

## LIFE CYCLE OF *VALVATA MACROSTOMA* MÖRCH, 1864 (GASTROPODA: HETEROBRANCHIA) IN THE LABORATORY

STANISŁAW MYZYK

Sapolno 14, 77-320 Przechlewo, Poland

**ABSTRACT:** Laboratory culture in 1994–1999 provided the following data on the life cycle of *Valvata macrostoma* Mörch: in favourable food conditions female maturity is attained in 60–250 days (mean: 108) at shell diameter 2.40–3.30 mm (rarely more: up to 4.10) and 2.50–3.00 whorls (rarely up to 3.37); in unfavourable conditions the snails mature later, at a somewhat smaller shell size. Snails kept in pairs/groups produce a maximum of 166 cocoons during their lifetime; the cocoons contain a total of up to 1,436 eggs, the number of eggs per cocoon ranging from 0 to 25 (rarely up to 34). Snails kept singly deposit few cocoons and eggs; and the eggs fail to develop. The average life span in favourable food conditions is 383 days, in unfavourable conditions 706 days (maximum 1,192 days). The mean shell size attained in favourable conditions is: diameter 4.46 mm, 3.46 whorls; in unfavourable conditions: diameter 3.65 mm, 3.09 whorls. Mortality of adult snails in laboratory shows a clear seasonal variation, with maximum in June and July.

**KEY WORDS:** Gastropoda, Heterobranchia, *Valvata*, life cycle, reproduction

### INTRODUCTION

The following valvatid species occur in Northern and Central Europe (FALKNER et al. 2001): *Valvata cristata* O. F. Müller, 1774, *V. macrostoma* Mörch, 1864, *V. piscinalis* (O. F. Müller, 1774), *V. sibirica* Middendorf, 1851, *V. studeri* Boeters et Falkner, 1998 and *Borysthenia naticina* (Menke, 1845). In the introduction to my earlier paper (MYZYK 2002) *V. studeri* was omitted.

*Valvata macrostoma* Mörch, 1864 (= *V. pulchella* Studer, 1820) occurs mainly in small, shallow water bodies, both temporary and permanent, with lush vegetation. According to literature data it inhabits considerable areas of Europe. Typical shells have a

relatively low spire, wide umbilicus and maximum diameter of 4.0–5.5 mm. The surface is covered by dense, regular, delicate ribs. Some authors (FRETTER & GRAHAM 1962, BINDER 1967, FALKNER et al. 2001) use the name *V. macrostoma*, others (PIECHOCKI 1979, FALNIOWSKI 1989a, b, 1990) – *V. pulchella*. Literature information on the biology of the species is very scanty (PIECHOCKI 1979, FALNIOWSKI 1989a). FALNIOWSKI (1989a, b, 1990) provides detailed descriptions and figures of its shell and anatomy. Some data contained in my earlier paper (MYZYK 2002) on the life cycle of *V. cristata* pertain also to *V. macrostoma* (e.g. embryonic development, variation of eggs).

### MATERIAL AND METHODS

The initial material for the laboratory culture included snails collected on the 7th of June 1994 in the Narew River valley near Łomża (NE Poland). Their shell measurements were: body whorl diameter 1.20–2.05 mm (mean 1.71 mm), number of whorls

1.62–2.25 (mean 2.03). Based on the growth rate in the laboratory, it could be estimated that they had hatched at the end of April 1994.

The snails were kept in closed containers of different size: smaller of 25 mm diameter, filled with water

up to ca. 25 mm (further called “small containers”) and larger of 60 mm diameter, filled with water up to 40 mm (further “large containers”). Leaves of trees (mainly black alder and oak), covered by periphyton, were placed in the containers as food source. The leaves were arranged loosely in a few layers. In small containers the combined surface of both sides of leaves was ca. 30–50 cm<sup>2</sup>, in large containers it ranged from 150 to 250 cm<sup>2</sup>, depending on the snails’ food requirements. New leaves were usually provided every 10 days, and in winter irregularly only during longer thaw periods. Water in the containers was changed every 3–5 days in summer, and every 10 days in the remaining period. Snails collected in the wild in 1994 were kept in the worst food conditions (testing various kinds of food), those hatched in 1997–1998 and kept in large containers had the best conditions.

The total number of snails in the culture was 147, including 24 collected in the field and 123 hatched in the laboratory. Twenty eight of these died as juveniles, and three reached only male maturity (partly excluded from the analysis). Eighty six adult snails were kept in pairs or groups, 30 were kept singly without a possibility to copulate. Snails collected near Łomża (23 adults) and most of those hatched in 1995 (47 adults) were kept in small containers throughout their lifetime. Some of the snails hatched in 1995 (18) were initially kept in small containers, and transferred to large containers after 1–1.5 year. Those hatched in 1996–1998 stayed in small containers till 2–3 mm diameter, and were then transferred to large containers (total of 28 adults). Snails kept in pairs/groups, whose partners died, were joined in new, permanent pairs or placed temporarily with other snails to enable copulation. Besides snails permanently residing in the laboratory, some observations were based also on snails kept in the laboratory only for a short time after hatching.

The water temperature in the laboratory in May–September was usually 15–25°C and thus close to the temperature of littoral water in the lake. In the remaining months it was higher than in natural conditions, mostly 8–18°C (sporadically it decreased below 5°C).

Measurements are presented in Figs 1–3. After removing the mucus layer the length of cocoon was measured parallel to the striae on the capsule, irrespective from the angle they formed with the substratum (Fig. 2). The filaments of capsule-forming substance which were sometimes present on the top of the cocoon, as well as the layer of cementing substance, were disregarded when measuring cocoon length. The width of cocoons was measured in the widest place, perpendicular to the striae. Young snails were measured in a drop of water, older individuals (from ca. 1.7 mm) – dry. Since it was rather difficult to measure shell diameter (Fig. 3 – Ds) in live snails, the diameter of the body whorl was measured instead (Db). Exact shell measurements were taken only after death of snails. Comparisons of shell diameter and the body whorl diameter showed that for most shells the two values were identical or nearly so (Fig. 4), and the greatest differences did not exceed 5.6% (at a

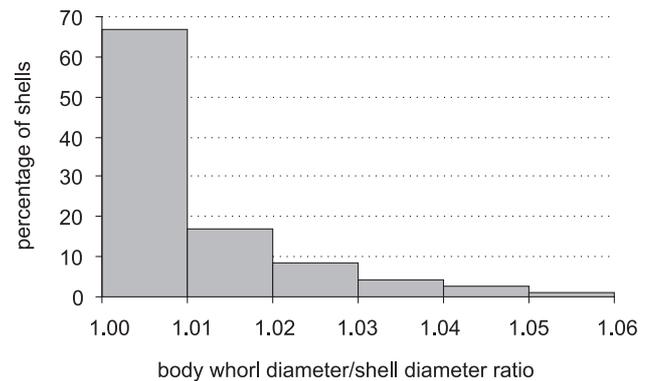
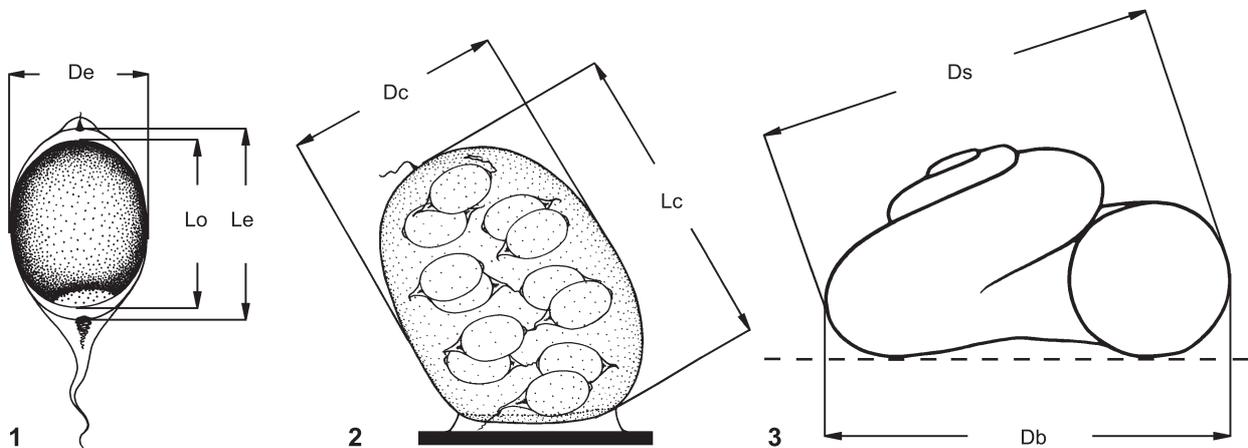


Fig. 4. Variation in the body whorl diameter/shell diameter ratio



Figs 1–3. Measurements: 1 – egg: De – egg chamber diameter, Le – egg chamber length, Lo – egg cell length; 2 – cocoon: Dc – maximum cocoon diameter at sutures, Lc – cocoon length measured parallel to the capsule striae; 3 – shell: Db – body whorl diameter, Ds – shell diameter



rather high spire). Measurements were taken with calibrated eyepiece, to the nearest 0.01 mm (range 0.1–1 mm) or 0.05 mm (above 2.3 mm). Whorls were counted to the nearest 1/8 (=0.125; error±0.062).

To facilitate description of embryonic development, the whole development period was divided in

## RESULTS AND DISCUSSION

### COPULATION

At room temperature (18–22°C), soon after attainment of male maturity, most snails kept in pairs or groups copulated, irrespective of the season. At temperatures below 15–16°C no copulation was usually observed, and the lowest temperature at which copulation occurred was slightly over 12°C (a pair of snails with numerous mature oocytes in their gonads). At high temperatures of over 26°C the snails failed to copulate.

The course of copulation resembled that described for *V. cristata* (MYZYK 2002). In the initial phase the “female” often moved and fed, while the “male” took a position on its shell, on the body whorl near the aperture. During copulation the “male” had its head strongly tilted to the left, its pallial tentacle was in a normal position, and the gill usually protruded beyond the shell margin. The total duration of copulation was 0.5–1.5 hrs, and frequently the partners changed their roles to copulate again.

The snails reached male maturity slightly earlier than female maturity, and sometimes copulated with other individuals which also had not attained female maturity. Three exclusively “male” snails with relatively small gonads copulated only in the initial period after attaining maturity. At the end of their life also hermaphroditic snails rarely played the male part during copulation.

### COCOON FORMATION

The yolk-containing oocytes were usually well visible in the gonad. During their movement they were also visible in the albumen gland and shell gland. Up to ten oocytes could be contained in the albumen gland at the same time (four in *V. cristata*; MYZYK 2002). Since consecutive oocytes usually moved every 3–5 minutes (sometimes longer, up to ca. 10 minutes), formation of larger cocoons in the shell gland took as long as up to 1–2 hours.

### COCOON DEPOSITION

About 10–20 minutes before cocoon deposition, the snail started to clean the selected place with its radula (also when the cocoon was to be deposited on the surface film). During deposition the snail tilted its

three phases: “egg” – from cocoon deposition to breaking the external egg envelope, “larva” – intermediate stage, “snail” – from shell formation till hatching (MYZYK 2002: Figs 43–51).

All the figures are original. The shells are in the author’s collection.

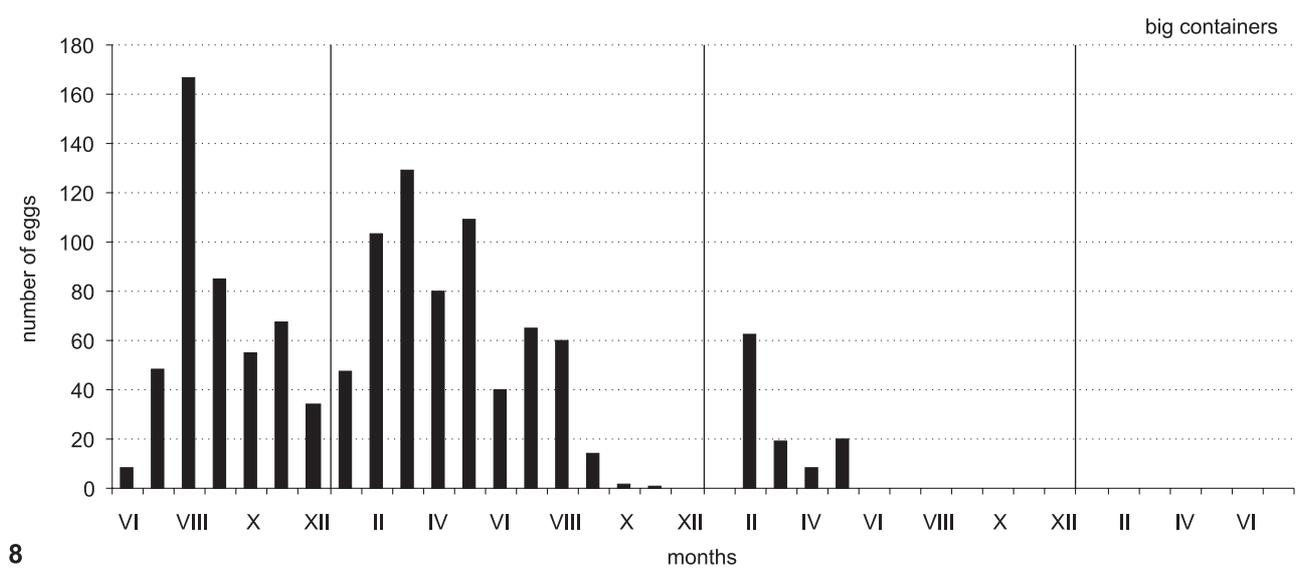
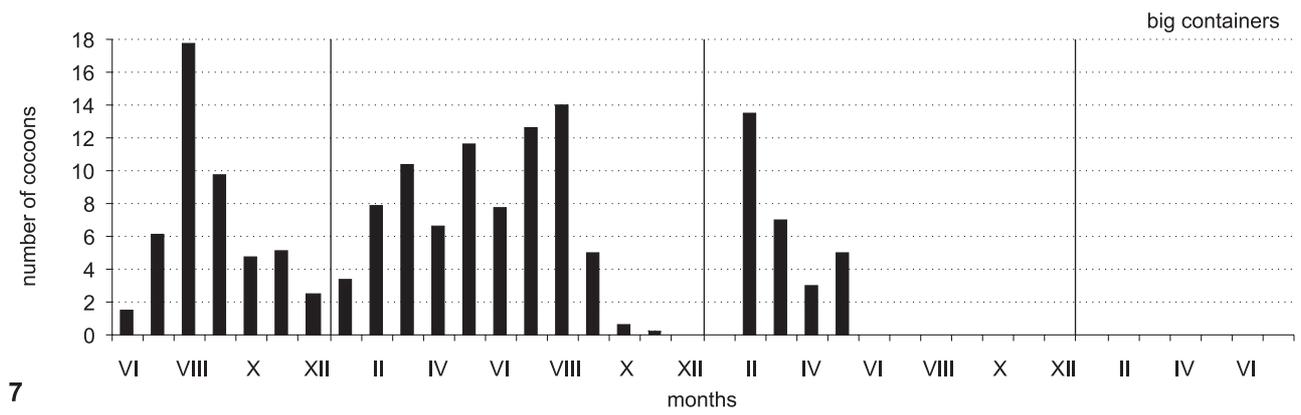
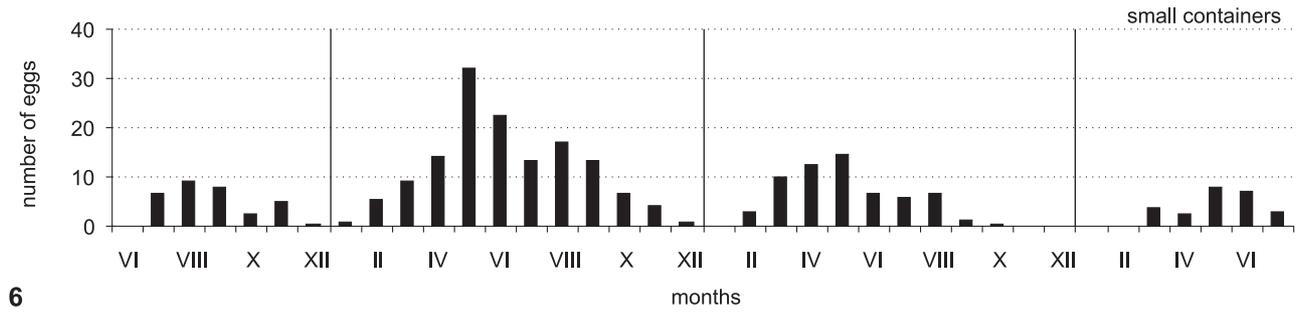
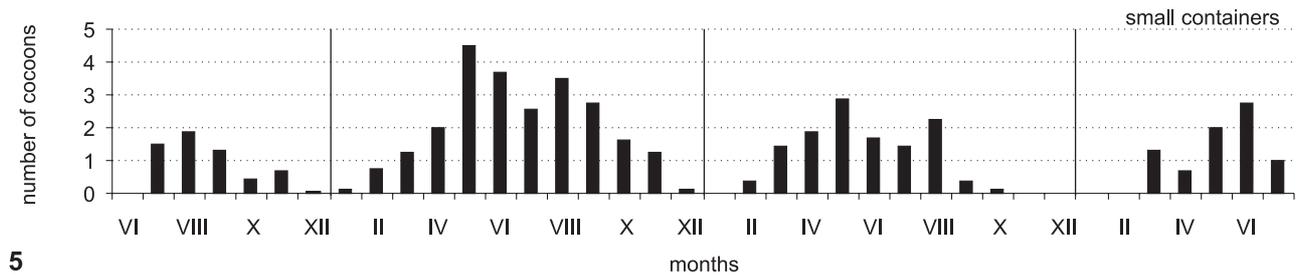
shell to the right, so that the aperture margin was positioned parallelly to the substratum, bent the anterior part of its body strongly to the left, and slightly contracted its head. The pallial tentacle, gill, and tips of cephalic tentacles and proboscis protruded beyond the shell margin. The cocoon, emerging from the reproductive ducts, had a drop of cementing substance on its base and was pressed to the substratum with the whole body. Only after fixing the cocoon the snail slowly tilted its shell to the left till it assumed the normal position. Usually after 6–10 minutes the snail moved away from the cocoon for a short distance to the left, and then crawled away.

