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# Large branchiopods in extensive carp ponds – morphometric analysis of *Triops cancriformis* (Bosc, 1801) and *Streptocephalus torvicornis* (Waga, 1842)

by

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## Abstract

Large branchiopods is a group of poorly understood crustaceans which is highly threatened by the impact of human activities. Currently, their protection is difficult due to large deficiencies in documentation of their places of occurrence and not well researched ecology. After rediscovery of *Streptocephalus torvicornis* in Poland by Cukier in 2019, we conducted a survey to identify the species found at the Łąki Jaktorowskie Fisheries Research Station, Poland. This study consisted of observations and measurements of animals reared from cysts which had been obtained from soil samples taken at the nursery ponds in the study area. The following species were found: *Limnadia lenticularis*, *Triops cancriformis*, and *S. torvicornis*. We are presenting results of the morphometric measurements of *T. cancriformis* and the morphometric measurements of *S. torvicornis* from their only known population in Poland. Males were found in the *T. cancriformis* population, contrary to what has been documented previously. Large branchiopods have been repeatedly found in fishponds, and this work confirms the potentially high importance of ponds in the conservation of large branchiopods. With this study, we are drawing attention to the necessity of research about the distribution of large branchiopods in Poland.

**Key words:** large branchiopods; fairy shrimp; nature conservation; ephemeral ponds

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## 1. Introduction

Large branchiopods are a group of crustaceans comprising the orders Anostraca (fairy shrimp and brine shrimp), Notostraca (tadpole shrimp), Spinicaudata, Laevicaudata and Cyclestherida (clam shrimp) (Brendonck et al. 2008, Dodson et al. 2010). Their occurrence in bodies of water with unstable water conditions is possible through specific survival strategies. Production of dormant cysts during reproduction allows the population to wait out adverse conditions. This adapts them to function in, for example, vernal pools, periodically flooded areas (Ramułt 1939), ruts in dirt roads (Gołdyn et al. 2007, Łaciak&Łaciak 2015) and Arctic and Antarctic glacial lakes frozen solid to the very bottom (Hawes et al. 2008, Lindholm et al. 2016). They have different feeding strategies: from predators feeding on insects and other small arthropods (Daborn 1975, Boix et al. 2006, Waterkeyn et al. 2011) to non-selective filter feeders sifting algal and bacterial plankton from the water using legs with numerous setae (Williams 2007, Celewicz et al. 2018). Large branchiopods can provide a food source for birds (Green et al. 2005; Rogers 2014; Redón et al. 2019, 2020), amphibians (Vanschoenwinkel et al. 2008, Schalk et al. 2014), insects (Beladjal&Mertens 2009), fish (Beladjal et al. 2007) and crayfish (Pérez-Bote et al. 2005, Lovas-Kiss 2018). Being a component of trophic networks of the aquatic as well as terrestrial ecosystems, they can be important in the nutrient cycling by protecting against eutrophication of small bodies of water.

Anostraca as well as other large branchiopods are considered to be at high risk of extinction worldwide (Petrov&Petrov 1997; Eder&Hödl 2002; Brendonck et al. 2008; Gołdyn et al. 2012, 2019). Representatives of this group are included in many national red lists and as protected species (Eder&Hödl 2002, Brendonck et al. 2008, Merta et al. 2016). Populations of large branchiopods are significantly affected by human activities, especially environmental transformations causing the disappearance of small bodies of water (Brendonck et al. 2008, Zierold et al. 2009). Climate change that modifies hydrological cycles may be another threat to large branchiopods populations (Stoch&Naselli-Flores 2014, Tuytens et al. 2014, Qvenild et al. 2018). There are serious gaps in information on the occurrence, distribution, population status and habitat preferences of large branchiopods, especially those species found in temporary waters in the northern parts of Europe (Gołdyn et al. 2019). This poses a serious problem when planning the activities needed for effective conservation of the populations of large branchiopods. Many studies indicate that branchiopods can be an important component of ecosystems subjected to periodic anthropopressure (Ramułt 1939, Hempel 1963, Gołdyn et al. 2012). There are 12 species of large branchiopods recorded in Poland (Zwolski 1956, Jankowiak 2011):

Anostraca

- *Branchinecta paludosa* (Müller, 1788)
- *Branchipus schaefferi* (Fischer, 1834)
- *Chirocephalus diaphanus* (Prévost, 1803)
- *Chirocephalus josephinae* (Grube, 1853)
- *Chirocephalus shadini* (Smirnov, 1928)
- *Eubranchipus grubii* (Dybowski, 1860)
- *Streptocephalus torvicornis* (Waga, 1842)

Laevicaudata

- *Lynceus brachyurus* (Müller, 1776)

Notostraca

- *Lepidurus apus* (Linnaeus, 1758)
- *Triops cancriformis* (Bosc, 1801)

Spinicaudata

- *Cyzicus tetracerus* (Krynicki, 1830)
- *Limnadia lenticularis* (Linnaeus, 1761)

Of this list of branchiopods in Poland, strict protection is given to: *B. paludosa*, *C. diaphanus*, *Pristicephalus shadini* (This is probably a synonym for *Pristicephalus josephinae shadini* Smirnov, 1928, the valid name of which is *C. shadini* [Smirnov, 1928] [Rogers, 2013]), *S. torvicornis*. Partially protected are: *B. schaefferi*, *C. tetracercus*, *L. brachyurus* and *T. cancriformis* (Regulation, 2016). One species *B. paludosa* is listed in the Polish Red Data Book of Animals (national red list) as extinct (Kownacki 2004).

Research in Poland on this crustacean group has been conducted since the mid-19th century. In the 20th century, numerous small bodies of water, inhabited by such species as *B. schaefferi*, *T. cancriformis*, and *L. lenticularis* were observed in various parts of Poland, including Mazovia (Hempel

1963, Hempel-Zawitkowska 1965), Lesser Poland (Ramułt 1939, Zwolski 1956), and Pomerania (Ramułt 1939). The current studies mainly concerns the regions of Greater Poland (Gołdyn et al. 2007, 2012), Podlachia (Biggs et al. 2004), Lesser Poland (Łaciak&Łaciak 2015), West Pomerania (Lukić et al. 2019) and, Mazovia (Cukier 2020). The largest and most thorough survey made by Gołdyn et al. (2012) concerns Greater Poland and revealed that species such as *L. apus* and *E. grubi* were quite common, but thermophilic species are extremely endangered because populations are very scarce. The study showed that some species are now found in water pools of anthropogenic origin namely in ruts on roads within military training grounds. Previously, species such as *B. schaefferi* were found in natural floodplains, e.g., in Cracow (Ramułt 1939). However, such habitats have been destroyed by drainage, eutrophication and changes of land use (Waldon 2012). Our paper deals with other anthropogenic habitats in which large branchiopods can occur are breeding ponds, or more precisely, nursery ponds which are periodically filled for the purpose of rearing carp (*Cyprinus carpio* Linnaeus, 1758) fry.

