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Short Research Note

pH-related hatching success of *Triops cancriformis* (Crustacea: Branchiopoda: Notostraca)

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Abstract

Six different pH ranges were tested for the hatching rate of *Triops cancriformis* cysts. Cumulative hatching numbers approach to approximately 50% of cysts. The highest hatching rates, co-occurring with the shortest hatching times, were recorded in the neutral range. No hatching took place at pH values 9 and above. This corresponds with the most frequent habitats of this species, snowmelt pools of river flood plains as well as aestival pools caused by heavy rainfalls. Occurrences in sodic alkaline pans can be explained by relatively low pH values at the time of inundation.

Introduction

The tadpole shrimp *Triops cancriformis* (Bosc, 1801), an inhabitant of temporary freshwater pools, is widely distributed in Eurasia and North Africa (Longhurst, 1955; Zaffagnini & Trentini, 1980; Brtek & Thiéry, 1995). The subspecies *T. cancriformis cancriformis* occurs in Austria primarily along the Morava and Danube rivers, and the Wiener Becken Depression as well as the National Park Neusiedler See – Seewinkel (Eder & Hödl, 2003). It is usually found in temporary pools filled by rain or rising water tables, but has also been detected in a few alkaline pans where it co-occurs with the anostracan genus *Branchinecta* (Eder et al., 1997).

Like most other "large branchiopods" (Anostraca, Notostraca, Laevicaudata, Spinicaudata), *T. cancriformis* survives long drought periods by producing resistant cysts that remain viable after even prolonged periods (Lauterborn, 1921, reported 27 years) of diapause. Shortly after inundation nauplius larvae hatch and develop to a new reproductive generation. Hatching conditions play a key role for understanding the mechanisms of diapause and hatching (Brendonck, 1996; Gyllström & Hansson, 2004), as well as the species' local distribution (Eder & Hödl, 1996). However, little is known about the various environmental parameters of astatic water bodies affecting cyst hatchability of "large branchiopods" (see Table 1). Light, pH values and salinity have been shown to influence the hatching success of several notostracan and anostracan species. Some biotic parameters have been investigated, such as the presence of nutrients (Spencer & Blaustein, 2001), conspecific adults (Hall, 1959a; Bishop, 1967; Mitchell, 1990) or predators (Spencer & Blaustein, 2001).

For *Triops longicaudatus* (LeConte, 1846), various parameters influencing hatching, such as pH, temperature, salinity/osmolarity, pool and substrate depth, as well as drought period and substrate moisture, have been investigated with partly contradictory results (Scott & Grigarick, 1979; Fry & Mulla, 1992; see Table 1). *Triops*

Table 1. Studies on abiotic factors affecting cyst hatching in Notostraca, Spinicaudata and Anostraca (except for *Artemia* sp.)

