Predation Risk Perception in *Daphnia carinata* Induced by the Milt of Common Carp (*Cyprinus carpio*)

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**ABSTRACT**

Numerous experimental studies suggest that many organisms can sense predation risk and subsequently adjust their life history, behavior, and morphology as adaptations to predators. The aim of the present study was to test if fish milt would induce changes in *Daphnia carinata*. We exposed *D. carinata* to the milt of fish and then measured parameters of growth and reproduction until the offspring of the third clutch were released. Individual growth rate was higher in the treatment with fish milt than in the control. Size at the first three clutches was greater in the milt treatment than in the control. Age at the first three clutches was significantly younger in the high milt treatment than in the control. Moreover, *D. carinata* produced more offspring in the presence of fish milt. The degree of response by *D. carinata* was dose-dependent, correlating with the concentration of milt. *D. carinata* can use some substance originating from fish milt to sense risk of predation risk and change its life history to increase its fitness.

**INTRODUCTION**

Predation arguably serves as one of the key mechanisms that structure aquatic communities (Brooks and Dodson 1965, Wellborn et al. 1996). Predators may affect prey populations through direct consumption. On the other hand, to the prey, many anti-predator defensive reactions can be induced by the predation risk for better survival. In freshwater ecosystems, many studies have demonstrated that planktonic organisms can detect predators through chemical cues called kairomones, which enable them to express predator-specific responses for reducing predation risk (Stibor 1992, Larsson and Dodson 1993, Tollrian 1995).

The influences of fish kairomones on daphnids have been documented particularly well. Field observations and laboratory experiments suggest that daphnids can sense the presence of predators through kairomones and respond with changes in morphology, behavior, filter-feeding rate, or life history (Larsson and Dodson 1993, Tollrian 1995). Life-history changes in *Daphnia* spp. in response to predator kairomones have become a paradigm for chemically induced anti-predator defenses (Lass and Spaak 2003). In general, previous studies found the life-history responses of *Daphnia* spp. at risk from fish predators induced reduction in age and size at maturity, increased allocation of resources to reproduction, production of greater numbers of smaller offspring at a younger age (Hanazato et al. 2001, Sakwińska 2002), and increased growth rate and size at first reproduction when facing the risk from invertebrate predators (Walls et al. 1997, Stibor and Lampert 2000). Chemical signals from invertebrate and vertebrate predators can modify resource allocation. Stibor (2002) indicated that the yolk protein dynamics of *Daphnia magna* and its ability to exhibit flexible brood reduction behavior can help to explain kairomone-induced life history modifications of daphnids in numerous life history experiments.

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When confronted with a predator, individuals often alter their behavior to minimize predation risk (Szulkin et al. 2006). Kairomones from predators can lead large-bodied zooplankton to perform diel horizontal or vertical migration (Bollens and Frost 1991, De Meester and Cousyn 1997, Burks et al. 2002). Pijanowska and Kowalczewski (1997) reported kairomones released by predators can induce a significant decrease in daphnid swimming speed and an increased readiness to perform somersaulting escape behavior. Morphological responses to the presence of predator kairomones also have been described for many Daphnia species. Daphnia galeata can modify its head width, helmet length, and body curvature in the presence of fish kairomones (Weber and Vesela 2002). Daphnia lumholtzi neonates produced from individuals exposed to predator kairomones have significantly longer head and tail spines (Dzialowski et al. 2003). Tollrian and Heibl (2004) indicated it might be common that daphnids can reduce their pigmentation in the presence of fish kairomones to avoid the visually orienting predators. Phenotypic plastic responses to predators in ecological time may be a result of the long-term evolution and escalation of interactions between predators and prey (Agrawal 2001). These abilities of Daphnia spp. to produce some different phenotypes in multiple environments are thought to be important to ensure their survival.