Cocoon deposition took place both in the daytime and in the night, in the whole volume of the containers: on the walls, leaves, surface film and even shells of other individuals. However, the most often selected place was the narrow zone of ca. 2 mm just below the water surface (on the container’s walls and leaves protruding from the water), where ca. 45% cocoons were deposited. Fairly many cocoons were placed on the remaining surface of the walls (20.6%) and the lower and upper surface of submerged leaves (20.8%). About 7.1% cocoons were deposited on the surface film, and 1.9% above the water table. Sporadically, cocoons were deposited on the bottom or on shells of other individuals. About 4.1% cocoons were not fixed to any substratum, and lied loosely on the bottom or on the leaves.

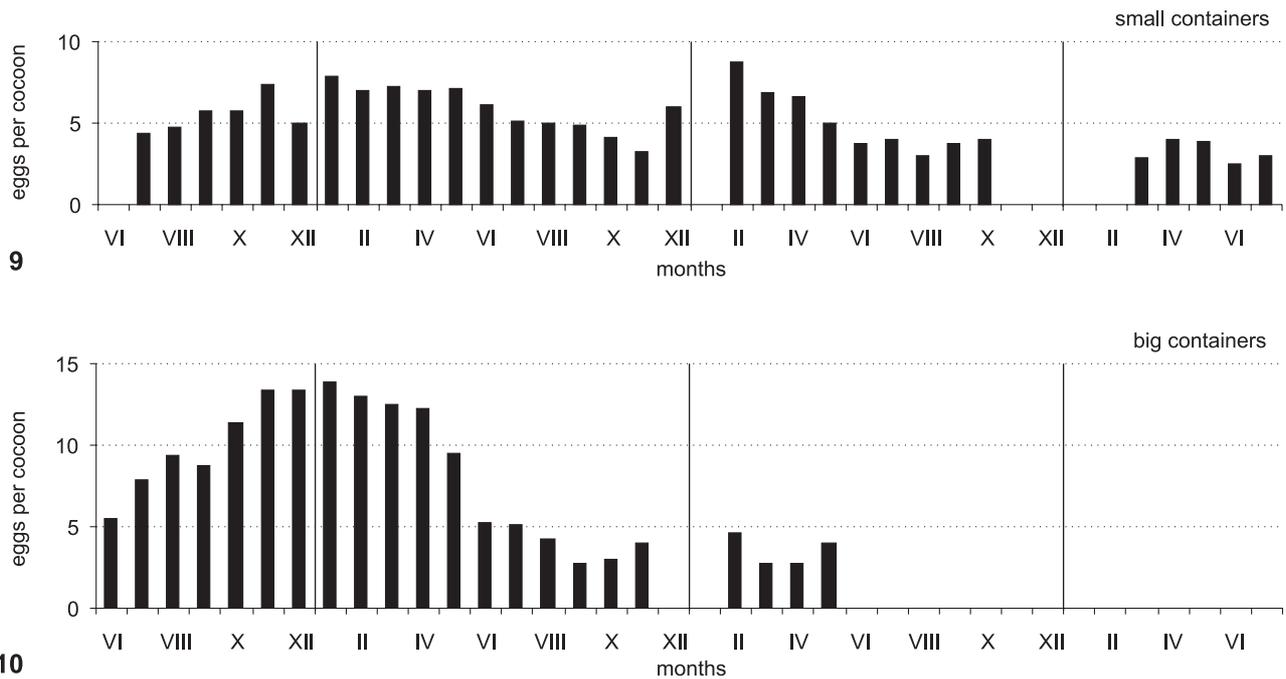
From time to time some of the snails produced blobs of mucus, sporadically with small fragments of egg cord.

### TIMING AND DURATION OF REPRODUCTION

Reproduction of snails kept in pairs or groups was influenced, among others, by food, temperature and possibility to copulate. In the laboratory reproduction in autumn and winter was possible due to the much higher temperature, compared to natural conditions. Seasonality of reproduction was very clear in unfavourable food conditions (small containers), while in favourable conditions (large containers) it was less pronounced (Figs 5–10). In small containers the first cocoon was deposited 102–414 days from hatching (mean 182, SD=74, n=25), in large containers – 76–138 days (mean 112, SD=21, n=8). The reproduction usually started in the year when the snail hatched



Figs 5–8. Cocoons laid by snails kept in pairs or groups in consecutive months and years; mean number of cocoons and eggs converted to one snail living in a given month



Figs 9–10. Cocoons laid by snails kept in pairs or groups in consecutive months and years; mean number of eggs per cocoon

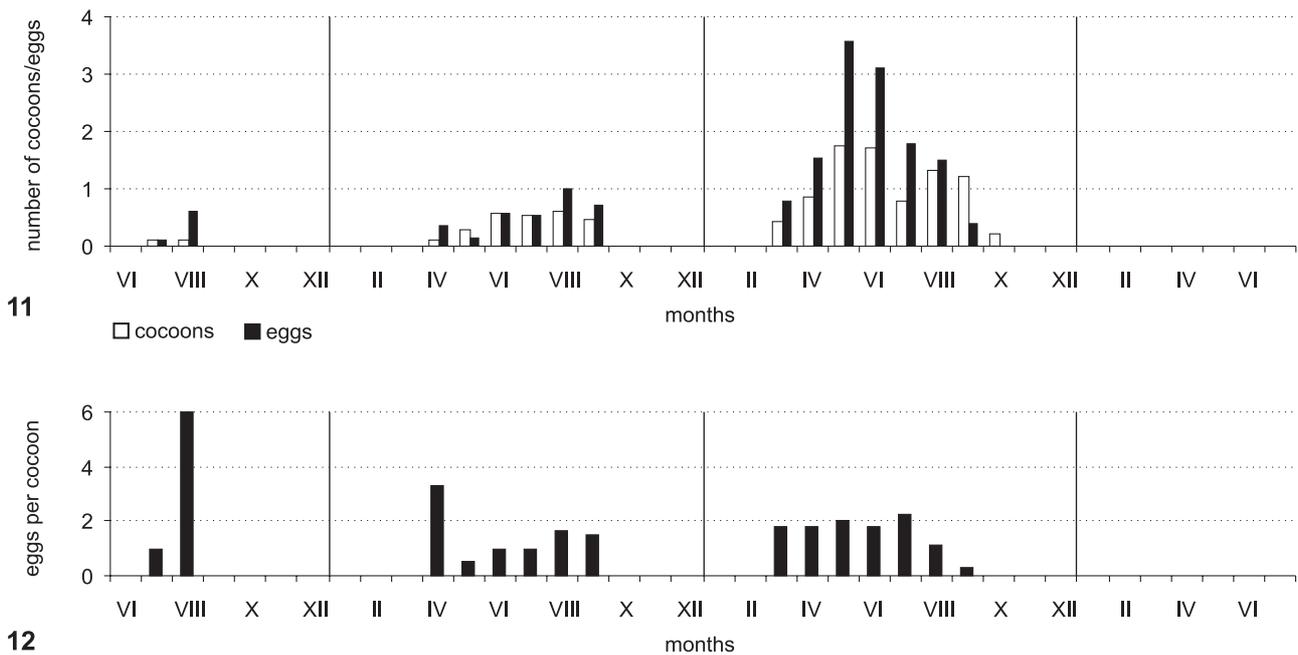
(July–November), and rarely (11.4% containers) only next year (January–May). Snails hatched in May and kept in large containers from the beginning deposited their first cocoons in July–September, those hatched in winter (February–March) – as early as the end of June. The winter pause in cocoon deposition started at different time, depending on the container (usually end of October–beginning of December) and its duration varied (37–170 days). In small containers its mean duration was 110 days (SD=28, n=30), in large containers – 86 days (SD=30, n=16). Maintaining temperature at a constant level below 15°C usually inhibited reproduction, though sporadically cocoons were produced even at 7–10°C (after an earlier copulation at a higher temperature). Likewise, unfavourable food conditions and small number of mature oocytes in the gonad often inhibited reproduction in the autumn-winter period, in spite of a rather high temperature (17–19°C). At room temperature and in favourable food conditions reproduction in some containers was uninterrupted from maturity (July) till death (end of June next year).

After the first wintering snails kept in large containers started reproducing already in January (ca. 67%) or February (ca. 33%), and intense deposition of cocoons containing oocytes, accumulated during the winter pause, lasted till March or April. By contrast, in small containers reproduction started much later (in January ca. 17%, in February ca. 39%, in March ca. 26%, in April ca. 13%, or even as late as May). In May (rarely already at the end of April or still at the beginning of June), in both large and small containers, there was a period of very intense reproduction, followed by a clear decrease in the number of deposited

cocoons. Snails with a longer life span (2 or 3 years) resumed intense reproduction at the end of July or in August. In September and October, with decreasing temperature, the number of deposited cocoons and eggs decreased gradually, but in some containers the reproduction continued till half of December. In consecutive years the onset and termination of reproduction had a course similar to that described above.

In most containers (59.5%) snails kept in pairs/groups started cocoon deposition short after attainment of female maturity (up to 60 days). For example, a pair of snails hatched on the 2nd of May 1997 attained their female maturity on the 12th of July 1997, and the first cocoons were deposited on the 23rd of July 1997. The mean time between the attainment of female maturity and the deposition of the first cocoon in small containers was 58 days (SD=49, n=25, range 1–214), and in large containers 28 days (SD=11, n=8, range 11–47). When snails had an opportunity to copulate, the reproductive period lasted almost till the end of their lifetime, and the longest time between the deposition of the first and last cocoon was 925 days. The time between the deposition of the last cocoon in the container and the death of the longest-lived snail depended on food conditions and was on average 21 days (SD=17, n=26, range 2–72).

Among the 30 snails kept singly (no possibility to copulate) only 15 deposited cocoons. In most containers (86.7%) the first cocoons were found 306–847 days from hatching, only in two as early as 99 and 135 days (mean 421, SD=193). The mean time between the attainment of female maturity and deposition of the first cocoon was 326 days (SD=191, n=15, range 19–762). Cocoon deposition was clearly seasonal:



Figs 11–12. Cocoons laid by snails kept singly in consecutive months and years: 11 – Mean number of cocoons and eggs converted to one snail living in a given month; 12 – Mean number of eggs per cocoon

from half of March till the end of September, only one cocoon with no eggs was deposited later (October 16th 1997) (Fig. 11–12). The mean time between the deposition of the last cocoon and death was 154 days ( $SD=137$ ,  $n=15$ , range 10–474 days).

The young snails which were the base of the culture, were collected near Łomża on the 7th of June and their shells were then 1.20–2.05 mm in diameter. Considering the growth rate, they were probably hatched at the end of April, from cocoons deposited at about half of April. This confirms the data of FALNIOWSKI (1989a), on the onset of reproduction in the second half of April. The time of appearance of juvenile snails reported by PIECHOCKI (1979) and FALNIOWSKI (1989a) differs from that observed in this study and perhaps actually pertains to adult individuals.

## FERTILITY

The mean number of cocoons and eggs deposited by snails kept in pairs/groups in particular months and years of life (converted to one snail alive in a given month) is presented in Figs 5–8. In favourable food conditions (large containers) a pair of snails laid a maximum of 53 cocoons containing 471 eggs during one month, and in unfavourable conditions (small containers) – a maximum of 18 cocoons with 132 eggs. Snails which lived for 2 or 3 years produced the most numerous cocoons and eggs in the first year of their life, and in the next years the number was increasingly smaller.

The maximum numbers of cocoons and eggs produced during 24 hours by a pair of snails kept in large containers were: August 10th 1997 – 4 cocoons with

33 eggs (10+9+7+7); December 12th 1997 – 4 cocoons with 56 eggs (18+15+13+10); April 30th 1998 – 2 cocoons with 48 eggs (24+24); February 23th 1998 – 3 cocoons with 44 eggs (17+14+13).

Snails kept singly after their partners died sporadically deposited within 24 hours even up to 3–4 cocoons, but these contained few eggs (e.g. 3+1+1+1 or 6+1+1).

Snails which copulated (once or several times), subsequently isolated, deposited in large containers mostly 23–36 cocoons with a total of 131–241 eggs, and those kept singly after death of their partners even up to 58 cocoons with a total of 346 eggs. Snails which could not copulate regularly (e.g. after the partner's death) laid increasingly smaller numbers of eggs and cocoons, and their gonads became filled with mature oocytes. However, some of the snails which copulated in summer and were then kept singly, still deposited cocoons with fertilised eggs in spring next year. Examples: I. a snail kept singly after its partner's death (26.07.1995), from August 27th till September 21st 1995, deposited 3 cocoons (6+6+5) and from March 6th till May 20th 1996 – 5 cocoons (5+1+1+2+1), from which juveniles hatched; II. a snail which copulated on the 12th of September 1995 (before attaining female maturity), from October 29th till November 18th 1995 deposited 5 cocoons with 38 eggs and from April 6th till July 17th 1996 – 18 cocoons with 57 eggs, from which juveniles hatched.

Cocoons deposited by snails kept in pairs/groups in small containers contained 0–16 eggs, and sporadically up to 20 (mean 5.8,  $SD=3.1$ ,  $n=1,762$ ). Cocoons deposited in large containers contained 0–25 eggs, and sporadically even up to 34 (mean 8.9,  $SD=5.0$ ,



n=2,715) (Fig. 13). The largest cocoon, with 34 eggs, was deposited on the 2nd of May 1997, after a three-week pause in reproduction (shells of 4.75 mm and 4.65 mm). Some of the deposited cocoons contained no eggs, but only an egg cord or only gelatinous substance. Cocoons with no eggs constituted ca. 1.5% of all cocoons in small containers, and ca. 0.5% in large containers. The mean number of eggs per cocoon depended on the snail size and age. Cocoons deposited at the end of lifetime contained usually few eggs. Variation in the mean number of eggs in cocoons deposited in particular months and years of life by snails kept in pairs/groups is presented in Figs 9–10.

During their lifetime snails kept in pairs/groups in the worst food conditions (collected in 1994, small containers) deposited 5–26 cocoons with a total of 44–153 eggs, in slightly better conditions (hatched in 1995, small containers) 12–52 cocoons with a total of 88–316 eggs. Snails kept initially in small, and then in large containers produced 22–166 cocoons with a total of 155–1,275 eggs. In the best food conditions (large containers from the beginning) the number of cocoons was 21–141 and they contained a total of 201–1,436 eggs. Variation in the number of cocoons and eggs produced during a lifetime by particular snails, and variation in the mean number of eggs per cocoon are presented in Figs 14–16. Table 1 shows variation in the mean number of cocoons and eggs

deposited during the lifetime depending on food conditions.

Among the 30 snails kept singly 15 deposited no cocoons, and 6 produced each only one cocoon with 0–2 eggs, in their lifetime. The remaining 9 snails deposited from 2 to 46 cocoons with a total of 2 to 99 eggs. The highest number of cocoons (46) and eggs (99) was produced by a snail kept initially in a small and then in a large container (life span 820 days). The number of deposited cocoons and eggs most often increased in consecutive years of life (Fig. 11), but no significant correlation was found between the total number of deposited cocoons and eggs, and life span (Pearson's correlation: number of cocoons/life span  $r=0.28$ ; number of eggs/life span  $r=0.18$ ,  $n=30$ ). Cocoons deposited by snails kept singly in small containers contained 0–6 eggs (mean 1.2,  $SD=1.1$ ,  $n=51$ ), and in large containers – 0–8 eggs (mean 1.8,  $SD=1.4$ ,  $n=86$ ) (Fig. 17). Cocoons with no eggs constituted ca. 25.5% all cocoons in small containers and ca. 16.3% in large containers. Variation in the mean number of eggs in cocoons deposited in particular months and years of life is presented in Fig. 12 (small and large containers combined).

