doi: 10.5281/zenodo.8081497

AGE STRUCTURE AND REPRODUCTIVE PATTERN OF THE ROCK-DWELLING LAND SNAIL *ALBINARIA ARCADICA* ALONG AN ELEVATIONAL GRADIENT

Paraskevi-Danai Androulidaki & Sinos Giokas

Department of Biology, University of Patras, Patras, 26500, Greece

Abstract *The age structure of the land snail* Albinaria arcadica *was studied in five populations on Mt. Chelmos (Peloponnisos, Greece), along an elevational gradient, between November 2018 and May 2019. Sampling took place every other month and shell height measurements were taken and analysed. Population growth rates varied between 11 months (one growing season) to 32 months (3 growing seasons) with the average rate being 18 months (two growing seasons). It was concluded that the species reproduces twice a year, in autumn and in spring.*

Key words *Albinaria, Greece, age structure, reproduction, elevational gradient*

Introduction

The analysis of age structure of a population, based on size measurements, can provide useful information for the biological cycle of a species. In many organisms, including land snails, life-history patterns vary due to the effects of environmental conditions, such as climatic altitudinal variation (Cook, 2001; Sulikowska-Drozd, 2011).

Albinaria Vest, 1867 is the most speciose clausiliid genus (c.a. 120 species), with a high degree of genetic and morphological differentiation (Giokas *et al.*, 2014; Schilthuizen, 2018). However, it has been suggested that its high taxonomic variation does not match with its low ecological differentiation (Gittenberger, 1991). The genus is distributed mainly in Greece, and is also found in Albania, Cyprus, and Turkey (Welter-Schultes, 2012). However, although the biogeography and systematics of *Albinaria* have been well studied, and its species constitute about 15% of the total Greek land-snail species (Vardinoyannis *et al.*, 2018), there are few studies of the life history and age structure of this genus.

Albinaria is an oviparous, iteroparous genus (Giokas & Mylonas, 2002; Giokas *et al.*, 2005) that lives on limestone substrates feeding on the microflora, i.e. lichens and bryophytes. They are active only during the wet season, that is, in southern Greece, from late October through the end of April. During the dry period individuals aestivate on the rock surfaces, in rock crevices, and occasionally on shrubs, or under stones. Especially during aestivation, aggregates are often formed, sometimes including many tens of individuals. Copulation then takes place during the first weeks of autumn rains. Eggs are laid in clutches of about 5–8 eggs, generally shortly (within 2–3 weeks). The duration of the oviposition and hatching period is short, but oviposition can be prolonged under favourable climatic conditions. The development from a juvenile (no lip or well-formed internal lamellae) to a fully-grown adult snail takes two and occasionally three wet seasons. During aestivation shell growth is suspended (Giokas *et al.*, 2005). Shell development precedes maturation, which occurs during the last dry season after enlargement of genitalia and its life span is estimated to be about seven years (Giokas & Mylonas, 2002).

In this study we examined variation of the age structure of *Albinaria arcadica* (Boettger, 1878) along an elevational gradient. *Albinaria* species are distributed in central Greece and Peloponnisos and *A. arcadica* is a rock-dwelling endemic to Greece. We aimed to examine patterns in life cycle, growth rate and reproductive behaviour among five populations.

Methods

Sampling and Data collection

The study was conducted on Mt. Chelmos (Peloponnisos, Greece). Five stations (populations) were selected along an altitudinal gradient of 1400m, ranging from 38m to 1478m. The rocky area within each sampling station that was scanned for snails varied between 40–60m^2.

Contact author : danae.biologia@gmail.com

Sampling took place every other month, from November 2018 to May 2019, except for the 5th station that we sampled only in November and May, because in the in-between months that station was covered by snow. All the individuals found were collected and shell height measurements taken from at least 15 juveniles and 15 adults (randomly chosen) from each population using a digital calliper to the nearest millimetre. In total, shell height measurements were taken from 583 individuals (240 juveniles and 343 adults). Afterwards the snails were returned to the rocks.

Data analysis

To identify the age structure of juveniles in each sampling occasion, the data taken were analysed using the Bhattacharya method with the FiSAT II, a package developed for the analysis of size-frequency data (Gayanilo *et al.*, 2005). This method fits normal distributions to the height-frequency histograms produced from the data, resulting in one normal distribution representing one age class (Bhattacharya, 1967). The histograms required for the analysis were derived using the Freedman & Diaconis method (Freedman & Diaconis, 1981). The adults were excluded from this analysis, assuming that they all belong in one age class. A separation index (SI) is calculated between the successive age classes created, indicating that the two age classes are confidently separated from each other if its value is >2. For the adult age classes or for the juveniles when no juvenile age class could be derived, the mean value and the standard deviation were calculated in each sampling occasion using IBM SPSS Statistics (Version 20).

