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REPRODUCTIVE SYSTEM DEVELOPMENT AND ANNUAL CYCLE OF GONAD ACTIVITY IN *HELICODONTA OBVOLUTA* (O. F. MÜLLER, 1774) (GASTROPODA: PULMONATA: HELICIDAE S. LAT.)

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ABSTRACT: The reproductive system structure in *H. obvoluta* follows the helicid pattern except for the dart sac which is absent. In juvenile snails the gonad is the first to develop; the later development of male and female reproductive organs is synchronous. At the stage of lip formation the reproductive system is fully developed. At an initial stage of the gonad development (3 and 4 whorls) mitotically dividing cells prevail, and the first pools of oocytes and spermatocytes in meiotic prophase appear. At the stage of 5 whorls the number of mitoses decreases, while the number of cells in meiotic prophase increases considerably, resulting in an increase in the gonad volume. The first growing oocytes appear, while spermatids and mature spermatozoa are absent. The gonad in lip-building snails contains all the cells characteristic of mature hermaphroditic gland. In mature gonad oocytes and spermatozoa are present throughout the year. The number and size of oocytes vary seasonally. Large vitellogenic oocytes are present from April till the beginning of July and from the end of August till October. The pool of growing oocytes appears in August and in November-December. The intensity of divisions leading to formation of the respective gametes changes in a similar way. The development of female lineage cells seems to determine the timing of reproduction, since spermatozoa are present throughout the year.

KEY WORDS: Helicidae, *Helicodonta obvoluta*, reproductive system, development, gametogenesis

INTRODUCTION

Studies on the development of gastropod reproductive system and seasonal changes in the gonad activity may provide valuable information on the biology of the species. They constitute an essential supplement to data obtained through field and laboratory observations, resulting in a more complete picture of the life cycle and reproductive biology.

Besides information on the morphology of the reproductive system and on the histological structure of the gonad (SHILEYKO 1978, SEMBRAT 1981, TOMPA 1984, URBAŃSKI 1989, KORALEWSKA-BATURA 1999), data on the development of the reproductive system in Helicidae sensu lato are limited to *Helix aspersa* (ENÉE & GRIFFOND 1983) and *H. lutescens* (KORALEWSKA-BATURA 1994), while there is no information on the gametogenic activity of the mature gonad.

The objective of this paper was to trace the development of the reproductive system and the activity of mature gonad in annual cycle in *Helicodonta obvoluta* (O. F. Müller, 1774) in order to better understand the biology of this threatened species.

MATERIAL AND METHODS

The age classes were based on the number of whorls in juvenile and on the degree of damage to periostracum in adult individuals (Table 1).

ANATOMICAL STUDIES

Anatomical studies involved examination of the structure of the reproductive system of adult individuals, and analysis of structural changes of that system during ontogeny. The material included 10 adult specimens and 10 juvenile specimens of age classes J3, J4 and J5, collected in the nature reserve Muszkowicki Las Bukowy (SW Poland). Dissection of the reproductive system in specimens smaller than J3 class proved impossible. The snails were preserved in 70% ethyl alcohol and dissected under stereomicroscope Nikon SMZ-U.

HISTOLOGICAL STUDIES

In order to trace seasonal changes in the gonad activity of adult *H. obvoluta* and the ontogenetic changes of the organ, as well as histological differences between the mucus gland and the spermatheca, the following histological techniques were used.

1. Histological analysis of gonads fixed with Bouin's liquid (light microscope – Nomarski contrast)
2. Histological analysis of spermatheca and mucus gland fixed with glutaraldehyde (light microscope)

Studies on the gonad activity in annual cycle were carried out in 1997–1999, and on the ontogenetic development in 1999–2000. The snails were collected in the nature reserve Muszkowicki Las Bukowy. Adult individuals were collected monthly in the field during the vegetation season; those which constituted winter material were kept in the laboratory prior to dissection. The studies included 15 consecutive months, and the total number of adult specimens was 90. Fifteen juvenile specimens (five of each of the studied age classes) were collected in the same nature reserve in May 1999 and kept in the laboratory till dissection.

Table 1. Age classes of *H. obvoluta*, based on the number of whorls in juvenile and the degree of damage to periostracum in adult individuals

Age class	Description
J1	1.0–2.0 whorls
J2	2.1–3.0 whorls
J3	3.1–4.0 whorls
J4	4.1–5.0 whorls
J5	5.1 till lip completion (c. 6 whorls)
A1	1 year old (since lip completion), periostracum undamaged, with distinct hairs
A2	Over 1 year old, periostracum partly or wholly damaged

The gonads were dissected in Bouin's fixative in which the whole snails were kept for 2 hrs before dissection. Following dissection the gonads were kept in Bouin's fixative for 24 hrs, rinsed in 80% ethanol and left in ethanol for another 24 hrs till the yellow colour disappeared. Then the gonads were dehydrated in ethanol series, rinsed in xylen three times and embedded in paraffin for at least 24 hrs, and cut with rotation microtome into sections 7 μm thick. Following re-hydration the sections were stained with Delafield haematoxilin and 25% aqueous solution of eosin (ZAWISTOWSKI 1986). After rinsing and dehydration in ethanol series permanent slides were made, examined and photographed in light microscope Olympus BHS with Nomarski contrast.