After death, gonads of nearly all snails (except those infected with trematode larvae) still contained oocytes with yolk. The number of oocytes in snails kept in pairs/groups was most often 2–20, rarely up to 150 (no copulation?). In most (90%) snails kept sin-

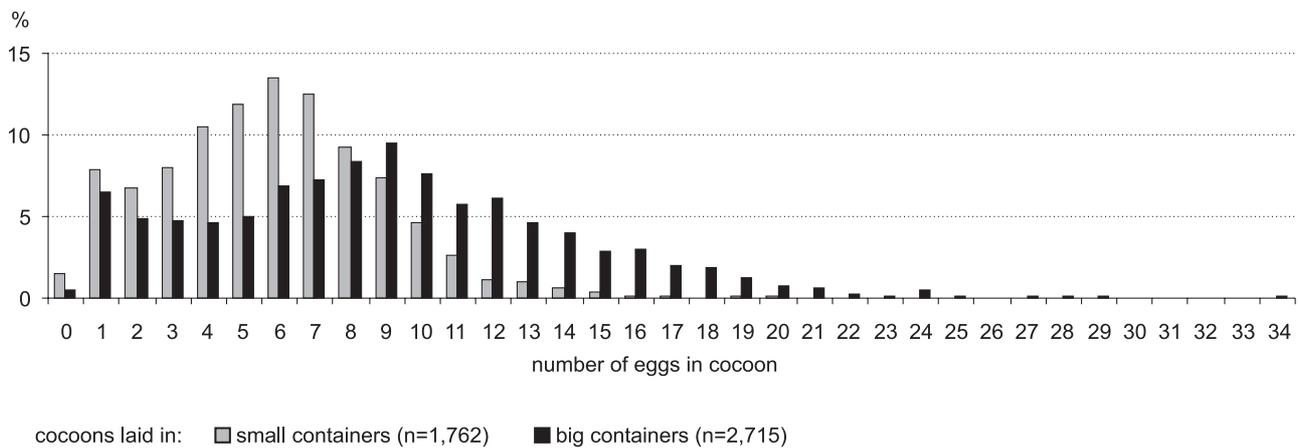
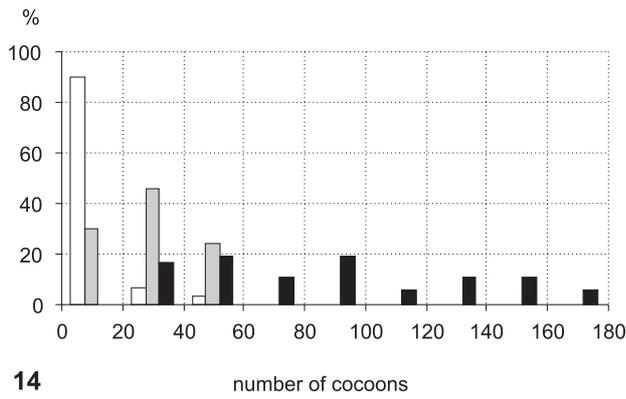


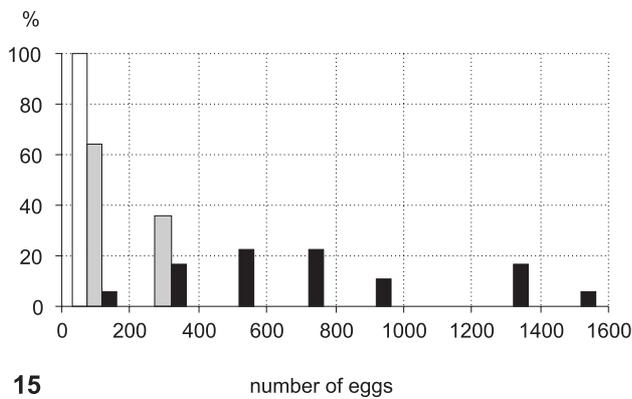
Fig. 13. Number of eggs in cocoons laid by snails kept in pairs or groups; number of cocoons given in parentheses

Table 1. Mean number of cocoons and eggs deposited per lifetime (pairs/groups converted to one snail); s – small containers, s/b – initially small, later big containers, b – big containers

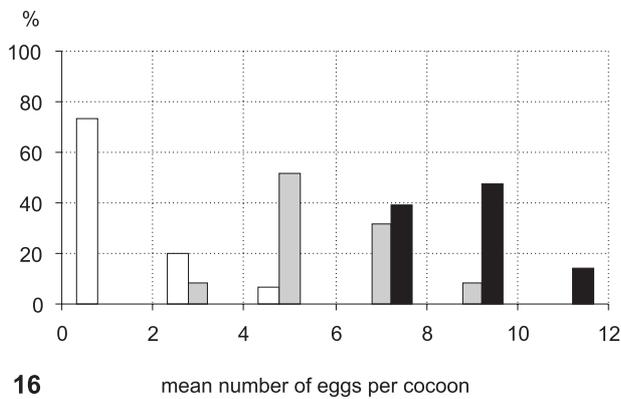
Year of hatching	Kept in pairs or groups			Kept singly		
	Number of snails	cocoons mean $\pm$ SD	eggs mean $\pm$ SD	Number of snails	cocoons mean $\pm$ SD	eggs mean $\pm$ SD
1994 s	19	16.8 $\pm$ 7.1	100.9 $\pm$ 35.9	4	1.2 $\pm$ 1.6	1.0 $\pm$ 1.0
1995 s	36	35.2 $\pm$ 9.7	198.8 $\pm$ 50.4	11	2.9 $\pm$ 4.6	3.3 $\pm$ 6.1
1995 s/b	13	96.2 $\pm$ 44.2	765.4 $\pm$ 326.1	5	18.6 $\pm$ 16.7	31.4 $\pm$ 37.0
1996–1998 b	18	91.1 $\pm$ 43.5	845.8 $\pm$ 427.1	10	0.7 $\pm$ 1.6	1.6 $\pm$ 3.2



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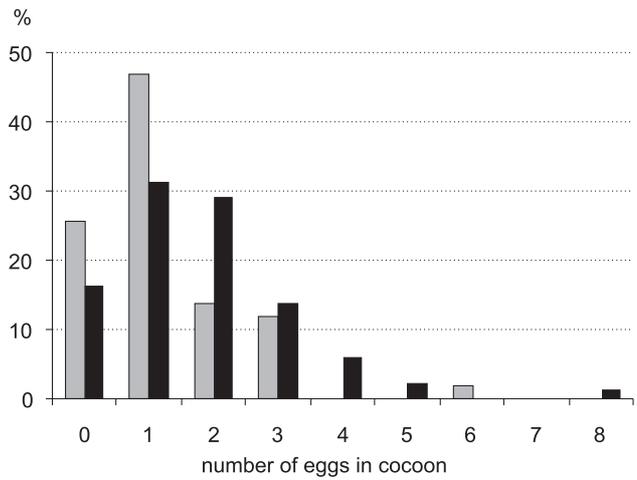
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□ kept singly  
 □ pairs/groups kept in the small containers  
 ■ pairs/groups kept in the big containers

Figs 14–16. Variation in the number of cocoons and eggs deposited during lifetime, and in the mean number of eggs per cocoon

gly the gonads were completely filled by vitellogenic oocytes (maximum up to ca. 150).

No negative effect of inbreeding (joining siblings from the same cocoon in pairs) on the reproduction was observed. In consecutive generations, in similar



cocoons laid in: □ small containers (n=51)  
 ■ big containers (n=86)

Fig. 17. Number of eggs in cocoons laid by snails kept singly; number of cocoons given in parentheses

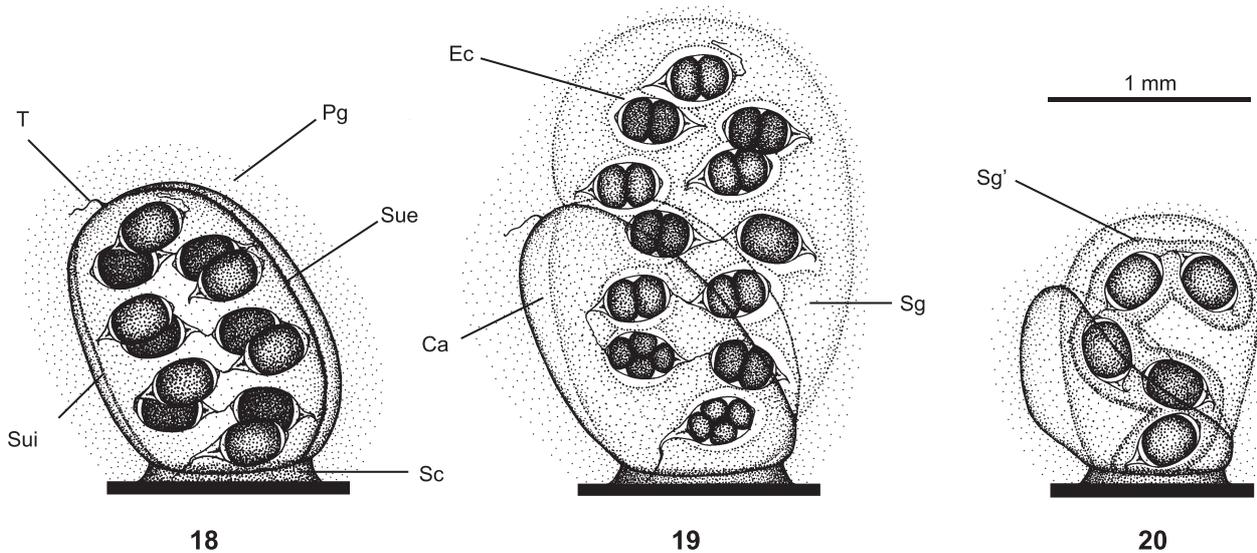
food conditions, the number of eggs and cocoons remained at the same level or even increased. Example: parents – 166 cocoons/1,275 eggs; generation I – 140 cocoons/1,332 eggs; generation II – 132 cocoons/1,436 eggs (converted to one snail).

COCOON STRUCTURE

Cocoon structure is diagrammatically presented in Figs 18–33. The cocoons had usually the shape of an oval pouch of variable proportions (Figs 18, 21, 26, 27, 29, 34). In transverse section they were usually slightly flattened, and the major diameter of the ellipse was in the plane of sutures. Cocoons of different shape were rare (a total of ca. 2.5%): e.g. pear-shaped (Fig. 25), conical (Fig. 32), constricted (Figs 23, 28, 35) or composed of 2–3 connected capsules (Figs 22, 24, 30, 33). Sporadically (0.3%) a single mucus layer contained two unconnected capsules which were treated as two separate cocoons (Fig. 31). Additional capsules were not provided with cementing substance and contained most often one egg (less often 2 or 3).

The length/diameter ratio of the capsule (Lc/Dc) in egg-containing cocoons ranged from 0.55 to 3.05 (only in one atypical cocoon with strongly elongated apex it was 4.35). Among cocoons containing one egg, 65.9% were somewhat flattened (Lc/Dc values 0.55–0.99), and 29.6% were elongated (Lc/Dc values 1.01–2.29). Among cocoons with 9 eggs 17.0% were flattened (0.75–0.99), and 70.6% elongated (1.01–2.00). Cocoons with higher numbers of eggs were usually elongated. Cocoons containing no eggs were often much elongated, with mean Lc/Dc value 2.31 (range 0.78–10.22).

Most cocoons were covered by a mucus layer (palium gelatinosum – Pg) of varied thickness. In



Figs 18–20. Cocoon structure: 18 – newly deposited cocoon: Pg – mucus layer covering cocoon (palium gelatinosum), Sc – cementing substance, Sue – suture located on the external (more convex) margin of the cocoon, Sui – suture located on the margin opposite to Sue (sometimes not visible), T – filament extending from the capsule; 19 – cocoon disrupted shortly after deposition: Ca – cocoon capsule, Ec – cavities around eggs, formed in the gelatinous substance, Sg – gelatinous substance filling the space between the eggs and the capsule; 20 – cocoon with two different kinds of gelatinous substance: Sg' – layer of thick gelatinous substance surrounding the row of eggs

some cocoons it reached 1 mm, in others it was very thin or even absent. A gelatinous layer of medium thickness protected the cocoon removed from water against desiccation for 15–25 minutes, and only then the capsule began to shrink. The mucus layer was produced in the shell gland which is indicated by sporadic presence of drops of capsule-forming substance in the layer. Fairly often pieces of faeces adhered to the mucus layer.

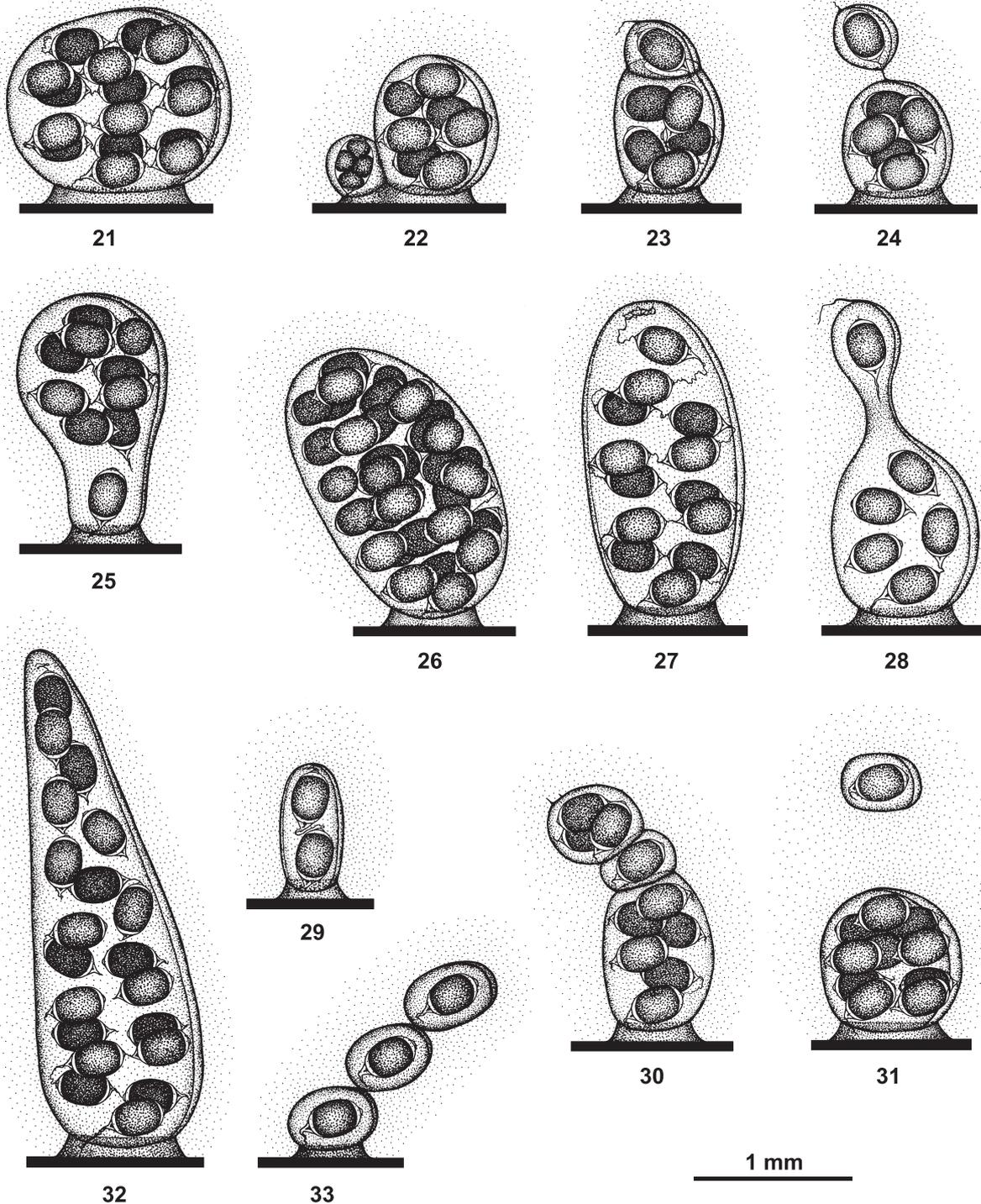
The external surface of the capsule was covered by more or less distinct striae extending from the base to the apex. Sometimes also striae parallel to the substratum were visible at the base. Ca. 1.4% cocoons bore on their apices short filaments (one or several, 0.1–0.4 mm long, rarely more) of the capsule-forming substance (Figs 18, 23, 24, 28, 30). The thickness of the measured capsules was 0.001–0.005 mm. Immediately after deposition the capsule was colourless but in a few hours it usually became yellow or brown (rarely remained colourless). The capsule was composed of two parts joined by sutures. The suture on the outer (more convex) side of the cocoon (Sue) was nearly always more clearly marked, and sometimes a narrow membrane ridge extended along it. The suture on the opposite side of the capsule (Sui) was distinct only in some cocoons, in others its position was indicated by the line of convergence of the striae on the capsule. Very few cocoons were devoid of the capsule or had it deformed (e.g. with orifices, without apex etc.).

