Unusual reproductive strategy of pulmonate gastropods in the Balkan ancient Lake Prespa

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Abstract. In freshwater pulmonates, egg masses are usually deposited on hard substrates such as macrophytic plants, rock debris or wood. An unusual reproductive strategy in *Planorbis presbensis* and *Radix* cf. *auricularia* was found in the Balkan ancient Lake Prespa. These two species oviposite on their own shells and carry the egg masses. Selected reproductive parameters are compared with related species. Potential reasons causing the observed behaviour in an ancient lake environment are discussed.

Kurzfassung. Eine ungewöhnliche Reproduktionsstrategie von Pulmonaten im Balkan-Langzeitsee Prespa. – Süßwasser-Lungenschnecken laichen auf Hartsubstrat wie z. B. Steinen, Wasserpflanzen oder Holzstücken. Eine ungewöhnliche Reproduktionsstrategie wurde für *Planorbis presbensis* und *Radix* cf. *auricularia* im Prespasee, einem Langzeitsee auf dem Balkan, gefunden. Beide Arten tragen ihre Gelege auf der Schale. Ausgewählte Reproduktionsparameter werden mit denen verwandter Arten verglichen. Potentielle Ursachen für ein derartiges Verhalten in einem Langzeitsee werden diskutiert.

Key words. Reproduction, *Planorbis*, *Radix*, Lake Prespa, ancient lake.

Introduction

Oviparity with eggs laid as egg masses (spawn) containing capsules dominates as mode of reproduction among Pulmonata (GERAERTS & JOOSSE 1984; TOMPA 1984). In freshwater pulmonates, egg masses are usually deposited on hard substrates such as macrophytic plants, rock debris or wood (e. g. BONDESEN 1950; DUNCAN 1975; TOMPA 1979a,b; GERAERTS & JOOSSE 1984). Morphology and structure of these egg masses have been occasionally studied and their phylogenetic implications discussed by several authors (e. g. NEKRASSOW 1929; BONDESEN 1950; PARAENSE 1959; BEREZKINA & STAROBOGATOV 1986, 1988).

Particular reproductive strategies like brooding in prosobranch snails have been suggested to be involved in speciation processes in ancient or long-lived lakes. Viviparous gastropods are thought to be more speciouse due their limited dispersal abilities and brooding habit (COHEN & JOHNSTON 1987; MICHEL 1994, 2004; MARTENS 1997). Special brooding strategies were also reported in taxa endemic to the Sulawesi (Indonesia) ancient lakes like for pachychilid gastropod *Tylomelania* (e. g. RINTELEN & GLAUBRECHT 2004, 2006, BOGAN & BOUCHET 1998). Studies on reproduction in Baicaliidae from ancient Lake Baikal suggested a link between reproduction, hatching and feeding in these gastropods (SITNIKOVA et al. 2001).

In contrast, data on reproductive strategies of pulmonate gastropods from ancient lake environments are rather scarce with some notable exception like the acroloxid limpets in Lake Baikal (e. g. SHIROKAYA & RÖPSTORF 2003). Recently, a novel and unique reproductive strategy including retention of spawn and brood care in the lacustrine freshwater limpet genus *Protancylus* was reported from an ancient lake system on Sulawesi (ALBRECHT & GLAUBRECHT 2006).

A recognized European ancient (long-lived) lake is Lake Prespa in the Macedonia-Albania-Greece border region. Lake Prespa – also called Megali Prespa, Prespanskoye Jezero, and Prespës së Madhe (South Adriatic-Ionian biogeographic region) – is located at an altitude of 853 m a.s.l. It covers 253 km² and is the highest major lake in the Balkan. Subterranean karstic channels are believed to connect the lakes Ohrid and Prespa. The maximum depth of Lake

Tab. 1. Reproductive parameters of *Planorbis presbensis* and *Radix* cf. *auricularia*. Footnotes: (1) not measurable due to 3-dimensional warps of the egg masses on the shell surface; (2) minimum time span, covered by collections during these months in the field.

	Planorbis presbensis	*Radix* cf. *auricularia*
No. egg masses examined	4	11
No. eggs per egg mass	12 (7-22)	111 (73 – 135)
Average length of egg mass [mm]	2.05	– (1)
Average length of egg mass carrying individual [mm]	8.6 (8.2 – 9.0)	15.5 (14.33 – 20.22)
Egg mass length/length mother	0.24 (0.19 – 0.30)	– (1)
Average size of egg [mm]	0.65 (0.50 –0.72)	0.62 (0.54 –0.73)
Time of reproduction	May (2)	September/October (2)

Prespa is approximately 55 m. Lake Prespa is considered as oligomesotrophic with an average Secchi depth of 6.2 m and a pH of 8.3 (ZACHARIAS et al. 2002). Summer thermal stratification is pronounced and eutrophication problems are known as well as from many other Balkan lakes (e. g. ALBRECHT et al. 2006).
I here report on two cases of an unusual pulmonate reproductive strategy involving carrying egg masses in the Balkan ancient Lake Prespa.

