae species (Aves, Ciconiiformes) in the Axios Delta, Greece. Belgian Journal of Zoology, 135: 165-170.

Kiefer F. (1978) Das Zooplankton der Binnengewässer. Freilebende Copepoda. Die Binnengewässer, Band 26 Teil 2. E. Schweizerbart’sche Verlagbuchhandlung, Stuttgart.

Korn, M., Marrone, F., Perez-Bote, J.L., Machado, M., Da Fonseca, L.C. & Hundsdoerfer, A. (2006) Sister species within the Triops cancriciformis lineage (Crustacea, Notostraca). Zoologica Scripta, 35, 301-322. DOI: 10.1111/j.1463-6409.2006.00230.x

Marrone, F. (2006) The microcrustacean fauna of Sicily and the Central Mediterranean Sea area - current knowledge and gaps to be filled. Polish Journal of Ecology, 54 (4), 681-685.

Marrone, F., Havenstein, K., Tiedemann, R. & Ketmaier, V. (2016) Identification and characterization of five polymorphic microsatellite loci in the freshwater copepod Hemidiaptomus gurneyi (Copepoda: Calanoida: Diaptomidae). Italian Journal of Zoology, 83, 146-150. DOI: 10.1080/11250003.2015.1126363

Marrone, F., Alfonso, G., Naselli-Flores, L. & Stoch, F. (2017) Diversity patterns and biogeography of Diaptomidae (Copepoda, Calanoida) in the Western Palearctic.
Marrone, F., Alfonso, G., Stoch, F., Pieri, V., Alonso, M., Dretakis, M. & Naselli-Flores, L. (2019) An account on the non-malacostracan crustacean fauna from the inland waters of Crete, Greece, with the synonymization of *Arctodiaptomus piliger* Brehm, 1955 with *Arctodiaptomus alpinus* (Imhof, 1885) (Copepoda: Calanoida). Limnetica, 38, 1-21. DOI: 10.23818/limn.38.01

Meisch, C. (2000) Freshwater Ostracoda of Western and Central Europe. Spektrum Akademischer Verlag, Heidelberg, Berlin.

Nylander, J.A.A. (2004) MrModeltest 2. Program Distributed by the Author. Evolutionary Biology Centre, Uppsala University

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., & Wagner, H. (2015) Vegan: Community Ecology Package. R package version 2.2-1. Available at: http://CRAN.R-project.org/package=vegan

Pesta, O. (1921) Kritische Revision der Branchipodidensammlung des Wiener naturhistorischen Staatsmuseums. Annalen des Naturhistorischen Museums in Wien, 34, 80-98.

R Development Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/

Ronquist, F., Teslenko, M., Van der Marl, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes v. 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61, 539–542. DOI: 10.1093/sysbio/sys029

Sahuquillo, M. & Miracle, M.R. (2013) The role of historic and climatic factors in the distribution of crustacean communities in Iberian Mediterranean ponds. Freshwater Biology, 58, 1251-1266. DOI: 10.1111/fwb.12124

Stephanides, T. (1948) A survey of the freshwater biology of Corfu and of certain other regions of Greece. Praktika of the Hellenic Hydrobiological Institute, 2, 1–263.

Tziortzis, I., Zogaris, S., Papatheodoulou, A. & Marrone, F. (2014) First record of the Tadpole Shrimp *Triops cancriformis* (Branchiopoda, Notostraca) in Cyprus. Limnetica, 33, 341-348. DOI: 10.23818/limn.33.26

Van Damme, K. & Dumont, H.J. (2010) Cladocera of the Lençóis Maranhenses (NE-Brazil): faunal composition and a reappraisal of Sars’ Method. Brazilian Journal of Biology, 70, 755-779.

Williams, D.D. (2005) The Biology of temporary waters. Oxford University Press. DOI: 10.1093/acprof:oso/9780198528128.001.0001

Zarfdjian, M.H. & Economidis, P.S. (1989) Listes provisoires des rotifers, cladocéres et copepods des eaux continentales grecques. Biologia Gallo-gellennica, 15, 129-146.

Zogaris, S., Economou, A.N. & Dimopoulos, P. (2009) Ecoregions in the Southern Balkans: should their boundaries be revised? Environmental Management, 43, 682-697. DOI: 10.1007/s00267-008-9243-y

Submitted: 27 May 2019

First decision: 4 July 2019

Accepted: 26 July 2019

Edited by Nico Cellinese
Appendix 1. Environmental variables registered in the studied water bodies. Temp: water temperature (°C); Cond: electrical conductivity (µS/cm); Turb: water turbidity; Macr: presence of aquatic macrophytes (see text for details).

| Sites                              | Habitat | Code  | Date       | Temp | Cond | Turb | Macr |
|------------------------------------|---------|-------|------------|------|------|------|------|
| Pond 1 Anopia                      | NO      | GR160 | 28/05/2017 | 14.4 | 460  | 1    | 2.5  |
| Limni Nevropolis                   | YES     | GR161 | 05/05/2017 | nd   | nd   | nd   | nd   |
| Limni Nevropolis                   | YES     | GR161 | 28/05/2017 | 14.8 | 260  | 2    | 2    |
| Nevropolis pool                    | NO      | GR162 | 28/05/2017 | 12   | 140  | 3    | 1    |
| Limni Louka                        | YES     | GR163 | 05/05/2017 | nd   | nd   | nd   | nd   |
| Limni Louka                        | YES     | GR163 | 29/05/2017 | 15   | 68   | 1    | 1.5  |
| Anthili rice fields                | NO      | GR164 | 29/05/2017 | nd   | nd   | 3    | 1    |
| Pond 1 Katavothra                  | NO      | GR165 | 30/05/2017 | 12   | 84   | 1.5  | 2    |
| Artificial pond on the path to Livadies | NO | GR166 | 30/05/2017 | 10   | 39   | 1    | 2    |
| Limni Greveno                      | YES     | GR167 | 19/05/2017 | nd   | nd   | nd   | nd   |
| Limni Greveno                      | YES     | GR167 | 30/05/2017 | 19   | 37   | 1    | 1.5  |
| Limni Alikaina                    | YES     | GR168 | 19/05/2017 | nd   | nd   | nd   | nd   |
| Limni Alikaina                    | YES     | GR168 | 30/05/2017 | 22   | 24   | 1    | 1    |
| Limni Livadies                     | YES     | GR169 | 19/05/2017 | nd   | nd   | nd   | nd   |
| Limni Livadies                     | YES     | GR169 | 30/05/2017 | 23   | 18   | 1    | 2    |
| Limni Livadies                     | YES     | GR169 | 23/11/2017 | nd   | nd   | nd   | nd   |
| Pond at the mouth of Spercheios river | NO | GR170 | 31/05/2017 | nd   | nd   | 1    | 1    |
| Reservoir at the mouth of the Spercheios river | NO | GR171 | 31/05/2017 | 23   | 2700 | 2    | 2    |
| Pond close to the village of Kastriotissa | NO | GR172 | 31/05/2017 | 22   | 670  | 1    | 1.5  |
| Mourouza                           | YES     | GR173 | 23/11/2017 | nd   | nd   | nd   | nd   |
| Mourouzos                          | YES     | GR174 | 23/11/2017 | nd   | nd   | nd   | nd   |
| Mourouzos                          | YES     | GR174 | lab culture| nd   | nd   | nd   | nd   |