

Intraspecific differences in hatching phenology of the fairy shrimp *Chirocephalus diaphanus* Prévost, 1803 (Crustacea, Anostraca) in relation to habitat duration

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ABSTRACT

The influence of environmental characteristics on hatching phenology in the fairy shrimp *Chirocephalus diaphanus* Prévost, 1803 was investigated by comparing the hatching behaviour of two populations living in mountain pasture pools differing in size and in duration of filling. Significant differences observed at population level confirmed that an influence of the environment in determining hatching pattern does actually exist. *C. diaphanus* seems to be able to adjust the cyst reactivity to the stochastic nature of its habitat. The cysts laid by females adapted to a predictable and relatively constant environment are characterized by higher hatching percentages with respect to those produced in a temporary pool having erratic hydrocycles.

Key words: hatching behaviour, temporary waters, environmental diversity

1. INTRODUCTION

Anostraca, which typically inhabit temporary pools, survive adverse periods by producing "dormant eggs" (cysts). A certain fraction of them resumes metabolism when favourable environmental conditions are restored, while others remain paused for one or more seasonal cycles passed. This observed delay in cyst hatching is supposed to be an adaptation to overcome predictable and unpredictable seasonal changes that could be fatal for the adult life-phase. In this sense, the existence of a marked inter- and intraspecific variation observed in the hatching pattern of fairy shrimps (see review in Brendonck 1996; Simovich & Hathaway 1997; Van Dooren & Brendonck 1998; Mura & Zarattini 1999; Brendonck & Riddoch 2001), provided evidence that a different cyst reactivity related to the degree of environmental unpredictability exists (Belk & Cole 1975).

We investigate the influence of biotope diversity by collecting cysts produced by two populations of *Chirocephalus diaphanus* living in pools with different hydrological cycles.

2. MATERIALS

The populations considered in this study belong to the broadly tolerant *Chirocephalus diaphanus* Prévost, 1803, the most common and widespread species in Italy (Mura 1999). We selected two sites located in the Central Apennine Mountains, an area typically characterized by snowy winters and dry summers. Lake Tilia (1591 m a.s.l.) is a semipermanent pasture pool on Mt. Tilia (Leonessa, Rieti) with a maximum depth of 97 cm and a roughly rectangular shape (54 × 48 m). The Illica

Pool (1485 m a.s.l.) is a small temporary water body on Mt. Inversaturo (Ascoli Piceno) with a maximum depth of 50 cm and a dimension of 25 × 16 m. Because of the differences in dimension and maximum depth, they differ significantly in daily water temperature excursion, in fluctuations of the surface area/volume ratio and in water phase duration (Mura *et al.* 2003).

3. METHODS

In each pool a constant number of ovigerous females was collected at the beginning and at the end of their life cycle. They were transferred to the laboratory and isolated in deposition chambers (100 ml volume) under controlled conditions (18 ± 1 °C, 12L/12D). Immediately after deposition, cysts were removed, counted and subsequently used for hatching tests. Broods were kept separate and divided over three different hatching treatments. In the first test (hereafter referred to as "natural dehydration") cysts were dried for 10 days at room temperature and humidity and subsequently inundated under controlled conditions (18 ± 1 °C, 12L/12D). Cysts remained submersed for 4 weeks and were checked daily for hatching. After that, we performed two more inundations (each lasting 4 weeks each, with daily counts of hatchlings) separated by drought intervals of 10 days.

In the second test (hereafter referred to as "dehydration in oven"), freshly laid cysts were dried for 10 days in an oven (30 ± 1 °C) and subsequently inundated under controlled conditions (18 ± 1 °C, 12L/12D). Cysts remained submersed for 4 weeks and were checked daily for hatching. As in the previous case, we repeated two more inundations (each lasting 4 weeks, with daily scoring of hatchlings) separated by

drought intervals of 10 days. In the last test (hereafter referred to as "no dehydration") fresh cysts, deposited under controlled conditions (18 ± 1 °C, 12L/12D), were subjected to a 1:10 dilution of parental water without any temperature variation and checked daily for hatching. After 4 weeks, they were subjected to a thermal shock by reducing temperature (5 ± 1 °C) for 10 days and raising it for a further 4 weeks (with daily counts of hatched nauplii) to the initial thermal regime.