Material and methods

Material was collected during field trips in May and September/October 2005 to the lakes in the southern Balkan region. This paper is concerned with two species collected by hand in Lake Prespa: *Planorbis* (*Crassiplanorbis*) *presbensis* Sturany, 1894 (locality 1) and *Radix* cf. *auricularia* (Linnaeus, 1758) (locality 2).
Planorbis presbensis was often considered to belong to *Gyraulus*. MEIER-BROOK (1976) in his detailed account showed that *P. presbensis* indeed is a true *Planorbis s. str.* species according to reproductive organ characteristics. *Planorbis presbensis* is distinguished from *Gyraulus* spp. by a terminal penis opening and absence of the characteristic stylet (MEIER-BROOK 1976). Genetic data also indicate a close relationship of *Planorbis presbensis* and *Planorbis planorbis* (ALBRECHT, unpublished data). The clearly pseudodextral species with thickened shell and absent angle is endemic to Lake Prespa and was placed in a separate subgenus *Crassiplanorbis* Meier-Brook, 1976.
The collecting localities are:
1. Greece, Dytikí Makedonía, Psarades, between Psarades and Albanian border, Lake Megali Prespa, bay at cliff-like coast, 40.82032° N, 21.01939° E, calcareous rocks with interlithonic spaces (at least in part), 0–0.6 m depth (code BA05.45), leg.: 16 May 2005, C. Albrecht, T. Wilke;
2. Republic of Macedonia, Resenski oblast, Asamati, Lake Prespa NE edge, 40,98916°N, 21,04429°E, 0–0.2 deep littoral mud (code BA.05.103), leg.: 1 October 2005, C. Albrecht, T. Wilke, R. Schultheiß, T. Geertz, A. Hauswald.
Snails were preserved in 80% ethanol. Individuals and egg masses were studied using a Olympus SZX 12 stereo microscope. Digital images with scale were taken with a ColorView FW camera system. All measurements were done utilizing the analySIS 5.0 software package (Soft Imaging System GmbH 2004). Terminology follows Klussmann-Kolb and Wägele (2001). Statistical analyses were performed using STATISTICA (StatSoft. Inc. 1995). All material is deposited in the Malacological collection of the Institut für Allgemeine und Spezielle Zoologie Giessen, inventorized under the respective catalogue numbers.

