

Behavioral response of Lake Michigan *Daphnia mendotae* to *Mysis relicta*

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ABSTRACT. We performed laboratory experiments to determine if *Mysis relicta* induce changes in the behavior of *Daphnia mendotae* collected from Lake Michigan. Laboratory results indicate that *Daphnia* perceived *Mysis* kairomones and responded by changing their vertical position in cylinders. Experiments using different resource levels, and two procedures to examine the potential effects of the chemical cues from *Mysis* or from particulate matter or bacteria associated with capture and defecation of prey, suggest that *Daphnia* detect *Mysis* via a chemical cue. This is the first laboratory study that we are aware of that indicates that a zooplankton species from the Great Lakes responds behaviorally to an invertebrate predator. Our findings support the hypothesis that changes in vertical distribution of zooplankton associated with changes in invertebrate predator density, observed in previous Great Lakes studies, is due to behavioral responses to reduce predation risk. It is important to understand and quantify such responses, because predator-induced changes in prey behavior represent trait-mediated interactions that can potentially strongly affect prey growth rates, and indirectly affect resources, competitors, and predators of the prey.

INDEX WORDS: *Daphnia mendotae*, *Mysis relicta*, Lake Michigan, vertical migration, phenotypic plasticity, kairomone.

INTRODUCTION

In aquatic systems, predation risk is believed to be an important factor in determining the vertical position of many species in the water column (Zaret and Suffern 1976, Bowers and Vanderploeg 1982, Ohman *et al.* 1983, Hayes 2003). Predation provides a strong selective force on behavior, and many species respond to environmental cues, migrate according to diel cycles, or respond to changes in light level, in a manner that reduces spatial overlap with visual predators. More recently, researchers have found that species can perceive the presence of predators, often via chemical cues, and change the degree to which they migrate in an adaptive manner to increase fitness (Dodson 1988,

Ramcharan and Sprules 1991, Loose and Dawidowicz 1994, Van Gool and Ringelberg 1998, reviewed in Tollrian and Harvell 1999). Thus, not only has behavior (and other traits) been selected to reduce predation risk, but behavior can be modified depending on short-term changes in predation risk.

Invertebrate predators are recognized as playing a critical role in the structure of aquatic food webs, including those of the Great Lakes food webs (Bowers and Vanderploeg 1982, Branstrator and Lehman 1991, Johannsson *et al.* 1994, Schulz and Yurista 1998). To fully understand the effects of invertebrate predators it may be important to determine if they affect zooplankton prey phenotype such as vertical position. If zooplankton prey respond to invertebrate predators by modifying their behavior and other phenotypic traits to reduce predation risk, this could affect prey consumption rates

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of resources and vulnerability to predators (i.e., to the predator inducing the change and to other predators). By inducing change in zooplankton prey phenotype, a predator can therefore affect the fitness of both the responding prey, and species with which the prey interacts. Such interactions are termed trait-mediated interactions (Abrams *et al.* 1996) in order to differentiate them from density-mediated interactions that occur through density effects (i.e., predation). Trait-mediated interactions can contribute strongly to the net effect of a predator (Turner and Mittlebach 1990, Huang and Sih 1991, Peacor and Werner 2001, Peacor and Werner 2004, Romare and Hansson 2003, reviewed in Dill *et al.* 2003, and Werner and Peacor 2003). Further, theory predicts that trait-mediated effects will affect population dynamics and the stability of communities (reviewed in Bolker *et al.* 2003).

In Lake Michigan, the invertebrate predator *Mysis relicta* is a voracious predator of cladocerans, including *Daphnia* spp. (Bowers and Vanderploeg 1982). It has been hypothesized that this vulnerability to *Mysis* contributes to a vertical diel migration by *Daphnia* spp. that minimizes spatial overlap, and therefore vulnerability (Bowers and Vanderploeg 1982, Spencer *et al.* 1999, Clarke and Bennet 2003). Based on these patterns, we hypothesized that *Mysis* induce a change in *Daphnia mendotae* behavior, in particular vertical migration patterns, and we tested this hypothesis with laboratory experiments. Our results indicate that *D. mendotae* changed vertical position in experimental columns in response to *Mysis* kairomones (i.e., chemical cues produced by *Mysis*). Our results suggest that *D. mendotae* potentially modify their vertical migration patterns in the field in response to *Mysis* presence and absence. Such induced changes in behavior could contribute to the net effect of *Mysis* on *D. mendotae* density and also indirectly affect other species in Lake Michigan food web through trait-mediated interactions.

METHODS

General Experimental Approach

We conducted four laboratory experiments to investigate the behavioral response of *D. mendotae* to *Mysis* using two isofemale lines of *D. mendotae*, labeled clone-A and clone-B, originating from two females collected in Lake Michigan approximately 8 km offshore of Muskegon, Michigan. *D. mendotae* clones were transported to the Great Lakes Environmental Research Laboratory (NOAA) in Ann

Arbor, Michigan, and cultured separately in 4-L vessels in aged Lake Michigan water under experimental temperatures (20°C). Food conditions consisted of 6 mg/L of *Nanochloropsis limnetica* (SAG 18.99, University of Gottingen, Sag, Germany), and lighting was maintained at a 14 h light:10 h dark regime. *Mysis* were also collected in Lake Michigan near Muskegon, at sites approximately 75–100-m total depth, using a 1-m diameter net with 1,000- μ m mesh made after sunset. *Mysis* were maintained in the laboratory in a 30-L aquarium at 4°C.

In general, the experiments were designed to evaluate the vertical movement (i.e., upward or downward) of *D. mendotae* in response to *Mysis* kairomones in clear acrylic cylinders (e.g., Loose and Dawidowicz 1994, Pijanowska 1997, and Van Gool and Ringelberg 1998). *D. mendotae* (standard body length range, 1.5–2.2 mm) were transferred with a wide bore pipette into 60-cm tall, 26-mm diameter, clear acrylic cylinders that were filled with experimental water (e.g., water with *Mysis* kairomone and control water, see below) and submerged vertically in a transparent, 40-L aquarium. The aquarium acted as a water bath, and uniform water temperature was regulated at 20°C using an external chiller unit. The cylinders were illuminated by diffused light from directly above using three, 50W halogen bulbs. Light intensity was kept constant over the duration of the experiment, with a photon flux density of 16.0 μ Einst \cdot m⁻² at the top of the cylinder, and 4.0 μ Einst \cdot m⁻² at the bottom. No resources were added to the cylinders, except in Exp. 2 in which the effect of resource level was examined.