Eggs in the cocoon were surrounded by a transparent, colourless gelatinous substance, which tightly filled the whole capsule. After breaking the capsule

the substance increased its volume by ca. 50% and very easily adhered to various objects. Several snails, originating from the same parents, deposited cocoons in which the gelatinous substance had a complex structure: the row of eggs was surrounded by a dense substance 0.30–0.60 mm in diameter, while a slightly more liquid substance filled the remaining space in the capsule (Fig. 20). The row of eggs in the cocoon (both connected and unconnected by a cord) sometimes formed a dextral spiral. Some snails with numerous mature oocytes in their gonads deposited cocoons containing, besides eggs, grains of yolk from other, damaged eggs. The first egg in the cocoon was often connected by a cord with the base of the capsule. In cocoons containing more than one egg the cord often connected also the other eggs (all in 19% cocoons, some in 26% cocoons). About 0.9% cocoons deposited by snails kept in pairs/groups and ca. 19.7% of those deposited by snails kept singly contained no eggs, but sections of egg cord of variable thickness and structure, or only gelatinous substance.

The cocoon was fixed to the substratum with a layer of cementing substance, placed on its base centrally or slightly shifted toward the Sui suture. Very rarely cocoons (with 7–9 eggs) were observed to have no cementing substance or the substance was placed on the mucus layer. Small cocoons (1–2 eggs), found sometimes on the bottom or leaves, with no cementing substance, resembled extra capsules presented in Fig. 31.

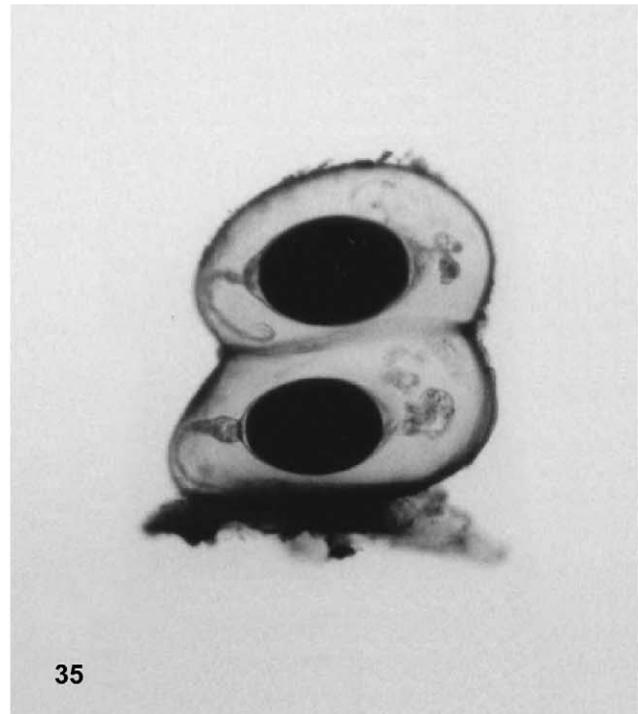
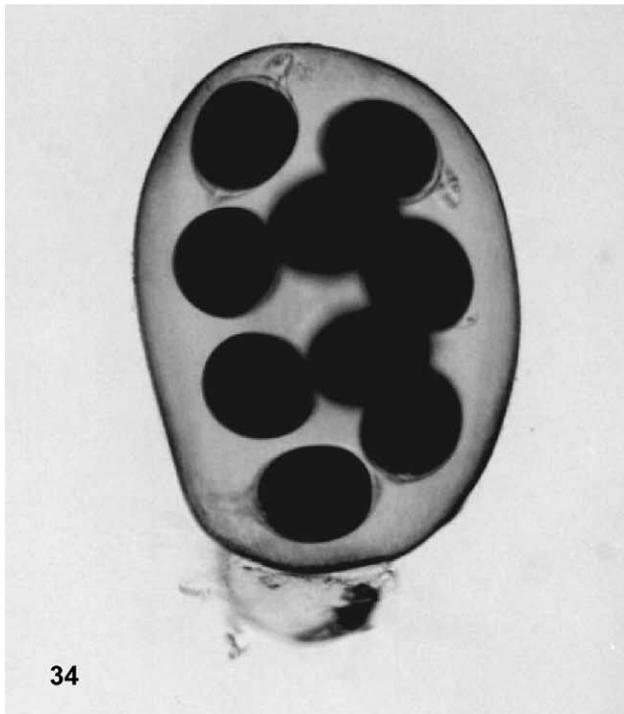
The smallest cocoons with 1 egg measured: I. length 0.30 mm, diameter 0.45 mm; II. length 0.40



Figs 21–33. Examples of variation of cocoon shape and size

mm, diameter 0.25 mm. Minimum measurements of the cocoons depended on the number (volume) of the contained eggs; the maximum measurements varied rather widely. Cocoons of similar size could contain very different numbers of eggs (examples: Figs 26, 27). The longest cocoons containing eggs were: I. with 15 eggs – 3.70 mm × 0.85 mm (much deformed,

with an elongated apex), II. with 20 eggs – 3.20 mm × 1.05 mm (Fig. 32). A cocoon with 21 eggs had the largest diameter (1.50 mm) and length of 1.65 mm. The longest cocoon containing the egg cord only was 4.60 mm in length and 0.45 mm in diameter. Length and width values for typically structured cocoons (single capsule) are presented in Table 2. The cocoon



Figs 34–35. Light microscope, 44×: 34 – cocoon deposited under the surface film; 35 – constricted cocoon

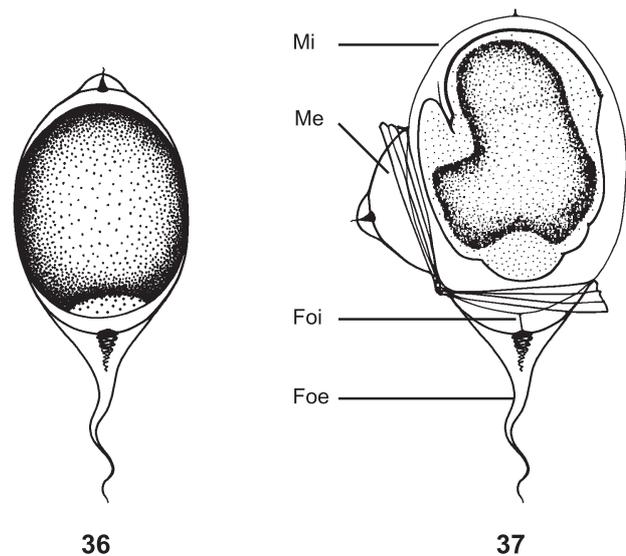
measurements were strongly correlated with the number of contained eggs: Pearson's correlation: cocoon length/number of eggs  $r=0.83$ , cocoon diameter/number of eggs  $r=0.83$ , cocoon length/cocoon diameter  $r=0.68$ ,  $n=2,658$ .

#### EGG STRUCTURE

The egg was surrounded by two colourless, translucent envelopes, strongly adhering to each other (Figs 36–37). The rather stiff external envelope had a varied thickness – near the egg equator it was very thin, and became increasingly thicker towards each pole. The thin and elastic internal envelope most often became visible only during the embryonic development: phases of “larva” and “snail”. The yolk-rich oocyte was covered by a very thin vitelline membrane. The yolk contained in the oocyte was usually yellow, but a slight admixture of “green” blue-green algae (e.g. *Oscillatoria*) in the food resulted in yellow-green or green colour. The oocyte was usually located centrally and touched the envelopes near the egg equator. Its length was 66.7–93.7% length of the egg chamber (mean 86%,  $SD=3.5\%$ ,  $n=573$ ). At the anterior pole of the egg there was usually a distinct, conical thickening which passed into the egg cord; the posterior pole bore a slight tubercle. Compared to *V. cristata*, the eggs were somewhat larger and had more marked poles, but the shape variation was similar (cf. MYZYK 2002: Figs 52–67).

Since there was no lower limit to the egg size, eggs with oocyte diameter of 0.12 mm (sporadically observed cleavage) were regarded as the smallest.

Smaller, egg-like accumulations of yolk were regarded as variations of the egg cord. The measurements of the largest eggs were: chamber length 0.67 mm (constricted eggs even up to 0.78 mm), chamber diameter 0.32 mm. Large or constricted eggs usually contained two oocytes. Juvenile snails hatched only from eggs containing a single oocyte of 0.22–0.35 mm length and 0.20–0.27 mm diameter. The remaining eggs (e.g. smaller or larger, containing two oocytes, con-



Figs 36–37. Egg structure: 36 – newly laid egg; 37 – egg after disruption of external membrane (first day of “larva” stage): Foe – egg cord extending from external membrane, Foi – egg cord extending from internal membrane, Me – egg external membrane, Mi – egg internal membrane

Table 2. Length and diameter of cocoons (containing no eggs – all measured, remaining – only single-capsule cocoons)

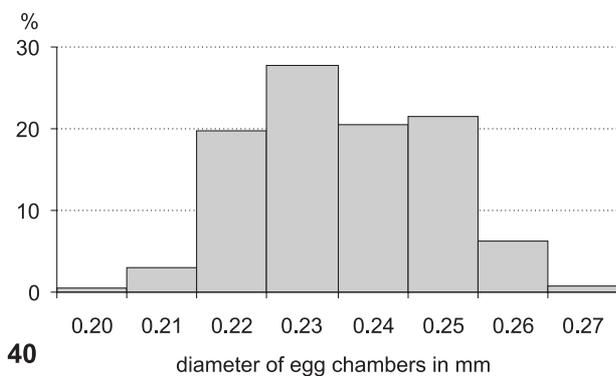
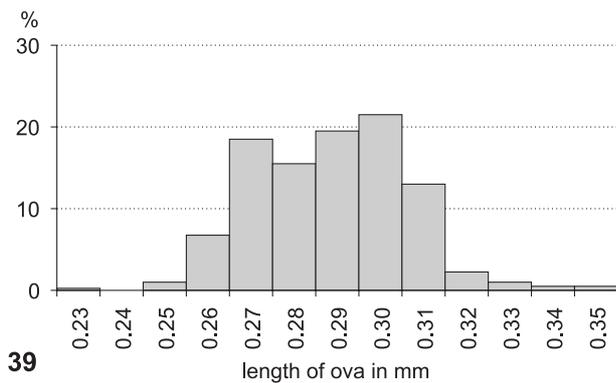
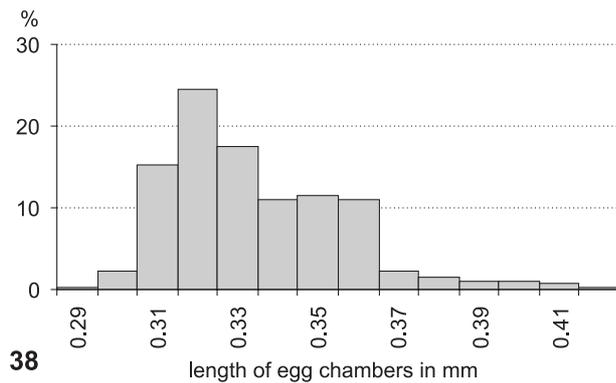
Number of eggs in cocoon	Number of measured cocoons	Length of cocoons in mm				Diameter of cocoons in mm			
		mean	SD	minimum	maximum	mean	SD	minimum	maximum
0	36	1.35	1.01	0.50	4.60	0.70	0.25	0.25	1.30
1	220	0.51	0.12	0.30	1.25	0.58	0.16	0.25	1.00
2	177	0.60	0.10	0.40	1.05	0.67	0.12	0.30	1.00
3	167	0.68	0.10	0.40	1.20	0.68	0.10	0.40	1.10
4	181	0.73	0.11	0.50	1.45	0.70	0.09	0.55	1.15
5	164	0.79	0.11	0.60	1.35	0.73	0.09	0.50	0.95
6	178	0.88	0.14	0.65	1.45	0.77	0.09	0.55	1.10
7	205	0.92	0.12	0.70	1.50	0.81	0.08	0.60	1.10
8	195	0.95	0.13	0.75	1.55	0.83	0.09	0.65	1.25
9	218	1.00	0.15	0.70	1.70	0.87	0.09	0.65	1.30
10	187	1.03	0.13	0.75	1.40	0.90	0.09	0.70	1.15
11	122	1.08	0.19	0.80	2.00	0.91	0.09	0.70	1.35
12	123	1.07	0.11	0.80	1.55	0.94	0.08	0.70	1.15
13	110	1.12	0.16	0.85	2.05	0.99	0.10	0.75	1.25
14	91	1.15	0.14	0.95	1.65	1.04	0.10	0.80	1.30
15	65	1.15	0.13	0.90	1.60	1.06	0.08	0.90	1.20
16	68	1.22	0.19	0.95	2.35	1.09	0.11	0.85	1.40
17	45	1.22	0.15	1.00	1.70	1.10	0.10	0.80	1.40
18	47	1.27	0.16	1.00	1.80	1.11	0.08	0.90	1.25
19	31	1.33	0.23	1.05	2.30	1.12	0.12	0.85	1.30
20	20	1.36	0.45	1.10	3.20	1.14	0.07	1.05	1.30
21	16	1.34	0.14	1.15	1.65	1.19	0.11	1.05	1.50
22	8	1.33	0.09	1.20	1.45	1.18	0.11	1.00	1.35
23	4	1.37	0.06	1.30	1.45	1.19	0.04	1.15	1.25
24	10	1.38	0.07	1.25	1.50	1.19	0.11	1.00	1.40
25	1	1.35	–	1.35	1.35	1.20	–	1.20	1.20
26	–	–	–	–	–	–	–	–	–
27	2	1.85	0.25	1.60	2.10	1.12	0.07	1.05	1.20
28	1	1.80	–	1.80	1.80	1.15	–	1.15	1.15
29	1	1.95	–	1.95	1.95	1.10	–	1.10	1.10
–	–	–	–	–	–	–	–	–	–
34	1	1.85	–	1.85	1.85	1.10	–	1.10	1.10

stricted or with damaged envelopes) were regarded as abnormal. About 1.1% eggs deposited by snails kept in pairs/groups were abnormal. In the worst food conditions the mean proportion of abnormal eggs was 3.9%, in the best conditions in large containers – 0.5%. On an average 45.5% (usually 38–71%) eggs deposited by snails kept singly were abnormal.

The mean measurements of eggs with single oocytes were (n=573): egg chamber length 0.337 mm (SD=0.023 mm), oocyte length 0.290 mm (SD=0.018 mm), egg chamber diameter 0.235 (SD=0.014 mm), the correlation coefficients (Pearson) were (n=573): egg chamber length/chamber diameter r=0.40,

oocyte length/egg chamber diameter r=0.46, egg chamber length/oocyte length r=0.80. Variation of measurements of eggs with single oocytes is presented in Figs 38–40. The mean size of eggs deposited by different pairs of snails differed at most by ca. 12% but showed no correlation with the parent shell size.

The egg cord extending from the external envelope (Foe) near the anterior pole was often rather thick, with another cord extending from the internal envelope visible inside (Foi) (Figs 36–37). Sometimes the cords contained chambers of different size and contents (empty, with single yolk granules, entirely filled with yolk or with small lumps of yolk sur-



Figs 38–40. Size variation of eggs containing one oocyte (n=573)

rounded by vitelline membrane). The egg cord (Foe), located between the base of the cocoon and the first egg, and between the eggs, was often spirally twisted (dextral). Also the cord inside it (Foi) sometimes formed a tight dextral spiral. In “larva” and “snail” phases (Fig. 37) a short section of the cord (Foi) often connected the external and internal egg envelopes. The observed variation of the egg cord was even wider than in *V. cristata* (MYZYK 2002: Figs 41–42) because of its larger diameter. Sometimes the cord diameter in *V. macrostoma* was so large, that whole eggs could be contained in it.

#### EMBRYONIC DEVELOPMENT

Immediately after cocoon deposition, the oocytes were most often at stage I. In some cocoons, built of

2–3 connected capsules (Figs 22, 24, 30, 33), oocytes were at different development stages in different capsules. In an extreme case, presented in Fig. 22, when the cocoon was found, in the capsule on the left the oocyte was already divided twice, while in the capsule on the right the oocytes were still at stage II. Smaller differences between oocytes located near the base and the apex could sometimes be observed in elongated cocoons which contained numerous eggs. Falling out of the gelatinous substances did not affect the development of eggs. The absence of or too early damage to any egg envelope caused death of the embryo.

The total number of incubated eggs was 4,716; they originated from snails kept in pairs/groups. At temperatures of 26–27°C hatching took place between the 5th and the 9th day of incubation (usually on the 6th and 7th day), at temperatures of 7–8°C as late as between the 54th and 75th day of incubation (most often between the 61st and 65th day). The duration of embryonic development showed a strong negative correlation with the mean incubation temperature (Pearson’s  $r=-0.83$ ,  $n=3,044$ ). The dependence between the mean duration of embryonic development and the mean incubation temperature is presented in Fig. 41. For temperatures of 13, 18 and 26°C also individual variation in duration of embryonic development is shown (Fig. 42).

