

## Trimethylamine does not trigger antipredatory life history shifts in *Daphnia*

**Abstract**—I studied the responses of *Daphnia galeata* and *Daphnia hyalina* × *galeata* life history traits to trimethylamine (TMA) and fish kairomone. TMA and fish exudate had opposite effects on age at maturity and fecundity in two different experiments. The effects on size at maturity and offspring size were similar. Specific antipredatory life history shifts occurred only in response to fish exudate, whereas TMA caused changes that could be expected from any mildly harmful substance.

It is well established that antipredator defenses in *Daphnia*, diel vertical migration (DVM) and life history shifts, can be induced by fish-exuded kairomones (Tollrian and Dodson 1999). Attempts to identify the chemical identity of the kairomone triggering these responses resulted in its preliminary chemical characterization (Loose et al. 1993; Von Elert and Loose 1996).

A recent paper by Boriss et al. (1999) reported that trimethylamine (TMA) induces diel vertical migration in *Daphnia* and it is a biologically active component of the fish-exuded kairomone. If indeed TMA were an ecologically relevant cue, it should also induce characteristic antipredator life history shifts. The best known life history shifts in *Daphnia hyalina* and *Daphnia galeata* × *hyalina* in response to fish exudates are earlier maturation at smaller size and larger clutches of smaller offspring (Stibor 1992; Reede 1995). These shifts are considered adaptive because they increase the probability of survival and successful reproduction.

The *Daphnia* clones used for this study originated from Lake Constance, Germany, and were kept in culture for about a year before the experiments started.

There were two experiments, each with two TMA treatments (two concentrations): a fish treatment and a control. Modified AdaM medium was used (Klüttgen et al. 1994) (modified by using 0.05 SeO<sub>2</sub> concentration and adding 20% water from a local well). The water for the fish treatment was obtained by keeping three fish (*Leuciscus leuciscus*, body length 7 cm) in a 10-liter aquarium for 24 h, whereas the control treatment received water from an identical aquarium but without fish. Both control and fish water were filtered through 0.45- $\mu$ m filter. TMA treatments were made from the control medium. The aquaria were cleaned and refilled daily. The fish were fed frozen Chironomidae every second day.

In experiment 1, TMA concentrations were 10 and 50  $\mu$ M. Four clones of *D. galeata* were used, with four to six replicates per clone. The replicates were the lines grown for three generations in conditions identical to the experimental control. From each female, four offspring from the third clutch were taken and each was randomly assigned to the four experimental treatments. The animals were fed  $2.5 \times 10^4$  cells ml<sup>-1</sup> of *Scenedesmus* algae per day.

In experiment 2, TMA treatments were 20 and 200  $\mu$ M. A single *Daphnia galeata* × *hyalina* hybrid clone was used. The mothers of the experimental animals were kept in con-

ditions identical to the control for a week until they produced two clutches. Four offspring from each of 17 females were assigned randomly to each experimental treatment. The daily food supply was  $3.5 \times 10^4$  cells ml<sup>-1</sup>.

Both experiments followed similar procedures. They were conducted at 16°C and 16:8 light:dark (L:D). The animals were less than 24-h old at the beginning of the experiments. They were kept individually in 95 ml of medium. The media and food were refreshed daily. When the animals started maturing, they were checked every 12 h for the time of release of eggs in the brood chamber (age at maturity) and time of release of neonates (age at first reproduction). Size at maturity was the body length (measured from the top of the eye to the base of the tail spine) at the instar where the eggs were deposited to the brood chamber. The offspring from the first and the second clutch were counted, and the body size of the neonates from the first clutch was measured.

The analyzed traits were: age and size at maturity, body size of offspring from the first clutch, and the number of offspring in the first two clutches. The effect of the treatments was tested by two-way analysis of variance (ANOVA) in experiment 1, with treatment and clone as the main effects. No interaction was included in the model because clones were considered as blocks, and I was interested in testing the hypothesis that there was no treatment effect on any clone (Newman et al. 1997). In experiment 2, one-way ANOVA was carried out with treatment as the main factor.