The aim of this study is to extend the findings of Cukier (2020) by precise identification of the species and further analysis of the morphometric parameters of large branchiopods which occur in the Polish Lowlands under conditions of extensive carp aquaculture. The research was conducted at the Łąki Jaktorowskie Fisheries Research Station, where occurrence of the large branchiopods were reported (Hempel 1963, Hempel-Zawitkowska 1965, Cukier 2020). We draw the special attention to the methodological details that make it possible to discern anatomical features of diagnostic significance. We focus on morphometric parameters in the population of *T. cancriformis* and the only currently known population of *S. torvicornis* in Poland. Our study examines the efficacy of morphometric measurements in identifying and comparing populations of large branchiopods, as well as the potential impact of individual age on the validity of these measurements. To assess our findings, we compared our data to previously published studies. Additionally, the study aims to discuss the potential role of fish farms in maintaining large branchiopods populations.

## 2. Materials and methods

Preliminary observations were carried out in June 2018 on flooded nursery ponds of the Łąki Jaktorowskie Fisheries Research Station (Fig. 1) to determine the presence of large branchiopods at the site. In October 2019, soil samples were collected from each of the three nursery ponds using a systematic random sampling method. Ten subsamples, each approximately 200 ml in volume, were taken from each pond yielding three aggregate samples of approx. 4 kg – one from each pond. Soil was taken from the top layer, no deeper than 5 cm and from approximately 200 cm$^2$ area, using a small spatula. The collected material was spread out on sheets of paper and left to dry for two weeks. 0.2 kg portions of each sample were then flooded with tap water, and using flotation and filtration through a mesh of approximately 0.1 mm diameter, large branchiopods cysts were separated from the soil elements. The flotation process was carried out using water without the addition of sugar or salt. Density differences were solely relied upon to separate the dehydrated cysts from the sand and clay suspension. The flooded sediment was promptly stirred, and a sieve was employed to retain elements larger than 0.1 mm, including cysts while allowing the water with light sediment elements, to pass through. Each 200 g sample was subjected to this procedure ten times.

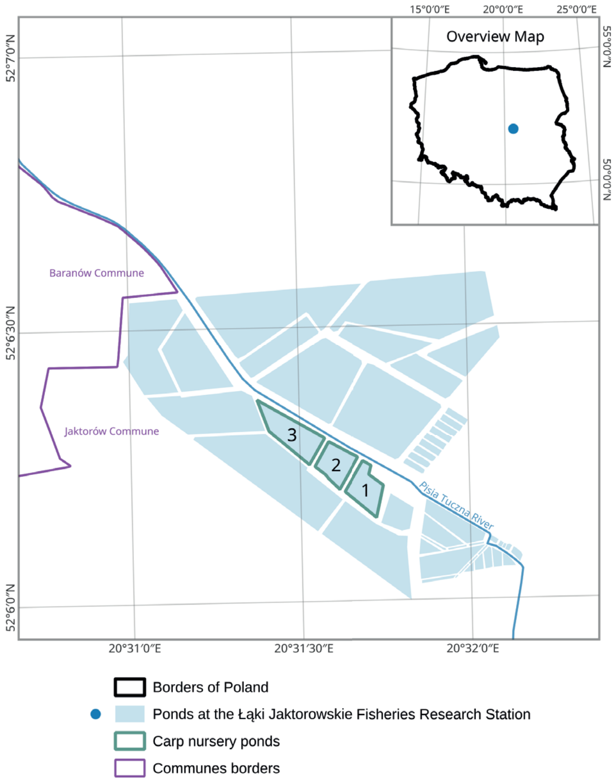

**Figure 1**

Study area location and map of fishery ponds at Łąki Jaktorowskie Fisheries Research Station

The mixture of cysts and non-separable soil elements was transferred to Petri dishes. Using a Nikon SMZ1000 light microscope (Tokyo, Japan), morphological features of the cysts were analysed to assess species affiliation (Czyż et al. 2016, Kappas et al. 2017). Cysts from samples of a certain mass were counted to estimate the number of cysts of each species per unit of soil mass.

Cysts partially separated from 0.2 kg soil, as well as 0.2 kg soil from the Łąki Jaktorowskie Fisheries Research Station, were placed into each of the three 20 l aquariums. They were flooded with tap water and distilled water in a ratio such that the electrical conductivity was close to that recorded at the ponds (550 μS cm$^{-1}$). The compacted soil fragments were crushed, and the entire contents of the tanks were mixed. The tanks were illuminated using LED lamps of 6 W each on a day : night / 12 h : 12 h cycle. Room temperature (20 - 24°C) was maintained in the aquariums. After about 24 hours, larvae hatched. The branchiopods larvae were fed with an aqueous suspension of dried spirulina (*Arthrospira platensis*). Mature fairy shrimps were fed twice a week a suspension of live *Scenedesmus* sp. algae. The algae were obtained from cultivation carried out in 20 l culture tanks using liquid medium. The dose was selected so that the animals did not show signs of starvation, that is, a digestive tract filled with food content and no or sporadic intake of food from the bottom of the aquarium. Microalgae were chosen as food because they are considered suitable food for fairy shrimp (Namin et al. 2007). In the first few days, spirulina was used as feed instead, because it appears, based on personal observation, that feeding live microalgae causes high mortality in fairy shrimp larvae. *T. cancriformis* in aquarium were fed daily by TetraPro Energy Tetra GmbH (Melle, Germany) fish food. To prevent predation, species were placed into separate aquariums.