The course of embryonic development resembled that described for *V. cristata* (MYZYK 2002: Figs 43–51). In “egg” phase the development took place inside the slightly elongated egg chamber. At the end of this phase the embryo head was located next to one pole, the larval shell close to the other, and the whole embryo rotated slowly clockwise around the long axis of the egg. After breaking of the external envelope (“larva” phase), the internal envelope gradually increased its size (to 0.50–0.65 mm diameter just before hatching). In this phase shell growth allowed for formation of a small pallial cavity, eyes, heart beat and pulsations of the back became visible, but the embryo body was still not retracted into the shell (Fig. 37). In “snail” phase the shell covered the whole back, and the embryo body could fit into it when contracted. Further development resulted in formation of stomach, hind gut and the pallial branch of kidney. Gill bud (from a small tubercle to an elongated, finger-like process), a short pallial tentacle and radula were visible on the last day before hatching. Single scattered grains of brown pigment were often visible on the head and foot. The mean relative duration of particular stages of embryonic development was (n=3,044): “egg” – 50.7% (SD=5.5%), “larva” – 16.0% (SD=5.5%), “snail” – 33.3% (SD=6.2%). Since the capsule usually broke as late as “snail” stage, in tightly filled cocoons some embryonic shells were more or less deformed between 0.37 and 0.50 whorl. The moment of disruption of the capsule was often marked on the shells as a radial stria. In large cocoons with

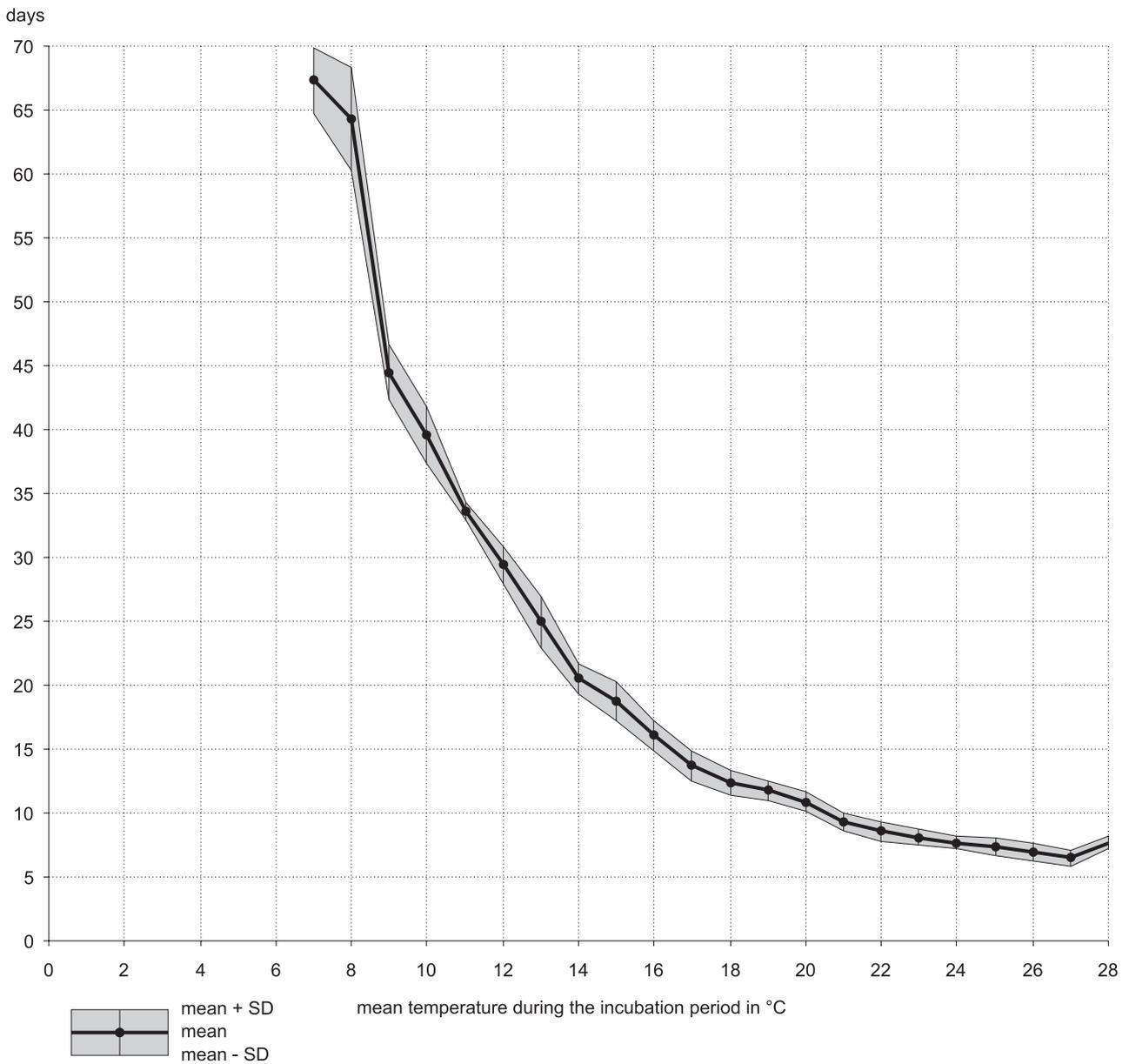


Fig. 41. Dependence between the mean temperature and the mean duration of development

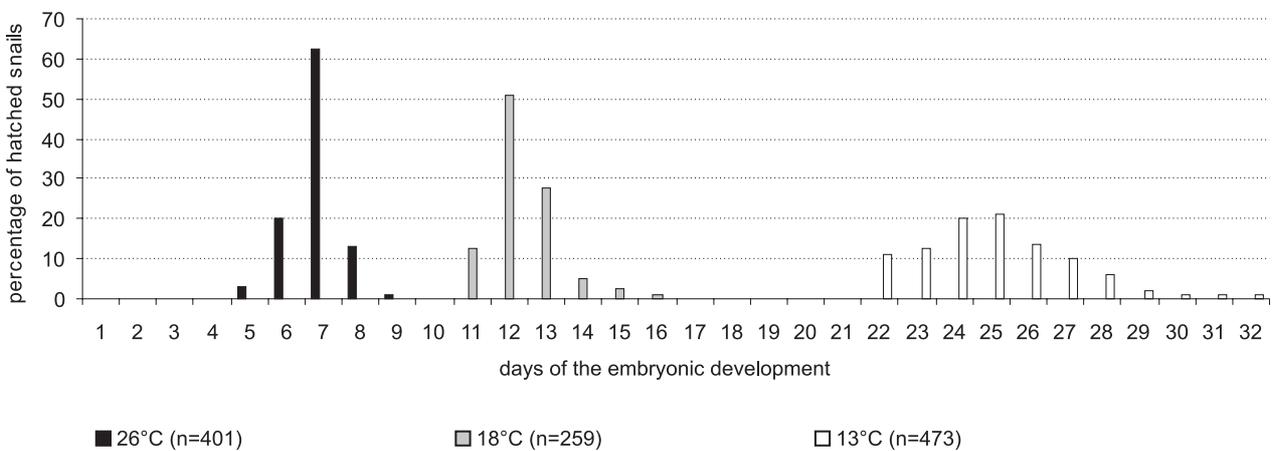


Fig. 42. Variation in incubation time at constant temperature (13°C, 18°C and 26°C) – percentage of snails hatched on consecutive days

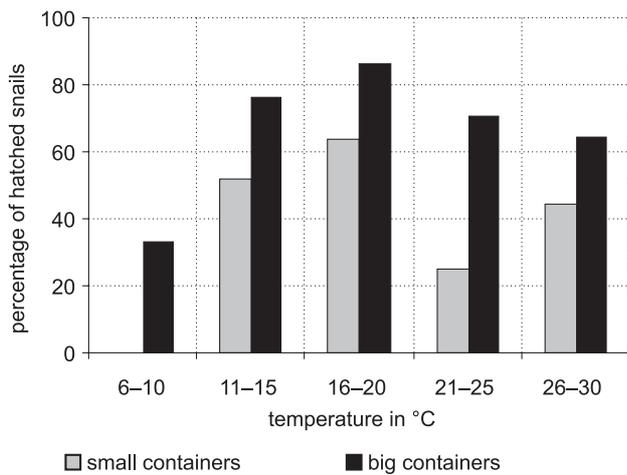


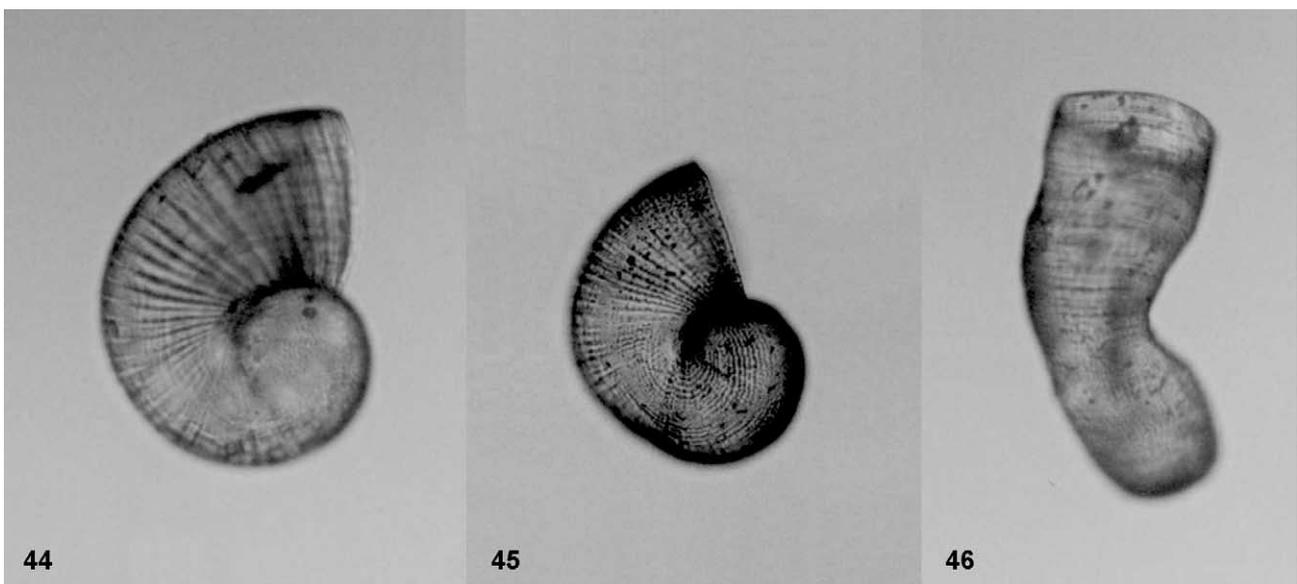
Fig. 43. Variation in the percentage of hatched snails (with normal shell) depending on the mean incubation temperature and origin of eggs

few eggs the capsules sometimes remained unbroken even after hatching, and the imprisoned snails died after the yolk reserves were exhausted.

The percentage of hatching snails (without osmotic disturbances and with normal shells) varied, depending on the incubation temperature and origin of eggs (Fig. 43). It reached the highest mean value (86%) during incubation at 16–20°C of eggs deposited in large containers. During incubation below 10°C the percentage of snails with osmotic disturbances increased, as well as the proportion of premature hatchings with poorly developed internal organs. The percentage of embryos which died at “larva” and “snail” stages was 31.4% (at higher temperatures 7.1–16.9%), and the percentage of hatched snails with small shell not covering the body, or with osmotic disturbances – 12.5% (at higher temperatures 3.2–6.4%). However,

even at temperatures close to 0°C initial divisions of the oocyte occurred, though they took a long time. For example, at ca. 2°C the first division was completed only 7–8 days from cocoon deposition, while at ca. 19°C – after 7–7.5 hours (consecutive divisions were ca. 3 times faster). Relatively high incubation temperature (mean above 27°C) also had a negative effect on the embryonic development (higher mortality of embryos at “egg” stage, a slightly higher frequency of developmental anomalies). However, a short-lasting increase in temperature even up to 32–34°C did not cause death of the embryos. When prior to cocoon deposition adult snails spent a longer time at a rather high temperature (over 25°C), some embryos often died at “egg” stage, while the remaining ones showed more or less pronounced osmotic disturbances (irrespective of incubation temperature).

The embryonic shell was usually 0.62–0.87 whorl (rarely less, from ca. 0.37 whorl, or more, up to ca. 1 whorl) (Figs 44–48). The shell surface close to apex was covered by spiral striae, and near the aperture – by radial striae. The spiral striae were composed of rows of granular thickenings separated by narrow strips of nearly smooth surface. About 0.25 whorl on the outer margin (later most often covered by the next whorl) the width of spiral striae was 0.006–0.010 mm, and toward the inner margin they were slightly larger, which is compatible with BINDER’s (1967) data. The size of the area covered by spiral striae varied individually rather widely. In most cases they were well visible till ca. 0.37–0.50 whorl, but on some shells they started to disappear already at ca. 0.12–0.25 whorl. Rarely the spiral striae reached beyond the initial 0.50 whorl, and sporadically covered all the embryonic shell, near the aperture intersecting with radial striae.



Figs 44–46. Light microscope, embryonic shells, 88x: 44 – correctly developed shell, 45 – slightly deformed shell, 46 – uncoiled, tubular shell

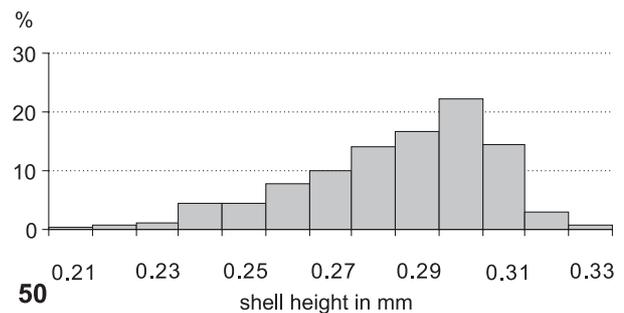
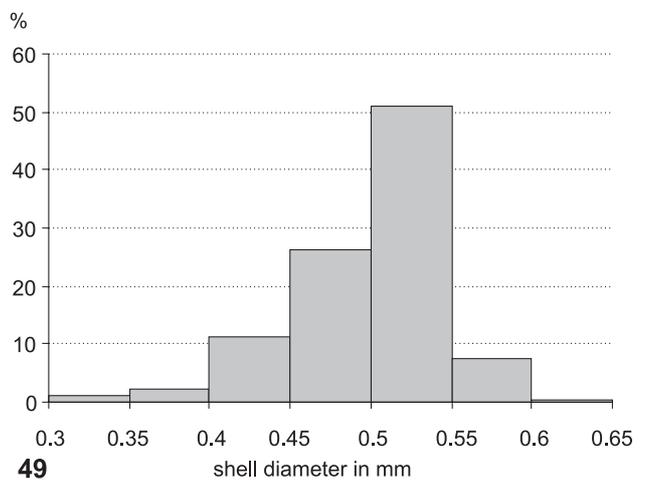
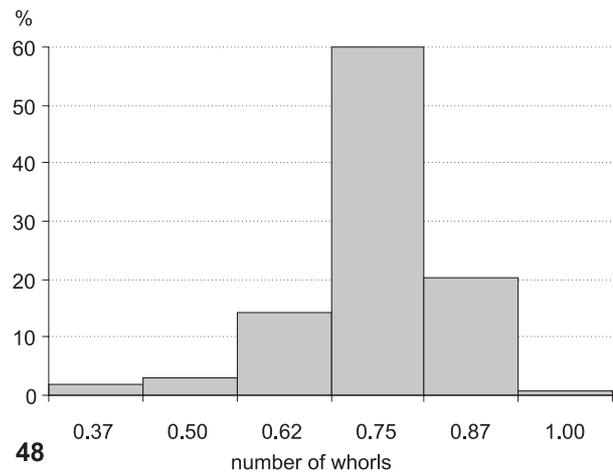


Fig. 47. Light microscope, shell of a young snail, 88x. The border of embryonic shell indicated with arrow

Sometimes the area covered by the spiral striae ended with a distinct radial stria, but more often the spiral striae disappeared gradually till the surface became almost completely smooth. The apex of the shell, ca. 0.10–0.12 mm in diameter (later covered by younger whorls), was covered by irregular granules, on the margins passing into spiral striae. Very rarely, instead of spiral striae, only irregular granularities were visible on the entire shell surface. Narrow and irregular radial striae appeared rather often already starting from 0.25–0.37 whorl (rarely 0.12 whorl). From ca. 0.62 whorl the radial striae were nearly always strongly marked and fairly regular (Figs 44, 45, 47).

Besides typical shells, with adhering whorls, there were some which were scalariform to various degree (fissure between whorls) and, sporadically, not coiled (Fig. 46). The mean size of typical embryonic shells was (n=444): diameter 0.492 mm (SD=0.047 mm), height 0.286 mm (SD=0.023 mm), number of whorls 0.74 (SD=0.10). Variation of embryonic shell size is presented in Figs 48–50, Pearson’s correlation coefficients were (n=444): diameter/height  $r=0.74$ , diameter/number of whorls  $r=0.82$ , height/number of whorls  $r=0.58$ . Though the smallest shell was 0.30 mm in diameter and had ca. 0.37 whorl, the contracted body of normally developed snails rarely fit into a shell of a diameter below 0.37 mm and 0.50 whorl. Sometimes during the embryonic development a portion of yolk was released from the body which resulted in a smaller snail size (including shell). The largest embryonic shells were 0.60 mm in diameter and had ca. 1 whorl. The initial 0.5 whorl was colourless, further increments were most often light brown or yellowish.

