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Developmental Rates of the Native Milfoil Weevil, *Euhrychiopsis lecontei,* and Damage to Eurasian Watermilfoil at Constant Temperatures

Kristine C. Mazzei,*[,1] Raymond M. Newman,*[,2] Alyson Loos,† and David W. Ragsdale†

Department of Fisheries and Wildlife and †Department of Entomology, University of Minnesota, St. Paul, Minnesota 55108

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The native aquatic weevil *Euhrychiopsis lecontei* (Dietz) is a potential biological control agent of Eurasian watermilfoil (*Myriophyllum spicatum* L.). The weevil reduces the viability of milfoil by mining plant stems. We determined the influence of temperature on the developmental rates of the weevil and damage to Eurasian watermilfoil stems. Single *E. lecontei* eggs were laid on rooted plants in individual tubes filled with water and 16 such tubes were randomly assigned to each of eight environmental chambers set at constant temperatures of 15, 19, 21, 23, 25, 27, 29, and 31°C with a 16-h daylength. Weevils and plants were monitored daily and development times were recorded for the egg, larval, and pupal stages. Length of watermilfoil stem damaged (cm) was estimated at 21, 25, and 29°C. Developmental rate was linearly related to temperature, up to 29°C; the developmental maximum appeared to be between 29 and 31°C. Average egg hatch occurred in 12.0 days at 15°C and in 4.2 days at 31°C. Average larval development time took 20 days at 15°C and 6.1 days at 31°C. Complete egg to adult development ranged from 16.6 days at 29°C to 61.7 days at 15°C. The lower developmental threshold was between 8.2 and 10.5°C, and egg to adult development required 309 ± 27.6 (2 SE) degree-days above 9.8°C. Daily stem damage increased with temperature but total damage (by larvae) was equal across temperatures and averaged 15.1 ± 1.9 cm. Field temperature data indicated that up to five generations could be completed in a typical summer in Minnesota lakes.

Key Words: **aquatic weevil; biological control; degree-days; *Euhrychiopsis lecontei;* Eurasian watermilfoil; *Myriophyllum spicatum;* development rate.**

[1] Current address: Evolution and Ecology, University of California, Davis, CA 95616.

[2] To whom correspondence should be addressed. Fax: (612) 625-5299. E-mail: rmn@fw.umn.edu.

INTRODUCTION

Eurasian watermilfoil, *Myriophyllum spicatum* L., is a troublesome submersed aquatic weed introduced to the United States in the 1940s (Aiken *et al.,* 1979; Smith and Barko, 1990). In addition to being a nuisance, it can out-compete native lake flora (Madsen *et al.,* 1991). The aquatic weevil, *Euhrychiopsis lecontei* (Dietz) (Coleoptera: Curculionidae), is being evaluated as a possible biological control agent of this pest species (Creed and Sheldon, 1995; Sheldon and Creed, 1995). The weevil is endemic to North America (Tamayo *et al.,* 1999) and has been recognized as an herbivore of watermilfoils and considered as a potential control agent of Eurasian watermilfoil since 1991 (Creed and Sheldon, 1995). The weevil's damage to milfoil has been shown to reduce the health and survival of milfoil in laboratory (Creed *et al.,* 1992), tank (Newman *et al.,* 1996), and field experiments (Creed and Sheldon, 1995; Sheldon and Creed, 1995), and weevils have been associated with Eurasian watermilfoil declines in the field (Creed and Sheldon, 1995; Lillie, 1996; Sheldon, 1997).

Female weevils lay their eggs near the tip of the milfoil meristem (Sheldon and O'Bryan, 1996). Upon hatching, *E. lecontei* larvae eat the meristem and damage the milfoil by mining down the stem, consuming the cortex (Newman *et al.,* 1996). The larvae bore a pupal hole further down on the stem where they complete development. The complete life cycle, which takes 22 to 30 days at 20 to 25°C (Newman *et al.,* 1997; Sheldon and O'Bryan, 1996), occurs underwater during the summer, and the weevils overwinter on the shoreline.

