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INVASION NOTE

Winter hatching in New England populations of invasive pheretimoid earthworms *Amynthas agrestis* and *Amynthas tokioensis*: a limit on population growth, or aid in peripheral expansion?

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Abstract Some Asian megascolecid earthworms, e.g. *Amynthas agrestis* and *Amynthas tokioensis*, are highly invasive and have only recently been reported from natural ecosystems in the northeastern USA. There, they are annual earthworms that survive the winter as cocoons (an egg in a tough covering). Hatching occurs in mid-April when temperatures rise consistently above 10 °C. In some years, winter temperatures also reach 10 °C during short warming episodes, but hatchlings then are likely to die when temperatures drop again. To test this hypothesis, soil was collected on 7 sampling dates during January–May 2016 at one site in the Champlain Valley, Vermont and extracted for the cocoons. Both hatched and unhatched cocoons were enumerated and identified to species by both size and sequencing of the mitochondrial cytochrome c oxidase I gene (COI). A regression model on the number of unhatched cocoons over time predicted that ∼ a quarter (*A. agrestis*) and ∼ a third (*A. tokioensis*) would hatch before mid-May; no hatchlings were observed. Thus, hatching during warming periods in winter seemingly resulted in high mortality. Such winter hatching, and loss, may increase with climate warming in the region because winter warming periods should become more common. Climate warming could therefore reduce ability of the invasive earthworms to persist in northern habitats. Conversely, rapid hatching when the soil warms in spring could have an ecological benefit by allowing opportunities to expand at the thermal edge of the range as the climate continues to warm.

Keywords Invasive earthworms · *Amynthas* spp. · Winter hatching rate · Climate change

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Asiatic earthworms in the genus *Amynthas* (Kinberg) are now present in all New England States (Reynolds 2010; Reynolds et al. 2015), arriving in northern New England (Vermont) only within the past 10–20 years (Görres and Melnichuk 2012). They were first noted by Gates (1954) as occurring in horticulture and zoos in New York in the 1940s. In the northeastern USA, *Amynthas agrestis*, *A. tokioensis* and *Metaphire hilgendorfi* frequently co-invade forested ecosystems

(Chang et al. 2017). They create an extensive layer of loose castings with ecosystem scale consequences (Greiner et al. 2012; Ziemba et al. 2015). These species have an annual life cycle and survive the winter as eggs within casings referred to as cocoons (Chang et al. 2016), likely by a dehydration process (Holmstrup and Westh 1994). Hatched worms must reach reproductive maturity by late November when they die. Freeze-free periods longer than the length of time between hatching and reproductive maturity supports their persistence in the northern hardwood forest (Görres and Melnichuk 2012; Moore et al. 2013). *Amynthas* cocoons hatch when temperatures reach above 10 °C (Blackmon 2009) and die when temperatures drop below 5 °C (Richardson et al. 2009). Correspondingly, mild winters with brief warm episodes may hinder persistence of *Amynthas* earthworms at the northern edge of their range because hatching during these episodes may be followed by cold temperatures lethal to hatchling. The frequency and duration of warm winter episodes are likely to increase with climate warming (Higgins et al. 2002), and thus warming may well hinder, not favor, persistence of the invasive earthworms.

We conducted cocoon surveys during the winter and early spring of 2016. The National Oceanographic and Atmospheric Administration had predicted higher than normal winter temperatures for that winter season (NOAA 2015). This presented us with an opportunity to explore whether *Amynthas* hatched during warm weather episodes. We were particularly interested in whether the two species found at the site, *A. agrestis* and *A. tokioensis*, hatched at different rates over the winter and early spring. The species were identified by sequencing the mitochondrial COI gene, and their presence confirmed by morphological taxonomy. The two species share the top 10 cm of mineral soil at our study sites (Görres et al. 2016). Differential hatching and survival of premature hatchlings could tilt competition between the two species. New observations made in 2017 showed that embryos were at a wide range of developmental stages during the winter months, including hatchling-sized embryos (data not shown).