The upper margin of the aperture was always slightly produced relative to the lower and sometimes slightly descending relative to the apex (difficult to



Figs 48–50. Size variation of embryonic shells (n=444)

measure in live snails). Thus the maximum height of the embryonic whorl was adopted as the shell height. The height of the whorl was most often the greatest near the aperture, less often at ca. 0.37–0.50 whorl, and then decreased somewhat. A normal operculum of the embryonic shell had usually 2.00–2.75 whorls and was 0.21–0.33 mm in diameter, corresponding to the aperture size. The beginning of the first whorl of the operculum was 0.04–0.06 mm wide, but further increments sometimes appeared narrower (0.02–0.03 mm) since they partly overlapped.



Though all the eggs deposited by snails kept singly were incubated, no juveniles hatched from them. In most of such eggs no development was observed, and only rarely (4.7% eggs) a few initial divisions took place.

#### POST-EMBRYONIC DEVELOPMENT AND MATURATION

Soon after breaking the internal envelope the juvenile snails left the gelatinous substance and the cocoon. Nearly all newly hatched juveniles still had small quantities of yolk which most often disappeared within 3–7 days. When the snails were little active, single yolk granules remained in their bodies for a much longer time, sporadically even up to 3 weeks after hatching.

According to FALNIOWSKI (1989a, b, 1990) the embryonic shell is clearly delimited from later increments which have a completely different sculpture. However, the laboratory observations show that the initial ca. 0.50 whorl with more or less clear spiral striae in nearly all the hatched snails (95.3%) constituted only a part of the embryonic shell. The radial striae which were present near the aperture in most embryonic shells (0.62–1 whorl) were identical with those found on post-embryonic increments; consequently the border of the embryonic shell was only rarely visible (e.g. damaged aperture edge – Fig. 47).

Among the snails kept in the laboratory, 14.6% died within 30 days from hatching, and the total proportion of snails which died at juvenile stages was 19%. The longest-lived (240 days) juvenile snail had a shell 2.30 mm in diameter and 2.37 whorl when it died.

Some juvenile snails grew very fast, other individuals (even in the same container) showed a much slower growth. Thirty days from hatching the diameter of the body whorl was 0.67–2.02 mm, after 60 days – 0.92–3.00 mm, after 90 days – 1.43–3.65 mm, and the growth continued, very often till the end of lifetime.

Attainment of maturity usually did not affect the growth rate to any greater extent. This is in disagreement with the opinion of PIECHOCKI (1979) and FALNIOWSKI (1989a) who report that in the laboratory *V. macrostoma* reaches its maximum size in ca. 3 months.

Penis, as a small colourless tubercle at the base of the right cephalic tentacle, appeared on an average 31 days from hatching (SD=10, n=56, range 19–60). Roughly at the same time the gonad became visible (a lighter spot at ca. 0.1–0.2 whorl) and outlines of the pallial parts of the female reproductive organs. When penis appeared, the shell (n=56) was 1.13–1.55 mm in diameter (mean 1.33 mm, SD=0.09 mm) and the number of whorls was 1.62–1.87 (mean 1.78, SD=0.09) (Figs 51–53).

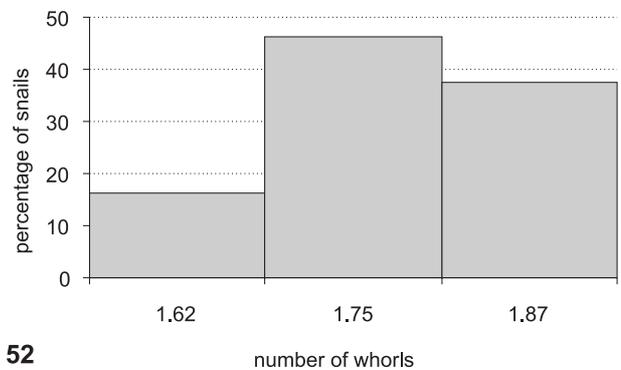
Further growth of reproductive organs (e.g. gonad, penis) was rather fast. Examples: 30th day after

hatching – shell 1.33 mm/1.75 whorl – gonad 0.12 whorl, penis – tubercle; 40th day – shell 1.70 mm/2 whorls – gonad 0.37 whorl; 60th day – shell 2.20 mm/2.37 whorls – gonad 1 whorl; 80th day – shell 2.55 mm/2.62 whorls – gonad 1.50 whorl; 92nd day – shell 2.75 mm/2.75 whorls – gonad 1.75 whorl, in the gonad 2 yolk-containing oocytes. In adult snails the gonad occupied all the whorls except for the body whorl (sometimes slightly more or less, within 0.12 whorl).

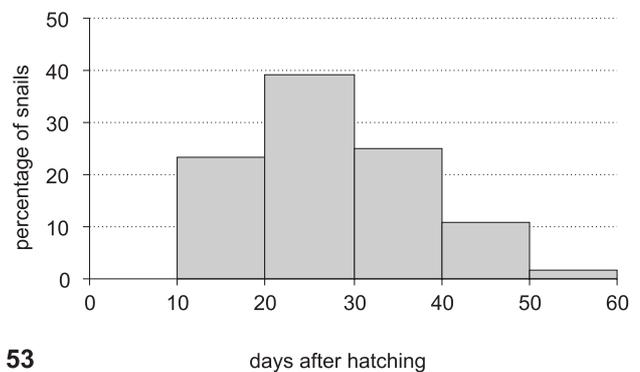
The snails reached their male maturity somewhat earlier than the female maturity, but the moment they attained male maturity could be identified only approximately in live snails. From the first copula-



51



52



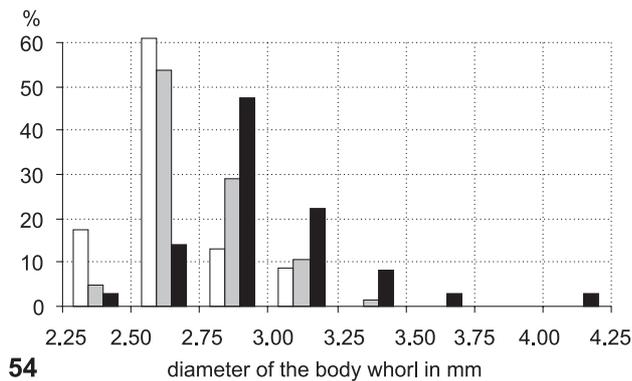
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Figs 51–53. Variation in shell size and the time of appearance of penis

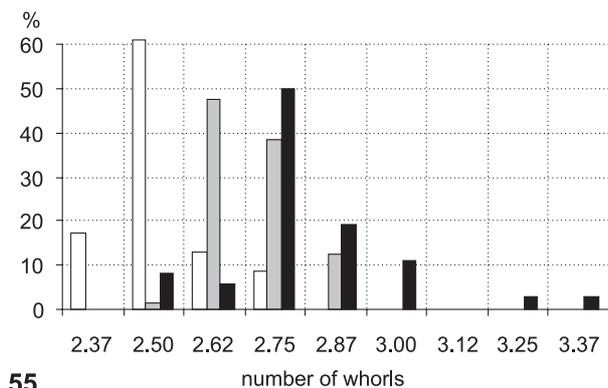
tion as “male”, till the appearance of the first vitellogenic oocytes in the gonad (female maturity), the shell diameter increased mostly by ca. 0.10–0.15 mm (ca. 0.12 whorl). Examples: I. The smallest snail copulating as “male” had a shell of 2.35 mm/2.50 whorl, and attained female maturity after 20 days, at shell diameter 2.50 mm/2.62 whorls; II. Two snails of shells 2.70 mm/2.62 whorls, which copulated: the “male” reached female maturity after 30 days at shell diameter 2.80 mm/2.75 whorls, the “female”

reached female maturity after 16 days at 2.85 mm/2.75 whorls.

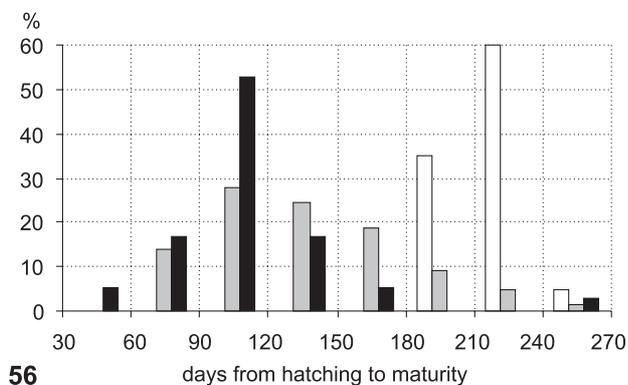
Female maturity was usually attained at shell diameter of 2.40–3.30 mm (rarely less or more, within 2.30–4.10 mm) and 2.37–3.00 whorls (sporadically more, up to 3.37 whorls) (Figs 54–55). In the worst food conditions (snails from Łomża) the shell size at the time of appearance of the first yolk-containing oocytes in the gonad was: mean diameter 2.62 mm (SD=0.17, range 2.30–3.00), mean number of whorls 2.52 (SD=0.10, n=23, range 2.37–2.75). Snails hatched in the laboratory in 1995 reached female maturity at shell size: mean diameter 2.73 mm (SD=0.19, range 2.45–3.25), mean number of whorls 2.70 (SD=0.09, n=65, range 2.50–2.87), whereas those hatched in 1996–1998 – at mean shell diameter 2.95 mm (SD=0.32, range 2.40–4.10) and mean number of whorls 2.81 (SD=0.17, n=28, range 2.50–3.37). The time between hatching and female maturity was the longest in the worst conditions, and for snails collected in the wild it was approximately 7–9 months. Snails hatched in the laboratory in 1995 reached maturity on an average after 136 days (SD=42, n=65, range 68–251), and those hatched in 1996–1998 on an average after 108 days (SD=36, n=28, range 60–250) (Fig. 56).



54



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56

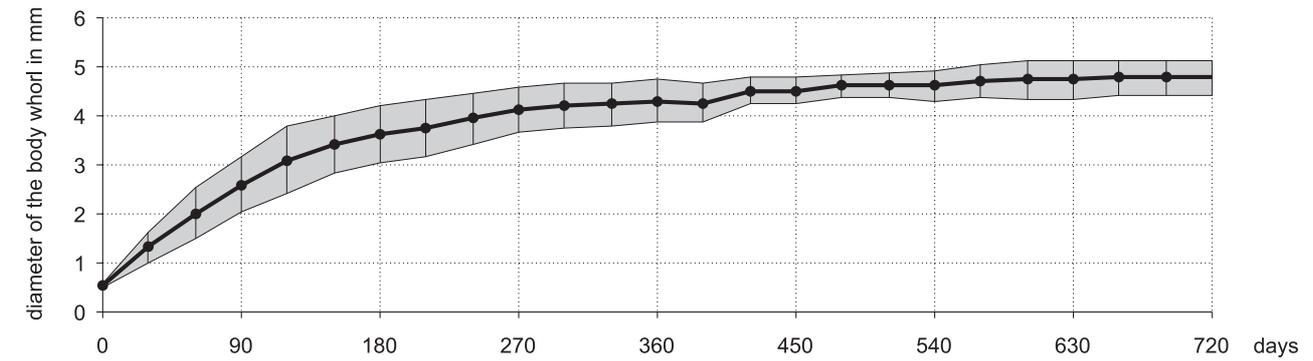
□ 1994    ■ 1995    ■ 1996–1998

Figs 54–56. Variation in shell size and the time of female maturation depending on food conditions – initially the worst, then gradually improving

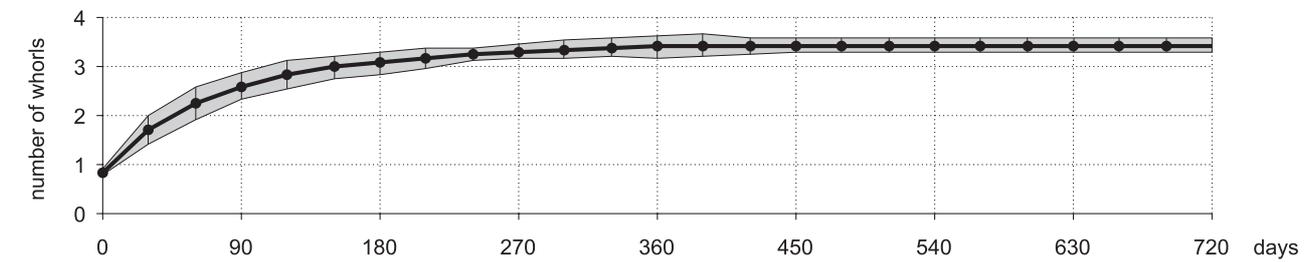
#### ADULT GROWTH AND MORTALITY

Shell growth continued in sexually mature snails and usually lasted till the end of life, though it became increasingly slower. In both small and large containers mean growth curves were roughly parabolic (Figs 57–60), but in large containers snails reached larger size. Examples of individual variation in growth curves in small and large containers are shown in Figs 61–63. Transfer of adult snails from small to large containers was followed by a period of intense growth and the shells reached a size similar to that typical of snails kept from the start in large containers (Fig. 62). In most snails a slow shell growth was observed even during intense reproduction. In small containers (unfavourable food conditions) snails kept singly reached a slightly larger average shell size, compared to those kept in pairs/groups (diameter by 11.5%, number of whorls by 0.5% larger). In large containers snails kept singly and those kept in pairs/groups reached a similar mean shell size.

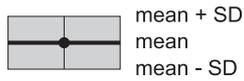
Examples of shell structure variation are presented in Figs 64–74. Shells of adult snails ranged in shape from conical (tapered apex and regularly increasing whorls) to flattened (apex flat or slightly convex), with a descending wide body whorl. The spire height was 21.1–56.8% shell height, and in the single flat shell the apex was depressed (Fig. 72–73). The aperture was most often oval (long axis positioned horizontally, vertically or obliquely in the plane of suture), less often circular or with a poorly marked angle near



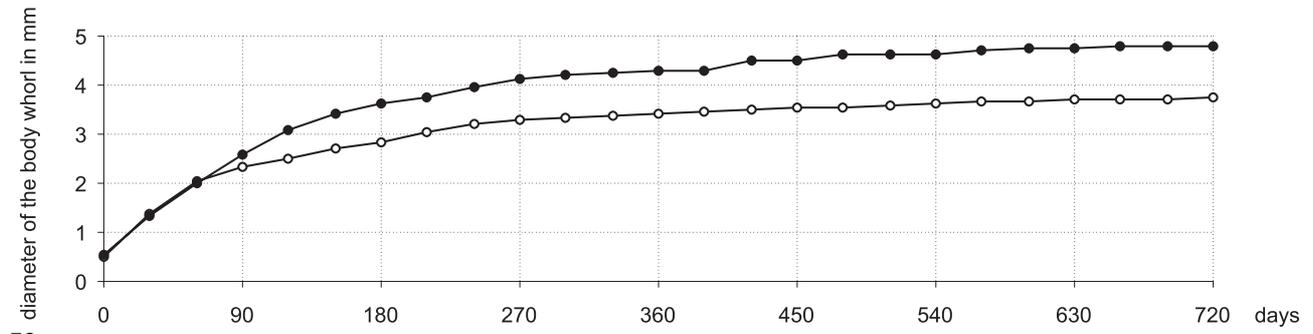
57



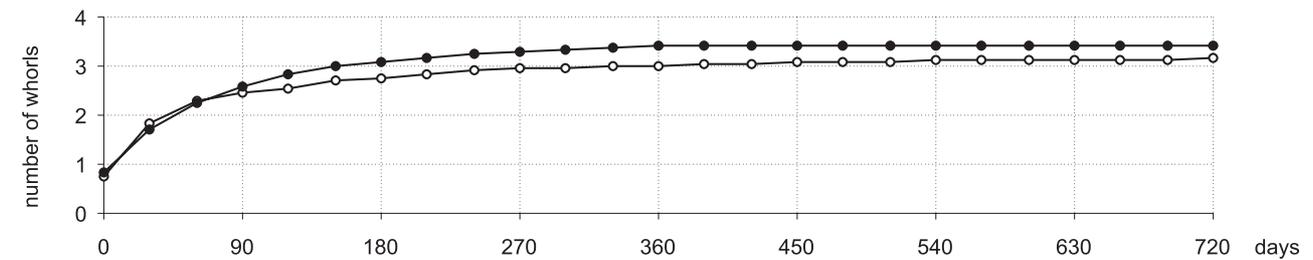
58



Figs 57–58. Mean growth curves for the body whorl diameter (57) and number of whorls (58) – snails kept in big containers (favourable food conditions)