The results of hatching tests, expressed as a percentage of hatched nauplii/brood, were analysed for significant differences by means of ANOVA (STATISTICA '98 for Windows, STATSOFT Inc.) after arc-sine transformation of the data (Sokal & Rohlf 1995).

4. RESULTS

Mean brood size and mean number of nauplii/brood obtained at the beginning and at the end of the water phase in biotopes are shown in table 1. Overall significant differences ($P < 0.01$) were recorded in hatching data within and among treatments as illustrated in figure 1.

In Lake Tilia, cysts produced at the beginning of the water phase showed a higher cumulative hatching rate (78.5%) than those laid at the end of life cycle (36.5%). The cysts undergoing natural dehydration showed a high overall hatching rate (80.7%) and the sequence of three inundations resulted in 47.5%, 30.0% and 3.2% hatching, respectively. Those produced at the end of the water phase responded in a similar way when subjected to the stimuli described above: 42.2% after the first inundation, 14.1% after the second and 2.3% after the third. Freshly laid cysts collected at the beginning of the life cycle and subjected to dehydration in an oven hatched more successfully (82.8%) than all the others. Moreover, in this group the reactivity to three successive inundations was observed to vary: 53.2%, 22.5% and 7.1%, respectively. Striking differences between the last percentages and those shown by the cysts produced at the end of the water phase (36.6%) emerged from the same hatching test. In this case, hatching responses varied from a maximum of 19.9% recorded after the first inundation to a minimum of 0.2% obtained after the third inundation. No dehydration proved to be an effective stimulus only for the cysts produced by already mature females (overall hatching rate 72.3%), whereas 80% of those laid at the end of the life cycle did not hatch.

The cumulative hatching rate of nauplii hatched at the beginning of the water phase in the Illica Pool was 66.7%. Conversely, only a very low proportion (4.6%) of the total cysts produced at the end of life cycle hatched. As regards the first sampling, natural dehydration determined the greatest overall hatching (73.9%), in particular 34.7% after the first inundation, 33.3% after the second and only 5.8% after the third. Conversely, oven incubated cysts initially showed a minimal hatching (8.8%), which took place only after

the second inundation (32.0%), whereas no hatching at all was observed after the third stimulus. In the last hatching test (no dehydration), initial dilution resulted in 24.8% hatching and the second stimulus (thermal shock) in 46.8% hatching. The total hatching percentages obtained during the second sampling were 6.2% for natural dehydration, 8.0% for oven dehydration and 0.8% for no dehydration. The partial hatching percentages recorded after each single stimulus were low, varying between 0.1% and 0.5%.

5. DISCUSSION AND CONCLUSIONS

The two high-altitude populations of *Chirocephalus diaphanus* investigated by us showed significant differences in hatching patterns and requirements. Once Mura and coworkers (2003) recently showed that the two populations, which inhabit biotopes differing in size and predictability, adopt distinct life-cycle strategies. Predictability and the long lasting stability of Lake Tilia resulted in slower growth, later reproduction and a longer life cycle compared to those observed in Illica, which is characterised by practically no stable period and behaves erratically, differing in its filling time from year to year.

The present data, while supporting recent findings (Brendonck 1996; Hathaway & Simovich 1996; Simovich & Hathaway 1997; Brendonck *et al.* 1998; Brendonck & Riddoch 2001), provide additional information by suggesting the existence of a relationship between hatching phenology and habitat stochasticity.