Over the past few years researchers have been trying to ascertain the success of the weevil as a control agent. Newman *et al.* (1996) postulated that weevil densities in Minnesota often have not reached high enough levels to control Eurasian watermilfoil in the field. Sutter and Newman (1997) suggested that fish predation might be an important factor controlling weevil populations when weevil densities are low. Also, temperature influences insect developmental rates and ultimately the number

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of generations produced in a summer. Our research was conducted to assess the development times of the weevil on Eurasian watermilfoil and the amount of damage incurred at different temperatures. Specifically, the aim was to determine the number of days and degree-days it takes for the weevil to develop from egg to larva, larva to pupa, and finally, pupa to adult. Determination of developmental rates and damage incurred at various temperatures will provide an additional clue to the success of the aquatic weevil on milfoil in nature and aid in the development of weevil population models (Godfrey and Anderson, 1994).

MATERIALS AND METHODS

The experiment was conducted over two consecutive summers. During the summer of 1996 data were collected for the development times of weevils at 15, 19, 23, 27, and 31°C. In 1997 weevils were reared at three different temperatures, 21, 25, and 29°C, and amount of stem damage was also determined.

In the summer of 1996, approximately 150 Eurasian watermilfoil cuttings were grown in 0.38-m^3 outside tanks with fertilized lake sediment (following methods of Newman *et al.,* 1996) until they reached the height of 35 cm. Over a period of 3 weeks, rooted plants were removed from the stock tanks and placed in one of 10 individual Mason jars containing a single mated female *E. lecontei.* The weevils were left to lay eggs on the individual plant meristems, generally within 0.5–2 days. As soon as an egg was oviposited on the meristem, the plant was removed from the jar and replaced with a fresh plant.

Each egg-bearing plant was rooted in a 45-cm-high clear acrylic tube with 5 cm of lake sediment and filled with well water. The tubes were randomly assigned to one of five constant temperature regimes in five environmental chambers (Sherer RFHTT-1 and Percival I35LL) set at 15, 19, 23, 27, and 31°C (±1°C) with a 16-h photophase and an average light intensity of 65 µE/m^2/s. This procedure was repeated until all chambers were filled with 16 tubes and had at least one egg from each of the 10 females.

Tubes were monitored daily for the presence of eggs, larvae, pupae, and adults (following methods of Newman *et al.,* 1997). Eggs were visible on the plant tip and their location was noted. As soon as the egg had disappeared, the weevil was recorded as entering the larval stage of development; continuing larval development was noted by characteristic stem damage. The pupal stage was noted with the presence of a pupal hole. Emerging adults were removed from the chamber and weighed.

This entire procedure was repeated in the summer of 1997 for three new temperatures: 21, 25, and 29°C. These temperatures were chosen following analysis of the previous summer's data to further define the maximum development rate and to provide more estimates at the range of temperatures weevils typically encounter in the field. In addition to noting development, the length of stem damaged each day by larva was measured with a ruler (nearest mm) and recorded.

The average rate of development (1/d, where d = time in days) for each stage and complete development was plotted versus temperature (T, °C). The slope and intercept were estimated by linear regression for the points which appeared to be linearly related. In the current study, all points for temperatures ≤29°C were included. The 31°C point was identified as an outlier (*t* test of Studentized residual) for complete egg to adult development and was clearly below the maximum rate. This point was therefore deleted from the regressions. The reciprocal of the slope was used to determine degree-days (DD) over the minimum threshold required for each of the developmental stages and the *x* intercept was an estimate of the minimum threshold temperature (T_0: calculated as DD · *y*-intercept) (Giberson and Rosenberg, 1992). Standard errors of the slope and *x* intercept were used to estimate standard errors for DD and T_0 (from Campbell *et al.,* 1974; corrected by R. D. Moon, University of Minnesota). All statistics were calculated in SYSTAT 5.1 (Wilkinson, 1991).

Because it is not known how soon upon entry to the water from shore in the spring that female weevils develop eggs, we conducted an experiment in 1998 with females collected from the shoreline leaf litter at Lake Auburn and Smith's Bay of Lake Minnetonka. Weevils were sifted from the soil in mid-April and placed in containers with Eurasian watermilfoil and held at 10°C for 5 days. Then, 50–60 weevils were placed in each of three buckets containing rooted Eurasian watermilfoil and buckets were assigned to growth chambers at 10, 15, and 20°C with a 16-h photophase. Every 3–5 days, a sample of weevils was collected and dissected to determine ovarian development. Females with distinct ova developed within the ovarioles were judged to have developed eggs.