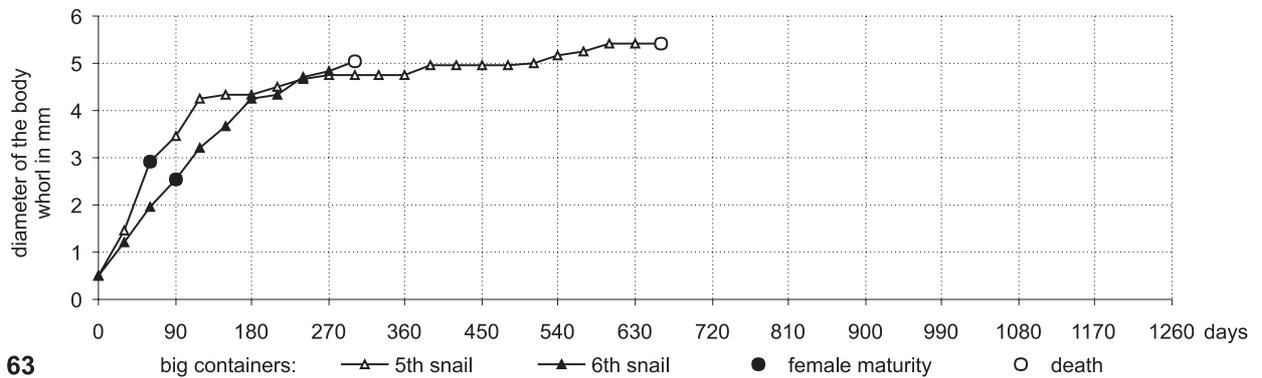
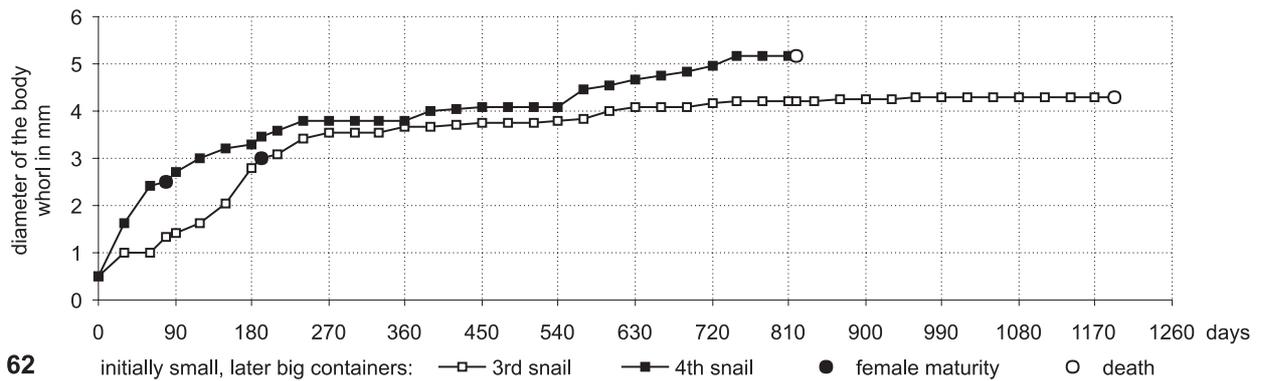
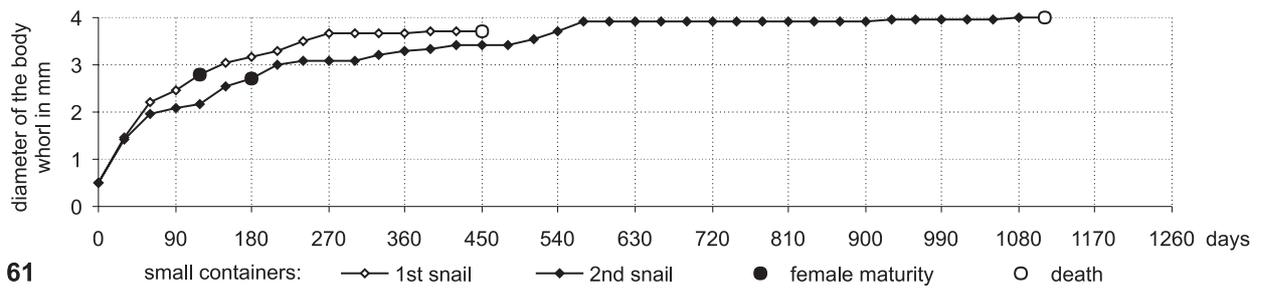
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60



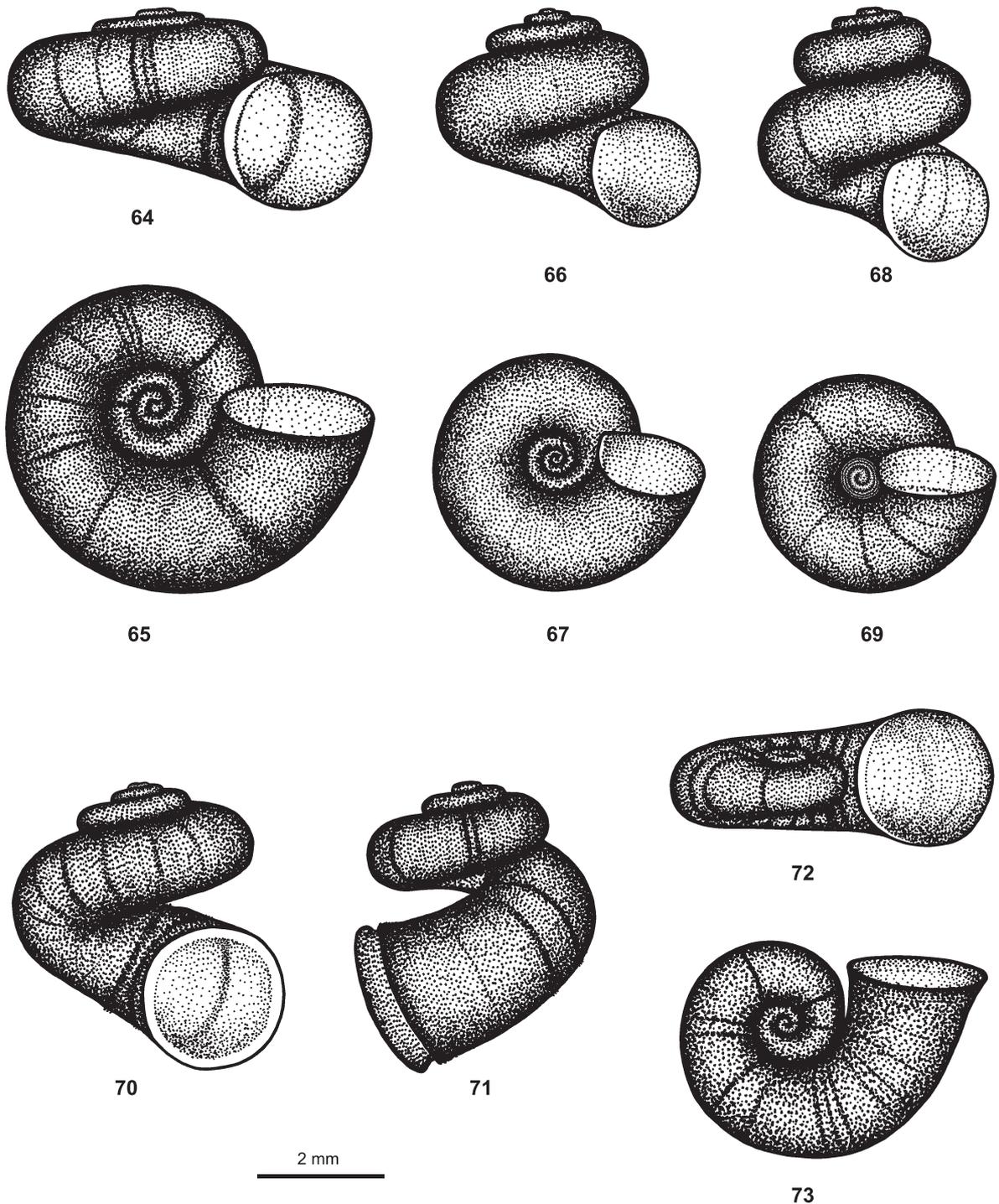
Figs 59–60. Comparison of mean growth curves of snails kept in small and big containers (different food conditions)



Figs 61–63. Examples of individual growth curves in different food condition

the suture. In older snails (2, 3 years old) sometimes the aperture margin was slightly reflexed, or inside the aperture there was a distinct thickening parallel to the margin. In some shells the terminal part of the body whorl was detached from the penultimate (Figs. 70–73), or sporadically there was a fissure between the earlier whorls. The upper margin of the aperture was always slightly produced relative to the lower margin. The umbilicus constituted usually 25–32% shell diameter (rarely less, minimum ca. 19%) and all the earlier whorls could be seen in it (Figs 65, 67, 69, 73). In some shells it was partly hidden by the body whorl. Shells of snails kept in small containers bore traces of rather regular periods of intense growth after new leaves were supplied (Fig. 74), in the form of wide light stripes, and periods of inhibited growth (dark striae or stripes). In large containers the quantity of food was usually sufficient and the dark stripes or

bands were rare and irregular. During fast growth the shell surface was smooth or covered by weak, irregular radial striae. Sometimes small smooth fragments of the shell surface bore traces of very weak spiral striae. Moderate growth rate was marked on the shells as rather regularly arranged radial striae or delicate ribs. At the end of the growth period there usually appeared ridge-like growth lines (dark striae or stripes). They were densely arranged and had a shape of thin ridges positioned perpendicular to the shell surface – the long time of food assimilation resulted in a reflexed aperture margin, while at the same time the whorl increment was small. Wide dark stripes composed of numerous ridge-like growth lines (especially frequent on the body whorl) arose also in periods of intense reproduction. The shells were most often creamy, less often milky-white or yellow-brown.



Figs 64–73. Examples of shell variation: 64–65 – a typical shell; 66–69 – shells with the highest spire; 70–71 – a shell with the body whorl detached from the penultimate; 72–73 – a flat shell; 68–69, 72–73 – extremes of shell variation in the laboratory

Shells of snails kept in small containers (n=71) reached a diameter of 2.81–4.48 mm (mean 3.65, SD=0.30) and were composed of 2.62–3.62 whorls (mean 3.09, SD=0.17). In large containers (n=48) the maximum diameter was 3.27–5.82 mm (mean 4.46,

SD=0.49 mm) and the maximum number of whorls 2.87–4.00 (mean 3.46, SD=0.22) (Figs 75, 76). Pearson's correlation coefficient shell diameter/number of whorls was  $r=0.66$ ,  $n=119$ .

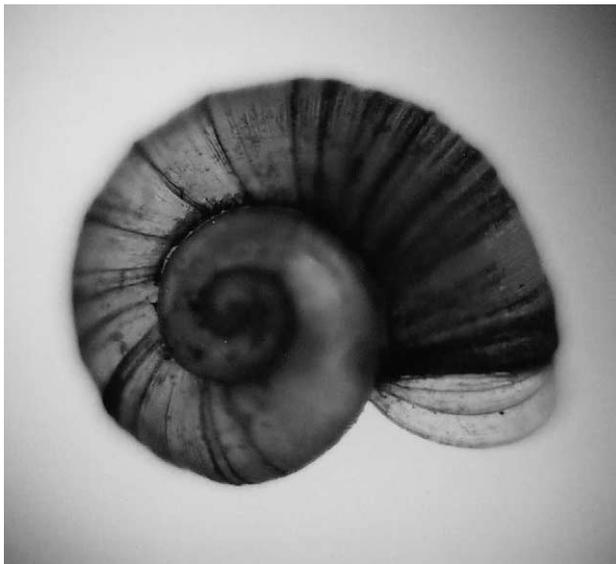
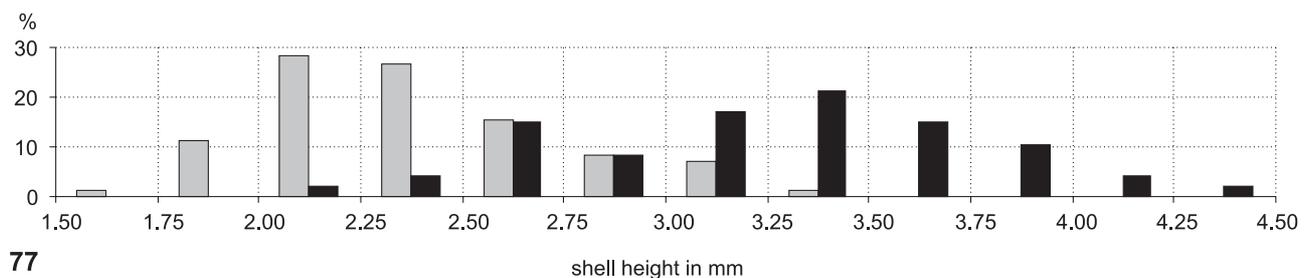
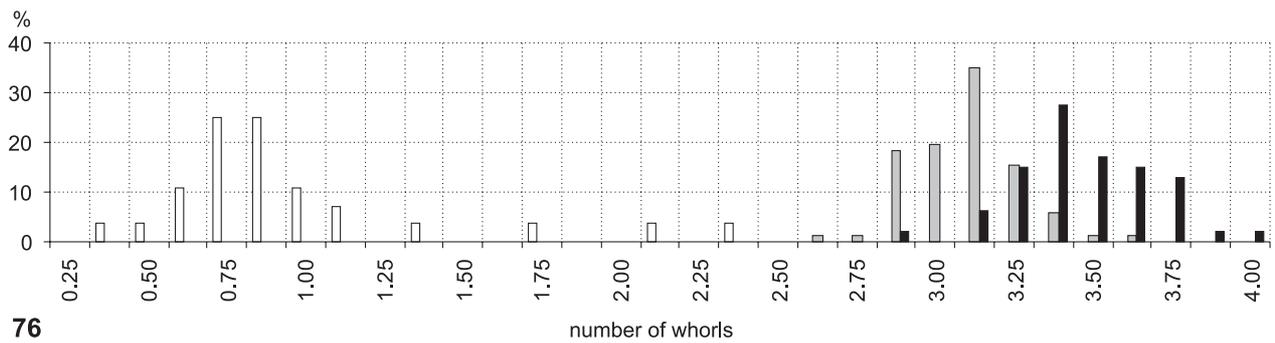
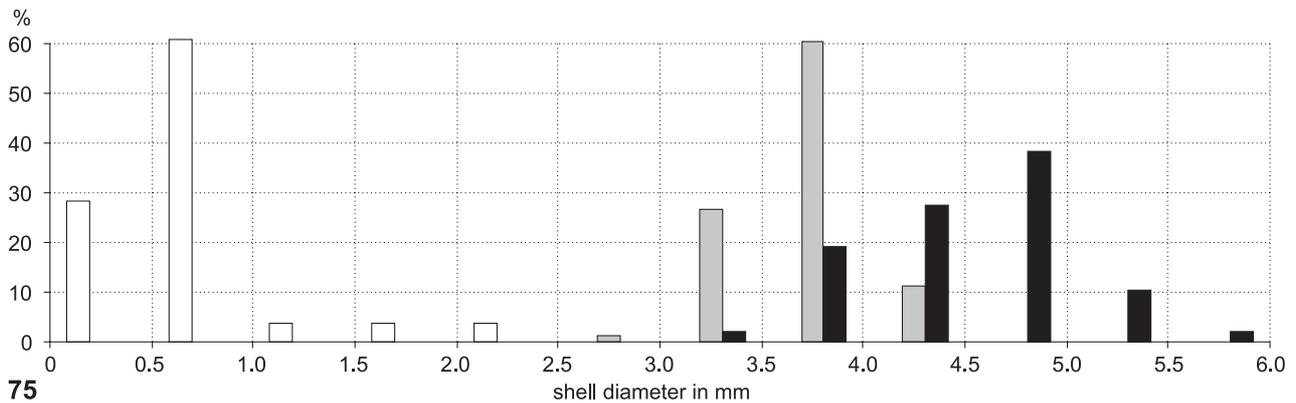


Fig. 74. Light microscope, shell of trematode-infected snail kept in a small container, 20×

The shell height depended on the position of particular whorls and on the maximum height of the body whorl. Shell of snails kept in small containers reached a height of 1.56–3.38 mm (mean 2.38, SD=0.36, n=71), in large containers 2.18–4.44 mm (mean 3.26, SD=0.49, n=48) (Fig. 77). Pearson's correlation coefficients were: shell height/diameter  $r=0.74$ , shell height/number of whorls  $r=0.91$ ,  $n=119$ . The shell height/diameter ratio ranged from 0.437 (Fig. 72) to 1.069 (Fig. 68), the mean being 0.685 (SD=0.103,  $n=119$ ) (Fig. 78). In small containers shells were on an average more flattened than in large containers (height/diameter ratio 0.650 and 0.737, respectively). The aperture height was strongly positively correlated with the shell diameter (Pearson's  $r=0.90$ ) and number of whorls ( $r=0.71$ ,  $n=119$ ). The mean ratio aperture diameter/shell diameter was 0.414 (SD=0.026,  $n=119$ , range 0.325–0.499) and showed no dependence with the shell size (diameter or number of whorls) (Fig. 79). The observed variabil-



□ dead as juveniles      □ adult – kept in the small containers      ■ adult – kept in the big containers

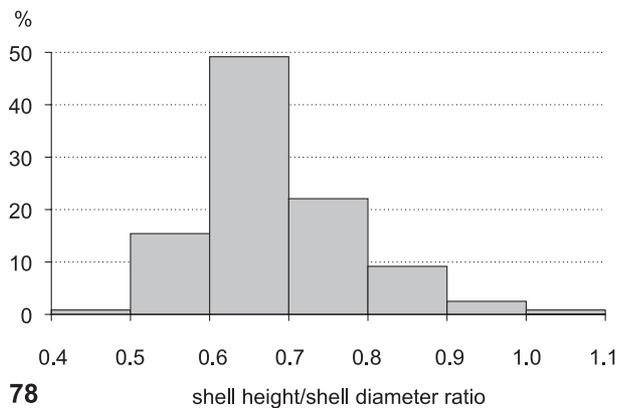
Figs 75–77. Variation in shell size reached in the laboratory



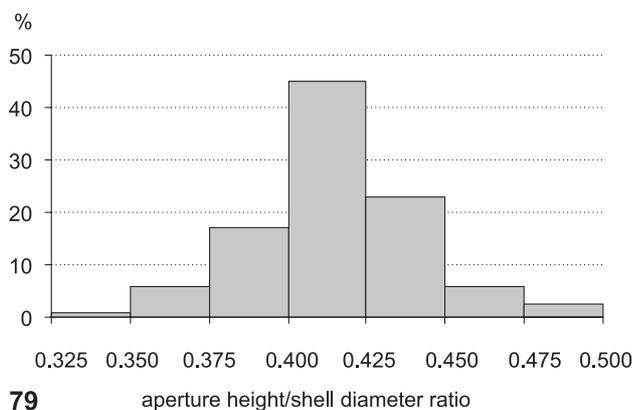
ity range of particular shell characters from the laboratory culture was slightly wider than that given by PIECHOCKI (1979) and FALNIOWSKI (1989a).