The following criteria were used when estimating the gonad activity:

* Oocytes: [1] – small, growing oocytes; [2] – oocytes during early vitellogenesis; [3] – large, vitellogenic oocytes;
* Mitoses: [1] – few divisions (1–2 per slide); [2] – moderately numerous divisions (several per slide); [3] – numerous divisions (about a dozen per slide);
* Meioses: [1] – few divisions (several dozen per slide); [2] – moderately numerous divisions (hundreds per slide); [3] – numerous divisions (thousands per slide);
* Spermatids: [1] – rosette-forming spermatocytes, single spermatids; [2] – spermatocyte rosettes, numerous spermatids, initial stages of spermiogenesis; [3] – spermatocyte rosettes, numerous spermatids, advanced spermiogenesis;
* Spermatozoa: [1] – no mature spermatozoa, initial stages of spermiogenesis; [2] – few packets of spermatozoa; [3] – numerous packets of spermatozoa.

Spermathecae and mucus glands were obtained from 5 adult specimens collected in the nature reserve in October 2000. The organs were dissected and fixed for 24–48 hrs in 2.5% glutaraldehyde, 0.1 M phosphate buffer (pH 7.4), repeatedly rinsed in the same buffer and fixed during 1 hr in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4. Following rinsing in the buffer the material was dehydrated in acetone series and embedded in epoxy resin Epon 812. The material was cut into semi-thin sections (0.6 μm) with ultramicrotome Reichert Ultracut E, stained in 1% methylene blue in 1% borax, and examined and photographed in light microscope Olympus BHS.

RESULTS

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

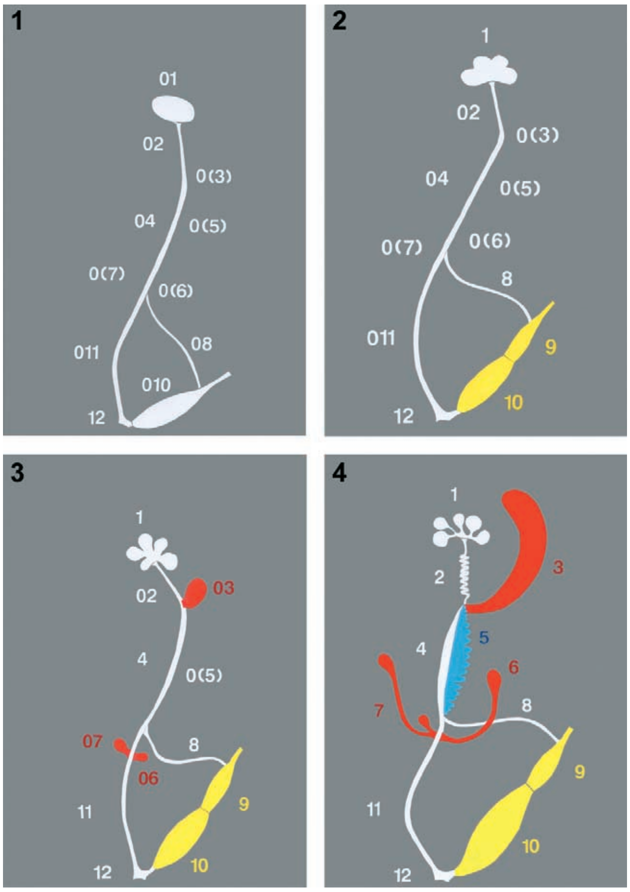
Stage J3 (3.1–4.0 whorls) (Fig. 1). At that stage the following primordial structures can be distinguished: gonad, hermaphroditic duct, spermoviduct, vagina and vas deferens; the male copulatory organ is not differentiated into penis and epiphallus.

Stage J4 (4.1–5.0 whorls) (Fig. 2). The gonad shows primordia of lobes while the male copulatory organ is differentiated into penis and epiphallus which are of a similar size.

Stage J5 (5.1 till lip completion) (Fig. 3). The gonad lobes are distinct, and primordia of albumen gland, mucus gland and spermatheca appear, thus resulting in a division of vagina into the upper and lower section; the penis becomes larger than epiphallus.

Adult (Figs 4–5). In individuals which have completed their lip formation, the following structures are present: gonad composed of lobes divided into acini, much folded hermaphroditic duct, well-developed albumen gland, spermoviduct with prostate gland, mucus gland composed of a shorter and longer branch, spermatheca, vas deferens, penis, epiphallus and vagina.

The spermatheca and the mucus gland, which are sometimes difficult to distinguish upon dissection of alcohol-preserved specimens. However, they are well-distinguishable during dissection in other fixatives, e.g. glutaraldehyde. Histological differences in the structure of these two organs are shown in Figs 6–10.



Figs 1–4. Reproductive system development of *Helicodonta obvoluta*, diagrammatic: 1 – Stage J3; 2 – Stage J4; 3 – Stage J5; 4 – Adult.
Organ symbols: 1 – gonad, 2 – hermaphroditic duct, 3 – albumen gland, 4 – spermoviduct, 5 – prostate, 6 – spermatheca, 7 – mucus gland, 8 – vas deferens, 9 – epiphallus, 10 – penis, 11 – vagina, 12 – atrium
Explanation for numerical symbols: 0(X) – organ absent, 0X – primordial organ, X – developed organ