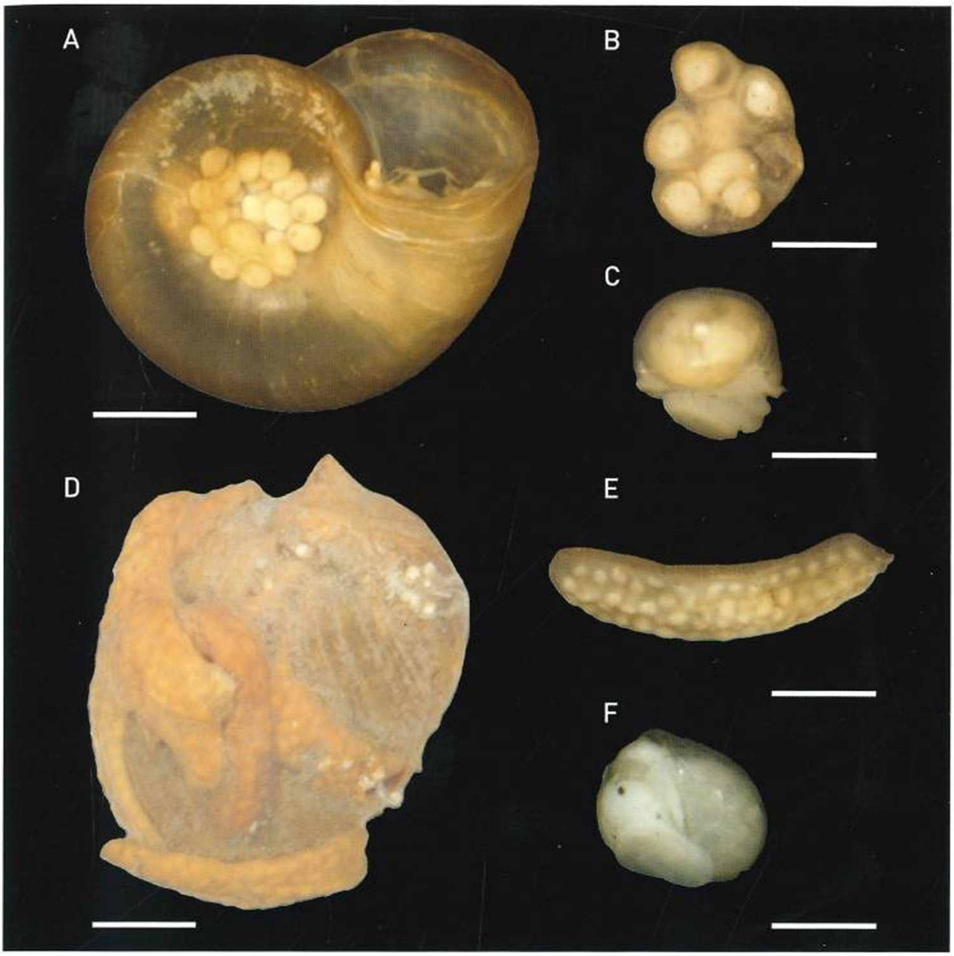

Fig. 1. Reproductive strategy of *Planorbis presbensis* and *Radix* cf. *auricularia* in Lake Prespa. *Planorbis presbensis*: A – Position of egg mass in concave side of the shell, scale bar 2 mm, B – Egg mass containing embryos with eyes visible, scale bar 1 mm, C – Freshly hatched individual, scale bar 0.2 mm. *Radix* cf. *auricularia*: D – Snail covered with several egg masses, scale bar 5 mm, E – Horseshoe shaped egg mass, scale bar 5 mm, F – Freshly hatched individual, scale bar 0.2 mm.

Results

Planorbis presbensis.

In the population studied, a total of 6 egg masses was found of which 4 where still intact (Tab. 1). In *Planorbis presbensis*, egg masses are firmly attached to and carried in the concave side of the shell. This is the functional umbilical position since *Planorbis presbensis* is pseudodextral (Fig. 1A). The roundish egg masses fit the shape of the concave side of the shell. The egg masses contain polygonal eggs laid in one layer (Fig. 1A, B). Most egg masses appear to be slightly yellowish in colour.

Up to 22, but on average 12 eggs (Tab. 1) are enveloped by a solid but transparent membrane forming the egg masses (Fig. 1B). The smallest snail carrying an egg mass was 8.2 mm in maximum diameter (Tab. 1). A freshly hatched embryonic snail was 0.33 mm in length (Fig. 1C).

Radix cf. *auricularia*.
A total of 11 egg masses (Tab. 1) was found which are carried on all possible position of the teleconch (Fig. 1D). Usually, one individual carried 1–2 egg masses on its shell. Eggs are oviposited in 1 to 2 layers in a certain direction producing egg masses with spiral torsion (Fig. 1D, E). Egg masses appeared to be whitish but had an ochre colour incrustation because of the substrate the snails live at (Fig. 1D). The number of eggs per egg mass varied widely with an average of 111 eggs (Tab. 1) These eggs were enveloped by a solid, not entirely transparent membrane (Fig. 1E). An embryo was 0.55 mm long (Fig. 1F).

Discussion

Comparison of reproductive strategies. The *Planorbis presbensis* egg mass is a typical "capsula plana" (NEKRASSOW 1929). These egg masses (= capsules) are found throughout members of the Planorboidea showing both spiral torsion and a characteristic operculate suture (BONDESEN 1950; BEREZKINA & STAROBOGATOV 1986). *Planorbis planorbis*, the widespread European species, produces 30 eggs on average (BONDESEN 1950). Other literature records report lower numbers e.g. 10 to 20 eggs (PFEIFFER 1821), up to 21 eggs (MOQUIN-TANDON 1855). NEKRASSOW (1929) found 25 eggs while PRECHT (1936) found 12.5 eggs per capsule on average. Therefore, the number of eggs or juveniles produced in *Planorbis presbensis* is within the range typical for the superfamily (BONDESEN 1950). In contrast, other freshwater basommatophoran families like Physidae and Lymnaeidae produce up to 10–20 times more eggs per clutch (GERAERTS & JOOSSE 1984).
Planorbis planorbis is characterized by an pronounced operculate suture, while both *Planorbis carinatus* and *Planorbis presbensis* lack such a structural feature. *Planorbis carinatus* and *Planorbis planorbis* have similar egg masses. MOQUIN-TANDON (1855) found 10 to 20 eggs per capsule, 4 to 7 were reported by PRECHT (1936) and BONDESEN (1950) gives up to 28 eggs. Egg masses are laid on macrophytes like *Myriophyllum* sp. (BONDESEN 1950). The variation in egg numbers were attributed to the size (age) of the ovipositing snail (FRÖMMING 1956). This author found hatchlings 0.8 to 0.9 mm large which is considerably bigger than the individual found in *Planorbis presbensis*. However, the latter species is considerably smaller than *Planorbis planorbis*.
The way how egg masses are attached to the functional underside of the snails makes oviposition by another animal of the same population unlikely.
The *Radix* species was attributed to the *auricularia*-type according to preliminary morphological and prior to genetic examination. Problems in distinguishing *Radix* species are pertinent (e. g. BARGUES et al. 2001) and genetic variability is pronounced and complex (ALBRECHT, unpublished data). While some species of *Radix* in Central and Northern Europe can be distinguished by a combination of morphological and anatomical characters, there are not enough data available yet for the circum-Mediterranean lineages. Nevertheless, the amount of eggs per egg mass is well within the range given for *Radix auricularia*. Large egg masses up to 70 mm have been reported for that species with up to 338 eggs (BONDESEN 1950). Two egg layers are usually found (e. g. MOQUIN-TANDON 1855). The regularity of egg placement appearing as egg rows is long known for *Radix auricularia* (BONDESEN 1950). Despite the usual oviposition on plants or rocks, HAZAY (1881) published his observation of oviposition of 8 to 12 egg masses on the shell of a single individual. NEKRASSOW (1929) described the horseshoe-shape of the egg mass. This could be found in only very few cases in the population from Lake Prespa. It is impossible to decide whether the egg masses on the shells were laid by the very carrying or conspecific individuals.
Little is known about the reproductive strategy of Lake Prespa endemic *Radix pinteri* Schütt, 1974. However, I did not find *Radix pinteri* carrying egg masses. This is also true for the Lake Ohrid endemic species *Radix relicta* (Polinski, 1929).

