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CIRCANNUAL GONAD ACTIVITY IN TWO SPECIES OF THE GENUS *VESTIA* P. HESSE (GASTROPODA: PULMONATA: CLAUSILIIDAE)

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Abstract.— *Vestia gulo* and *V. turgida* are iteroparous simultaneous hermaphrodites. In the wild they are active from April till October; they reproduce in the spring and summer (egg retention from May to August), and hibernate from November till March. Their gonads show the greatest activity in the spring and summer (maturation of oocytes, intensive vitellogenesis: March–May, numerous mature oocytes: May–July; production and maturation of spermatozoa: March–May; numerous packets of mature spermatozoa: May–October), which coincides with the reproductive season. The onset of reproduction is determined by the size of the pool of vitellogenic and mature oocytes; oocyte production starts in the summer of the previous vegetation season and lasts till next spring. Mature spermatozoa are present in the gonad from spring till autumn which indicates an ability to mate during the whole active period. During hibernation the gonads contain no spermatids, mature spermatozoa or advanced vitellogenic oocytes.

Key words.— land snails, Clausiliidae, *Vestia*, species biology, ovotestis, gametogenesis.

INTRODUCTION

The knowledge of life cycles and reproductive biology is useful when studying phylogeny, evolution and ecology. In the case of land pulmonates, several comprehensive works contain valuable information on their biology (e.g. Frömming 1954, Runham and Hunter 1970, Tompa 1984, Baur 1994, Heller 2001, Jordaens *et al*. 2007). Apart from observations of individuals or population dynamics, another source of important data is histological and cytological research. It provides an insight in the structure and function of the reproductive system, most of all gonad, and processes that take place in it (e.g. Tompa 1984, Gómez 2001, Gomot de Vaufleury 2001, Healy 2001 and references contained therein).

Histological studies on the gonad activity in annual cycle supplement life history information; in the case of terrestrial pulmonates such publications are few, and the information pertains to single, often phylogenetically remote species, for example *Lauria cylindracea* (Pupillidae), *Vertigo pusilla* (Vertiginidae), *Scutalus tupacii* (Bulimulidae), *Achatina fulica* (Achatinidae), *Megalobulimus abbreviatus* (Strophocheilidae), *Oxychilus atlanticus* (Zonitidae), *Deroceras reticulatum* (Agriolimacidae), *Arion ater*, *Arion circumscriptus* (Arionidae), *Helicodonta obvoluta* or *Helix pomatia* (Helicidae) (Luchtel 1972a, b, Runham and Hogg 1979, Ngowsiri *et al*. 1989, Cuezzo 1993, Heller *et al*. 1997, Rodrigues *et al*. 1998, Juchno 1999, Maltz 2003, Horn *et al*. 2005, Mazurkiewicz and Pokryszko 2005).

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Because of the lack of information on the circannual activity of mature clausiliid gonad, we included such studies in a project on the reproductive biology of two Carpathian species of the genus *Vestia* (see below).

Reproductive biology of the species

Both *Vestia gulo* and *V. turgida* retain their eggs in the oviduct, but their reproductive strategies differ significantly (Sulikowska-Drozd 2009). *V. gulo* retains eggs for a short time and the juveniles hatch in 7–10 days from egg laying (room temperature). The largest embryos in the retained eggs have embryonic shells 1.3 whorl. In the field, gravid individuals are found from half of May till the end of July (Fig. 1). Neonates – juveniles of 2.3–3 whorls – appear in June and July. *V. turgida* is fully ovoviviparous; the embryos remain in the oviduct till hatching, or hatch within 1–2 days from egg laying. The embryos grow shells of 2.9 whorls inside the parent's body. Gravid individuals are observed from the beginning of May till the end of August, with two periods when developmentally advanced embryos prevail (June and August). Neonates appear starting with half of June. The maximum size reached by the embryos inside the parent's oviduct limits the number of offspring per litter. *V. gulo* usually retains 12–15 eggs, *V. turgida* – 5–8 (Sulikowska-Drozd 2009).

In the wild the activity and growth of juveniles depend mainly on air temperature (Sulikowska-Drozd, in prep.). In the winter (end of October – half of April) the growth is arrested and the aperture is covered by a thin membrane. However, active individuals are observed on warmer days in late autumn and early spring. Mating in *V. gulo* and *V. turgida* was rarely observed in the wild; single cases were noted in the autumn – September and October (Sulikowska-Drozd, in prep.).

The total time necessary to complete shell growth ranges from one to more than three years, depending on the population. In the laboratory, with constant temperature and high humidity, that time is shortened to 3.5–4 months. Anatomical and histological examination of the reproductive system shows that full sexual maturity is attained later than ultimate shell size (Maltz and Sulikowska-Drozd, in prep.). At the stage of lip formation the reproductive system is still not fully formed, the gonad contains first spermatids and spermatozoa and growing previtellogenic oocytes. The snails start producing offspring 5–6 months after shell growth completion. At that time the histological image of the gonad is identical with that of adults aged one year and older. In the wild, adults of both species live and reproduce during at least four years (Sulikowska-Drozd, in prep.).

Material and methods

The studies were carried out from September 2006 till October 2008 at the Museum of Natural History, Wrocław University. The material included adult, sexually mature snails of both species (maturity criteria: spindle-shaped shell with complete closing apparatus, developed reproductive system, gonad overgrowing

