

## Constitutive but no *Triops*-induced differences in bet-hedging strategies for hatching in *Daphnia*

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**Abstract** Since cladocerans from the genus *Daphnia* are known to have evolved several inducible defenses (morphological and life history shifts) against the notostracan predator *Triops*, we investigated whether hatching was also altered in response to *Triops*. We tested whether dormant eggs of *Daphnia magna* are able to detect *Triops cancriformis* kairomones in the water as a signal of predation pressure and alter their hatching response accordingly to avoid predation. We predicted that, in the presence of *Triops* kairomones, hatching fractions might be reduced (postponing hatching to a next growing season) and/or that hatching might peak earlier (increasing chances to reproduce before *Triops* becomes predatory). We also tested whether this response depended on the origin of the population. Ehippia from three *D. magna* populations, originating from one permanent lake and two temporary pond systems, were

exposed to *Triops* kairomone and control treatments. We observed significant population differences in hatching patterns, both in terms of the fraction of eggs that hatch as well as the timing of hatching, with evidence for within-season bet-hedging through delayed hatching in the populations inhabiting temporary habitats. However, no indication was found that the populations also adjust their hatching pattern to the presence of *Triops* kairomones.

**Keywords** Predation · Bet-hedging · Hatching · *Daphnia magna* · Dormant egg bank · Kairomones

### Introduction

Predation is one of the most important drivers of population and community structure in aquatic ecosystems. Predators affect prey populations not only by direct consumption but also in a variety of non-consumptive ways (Preisser & Bolnick, 2008). Prey have evolved a broad range of defense mechanisms to cope with predation pressure (Tollrian & Harvell, 1999). The development of phenotypically plastic defense mechanisms often relies on the presence of reliable cues for assessing predation pressure, such as predator-released kairomones (i.e., chemical cues released by the predator). Three main chemically induced anti-predator defenses have been recognized: behavioral, morphological, and life history changes (Kats & Dill, 1998; Lass & Spaak, 2003). The impact

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of predator-released kairomones on life history switch points, such as the production of dormant stages and their hatching, are especially interesting, but not fully understood (Lass et al., 2005).

Dormancy is considered an efficient mechanism to escape periods of adverse abiotic and biotic conditions and is especially common in organisms inhabiting variable and unpredictable environments, such as certain freshwater habitats (Brendonck & De Meester, 2003; Gyllström & Hansson, 2004). Although the production of dormant stages as response to predation is generally well accepted (Hairston, 1987; Slusarczyk 1995; Pijanowska & Stolpe, 1996), there is, however, some controversy on whether the hatching of these dormant stages is also modulated by predation cues. A few studies have demonstrated that dormant stages can detect the presence of predators and adjust their hatching accordingly. Hansson (1996) and Rengefors et al. (1998) showed that dinoflagellates reduced recruitment from the dormant egg bank in the presence of herbivorous zooplankton. De Roeck et al. (2005) and Beladjal et al. (2007) found that anostracan hatching fractions were reduced in the presence of turbellarians and heterospecific fairy shrimp kairomones, respectively. Spencer & Blaustein (2001a) demonstrated that spinicaudatan eggs hatched in lower numbers in the presence of salamander larvae. Finally, Lass et al. (2005) and Bozelli et al. (2008) showed that *Daphnia* can tune their hatching to the risk of predation by fish. Other studies did not find any effect of predator-released kairomones on prey hatching, such as for several zooplankton species in response to salamander larvae (Spencer & Blaustein, 2001b) or fish (Angeler, 2005; Santangelo et al., 2010).