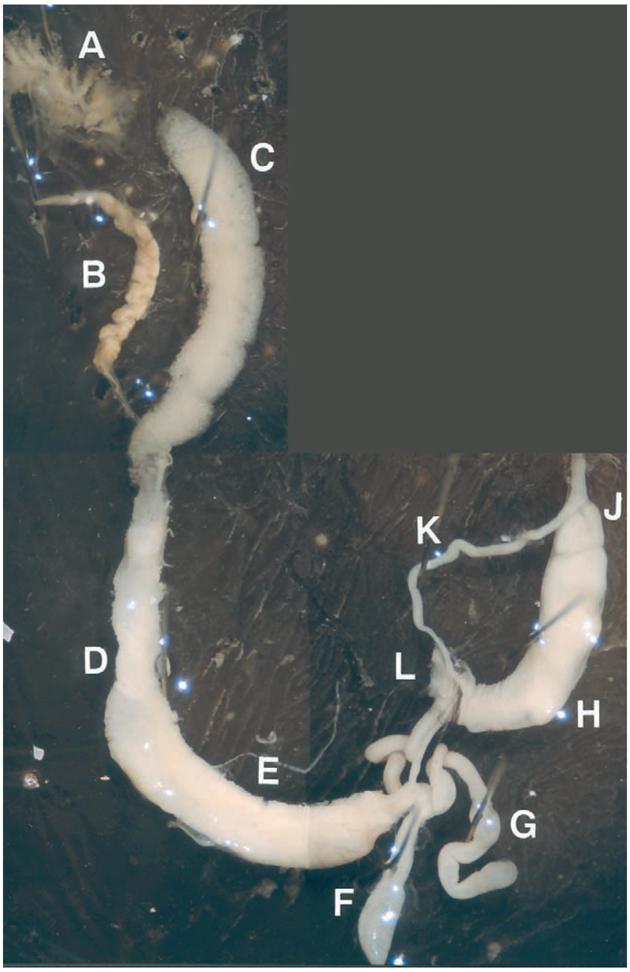


Fig. 5. Reproductive system of adult *Helicodonta obvoluta*: A – gonad, B – hermaphroditic duct, C – albumen gland, D – spermoviduct, E – prostate, F – spermatheca, G – mucus gland, H – penis, J – epiphallus, K – vas deferens, L – atrium

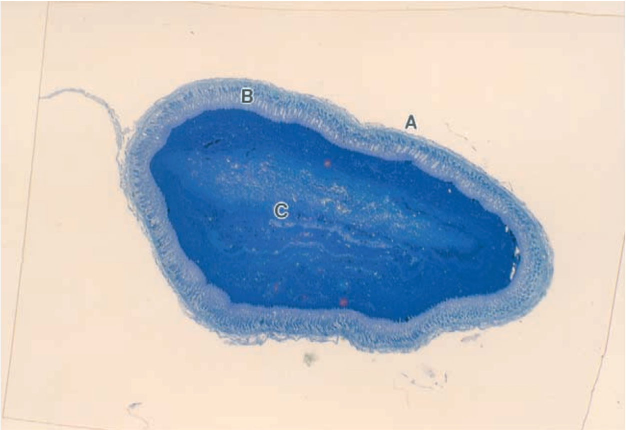


Fig. 6. Transverse section through the spermatheca of *Helicodonta obvoluta*. A – covering tissue (connective tissue + epithelium), B – epithelium of spermatheca wall, C – lumen. Semithin section, methylene blue, 93×

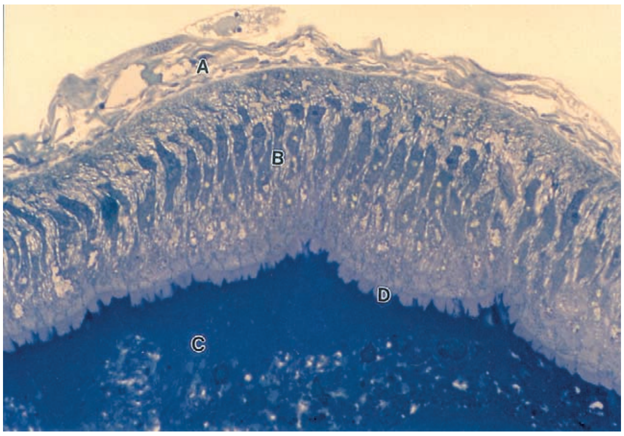


Fig. 7. Transverse section through the spermatheca of *Helicodonta obvoluta*. A – covering tissue (connective tissue + epithelium), B – epithelium of spermatheca wall, C – lumen, D – ciliae. Semithin section, methylene blue, 555×

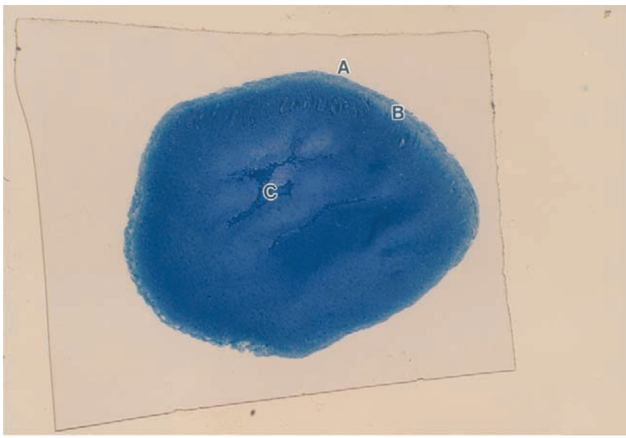


Fig. 8. Transverse section through the mucus gland of *Helicodonta obvoluta*. A – covering tissue (connective tissue + epithelium), B – epithelium of gland wall, C – mucus-filled lumen. Semithin section, methylene blue, 93×