RESULTS

Development times decreased with increasing temperature (Table 1); colder temperatures slowed the development of the weevils. For instance, complete development took 61.7 days at 15°C and 17.3 days at 31°C. This trend was matched through all stages of development. The maximum developmental rate appeared to occur between 29 and 31°C for egg and pupal stages and for development from egg to adult. The maximum rate for larvae appeared to be lower, between 27 and 31°C. However, the larval development time at 27°C may be anomalously short, as it was identified as an outlier in the full data set (*t* test of Studentized residuals).

TABLE 1

Mean Number of Days ± 2 SE to Complete Egg, Larval, Pupal, and Egg to Adult Development and Percentage Survival in Each Stage for *Euhrychiopsis lecontei* at Eight Constant Temperatures (T °C)[a]

T (°C)	Egg	Larva	Pupa	Egg-Adult
15	12.0 ± 0.8 75%	20.0 ± 4.1 42%	29.0 ± 5.0 60%	61.7 ± 7.7 19%
19	6.6 ± 0.8 88%	12.7 ± 2.4 93%	13.6 ± 1.0 77%	31.8 ± 1.1 63%
21	7.1 ± 0.5 100%	9.9 ± 1.0 69%	11.3 ± 1.2 82%	28.2 ± 1.1 56%
23	5.1 ± 0.7 77%	9.4 ± 1.1 69%	9.8 ± 0.5 89%	23.9 ± 1.0 47%
25	5.2 ± 0.5 94%	7.3 ± 0.7 67%	8.2 ± 0.6 90%	20.6 ± 0.8 56%
27	4.6 ± 0.4 94%	5.5 ± 0.9 80%	7.2 ± 0.6 100%	17.2 ± 0.6 75%
29	3.6 ± 0.3 88%	6.3 ± 0.9 79%	6.9 ± 1.0 82%	16.6 ± 0.7 56%
31	4.2 ± 0.5 89%	6.1 ± 0.8 88%	7.4 ± 0.9 71%	17.3 ± 1.4 56%

[a] Initial number of eggs was 16 at each temperature.

The survival of *E. lecontei* varied across the temperatures and the stages of development (Table 1). Hatch success varied between 75 and 100%. Larval and pupal survival was generally high, between 67 and 100% for temperatures above 15°C, yet it was clear that survivorship at 15°C was very low (Table 1); only 19% of eggs tested at 15°C completed development to adult eclosion compared to 47–75% at the higher temperatures.

Development rates (1/d) from egg to adult were linear with temperature, increasing steadily until 29°C followed by a decline at 31°C (Fig. 1); similar trends were seen for egg and pupal stages (Table 1). Based on a regression of development rate and all temperatures between 15 and 29°C (the linear portion), *E. lecontei* has a minimum temperature threshold between 8.2 and 10.5°C, depending on life stage (Table 2). The lower threshold was for egg hatch while the upper value was for the pupal stage. Complete development to adult eclosion indicates a lower threshold of 9.8 ± 1.2 (2 SE)°C. Using the inverse of the slopes of the regression lines we calculated that *E. lecontei* require 78 degree-days for egg development, 104 degree-days for larval development, 119 degree-days for pupal development, and 309 degree-days for complete development (Table 2).

TABLE 2

Parameter Estimates from Regression of Development Rate (1/d) on Temperature (°C), Estimated Lower Threshold Development Temperature (T_0), and Cumulative Degree-Days (DD) Required for Development of *Euhrychiopsis lecontei*[a]

Stage	Y-Intercept	Slope	r^2	T_0 (°C)	DD (°C-day)
Egg	−0.1048	0.01281	0.91	8.2	78.0
(SE)	(0.0409)	(0.00177)		(2.1)	(10.8)
Larva	−0.0960	0.00959	0.89	10.0	104.3
(SE)	(0.0353)	(0.00153)		(2.1)	(16.6)
Pupa	−0.0887	0.00844	0.99	10.5	118.5
(SE)	(0.0061)	(0.00026)		(0.4)	(3.7)
Complete	−0.03156	0.00323	0.99	9.8	309.2
(SE)	(0.0033)	(0.00014)		(0.6)	(13.8)

[a] Regressions (all $P \leq 0.005$) are based on mean rates over seven temperatures (15–29°C).