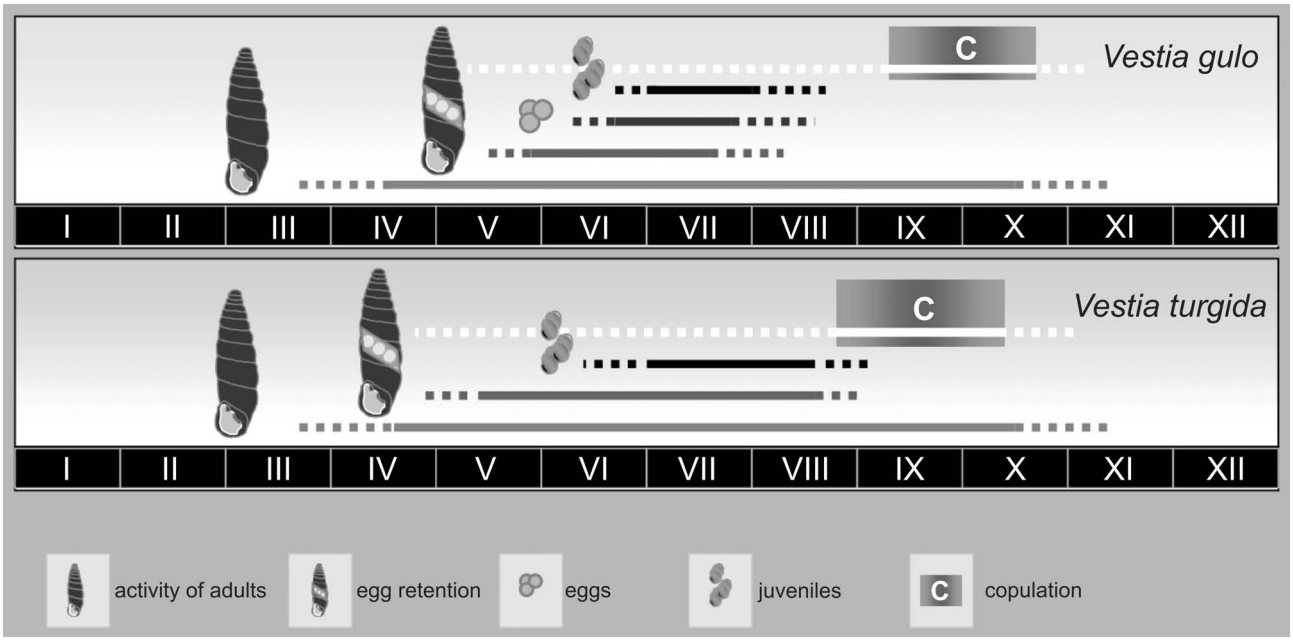

Figure 1. Annual activity of *Vestia gulo* and *V. turgida* (based on field observations).

hepatopancreas, black-pigmented, with distinct division into lobes and lobules). Adults were collected each month in the site of field observations during the vegetation season (April–October) (Krościenko on the Dunajec, 49°25'52.8''N, 020°26'14.0''E; 425 m a.s.l.; mean annual temperature 7°C), while in the remaining months (November–March) a group of individuals collected in October was kept in a cool room (temperature 3°C, humidity close to 100%, dark; the snails were retracted into their shells but with no membrane in the aperture), and a few snails were used for the studies each month.

The gonad with a fragment of hepatopancreas was dissected and fixed in the Bouin solution. The material was dehydrated in a graded alcohol series and xylene, embedded in paraffin (Paraplast Plus with 8% DMSO) and cut on a rotational microtome into 7 μm sections (serial sections attached to hot microscopic slides). Following rehydration, the slides with sections were stained with Delafield hematoxylin and 25% aqueous solution of eosin (Zawistowski 1986), dehydrated, cleared and coverslipped with DPX mounting medium for light microscope observations (Olympus BHS). A total of 137 gonads were fixed and stained from September 2006 till February 2008 (each month at least 3–5 gonads of each species).

Classification of cells of male and female germinal line necessary for the interpretation of the histological image of the gonad was based on available literature (Noyce 1973 after Tompa 1984, Kubrakiewicz 1985, Griffond and Bolzoni-Sungur 1986, Rodrigues *et al*. 1998, Maltz 2003).

Five stages of spermatogenesis were identified:

sc1 – spermatocytes I in meiotic prophase I (sphaeroidal cell, ca. 8–10 μm in diameter, nucleus large in relation to the small quantity of cytoplasm);
sc2 – spermatocytes II (ca. 11–17 μm in diameter, with a more abundant cytoplasm, rosette-forming);
st1 – early spermatids (small cells – ca. 8–10 μm in diameter, sphaeroidal in shape);
st2 – late spermatids with thick flagella;
s – mature spermatozoa forming packets.

Four stages of development have been distinguished during oogenesis:

po – growing previtellogenic oocytes (ranging from spherical to oval, up to ca. 30 μm diameter, no visible granules in the cytoplasm);
ev – early vitellogenic oocytes – cells in initial phase of vitellogenesis (ranging from oval to elongate, up to ca. 80–90 μm diameter; cytoplasmic inclusions – granules appear);
vo – vitellogenic oocytes (elongated to rounded, ca. 90–120 μm diameter; cytoplasmic inclusions – increasing number of granules);
mo – mature oocytes (large, rounded cells, of more than 120 μm diameter, most often 125–135, cytoplasm filled with numerous yolk platelets and lipid droplets).

Cell diameter was measured with calibrated eyepiece (WF DIN 10x).

Criteria considering the kind of germinal line cells and their estimated number (scale 0–2) were used to assess the gonad activity (Table 1).

Table 1. *Vestia gulo* and *V. turgida*. Criteria used for gonad activity assessment.

CELL TYPE	CRITERIA			
	0	0.5	1	2
mitotically dividing cells (m)	cells absent	few divisions (1–2 per slide)	moderately numerous divisions (several per slide)	numerous divisions (about a dozen per slide)
spermatocytes I (sc1)	cells absent	few cell clusters (1–2 per slide)	moderately numerous clusters (several per slide)	numerous clusters filling inside of acinus
spermatocytes II – rosette-forming (sc2)	cells absent	few rosettes (1–2 per slide)	moderately numerous rosettes (several per slide)	numerous rosettes (about a dozen per slide)
early spermatids (st1)	cells absent	few cell clusters (1–2 per slide)	moderately numerous clusters (several per slide)	numerous clusters (about a dozen per slide)
late spermatids (st2)	cells absent	few cell clusters (1–2 per slide)	moderately numerous clusters (several per slide)	numerous clusters filling inside of acinus
spermatozoa (s)	cells absent	few packets of spermatozoa	moderately numerous packets (several per slide)	numerous packets filling inside of acinus
oocytes (po, ev, vo, mo)	cells absent	few oocytes (po, ev, vo: several per gonad; with reference to mo – 1–2 per gonad)	moderately numerous oocytes (po, ev, vo: several dozen per gonad; with reference to mo – about 3–6)	numerous oocytes (po, ev, vo: about a dozen per gonad; with reference to mo – over 6)

