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MORTALITY PATTERN RELATIVE TO SIZE VARIATION WITHIN *AMYNTHAS AGRESTIS* (GOTO & HATAI 1899) (OLIGOCHAETA: MEGASCOLECIDAE) POPULATIONS IN THE CHAMPLAIN VALLEY OF VERMONT, USA.

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ABSTRACT

Amynthas agrestis is a summer-active, annual species in Vermont. This invasive earthworm hatches in April and most individuals die by the beginning of November. Populations develop a broad length distribution with adult length varying between 2 and 15 cm. However, several individuals survive until late November. These late surviving *A. agrestis* tend to be large individuals, greater than 8 cm. Large worms may not be as susceptible to cooling as smaller ones. However, predation by Red-backed Salamanders (*Plethodon cinereus*) may also explain this phenomenon.

Key words: Oligochaeta, Megascolecidae, temperature, mortality, size distribution, *Amynthas agrestis*.

RÉSUMÉ

Amynthas agrestis est une espèce annuelle, active durant l'été dans le Vermont. Ce ver de terre envahissant éclos en avril et la plupart des individus meurent avant le début novembre. Les populations présentent une grande variation dans la répartition des tailles, avec la longueur des adultes variant entre 2 et 15 cm. Cependant, plusieurs individus survivent jusqu'à la fin de Novembre. Ces survivants tardifs ont tendance à être des individus de grande taille, soit plus de 8 cm. Les gros vers peuvent ne pas être aussi sensibles que les petits au refroidissement. Toutefois, la prédation par les salamandres rayées (*Plethodon cinereus*) peut également expliquer ce phénomène.

Mots-clé: Oligochaeta, Megascolecidae, température, mortalité, répartitions des tailles, *Amynthas agrestis*.

RESUMEN

Amynthas agrestis es una especie activa anualmente en el verano en Vermont. Estas invasivas lombrices de tierra eclosionan en abril y la mayoría de los individuos mueren a principios de noviembre, aunque varios individuos sobreviven hasta finales de noviembre. Las poblaciones desarrollan una amplia variación en su longitud, en el adulto varía entre 2 y 15 cm. Estos sobrevivientes de *A. agrestis* tienden a ser de gran tamaño, superior a 8 cm. Las grandes gusanos no son tan susceptible a los fríos como los más pequeños. Sin embargo, la depredación por parte de las salamandras dorso-rojo, (*Plethodon cinereus*) también puede explicar este fenómeno.

Palabras claves: Oligochaeta, Megascolecidae, temperatura, mortalidad, distribución de tamaño, *Amynthas agrestis*.

ZUSAMMENFASSUNG

In Vermont ist *Amynthas agrestis* eine einjährige Regenwurmart, die im Gegensatz zu europäischen Regenwurmarten im Sommer aktiv ist. In dieser Studie entwickelte die untersuchte Population eine disperse Längenverteilung adulter Individuen mit Längen zwischen 2 und 15 cm. Diese invasorische Regenwurmart schlüpft im April und der Großteil der Population stirbt Anfang November. Einige Individuen überlebten allerdings bis Ende November. Die überlebenden Regenwürmer waren größtenteils länger als 8 cm. Wegen des kleineren Verhältnis zwischen Oberfläche und Volumen der größeren Tiere mögen ihre Körpertemperaturen langsamer abkühlen als die der kleineren Würmer. Ein ähnlicher Effekt könnte allerdings auch durch Prädation durch Rotrücken-Waldsalamander (*Plethodon cinereus*) verursacht worden sein.

Stichwörter: Oligochaeta, Megascolecidae, Temperatur, Sterblichkeit, Größe-Vertrieb, *Amynthas agrestis*.

INTRODUCTION

Amynthas agrestis (Goto and Hatai, 1899) is a relatively new colonizer to Vermont (Görres and Melnichuk, 2012; Reynolds, 2012). In the state's deciduous and mixed-deciduous woodlands it is an annual species, with high peak abundances during the summer exceeding 100 individuals per m^2. As a species that is mainly active in summer, it appears to avoid direct competition with aestivating European Lumbricidae and often is found as the sole earthworm species in invaded habitats in Vermont.