Mature *S. torvicornis* individuals (n = 124) at 60 and 90 days post-hatching (2 age groups) were fixed in 4% paraformaldehyde solution. The sex of all individuals was determined based on the presence of the brood pouch and the structure of the second antennae (Waga 1842, Belk 1991, Kraus et al. 2004). In males (n = 55) of *S. torvicornis* the following morphometrics were measured: total length (tl), standard length (sl), basal joint length (bj), apical antennal joint length (aj), peduncle of the distal antenna outgrowth length (pd), spur length (sp) and thumb length (th) (Fig. 2). Based on the measurements, the parameters aj/pd, pd/bj, pd/sl th/sl and th/sp were calculated. This process has been based on the methodology described by Maeda-Martinez & Dumont (1995) and Dumont et al. (1991). The presence of crenation on the dorsal margin of the antennulae was verified in males and females (Fig. 7). Photographic documentation of specimens was also made using a stereo microscope with a Nikon SMZ1000 camera (Tokyo, Japan). Measurements were taken under transmitted light in a Petri dish. During measurements, individuals were immersed in water except for measurements bj, aj, pd, sp and th, when males were placed on a basal slide and gently dried with a paper towel. The aforementioned measurements had to be taken on the outstretched 2nd antennae. The positioning of the antennae was much easier after drying. The tadpole shrimps 60 days after hatching were also fixed in 4% paraformaldehyde. Sex was assessed based on the structure of the 11th pair of legs. The following morphometric measurements were made of the carapace length: (ca), eye width (ye), number of spines on the carina (spi) and

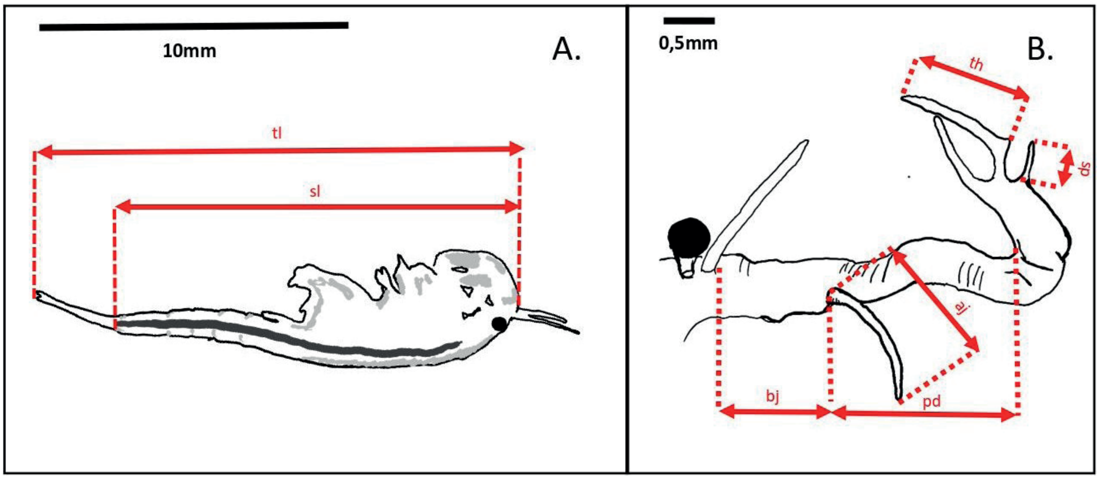

**Figure 2**

Morphometric measurements of *Streptocephalus torvicornis*

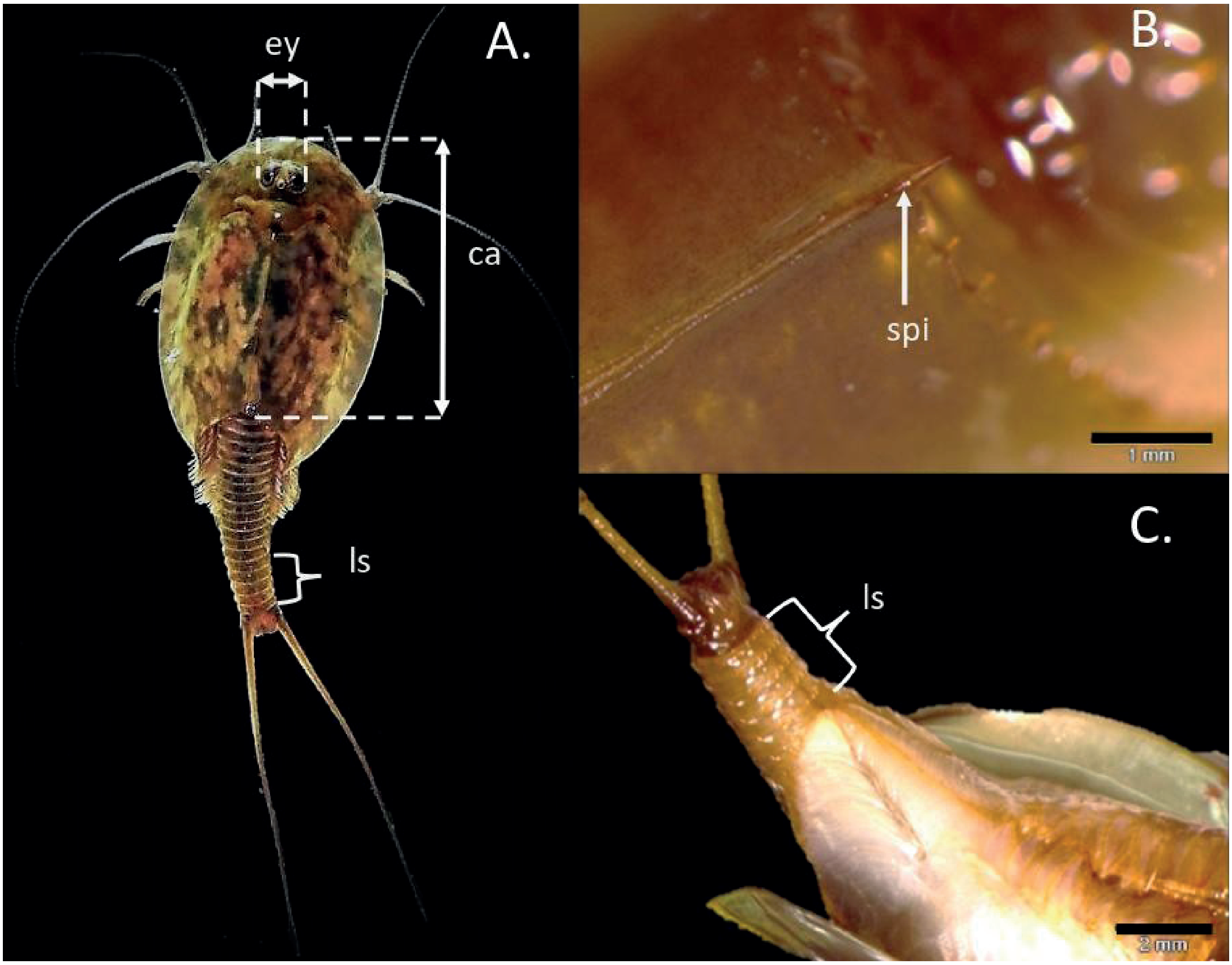

**Figure 3**

Morphometric measurements of *Triops cancriformis*

number of legless segments (ls) (Fig. 3). Measurements were taken using Pixelink Capture SE software.