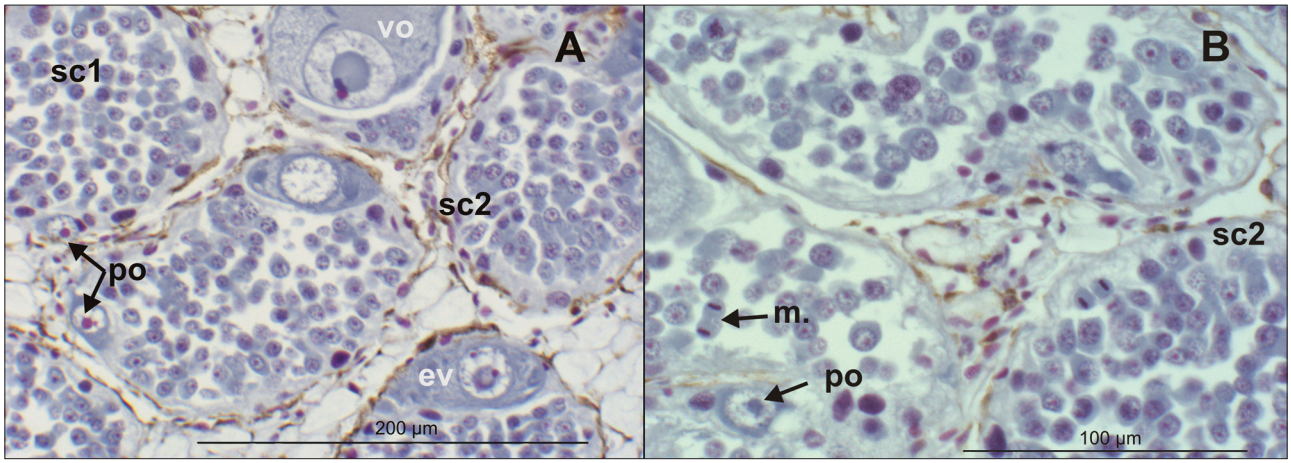

Figure 2. Hermaphroditic gland of *Vestia gulo* (A–B) [28 II 2007]: ev – early vitellogenic oocytes; m – mitoses; po – growing previtellogenic oocytes; sc1 – spermatocytes I in meiotic prophase I; sc2 – rosette-forming spermatocytes II; vo – vitellogenic oocytes.

Results

Histological examination showed that the circannual activity of mature gonad of *V. gulo* (VG) and *V. turgida* (VT) underwent some seasonal fluctuations so the results chapter was divided into three parts according to phenological periods. The description below pertains to both species, with only significant differences indicated.

End of winter and early spring. On the boundary of February and March numerous growing previtellogenic and early vitellogenic oocytes were observed in the gonad, first vitellogenic oocytes were also visible (28.6% of VG gonads, 85.7% of VT gonads). In that period the gonads contained no spermatids, spermatozoa or mature oocytes; mitotically dividing cells, spermatocytes I in meiotic prophase I and rosette-forming spermatocytes II were observed (Fig. 2A–B). At the end of March the number of rosete-forming spermatocytes increased; early spermatids, late spermatids with thick flagella and first packets of spermatozoa appeared (Fig. 3). The number of early vitellogenic and vitellogenic oocytes remained similar to that in the preceding month, while the number of growing previtellogenic oocytes decreased. On the boundary of April and May, rosette-forming spermatocytes II, early and late spermatids predominated; the number of packets of spermatozoa increased (Fig. 4A–B). In that period also single mature oocytes were observed (25% of VG gonads, 50% of VT gonads). The pool of oocytes present in the gonads was dominated by vitellogenic cells (Fig. 4A).

Late spring and summer. At the end of May the gonads were filled by numerous packets of spermatozoa, spermatocytes and spermatids (Fig. 5A–B); mitotically dividing cells were also visible (Fig. 6). The number of mature oocytes increased (present in all gonads of both species). The number of vitellogenic oocytes was still high. At the end of June mature spermatozoa predominated in the gonad (Fig. 7). The number of mature oocytes was then the greatest while early vitellogenic oocytes were fewer. On the boundary of July and August numerous packets of spermatozoa still prevailed in the gonads, while rosette-forming spermatocytes II and spermatids were fewer. The number of mature oocytes also decreased; single early vitellogenic oocytes were observed, the number of which increased at the end of August (Fig. 8). The number of spermatocytes II and spermatids also increased at that time. Packets of mature spermatozoa were still very numerous.

Autumn and winter. In September the gonads often contained spermatozoa in the collecting duct

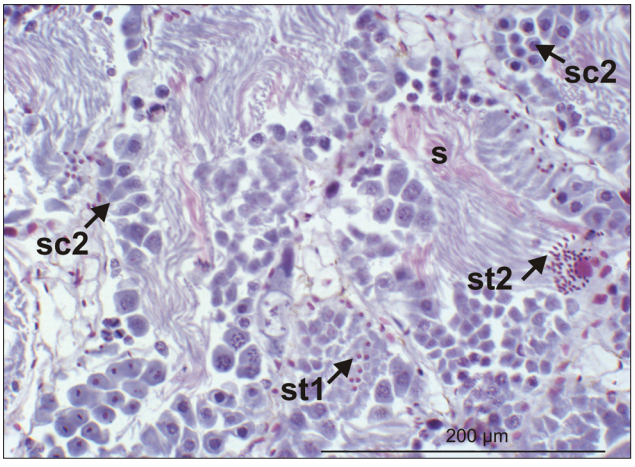

Figure 3. Hermaphroditic gland of *Vestia turgida* [29 III 2007]: s – spermatozoa; sc2 – spermatocytes II; st1 – early spermatids; st2 – late spermatids.

