Experimental hatching of resting eggs of large Branchiopoda from temporary ponds in the steppe Biosphere Reserve "Askania Nova" (Ukraine)

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With 3 figures

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In the most famous Great Chapli Depression of the Ukraine under protection within the Biosphere Reserve "Askania Nova", seven species of large Branchiopoda were recorded (Streptocephalus torvicornis, Branchipus schaefferi, Leptestheria dabalacensis, Limnadia lenticularis, Cyzicus tetracerus, Lepidurus apus, Triops cancriformis) by means of incubating and hatching resting eggs, inventorying old collections taking pictures and filming. These species of the Ukrainian fauna are rare and little studied.

1 Introduction

Branchiopoda are an archaic group of invertebrates widespread in temporary ponds. They rely on the stock of resting eggs (egg banks) that can remain viable for decades or possibly centuries without re-hydration (Brendonck et al. 2008). Such latent periods of the life cycle lead to the fact that large Branchiopoda species are the least studied group of aquatic macroinvertebrates. Localities with Branchiopoda can be repeatedly studied with traditional monitoring methods, but that can not reveal the presence at rest stage (Martin et al. 2016).

In Ukraine, the most of large Branchiopoda are only known from one or two records. At the same time, these are precisely the species, which are adapted to drought and contribute to the diversity of ephemeral freshwater and brackishwater communities in the steppe zone. At present, the most of European steppe landscapes are destroyed due to agricultural activity, aquatic ecosystems specific for this zone (temporary rivers and ponds) remain little studied. Studies of a few steppe water bodies still existing in their natural state show that they are inhabited by original faunal complexes including rare and relic species (Vinarsky et al. 2010, Kovalenko & Utevsky 2012).

However, with the exception of insects, aquatic invertebrates of the most interesting original water bodies of the steppe zone, “pods” or “steppe saucers”, have never been studied. In large depressions of flat steppe melt water accumulates from a vast area in case of a coincidence of many weather factors. Such flood waters can form huge temporary ponds in the centre of a depression, but in dry periods (usually 5-10 years) no ponds appear, except for rain puddles (Fig. 1). This distinguishes them from lowlands with a high level of groundwater, where conditions of steppe ponds are less extreme, such as Horila Dolyna, Kharkiv Region (Kovalenko & Utevsky 2012).

The most famous large steppe depression of the Ukraine is located in an undisturbed territory, Great Chapli Depression (Bolshoy Chapelskiy Pod, Velykyj Chapelskyj Pid) and is protected within the Biosphere Reserve “Askania Nova” and included to “Ramsar sites” designated as “Wetlands of International Importance”. Aquatic invertebrates of this water...
body have not been studied before. Photographs and video recordings show the presence of large Branchiopoda species during the late flooding in 2010 (Fig. 2).

Studying the biodiversity of this locality, we used experimental hatching of resting eggs of Branchiopoda from the surface layer of soil sampled in dry stage of this ecosystem.

Fig. 1: Dry (A) and submerged (B) stages of the Great Chapli Depression in 2015 and in 2010

Fig. 2: Large Branchiopoda from the Great Chapli Depression during the last flooding in 2010
A: *Triops cancriformis* (Bosc, 1803), B: *Cyzicus tetracerus* (Krynicki, 1830)
2 Methods

Was used authoring algorithm developed by A.V. Koshelev that includes:
(1) sampling of resting eggs in places of their accumulation, with surface layer of the soil;
(2) washing the selected soil sample through a set of laboratory sieves to concentrate resting stages (in the field);
(3) activation of resting eggs by means of short-term freezing in a refrigerating chamber (24 hours);
(4) exposure of resting eggs in distilled water under conditions of round-the-clock illumination at a temperature of 20-25 °C.

At these conditions, experimental hatching occurs within 1-3 days. After that, organisms are grown to stages used for identification. At the stage of sampling, large *Daphnia ephippia* visible to the naked eye can serve as indicators of an egg bank with different resting eggs (Fig. 3).

Fig. 3: Large *Daphnia ephippia* on a surface layer of the soil

We used samples from three different steppe localities: Great Chapli Depression (46°29'01; N 33°51'10 E), artificial pond (46°27'45 N; 33°51'33 E) and the dry bottom of a small steppe pool (46°27'28 N 34°06'33 E), but large Branchiopoda were obtained only from the first locality. Also we examined I. K. Polishchuk’s collections and photo and video materials made during the previous flooding of the Great Chapli Depression in 2010.

3 Results

Seven species of large Branchiopoda were collected in the Great Chapli Depression: *Streptocephalus torvicornis* (Waga, 1842), *Branchipus schaefferi* Fischer, 1834, *Leptestheria dahalacensis* (Rüppel, 1837), *Limnadia lenticularis* (Linne, 1761), *Cyzicus tetracerus* (Krynicki, 1830), *Lepidurus apus* (Linnaeus, 1758) and *Triops cancriformis* (Bosc, 1803).
Four species (S. torvicornis, B. schaefferi, L. dabhacensis, L. lenticularis) were obtained using experimental hatching. Cyzicus tetracerus, L. apus and T. cancriformis were photographed and filmed in 2010 during the previous flooding of the Great Chapli Depression. Both L. apus and T. cancriformis were also repeatedly observed in diverse small pools in the surrounding area.

Additionally, C. tetracerus and L. dabhacensis were present in another nearby pond, the Small Chapli Depression (near the village of Krestovka, 15.06.1985; coll. I. K. Polishchuk). Now, this depression, which is situated outside the reserve, is totally transformed into an agricultural land.

Both species of Notostraca found, L. apus and T. cancriformis, occur sporadic but widespread in Ukraine. Unlike them, all Anostraca and Spinicaudata species are rare and threatened. Branchipus schaefferi has been known in Ukraine only from the Crimean Peninsula (Samchhya & Turbanov 2008). Another Anostraca species, S. torvicornis, is more widespread, but its occurrence in Ukraine is not properly studied. Within the last decades its presence in active stage were reliably confirmed only for one locality in eastern Ukraine (Sidorovskiy 2011), but communications by amateur scientists and experimental hatching show that it is present in many localities of the steppe zone in Ukraine (unpublished data). Cyzicus tetracerus is the only Spinicaudata species that has been repeatedly recorded in Ukraine (Shkorbatov 1950, Brtek & Thiery 1995, Sidorovsky 2014), but it has never been recorded from the steppe zone. Leptestheria lenticularis is a rare species in Eastern Europe (Brtek & Thiery 1995) and has been recorded, only for two localities in the steppe zone: temporary ponds in Odessa (Okul 1937) and an artificial fishery pond near the Dnieper Delta (Dobrynina 2010).

4 Discussion

The results show that the Great Chapli Depression is a very few known area with diverse communities of large Branchiopoda in Ukraine. As already noted, this area is well preserved in comparison with other steppe areas. On the other hand, the Biosphere Reserve “Askania Nova” is one from the largest protected natural territories important for many waterfowl species migration routes that pass along the northern shores of the Black Sea and Azov Sea (Havrylenko 2000). Large Branchiopoda inhabiting temporary ponds have developed diverse passive strategies to pass the barrier of the surrounding land such as dispersal of resting eggs by migrating birds (Rogers 2014, Incagnone et al. 2015). This locality may be favorable for large Branchiopoda both with respect to the survival of old populations and introductions of new species. Perhaps the massive use of experimental hatching for steppe sites will help to distinguish between these two possibilities.

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