For an invasive annual species it is important that life history cues are well synchronized with climate and resource availability. Hatching may be triggered by temperatures exceeding 10º C (Blackmon, 2009). Richardson *et al* (2009) conducted lab experiments which showed that 100% of *A. agrestis* kept in mesocosms at or below 5º C died within 3 days and that earthworms kept at 12º C had survival rates of between 43% and 80% after 28 days incubation. Richardson's *et al*. (2009) lab experiment left uncertain the effect of temperatures between 5º and 12º C on mortality. Survivorship was greatest when the mesocosms were kept at 12º and 23º C, with greater soil moisture increasing survival rates at temperatures above 20ºC. In an experiment examining earthworm stimulation of enzyme activity in woody mulches, we observed that 60 to 100% of *A. agrestis* survived 35 days of incubation, with all survivors maturing from juvenile to adult when kept at 20º C and approximately 30% moisture by mass (data not shown).

Little is known about how late into the season *A. agrestis* persists in the field when temperatures approach freezing and whether Richardson's mortality temperature benchmark of 5º C is also valid under field conditions. Temporal and spatial variations in temperature and moisture may provide temporary relief to *A. agrestis* in the fall. Field populations of *Amynthas* species show broad distributions in length and diameter (Tsai and Tsai, 2001; Blackmore 2013). We questioned whether size is a determining factor in survival, since an earthworm's area to volume ratio may govern heat loss. We investigated the time course of the decline of a population both in terms of total abundance and body size distribution. Our hypotheses were: (1) *A. agrestis* decline when air temperatures dropped below Richardson's *et al* (2009) survival threshold of 5º C and (2) large specimens survive longer.

MATERIALS AND METHODS

A population of *A. agrestis* was surveyed in a mixed deciduous forest stand dominated by Sugar Maple (*Acer saccharum*, Marshall) at the Horticultural Research Center of University of Vermont (44º 25' 53" N, 73º 11' 58" W) from the end of July to the end of November, 2013. The soil was a Windsor sandy loam on a 2-3% slope.

Of particular interest to sampling design were times when minimum ambient temperatures dropped below 5º C for longer than 3 days or when they dropped below freezing. Figure 1 shows the ambient temperatures for the National Weather Service station at Burlington International Airport (KBTV) located 5.5 km from the study site. We sampled on September 16, September 26 and October 5, 2013 to obtain a baseline where temperature was unlikely to trigger mortality. No frost had occurred during this period, but minimum temperatures dipped below 5º C briefly on three days between September 16 and October 5. We also sampled on October 30, November 1 and November 21, 2013, after the first frosts had occurred and minimum temperatures stayed below 5º C more persistently.

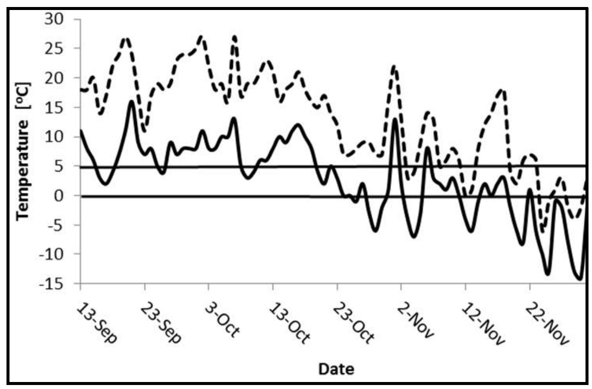

Fig. 1. Temperature variation during the study period. Stippled line indicates maximum daily temperature and solid line minimum daily temperature. The two horizontal lines indicate 5º C and 0º C.