Results were statistically analysed using R (R Core Team, 2022). Conformity to a normal distribution was tested using the Shapiro-Wilk test. Equality of means for the variables aj/pd, pd/bj, pd/sl, th/sl and th/sp were compared between age groups using the *t*-test. The result of Levene's test of homogeneity of variance was included. It should also be noted that pd/bj was the only parameter that did not have a normal distribution ($p = 0.03$ for the 60-days sample, $p < 0.001$ for the 90-days sample), and the hypothesis of equality of means was tested using the Wilcoxon test.

The significance of the sex difference for morphometric parameters was tested in *T. cancriformis*. Due to the unequal group sizes, the Wilcoxon test was used. For a clear visualisation of the results, box plots were made for morphometric parameters in fairy shrimp and tadpole shrimp.

## 3. Results

Two species of large branchiopods, *T. cancriformis* and *S. torvicornis,* were identified based on the ornamentation analysis of the cysts isolated from the soil. In addition, 2 specimens of *L. lenticularis* were found during the preliminary observations. The average number of *T. cancriformis* cysts from all ponds was 355 cysts kg$^{-1}$ and *S. torvicornis* 1319.67 cysts kg$^{-1}$. The total numbers of cysts in each pond are shown in Table 1.

Under aquarium conditions, 64 females and 60 *S. torvicornis* males, and 19 females or hermaphrodites and 9 *T. cancriformis* males, were successfully kept to sexual maturity.

Based on the analysis of diagnostic characters described in Maeda-Martinez & Dumont (1995), the affiliation of individuals of the order Anostraca to the species *S. torvicornis* was confirmed. Crenation was

**Table 1**

*Streptocephalus torvicornis* and *Triops cancriformis* total count of cysts per 1 kg for nursery ponds in Łąki Jaktorowskie Fisheries Research Station.

| Species | Pond I | Pond II | Pond III |
|---|---|---|---|
| *Triops cancriformis* | 331 | 365 | 369 |
| *Streptocephalus torvicornis* | 1226 | 1309 | 1421 |

observed on the 2nd antennae only in males as shown in Figure 7. The values of morphometric parameters are shown in Table 2 and in the Figure 8, along with the *p*-value for the *t*-test of comparison between age groups. Maeda-Martinez & Dumont (1995) and Dumont et al. (1991) validated selected parameters by assessing the relationship with body length. In Figure 4, we presented the results of comparing our results with body length. The only parameter whose variability can be significantly explained by body length is th/sl.

A comparison of the values of the th/sp parameter for the two age groups with the values calculated from the data made available by Kappas et al. (2017) is shown in Figure 6. A large scatter for the Jaktorów study sample in comparison with other locations is likewise shown in Figure 6. In the same graph, we have colour-coded the ranges that were used to delineate the division between subspecies according to Dumont et al. (1991). According to this criterion, the majority of individuals from many locations fall into the intermediate zone.

In addition, we observed the occurrence of denticulation on the thumb, extending above the thumb major inflexion and protuberances on the peduncle of distal outgrowth in all males studied (Fig. 5).

The results of morphometric parameters for *T. cancriformis* are shown in Figure 9 and in the Table 3, along with *p*-values for the Wilcoxon test for sex comparisons. As show in this figure, females presented longer carapaces and wider eye plates, and the ratio of carapace length to eyes was also greater in females. However, no clear difference could be observed in terms of the number of legless segments.

**Table 2**

Results of morphometrics measurements by age group. tl – total length, aj – apical antennal joint length, pd – peduncle of the distal antenna outgrowth length, bj – basal joint length, th – thumb length, Avg. – average, N – number of measured specimens, SD – standard deviation, test – applied test, *p*-value – *p*-value for applied test.

|  | Age [days] | tl [mm] | aj/pd | pd/bj | pd/sl | th/sl | th/sp |
|---|---|---|---|---|---|---|---|
| Avg. | 60 | 15.6 | 0.615 | 1.55 | 0.208 | 0.133 | 2.99 |
|  | 90 | 18.2 | 0.681 | 1.58 | 0.188 | 0.114 | 2.73 |
| SD | 60 | 0.866 | 0.074 | 0.316 | 0.019 | 0.014 | 0.513 |
|  | 90 | 0.694 | 0.075 | 0.721 | 0.017 | 0.009 | 0.288 |
| N | 60 | 30 | 25 | 26 | 26 | 25 | 25 |
|  | 90 | 25 | 24 | 24 | 24 | 25 | 25 |
| test | – |  | *t*-test | Wilcoxon | *t*-test | *t*-test | *t*-test |
| *p*-value | – |  | 0.003 | 0.330 | < 0.001 | < 0.001 | 0.033 |

**Table 3**

*Triops cancriformis* morphometrics. ca – carapace length, ey – eye width, Avg. – average, N – number of measured specimens, SD – standard deviation, p-value – p-value for Wilcoxon test. Sex values: m – male, f/h – female/hermaphrodite.

|  | sex | ca [mm] | ey [mm] | ey/ca | ls | spi |
|---|---|---|---|---|---|---|
| Avg. | f/h | 15.4 | 2.24 | 0.147 | 4.92 | 1 |
|  | m | 13.4 | 2.08 | 0.155 | 5.39 | 1 |
| SD | f/h | 1.61 | 0.155 | 0.010 | 0.534 | 0 |
|  | m | 1.49 | 0.193 | 0.007 | 0.601 | 0 |
| N | f/h | 19 | 19 | 19 | 19 | 19 |
|  | m | 8 | 8 | 8 | 9 | 9 |
| *p*-value |  | 0.014 | 0.075 | 0.016 | – | – |

