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Demonstration of Sex Pheromones in *Anabolia Bimaculata, Hydatophylax Argus,* and *Nemotaulius Hostilis* (Trichoptera: Limnephilidae)

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Demonstration of Sex Pheromones in *Anabolia bimaculata*, *Hydatophylax argus*, and *Nemotaulius hostilis* (Trichoptera: Limnephilidae)

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Abstract

The presence of extractable semiochemicals for mate attraction is demonstrated experimentally for the first time in *Anabolia bimaculata* (Walker), *Hydatophylax argus* (Harris), and *Nemotaulius hostilis* (Hagen), three species of limnephilid caddisfly. Each species is also the first member of its respective genus to demonstrate pheromone presence. Sex pheromones appear to be widespread within the Trichoptera, although < 1% of species have been tested and there remains substantial research still to be done on pheromone demonstration, chemical composition, and release behavior.

This note describes the recent discovery of female-produced sex pheromones attracting males within three caddisfly species of the family Limnephilidae—*Anabolia bimaculata* (Walker), *Hydatophylax argu*s (Harris), and *Nemotaulius hostilis* (Hagen)—based on field trials conducted in northern Lower Michigan in 2011–2012. Pheromones are known to be part of the mating systems of eight insect orders (Pherobase 2012), including 40 caddisfly species within 15 families (Houghton et al. 2009 and references therein). Although much of the recent work on caddisfly pheromones involves physiological and chemical studies (Ivanov and Löfstedt 1999; Bergmann et al. 2001, 2002, 2004; Löfstedt et al. 2008), basic pheromone biology is still in its relative infancy, as their presence has been demonstrated in < 1% of the described caddisfly fauna.

Adults of *N. hostilis* and *A. bimaculata* were collected in 2011 from ultraviolet lights placed near Fairbanks Creek (N 44.04°, W 85.64°) in late May and early June, respectively. Previous research (Houghton 2002) has suggested no significant difference in pheromonal attractiveness between virgin and post-oviposition females, thus the status of females was not checked. Fifth instar larvae of *H. argus* were collected in mid May 2012 from the Little Manistee River (N 44.03°, W 85.73°) and reared to adult in a Frigid Units Living Stream™ set to ambient temperature and photoperiod. Adults emerged in late May and were maintained in the laboratory until enough specimens for a trial had emerged (< 72 h).

Following the procedure outlined by Wood and Resh (1984), individual specimens of each species were placed into individual 12 ml glass vials with 2–3 ml of HPLC-grade dichloromethane (Fisher Scientific, Pittsburg, PA) for 1–2 hours. Foam *Drosophila* vial plugs (Carolina Biological, Burlington, NC) were infused with the 2–3 ml extract from each individual specimen (1 female equivalent) and placed into a Trécé Pherocon 1C wing trap (Gempler's, Belleville, WI). All pheromone trapping occurred at Fairbanks Creek. This stream was the natal habitat for the extracted adults of *A. bimaculata* and *N. hostilis*, and

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was approximately 12 km from the natal habitat for the *H. argus* specimens. Populations of *H. argus* were previously known to exist in Fairbanks Creek. Extracts of males, females, and dichloromethane controls were all tested for their ability to attract conspecific males. In all trials, traps were hung in random order from riparian vegetation approximately 1–2 m above the water, and 10 m apart from each other. Each trial began at dusk and lasted 24 hours. Voucher specimens of all tested species have been deposited in the Hillsdale College insect collection.

For all three species, the number of conspecific males caught in traps baited with female extracts was significantly higher than the number caught in control traps or those baited with male extracts (Table 1), demonstrating the presence of an extractable female semiochemical for male attraction. Each species is the first within its respective genus shown to use sex pheromones. Seventeen other limnephilid species, however, have previously been documented to use them. Several species in the genus *Pycnopsyche*, which is the sister genus to *Hydatophylax*, have been well-studied in their pheromone use (Houghton 2002, Houghton et al. 2009). Likewise, several species in the limnephilid tribe Limnephilini—which includes the genera *Anabolia* and *Nemotaulius*—have been demonstrated to use pheromones (Pherobase 2012). It is likely that pheromone use in the Trichoptera is more prevalent than is known, especially when considering the common and widespread use in their sister taxon, the Lepidoptera. Thus, basic caddisfly pheromone demonstration studies remain important.

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Table 1. Mean (± SD) number of conspecific males caught in traps baited with various extracts from field trials. Each series of three extracts constitutes a separate experiment. Superscript numbers denote statistically distinct groups of means (One-way Analysis of Variance on ranked data with *post-hoc* Tukey test).

Date	Extract	*n*	# caught	*df*	*F*	*p*
May 2011	Dichloromethane only	3	0.0 (± 0.0)^{A}			
	N. hostilis males	3	0.0 (± 0.0)^{A}			
	N. hostilis females	4	2.75 (± 0.96)^{B}			
			Summary data:	2	56.5	< 0.001
June 2011	Dichloromethane only	3	0.0 (± 0.0)^{A}			
	A. bimaculata males	4	0.0 (± 0.0)^{A}			
	A. bimaculata females	5	2.4 (± 1.82)^{B}			
			Summary data:	2	9.27	0.007
June 2012	Dichloromethane only	3	0.0 (± 0.0)^{A}			
	H. argus males	4	0.0 (± 0.0)^{A}			
	H. argus females	3	3.33 (± 0.58)^{B}			
			Summary data:	2	122.5	< 0.001

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