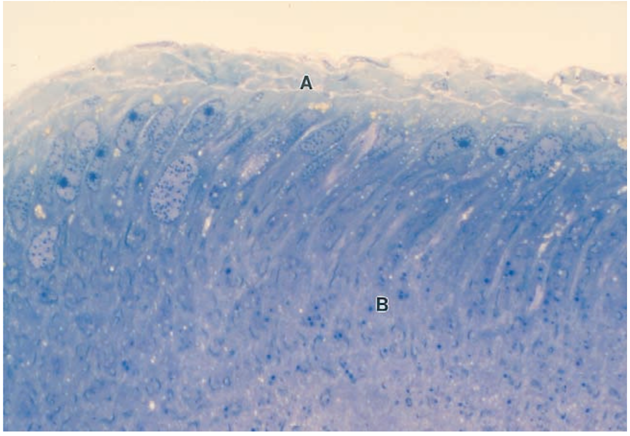


Fig. 9. Transverse section through the mucus gland of *Helicodonta obvoluta*. A – covering tissue (connective tissue + epithelium), B – epithelium of gland wall. Semithin section, methylene blue, 555×

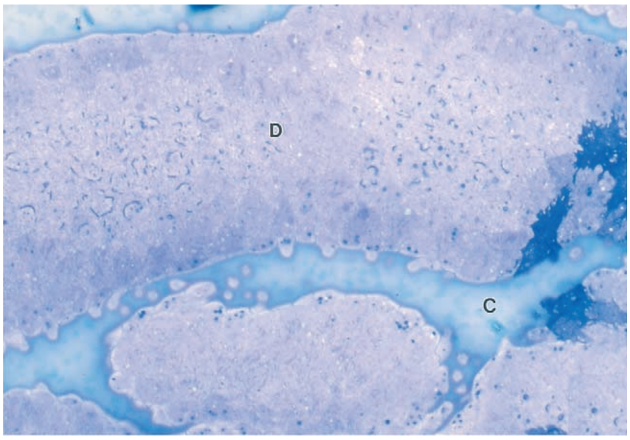


Fig. 10. Transverse section through the mucus gland of *Helicodonta obvoluta*. C – mucus-filled lumen, D – gland parenchyma. Semithin section, methylene blue, 555×

GONAD DEVELOPMENT

The sequence of events in the ontogenetic development of the gonad is present in Table 2 and Figs 11–16.

The gonad activity in juvenile *H. obvoluta* is presented in Fig. 17. For explanation of the values on the abscissa see criteria of gonad activity. At stage J3 mitotically dividing cells predominate, and some cells in meiotic prophase appear; the latter clearly prevail at stage J4. At that stage the number of oocytes increases as well. At stage J5 the gonad contains all the cells characteristic of mature gonad.

MATURE GONAD ACTIVITY IN ANNUAL CYCLE

Divisions. The intensity of mitotic divisions increases in March (Fig. 18), then in July and August (Fig. 20) and in December. Numerous cells in meiotic prophase arising as a result of mitotic divisions appear in April, September, and then in November/Decem-

Table 2. Sequence of events in the ontogenetic development of the gonad in *H. obvoluta*

Stage	Gonad development advancement
J3	Divisions: mitotic divisions, numerous cells in meiotic prophase Oogenesis: few growing oocytes Spermatogenesis: spermatids and spermatozoa absent
J4	Divisions: mitotic activity inhibited, number of cells in meiotic prophase increases, distinct increase in gonad volume compared to J3 as a result of large number of cells resulting from the mitoses characteristic of J3 Oogenesis: number of growing oocytes increasing Spermatogenesis: spermatids and spermatozoa absent
J5	Divisions: cells in meiotic prophase, sporadic mitoses, cells in consecutive stages of spermato- and spermiogenesis appear; further increase in gonad volume resulting from oocyte growth and increasing number of spermatids and spermatozoa Oogenesis: vitellogenic oocytes appear Spermatogenesis: spermatocytes forming characteristic rosettes; spermatids and packet-forming spermatozoa present

ber and in January (Fig. 23). In the remaining months the number of divisions is low.

Oogenesis. Oocytes are present in the gonad throughout the year, though their number and development stage vary seasonally. In March small growing (previtellogenic) oocytes appear, in April their number keeps increasing and also first vitellogenic oocytes are visible (Fig. 18). From May till July the number of the latter cells increases (Fig. 19), while growing oocytes are absent and will not appear till August. In September the growing

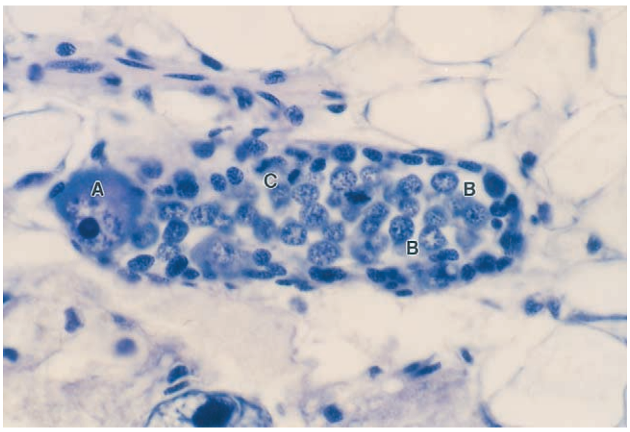


Fig. 11. Section through the gonad of *Helicodonta obvoluta*. Stage J3. A – growing oocyte, B – cells in meiotic prophase, C – mitoses. Paraffin section 7 µm, haematoxilin and eosin, 536×