Recently, several studies have suggested that benthic omnivorous notostracans (tadpole shrimp) are keystone species in temporary waters (Yee et al., 2005; Waterkeyn et al., 2011a), and the number of studies documenting their status as important predators (feeding on zooplankton, anostracans, dipterans, mayflies, annelids, and amphibian eggs/larvae) is growing (Pont & Vaquer, 1986; Boix et al., 2006). Recent findings indicate that notostracans are also able to feed on the dormant stages of zooplankton (Waterkeyn et al., 2011a, b), but relative predation rates when both dormant and mobile stages are available are currently unknown. In this study, we assess whether *Triops* can also alter the benthic–pelagic coupling in an indirect way. We therefore investigate whether

*Daphnia* ephippia are able to sense *Triops* kairomones as a cue for predation pressure, and respond by adjusting their hatching patterns (timing and total hatching fraction). *Triops* kairomones are already known to trigger strong morphological and life history responses in several *Daphnia* species (Petrušek et al., 2008; Rabus & Laforsch, 2011; Rabus et al., 2011; Waterkeyn, unpublished data), but hatching responses remain unstudied. For this experiment, we use *Daphnia* populations from three different habitats: a Belgian permanent lake, a French temporary pond, and a Hungarian temporary pond. Our prediction was that, in the presence of *Triops* kairomones, hatching fractions would be reduced and/or hatching might start earlier and peak in the first days. Both responses can be seen as risk-avoidance strategies; the former by postponing hatching to a next growing season or inundation event, the latter by increasing the chances to reproduce before *Triops* becomes predatory. We also anticipated that responses to *Triops* kairomones would be more pronounced in populations from the temporary ponds than in the one from a permanent lake, as *Triops* is a typical temporary ponds inhabitant. For the two temporary pond populations, we furthermore expected a more pronounced response in the population from the French than from the Hungarian pond, since the former houses *Triops cancriformis*, while the latter does not.

## Methods

Ephippia from three *Daphnia magna* populations were used: a Belgian (permanent Lake Blankart in the Ijzer valley, 50°59'02"N–02°51'34"E), a French (temporary pond Cerisières sud in Tour du Valat nature reserve in the Camargue, 43°29'24"N–04°40'29"E), and a Hungarian population (semi-permanent pond Kelemen-szék in Kiskunság National Parc, 46°47'46"N–19°11'04"E). Only the French pond housed *T. cancriformis*. *D. magna* ephippia were isolated from sediment samples that were taken from the upper 4 cm of the dormant egg bank. During all steps of the isolation process, precautions were taken to maintain the eggs in a dormant state. The sediment was stored in the dark at 4°C and the ephippia were isolated on ice and illuminated only by monochromatic red light ( $\lambda = 620\text{--}750\text{ nm}$ ). After determining the degree of filling of the ephippia (one ephippium

can contain up to two eggs) for the three populations (by decapsulating 100 ephippia), we collected enough ephippia to obtain 400 eggs for each population (i.e., 280 ephippia for the Belgian population and 420 ephippia for the French and Hungarian populations). These were then stored in a dark refrigerator (4°C) for 2 weeks.

For the hatching experiment, the ephippia were incubated in six well plates (10 ephippia per well of 10 ml each) at 18°C and a long-day photoperiod (16L:8D). Half of the ephippia of each population were incubated in *Triops* conditioned medium and the other half in control medium (i.e., 14 replicates for the Belgian population and 21 replicates for the French and Hungarian populations). *Triops* conditioned medium was obtained by allowing one *T. cancriformis* (mean carapax length  $1.55 \pm 0.25$  cm, hatched from sediment originating from a temporary pond in the Camargue, France) to swim for 24 h in 1 l dechlorinated tap water. A standardized amount (5 pellets) of commercial fish food (TetraMin Goldfish) was added as food. Afterward the medium was filtered by vacuum filtration with a 11- $\mu$ m GFC Whatman filter. The control medium was prepared in exactly the same way (including fish food), but this time without *Triops*. The medium in the experimental wells was renewed daily. During 16 days, all wells were checked daily for hatched *D. magna* individuals, which were counted and subsequently removed. To calculate exact hatching percentages, we decapsulated all ephippia at the end of the experiment and counted the remaining unhatched healthy eggs within each well.

First, hatching success was analyzed using a generalized linear model with binomial error distribution and logit link function (STATISTICA 9.0). This model assessed for variation in total hatching linked to kairomone treatment, population and their interaction. The factor “well” was included as random factor to control for pseudo-replication with respect to hatching data from the same well. Second, the impact of kairomone treatment and population on the timing of hatching was tested by incorporating day of hatching as repeated measure in an ANOVA using arcsin-transformed daily hatching percentages (STATISTICA 9.0). The few individuals that hatched during the first 2 days of the experiment (Fig. 1) were considered an artifact and therefore excluded from the statistical analyses. Development after stimulation of the dormant egg to time of hatching indeed normally

takes at least 3 days under the given temperature conditions. Dormant eggs hatching earlier were hence probably triggered for hatching during the isolation process, despite all precautions.