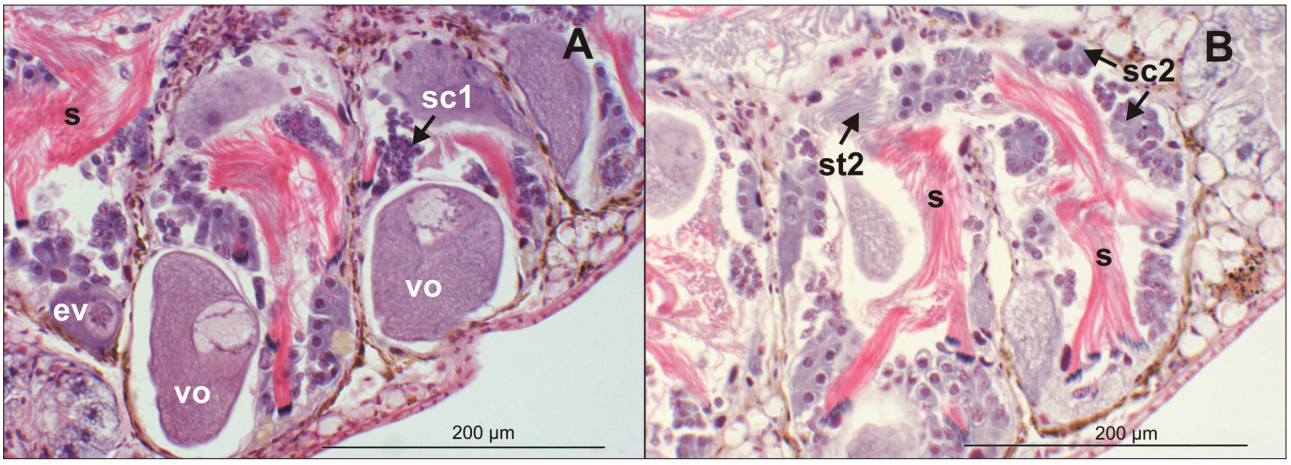

Figure 4. Hermaphroditic gland of *Vestia gulo* (A–B) [29 IV 2007]: ev – early vitellogenic oocytes; s – spermatozoa; sc1 – spermatocytes I; sc2 – spermatocytes II; st2 – late spermatids; vo – vitellogenic oocytes.

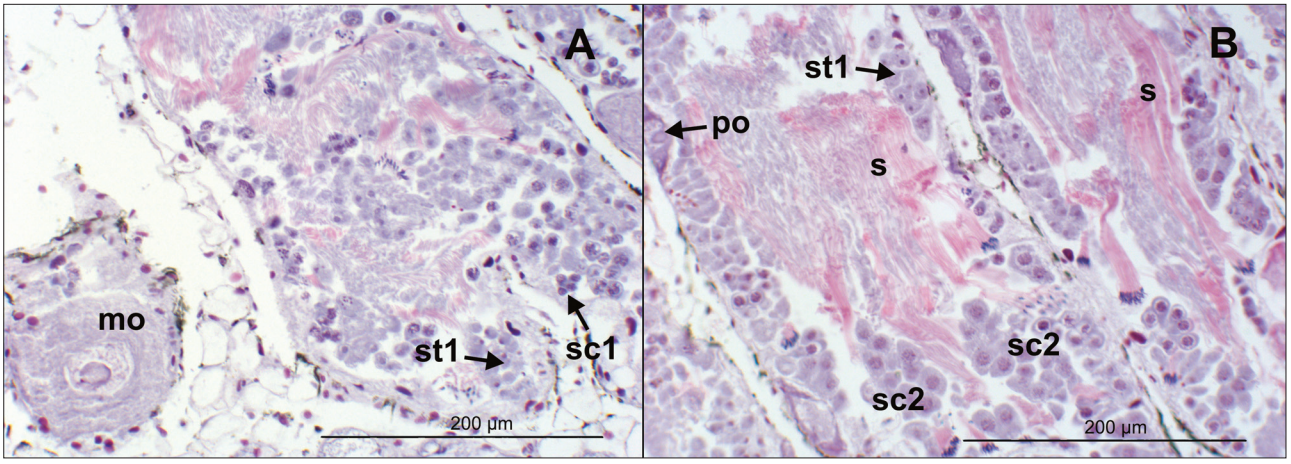

Figure 5. Hermaphroditic gland of *Vestia turgida* [30 V 2007]: mo – mature oocytes; po – growing previtellogenic oocytes; b2.2 – vitellogenic oocytes; s – spermatozoa; sc1 – spermatocytes I; sc2 – spermatocytes II; st1 – early spermatids.

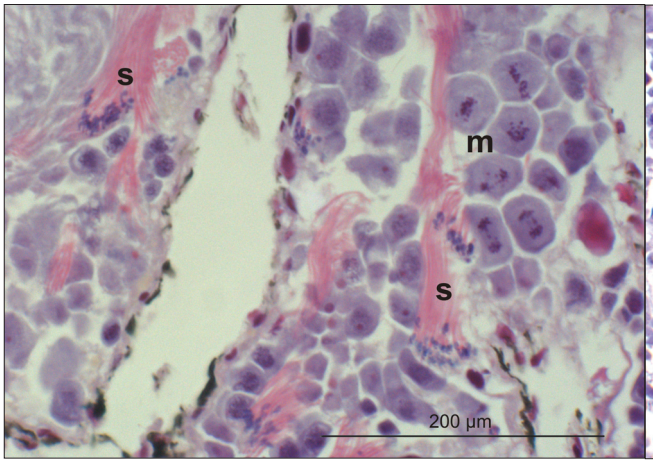

Figure 6. Hermaphroditic gland of *Vestia gulo* [30 V 2007]: m – mitoses; s – spermatozoa.

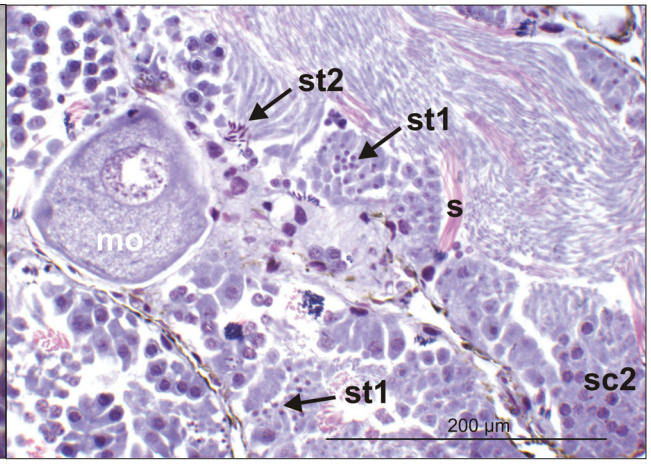

Figure 7. Hermaphroditic gland of *Vestia gulo* [29 VI 2007]: mo – mature oocytes; s – spermatozoa; sc2 – spermatocytes II; st1 – early spermatids; st2 – late spermatids.