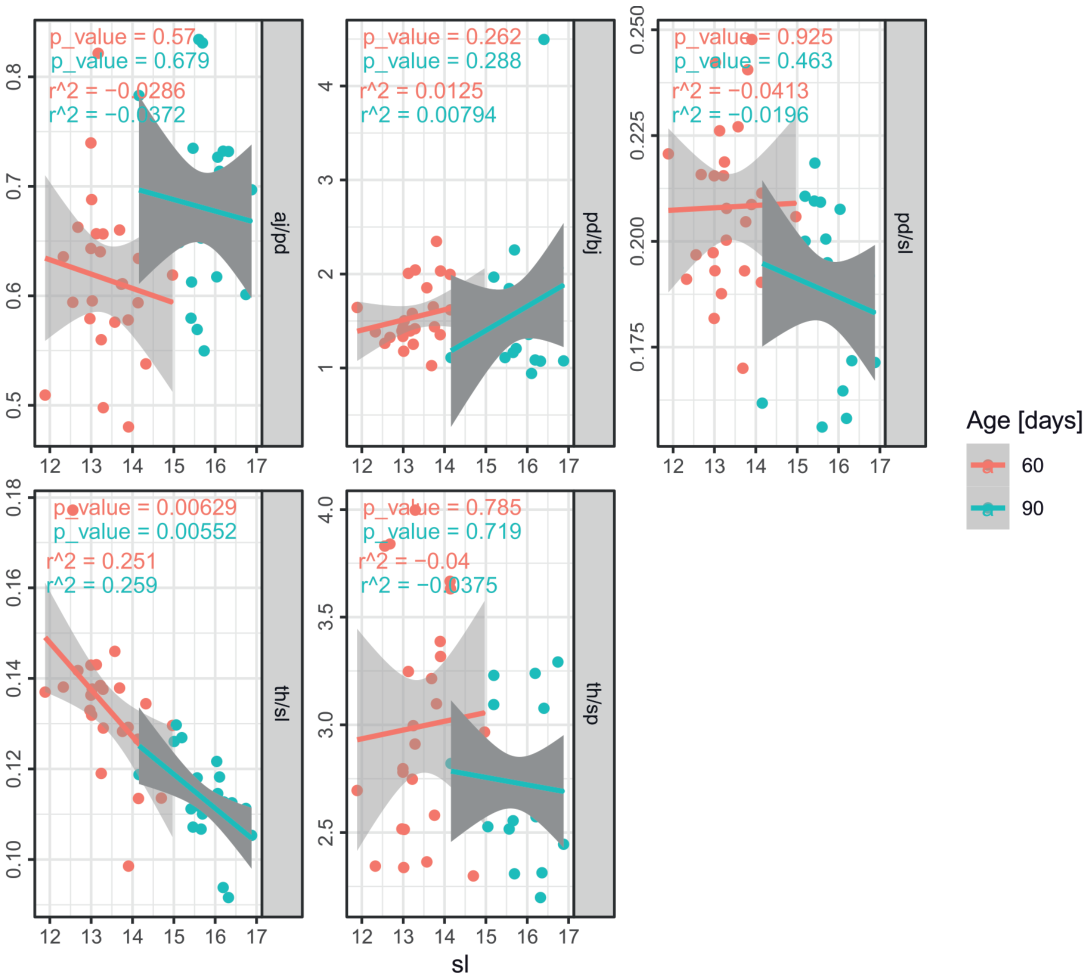

**Figure 4**

Relationship between standard length and parameters: aj/pd, pd/bj, pd/sl, th/sl, th/sp in two age groups of *Streptocephalus torvicornis*.

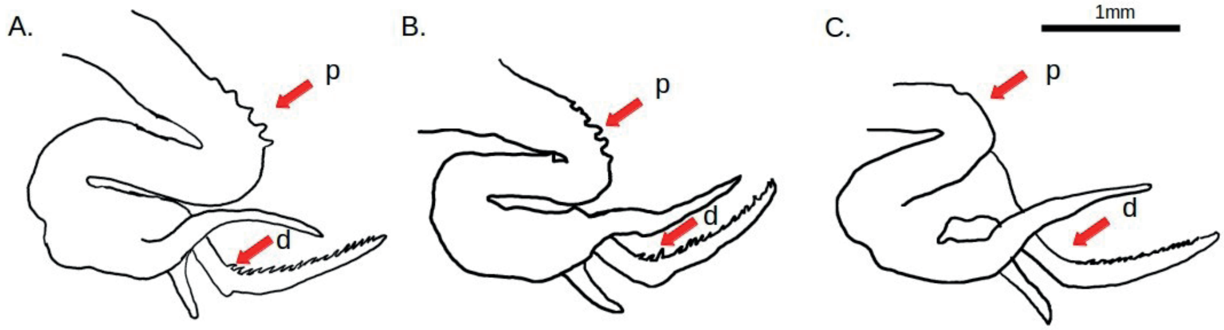

**Figure 5**

Male second antennae of *Streptocephalus torvicornis* in different populations. Differentiating features: denticulation on thumb (d), peduncle distal outgrowth (p). A. Jaktorów (Poland) B. Malý Horeš (Slovakia) (Dumont et al. 1995), C. Kutab (Yemen) (Dumont et al. 1995). Differences: denticulation on thumb (d) – A and B – extending above thumb major inflexion, C – extending below thumb major inflexion; peduncle distal outgrowth (p) – A and B – protuberances, C – smooth.