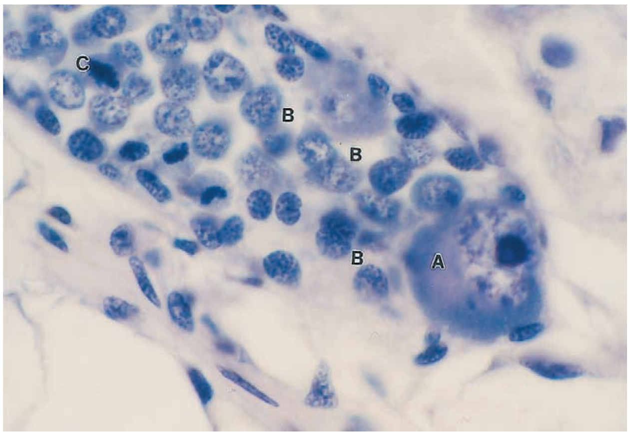


Fig. 12.Section through the gonad of *Helicodonta obvoluta*. Stage J3. A – growing oocyte, B – cells in meiotic prophase, C – mitoses. Paraffin section 7 µm, haematoxilin and eosin, 893×

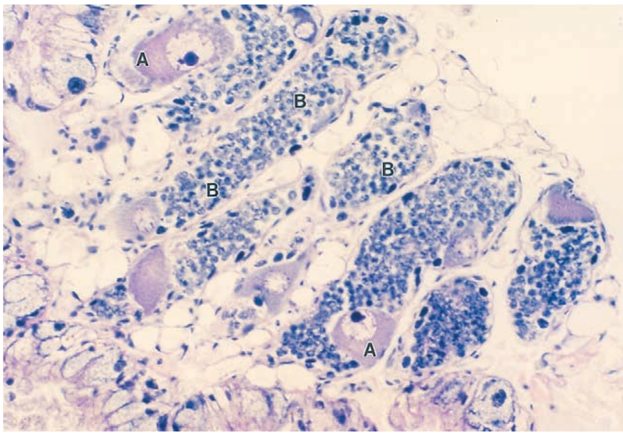


Fig. 13. Section through the gonad of *Helicodonta obvoluta*. Stage J4. A – numerous growing oocytes, B – numerous cells in meiotic prophase. Paraffin section 7 µm, haematoxilin and eosin, 179×

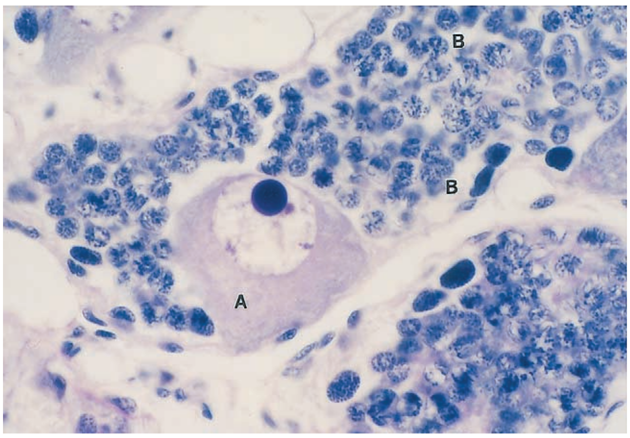


Fig. 14. Section through the gonad of *Helicodonta obvoluta*. Stage J4. A – early vitellogenic oocyte, B – numerous cells in meiotic prophase. Paraffin section 7 µm, haematoxilin and eosin, 536×

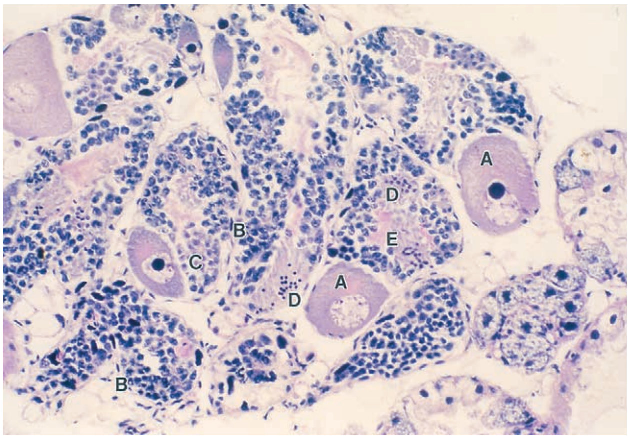


Fig. 15. Section through the gonad of *Helicodonta obvoluta*. Stage J5. A – vitellogenic oocytes, B – cells in meiotic prophase less numerous. Paraffin section 7 µm, haematoxilin and eosin, 179×

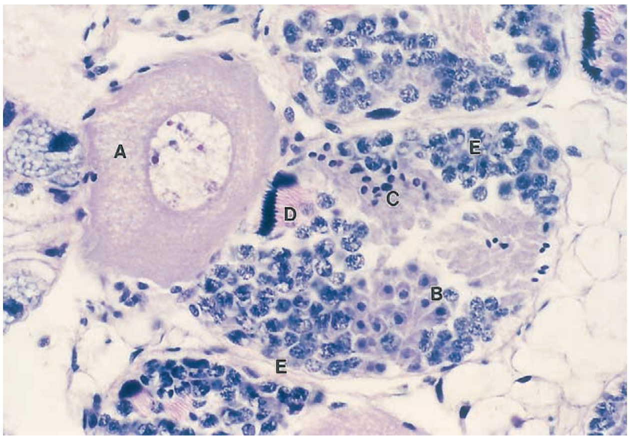


Fig. 16. Section through the gonad of *Helicodonta obvoluta*. Stage J5. A – large vitellogenic oocyte, B – spermatocyte rosettes, C – spermatids, D – spermatozoa, E – cells in meiotic prophase. Paraffin section 7 µm, haematoxilin and eosin, 357×