## Results

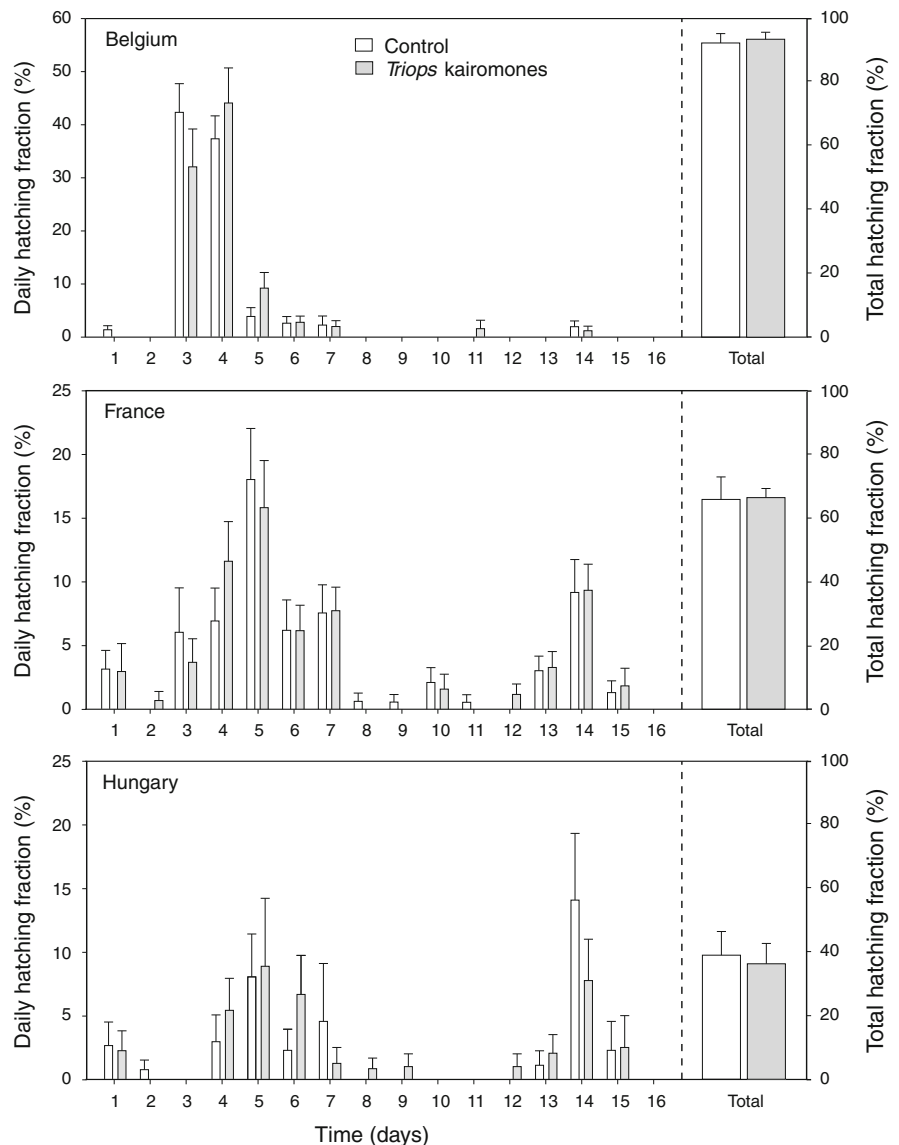
The three populations differed significantly in their total hatching fractions (Wald stat. = 147.216; df = 2;  $P < 0.0001$ ) (Fig. 1). The Belgian population had the highest (av.  $92.4\% \pm 11.7\%$  st. dev.), the French population an intermediate ( $65.9 \pm 19.7\%$ ) and the Hungarian population the lowest hatching percentage ( $39.1 \pm 31.6\%$ ). The presence of *Triops* kairomones had no significant effect on the total hatching fraction (Wald stat. = 0.600; df = 1;  $P = 0.439$ ), nor was there a significant population  $\times$  kairomone interaction effect (Wald stat. = 1.016; df = 2;  $P = 0.602$ ).