We hand-sorted earthworms from litter and soil taken from five 50 cm × 50 cm quadrats. Each quadrat was sampled only once. Newly explored quadrats were at least 5 m apart from any previously explored quadrats. *A. agrestis* is an epi-endogeic species which we found to a depth of 5 cm in the summer. For this study we examined the top 20 cm of soils to make sure that we did not miss any *A. agrestis* that sought refuge from the cold deeper in the soil. When soils showed disturbance at 20 cm depth we excavated soils to 30 cm. We classified individuals of *A. agrestis* into clitellate and aclitellate. The length of each earthworm was measured in the field to the nearest 0.5 cm. The earthworms were returned to their previous quadrat post data collection.

Thirty specimens were collected in August 2013 for biometric work. They were euthanized and preserved in ethanol. The projected area and length of these preserved earthworms were measured using ImageJ (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland). Length, L, was measured using ImageJ's line tool by tracing the length of the earthworms in small increments and subsequently adding all increment lengths. The number of segments was counted and the average segment length calculated, so that individuals with autotomized tails could be excluded from the analysis according to the method of Tsai and Tsai (2001). Projected area, A_p, was measured by ImageJ after thresholding the image on brightness thus distinguishing the earthworm body from the background. The average diameter, D, of the earthworm was approximated as the ratio of A_p and L. A regression equation was established between D and L to find D for field populations based on their length. It is likely that live specimens have slightly different body dimensions, but we assumed that the ratio of dimensions was nearly the same for live and preserved earthworms. Using cylindrical dimensions as a model for the earthworm body geometry, we derived body area-to-volume ratios from L and D for the field populations. We also calculated the ratio of the number of earthworms longer than 8 cm to total abundance, R(>8).

RESULTS

The abundance of *A. agrestis* varied from 184 m^{-2} on July 31 (data not shown) to 9 m^{-2} on November 26, 2013 (Table 1). The population declined after the first frosts to fewer than 10 m^{-2} by the end of November (Table 1). The first adults in July were large (approximately 11 cm, data not shown) but by October 5 the average adult size was a mere 6 cm and the minimum length 2 cm (Figure 1C). When mortality rates increased at the end of October the average earthworm size increased to just less than 10 cm by November 21. The biometric work on preserved specimens showed a bimodal distribution of length. The longer specimens had an average length of 8.4 cm with additional segments anterior of the clitellum a total of 109-111 segments. The shorter earthworms had an average length of 5.5 cm and between 98 and 103 segments.

The ratio R(>8) increased from 13% on October 5 to 100% on November 26 (Table 1), suggesting that the earthworms surviving later from October onward were of the larger phenotypes. We also found dead, decaying earthworms on October 30 to November 21. From October 30 to November 1, deceased *A. agrestis* varied between 4 and 8 cm length. After November 21, deceased earthworm bodies were exclusively longer than 8 cm.

In September and October before frosts occurred, small (<8cm) clitellate and aclitellate individuals were still observed (Figure 2 a-c). Soon after the first frost, the size distribution became more clearly bimodal, but with greatly reduced overall abundance. Eventually, in late November the distribution was skewed towards large (>8cm) individuals.

Of note, we observed some qualitative changes in the population from September to November. As of late September, the skin of some *A. agrestis* had a greenish, purple sheen even in diffuse light (Figure 3). In the summer and spring the colour was grey, with some iridescence displayed in narrow longitudinal stripes when bright light struck the skin. In November, surviving large earthworms were found in clumps of 2 to 4 individuals.

DISCUSSION

The breadth of the length distribution of *A. agrestis* observed in September and early October (Figure 2 a-c) is not unexpected, as it is similar to distributions observed for other *Amynthas* species (Blackmore, 2013; Tsai and Tsai, 2001). However, to our knowledge, the presence of very small clitellate *A. agrestis* has not been noted elsewhere. The development of small clitellated individuals may be a result of resource scarcity due to the depleted litter layer.