As suggested by Simovich & Hathaway (1997), there is a range of cyst reactivity from the near 100% hatch of the species living in pools with a high probability of filling to species inhabiting highly unpredictable pools. Owing to its high hatching rate, the Lake Tilia population belongs to the first group, which includes the European *Eubranchipus* species (Belk 1977; Mossin 1986) together with *Branchinecta mackini* from the Arizona mountains (Gonzalez *et al.* 1996). Conversely, the erratic hatching patterns shown by cysts produced in the Illica Pool are in good agreement with previous observations concerning two southern California species, *Branchinecta sandiegonensis* and *Streptocephalus woottoni* (Simovich & Hathaway 1997). Both exhibit a life history in which their reproductive effort is spread over more than one pool hydration event, which effectively reduces the probability of complete reproductive failure if a pool dries up prematurely. The low initial hatching rate (6% and 0.2%, respectively) and the cumulative values over three hydrations (28% and 2.8%) for these species are among the lowest values reported for anostracans (Brendonck 1996). As suggested by Brendonck *et al.* (1998) and by Brendonck & Riddoch (2001) a low hatching rate means little chance of successful production of resting eggs due to frequent early drying of the short-lived desert rock pools colonized by *Branchipodopsis wolffi*.

Tab. 1. Quantitative data on cyst production (mean brood size) and hatching (mean number of nauplii) for the females of *Chirocephalus diaphanus* collected at the beginning and at the end of the water phase in the two biotopes under study. For details on hatching treatments see Methods.

	natural dehydration			dehydration in oven			no dehydration							
	N.	N. of nauplii			N.	N. of nauplii			N.	N. of nauplii				
		brood size	1° inundat.	2° inundat.		3° inundat.	brood size	1° inundat.		2° inundat.	3° inundat.	dilution	themat shock	
Tilia	30	41.6 (30.7)	19.7 (24.0)	12.5 (13.2)	1.4 (5.0)	29	38.3 (23.3)	20.3 (16.8)	8.6 (10.0)	2.7 (4.9)	30	41.2 (31.0)	29.0 (28.1)	29.0 (28.1)
end	28	607.6 (170.5)	256.1 (117.6)	85.7 (79.9)	14.1 (23.9)	24	541.4 (169.0)	107.8 (82.8)	89.1 (41.3)	1.3 (3.2)	27	558.3 (183.7)	44.4 (38.3)	19.7 (19.7)
Illica	29	29.5 (17.3)	10.2 (11.3)	10.6 (14.4)	2.5 (4.9)	29	26.8 (17.4)	2.3 (4.9)	8.6 (11.3)	0	28	29.5 (16.7)	7.1 (12.8)	13.8 (11.9)
end	7	564.0 (201.7)	2.6 (5.6)	2.3 (4.8)	29.9 (27.5)	7	455.3 (265.8)	0.1 (0.4)	0.3 (0.8)	35.9 (91.4)	8	564.4 (200.4)	0	4.8 (13.0)