RESULTS

The number of age classes detected in each sample differed between the five stations (Table 1). See Table 1 for the abbreviations used for the age classes. In six samples, even though juveniles were encountered and measured, their number was not sufficient for the applied method to produce any juvenile age class. The relative absence of juveniles, especially in the May samples, must be attributed in their cryptic behaviour, compared to adults, in rock crevices. However, in the other 12 samples the separation index was always satisfactory (>2), apart from one juvenile class (4S-Nov-J3) in the 4th station's November sample.

From the age classes derived (Table 1), the common pattern of almost all samples was the presence of one adult and one juvenile age class, with the exceptions of (a) the six aforementioned samples where only one adult class was detected, (b) four samples where two juvenile classes were produced, and (c) the 4th station's November where three juvenile classes were detected. The mean height for the juvenile classes varied between 4.25–16.68mm and for the adults between 15.94–19.15mm.

In the first station two juvenile classes and one adult were detected. The first juvenile class (S1-Nov-J1) had a mean height of 6.82mm in November, reached 14.42mm in January (S1-Jan-J2) and attained the final adult shell size (16.86mm) in March (S1-Mar–A). The second detected juvenile class (S1-Jan-J1) measures 6.54mm in January, grew to 7.91mm in March (S1-Mar-J1), and should attain a fully-grown shell in the following year.

In the second station there were two juvenile classes in November, and one in March. The first November's juvenile class (S2-Nov-J1) measured 12.45mm and most likely reached the final shell size in May (S2-May–A), measuring 18.41mm. The second November's juvenile class (S2-Nov-J2) had a mean height of 16.68mm and will be fully-grown (S2-Jan–A) by January (19.15mm). The third juvenile class (S2-Mar-J1) shows up in March with a mean height of 6.54mm and will attain a fully-grown shell the following year.

Regarding the 3rd station, the 3S-Nov-J1 age class (4.25mm) on November, will likely to become 3S-Jan-J1 (8.23mm) on January, then 3S-May-J1 (11.61mm) on May and to reach its final shell size the following year.

In the 4th station, unlike the previous stations, three juvenile classes were detected in November, with high differences in their mean heights: 8.23mm (4S-Nov-J1), 13.89 (4S-Nov-J2) and 16.68mm (4S-Nov-J3). The mean height (16.68mm) of 4S-Nov-J3 age class almost reaches the adult classes mean height, meaning it is close to fully-grown shell and will most probably reach it (18.57mm) on January (4S-Jan–A). The 4S-Nov-J1 age class will grow up to be 4S-Mar-J1 on March (12.17mm) and will reach its final shell size next year. The 4S-Nov-J2 age class will become the 4S-Jan-J2 (14.85mm) on January and

Table 1 The age classes of *Albinaria arcadica* detected in the five stations studied along an altitudinal gradient on Mt. Chelmos. For each station are given data concerning coordinates and elevation, the sampling month, the age classes detected (J for Juveniles, A for Adults), the mean height (mm) and standard deviation SD (mm) of each class, the sample size (N) and the separation index (SI). Due to the small sample in some cases (NA – non applicable), no juvenile age class could be derived by the method applied. Mean height and SD were calculated using Bhattacharya's method for the juveniles and SPSS for the adults or for the juveniles when no juvenile age class could be derived.