In many previous studies, fish kairomones were used to refer to all chemicals directly or indirectly associated with the presence of predators. However, the chemical composition of kairomones remains unclear. Weber (2003) found that life history traits of D. galeata were affected differently by kairomones exuded from two fish species. There may be more than one fish kairomones. The different chemical compounds (such as mucus or faeces) released by fish predators may have different influences on daphnid (Śluarczyk and Rygielska 2004). During the reproductive season of fish, large amounts of eggs and milt are spawned into the lake water, and this will be followed by high predation pressure on zooplankton. Many studies have reported the influence of larval fish on the crustacean zooplankton through predation and kairomones (Vijverberg and Richter 1982, Hülsmann et al. 2004). However, it is not known whether Daphnia spp. can sense the predation risk through some chemical cues released by fish reproductive activity. In this study, we investigated the influence of kairomones exuded by the milt of common carp (Cyprinus carpio) on the life history of Daphnia carinata. The purpose of this study was to examine if fish milt is used by Daphnia carinata as a cue announcing fish predation risk.

METHODS AND MATERIALS

In the reproduction season, two spawning male common carp were collected from Lake Donghu, and a total of 8 mL of milt was obtained by abdominal compression. Milt was stored at -20 °C until used. A clone of D. carinata which was originally collected from Lake Caohu and maintained in our laboratory for more than four years, was used in the experiment. D. carinata was cultured at 25±0.5 °C under a photoperiod of 14 h light/10 h dark. We added 0.25 mL l⁻¹ and 0.025 ml l⁻¹ fish milt to aged lake water as the culture medium for the high and low milt treatment, respectively. These dilutions of milt were membrane filtered (0.45 μm) before use. In the control, the similarly filtered aged lake water was culture medium. The green alga Scenedesmus obliquus grown in an incubator was used as the food resource at a concentration of 10⁵ cell mL⁻¹. Scenedesmus obliquus was cultured in MA medium (Ichimura 1979).

Daphnia carinata neonates, not older than 8 h, were raised individually in 50 mL beakers containing 40 mL medium. There were 15 replicates per experimental treatment.
Initial neonate size was not significantly different according to one-way analysis of variance (ANOVA, \( p < 0.05 \)). Experimental media were renewed daily, at which time the body lengths of the daphnids (from the top of the head to the posterior of the carapace) were measured to the nearest 0.025 mm under a microscope at 40× magnification. Age and size at the first three clutches were recorded, as was the number of offspring. The newly born offspring were removed every day. We continued our observations until the offspring of the third clutch were released. Growth rate of body length (\( r, \text{day}^{-1} \)) was calculated according the following formula: 
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 r = \frac{\ln(L_2) - \ln(L_1)}{t_2 - t_1},
\]
where \( t_1 \) and \( t_2 \) are sampling days and \( L_1 \) and \( L_2 \) are body lengths at \( t_1 \) and \( t_2 \). Age at successive reproductive events was determined with the accuracy of 4 h (except for 8 h at night from 23:00 to 7:00). The effects of experimental treatment on observed life history parameters were statistically compared by one-way ANOVA.

RESULTS

The results showed that fish milt treatment can obviously promote the growth of \textit{D. carinata} (Fig. 1). In the beginning of our experiment, body length of \textit{D. carinata} neonates was no different between the experimental treatments (ANOVA, \( P > 0.05 \)). During the first 11 days, growth rate of \textit{D. carinata} was significantly lower in the control than in the high milt treatment (\( F = 19.889, P < 0.01 \)) and the low milt treatment (\( F = 5.758, P < 0.05 \)). The growth rate did not show a significant difference between the low milt treatment and the high milt treatment (\( F = 2.235, P = 0.146 \)). Presence of fish milt significantly influenced the size at first three clutches (Fig. 2). In the high milt treatment, \textit{D. carinata} sizes at the first three clutches all were greater than in the control (\( P < 0.01 \)). In the low milt treatment, \textit{D. carinata} size at the first reproduction was significantly larger than in the control (\( F = 5.7, P < 0.05 \)). However, in the subsequent two clutches, size was not significantly different from that in the low milt treatment or the control (\( P > 0.05 \)).