To quantify the vertical position of *D. mendotae*, the cylinders were demarcated in 10-cm intervals, creating six vertical sections that were assigned the values 1 to 6, with the lowest being closest to the bottom. *D. mendotae* were allowed to acclimate to experimental conditions for 1 h before observations were initiated. In Experiment 1, the vertical position of all *D. mendotae* in each cylinder was recorded every 20 min over a 4-h period (12 observations total). Experiments 1–3 were similarly conducted, however over a 4.5-h period, with observations recorded every 30 minutes (10 observations total).

Specific Experimental Treatments

Experiment 1 was conducted on 23 Jul 03 to evaluate the behavioral response of clone-A and clone-B *D. mendotae* to *Mysis* kairomones. The ex-

periment was carried out as a 2×2 factorial design with two clones and treatments with and without *Mysis* cue, thus four treatments total. In the treatment with *Mysis* cue, which we denote the “*Mysis* water” treatment, *Mysis* were transferred into refrigerated well water in 1-L containers (density, $10 \text{ Mysis} \cdot \text{L}^{-1}$) 5 days prior to the experiment, and fed on the first and last day of this period with both clone-A and clone-B *D. mendotae* (approximately three *D. mendotae* $\cdot \text{Mysis}^{-1}$). In the treatment without *Mysis* cue, the control treatment was well water maintained under similar condition with no *Mysis* or *D. mendotae* added. *Mysis* were held at a lower temperature (4°C) than used in the experiment (20°C) and would die if quickly transferred to this warm temperature. In order to allow the temperature of the experimental water to warm to experimental conditions, we therefore removed *Mysis* from the water, and transferred the remaining water, denoted “*Mysis* water,” and the control water to a 20°C incubator the evening before the experiment. Two hours prior to the experiment, the *Mysis* water and control water were passed through a $64\text{-}\mu\text{m}$ filter to remove large debris and then transferred into respective cylinders. We used a randomized factorial block design; each of the four treatments was represented in each of five spatial blocks, with each consecutive four cylinders representing a block (20 cylinders aligned side by side in total). Each cylinder received either five clone-A or six clone-B *D. mendotae* (we used different numbers depending on availability).

Three additional experiments were conducted on 5 Aug 03, 29 Aug 03, and 7 Nov 03, to examine if the presence of bacteria or debris associated with *Mysis* consumption of *D. mendotae* served as a resource that could contribute to the behavioral responses of *D. mendotae* to *Mysis*. Resource conditions can affect the magnitude of predator-induced changes in prey behavior (Werner and Anholt 1993, Grand and Dill 1999, Weber 2001, Weetman and Atkinson 2002). These experiments also served to test the robustness of the results of Experiment 1 and to shed insight into the origin of the kairomone to which *D. mendotae* respond. The general methods were similar to that of Experiment 1, and some minor deviations are listed in Table 1.

Experiment 2 consisted of a $2 \times 2 \times 3$ factorial design with two clones (A and B), two predator cue levels (control water and *Mysis* water), and three resource levels. We used 0, 0.5, and 3 mg/L of *N. limnetica* to represent low, intermediate, and high resource level treatments. Two replicate cylinders were designated to each treatment (24 cylinders total), and each cylinder received either eight clone-A or eight clone-B *D. mendotae*.

Experiment 3 consisted of three treatments: control, *Mysis* water, and a third treatment denoted “ultra-filtered *Mysis* water.” As in other experiments, all experimental water was passed through a $64\text{-}\mu\text{m}$ filter prior to the experiment; however, the ultra-filtered *Mysis* water was passed through an additional $0.46\text{-}\mu\text{m}$ filter. Past research has found that *D. mendotae* can selectively filter particles

TABLE 1. Summary of methods that varied in Experiments 1, 2, 3, and 4.

Procedures	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Treatments (in addition to control)	<i>Mysis</i> water	<i>Mysis</i> water and three resource levels	<i>Mysis</i> water and ultra-filtered <i>Mysis</i> water	<i>Mysis</i> water and particulate-free <i>Mysis</i> water
Clones	A and B	A and B	B	B
<i>Mysis</i> water production period (d)	5	4	4	2
<i>Mysis</i> diet during incubation	3 <i>Daphnia</i> $\cdot \text{Mysis}^{-1}$ on days 1 and 5	4 <i>Daphnia</i> $\cdot \text{Mysis}^{-1}$ on days 1 and 4	4 <i>Daphnia</i> $\cdot \text{Mysis}^{-1}$ on days 1, 2, 3, and 4	4 <i>Daphnia</i> $\cdot \text{Mysis}^{-1}$ on day 1
Experimental water thermal acclimation*	17-h period in incubator at 20°C	14-h period in incubator at 20°C	1-h period using warm-water bath	13.5-h period in incubator at 20°C

* Time period and method used to warm water originally at 4°C to 20°C used in experiment.

down to approximately 0.6 microns (Gophen and Geller 1984); therefore, ultra-filtration should eliminate all potential food resources for *D. mendotae*. Four replicate cylinders were designated to each treatment, and each cylinder received six clone-B *D. mendotae*. Note that in Experiment 3, *Mysis* and control water were warmed with a warm water bath (Table 1), and therefore warmed more quickly than in the other experiments in which the water was placed in the incubator to slowly warm over night. This difference in protocol was due to logistical constraints, but we believe it unlikely to affect the qualitative nature of the results.