Station	Coordinates	Elevation (m)	Sampling Month	Age class	Mean Height (mm)	SD (mm)	N	SI
1st	38° 10' 44.7''N, 22° 11' 35.6''E	38	November	S1-Nov-J1	6.82	1.85	26	-
				S1-Nov-A	15.94	1.03	42	-
			January	S1-Jan-J1	6.54	2.03	20	-
				S1-Jan-J2	14.42	2.7		2.66
				S1-Jan-A	16.53	1.45	20	-
			March	S1-Mar-J1	7.91	1.14	15	-
				S1-Mar-A	16.86	1.01	15	-
			May	S1-May-J1	8.32	0.93	3	NA
				S1-May-A	16.33	0.73	15	-
2nd	38° 09' 05.8''N, 22° 10' 23.9''E	137m	November	S2-Nov-J1	12.45	1.44	11	-
				S2-Nov-J2	16.68	1.33		2.21
				S2-Nov-A	18.99	1.16	12	-
			January	S2-Jan-J1	9.08	5.11	6	NA
				S2-Jan-A	19.15	1.16	20	-
			March	S2-Mar-J1	6.54	2.28	15	-
				S2-Mar-A	18.72	1.2	15	-
			May	S2-May-J1	-	-	0	NA
				S2-May-A	18.41	0.66	8	-
3rd	38° 03' 35.9''N, 22° 09' 17.4''E	668m	November	3S-Nov-J1	4.25	1.88	10	-
				3S-Nov-A	17.34	0.9	30	-
			January	3S-Jan-J1	8.23	2.03	6	-
				3S-Jan-A	16.75	0.87	16	-
			March	3S-Mar-J1	12.67	2.92	4	NA
				3S-Mar-A	17.23	1.03	21	-
			May	3S-May-J1	11.61	2.03	5	-
				3S-May-A	17.46	1.01	15	-
4th	38° 07' 33.5''N, 22° 11' 28''E	907m	November	4S-Nov-J1	8.23	1.47	52	-
				4S-Nov-J2	13.89	1.91		2.47
				4S-Nov-J3	16.68	1.81		1.88
				4S-Nov-A	18.24	1.18	22	-
			January	4S-Jan-J1	7.38	1.44	17	-
				4S-Jan-J2	14.85	2.29		2.85
				4S-Jan-A	18.57	0.53	16	-
			March	4S-Mar-J1	12.17	2.28	20	-
				4S-Mar-A	18.44	0.85	18	-
			May	4S-May-J1	9.51	1.22	7	NA
				4S-May-A	17.12	1.2	8	-
5th	38° 01' 13.1''N, 22° 10' 37''E	1478m	November	5S-Nov-J1	6.54	1.77	20	-
				5S-Nov-J2	11.7	1.57		2.44
				5S-Nov-A	17.51	0.76	36	-
			May	5S-May-J1	8.47	1.51	3	NA
				5S-May-A	17.43	1.25	14	-

will have a full-grown shell (17.12mm) on May (4S-May–A).

Concerning the 5th station, two juvenile classes were derived for November (5S-Nov-J1 and 5S-Nov-J2) and none for May and no comment about their growth can be made. Regarding the adults, their mean height is 17.47mm (SD: 1.38mm). However, it is noticeable that they exhibit different mean sizes in each station. The highest mean heights occur in the 2nd station (18.89mm, SD: 1.12mm) and the lowest in the 1st (16.29mm, SD: 1.13mm). In between are the 4th station (18.24, SD: 1.05mm), the 5th station (17.50, SD: 0.92mm) and 3rd station (17.22, SD: 0.97mm). These height differences of adults among station where significant ($F=64.61$, $p<0.0001$).

It should be mentioned that juveniles below 6mm were encountered at every station. They were found in November in all of the five stations and also in January for the 1st station and January-March for the 2nd station. Nevertheless, only one age class with such low mean height was obtained, probably due to the small number of individuals found and measured.

DISCUSSION

The differences found in growth rate can be attributed to varying environmental conditions. At the first four stations higher growth rates were observed at the lower altitudes. A possible explanation for this difference could be more favourable weather conditions, such as temperature and humidity, at the lower altitudes. Similarly, Sulikowska-Drozd (2011) found higher growth rates in populations at lower altitudes in the hygrophilous, egg-retaining clausiliid *Vestia gulo*, that was studied in the Carpathians. High humidity has been reported to increase the snails' growth rate. In the study of Staikou *et al*. (1988) it was suggested that the increased growth rate of *Helix lucorum* juveniles measured over two years was due to the higher humidity. The same hypothesis was also made for the *Bradybaena fruticum* juveniles (Staikou *et al.*, 1990). In the study of Prockow *et al*. (2012) it was suggested that low precipitation resulted in decelerated growth rate for the *B. fruticum* juveniles.

It is rational to claim that the juveniles with height around 7mm in November could not have been hatched that same month, but rather on the preceding spring, perhaps around March. This is based upon the fact that the eggs observed in November did not have such a large diameter, meaning that the hatchlings could not measure 7mm. Having been hatched in March would allow for these hatchlings to grow until April, when aestivation starts, and to continue growing when the rainfalls start around October–November. Moreover, as mentioned before, juveniles with shell height around 4mm were found which must be November's hatchlings. On the contrary, the juveniles with a mean height of about 7–8mm in March must be the those that hatched in November and gained on average 3mm in 4 months. Furthermore, the juveniles which measured 12mm or more on November may have been hatched on the previous November in order to have time to grow and will reach the adult shell size until May.