Authors	Species	Tem	pH	O_2	CO_2	Sal	Dro	Hum	Fro	Lig	Dep	Mud
Notostraca:												
Scott & Grigarick, (1979)	*Triops longicaudatus* (LeConte, 1846)	22 °C	5.6	–	–	neg	no	pos	–	–	no	neg
Hann & Lonsberry, (1991)	*Lepidurus couesii* Packard, 1875	20 °C	–	–	–	–	–	–	pos	–	–	–
Fry & Mulla (1992)	*T. longicaudatus*	–	–	–	–	–	pos	neg	–	–	–	–
Kuller & Gasith (1996)	*Lepidurus apus lubbocki* Brauer, 1873	16–20 °C	–	–	–	–	–	–	–	pos	–	–
Kuller & Gasith (1996)	*Triops cancriformis*	24–32 °C	–	–	–	–	–	–	–	pos	–	–
Spinicaudata:												
Bishop (1967)	*Limnadia stanleyana* (King, 1855)	20 °C	–	pos	–	–	no	–	–	pos	–	–
Khalaf & Al–Jaafery (1985)	*Eocyzicus spinifer* Prasad et al. 1981	28–30 °C	–	–	–	–	–	–	–	–	–	–
Brendonck et al. (1993)	*Caenestheriella australis* (Loven, 1847)	–	–	–	–	–	pos	–	–	–	–	–
Brendonck et al. (1993)	*Eocyzicus klunzingeri* (Wolf, 1911)	–	–	–	–	–	no	–	–	–	–	–
Brendonck et al. (1993)	*Leptestheria aegyptiaca* Daday, 1923	–	–	–	–	–	pos	–	–	–	–	–
Brendonck et al. (1993)	*Eulimnadia africana* (Brauer, 1877)	–	–	–	–	–	pos	–	–	–	–	–
Anostraca:												
Weaver (1943)	*Eubranchipus vernalis* (Verril, 1869)	–	–	–	–	–	pos	–	pos	–	–	–
Hall (1959a)	*Chirocephalus diaphanus* (Prévost, 1803)	22 °C	–	–	–	?	–	–	–	–	neg	no
Hall (1959b)	*C. diaphanus*	–	–	–	–	–	–	–	–	–	neg	–
Prophet (1963)	*Eubranchipus serratus* Forbes, 1876	6–15 °C	–	–	–	–	–	–	–	–	–	–
Brown & Carpelan (1971)	*Branchinecta mackini* Dexter, 1956	no	–	pos	–	neg	no	–	–	–	–	–
Bernice (1972)	*Streptocephalus dichotomus* Baird, 1860	30 °C	–	–	–	neg	10 to 20 d	–	–	–	no	–
Mossin (1986)	*Eubranchipus (Siphonophanes) grubii* (Dybowski, 1860)	–	5.5	pos	pos	–	–	–	–	–	–	–
Mitchell (1990)	*Streptocephalus macrourus* Daday, 1908	14–20 °C	–	pos	–	–	pos	–	–	pos	–	–
Brendonck et al. (1993)	*Streptocephalus proboscideus* (Frauenfeld, 1873)	–	–	–	–	–	pos	–	–	–	–	–
Brendonck et al. (1993)	*Streptocephalus sudanicus* Daday, 1910	–	–	–	–	–	pos	–	–	–	–	–
Murugan & Dumont (1995)	*Thamnocephalus platyurus* Packard, 1877	–	–	–	–	–	–	–	–	pos	–	–
Merta (2003)	*E. grubii*	2–4 °C	–	–	–	–	no	no	no	–	–	–

Tem – temperature; pH – pH value; Sal – salinity; Dro – length of drought period; Hum – humidity of substrate; Fro – frost period; Lig – light intensity; Dep – depth of water column; Mud – depth of mud coverage; pos – positive correlation with hatching rate; neg – negative correlation with hatching rate; no – no correlation found.

cancriformis and *Lepidurus apus* have long been observed to have different temperature preferences, but evidence is weak as there is only one systematic study on temperature effects on hatching (Kuller & Gasith, 1996, for a North African population).

For Austrian species, only semi-quantitative observations have been made (e.g. Metz & Forró, 1989; Hödl & Rieder, 1993; Eder et al., 1997); some species were considered to be relatively stenoecious, others euryecious, among these *T. cancriformis* (e.g. Eder & Hödl, 2003). Eder et al. (1997) suggested temperature, salinity and pH-values as the most probable abiotic environmental factors affecting the occurrence of Austrian "large branchiopods".

The occurrence of adult *T. cancriformis* specimens in alkaline pans at pH values of 8.8 and 9.2 (Eder et al., 1997) – this species is usually found in rain pools and flood plain pools – encouraged us to examine the environmental pH-range of hatching in this species.

As this study was performed in the course of a "Fachbereitsarbeit" (graduation paper) at a grammar school in Vienna, we chose simple methods. Sand with dry cysts was obtained from a laboratory culture maintained since 1996 by the second author. The initial cultures were collected in the Morava river flood plains, Lower Austria. Twenty grams of sand containing approximately 100 *T. cancriformis* cysts were added to each of 18 1.0 l plastic aquaria. 750 ml of distilled room temperature water was added to each aquarium. Six groups with different pH values were tested for hatching of *Triops* nauplii: pH 4–4.9, 5–5.9, 6–6.9, 7–7.9, 8–8.9, 9–9.9. pH values were measured with Merck pH indicator strips and adjusted using $NaHCO_3$ (> pH 5.9) and 15% CH_3COOH (< pH 5). To ensure an approximately equal number of cysts in each group, the sand for all samples was taken from the same breeding aquarium and each test was performed three times per group. A commercially available aquarium aeration pump supplied air.