Experiment 4 consisted of three treatments: control water, *Mysis* water, and a third treatment denoted "particulate-free *Mysis* water" treatment. This latter treatment was designed to include kairomones but no particulate matter. The *Mysis* water treatment was created using protocols similar to those described in Experiment 1. To create the particulate-free *Mysis* water treatment, 80 *D. mendotae* ($4 D. mendotae \cdot Mysis^{-1}$) were added to two 1-L beakers 3 days prior to the experiment, and *Mysis* were added. After 2 hours, the *Mysis* from both beakers were transferred into new beakers with well water in order to separate the *Mysis* from large particulate matter associated with consuming *Daphnia*. After an additional 24 hours (sufficient time for any consumed daphnia to have passed through the *Mysis*), the *Mysis* were again transferred into final beakers with well water which served as the particulate-free *Mysis* water treatment. This procedure ensured that the particulate-free *Mysis* water contained *Mysis*, but contained much less (almost no) particulate matter or bacteria associated with capture and defecation of *D. mendotae* relative to the regular *Mysis* water treatment. Indeed, fecal material was visually apparent at the bottom of the *Mysis* water treatment beakers, but not in the particulate-free *Mysis* water beakers. *Mysis* were removed from both the regular *Mysis* water treatment and the particulate-free *Mysis* water 13.5 h prior to the experiment (approximately 2 days after *Mysis* were isolated from particulate matter in the manner describe above). Seven cylinders were designated for each treatment (21 cylinders total) and six clone-B *D. mendotae* were transferred into each cylinder.

Quantification of Behavioral Response

The number of *D. mendotae* counted in each interval in each cylinder was averaged over the experimental observations. In each experiment, the

behavioral response of each treatment was evaluated based on two parameters: 1) the average interval occupied by *D. mendotae* in a cylinder (this metric is nearly equivalent to the average height of the *D. mendotae*), and 2) the fraction of the *D. mendotae* in the bottom half of the cylinder.

In each experiment, a fraction of the *D. mendotae* were not included in the observations because they either died or adhered to air bubbles on the side of the cylinder during the observation period. We quantified this loss using the metric, percent loss (l), which was calculated as $l = 100 \cdot (1 - N_f / N_i)$, where N_i was the initial number of individuals counted in each cylinder, and N_f was the final number of individuals counted in each cylinder. The percent loss was 1.71 ± 5.26 (mean \pm stdev), 4.6 ± 8.2 , 17 ± 13 , and 9.8 ± 11.5 , in Experiments 1, 2, 3, and 4, respectively. Percent loss was analyzed in two ways to determine if it created confounding effects on experimental parameters. First, we analyzed if there were treatment effects on percent loss using analysis of variance (ANOVA). Second, the two behavioral responses (above) were analyzed using analysis of covariance (ANCOVA), with percent loss designated as a covariate to experimental parameters. Both tests revealed no significant effects caused by percent loss, and therefore, factors that contributed to loss of *D. mendotae* did not confound the results on behavior.

Statistical Analysis

We analyzed untransformed data using a full factorial ANOVA following the generalized linear model (GLM) procedures (SAS Institute 1990). Data from Experiments 1 and 2 were blocked by clonal group and simple main effects of *Mysis* were evaluated within each block. Our null hypothesis for all experiments was that there would be no significant difference ($\alpha = 0.05$) among treatments. When significant differences among treatments were detected, a Tukey pair-wise comparison was used to separate the means. Based on Lilliefors tests, the data did not deviate from normality.

RESULTS

The experiments indicated that *D. mendotae* collected from Lake Michigan changed their position in the experimental cylinders in response to *Mysis* water, a result consistent with *D. mendotae* response to *Mysis* kairomones. In Experiment 1, both clones placed in *Mysis* water occupied lower posi-

TABLE 2. Factorial ANOVA on treatment effects on average interval position and fraction of *Daphnia* in the bottom half of the cylinder in Experiment 1. Simple main effects in *Mysis* treatments were also determined within each clone. Asterisks denote significant effects ($P < 0.05$).

Sources	df	Interval position		Fraction in bottom half	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
<i>Mysis</i>	1, 16	33.06	< 0.01*	19.43	< 0.01*
Clone	1, 16	30.81	< 0.01*	22.47	< 0.01*
<i>Mysis</i> × Clone	1, 16	5.56	0.031*	4.5	0.05*
<i>Mysis</i> (clone A)	1, 16	6.46	0.02*	2.44	0.11
<i>Mysis</i> (clone B)	1, 16	24.04	< 0.01*	22.96	< 0.01*

tions in the cylinders (Table 2, Fig. 1), on average, than in control water. Figure 1a illustrates the percentage of *D. mendotae* observed at each of the six demarcated intervals. In the *Mysis* water, *D. mendotae* were much more unevenly distributed than in the control treatment, with a much higher percentage (77% higher) of *D. mendotae* occupying the lowest 10-cm section. The difference in distribution is further shown in Figures 1b and 1c, which illustrate the effect of treatment on the two metrics we used to quantify the vertical distribution. In *Mysis* water, the average interval occupied by *D. mendotae*, and the fraction of *D. mendotae* in the bottom half of the cylinder, were significantly lower and higher than in the control, respectively.

Experiment 2 did not reveal effects of resource level on *D. mendotae* behavior (Fig. 2a, Table 3). Unfortunately, the difference in *D. mendotae* position in the cylinders within a treatment showed higher variation in this experiment for unknown reasons, and therefore, any potential effects were less likely to be evident. However, there clearly was not a very strong effect of the resource. Because there was no effect of resource, we collapsed the design and combined all different resource levels as one treatment. Examined in this way, the main effect of the *Mysis* on both measures of *D. mendotae* distribution was not significant (Table 3). However, there was a significant clone by *Mysis* interaction. This interaction arose because clone-A was significantly higher in the *Mysis* water treatment (Figs. 2b and 2c, Table 3) than in the control, but there was a non-significant effect of *Mysis* in the opposite direction on clone-B (Figs. 2b and 2c, Table 3).