In conclusion, if we assume a stable growth rate per station and that shell growth is suspended during the 6-month aestivation period, it seems that it takes, on average, 18 months (two growing seasons) for a hatchling to reach adult shell size. This outcome is similar to other four *Albinaria* species studied by Giokas & Mylonas (2002). Yet, this estimation could be as low as 11 months (one growing season) in the 1st station, and as high as 32 months (3 growing seasons) in the 4th station. Analogous studies in other clausiliids show contradictory results. *Cristataria genezarethana* (Heller & Dovel, 1994) studied in Israel, had three age groups (young, sub–adults and adults). Hatching occurred in February–March, but no shift from the juvenile to the adult age class was noted within 3 years, and it was suggested that the species matures in the eleventh year, due to adverse very arid conditions. Regarding *Alinda biplicata* (Kuznik-Kowalska, 1998), studied in SW Poland, new hatchlings appeared from June to August and the snails reach maturity after three or four years. In total, ten juvenile age classes were observed in different stages of growth, as well as one subadult and one adult. We must note that in this study immature individuals obtained from the samples were divided into age classes according to the number of whorls (each age class corresponds to one whorl increment). Finally, Sulikowska-Drozd (2011) suggest that *Vestia gulo*, studied in the Carpathians, reaches ultimate size in two or three seasons.

There is no research concerning the reproduction of *A. arcadica* and the only study on reproduction of the genus (Giokas & Mylonas, 2002) suggests that they usually copulate after the end of aestivation in autumn and lay the first eggs shortly (within two weeks) after copulation. Yet, Giokas & Mylonas (2002) also advocate that oviposition can be prolonged under favourable climatic conditions. However, our study suggests that *A. arcadica* lays eggs in spring as well as autumn and that can advocate either that the species has two reproductive periods or that it has one long reproductive period, lasting from October to March. Even though many land snail species have one reproductive event per year, there have been cases where reproduction takes place twice a year, like *Helicodonta obvoluta* (Maltz, 2003). According to Maltz (2003) *H. obvoluta* hibernates in winter and reproduces in spring (April–beginning of June) and in autumn (end of August–beginning of October). Another explanation could be that *A. arcadica* has one long reproductive period, like some other snail species (Kiss *et al*., 2005; Sulikowska-Drozd *et al.,* 2013). In the case of *Xeropicta derbentina*, Kiss *et al.* (2005) found that the species has a long five-month reproductive period, lasting from September to January. Similar observations were made by Sulikowska-Drozd *et al.* (2013) who found that *Alinda biplicata* can reproduce for seven months, from March to September.

The scenario of *A. arcadica* having one long reproductive period, lasting six months, from October to March, seems unlikely. In that case, the individuals reproducing during the six months should be egg-retaining and that would result in more juvenile classes, with lower mean heights than those observed. Even though egg-retention has been documented in some clausiliids (Sulikowska-Drozd, 2009) it has to be confirmed in *Albinaria*. Therefore, our data support the existence of two reproductive periods and not a single long one. To conclude, this study suggests that *A. arcadica* reproduces twice a year, in autumn and in spring. That conclusion is a novel one concerning *Albinaria* and suggests that more research is needed for other species in the genus, in order to see whether they also exhibit a similar reproductive pattern.

ACKNOWLEDGMENTS

We would like to express our gratitude to Grigoris Nikolopoulos and Vasilis Kypraios-Skrekas for their field assistance. We also thank Nikos Chelmis for providing information about the locations of the stations examined.

REFERENCES

BHATTACHARYA CG 1967 A Simple Method of Resolution of a Distribution into Gaussian International *Biometrics* **23**: 115–135.

COOK A 2001 Behavioural ecology: on doing the right thing, in the right place at the right time In: The biology of terrestrial molluscs (G.M. Barker, ed.) 447–487. CABI Publishing, Oxford.

FREEDMAN D & DIACONIS P 1981 On the histogram as a density estimator: L_2 theory *Zeitschrift für Wahrscheinlichkeitstheorie und Verwandte Gebiete* **57**: 453–476.

GAYANILO FC, SPARRE P & PAULY D 2005 FAO-ICLARM stock assessment tools II (FiSAT II) Revised Version User's guide FAO Computerized Information Series (Fisheries), No.8, Rome, 168 pp.