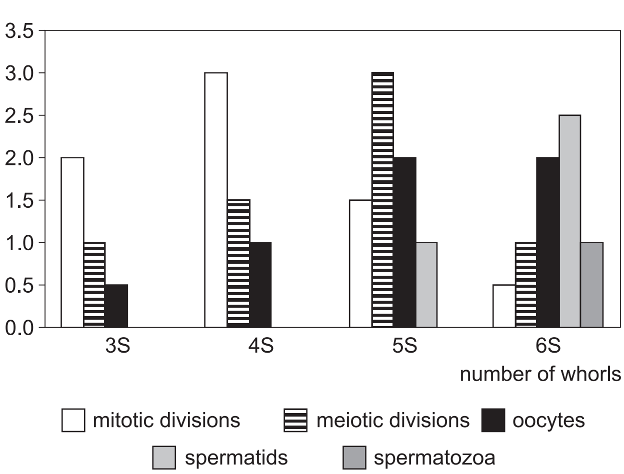


Fig. 17. Gonad activity of juvenile *Helicodonta obvoluta*

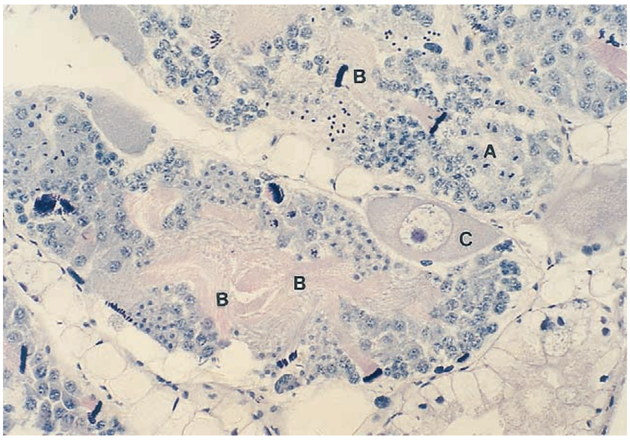


Fig. 18. Transverse section through the mature gonad of *Helicodonta obvoluta*. March. A – intense mitotic division, B – numerous spermatozoa, C – growing oocytes. Paraffin section 7 µm, haematoxilin and eosin, 179×

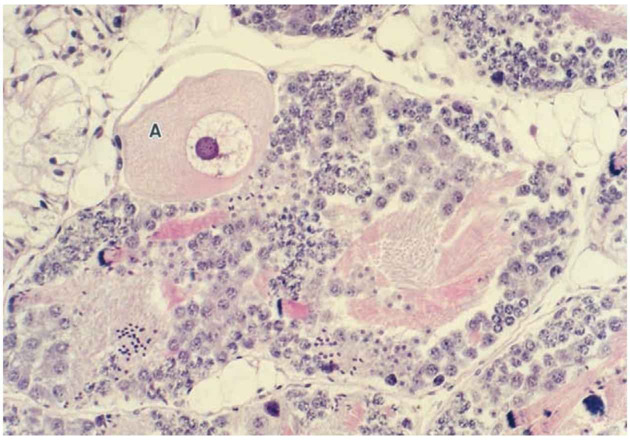


Fig. 19. Transverse section through the mature gonad of *Helicodonta obvoluta*. May. A – large vitellogenic oocyte. Paraffin section 7 µm, haematoxilin and eosin, 179×

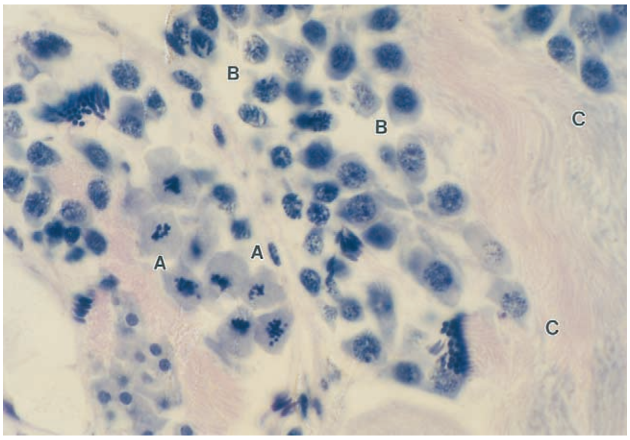


Fig. 20. Transverse section through the mature gonad of *Helicodonta obvoluta*. July. A – mitoses, B – cells in meiotic prophase, C – spermatozoa. Paraffin section 7 µm, haematoxilin and eosin, 179×

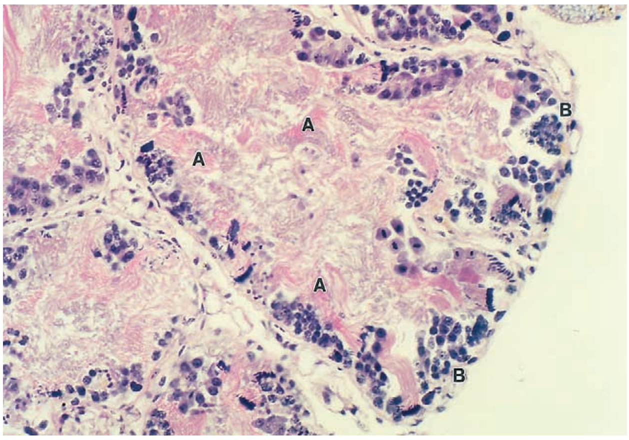


Fig. 21. Transverse section through the mature gonad of *Helicodonta obvoluta*. August. A – mitoses, B – cells in meiotic prophase, C – spermatozoa. Paraffin section 7 µm, haematoxilin and eosin, 179×