It was predominantly large *A. agrestis* that persisted when ambient temperatures fell below freezing (Figure 2 d-f). Our results can still be reconciled with Richardson *et al.* (2009), as freshly fallen leaf litter and the top few centimetres of soil where most of *A. agrestis* were found may have provided thermal insulation. However, during the last two sampling events, soil temperatures at sampling time were below 5º C, *i.e.*, below the lab mortality threshold for this species in the experiment of Richardson *et al.* (2010). Yet, some larger individuals still persisted. It is possible that *A. agrestis* populations in southern locations, where Richardson *et al.* (2009) harvested their earthworms, differ from those in Vermont. Genetic variations in populations that are geographically separated have been observed for other earthworm species (Shen *et al.*, 2002; Heethoff *et al.*, 2004).

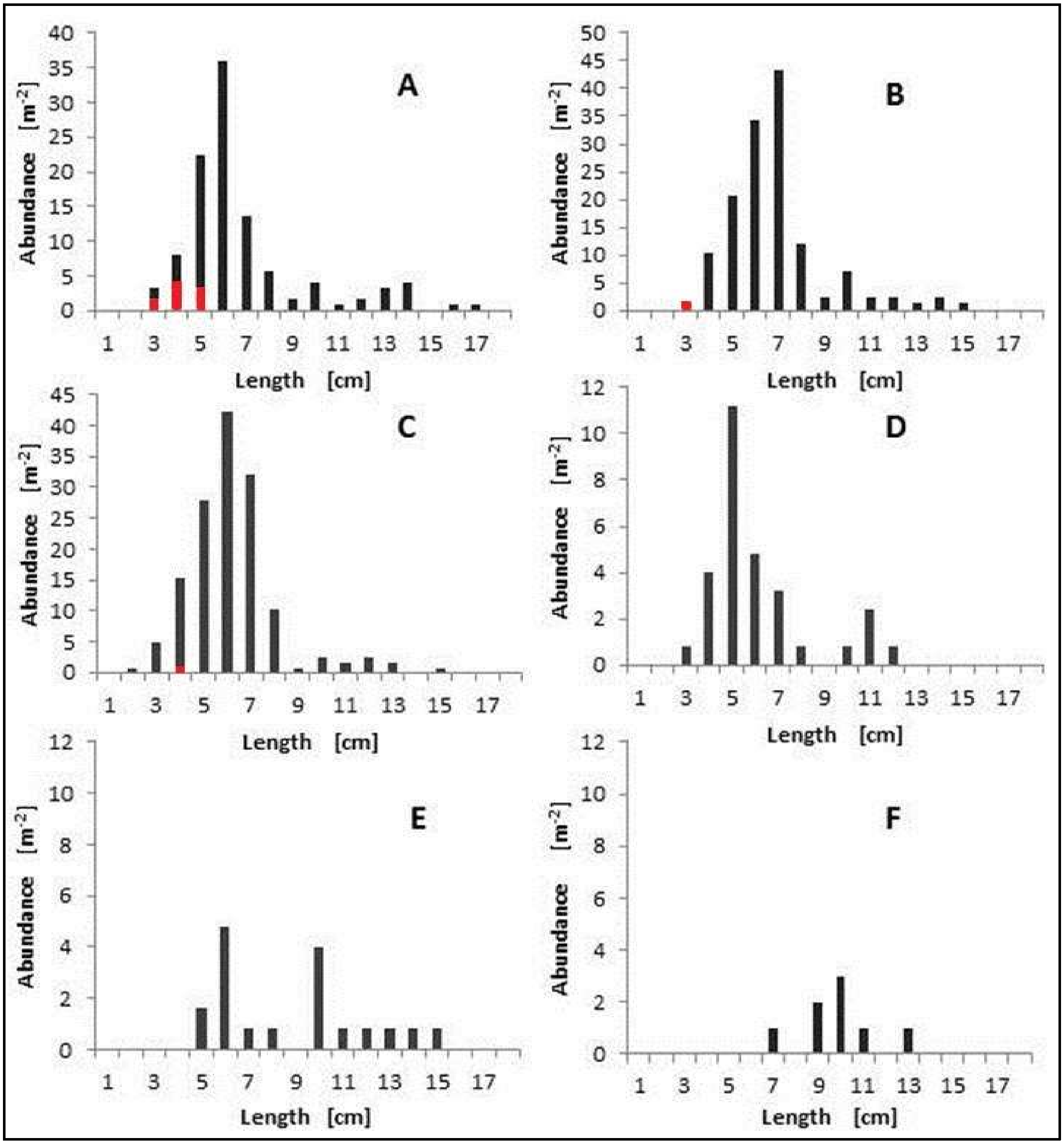

Fig. 2. Size variation of *Amynthas agrestis* on the sampling dates (A - September 16, B - September 26, C - October 5, D - October 30, E - November 1 and F - November 21). Red bars indicate aclitellate and dark bars clitellate earthworms.