GIOKAS S & MYLONAS M 2002 Spatial distribution, density and life history in four *Albinaria* species (Gastropoda, Pulmonata, Clausiliidae) *Malacologia* **44**: 33–46.

GIOKAS S, PAFILIS P & VALAKOS E 2005 Ecological and physiological adaptations of the land snail *Albinaria caerulea* (Pulmonata: Clausiliidae) *Journal of Molluscan Studies* **71**: 15–23.

GIOKAS S, PÁLL-GERGELY B & METTOURIS O 2014 Nonrandom variation of morphological traits across environmental gradients in a land snail *Evolutionary Ecology* **28**: 323–340.

GITTENBERGER E 1991 What about non-adaptive radiation? *Biological Journal of the Linnean Society* **43**: 263–272.

HELLER J & DOLEV A 1994 Biology and population dynamics of a crevice-dwelling landsnail, *Cristataria genezarethana* (Clausiliidae) *The Malacological Society of London* **60**: 33–46.

IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

KISS L, LABAUNE C, MAGNIN F & AUBRY S 2005 Plasticity of the life cycle of *Xeropicta derbentina* (Krynicki, 1836), a recently introduced snail in Mediterranean France *Journal of Molluscan Studies* **71**: 221–231.

KUZNIK-KOWALSKA E 1998 Life cycle and population dynamics of *Alinda biplicata* (Montagu, 1803) (Gastropoda: Pulmonata: Clausiliidae) *Folia Malacologica* **6**: 33–37.

MALTZ TK 2003 Life cycle and population dynamics of *Helicodonta obvoluta* (O. F. Müller, 1774) (Gastropoda: Pulmonata: Helicidae) *Folia Malacologica* **11**: 63–88.

PRÓCKÓW M, KUZNIK-KOWALSKA E & LEWANDOWSKA M 2012 Differences in population dynamics of *Bradybaena fruticum* (O. F. Müller, 1774) (Gastropoda: Pulmonata: Bradybaenidae) in a submontane and lowland area of Poland *Animal Biology* **62**: 451–462.

SCHILTHUIZEN M 2018 The *Albinaria* enigma. *In*: Biogeography and Biodiversity of the Aegean. pp.67–78. In: Biogeography and Biodiversity of the

Aegean. In honour of Prof. Moysis Mylonas (Eds. Sfenthourakis S, Pafilis P, Parmakelis A, Poulakakis N, Triantis K). Broken Hill Publishers Ltd, Nicosia, Cyprus.

Staikou A, Lazaridou-Dimitriadou M & Farmakis N 1988 Aspects of the life cycle, population dynamics, growth and secondary production of the edible snail *Helix lucorum* Linnaeus, 1758 (Gastropoda, Pulmonata) in Greece *Journal of Molluscan Studies* **54**: 139–155.

Staikou A, Lazaridou-Dimitriadou M & Pana E 1990 The life cycle, population dynamics, growth and secondary production of the snail *Bradybaena fruticum* (Müller, 1774) (Gastropoda Pulmonata) in Northern Greece *Journal of Molluscan Studies* **56**: 137–146.

Sulikowska-Drozd A 2009 Egg retention and ovoviviparity in clausiliids of the genus Vestia P. Hesse (Gastropoda: Clausiliidae). *Journal of Molluscan Studies*, **75**: 351–359.

Sulikowska-Drozd A 2011. Population dynamics of the Carpathian clausiliid *Vestia gulo* (EA Bielz 1859) (Pulmonata: Clausiliidae) under various climatic conditions. *Journal of Conchology* **40**: 462–470.

Sulikowska-Drozd A, Maltz TK & Kappes H 2013 Brooding in a temperate zone land snail: seasonal and regional patterns *Contributions to Zoology* **82**: 85–94.

Vardinoyannis K, Parmakelis A, Triantis K & Giokas S 2018 Land Molluscs in Greece: The rich, unique, diverse and unprotected animal models. pp. 47–66. In: Biogeography and Biodiversity of the Aegean. In honour of Prof. Moysis Mylonas (Eds. Sfenthourakis S, Pafilis P, Parmakelis A, Poulakakis N, Triantis K). Broken Hill Publishers Ltd, Nicosia, Cyprus.

Welter-Shultes FW 2012 European non-marine molluscs, a guide for species identification Planet Poster Editions, Gottingen, 760 pp.