Multivariate analysis of variance (MANOVA) indicated that the life history traits differed significantly among the treatments in both experiments. (Experiment 1: Wilks' Lambda = 0.4517,  $F = 3.41$ ,  $df = 12$ , 116.71,  $p = 0.0003$ . Experiment 2: Wilks' Lambda = 0.4492,  $F = 2.81$ ,  $df = 12$ , 95.54,  $p = 0.0025$ .)

In experiment 1, the animals from both TMA treatments

Table 1. Results of two-way ANOVAs testing for the effect of treatment (control, fish cue, and TMA in two concentrations) on life-history traits of four *D. galeata* clones.  $Df$  for treatment and clone = 3, error  $df = 57$  for size and age at maturity, and 54 for neonate size and 47 for clutch size.

Trait	Source	MS	$F$	$p$
Age at maturity	Treatment	2.7	0.84	0.478
	Clone	18.9	5.89	0.0014
	error	3.2		
Size at maturity	Treatment	0.019	5.19	0.0031
	Clone	0.082	22.06	<0.0001
	error	0.0037		
Offspring 1–2	Treatment	51.6	5.29	0.0032
	Clone	122.3	12.53	<0.0001
	error	9.76		
Offspring size	Treatment	0.0028	9.22	<0.0001
	Clone	0.0049	16.47	<0.0001
	error	0.00030		

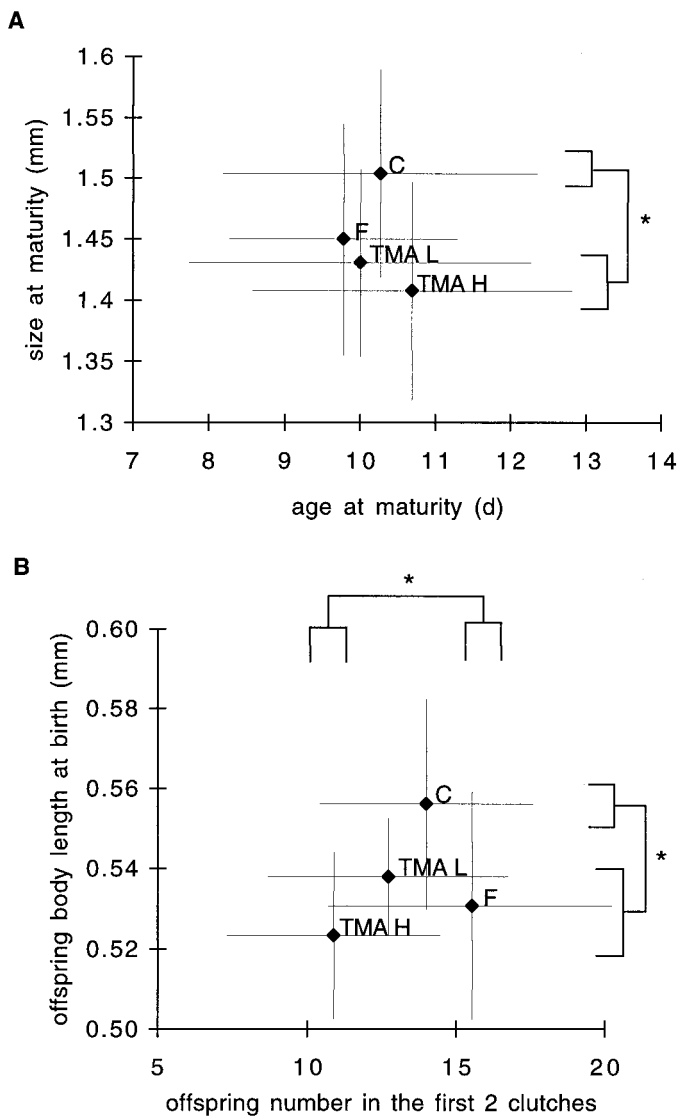


Fig. 1. Life history traits in control (C), fish (F), and two TMA treatments (TMA L – 10  $\mu$ M, TMA H – 50  $\mu$ M) in experiment 1 (means  $\pm$  SD for four clones pooled). (A) Age and size at maturity. (B) Offspring body length at birth and offspring number produced in the first two clutches. Treatments significantly different from each other according to Tukey's honestly significant difference (HSD) test ( $p < 0.05$ ) are indicated by asterisks.