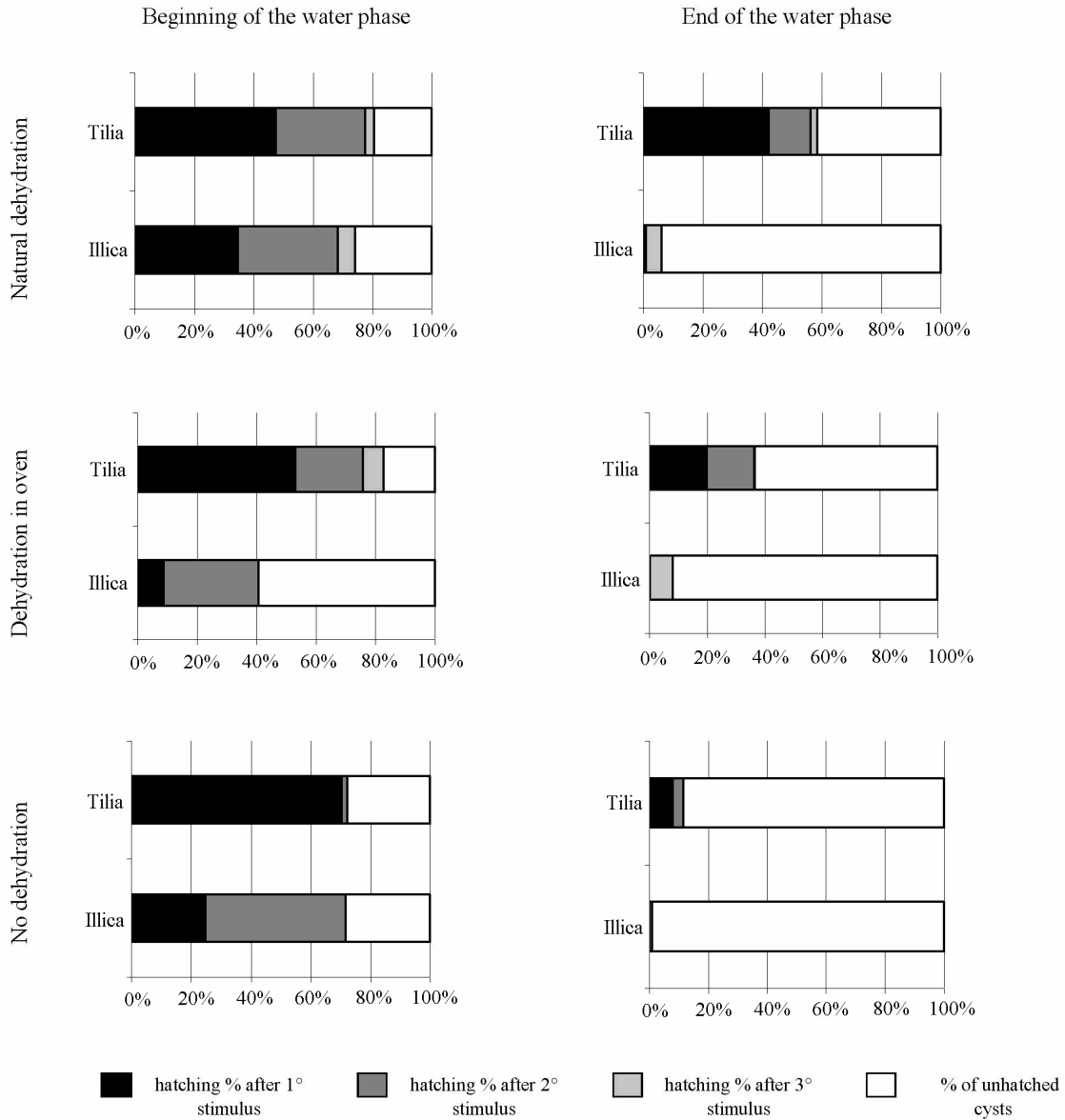


Fig. 1. Cumulative hatching (%) for each of the three samplings performed in the pools under study in relation to the different hatching tests (for details see Methods).

The fraction of cysts that hatches yearly should actually be approximately equal to the probability of a large production of these eggs, and the fraction which does not hatch should be almost equal to the probability of a total or near total failure to produce propagules (Cohen 1966, 1967, 1968). In our case, we observe that both populations show a higher hatching rate at the beginning of the water phase (75% for Tilia and 62% for Illica) than at the end of the life cycle (38% and 12%, respectively), when the environmental conditions begin to deteriorate.

In the same hatching test, the capacity to react was observed to a different extent in the two populations

under study. Since it is generally assumed that hatching is affected by a combination of environmental conditions related either to climatic region or type of habitat (Brendonck 1996), the occurrence of intra-specific differences in hatching suggests the existence of a local adaptation. Dilution of the medium proved to be an effective stimulus only for the cysts laid by the Lake Tilia females, whereas those produced in the Illica Pool were more sensitive to thermal shock. Differences between the two populations are even more evident when considering hatching patterns observed after each of the three dehydration cycles. On one hand, the Lake Tilia was always characterised by a high initial hatch-

ing compared to the total, in relation to reduced risk of pool drying, on the other hand the Illica Pool exhibited an initially low hatching rate which increased upon subsequent stimulation to cope with the risk of untimely pool extinction.

Although the results of the present study appear significant, they are not exhaustive. As already observed in the case of *Artemia* (Drinkwater & Clegg 1991), our knowledge of hatching phenology may be increased only by standardizing adequate culture conditions for rearing populations in the laboratory. This kind of experimental work would provide further support for the demonstration of the existence of true local adaptation, since one would expect the differences in hatching rates to be maintained across generations in laboratory cultures.

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