Ambient average monthly maximum and minimum temperatures for the winter of 2015/2016 were 2–7 °C greater than tri-decadal (1981–2010; NOAA 2016) average monthly maximum and minimum temperature normals at the NOAA station at Burlington

Airport, Vermont (KBTV) 5.9 km from the study site. Ambient temperatures exceeded 10 °C on 23 occasions with several multi-day exceedance events. However, after each of these warming episodes, temperatures fell below the 5 °C, temperatures purported to be lethal to these earthworms. As of mid-April temperatures stayed above 10 °C more consistently. Thus, the pattern of temperatures favoring hatching followed by conditions that should kill the worms was present during our study period.

To collect cocoons, three soil samples were taken from random locations in a woodland on seven sampling dates from January 8, to May 6, 2016. The sample locations were between 5 and 15 m apart. The only constraint on the sampling dates was that they had been preceded by at least one warming event where ambient temperatures rose of above 10 °C. In brief, 10-cm deep, 23-cm diameter steel rings, which served as sampling quadrats, were driven into the soil, the soil contained in these rings was removed and stored in the dark in a lab refrigerator at 4 °C until cocoons were extracted, usually 1 or 2 days later. Cocoons were found by hand-sorting using both visual and tactile cues.

Cocoons were inspected under a dissecting scope and sorted into small or large classes, and intact or open. Intact cocoons were classified as unhatched, open cocoons as hatched. The fraction of hatched cocoons was calculated as the ratio of the open cocoons to total cocoons in a sample. We assumed that decomposition of cocoon shells was slow in the winter. The diameters of 54 unhatched cocoons obtained on the May sampling date were measured as the mean of the minor and major axes of a projected ellipse (ImageJ version 1.48 V, NIH, Bethesda, Maryland, USA). The cocoon diameter distribution followed a bimodal distribution. COI sequence analysis of 50 cocoons determined that large cocoons were *A. agrestis* and small ones were *A. tokioensis*, with mean diameter of 2.13 mm $\pm$ 0.016 mm (se) and 3.12 $\pm$ 0.023 mm for *A. tokioensis* and *A. agrestis* respectively. Thus, we could compare cocoons by species based on their diameter.

Analyzing data from all quadrats and sampling dates, unhatched and hatched cocoons were abundant in the soil, but highly variable among the 21 samples taken over the 7 sampling dates (*A. tokioensis* x = 1097 $\pm$ 703 [standard error], *A. agrestis* x = 593 $\pm$ 307) The same was true for unhatched

cocoons (*A. tokioensis* x = 776 ± 135; *A. agrestis* x = 431 ± 52). The two-way ANOVA of the arcsine-transformed hatched cocoon fraction in the samples taken from January 2016 to April 2016 was significant ($df = 11$, F = 4.684, $p = 0.0015$), with significant effects of both variables (sampling date: $df = 5$, $p = 0.0025$; species: $df = 1$, $p < 0.0002$). The effect of sampling date was independent of species (no interaction, $df = 5$, $p = 0.7620$).

The inference from linear regression of the fraction of hatched cocoons versus time (Table 1) for the data set excluding the May sampling indicated that hatching occurred during the winter (slopes greater than 0, Fig. 1). We omitted May 6 data because hatching temperature was more consistently exceeded after mid-April. The mean hatching rate was 0.195% per day and did not differ for the two species ($p = 0.969$). The regressions (intercept) estimated that on January 1, 2016 1% and 14% of *A. agrestis* and *A. tokioensis* cocoons had already hatched ($p = 0.0006$). By winter's end, the model predicted that 24% and 37% (*A. agrestis* and *A. tokioensis,* respectively) had hatched.

