CONTRIBUTION TO KNOWLEDGE OF THE LIFE CYCLE OF *AEGOPINELLA EPIPEDOSTOMA* (FAGOT 1879) (GASTROPODA: PULMONATA: ZONITIDAE) IN THE LABORATORY

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Abstract *Laboratory observations made it possible to ascertain some life cycle parameters of* Aegopinella epipedostoma. *The eggs are laid in batches of 4–20 (mean 10); they are calcified, white and slightly flattened, of the mean size 1.6×1.3 mm; incubation takes 27 to 41 days (mean 35); hatchlings have shells of 1.3–2.0 whorls (mean 1.59). They reach adult size (4 whorls) in 220–298 days. The life cycle pattern is similar to that of* Aegopinella nitidula.

Key words *Zonitidae,* Aegopinella epipedostoma, *land snail, growth, reproduction*

INTRODUCTION

Aegopinella epipedostoma (Fagot 1879) is a montane species. Its distribution is still incompletely known because of frequent confusion with another, very similar species for example, *A. nitens* (Michaud 1831). It is known to occur in the Pyrenees and their northern foothills where it is common from Deux Sèvres (western France), the Taunus Mts (Germany), the Sudetes and the Carpathians. It is probably present also in Slovenia. An isolated northern outpost is located near Novgorod. The Pyrenees with their foothills are inhabited by the nominotypical subspecies; the form found in the east, including the Carpathians and the Sudetes, is *A. epipedostoma iuncta* Hudec (Riedel, 1988). The status of the two subspecies and their distribution require more detailed studies.

In Poland the species occurs in the Sudetes and their foothills, in the Carpathians (except for the Tatra) and in the Subcarpathian region (Riedel, 1988). It lives in damp litter and soil, mainly near streams, within montane forests. *Aegopinella epipedostoma* is regarded as rare and is red-listed in Poland (Wiktor & Riedel, 2002), although not included in the Polish Red Data Book (Głowaciński, 2004).

There is very little literature information on zonitid life cycles (see Heller, 2001; Maltz, 2003). The only published information on the biology of *A. epipedostoma* is a study on its age structure and growth rate in the field (Kuźnik-Kowalska, 2006). The present paper presents the results of laboratory observations on some life cycle parameters of the species.

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MATERIAL AND METHODS

The material for laboratory culture (72 snails of various age and 30 eggs) was collected on the 4 June 2005 in Muszkowice (SW Poland), close to the border of the nature reserve Muszkowicki Las Bukowy (for detailed habitat information see Kuźnik-Kowalska, 2006). The Muszkowice population represents subspecies *iuncta*. The laboratory observations lasted from 4 June 2005 to 28 February 2008. During the whole period of laboratory observations a total of 245 individuals were maintained, of which 72 were caught in the wild, 171 were hatched from eggs laid in the laboratory and 2 hatched from eggs collected in the wild.

The snails were kept in Petri dishes or in containers of a size depending on the number of individuals (dish diameter 5–14 cm, containers 6×7×7 cm, 7×11.5×7 cm and 12×15×7 cm). Damp tissue paper was used as substratum, with pieces of rotting timber and litter brought from the habitat where the snails were collected. The plant debris provided places for egg-laying and food (microflora). Additionally the snails were fed lettuce, cabbage, carrot, cottage cheese and boiled egg yolk. Dolomite tablets were provided as the source of calcium.

The temperature in the room ranged from 17°C (winter) to 25°C (summer). Humidity in the dishes and containers was maintained at a constant level of ca. 80%. The containers were aired at least once a week, and during periods of intense observations – every day or two days; water and food were supplied as needed.

The laboratory observations included growth rate, possibility of uniparental reproduction, fecundity, egg-laying, egg morphometrics, incubation and hatching. In order to ascertain the growth rate whorls were counted every 7 days (in the first month of life) and every 30 days (in the next months) by Ehrmann's (1933) method.

To confirm or exclude the ability to reproduce uniparentally ten individuals were kept singly from the egg stage or early juvenile stages until death.

Attempts at observing mating behaviour required keeping snails in pairs (20 kept in 10 pairs) and groups (143 in groups of 4 to 20 individuals: 8 groups of 4 individuals, 4 groups of 5 individuals, 6 groups of 5 individuals, 1 group of 10 individuals, 1 group of 11 individuals, 2 groups of 20 individuals).

In total, 315 eggs were observed. Eggs of individuals kept in pairs and in groups were measured with a calibrated eye-piece to the nearest 0.025 mm (n=60). Statistical analyses of the data were performed using Statistica PL 8.0 and Microsoft Excel 2007.