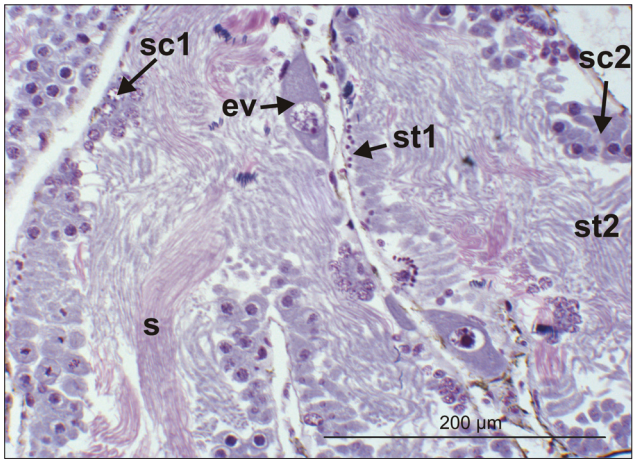

Figure 8. Hermaphroditic gland of *Vestia gulo* [29 VIII 2007]: ev – early vitellogenic oocytes; s – spermatozoa; sc1 – spermatocytes I; sc2 – spermatocytes II; st1 – early spermatids; st2 – late spermatids.

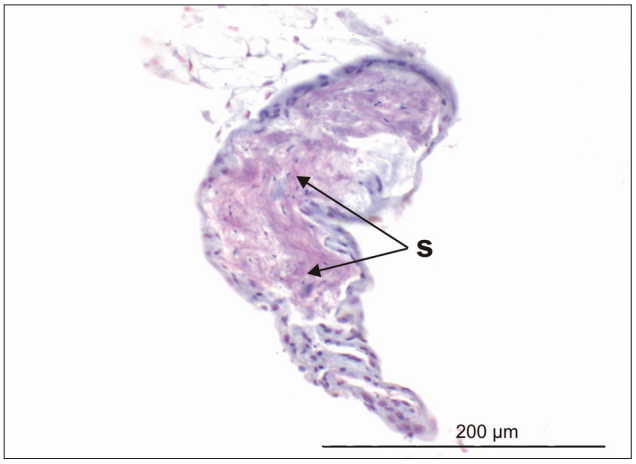

Figure 9. Initial part of hermaphroditic duct of *Vestia turgida* [19 IX 2007]: s – spermatozoa.

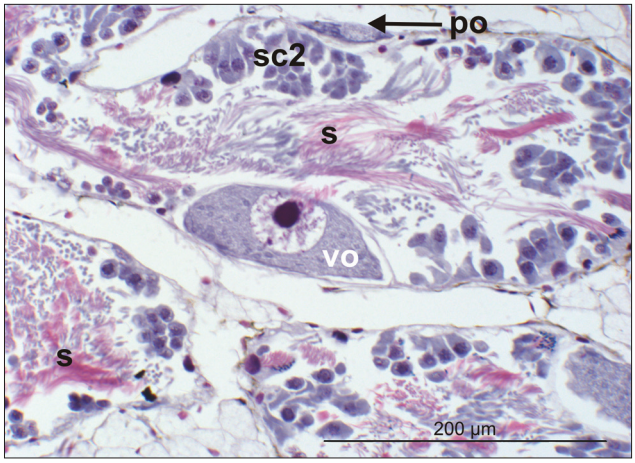

Figure 10. Hermaphroditic gland of *Vestia turgida* [19 IX 2007]: po – growing previtellogenic oocytes; s – spermatozoa; sc2 – spermatocytes II; vo – vitellogenic oocytes.

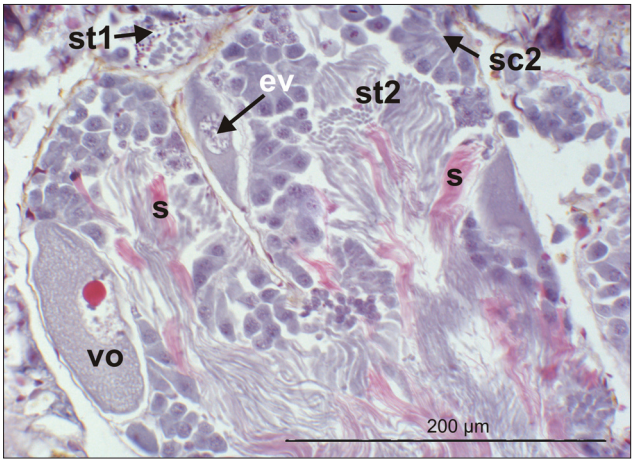

Figure 11. Hermaphroditic gland of *Vestia gulo* [29 X 2007]: ev – early vitellogenic oocytes; s – spermatozoa; sc2 – spermatocytes II; st1 – early spermatids; st2 – late spermatids; vo – vitellogenic oocytes.

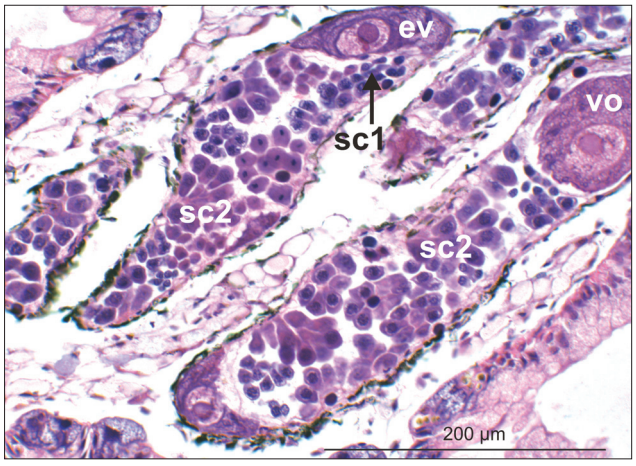

Figure 12. Hermaphroditic gland of *Vestia gulo* [20 XII 2007]: ev – early vitellogenic oocytes; sc1 – spermatocytes I; sc2 – spermatocytes II; vo - vitellogenic oocytes.