The appearance of nauplii was checked regularly, at least three times per day, and documented. Hatched nauplii were removed from the aquaria to avoid multiple counts. As our previous tests have shown that more than 95% of hatches take place within the first two weeks (own unpublished results), the tests were stopped fourteen days after water addition. In total, 18 tests resulting in 408 hatched nauplii were performed. Statistical examination was performed using the Statistical Package for the Social Sciences (SPSS), version 11.5.1. The results (hatching number, time, hatching rate) of the 18 groups were compared with the non-parametric test Kruskal–Wallis–H (Kruskal & Wallis, 1952).

Hatching number

408 nauplii hatched, more than 50% of them within the first five days (Fig. 1a). The greatest number (97.6%) hatched between pH 5 and 7.9, the maximum recorded in pH 6–6.9 ($\chi^2 = 11.091$, $p = 0.05$). The general appearance of the hatching success closely resembles an optimum curve (Fig. 1b).

Hatching (i.e. appearance) time

In general, average hatching time appears to be indirectly proportional to hatching numbers (Fig. 1b, c) ($\chi^2 = 14.046$, $p = 0.015$). Fastest hatching (116.8 h) took place in the group pH 6–6.9, except for the group pH 4–4.9 (59.3 h), which was the average of only three hatched specimens. No hatching took place at pH values of 9 and higher.

Hatching rate

The hatching rate (hatching number divided by time of appearance) shows significant differences between the tested groups ($\chi^2 = 14.666$, $p = 0.012$).

The highest hatching rate was observed at pH 6–6.9, followed by pH 7–7.9. However, the three groups from pH 5–7.9 did not differ significantly ($p = 0.193$).

The number of hatched cysts in most "large branchiopod" species hardly ever exceeds 50% (e.g. Van Stappen et al., 1998; Philippi et al., 2001; Ali & Dumont, 2002). This is supported by our results and can be interpreted as an adaptation to the unpredictable conditions of temporary water bodies: a relatively high percentage of cysts remains in the ground as a potential reserve for future inundations (Brendonck, 1996; Philippi