Ages at the first three clutches of \textit{D. carinata} all were shown to be significantly younger in high milt treatment than in the control (\( P < 0.01 \)) (Fig. 2), but age at each reproduction was not significantly different between the low milt treatment and the control (\( P > 0.05 \)). On average, offspring number of the high milt treatment was more than the low milt treatment and the control. However, there was no significant difference among the three treatments in the first two clutches (\( P > 0.05 \)). In the third reproduction,

![Figure 1. \textit{D. carinata} body length during the experimental period in the three treatments (control, low treatment, high treatment). Error bars represent 1 SD.](image-url)
the high milt treatment produced significantly more offspring than the low milt treatment \((F = 5.65, P < 0.05)\) and produced nearly significantly more offspring than the control \((F = 3.88, P = 0.06)\).

**DISCUSSION**

We found *Daphnia carinata* can change its life history in the presence of kairomones originating from the milt of common carp. The results suggest that milt spawned by fish may act as a danger signal for daphnids in lake water.

Our results showed that age at the first three clutches of *D. carinata* decreased and the offspring numbers increased in the presence of kairomones originating from fish milt, which was consistent with the previous studies using fish-exposed water as the treatment. Fish kairomones generally cause daphnids to mature at smaller sizes and younger ages and to produce larger first clutches (Stibor 1992, Hanazato et al. 2001, Sakwińska 2002). For relatively large-bodied zooplankton species, the probability of successful reproduction in the presence of visually hunting fish predators might be very small (Hall et al. 1976). A decreased age of first reproduction and increased offspring number might be advantageous for better survival when facing a higher predation risk.

Dissimilar to previous studies, our results showed an increased growth rate of *Daphnia carinata* and a larger size at the first reproduction in the treatment with fish milt. Many previous studies reported *Daphnia* spp. decrease their size at first reproduction in

![Figure 2. Body length, age, and offspring number of *D. carinata* at the first three clutches in the three treatments. Error bars represent 1 SD. Columns denoted by different letters are significantly different from each other (ANOVA, \(p < 0.05\)).](image-url)
the presence of fish predation and suggested that it is an energy reallocation with the trade-off between growth and reproduction for antipredation (Stibor 2002, Sakwińska 2002). However, the influence of kairomones on Daphnia spp. growth is associated with many factors, such as the initial neonate size and the food level (Reede 1997, Gliwicz and Maszczyk 2007). The response of life history to kairomones is not simply the result of a shift in resource allocation patterns (Reede 1997, Rinke et al. 2007). Weber (2003) indicated that there is substantial heterogeneity in life history response to different fish species and the presence of fish kairomone does not necessarily lead to the predicted trade-off between growth and reproduction. Kairomones originating from different predators might have opposite effects on the size of daphnids at first reproduction (Walls et al. 1997, Stibor and Lampert 2000). In general, a large size at first reproduction may mean a large offspring number. In the present study, it seems that increasing growth rate and maturity size is also likely an adaptation for the potentially higher predation risk during the period of fish spawning. However, the cost of these life history changes is not known. One possible interpretation is that D. carinata increases its feeding rate in the presence of milt-exuded kairomones. As we know, fish spermatozoa will die and be hydrolyzed after a short time (from 30 seconds to several minutes) when released into lake water (Scott and Baynes 1980). Recent research found that proteases and protease inhibitors widely exist both in spermatozoal extracts and seminal plasma of many fish species (Kowalski et al. 2003, Wojtczak et al. 2007). These enzymes or amino acids may be assimilated directly by daphnids or act as stimulants to increase the filtration and ingestion rates. And as a result, D. carinata demonstrated a fast growth rate in the milt treatments.

Phenotypic plasticity of prey may depend upon the concentration of kairomones originating from the predator as an indication of the proximity of threat (Reede 1995, Relyea 2002). In the present study, our results showed that the influence of milt-exuded kairomones was different in high treatment and low treatment. Thus, the degree of life history response by Daphnia may be dose-dependent and correlates with the strength of stimulus of fish milt.

Numerous experimental studies suggest that many organisms can predict predation risk and subsequently adjust their life history, behavior, and morphology as an adaptation to the predator (Larsson and Dodson 1993, De Meester 1996, Agrawal 2001). During late spring and early summer, juvenile fish play an important role in the mortality of Daphnia spp. (Vijverberg and Richter 1982). In the present study, it seems that D. carinata can use some substance originating from fish milt to sense risk of predation and change its life history for better reproductive output.

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