The presence of *Triops* kairomones did not affect the timing of hatching of *D. magna* dormant eggs ( $F = 0.971$ ; df = 12;  $P = 0.475$ ) (Fig. 1). Yet, the three populations strongly differed in the distribution of hatching across incubation days ( $F = 19.351$ ; df = 24;  $P < 0.001$ ). The Belgian population already attained its hatching peak on days 3 and 4, while the French and Hungarian populations both had their hatching peaks on day 5. The latter two populations also showed a considerable second hatching peak at days 12–15, which was almost non-existent in the Belgian population. In this population, most of the hatching (84%) already happened in the first 2 days after the start of hatching (i.e., day 3 and 4), while in the French and Hungarian population hatching was spread over 2 weeks. The three-way interaction between time, population, and kairomone treatment was not significant ( $F = 0.491$ ; df = 24;  $P = 0.982$ ).

## Discussion

Dormant eggs can remain viable for decades or longer (Hairston et al., 1995) and hatching patterns of populations may be adjusted to environmental cues (Gyllström & Hansson, 2004; Vanoverbeke & De Meester, 2009; Warkentin, 2011). When the fitness cost/benefit ratio is more favorable outside than inside the egg, embryos should hatch (Warkentin, 2011).

**Fig. 1** Daily and total hatching percentages (mean and standard error) of *D. magna* populations in the presence (gray bars) and the absence (white bars) of *T. cancriformis* kairomones



Ideally, hatching fractions of populations should be adjusted to the reigning conditions and its associated risks, such as desiccation risk or predation risk (“environmentally cued hatching,” Warkentin, 2011). In case of predation risk, we should make a distinction between predation of hatched and unhatched individuals. If predation risk in the active part of the population is high, but predators are not or barely capable of consuming dormant eggs, prey may respond by delaying their hatching. This was found in studies on dinoflagellates (Hansson, 1996; Rengefors et al., 1998), anostracans (De Roeck et al., 2005; Beladjal et al., 2007), spinicaudatans (Spencer & Blaustein, 2001a), and

cladocerans (Lass et al., 2005; Bozelli et al., 2008). In contrast, if predation risk for dormant eggs is higher than for hatchlings, prey may respond by increasing hatching rates. Some studies, however, did not find changed hatching responses in the presence of predator-released kairomones (Spencer & Blaustein, 2001b; Angeler, 2005; Santangelo et al., 2010).

In our study, we did not find a significant effect of *T. cancriformis* kairomones on the hatching fraction or pattern of the cladoceran *D. magna* in any of the three studied populations, even though kairomone concentrations (produced by 1 *Triops* l<sup>-1</sup>) were rather high compared to natural circumstances (Boix et al., 2002).

*Daphnia* and *Triops* have co-existed in temporary ponds for millions of years and several *Daphnia* species, including *D. magna*, were already shown to have evolved inducible defenses against *Triops* (Petrušek et al., 2008; Rabus & Laforsch, 2011; Rabus et al., 2011; Waterkeyn, unpublished data). However, these responses seem not to include changes in hatching dynamics. We see different potential explanations. First, *Triops* hatches synchronously with *Daphnia* and predates both on dormant and active individuals (Waterkeyn et al., 2011a, b). Adjusting hatching responses might, therefore, be a useless strategy to avoid predation. A second reason for the lack of hatching response to *Triops* kairomones may be that the presence of *Triops* within a given habitat is not variable enough through time. A third explanation is that there may be strong clonal variation in the *Triops*-induced hatching responses of *D. magna*, resulting in no visible overall population effect. Several other *D. magna* life history and morphological responses to *Triops* kairomones have been shown to be genotype-specific (Rabus et al., 2011; Waterkeyn, unpublished data). Clonal differences in hatching responses might indeed be traded off with other predation avoidance strategies, such that clones that do hatch in the presence of *Triops* are better in avoiding predation in the active population. Lass et al. (2005) found genotype-specific hatching responses of *D. magna* in response to fish kairomones, with some clones reducing hatching success and others showing no response, offsetting an overall population effect. It is unlikely, however, that this would not translate into differences among the three study populations in their response to *Triops* kairomone, given that the habitats inhabited by these populations are ecologically very different. Moreover, this hypothesis, is only applicable for the (French and Hungarian) populations from temporary ponds, where total hatching percentages are relatively low. The high hatching percentage in the Belgian population provides little room for allowing differential responses and compensation. A fourth reason, De Roeck et al. (2005) found that hatching percentages of the anostracan *Branchipodopsis wolffi* were only lowered in the presence of turbellarians when these originated from the same pond and suggested that this might reflect co-evolution. In our experiment, prey and predator did not originate from the same pond, albeit the *Triops* we used were isolated from a pond very near (c. 500 m) to the pond inhabited