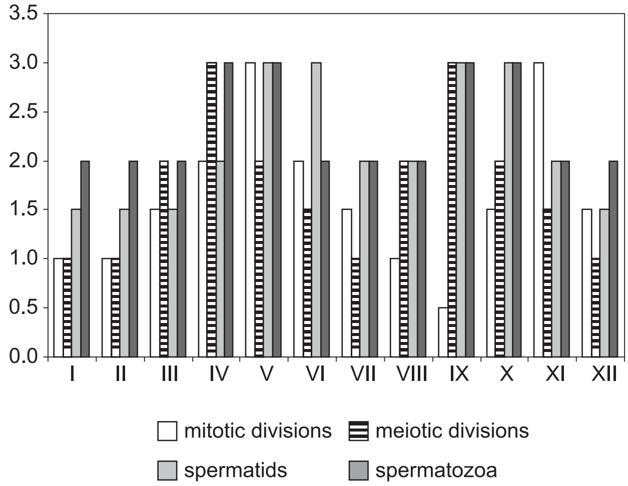


Fig. 24. Gonad activity of juvenile *Helicodonta obvoluta* in annual cycle (division, spermatogenesis)

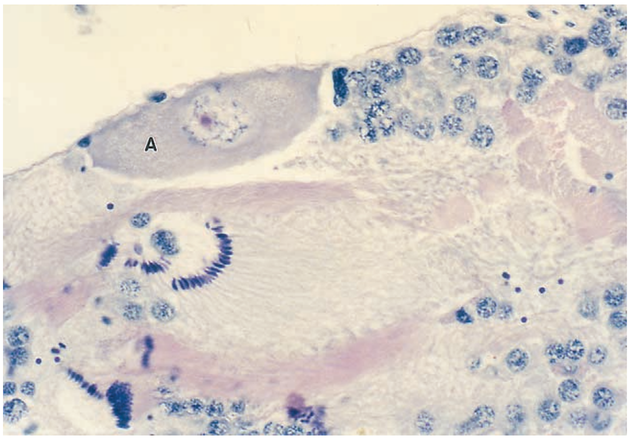


Fig. 22. Transverse section through the mature gonad of *Helicodonta obvoluta*. November. A – small growing oocyte. Paraffin section 7 µm, haematoxilin and eosin, 536×

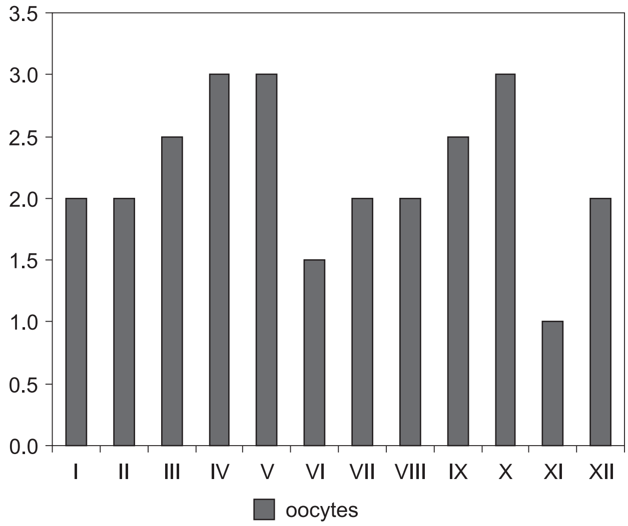


Fig. 25. Gonad activity of juvenile *Helicodonta obvoluta* in annual cycle (oogenesis)

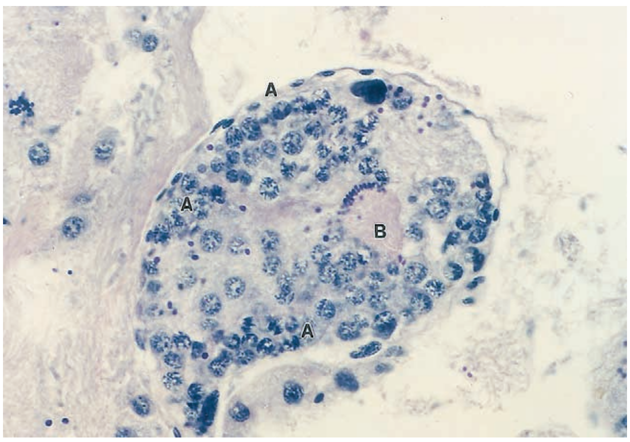


Fig. 23. Transverse section through the mature gonad of *Helicodonta obvoluta*. December. A – cells in meiotic prophase, B – spermatozoa. Paraffin section 7 µm, haematoxilin and eosin, 357×

oocytes enter vitellogenesis, so that in October large, vitellogenic cells predominate. They are no longer present in November, when consecutive generations of previtellogenic oocytes appear (Fig. 22) which prevail in December and January. Only in February few vitellogenic oocytes are observed, their number increasing in March and April.

Spermatogenesis. Mature spermatozoa are present in the gonad throughout the year, being however especially numerous from January till April (Fig. 18) and from June till October (Fig. 21). The number of spermatids increases from May till July and from August till October (Fig. 21).