were smaller at maturity than the control animals. The treatment had no effect on age at maturity (Fig. 1A, Table 1). TMA at 50- $\mu$ M concentration resulted in fewer offspring than did the fish treatment. The animals in both TMA and fish treatments produced smaller offspring than in the control (Fig. 1B, Table 1). There was a significant clone effect on all life history traits (Table 1).

In experiment 2, TMA at 200  $\mu$ M caused maturation at smaller size than the control treatment (Fig. 2A) ( $df = 3.46$ ,  $F = 6.4$ ,  $P < 0.002$ ). Fish treatment resulted in earlier maturation than the control (Fig. 2A) ( $df = 3.46$ ,  $F = 4.2$ ,  $P < 0.01$ ). TMA in high concentration brought about a decrease in number of offspring in the first two clutches compared to the fish treatment, but not to control (Fig. 2B) ( $df = 3.43$ ,

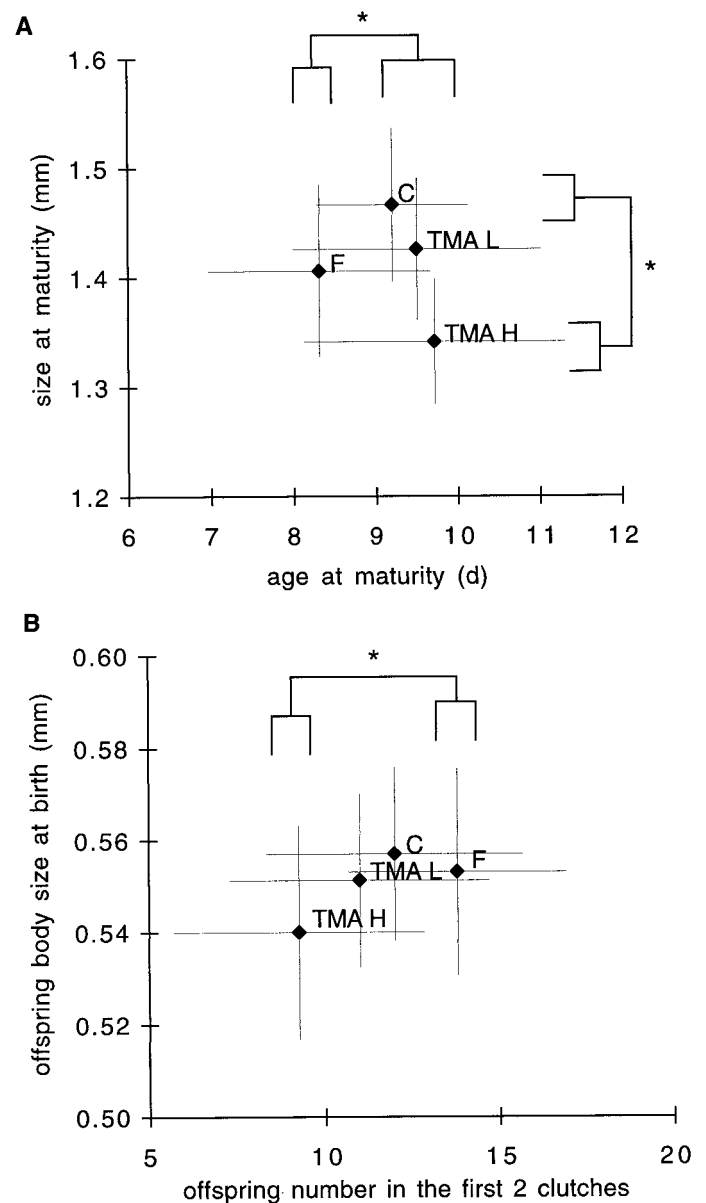


Fig. 2. Life history traits in control (C), fish (F), and two TMA treatments (TMA L – 20  $\mu$ M, TMA H – 200  $\mu$ M) in experiment 2 (means  $\pm$  SD). (A) Age and size at maturity. (B) Offspring body length at birth and offspring number produced in the first two clutches. Treatments significantly different from each other according to Tukey's HSD test ( $p < 0.05$ ) are indicated by asterisks.