In Experiment 3, *Mysis* water caused clone-B *D.*

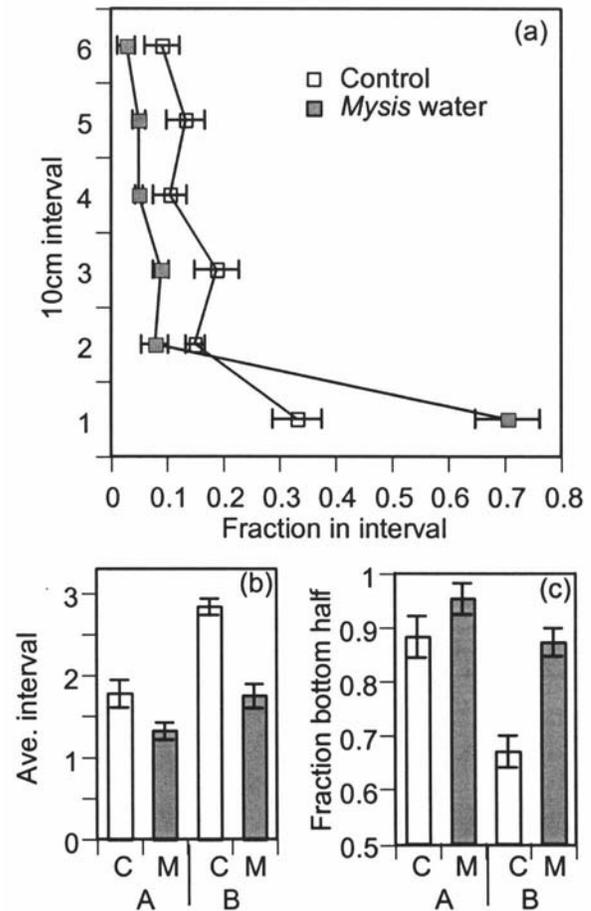


FIG. 1. Vertical distribution of clone-A and clone-B *D. mendotae* in control water (C) and *Mysis* water (M) in Experiment 1. (a) Fraction of clone-B *Daphnia* that occupied each of the 6 10 cm sections, where 1 was the lowest section (results of clone-A similar). (b) Average section occupied by *Daphnia* in a cylinder, where lower numbers represent *Daphnia* lower in the cylinder. Error bars are the standard error (SE) between cylinders within a treatment. (c) Fraction of the Clone A (A) and Clone B (B) *Daphnia* in the bottom half (mean \pm SE) of the cylinder (i.e., *Daphnia* that occupied one of the bottom 3 of 6 segments).

mendotae to move higher (Fig. 3) than in the control, however, this response was much weaker or non-existent in the ultra-filtered *Mysis* water treatment. The average interval occupied by *D. mendotae* in *Mysis* water was significantly greater ($F_{2,3} = 9.75$; $P = 0.01$) than in control and ultra-filtered *Mysis* water. In addition, the fraction of *D. mendo-*

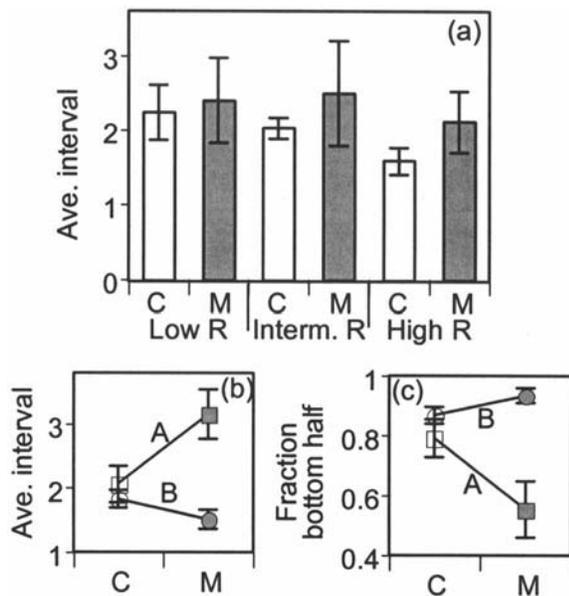


FIG. 2. Vertical distribution of clone-A and clone-B *Daphnia* in Experiment 2. (a) Vertical distribution of *Daphnia* (response of clone-A and clone-B averaged) in *Mysis* water (M) and control water (C) at the low (Low R), intermediate (Interm. R) and high (High R) resource level. (b) Effect of *Mysis* water on the average interval position of clone-A and clone-B. Squares and circles for clone-A and clone-B, respectively, and empty and filled symbols for control (C) and *Mysis* (M) water, respectively. (c) The effect of *Mysis* water on the fraction of clone-A and clone-B *Daphnia* in the bottom half of the cylinder. Error bars represent SE.

tae in the bottom half of the cylinder in *Mysis* water was significantly lower than in control and ultra-filtered *Mysis* water ($F_{2,3} = 7.59$; $P = 0.01$). In contrast, while there was a small trend for the ultra-filtered *Mysis* water in the same direction as the *Mysis* water, neither the average interval occupied, nor the fraction in the bottom half, were significantly different than the control ($P > 0.4$ for both responses).

In Experiment 4, *Mysis* water also caused clone-B *D. mendotae* to move higher into the water cylinder (Fig. 4). There was a high percentage of *D. mendotae* in the bottom 10-cm interval in the control treatment, but this pattern was reversed in the regular *Mysis* water and particulate-free *Mysis* treatments, in which the proportion of *D. mendotae* was much higher in the top two 10-cm sections

TABLE 3. Factorial ANOVA on treatment effects on average interval position and fraction of *Daphnia* in the bottom half of the cylinder in Experiment 2. Resource effects on both parameters were not significant ($F = 1.08$, $P = 0.37$; $F = 0.72$, $P = 0.51$, respectively), so they were removed from the analysis. Simple main effects in *Mysis* treatments were also determined within each clone. Asterisks denote significant effects ($P < 0.05$).

Sources	df	Interval position		Fraction in bottom half	
		F	P	F	P
<i>Mysis</i>	1, 20	2.50	0.13	2.31	0.14
Clone	1, 20	14.58	< 0.01*	16.38	< 0.01*
<i>Mysis</i> × Clone	1, 20	8.30	0.01*	7.28	0.01*
<i>Mysis</i> (clone A)	1, 20	9.96	< 0.01*	8.90	< 0.01*
<i>Mysis</i> (clone B)	1, 20	0.84	0.37	0.69	0.42

(Fig. 4a). As a result, *D. mendotae* in both *Mysis* water treatments occupied a significantly higher average interval ($F_{2,6} = 11.62$; $P < 0.01$) than those in control water. *D. mendotae* in both *Mysis* water treatments also had a significantly lower ($F_{2,6} = 9.88$; $P < 0.01$) fraction of individuals in the bottom half of the cylinder than those in the control water. Importantly, the particulate-free *Mysis* water treatment, which was designed to greatly reduce particulate matter, had a strong effect on behavior and was not significantly different from the regular *Mysis* water treatment (Fig. 4).