Daily stem damage per larva also increased with temperature ($r^2 = 0.19$; $P < 0.01$), from 1.6 ± 0.2 cm/day at 21°C to 2.1 ± 0.5 cm/day at 25°C and 2.4 ± 0.6 cm/day at 29°C; damage rate at 29°C was significantly higher than at 21°C ($P = 0.02$; Tukey's HSD). However, there was no significant difference in total damage resulting from complete larval development at the three temperatures (all $P > 0.8$, Tukey's HSD). Total damage per larva averaged 15.1 ± 1.9 cm.

Overwintering females incubated in water with Eurasian watermilfoil at 15 and 20°C developed eggs within 6–9 days, with faster development at 20°C. Weevils incubated at 10°C failed to develop ova after more than 40 days. Thus, it appears that females will not develop eggs at temperatures ≤10°C but develop eggs within 1 to 2 weeks of feeding at 15–20°C.

DISCUSSION

Our estimates of development rates and survival for *E. lecontei* compare favorably with other published data for which temperature was less rigorously controlled over much narrower ranges (Sheldon and O'Bryan, 1996; Newman *et al.,* 1997). At 22–25°C, typical lake temperatures, complete development can occur in 21–28 days. At temperatures between 27 and 31°C, however, egg to adult development can occur in 17 days. For temperatures above 15°C, survival was also high; generally >50% of eggs successfully developed to adult.

The upper or maximal developmental rate appears to be between 29 and 31°C. The developmental rates leveled off or declined in that temperature range. This matches Sheldon's (1997) findings that at 34°C hatching success and larval survival of *E. lecontei* were much lower (≤3%) than at cooler temperatures. Furthermore, she found that larval survival was significantly lower for both 31 and 34°C than for cooler temperatures. Although we did not see lower survival at 31°C, these observations suggest that sustained temperatures above 31–32°C are not conducive to weevil development and survival. Temperatures can exceed these values in watermilfoil surface mats; however, cooler water is usually available underneath the mat.

Our minimum temperature threshold for development was near 10°C. Although linear estimates of

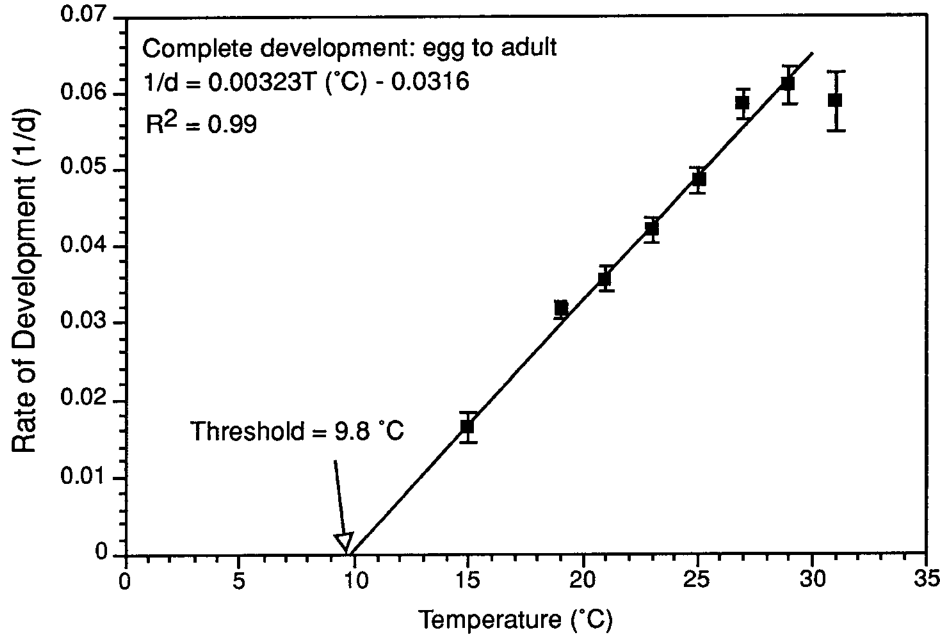

FIG. 1. Average rate (1/d; ±2 SE) of *E. lecontei* complete development versus temperature. The line is based on a linear regression of the mean rates ($n = 7$) for 15 to 29°C; the lower developmental threshold is indicated by the X intercept. Statistics are provided in Table 2.