$F = 4.0$ ,  $P < 0.02$ ). The treatments did not have a significant effect on offspring size (Fig. 2B) ( $df = 3.44$ ,  $F = 1.5$ , ns, MS error = 0.00046).

The most important finding of this study is the discrepancy of TMA and fish kairomone effects on *Daphnia* life history traits. Fish kairomone resulted in higher fecundity and earlier maturity, whereas TMA had the opposite effect on fecundity and no effect on age at maturity. TMA and fish exudate affected size-related traits similarly. However, a decrease in offspring size caused by TMA was never accompanied by a corresponding increase in clutch size, as was

the case with fish exudate. Similarly, reduced size at maturity in the TMA treatment was not associated with earlier maturation, as it was for fish exudate. TMA caused a decrease in fitness-related traits, as might any unspecific, mildly toxic chemical substance. In contrast, fish exudate brought about the expected shifts toward earlier reproduction and more but smaller offspring. Although the experiments differed in several respects (clones used, food level), the direction of responses was consistent in both.

The concentrations used in this study were based on the results of Boriss et al. (1999), so that the lower level was chosen to represent TMA concentration in naturally produced fish exudate and the higher to match that which was reported to induce pronounced migration behavior. The lower concentrations used (10–20  $\mu\text{M}$ ) produced rather weak effects, but these effects were in the same direction as the effects of higher TMA concentrations. It is unlikely that even lower concentrations would produce effects similar to the effects of fish exudate. A study by Reede (1995) showed that the response of life history traits to fish kairomone increased with concentration, and the reversal of that trend was never observed.

I conclude that TMA is not the substance triggering antipredatory life history shifts in *Daphnia*.

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## Some considerations of the <sup>210</sup>Pb constant rate of supply (CRS) dating model

**Abstract**—One of the most widely used radionuclides in the study of recent sedimentation processes is <sup>210</sup>Pb. Its depth profile in sediments is used as input for various dating models, which provide chronologies, mass fluxes, and sedimentation rates. In this work we revisited the CRS (constant rate of supply) model, widely used for dating sediments through <sup>210</sup>Pb. A more general hypothesis (periodic flux [PF]) was proposed and, although it confirmed the validity of CRS chronology, a detailed analysis of the mass sedimentation formula pointed out an inconsistency of the cited model. A new mass sedimentation formula was proposed and validated with a lake sediment core and four marine sediment cores.

Lead-210 is one of the most widely used radionuclides in recent radio-geochronology studies. This is basically due to its appropriate half-life ( $T_{1/2} = 22.3$  yr), which is suitable for studying sedimentary processes that have taken place during the last 100–150 yr and to the assumption that its atmospheric flux is constant, which permits relatively simple

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modeling of the natural <sup>210</sup>Pb cycle. Lead-210 found in sediments has two components: the supported <sup>210</sup>Pb, which originates from the <sup>222</sup>Rn decay that occurs in the sediment, and the unsupported <sup>210</sup>Pb, which originates from the <sup>222</sup>Rn decay that occurs in the atmosphere and the water column. The activity profile of the unsupported component is the input data for <sup>210</sup>Pb dating models, which are used to estimate the chronology of the sediment (Appleby and Oldfield 1978). Although modeling of the <sup>210</sup>Pb cycle would be desirable for dating purposes, dating models generally use rough assumptions on the sedimentation processes.

The aim of this work is to revise the basic assumptions of the constant rate of supply (CRS) model (Appleby and Oldfield 1978) and to explore the validity of its mass flux (sedimentation rate) formula.

**Review of the CRS model**—One of the most widely used <sup>210</sup>Pb sediment dating models is the CRS model. This model is based on the assumption that there is a constant rate of