Although hatched cocoons were found in all samples, hatchlings were not directly observed in the field during sampling. We had not expected to see any hatchlings because winter warming events are followed by cooling to below freezing point, likely killing early hatchlings. In the lab, hatchlings were observed during enumeration of cocoons shortly after soil collection. These observations suggested that a cohort of cocoons was ready to hatch in the winter. Further evidence of cocoons being ready to hatch comes from dissection of 46 cocoons, several of the embryos were visually not different from hatchlings. Hatching phenology may be linked to the phenology of adult emergence. At the study site, the worms become morphologically adult (the clitellum was present) in July, and the last adults die in late
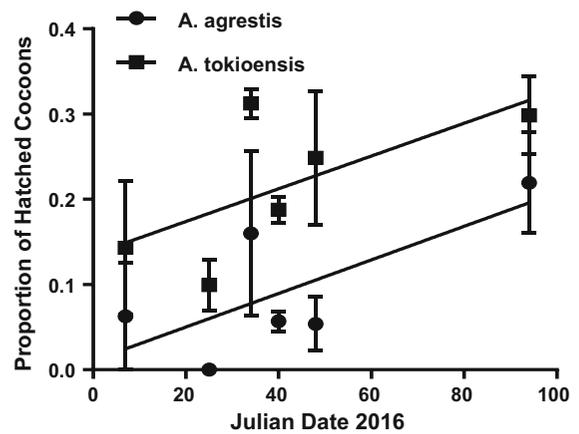

Fig. 1 Regression of the fraction of hatched cocoons versus Julian date. The rate of hatching is statistically indistinguishable for the two species. Error bars give standard errors

November (Görres et al. 2016). Cocoon production is thus spread over several months.

The substantial attrition due to winter hatching may well reduce population growth during the warm season. Yet, the ability of *Amynthas* to respond quickly to winter warming episodes may also have positive consequences for invasiveness and range expansion. Cocoons are most likely produced over a prolonged period (July to November) which would result in a range of embryonic development by winter. Hatching might thus be staggered, including the winter hatching we document here. Hatching in the winter, though, is unlikely to yield success when winter warming episodes are short lived and hatchlings are exposed to lethal temperatures shortly after hatching. The thermal temporal edge of the range is variable across years and perhaps even at a fine spatial scale. For example, the date of the last spring frost in Burlington, Vermont (NOAA station KBTV) varied over the last 75 years between April 15 (2015) to as late as May 31 (1961). Thus, early hatching pays off in

Table 1 Linear regression parameters of hatching with time for data sets excluding May 2016 samples (−May). m is the hatch rate (slope), and b is the percentage of cocoons in a soil sample that had already hatched at the beginning of the calendar year (intercept estimated for January 1, 2016). p (m > 0), p (m) and p (b) are the significance levels for slopes being different from 0 and between species and intercepts being different, respectively

Species	r^2 [p (m > 0)] − May data N = 18, df = 16	m (%/day^{-1})	p (m)	b (%)	p (b)
A. agrestis	0.245 (0.037)	0. 197 ± 0.09	0.969	1.0 ± 4.2	0.0006
A. tokioensis	0.233 (0.043)	0.192 ± 0.09		13.6 ± 4.3	

years when the last spring freeze date occurs early, adding time for growth and reproduction. Another consequence of this hatching phenology is that a lengthening of the freeze-free period due to climate change may extend the spatial range of the organisms. From 1970 to 2000, the average winter temperatures in the Northeast rose by 2.1 °C (Hayhoe et al. 2007; Rustad et al. 2012) and the growing season lengthened by 7 days, primarily due to a shift in the last freeze date in the spring. The lengthening of the growing season likely created opportunities for the survival of hatchlings earlier in the spring and consequently longer periods for maturation and reproduction. The growing season in the northeastern USA and north of the current geographic range limit in Canada is projected to lengthen by 29–40 days by 2100 (Hayhoe et al. 2007; Rochette et al. 2004). The accelerated warming reported in the northeast over the last decades (Rustad et al. 2012) may explain why *Amynthas* now occurs in northeastern hardwood forest (Chang et al. 2017). Expansion of these earthworms into more northerly latitudes is thus likely (Moore et al. 2017).

What are the consequences of winter hatching for summer abundance? We estimated that by May $\sim$ 320 and $\sim$ 490 cocoons m^{-2} remained for *A. agrestis* and *A. tokioensis*, respectively. In spite of sizable losses that occurs in the winter through early hatching and subsequent death of hatchlings, there would be ample cocoons to produce the maximum combined *Amynthas* abundance observed at this site in June 2016 (176 *Amynthas* spp. juveniles m^{-2}), not significantly different from that of previous years when it varied between 167 (2011) and 203 m^{-2} (2015). Thus, the large number of surviving cocoons found in the soil leads to the questions of how long cocoons remain viable and if there could be a persistent cocoon bank similar to the seed bank of plants.

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