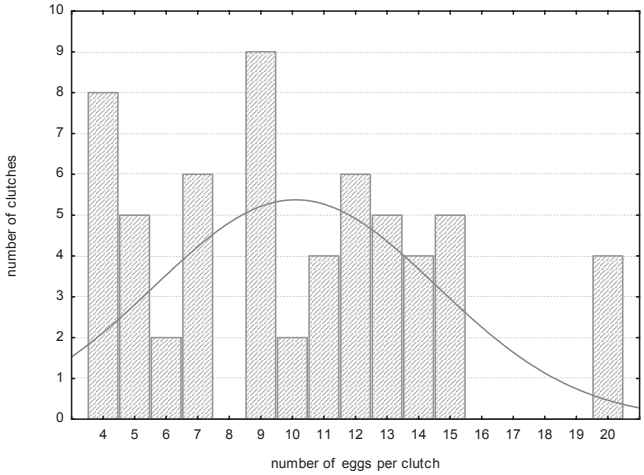

Figure 1 *Aegopinella epipedostoma.* Number of eggs per batch.

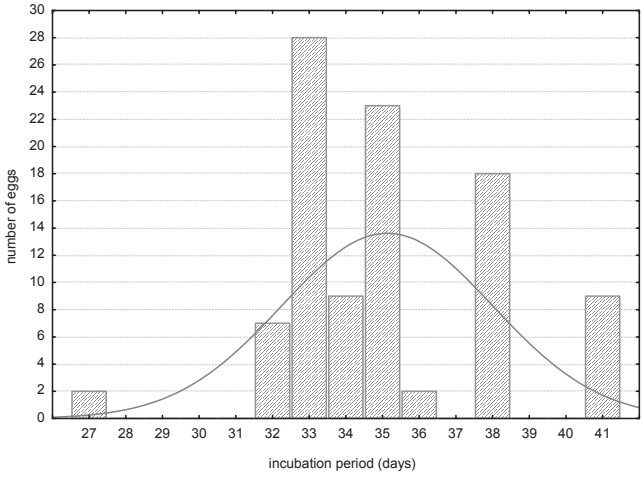

Figure 2 *Aegopinella epipedostoma.* Duration of incubation.

Results

No mating could be observed in the laboratory despite numerous attempts. *Aegopinella epipedostoma* laid eggs on or under pieces of rotting timber, tightly hidden in a corner of the container or among layers of tissue paper. The eggs were laid solely by individuals kept in groups and pairs, never by isolated individuals. Newly laid eggs were calcified, slightly ellipsoidal and flattened, milky white, initially shiny because of the fresh mucus covering; after 8–10 days they became matt. The egg size was: major diameter 1.13–1.85 mm (mean 1.59; SD 0.16; n=60), minor diameter 1.0–1.44 mm (mean 1.3; SD 0.1; n=60).

The number of eggs per batch ranged from 4 to 20 (mean 10; SD 4.45; n=60) (Fig. 1). One individual, observed closely from the start to the end of laying a batch of eggs, took 12 hours to produce the batch. In the laboratory the eggs were laid from June till August. There were no differences between size of eggs in relation to the number of eggs per batch (e.g. big eggs in the small batch, small eggs in the big batch).

Incubation took from 27 to 41 days (mean 35; SD=2.87; n=98) (Fig. 2). The hatchlings had translucent shells and bodies; their shells had 1.3–2.0 whorls (mean 1.59; SD 0.13; n=108) (Fig. 3). They were very active; at first they ate their own egg shells and then started looking for other food. The hatching success among the offspring of individuals kept in pairs or groups was 39.02%.

The growth of ten selected individuals is shown in Fig. 4. Since the smallest egg-laying individuals brought from the field had shells of four whorls, this size was accepted as the size at which *Ae.*

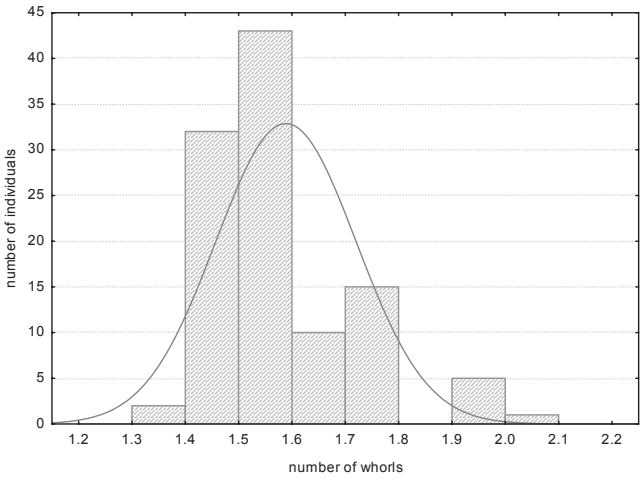

Figure 3 *Aegopinella epipedostoma.* Number of whorls in hatchlings.