The opercula were relatively thin (often with damaged margins) and usually slightly oval (rarely circular) (Figs 80, 81). The central part of the operculum was always somewhat concave, and the mean apical angle was  $136.3^\circ$  ( $SD=5.8^\circ$ ,  $n=96$ ). The spiral whorls of the operculum were sinistral. On most whorls dense spiral striae were visible (Fig. 82), and growth inhibitions were marked as oblique lines across the whorl. At an operculum diameter of ca. 2 mm the width of the last whorl (sometimes also of the earlier whorls) was 0.10–0.20 mm, and the maximum was 0.24 mm. The actual width of increments was even greater (up to 0.29 mm), since usually 25–30% width overlapped the previous whorl. The partial overlap of the whorls could compensate for any damage to the thin operculum edge, and make it possible to adjust the operculum size to the aperture size. Nearly always the operculum size was smaller than that of the aperture (very rarely equal). The largest opercula in the laboratory were: I.  $2.18 \times 2.05$  mm, II.  $2.14 \times 2.09$  mm. The number of opercular whorls was ca. 3 times larger than that of shell whorls (usually 2.6–3.6 times

depending on shell structure). Most opercula of 1.8–2.1 mm diameter were composed of 10–11 whorls, and the maximum observed was 12 (at 3.62

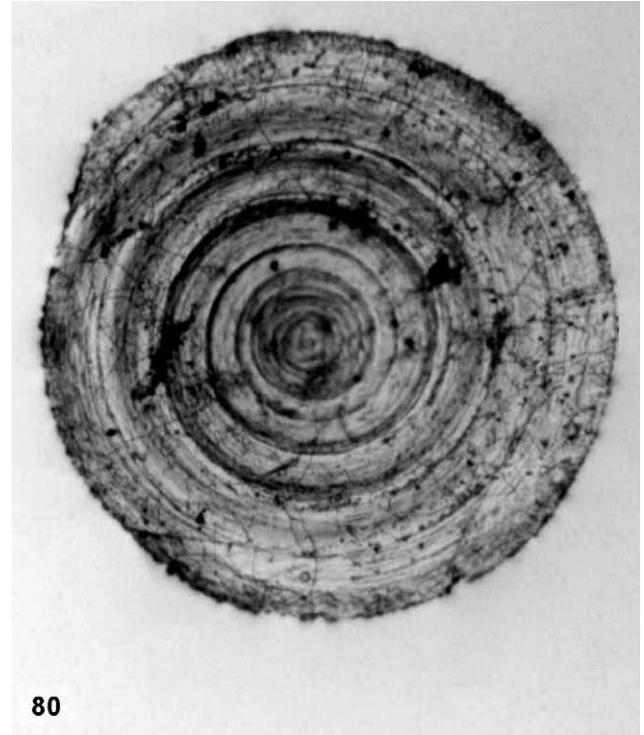


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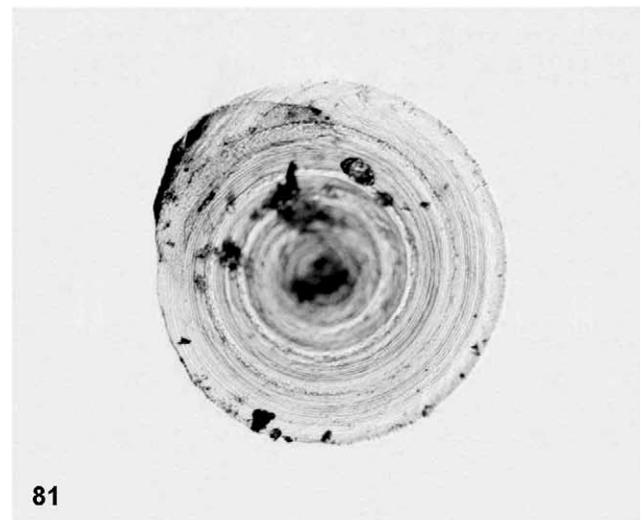


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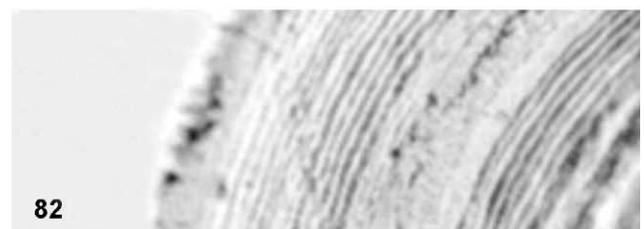
Figs 78–79. Variation in shell parameters: 78 – shell height/diameter ratio; 79 – aperture height/shell diameter ratio



80



81



82

Figs 80–82. Light microscope: 80 – operculum of adult snail, 44 $\times$ ; 81 – operculum of young snail, 44 $\times$ ; 82 – a fragment of operculum ultimate and penultimate whorls, 200 $\times$

shell whorls). The central part of the operculum was usually yellow-brown, the last whorl was almost colourless.

Joining in pairs of snails of similar shell structure caused gradual fixation of some characters, e.g. relative height of the spire. In extreme cases the shells reached a height larger than the diameter (Fig. 68) and had a very narrow umbilicus, or were planispiral (Fig. 72).

Mortality of adult snails showed distinct seasonal fluctuations (Table 3). In June and July 56% snails died, though the culture contained snails hatched in various months (from February till November). The high mortality in those months involved both snails aged one year and those aged two or three. Mass mortality in June and July was reported also by PIECHOCKI (1979) and FALNIOWSKI (1989a).

The life span of snails kept in pairs/groups and singly did not differ significantly (Fig. 83, Table 4). However, food conditions had a clear effect on the life span (Fig. 84). In unfavourable conditions (1995) the mean life span was 706 days (268–1,192). Rough estimates for the snails collected in the wild (1994) showed similar values (mean 787, range 240–1,200). In the worst food conditions (1994) each year ca. 1/3 snails kept in the culture died, and the mortality was higher for some age classes (“stairs” in the graph). The mean life span of snails hatched in 1996 was 561 days (275–817). In the most favourable conditions (1997–1998) the mean life span was 383 days (150–498) and all the snails died within a short period after the intense reproduction in spring. According to PIECHOCKI (1979) and FALNIOWSKI (1989a) *V. macrostoma* is an annual species, which in the laboratory was observed only in very favourable food conditions.

## DEVELOPMENTAL ANOMALIES

Some developmental anomalies appeared already at the “larva” and “snail” stages, e.g. osmotic disturbances causing swelling of entire body or only some organs (head, foot, mantle) and underdevelopment of shell or operculum (often combined with osmotic disturbances). Very rarely slight osmotic disturbances receded and the growth continued normally. Sporadically shell or operculum separated from the body. Most embryos with such anomalies died when still inside the egg envelopes, but some of them hatched. Examples of hatched snails with developmental anomalies: I. mantle swollen, head and foot normal, shell diameter 0.58 mm, height 0.42 mm, 0.37 whorl, operculum diameter 0.26 mm, II. whole body swollen, shell diameter 0.45 mm, height 0.42 mm, 0.25 whorl, no operculum, III. whole body strongly swollen, shell diameter 0.43 mm, height 0.47, aperture kidney-shaped, operculum diameter 0.10 mm, IV. body normal, shell diameter 0.54 mm, height 0.32 mm, operculum diameter 0.19 mm, V. body normal, mantle retracted backward, shell absent, operculum diameter 0.15 mm. Other developmental anomalies became apparent only after hatching, e.g. those of alimentary tract: no connection between oesophagus and stomach or stomach and intestine. Most snails with such anomalies died within a few days after hatching, but some survived even up to 20 days, and small postembryonic increments appeared on their shells.

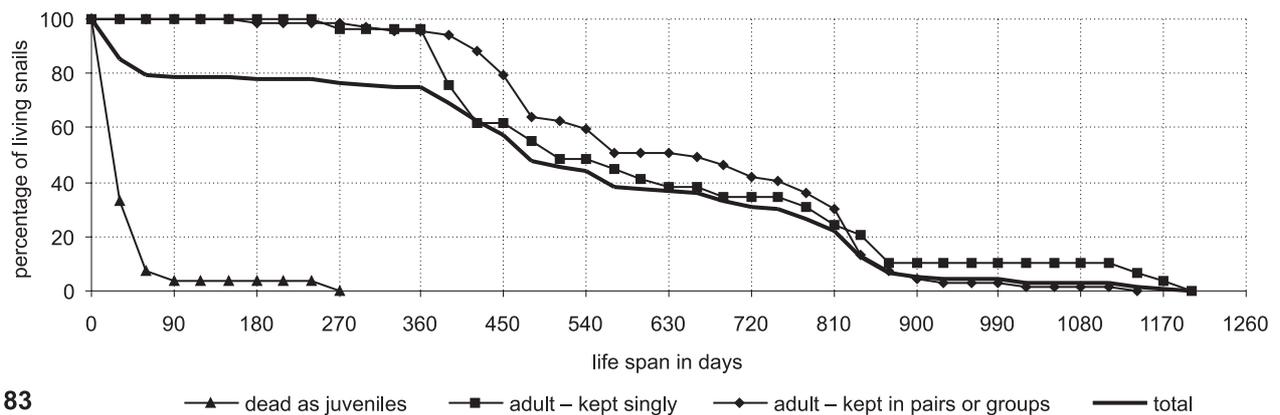
Shells of juvenile snails sometimes got damaged during observations. When the damage was located near the aperture, e.g. broken edge, it was rather quickly repaired. Damage to earlier whorls (e.g. apex) nearly always resulted in osmotic disturbances and death of the snail.

Table 3. Mortality of adult snails in laboratory; 0 – year of hatching; 1, 2, 3 – consecutive years

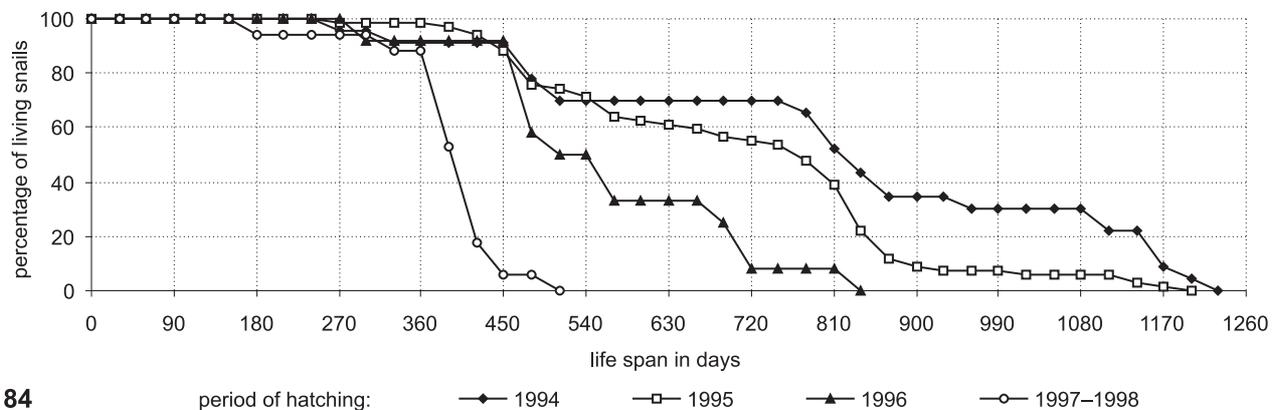
Years	Month of death												Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
0	–	–	–	–	–	–	–	–	–	1	–	1	2
1	–	1	1	1	2	19	14	6	4	3	–	2	53
2	–	2	3	2	5	6	19	3	5	2	–	1	48
3	–	1	–	1	3	5	2	–	1	–	–	–	13
Σ	–	4	4	4	10	30	35	9	10	6	–	4	116

Table 4. Mean life span in days; variable food conditions – initially the worst and then gradually improving; 1994 – rough estimate

Year of hatching	Kept in pairs/groups			Kept singly			Total		
	n	mean	SD	n	mean	SD	n	mean	SD
1994	19	747	295	4	975	196	23	787	293
1995	49	690	188	16	757	241	65	706	205
1996	10	567	150	1	498	–	11	561	144
1997–1998	8	377	98	9	388	13	17	383	68



83



84

Figs 83–84. Mortality of snails in the laboratory: 83 – all snails hatched in laboratory; 84 – only adult snails, variation in the mortality curves depending on food condition (initially the worst, then gradually improving)

Sometimes the shell of a young snail separated from the body. Within a few days (mostly 3–4) osmotic disturbances developed and the snail died.

Abnormal development of reproductive organs was rare. In three snails with normally developed gonad and female organs, the penis was very short (length to 0.20 mm) and distorted (only female?). Two of them, kept singly, deposited cocoons. One was initially kept singly (after copulation) and then joined with two other individuals. All the snails with underdeveloped penis originated from the same parents.

In another three snails the gonads were abnormally developed (only male). The gonad growth was rather slow and the organ did not reach the normal size (e.g. at 3.25 shell whorls the gonad occupied only the initial 1.37 whorl). No yolk-containing oocytes appeared, and after death only scattered yolk granules were found. Though the penis and the pallial part of female organs reached their normal size, the snails only rarely copulated and did it only during a short period after maturation (disregarded in the analyses of reproduction). These, only-male, snails originated from different pairs of parents.

Two hermaphroditic snails (kept singly) during their lifetime produced only single yolk-containing oocytes (I. shell diameter 3.60 mm – 1 oocyte, II. shell

diameter 4.75 mm – 2 oocytes). Their reproductive organs, both male and female, were normally developed.

#### PREDATORS AND PARASITES

In the laboratory culture incubating eggs, developing embryos and even adult snails were attacked by parasitic fungi. In polluted containers this led within a short time to all eggs or whole young snails being overgrown by the hyphae. Adult snails infected with the fungus lived for a short time, but with the growth of hyphae their bodies remained increasingly extended and before death could not fit into the shell.

Four snails in the second year of their life were infected with trematode larvae brought with the food. The parasites developed in their gonads and remained there till the death of the host. No infection of other snails in the same container was observed. Because of the rather late infection the effect of the parasite on reproduction was slight. Though production of new vitellogenic oocytes was inhibited, those already formed were placed in cocoons and deposited. The mean life span of the infected snails was similar to that of uninfected individuals. A permanent trace of the presence of parasites was atypical shell in-



crement near the aperture (Fig. 74). The infection caused first growth inhibition and a small decrease in the whorl diameter, and later increments were much greater on the dorsal side and relatively thin.

Another problem was dipteran larvae brought often with food. When left in the containers, they damaged cocoon capsules and consumed eggs.

#### FOOD AND FEEDING

During feeding the snails moved vigorously and scraped the substratum with their radulae. No filter-feeding was observed, and the pallial cavity almost always remained clean (sporadically containing faeces or periodically Ciliata). Trial and error method made it possible to establish that the best food in the laboratory were leaves of some trees covered by a fairly thick periphyton layer, collected in places of abundant occurrence of *V. cristata* (e.g. lake shores, oxbows). The largest shell increments and the highest numbers of deposited eggs were observed in snails fed with leaves of black alder *Alnus glutinosa* and oak *Quercus* sp., slightly smaller with poplar *Populus tremula* and birch *Betula* sp. Compared to *V. cristata*, the food spectrum was more diverse and the quantity consumed much greater. Feeding took place both in the night and in the daytime. However, the presence of regular ribs on the shells indicates periods of feeding and rest alternating every dozen or so hours, since in 10 days the snails usually produced 12–18 ribs. Most snails stopped feeding when

the temperature decreased to 6–7°C or increased to 27–28°C.

Faeces of *V. macrostoma* were short cylinders with a clear longitudinal groove (C-like in cross-section). Rarely they were not divided into short sections, but formed spirals of variable length. Like in *V. cristata*, live organisms, e.g. diatoms, were found in the faeces.

#### OTHER INFORMATION

In the absence of adequate food, young snails (juvenile and adult) often crawled above the water table and closed their shells with opercula. A longer period above water, of several days, often caused death through desiccation even at a very high humidity (tightly closed containers). Some adult snails crawled above the water table to deposit cocoons.

The maximum speed of crawling was ca. 0.5 mm s<sup>-1</sup> (1.8 m hr<sup>-1</sup>).

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