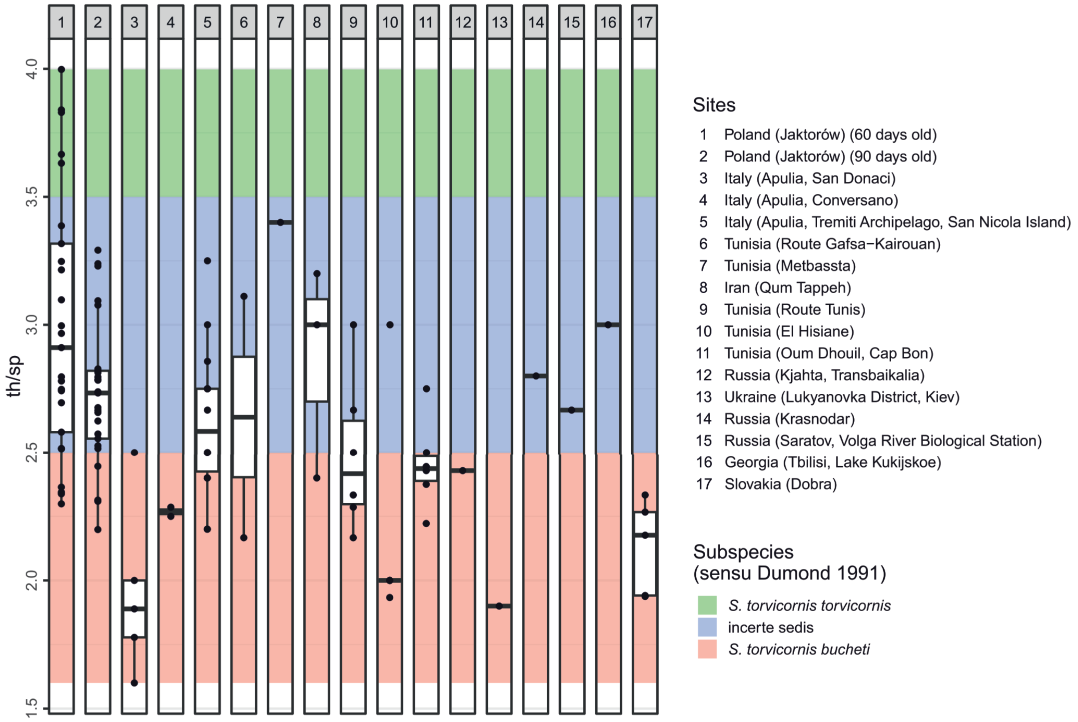

**Figure 6**

Boxplot of th/sp parameter of *Streptocephalus torvicornis* populations from Jaktorów (two age groups, this publication) and other from data by Kppas et al. (2017).

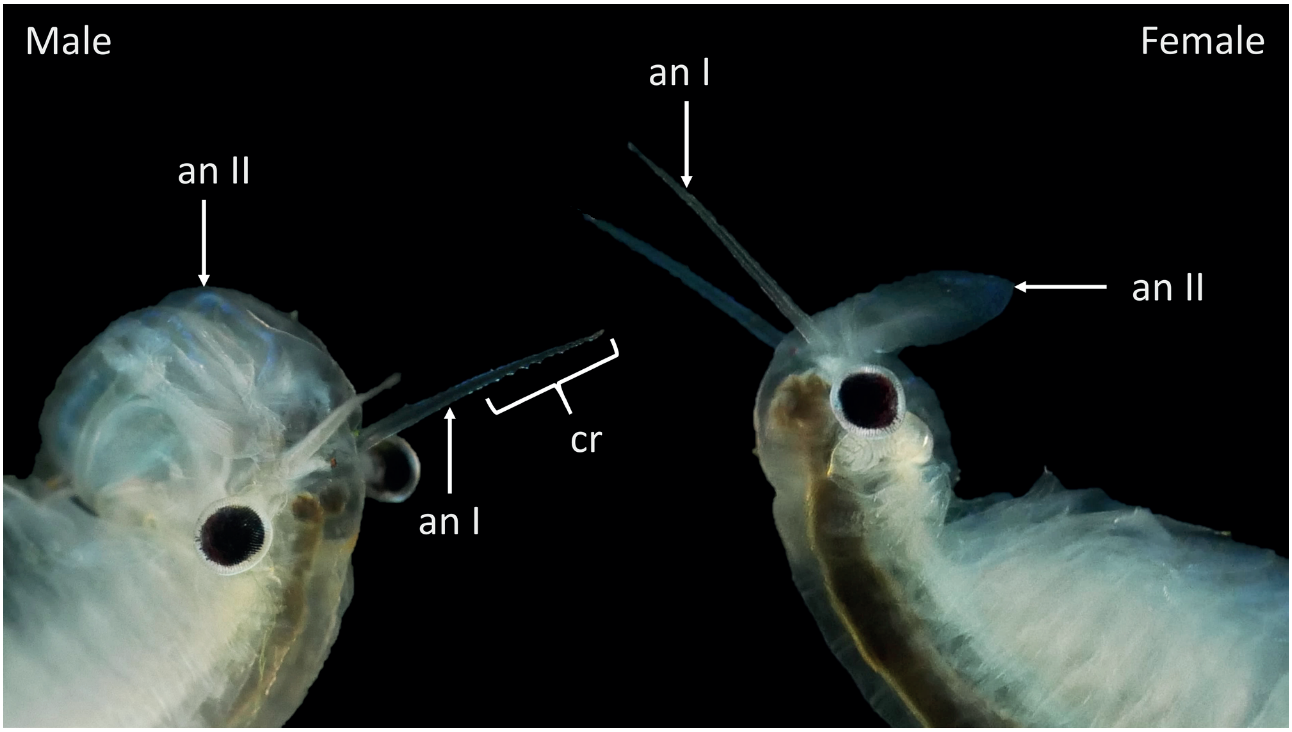

**Figure 7**

Photo showing the first antennae in both sexes in *Streptocephalus torvicornis*: **an I** – first antennae, **an II** – second antennae, **cr** – crenation. The female has a smooth edge of the first antennae (there is no crenation).

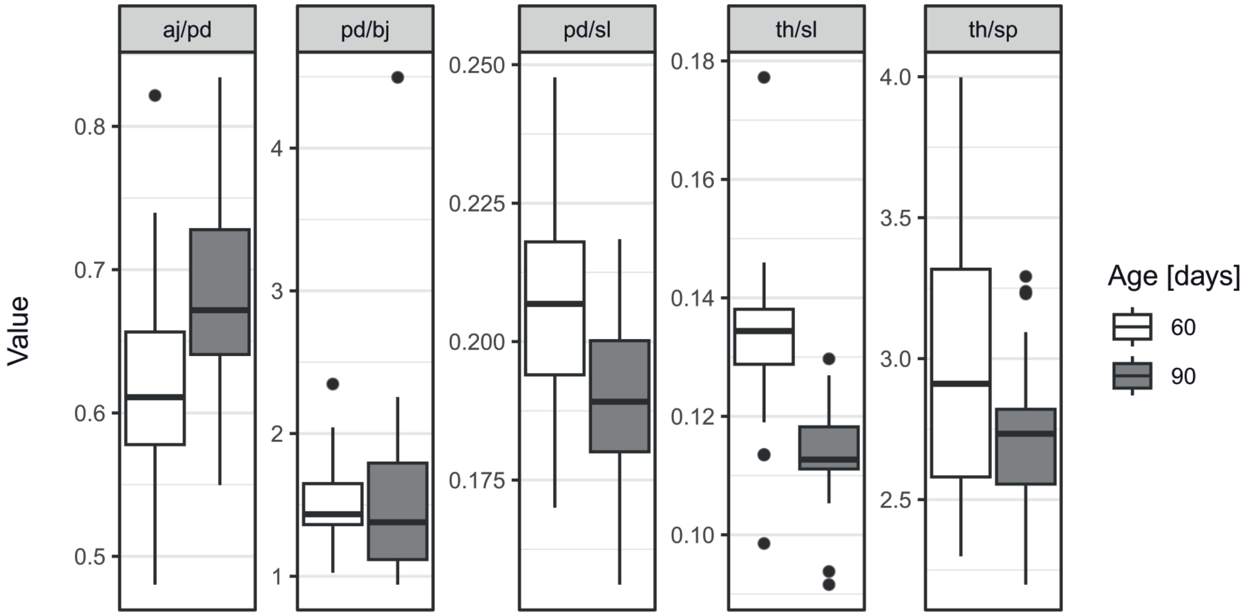

**Figure 8**

Boxplot of *Streptocephalus torvicornis* morphometrics parameters in different age.