Table 1. Abundance and body length and area-to-volume ratio (A/V) of *Amynthas agrestis* and soil and ambient temperatures at the study site for each sampling date.

Date	Abundance [m^{-2}] Total	Adult	Dead	Temperature [° C] Soil at sampling	Minimal ambient	R (>8)	Mean adult length (st. dev.) [cm]	A/V [cm^{-1}]
Sep -16	106	97	0	16.7	3	0.21	7.0 (2.7)	0.11
Sep. -26	141	142	0	14.4	1	0.22	6.5 (2.0)	0.12
Oct -5	142	142	0	13.8	10	0.13	5.6 (1.2)	0.16
Oct. -30	29	29	10	7.8	-2	0.17	6.1 (2.3)	0.13
Nov. -1	16	16	10	13.5	9	0.55	8.8 (3.1)	0.078
Nov -21	6	6	4	4.4	-8	0.83	9.8 (1.7)	0.068
Nov- 26	9	9	0	1.8	-1	1.00	9.6 (0.88)	0.069

Fig. 3. Iridescence of *Amynthas agrestis* in summer (image on left) and autumn populations (image on right).

A. agrestis abundance was severely reduced in our study after the first frost date at the end of October. Any dead earthworms found then were 8 cm or shorter in length. Late in November, dead earthworms were longer than 8 cm. As to why large individuals were favored to survive the prolonged cold snaps in October and November lends itself to speculation. Cold tolerance has been observed in other earthworm species. For example, high glucose concentrations lower the freezing point in *Aporrectodea caliginosa* (Holmstrup and Overgaard, 2007). However, body size may also matter as large body sizes in ectothermic animals retard cooling time (Ayers and Shine, 1997; Olalla-Tárraga and Rodríguez, 2007). It is possible that the small area-to-volume ratio of the large survivors (Table 1) protected them from cold damage in late October and early November by reducing heat loss per unit mass of the earthworm.

Preferential predation on small earthworms such as shown for the interaction between the red-backed salamander (*Plethodon cinereus*) and *Lumbricus terrestris* (Ransom, 2012) may contribute to the observed mortality pattern of small *A. agrestis*. *P. cinereus* is a common species and predator of earthworms in the Northeastern USA (Maerz *et al.*, 2005; Maerz *et al.*, 2009; Migge-Pleian *et al.*, 2006) and was present at the study site. In this context, it is interesting that the timing of peak activity of and earthworm predation by *P. cinereus* observed elsewhere (Taub, 1961; Maerz *et al.*, 2005) coincides with the decline of the *A. agrestis* population at our study site. Maerz *et al.* (2005) found that *P. cinereus* preyed on earthworms predominantly during the spring and autumn, especially after rainfall and at lower temperatures. Generally this assessment is supported by the activity pattern of *P. cinereus* at the soil surface as indicated by low capture rates in litter from June and August (Taub, 1961). However, if consumption by P. *cinereus* is a cause of mortality in *A. agrestis* in the fall, it only accounts for part of the loss in small juveniles, as dead, decomposing *A. agrestis* between 4 and 8 cm were found on October 30 and November 1.

CONCLUSIONS

Large individuals of *A. agrestis* survive in late autumn for longer than smaller ones. Dependence of mortality of small *A. agrestis* individuals in the field matched those observed in a lab study (Richardson *et al.*, 2009). However, mortality in large phenotypes did not follow this pattern.

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A catalogue of names, descriptions, and type specimens of the Oligochaeta
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