The seasonal activity of the gonad is presented in Figs 24 and 25.

DISCUSSION

DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Published information on the morphological changes during ontogenetic development of the reproductive system in pulmonates is fragmentary and pertains to very few members of this subclass: Lymnaeidae and Succineidae (JACKIEWICZ & ZBORALSKA 1994), Vitrinidae (UMIŃSKI 1975), *Helix aspersa* (ENÉE & GRIFFOND 1983) and *H. lutescens* (KORALEWSKA-BATURA 1994). Development of particular organs of the reproductive system in few stylommatophorans (*Arion* sp., *Deroceras reticulatum*, *Limax maximus*) was studied in view of hormonal regulation in tissue cultures (JOOSSE 1972, JOOSSE & GERAERTS 1983). The results of these studies indicate that the growth and differentiation of the remaining organs of the reproductive system depend on the hormones secreted by the gonad which differentiates earlier. The knowledge of the development of the reproductive system, contrary to conchological criterion, makes it possible to unambiguously determine the stage of sexual maturation of the gastropod.

In the ontogenetic development of *H. obvoluta* the most intense changes in the gonad take place at stages J4 and J5, while at stage J5 there is clear acceleration of the development of the remaining part of the reproductive system. The sequence of development of particular reproductive organs in *H. obvoluta* is in agreement with that sequence observed in other Stylommatophora which were studied with respect to hormonal regulation of the reproductive system. In the period immediately preceding lip formation the reproductive system is fully formed. This means that an individual which has completed lip formation is sexually mature. The situation is similar in *H. lutescens* (KORALEWSKA-BATURA 1994) while in Lymnaeidae, Succineidae or Vitrinidae the snails have fully developed reproductive system and are ready to copulate long before they reach their final size (UMIŃSKI 1975, JACKIEWICZ & ZBORALSKA 1994).

GONAD DEVELOPMENT

At stage J3 mitotically dividing cells (morphologically indistinguishable spermatogonia and oogonia) prevail in the gonad, as well as the first pool of spermatocytes and oocytes in meiotic prophase. Likewise, the first growing oocytes are present. At stage J4 mitoses are few while the number of cells in meiotic prophase increases considerably which results in a substantial increase in the gonad volume compared to the previous stage. Also growing oocytes are more numerous, while spermatids and mature spermatozoa are absent. The appearance of the pool of growing oocytes indicates the onset of activity of the gonad as ovary and is most probably associated with secretion of appropriate hormones stimulating the development of female reproductive organs (FRETTER & GRAHAM 1964, POKORA 1989 and references contained therein). Gonad hormones in the studied Stylommatophora take part, among others, in regulation of gametogenesis and maturation of reproductive glands (CSABA & BIERBAUER 1979, TAKEDA 1979, TAKAYANGI & TAKEDA 1985). Such an activity of the gonad seems to be confirmed by the timing of appearance of primordial albumen gland, spermatheca and mucus gland. At stage J5 all the cells characteristic of the mature hermaphroditic gonad of an adult individual are already present (large vitellogenic oocytes, rosette-forming spermatocytes, spermatids, packets of spermatozoa) (Figs 11–16). The onset of spermiogenesis probably indicates a full activity of the gonad (as ovary and testes), with respect to both gamete production and hormone secretion. At that stage development of prostate, further growth of penis and epiphallus, as well as full development of female reproductive organs are observed. At the stage of lip completion the gonad is fully developed which, combined with the completed morphological development of the reproductive system, implies full sexual maturity which is correlated with growth termination.

SEASONAL CHANGES IN ACTIVITY OF MATURE GONAD

Both oocytes and spermatozoa are present in the gonad throughout the year, but the number of oocytes changes seasonally depending on their development stage. The content of spermatozoa in the gonad increases periodically. The maximum numbers of mature spermatozoa fall on the period from January till April and from June till October which would indicate that in those periods the snails copulate. Appearance of large oocytes with accumulated yolk from April till the end of July and from the end of August till October would suggest that these are the periods of egg-laying. Growing oocytes appear twice a year (August and November-December), in the periods preceding the presence of mature oocytes. The intensity of cell divisions leading to formation of the respective gametes changes along a similar course (Figs 18–25). The observations suggest that the onset of reproduction is determined, directly or indirectly, by the development of female gametes, since spermatozoa are present throughout the year thus testifying to the ability to copulate.

The gonad activity in annual cycle or before and during the reproductive period was studied in few terrestrial pulmonates. The problem is only alluded to in the paper by HELLER et al. (1997), on the structure and functioning of the gonad in *Lauria cylindracea* in Israel. The activity of the snail starts with the onset of rainy season (autumn) and lasts till the beginning of dry season (summer). *L. cylindracea* breeds from early winter till spring. In October the gonad contains few oocytes, spermatogenesis and oogenesis start in December, in March mature spermatozoa appear, and in May mature oocytes become visible. With the increasing gonad activity, the percentage of gravid individuals increases from December till May. From July till October the gonad is inactive.

Considerable differences between the gonad activity cycle in *H. obvoluta* and phylogenetically remote, ovoviviparous *L. cylindracea* indicate that both the activity cycle itself, its regulating mechanisms and the mechanisms that trigger the onset of reproduction vary between gastropod taxa and/or differ between ovi- and ovoviviparous species. The problem requires further studies. In the few studied stylommatophorans (for literature review see POKORA 1989) spermatogenesis precedes oogenesis, and only in *Arion ater* ova and spermatozoa develop synchronously (PARIVAR 1978). *H. obvoluta* departs from both these patterns.

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