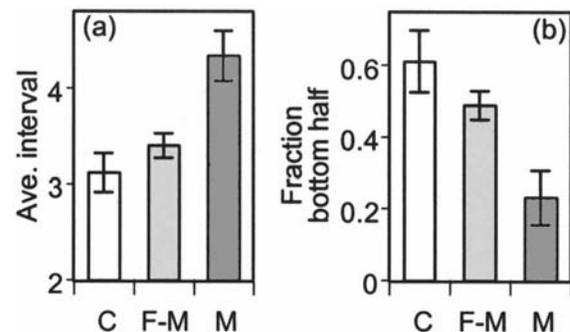


FIG. 3. Vertical position of clone-B *Daphnia* in control water (C), *Mysis* water (M), and *Mysis* water filtered with a fine filter (F-M). (a) Average interval position and (b) fraction of *Daphnia* in the bottom half of the cylinder. Bars are means \pm SE's of four replicates per treatment.

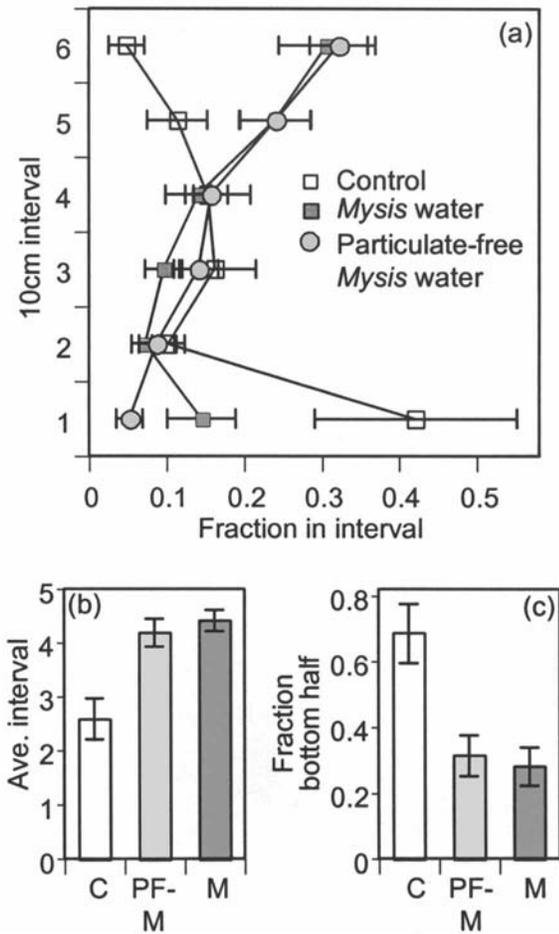


FIG. 4. Vertical position of clone-B *Daphnia* in control water (C), *Mysis* water (M), and “particulate-free *Mysis* water” (PF-M) in which particulate matter from consumption and defecation of the *Daphnia* they were fed before the experiment was greatly reduced (see text). Bars are means \pm SE's of seven replicates per treatment. Figures (a), (b) and (c) as in Figure 1.

DISCUSSION

The principal finding of this study is that *Mysis relicta* from Lake Michigan induced a change in the behavior of a prey species, *Daphnia mendotae*. Clearly this response must be olfactory, because visual or tactual cues were absent in the experimental cylinders. Experiments 2, 3, and 4 lend insight into the origin of the *Mysis* cue. It is possible that particulate matter and bacteria associated in the *Mysis* water served as a resource that could confound the response of the *D. mendotae* to kairomones, given that induced traits of *Daphnia* have been shown to be affected by resource level (Weber 2001, Weet-

man and Atkinson 2002). However, in Experiment 2, large changes in resource level had no effect on the behavior of the *D. mendotae*, nor on the response of the *D. mendotae* to the *Mysis* water. Further, in Experiment 4, the response of the *D. mendotae* without this particulate matter was strong, and response variables were not significantly different between the treatment with, and without, the particulate matter. This suggests that rather than responding to particulate matter, *D. mendotae* more likely responded to chemical cues (i.e., kairomones) emitted by *Mysis*. Experiment 3 suggests that these chemicals are large enough to be removed by a 0.46- μ m filter, as *Mysis* water that had been filtered through such a fine filter had little or no effect on *Mysis* vertical position. This may offer a clue into the identity and properties of the kairomones, which are not well-known or understood in invertebrate predator-prey systems (Lass and Spaak 2003). Note that Experiment 4 suggests that the cue is not associated with alarm signals from conspecifics (Pijanowska 1997) because *D. mendotae* responded to water that never contained conspecifics, but rather only contained *Mysis* previously fed *D. mendotae*.

While this is the first laboratory study we are aware of that demonstrates a behavioral response of a Great Lakes zooplankton species to a predator, several field studies have indicated shifts in zooplankton vertical position associated with changes in invertebrate predator density. For example, Benoit *et al.* (2002) used seasonal time series to examine the vertical distribution of zooplankton before and after the invasion of *Cercopagis pengoi* in Lake Ontario. They found that juvenile copepods occupied deeper waters after *Cercopagis* invaded in 1999. They hypothesized that this was due to either predator avoidance behavior, or localized depletion. Further, Lehman and Caceres (1993) showed that the cladocerans *Bosmina longirostris*, *D. mendotae*, and *D. pulicaria* shifted their vertical distributions following the invasion of *Bythotrephes longimanus* in Lake Michigan offshore from Grand Haven, Michigan. Similarly, Johannsson (O. Johannsson, Department of Fisheries and Oceans Canada, 3 Mar 04) has found that the average depth of *Bosmina* and *Daphnia* in Lake Erie is inversely related to *Bythotrephes* densities. These studies are suggestive of zooplankton response to invertebrate kairomones in the Great Lakes. Note, however, that other mechanisms could lead to these patterns. For example, zooplankton may be responding to other factors correlated with increases in predator density, or the

changes in distribution in predator presence could be due to density effects (i.e., differential predation on clones with higher spatial overlap with the predator).