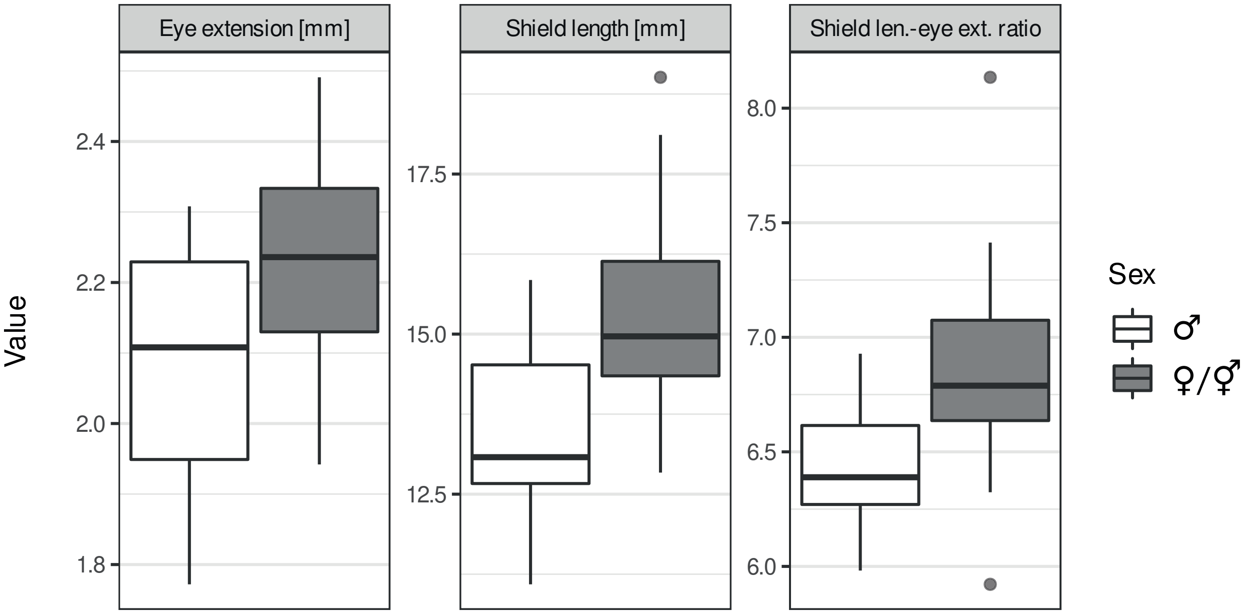

**Figure 9**

Boxplot of *Triops cancriformis* morphometrics parameters for each sex.

## 3. Discussion

Previous studies on branchiopods populations in the Łąki Jaktorowskie Fisheries Research Station were conducted in the 1960s and 1970s (Hempel 1962, 1963; Hempel-Zawitkowska 1965, 1972). These confirmed the occurrence of *T. cancriformis* and *L. lenticularis*. Populations of both species were identified as consisting of females only. However, results obtained in the present study indicate the presence of males in the population of *T. cancriformis*. The percentage of males in the sample was found to be 32.14%, which is higher than expected for the androdioecious population (Zierold et al. 2009). Within a single species from the Triopsidae family, different population types are encountered due to the respective reproductive strategy. In *T cancriformis*, a distinction is made between bisexual populations where females and males make up about 50% of the population, androdioeci where hermaphrodites make up the majority and males usually do not exceed 25%, and exclusively hermaphroditic populations. Cross-fertilisation between hermaphrodites does not occur in this species. It is also known that populations consisting of hermaphrodites occur in the northern part of the range. Bisexual populations are found in the Iberian Peninsula, which was probably a glacial refugium during the last glaciation. The current commonly accepted hypothesis to explain this distribution of populations with different reproductive strategies is that after the end of the glaciation, a strategy based on self-fertilisation was favoured during the colonisation of the numerous water bodies formed in the post-glacial landscape of Europe (Zierold et al., 2009). Studies have revealed a genetic basis for sex determination (Mathers et al. 2015). The population from Jaktorów is most likely to consist of hermaphrodites and males typical of central Europe. Interesting relatively high number of males in relation to females/hermaphrodites could be due to the higher survival rate of males during rearing under aquarium conditions or to a stochastic effect due to small sample size.

Hempel-Zawitkowska (1965) identified the presence of *B. schaefferi*, but this species was not found during the discussed research. It is possible that *S. torvicornis* was wrongly identified as *B. schaefferi* by Hempel-Zawitkowska. It is also possible that *B. schaefferi* has been displaced by *S. torvicornis* in recent years or that *B. schaefferi* is still present at the site but for other reasons was not found.

The possibility of human introduction of the species *S. torvicornis* should also be considered. From verbal information from employees associated with the Łąki Jaktorowskie Fisheries Research Station it seems that intentional introduction of this species has not occurred (R. Jończyk, M. Cieśla, personal communications). Stocking fish were not brought from regions where *S. torvicornis* is known to occur.

Until recently, there was virtually no contemporary data from Poland on the biology and ecology of large branchiopods and their distribution and the degree of threat faced by them (Gołdyn et al. 2019). The most extensively studied is the province of Wielkopolska (Gołdyn et al. 2012). In addition, there have been studies in eastern Poland (Biggs et al. 2004). The most up-to-date data from the central part of the Polish Lowlands comes from Cukier (2020) who proposed monitoring methods for *S. torvicornis* based on observations from the only currently known location of the species. *S. torvicornis* was found in 1938 in Kraków (Ramułt 1939) and in the village of Odolany, (which as of this writing has probably been absorbed into the Wola district of Warsaw) where the holotype had earlier been found (Waga 1842). On the basis of such truncated data, it is difficult to estimate the species' level of threat and its conservation strategy. Nevertheless, *S. torvicornis* is currently under strict protection under Polish law (Regulation, 2016). The *T. cancriformis* species we studied during this work was previously considered to be a common species in Poland (Zwolski 1956), but recent reports from the province of Greater Poland suggest that it is an endangered species in that area (Gołdyn et al. 2012). This species is under partial protection in Poland (Regulation, 2016). A declining trend in the number of *T. cancriformis* sites is also observed in other parts of Europe (Eder&Hödl 2002; Merta et al. 2016). *L. lenticularis* is another species we found at the study site. Surprisingly, it is not protected in Poland, but there is very little data on its occurrence. The last finding is from 1969 (Czeczuga&Czerpak 1969). During the most recent work in Wielkopolska, no specimens were found (Gołdyn et al. 2012).