(initial section of hermaphroditic duct, located within the gonad) (71.4% of VG gonads, 85.7% of VT gonads, in all 78.6% of gonads) (Fig. 9); mature spermatozoa, rosette-forming spermatocytes II and spermatids dominated in the lobules. The first vitellogenic oocytes appeared (Fig. 10); no mature oocytes were observed. Mature spermatozoa prevailed still in October (Fig. 11) and the number of previtellogenic oocytes increased. At the end of November the number of packets of spermatozoa decreased, and the number of growing previtellogenic oocytes increased further; also early vitellogenic cells were more numerous than in September. Gonads examined in December and January contained rather numerous mitotically dividing cells; spermatocytes I in meiotic prophase I and rosette-forming spermatocytes II were also present (Fig. 12), as well as previtellogenic, early vitellogenic and single vitellogenic

oocytes. No spermatids, spermatozoa or mature oocytes were observed in that period.

Using gonad activity criteria presented in Table 1 (scale 0–2) for interpretation of histological images, it can be seen that the circannual activity of mature gonad was similar in *V. gulo* and *V. turgida* (Table 2, Figs 13–14). Only slight differences were observed at the end of winter and in early spring: more gonads of *V. turgida* than *V. gulo* contained more vitellogenic oocytes in February/March and March/April; also more gonads of *V. turgida* than *V. gulo* contained single mature oocytes at the end of April. In both species the gonad activity was the greatest in the spring and early summer: in that period oocyte maturation and spermatocyte production and maturation were intensive (Figs 13A–B, 14A–B). At the end of summer and in early autumn a new pool of oocytes appeared in the gonads; they started growth and maturation at the end of the season, to become mature and be used for egg production in the late spring and summer of the next season. In the early autumn processes associated with gamete production and maturation were still intensive, and ceased with the onset of hibernation.

Two main cycles can be distinguished in the activity of mature gonads: one of their activity as testes, associated with spermiogenesis, from April/May till October/November (however spermatocytogenesis and spermatocyte stages take place uninterruptedly in all circannual cycle), and another, as ovaries, associated with oogenesis, starting in late summer of one season and ending in the summer next season (Figs 13C, 14C).

Discussion

Field and laboratory observations show that *Vestia gulo* and *V. turgida* are iteroparous (Maltz and Sulikowska-Drozd 2008, Sulikowska-Drozd 2009). Ontogenetically, mature spermatozoa appear in the gonad earlier than vitellogenic oocytes which was termed slight protandry (Maltz and Sulikowska-Drozd, in prep.). Later (completion of shell growth plus 5–6 months) the snails show simultaneous hermaphroditism. This is confirmed by the histological studies on the activity of mature gonad in circannual cycle. Cells of male and female germinal line, present in each gonad lobule, are observed throughout the year. Only the number of cells in different development stages undergoes some seasonal fluctuations indicating a certain periodicity in production of different cell categories. Similar results have been obtained from histological analysis of gonads of e.g. *Lauria cylindracea*, *Megalobulimus abbreviatus*, *Achatina fulica*, *Helicodonta obvoluta*, *Helix pomatia* and *H. aspersa* (Lind 1973, Bailey 1981, Ngowsiri *et al*. 1989, Heller *et al*. 1997, Juchno 1999, Maltz 2003, Horn *et al*. 2005). In semelparous species, like *Arion ater* or *Deroceras reticulatum* (Lusis 1961, Smith 1966, Runham and Laryea 1968, Parivar 1978), the gonad activity is limited to a certain period of life and involves a single act of production of a pool of male and female gametes. The situation is similar in *Oxychilus atlanticus* and *Vertigo pusilla* (Rodrigues *et al*. 1998, Mazurkiewicz and Pokryszko 2005), but in these species the gonad can resume its activity in favourable conditions which would indicate a reproduction mode intermediate between semelparity and iteroparity.

The activity of iteroparous terrestrial snails from areas with regularly alternating seasons (warm/cold or dry/wet seasons) is associated with environmental conditions: when the conditions are favourable, they feed and reproduce, when not – they hibernate or estivate. *Vestia*, inhabiting lower altitudes of the Carpathians, are active from half of April till the end of October, they reproduce in the spring and summer (May–August), and hibernate from November till March (Sulikowska-Drozd 2009, in prep.). Their gonads show the greatest activity in the spring and summer (oocyte maturation – intensive vitellogenesis: March–May, numerous mature oocytes: May–July, production and maturation of spermatozoa: March–April, numerous packets of spermatozoa: May–October). It can be conjectured that in clausiliids the onset of reproduction is directly or indirectly determined by the size of the pool of mature oocytes, which are absent in the gonads in late summer, autumn and winter. Mature spermatozoa are present in the gonad from April to November, which would indicate an ability to mate during the whole vegetation season though in the field mating was observed only in September and October (in these months spermatozoa were often observed in masses in the gonad collecting ducts). This is probably related to the fact that *V. gulo* and *V. turgida* stop being gravid at the end of August which may favour autumn mating. The spermatozoa acquired in the autumn will serve to fertilise the oocytes maturing next spring and/or summer. The production of the new pool of oocytes starts at the end of summer; they will serve for egg production in the next vegetation season. Only previtellogenic, early vitellogenic and single vitellogenic oocytes, as well as male cells at various stages of spermatocytogenesis, are present in the gonads during hibernation. A similar seasonality in gonad activity has been observed e.g. in *H. obvoluta*, *H. pomatia* and *H. aspersa* (Lind 1973, Bailey 1981, Juchno 1999, Maltz 2003), in the first case the gonad shows two peaks of activity (spring and autumn), while during hibernation it contains mature spermatozoa and cells of both germinal lines at various development stages. The gonads of the two species of *Helix* show one peak of activity (spring/summer), while in the autumn and winter they are either empty or contain only cells at early development stages.