Reproduction in ancient lake environment. *P. presbensis* lives in the littoral of Lake Prespa where enough hard substrate is available for oviposition in rocky or stony coastal parts. However, no oviposition on these substrates could be recognized during the field trips. In Lake Ohrid, a very similar habitat is populated by *Planorbis macedonicus* Sturany, 1894, a presumably closely related species. No oviposition like in *Planorbis presbensis* is known from Lake Ohrid. This is also true for the endemic pseudodextral *Gyraulus* (*Carinogyraulus*) sp. Therefore, it seems possible that annual fluctuations in water level could be a reason for the strategy observed. A highly unpredictable environment like the littoral zone of Lake Prespa which is known to vary by several meters (e. g. Löffler et al. 1998) should cause strategies for protection of sensitive egg masses and apparently offspring from dessication. Carrying the egg masses allows an adaptation to water level changes. Contrary in Lake Ohrid, annual water level fluctuations are much less pronounced thus providing a much more stabile habitat.
The above reasoning for the development of the observed reproductive strategy can also be involved when discussing the *Radix* behaviour, which showed an almost amphibious life style in the population described. Absence of submerse vegetation and predominance of soft and muddy substrates (at least in this part of the littoral) and rapidly changing water levels coincide in the particularly shallow NE part of the lake. These condition are most likely responsible for oviposition on the shells surface and carrying the egg masses.
It is not clear whether the ancient lake environment *per se* led to the evolution of the strategies described here. Oviposition on shells could also result in negative effects since due to necessary movements, egg masses would face a permanent danger of abrasion and contamination with debris and sand grains. In *Radix*, this was evident in the coloration of the egg masses caused by the substrate. In general, egg masses of gastropods are also in danger of predation (e. g. Rawlings 1990).
The reproductive strategy described in this paper concerns littoral species. On the other hand, deep-lake pulmonate gastropods of the genus *Choanomphalus* have been reported from Lake Baikal to oviposite on their own shells (Dybowski 1875). Egg masses contain 4–5 roundish eggs, they are whitish yellow and of a horny consistence. One to three egg masses are laid in the navel of the shell (Bondesen 1950). Nonetheless, more than one egg mass is laid on the outer surface of the *Choanomphalus* shell. The species mentioned live in 350 m depth of a cold north-hemisphaeric ancient lake, which characteristics Bondesen (1950) compares with ocean conditions. In addition, recently an acroloxid limpet *Gerstfeldtiancylus benedictiae* has been described to oviposite on shells of *Benedictia* spp. in the absence of hard substrate, another case involving a limpet in the ancient lake environment of Lake Baikal (Shirokaya & Röpstorf 2003).
It is not clear yet whether these examples hint at some congruent evolutionary forces acting on molluscs in these so-called ancient lakes. Boss (1978) suggested that toughness of egg membranes seems positively correlated with speciation potential in limnic basommatophorans. Matsuda (1987) proposed a selective advantage of brooding animals that depend on suitable habitat immediately after hatching. However, more data on pulmonate reproduction in different ancient lakes and especially on the Balkan are necessary to determine more precisely the role of life-history traits in the biological processes in these evolutionary theatres.

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