This raises the question of what role fish farms can play in maintaining populations of large branchiopods. In pond management advisories, the large branchiopods are presented as potential pests (Chebanov&Galich 2013, Horváth et al. 2015). They cite the negative impact of water turbidity on sturgeon fish fry caused by digging at the bottom by tadpole shrimp. The negative impact may be due to reduced visibility and inability to hunt effectively. Large branchiopods may also compete with fry for food (Chebanov&Galich 2013). It has been shown that *T. cancriformis* can significantly reduce populations of small crustaceans (Waterkeyn et al. 2011). Methods for control of large branchiopods are given in the manual for sturgeon fish farming published by the Food and Agriculture Organization of the United Nations (FAO) (Chebanov&Galich 2013). Suggested methods are chemical control with chlorine compounds, mechanical flushing of larvae before fish are allowed in, and allowing fish to feed on branchiopods larvae in a timely manner (Chebanov&Galich 2013). The authors of the present study encountered discussions on an internet forum that concerned the control of the *T. cancriformis* with pesticides such as trichlorophon.

However, it could be speculated that application of the chemical control methods are detrimental rather than beneficial. The harmfulness of the *T. cancriformis* on nursery ponds has not been well documented. From their diet, it might be expected that they feed on weakened or dead fish. Eradication of the large branchiopods can result in depleted food base for fry. *T. cancriformis*, *Leptestheria dahalacensis* (Rueppel, 1837) and *S. torvicornis*, in addition to cladocerans, form an important part of the diet of belugas in breeding tanks (Ruban 2020). *S. torvicornis* is sometimes deliberately introduced into ponds to increase the food base of sturgeon fish (Dulina 2008, Chebanov&Galich 2013).

We found differences in body proportions between the sexes in *T. cancriformis*. Zierold (2006) mentions sex differences in the number of legless segments. Our study does not confirm a clear relationship. In addition, we observed half legless segments in 2 specimens, similar to the one found in American *Triops* sp. by Macdonald et al. (2011). The difference in body proportions shown in the results of this paper may facilitate rapid sex recognition in *T. cancriformis* without having to observe the 11th pair of legs. The authors of the study had the opportunity to observe individuals of this species in aquaria. During these observations, differences in behaviour between the sexes were readily apparent. What was particularly interesting was that the males made characteristic vigorous movements at the same time embracing the females or the empty exoskeletons (after moulting) or another male. These movements were probably copulatory. By these behaviours it is also possible to quickly recognise males in a group of individuals of this species.

Morphometric measurements are still frequently used to determine phylogenetic relationships in wild Anostraca populations (Macdonald et al. 2011, Czyż et al. 2016, Kappas et al. 2017, Cohen et al. 2019). Comparisons of results from molecular data with those from morphometrics are not convergent (Zofkova&Timms 2009). It is believed that morphometric data can be useful, especially when it is not possible to obtain good-quality molecular data with adequate resolution, or as a supplement when molecular data do not allow unambiguous assessment of phylogenetic relationships. According to Ranta et al. (1993), environmental factors and age can strongly influence the phenotype of organisms, so comparing morphometric parameters between groups that developed under different conditions or were of different ages can generate serious errors. Our experiment revealed that there are significant differences between age groups in some parameters. The only parameter in which the difference was not significant was the ratio of antennal outgrowth peduncle length to the basal joint length (pd/bj). However, evaluation of the relationship between the parameters and body length showed a significant relationship only for the parameter th/sl. Knowing the possible influence of environmental conditions and age on morphology, we recommend taking morphometric measurements of individuals that were kept under the same conditions and were of the same age. An example of a study that used this method is Mayer (2002) , in which collected *Artemia* sp. cysts from different locations and then hatched and maintained the specimens under identical conditions, after which measurements were taken. This approach may give more reliable results in a phylogenetic context than measurements of specimens collected from the wild.

The morphology of *S. torvicornis* specimens studied is typical of European populations of this species. The denticulation on the thumb, extending above the thumb major inflexion and protuberances on peduncle of distal outgrowth that prior to the work of Dumont et al. (1991) were considered a feature of the subspecies *S. torvicornis torvicornis,* are present. However, the th/sp parameter does not give a clear picture rather. To the contrary, it is not adequate and would not allow the subspecies to be distinguished. The presence of subspecies in *S. torvicornis* in general is questioned on the basis of recent molecular and morphological data.

In our opinion, the methodology and the key for the recognition of representatives of the family Streptocephalidae presented by Maeda-Martinez & Dumont (1995) are not very helpful in the context of European faunal studies because probably *S. torvicornis* is the only representative of this family in the region. However, the scenario of an imported alien species and the need to verify the species affiliation cannot be excluded.

## 4. Conclusion

In summary, this study has provided important information on the presence of large branchiopod species in the Łąki Jaktorowskie Fisheries Research Station. Two species of large branchiopods, *T. cancriformis* and *S. torvicornis*, were identified based on the ornamentation analysis of the cysts isolated from the soil and on the morphology of adult specimens obtained from cysts. Additionally, the presence of males in the population of *T. cancriformis* was identified, which was unexpected given that previous studies had only found females.

Further, our results indicate that traditional semi-intensive carp aquaculture in ponds allows for the preservation of large branchiopods habitats and that carp farms have become a refugium for *S. torvicornis* and related species. Such measures may be necessary for the successful conservation of this group of organisms.

In addition, the results of the comparison of the two age groups of *S. torvicornis* suggest that individuals' age may have a slight effect on the parameters used for species identification and population comparison. This information may be useful when, in the future, plans are made for surveys that use morphometric measurements.

Overall, this study highlights the importance of continued research and monitoring of large branchiopod populations and their interactions with other aquatic species in order to better understand their ecological roles and potential impacts and role of extensive aquaculture in maintenance of the large branchiopods population which are currently under pressure from changes that threaten their very existence.

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