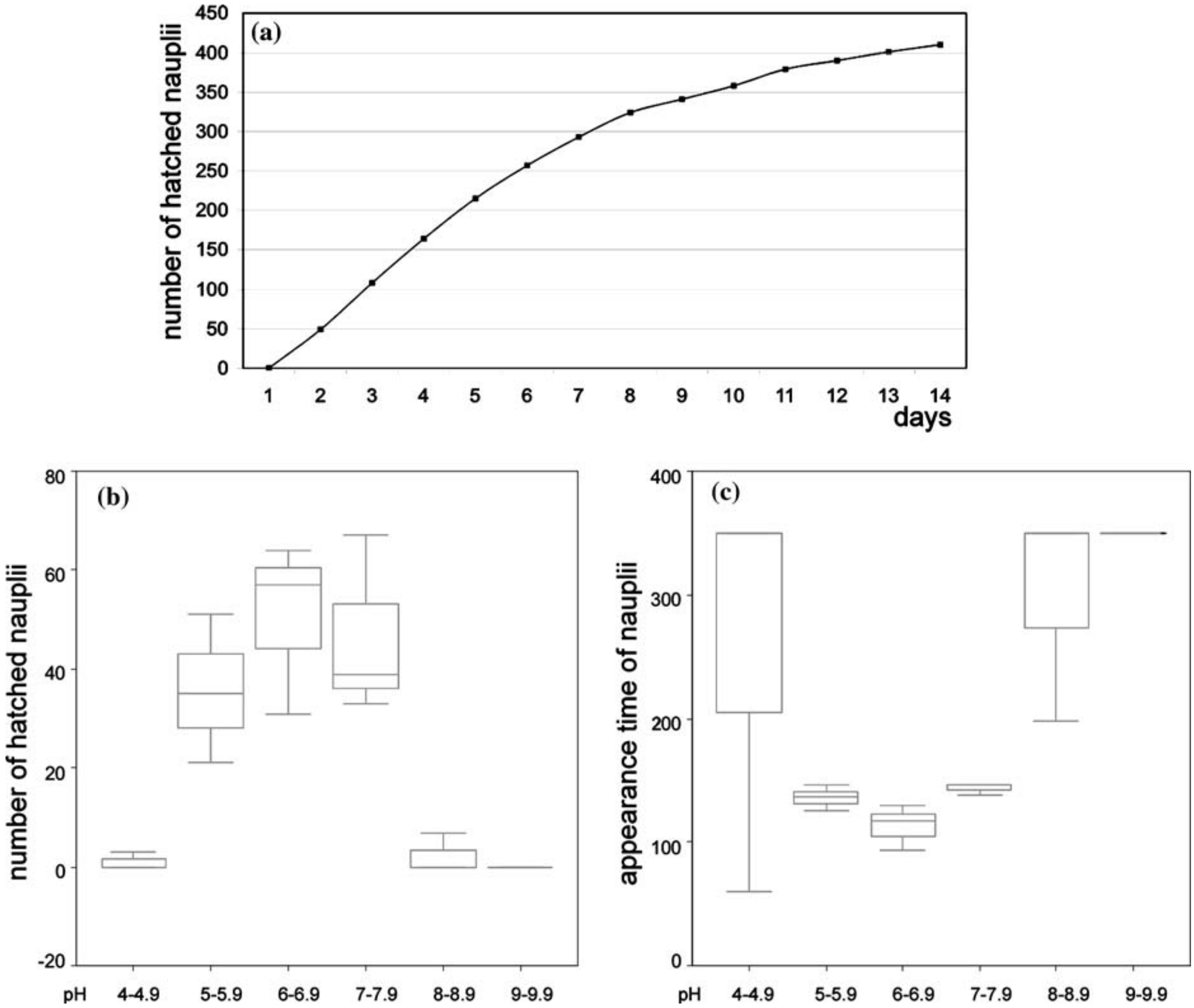

Figure 1. (a) Cumulative number of hatchlings in all test groups. The curve is close to asymptotic, more than 50% of specimens hatched within five days. (b) Number of hatched nauplii. The boxplot is closely similar to an optimum curve. (c) Hatching (i.e. appearance) time of nauplii. For groups where no hatching occurred, an artificial value higher than the maximum (350 h) was added. The high standard deviation in groups 4 and 8 is due to the low number of hatched specimens.

et al., 2001). During the first day after wetting, no nauplii at all hatched (cf. Belk, 1973), but within the following four days, more than 50% of all hatches occured (Fig.1a).

Apart from the stochastic nature of cyst hatching, a certain adaptive response to the local ecological conditions can be expected (Fryer, 1996). One of the parameters most examined is temperature: species occurring in early spring (e.g. *Lepidurus apus*) have a lower hatching temperature in laboratory than late spring or summer species (e.g. *T. cancriformis*, see Kuller & Gasith, 1996). Low salinity, usually a characteristic of recent inundations, is known to induce hatching (Bernice, 1972; Scott & Grigarick, 1979), whereas increasing salinity influences ovary maturation in adult specimens, which can be seen as a response to drying.

For some anostracan species, correlations with certain pH ranges are known. Most pools filled by rain water or snowmelt induced river inundations initially show relatively low pH values. *Eubranchipus* (*Siphonophanes*) *grubii* (Dybowski, 1860) has its optimal hatching pH value at 5.5 (Mossin, 1986), which can be interpreted as an adaptation to early spring snowmelt pools mostly covered with leaf litter, being the typical habitat for this species (Saiah & Perrin, 1990).

Both *Branchinecta orientalis* (Sars, 1901) and *Branchinecta ferox* (Milne-Edwards, 1840) are limited to steppic soda lakes in central Spain, the Pannonian lowlands and Eastern Europe, and have been documented in Austria at pH values up to 9.68 (Metz & Forró, 1989). Their decline in Austria is supposedly a result of the degradation of the alkaline pans in the Seewinkel region (Löffler, 1993). Only a few other "large branchiopod" species have been documented together with *Branchinecta*, such as *Chirocephalus carnuntanus* (Brauer, 1877) and *T. cancriformis*.

In Austria, *T. cancriformis* is primarily found in snowmelt-prone pools of river flood plains as well as in aestival pools filled by heavy rainfall (Eder & Hödl, 2003), which corresponds well with our present results. Our results show a clear preference of neutral environments for hatching of nauplii. We assume that immediately after inundations, sodic pools show relatively low pH values sufficiently suitable for hatching of *T. cancriformis* larvae, which could explain the occurrence of adults of this species in alkaline pans.

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