by the Camargue *Daphnia* population, i.e., from the same metacommunity. Finally, a last reason could be that the signal used in this study (filtered kairomone medium, renewed every 24 h) was not strong enough to significantly alter hatching patterns. Unfiltered kairomone medium might contain bacteria or other particles that increase the effectiveness of kairomones, which could result in a stronger (thus detectable) response. More frequent renewal of the *Triops* conditioned medium might also have increased the signal, since the activity window of *Triops* kairomones might be short. However, we argue that this is unlikely, since significant life history and morphological shifts were demonstrated using the same methods (Waterkeyn, unpublished data).

Although we did not find an effect of predator kairomones of *Triops* on *Daphnia* hatching, *Triops* may still strongly interfere with the recruitment from the dormant egg bank in *Daphnia* in several ways: through direct predation of dormant eggs and hatched juveniles and through bioturbation, with both the dislocation of eggs within the sediment (*cf.* Albertsson & Leonardsson, 2001; Gyllström et al., 2008) and increased turbidity changing light and oxygen conditions potentially leading to changed hatching dynamics.

The three studied populations strongly differed in their hatching pattern. The Belgian permanent lake population, had a very high hatching fraction (92%) and hatching was synchronized in time (most of them hatching during two consecutive days), in contrast to the observations for both temporary pond populations (French and Hungarian) which had considerably lower hatching fractions (66 and 39%, respectively) and longer hatching durations (spread over 13 days). Bet-hedging theory predicts that hatching fractions of dormant stages should be tuned to the probability of successful recruitment in the active population and on the survival probability of unhatched propagules (Cohen, 1966; Ellner, 1985). Our results, therefore, suggest that temporary pond populations hedge their bets by spreading hatching over several occasions (i.e., inundations), while this is not the case for the permanent population. The two temporary pond populations also appear to apply “short-term delayed hatching” as an additional, within-inundation cycle risk spreading strategy, since we observed a bimodal hatching pattern (first hatching peak after 5 days and second peak after 14 days) in these populations. Vanoverbeke & De Meester (2009) observed within-season delayed

hatching also in Belgian populations, especially when they inhabited unstable, temporary habitats. They linked the relative importance of immediate versus short-term delayed hatching (i.e., the relative importance of the first vs. the second hatching peak) to a trade-off between the advantages of quick niche filling and the risks associated with the unpredictability of reproductive success under unstable environmental conditions. Temporary ponds often have unpredictable hydrologic conditions and food availability at the start of the inundation, which may stimulate delayed hatching, while more stable conditions in permanent habitats promote high percentages of immediate hatching because small delays translate into large differences in fitness in an exponentially growing population. Both the among (hatching fraction) and the within-season delayed hatching are bet-hedging responses that represent strategies to cope with habitat uncertainty. It is striking that they show a nice tendency for joint variation across our three populations, with the Hungarian population showing the strongest risk spreading strategies for both responses and the Belgian population, the weakest (Fig. 1). An analysis of a wider range of populations is needed to confirm whether this is a general phenomenon.

To conclude, we found indications that *D. magna* populations may adjust their hatching pattern to the stability (permanent vs. temporary) of their habitat, while no indication was found that they also adjust their hatching pattern to the presence of *Triops* kairomones.

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