Surprisingly, the response of *D. mendotae* from the same clone was qualitatively different in different experiments. For example, in *Mysis* water (i.e., water that previously contained *Mysis*), clone-B occupied a lower position in Experiment 1 than in the control, did not respond significantly in Experiment 2, and occupied a higher position in Experiments 3 and 4. While we are aware of studies that show different predators can induce migration in different directions (Ohman *et al.* 1983, Hayes 2003) we are unaware of experiments that show the same predator species (and size class) can induce such a different response in the same prey. This ambiguity in the response is indicative of the complexity of the response of *Daphnia* to different environmental cues and/or internal states such as hunger level (Weetman and Atkinson 2002). While we attempted to hold all conditions constant (e.g., *Daphnia* were cultured in a similar manner and fed the day before each experiment), differences in the resources and light levels in the culturing environment of *Daphnia*, in their handling during transfer to the experiment, or in the preparation of *Mysis* cue, may have caused the *Daphnia* to respond differently (Weber 2001). While more research is required to understand the complexity of the response, the ambiguity does not change our principal result that Lake Michigan *D. mendotae* can perceive and respond to the presence of *Mysis* kairomones.

Note that the data (Figs. 1 and 2) suggest a difference in clonal response to a predator cue, which has been reported by other investigators for *Daphnia* (Dodson 1988, Weber and Declerck 1997, Weber and Van Noordwijk, 2002, Michels and De Meester 2004). We hesitate to make this claim here as the experiment was not designed to account for subtle differences in their culturing environment. A design which raises multiple groups of each clone using a factorial design would be needed to avoid pseudoreplication and thereby isolate clonal differences from those that arise from subtle differences in the culturing environment (Hurlbert 1984).

Predator-induced changes in vertical position and diel migration timing of *Daphnia* and other zooplankton in both freshwater and marine systems have been well documented (Frost and Bollens 1992, Fortier *et al.* 2001, Irigoien *et al.* 2004, reviewed in Hayes 2003, and Lass and Spaak 2003). Typically, movement is in a direction that is adap-

tive and reduces predation risk. In the case of the *Mysis relicta*-*Daphnia mendotae* interaction in Lake Michigan, we would predict that it would be adaptive for *D. mendotae* to migrate up in response to cues of *Mysis* presence. This is because, at night, *Mysis* migrate up from the hypolimnion and lower metalimnion, into the upper metalimnion and lower epilimnion. *D. mendotae* have been shown to simultaneously migrate away from the regions inhabited by *Mysis* to higher regions in the epilimnion (Bowers and Vanderploeg 1982). Bowers and Vanderploeg argue that this coordinated movement upward by *Mysis* and *D. mendotae* represents an evolutionary refugium of *Daphnia* from *Mysis* that minimize contact between the two species. Similar simultaneously migrations between *Daphnia* and *Mysis* have also been observed in Lake Pend Orielle, Idaho (Clarke and Bennet 2003) and Flathead Lake, Montana (Spencer *et al.* 1999).

Our study suggests that *D. mendotae* not only may have evolved to move upward to avoid *Mysis* at certain times of the day, but that they can also perceive *Mysis* presence, and thus modify the degree to which they migrate in an adaptive manner in response to variation in risk from *Mysis*. This is an important distinction, because if *D. mendotae* modify the degree to which they migrate as a function of *Mysis* density, then the immediate effects of *Mysis* on *D. mendotae* extend beyond the effect they have on *D. mendotae* density. Induced changes in behavior could affect *D. mendotae* population growth rates through changes in temperature (Lueke *et al.* 1990, Loose and Dawidowicz 1994), resource availability (Gielbelhausen and Lampert 2001) and risk to other predators (Liljendahl-Nurminen *et al.* 2003). For example, Loose and Dawidowicz (1994) have shown that in the presence of fish kairomone, *Daphnia magna* will migrate to colder temperatures that cause a 62% reduction in intrinsic growth rate, a value that most likely will contribute strongly to the net effect of the predator (Peacor and Werner 2004). Similarly, Winder *et al.* (2003) found that the vertical migration exhibited by *Daphnia galeata* was associated with a large thermal cost to growth and development rates in a high mountain lake. Further, such behavioral modifications can lead to trait-mediated indirect interactions, in which predators indirectly affect species through their effect on prey traits (such as behavior). Such indirect interactions are becoming increasingly recognized as playing a significant role in the net effect of predators (Turner and Mittlebach 1990, Huang and Sih 1991, Peacor and Werner

2001, Romare and Hansson 2003, reviewed in Dill *et al.* 2003, and Werner and Peacor 2003), and are predicted to affect population and community structure (Abrams 1984, reviewed in Bolker *et al.* 2003).

One factor that could influence an adaptive response of Lake Michigan *D. mendotae* to *Mysis* is the recent invasion of *Bythotrephes* (Lehman 1987). *Bythotrephes* is a visual predator, and therefore it may be adaptive for *D. mendotae* to respond to *Bythotrephes* by swimming lower in the water column. Indeed, Lehman and Cáceres (1993) found that *D. mendotae* was lower in the water column after *Bythotrephes* invasion. Whether the presence of this new species has affected the response to *Mysis* would depend on several factors, including how fast zooplankton evolve to a changing predator assemblage, and whether zooplankton such as *D. mendotae* can differentiate cues of different predators. There are several studies that indicate that *Daphnia* can differentiate between fish and invertebrate predators (e.g., Dodson 1988, Brett 1992, Weber and Van Noordwijk 2002, Dzialowski *et al.* 2003), and different invertebrate predators (e.g., *Chaoborus* and *Notonecta*, Dodson 1988, see also Nesbitt *et al.* 1996). We have recently conducted pilot studies (Pangle, unpublished data) that also suggest that *D. mendotae* can differentiate between *Mysis* and *Bythotrephes* cues, however more work is needed. Laboratory experiments could further shed insights into the interactive effects of cues from multiple predators, including fish (e.g., will cues fish overshadow those from *Mysis*?). In addition, laboratory experiments could be used to elucidate potential interactive effects of zooplankton responses to predator density and conspecific density, or even behavioral responses to the cues arising by other conspecific individuals responding to predation risk (Relyea 2002, Ralph Tollrian, personal communication, reviewed in Elgar 1989, Lima 1990, Roberts 1996, Peacor 2003). How Great Lakes zooplankton will respond to different predators, competitors, resources, and interactive effects between these factors, are complex and interesting questions that deserve further attention.