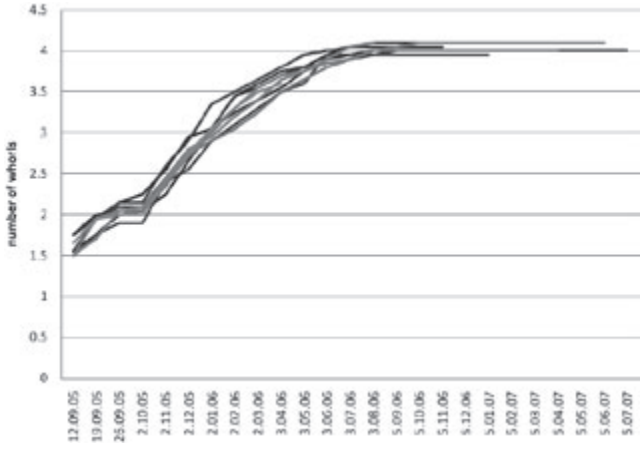

Figure 4 Growth curves of 10 selected individuals of *Aegopinella epipedostoma.*

epipedostoma reached maturity. The time between hatching and maturity (4.0 whorls) ranged from 220 to 298 days (mean 259.94; SD=28.16; n=40). Initially (from hatching to about 4 whorls) the growth was relatively fast, later there was almost no growth (see Fig. 4), though individuals much larger than 4 whorls were sometimes found in the field.

DISCUSSION

The knowledge of pulmonate life cycles is still far from complete, and varies much among all the various families (Heller, 2001). Zonitidae, with their 550 species and subspecies (Riedel, 1980), are among the least studied taxa. The list of papers dealing with various aspects of their biology includes Gassies (1849), Hazay (1881), Taylor (1906–14), Rigby (1963), Mordan (1978), Cameron (1982), Rodrigues *et al*. (1998, 2003) and Kuźnik-Kowalska (2006). The data contained therein pertain to *Aegopinella nitidula*, *A. nitens*, *A. epipedostoma*, *Oxychilus helveticus* and *O. atlanticus*, and are by no means complete. We managed to ascertain the structure of egg and batch, duration of incubation period and the appearance of hatchlings of *Ae. epipedostoma* in the laboratory, and – in the previous studies – its population dynamics and growth rate in the field (Kuźnik-Kowalska, 2006). Our observations pertained to only one population while it is known that populations may vary in some life cycle parameters according to their geographical location (Mordan, 1978). Nevertheless it is possible to attempt placing the information in the context of the family Zonitidae.

All the studied zonitids show indeterminate growth; even in species with unknown life histories such growth is evident from the shell structure and the wide range of variation in the number of whorls of mature individuals (see e.g. Riedel, 1966; Rodrigues *et al*., 1998). In this respect *A. epipedostoma* does not depart from the pattern set by other members of the family. Field studies on the population of the species from which the original laboratory material came indicate a life cycle of two, or at most, three years and a growth rate of about 0.5 whorl per month. The growth rate in the laboratory was ca. 0.5 whorl per month, also. Because the species is not very easy to keep in the laboratory, we could not ascertain the full life span. The duration of the life cycle is similar to that observed by Mordan (1978) in a British population of *A. nitidula* and by Gassies (1849) and Hazay (1881) for *A. nitens* in France and in Hungary – two species of size, shell structure and habitat requirements similar to those of *A. epipedostoma*. There is, however, some variation in the life cycle of *A. nitidula*, possibly depending on the geographical location and thus climate (Rigby, 1963; Mordan, 1978; Cameron, 1982). It is likely that studying more populations of *A. epipedostoma*, especially of its nominate subspecies, may reveal similar differences. Other representatives of Zonitidae, *Oxychilus helveticus* and *O. cellarius,* show a life cycle pattern similar to that of *Aegopinella* (Rigby, 1963; Cameron, 1982).

The hatchlings of *Ae. epipedostoma* in the field appeared from July till September, suggesting the egg-laying period was June-August. Laboratory individuals laid eggs in the same period. Mordan (1978) observed the peak of egg-laying in *A. nitidula* from March to August, though some eggs were recorded in other months.

The eggs of *Ae. epipedostoma* are calcified. There is no literature information on the egg structure in any other representative of the genus, but the only other zonitid studied in this respect, *Oxychilus atlanticus*, also has calcified eggs (Rodrigues *et al*., 2003).

Aegopinella epipedostoma lays eggs in batches of 4 to 20, the batch size being similar to that observed in *A. nitidula* (1–20; Mordan, 1978). The egg size (mean 1.59×1.30 mm in *A. epipedostoma* and 1.60×1.40 in *A. nitidula*) and incubation time (mean 35 days in *A. epipedostoma* and 38 days in *A. nitidula*) are also similar. The hatching success among the laboratory-bred *A. epipedostoma* was ca. 39%, in *A. nitidula* the percentage of infertile eggs in some batches was up to 47% (Mordan, 1978).

The life cycles of the few zonitids studied to date show a similar pattern of growth rate, fecundity and life span. All seem to be oviparous (or at least there is no information indicating egg retention or ovoviviparity; see Rodrigues *et al*., 1998; Mordan, 1978). It should be borne in mind, however, that the family includes many genera of very different shell size, habitat requirements and geographical distribution, and that large differences in life cycle patterns sometimes occur between representatives of such closely-knit taxa as Vertiginidae (Myzyk, 2011) or Clausiliidae (Maltz & Sulikowska-Drozd, 2008).

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