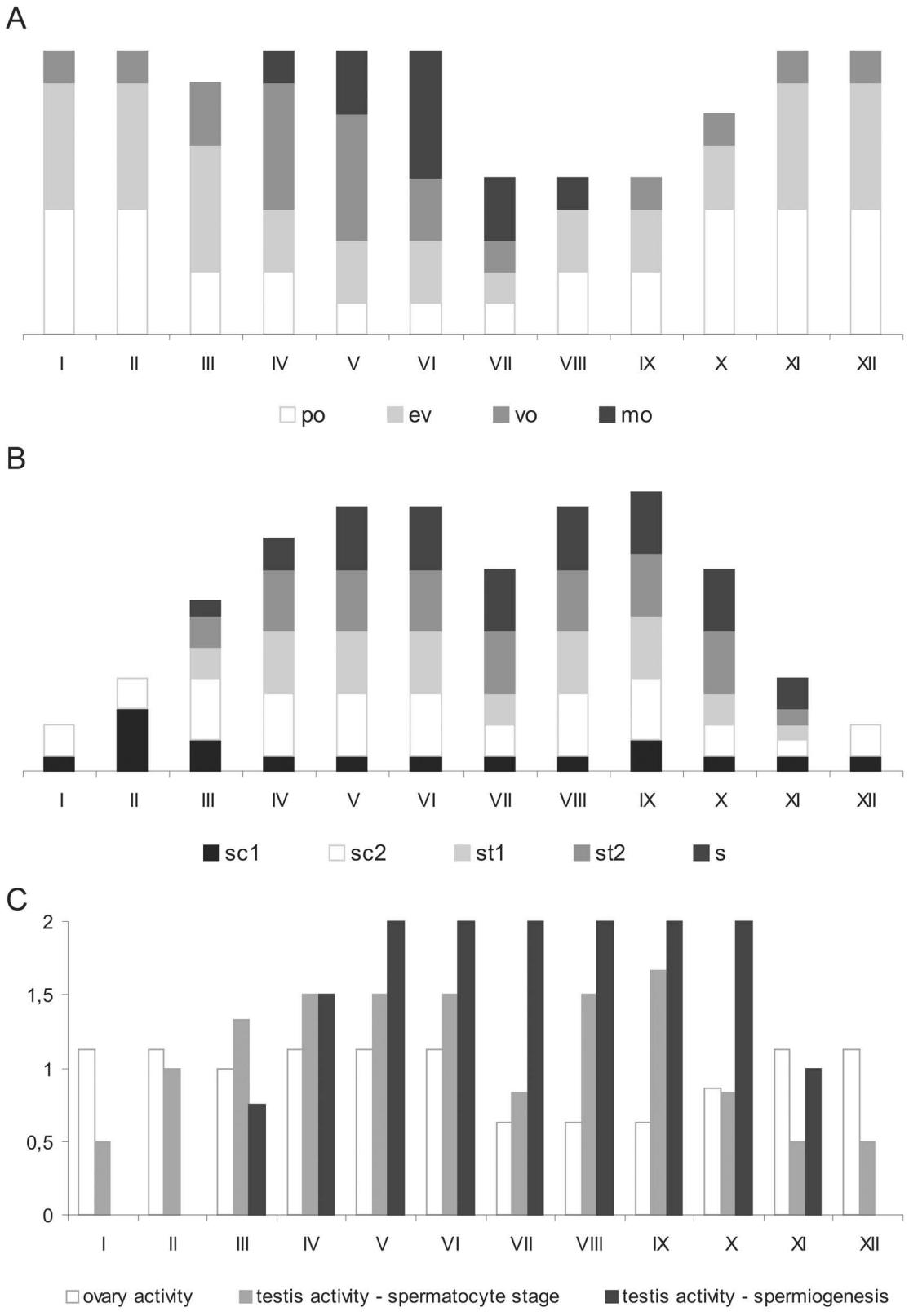

Figure 13. Annual gonad activity of *Vestia gulo* (based on Table 2): (A) – female germ line cells; (B) – male germ line cells; (C) – gonad activity as ovary and as testis: ev – early vitellogenic oocytes; mo – mature oocytes; po – growing previtellogenic oocytes; s – spermatozoa; sc1 – spermatocytes I; sc2 – spermatocytes II; st1 – early spermatids; st2 – late spermatids; vo – vitellogenic oocytes.