This study indicates that *Mysis* induce changes in behavior of Lake Michigan *Daphnia*. In the field, such a response could affect *Daphnia* population growth rate (e.g., through changes in temperature, resource availability, or predation from other predators), and indirectly affect resources, competitors, and predators of *Daphnia*. Clearly it is not possible to make direct inferences from laboratory experiments performed in small cylinders to interactions

that occur on the scale they do in the Lake Michigan. However, such laboratory experiments can contribute to our knowledge of the system and be an integral part of a larger comprehensive study (Werner 1998). But this is only a first step, and more studies are needed to gain further understanding and predictive capabilities. First, we must establish if the signals of *Mysis* are strong enough in the field to elicit strong responses. In our laboratory experiment, the concentration of the kairomone was likely much higher than that found in the field. One potential procedure is to use water from areas inhabited and not inhabited by *Mysis* in similar experiments as those performed here. It would also be helpful to examine the response to *Mysis* in more realistic conditions, across a range of light levels and temperature levels (*Mysis* are typically found at much lower temperatures and light levels than used in our experiment for convenience). Further, it will be important to combine survey and experimental studies to establish (1) how potential changes in vertical distribution will affect *Daphnia* growth rate in the field, and (2) how factors such as resource level and temperature affect the degree of migration, and in turn affect the consequences to growth rate. We believe that a combined field, laboratory, and modeling effort is required to understand the consequences of predator-induced behavioral responses of zooplankton prey in the Great Lakes.

CONCLUSION

To gain an increased understanding of the potential effects of invertebrate predators such as *Mysis* on their prey in the Great Lakes, it is important to understand the potential effects they exert by inducing changes in prey phenotype, including behavior. Ecologists are becoming increasingly aware that such responses can contribute to the net consequences of species interaction in many systems, including freshwater. That is, not only are such responses interesting from an evolutionary and behavioral standpoint, but they may play a critical role in the structure and dynamics of food webs. However, phenotypic responses to predators and potential consequent trait-mediated interactions have not received attention in the study of Great Lakes ecosystems. This may be particularly important in describing the effects of recent invasions of predatory cladocerans (i.e., *Bythotrephes longimanus* and *Cercopagis pengoi*) and of future invaders.

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REFERENCES

- Abrams, P.A. 1984. Foraging interactions and interactions in food webs. *Am. Nat.* 124:80–96.
- , Menge, B.A., Mittelbach, G.G., Spiller, D., and Yodzis, P. 1996. The role of indirect effects in food webs. In *Food webs: dynamics and structure*, eds. G. Polis and K. Winemiller, pp. 371–395. New York, NY: Chapman and Hall.
- Benoit, H.P., Johannsson, O.E., Warner, D.M., Sprules, W.G., and Rudstam, L.G. 2002. Assessing the impact of a recent predator invader: The population dynamics, vertical distribution, and potential prey of *Cercopagis pengoi* in Lake Ontario. *Limnol. Oceanogr.* 47:626–635.
- Bolker, B., Holyoak, M., Krivan, V., Rowe, L., and Schmitz, O. 2003. Connecting theoretical and empirical studies of trait-mediated interactions. *Ecology* 84:1101–1114.
- Bowers, J.A., and Vanderploeg, H.A. 1982. *In situ* predatory behavior of *Mysis relicta* in Lake Michigan. *Hydrobiol.* 93:121–131.
- Branstrator, D.K., and Lehman, J.T. 1991. Invertebrate predation in Lake Michigan—regulation of *Bosmina longirostris* by *Leptodora kindtii*. *Limnol. Oceanogr.* 36:483–495.
- Brett, M.T. 1992. *Chaoborus* and fish-mediated influences on *Daphnia longispina* population structure, dynamics, and life-history strategies. *Oecologia* 89:69–77.
- Clarke, L.R., and Bennet, D.H. 2003. Seasonal zooplankton abundance and size fluctuations across spatial scales in Lake Pend Orielle, Idaho. *J. Freshwater Ecol.* 18:277–290.
- Dill, L.M., Heithaus, M.R., and Walters, C.J. 2003. Behaviorally mediated indirect interactions in marine communities and their conservation implications. *Ecology* 84:1151–1157.
- Dodson, S. 1988. The ecological role of chemical stimuli for the zooplankton-predator-avoidance behavior in *Daphnia*. *Limnol. Oceanogr.* 33:1431–1439.
- Dzialowski, A.R., Lennon, J.T., O'Brien, W.J., and Smith, V.H. 2003. Predator-induced phenotypic plasticity in the exotic cladoceran *Daphnia lumholtzi*. *Freshw. Biol.* 48:1593–1602.
- Elgar, M.A. 1989. Predator vigilance and group size in mammals and birds: a critical review of the empirical evidence. *Biological Reviews of the Cambridge Philosophical Society* 64:13–33.
- Fortier, M., Fortier, L., Hattori, H., Saito, H., and Legendre, L. 2001. Visual predators and the diel vertical migration of copepods under Arctic sea ice during the midnight sun. *J. Plankton Res.* 23:1263–1278.
- Frost, B.W., and Bollens, S.M. 1992. Variability of diel vertical migration in the marine planktonic copepod *Pseudocalanus newmani* in relation to its predators. *Can. J. Fish. Aquat. Sci.* 49:1137–1141.
- Gielbelhausen, B., and Lampert, W. 2001. Temperature reaction norms of *Daphnia magna*: the effect of food concentration. *Freshwater Biol.* 46:281–289.
- Gophen, M., and Geller, W. 1984. Filter mesh size and food particle uptake by *Daphnia*. *Oecologia* 64:408–412.
- Grand, T.C., and Dill, L.M. 1999. The effect of group size on the foraging behaviour of juvenile coho salmon: reduction of predation risk or increased competition? *Anim. Behav.* 58:443–451.
- Hayes, G.C. 2003. A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migration. *Hydrobiol.* 503:163–170.
- Huang, C.F., and Sih, A. 1991. Experimental studies on direct and indirect interactions in a three trophic-level stream system. *Oecologia* 85:530–536.
- Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* 54:187–211.
- Irigoien, X., Conway, D.V.P., and Harris, R.P. 2004. Flexible diel vertical migration behaviour of zooplankton in the Irish Sea. *Mar. Ecol. Prog. Ser.* 267:85–97.
- Johannsson, O.E., Rudstam, L.G., and Lasenby, D.C. 1994. *Mysis relicta*—assessment of metalimnetic feeding and implications for competition with fish in Lakes Ontario and Michigan. *Can. J. Fish. Aquat. Sci.* 51:2591–2602.
- Lass, S., and Spaak, P. 2003. Chemically induced anti-predator defenses in plankton: a review. *Hydrobiol.* 491:221–239.
- Lehman, J.T. 1987. Palearctic predator invades North American Great Lakes. *Oecologia* 74:478–480.
- , and Caceres, C.E. 1993. Food-web responses to species invasion by a predatory invertebrate: *Bythotrephes* in Lake Michigan. *Limnol. Oceanogr.* 38:879–891.
- Liljendahl-Nurminen, A., Horppila, J., Malinen, T., Elooranta, P., Vinni, M., Alajarvi, E., and Valtonen, S. 2003. The supremacy of invertebrate predators over fish—factors behind the unconventional seasonal