threshold temperatures based on developmental rate (1/d) tend to overestimate the true threshold (Giberson and Rosenberg, 1992), the low hatch success and survivorship (18.8%) at 15°C suggests that a threshold of 10°C is quite plausible. Because females do not appear to develop eggs at 10°C, it is likely that eggs will not be laid in spring at temperatures ≤10°C. The lower minimum thermal threshold for successful hatch and higher egg survival at the lowest temperature relative to the other stages may make evolutionary sense. *E. lecontei* adults generally return to the water in April and May, although we have seen a few adults in the water soon after ice-out in late March and early April (personal observations). At this time, water temperatures are ≤5°C and it may be early to mid-May before daily mean water temperatures equal or exceed 10°C. Apparently, these adults are feeding and gaining energy stores before developing eggs once temperatures exceed 10–15°C. At least a week of feeding at 15°C appears to be required to develop eggs. Once the lake starts heating above 10–15°C, the temperature typically rises quickly and the later stages will thus likely be exposed to warmer temperatures. Thus, eggs may be better adapted to survive and develop in colder temperatures than the other stages.

Degree-days above 10°C were determined for two lakes that were monitored with temperature data loggers (Optic StowAway, Onset Computer, Pocasset, MA) from May through mid-October, 1996. Temperatures were recorded every 0.5 h at 0.75 m depth. By mid-May minimum temperatures exceeded 10°C in both lakes and by mid-June temperatures averaged 24–25°C. Temperature declined rapidly in mid-September, from 25 to around 15°C. Temperatures occasionally reached 30°C but did not exceed 31°C. To provide a conservative estimate of accumulated degree-days, we included data only from mid-May to mid-September, when mean daily water temperatures were above 15°C (and minima well above 10°C). In both lakes >1550 degree-days (>10°C, the lower thermal threshold) were accumulated, indicating a potential for development of five generations. Even when the accumulation was delayed for 10 days after a mean of 15°C was reached (to account for egg development), enough degree-days were accumulated to produce five generations. Because milfoil weevils stop laying eggs in early to mid-September, in response to declining temperatures or daylength, the final generation may not be produced. However, it is likely that four generations are regularly produced. Sheldon and O'Bryan (1996) suggested, based on field observations of patterns of egg and larval frequencies, that three generations were produced in Vermont lakes. Our results suggest that, at least in the Midwest, the first wave of overwintering weevils can produce four to five generations, although frequency distributions may reveal only three to four generations.

Stem damage rates (cm/day) increased with temperature but appeared constant for complete larval development at 15 cm. This estimate of total damage is consistent with results of a 4-week tank experiment in which final densities of about 200 weevils had mined much of the stems of 92 plants (40- to 50-cm tall), resulting in significant reductions in viable above- and below-ground biomass and carbohydrate stores of the watermilfoil (Newman *et al.,* 1996). This amount of damage per larva would make it unlikely that weevils could completely mine 2- to 3-m-tall watermilfoil plants. Furthermore, under ideal growing conditions, Eurasian watermilfoil can elongate 15 cm/week (e.g., Newman *et al.,* 1996) and can therefore grow as fast as weevils can mine. However, because mining starts at the meristem, one or two weevils per stem can inhibit elongation (Newman *et al.,* 1996) and also lead to a loss of buoyancy, which causes the photosynthetic tissue to sink (Creed *et al.,* 1992). The stem mining also reduces the translocation of carbohydrates and root stores (Newman *et al.,* 1996), which could explain field declines in the spring owing to depleted overwinter carbohydrate reserves (Creed and Sheldon, 1995). Our development rates also compare favorably with observations from the tank experiment (Newman *et al.,* 1996), in which we observed complete development (surplus weevils) in 21 days. Water temperatures averaged 25.2°C, which would accumulate 309 degree-days in just over 20 days.

Although simple linear estimates of developmental threshold and degree-days required for development may be oversimplifications (Wagner *et al.,* 1984), they adequately describe development within the ranges applicable to field conditions, for which other variables are probably more important (Higley *et al.,* 1986; Pruess, 1983). Furthermore, with high-frequency temperature data and the relative thermal stability of aquatic systems, this approach should accurately predict development.

Our estimates of *E. lecontei* development and damage rates will help us understand the potential population growth and effects on Eurasian watermilfoil in lakes of varying temperatures. These estimates can be used to model weevil population dynamics, predict effects on Eurasian watermilfoil, and assess the influence of weather and climate on the vitality of weevil populations.

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