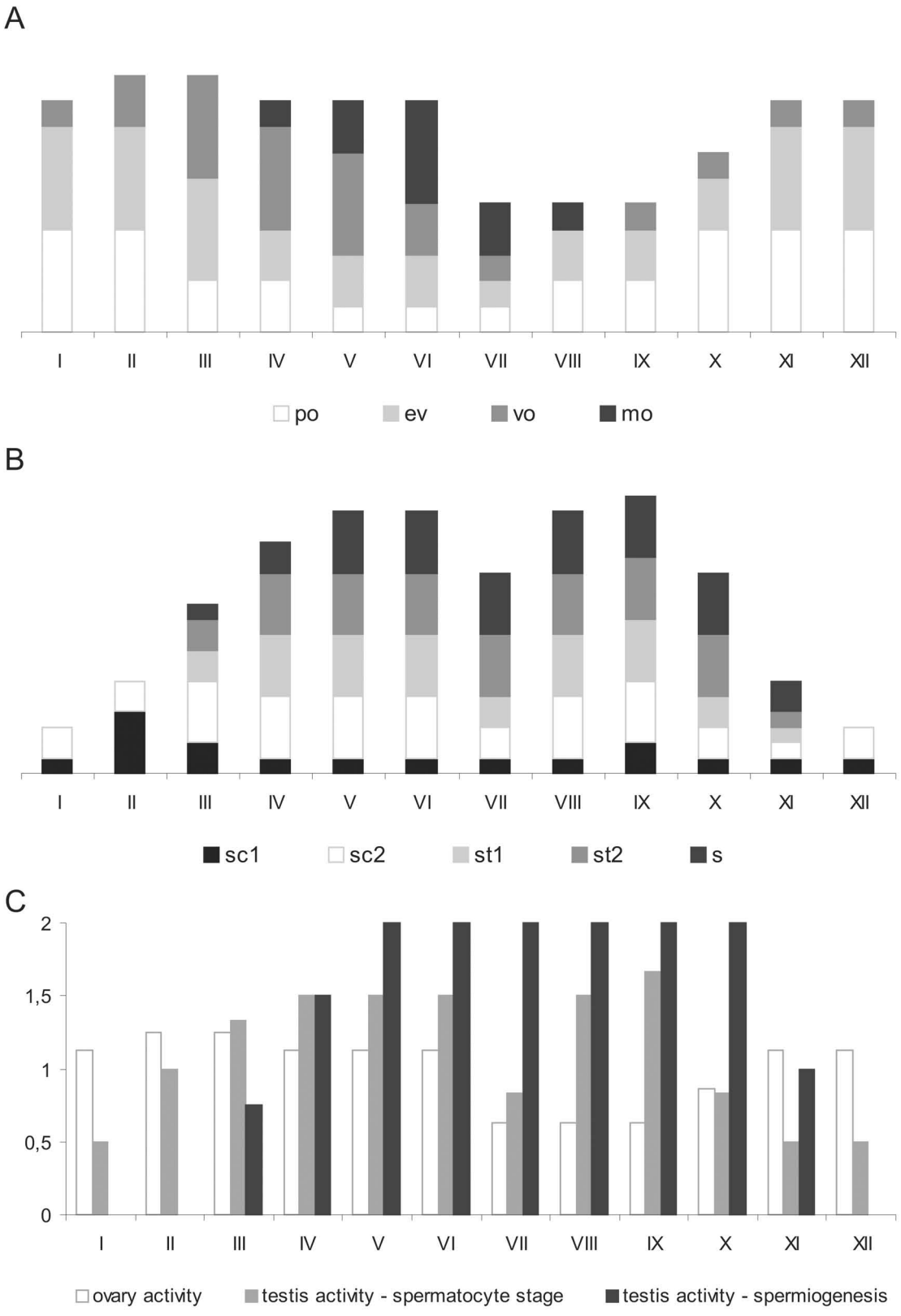

Figure 14. Annual gonad activity of *Vestia turgida* (based on Table 2): (A) – female germ line cells; (B) – male germ line cells; (C) – gonad activity as ovary and as testis: ev – early vitellogenic oocytes; mo – mature oocytes; po – growing previtellogenic oocytes; s – spermatozoa; sc1 – spermatocytes I; sc2 – spermatocytes II; st1 – early spermatids; st2 – late spermatids; vo – vitellogenic oocytes.

Table 2. *Vestia gulo* (VG) and *V. turgida* (VT). Quantitative interpretation of the gonad histological image (for criteria see Table 1)

cell type: / months:		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
po	**VG**	2	2	1	1	0.5	0.5	0.5	1	1	2	2	2
	VT	2	2	1	1	0.5	0.5	0.5	1	1	2	2	2
ev	**VG**	2	2	2	1	1	1	0.5	1	1	1	2	2
	VT	2	2	2	2	2	2	0.5	1	1	1	2	2
vo	**VG**	0.5	0.5	1	2	2	1	0.5	0	0.5	0.5	0.5	0.5
	VT	0.5	1	2	2	2	2	0.5	0	0.5	0.5	0.5	0.5
mo	**VG**	0	0	0	0.5	1	2	1	0.5	0	0	0	0
	VT	0	0	0	0.5	1	2	1	0.5	0	0	0	0
m	**VG**	1	1	1	0.5	1	1	0	0	0	0.5	0.5	1
	VT	1	1	1	0.5	1	1	0	0	0	0.5	0.5	1
sc1	**VG**	0.5	2	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5
	VT	0.5	2	1	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5
sc2	**VG**	1	1	2	2	2	2	2	2	2	1	0.5	1
	VT	1	1	2	2	2	2	2	2	2	1	0.5	1
st1	**VG**	0	0	1	2	2	2	1	2	2	1	0.5	0
	VT	0	0	1	2	2	2	1	2	2	1	0.5	0
st2	**VG**	0	0	1	2	2	2	2	2	2	2	0.5	0
	VT	0	0	1	2	2	2	2	2	2	2	0.5	0
s	**VG**	0	0	0.5	1	2	2	2	2	2	2	1	0
	VT	0	0	0.5	1	2	2	2	2	2	2	1	0
ovary activity													
(mean of values for po-mo)	**VG**	**1.13**	**1.13**	**1.00**	**1.13**	**1.13**	**1.13**	**0.63**	**0.63**	**0.63**	**0.88**	**1.13**	**1.13**
	VT	**1.13**	**1.25**	**1.25**	**1.13**	**1.13**	**1.13**	**0.63**	**0.63**	**0.63**	**0.88**	**1.13**	**1.13**
testis activity													
a) spermatocyte stage	**VG**	**0.50**	**1.00**	**1.33**	**1.50**	**1.50**	**1.50**	**0.83**	**1.50**	**1.67**	**0.83**	**0.50**	**0.50**
(mean of values for sc1-st1)	**VT**	**0.50**	**1.00**	**1.33**	**1.50**	**1.50**	**1.50**	**0.83**	**1.50**	**1.67**	**0.83**	**0.50**	**0.50**
b) spermiogenesis	**VG**	**0.00**	**0.00**	**0.75**	**1.50**	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**	**1.00**	**0.00**
(mean of values for st2-s)	**VT**	**0.00**	**0.00**	**0.75**	**1.50**	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**	**2.00**	**1.00**	**0.00**

In *L. cylindracea* from a population from Israel in the summer (estivation in dry season: July–October) the gonad is inactive but may contain single oocytes, while spermatogenesis and oogenesis start in December; mature spermatozoa appear in March, fully developed oocytes – in May (Heller *et al*. 1997). Since gravid individuals appear already in December, while mature spermatozoa are present in March and oocytes – in May, it can be supposed the offspring production at the beginning of the active season is based on gametes stored through the estivation period. The situation is very similar to that in *Vestia* where at the end of the active season the snails start producing a new pool of gametes (mainly oocytes) necessary to reproduce next spring. In the studied helicids – typically oviparous snails – the gamete production becomes intensified in the same season, just before the beginning of reproduction, which would indicate differences compared to ovoviviparous *Lauria* and *Vestia*.

Literature data indicate that an array of factors have a significant effect on reproductive processes in terrestrial pulmonates. They include abiotic factors, among others light, humidity or temperature (environmental factors), and internal factors, such as metabolic processes associated with hibernation/estivation or neurohormonal regulation (Gomot de Vaufleury 2001). Available information pertains to economically important species: edible snails (genus *Helix*) and agricultural pests (members of Arionidae, Limacidae, Agriolimacidae) (Henderson and Pelluet 1960, Bailey 1981, Bonnefoy-Claudet and Deray 1984, Gomot and Gomot 1985, 1989, Gomot *et al*. 1990, Griffond *et al*. 1992). In the studied clausiliids resumption of reproductive activity after hibernation (beginning of spermiogenesis in March/April) is associated on the one

hand with increasing day length in the spring, and with increasing air temperature on the other (mean monthly temperatures in the study site in Krościenko in 2007: March 3.5°C, April 7.7°C, May 13.3°C, June 15.2°C, July 15.6°C, August 15.5°C, September 9.7°C).

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