- dynamics of cladocerans in Lake Hiidenvesi. *Arch. Hydrobiol.* 158:75–96.
- Lima, S.L. 1990. The influence of models on the interpretation of vigilance. In *Interpretation and Explanation in the Study of Animal Behaviour: Vol. 2. Explanation, Evolution and Adaptation*, ed. M. Bekoff and D. Jamieson, pp. 246–267. Westview Press.
- Loose, C.J., and Dawidowicz, P. 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. *Ecology* 75:2255–2263.
- Lueke, C., Vanni, M.J., Magnuson, J.J., Kitchell, J.K., and Jacobson, P.T. 1990. Seasonal regulation of *Daphnia* populations by planktivorous fish: implications for the spring clear-water phase. *Limnol. Oceanogr.* 35:1718–1733.
- Michels, E., and De Meester, L. 2004. Inter-clonal variation in phototactic behavior and key life-history traits in a metapopulation of the cyclical parthenogen *Daphnia ambigua*: the effect of fish kairomones. *Hydrobiol.* 522:221–233.
- Nesbitt, L.M., Riessen, H.P., and Ramcharan, C.W. 1996. Opposing predation pressures and induced vertical migration responses in *Daphnia*. *Limnol. Oceanogr.* 41:1306–1311.
- Ohman, M.D., Frost, B.W., and Cohen, E.B. 1983. Reverse diel vertical migration: an escape from invertebrate predators. *Science* 220:1404–1407.
- Peacor, S.D. 2003. Phenotypic modifications to conspecific density: a new mechanism arising from predation risk assessment. *Oikos* 100:409–415.
- , and Werner, E.E. 2001. Effects of predators on ecological communities: the role of adaptive prey behavior. *Proc. Natl. Acad. Sci. U. S. A.* 98:3904–3908.
- , and Werner, E.E. 2004. How dependent are species-pair interaction strengths on other species in the food web? *Ecology* 85:2754–2763.
- Pijanowska, J. 1997. Alarm signals in *Daphnia*? *Oecologia* 112:12–16.
- Ramcharan, C.W., and Sprules, W.G. 1991. Predator-induced behavioral defense and its ecological consequences for two calanoid copepods. *Oecologia* 86:276–286.
- Relyea, R.A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. *Ecological Monographs* 72:523–540.
- Roberts, G. 1996. Why individual vigilance declines as group size increases. *Animal Behaviour* 51:1077–1086.
- Romare, P., and Hansson, L.A. 2003. A behavioral cascade: Top-predator induced behavioral shifts in planktivorous fish and zooplankton. *Limnol. Oceanogr.* 48:1956–1964.
- SAS Institute. 1990. *SAS/STAT user's guide, version 6, 4th edition*. Cary, North Carolina: SAS Institute.
- Schulz, K.L., and Yurista, P.M. 1998. Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. *Hydrobiol.* 380:179–193.
- Spencer, C.N., Potter, D.S., Bukantis, R.T., and Stanford, J.A. 1999. Impact of predation by *Mysis relicta* on zooplankton in Flathead Lake, Montana, USA. *J. Plankton Res.* 21:51–64.
- Tollrian, R., and Harvell, C.D. 1999. *The ecology and evolution of inducible defenses*. Princeton, N. J.: Princeton University Press.
- Turner, A.M., and Mittelbach, G.G. 1990. Predator avoidance and community structure: interactions among piscivores, planktivores, and plankton. *Ecology* 71:2241–2254.
- Van Gool, E., and Ringelberg, J. 1998. Light-induced migration behaviour of *Daphnia* modified by food and predator kairomones. *Anim. Behav.* 56:741–747.
- Weber, A. 2001. Interactions between predator kairomone and food level complicate the ecological interpretation of *Daphnia* laboratory results. *J. Plankton Res.* 23:41–46.
- , and Declerck, S. 1997. Phenotypic plasticity of *Daphnia* life history traits in response to predator kairomones: genetic variability and evolutionary potential. *Hydrobiol.* 360:89–99.
- , and Van Noordwijk, A. 2002. Swimming behavior of *Daphnia* clones: differentiation through predator infochemicals. *J. Plankton Res.* 24:1335–1348.
- Weetman, D., and Atkinson, D. 2002. Antipredator reaction norms for life history traits in *Daphnia pulex*: dependence on temperature and food. *Oikos* 98:299–307.
- Werner, E.E. 1998. Ecological experiments and a research program in community ecology. In *Experimental ecology: issues and perspectives*, eds. W.J. Reseterits and J. Bernardo, pp. 3–26. Oxford: Oxford University Press.
- , and Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *Am. Nat.* 142:242–272.
- , and Peacor, S.D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100.
- Winder, M., Boersma, M., and Spaak, P. 2003. On the cost of vertical migration: are feeding conditions really worse at greater depths? *Freshwater Biol.* 48:383–393.
- Zaret, T.M., and Suffern, J.S. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. *Limnol. Oceanogr.* 21:804–813.

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