LIFE CYCLE OF *VALVATA CRISTATA* O. F. MÜLLER, 1774 (GASTROPODA: HETEROBRANCHIA) IN THE LABORATORY

STANISŁAW MYZYK

Sąpolno 14, 77-320 Przechlewo, Poland

ABSTRACT: Laboratory studies in 1991–2001, supplemented with observations in natural habitat, made it possible to establish the following characteristics of the life cycle of *Valvata cristata* O. F. Müll.: female maturity at shell diameter of 2.05–2.95 mm, number of whorls 2.375–3, 55–533 days from hatching, depending mainly on food conditions. Mean life span in good food conditions 377 days, in bad food conditions 896 days (maximum 1,170 days). Snails kept in pairs deposited a mean number of 51 cocoons per lifetime (maximum 105), with a total of 134 eggs (maximum 353); the number of eggs per cocoon ranged from 0 to 10, rarely more than 16 (mean 2.6). Depending on life span there were 1–3 reproductive seasons. Of 20 adult snails kept singly, only one laid eggs which hatched. The mean maximum shell size for snails kept in pairs: 3.28 mm, 3.125 whorls, for snails kept singly: 3.93 mm, 3.375 whorls (maximum 4.70 mm, 3.750 whorls). Snails with shells of strongly descending whorls were almost always infected with trematode larvae located in their gonads. Snails infected when young never reached female maturity.

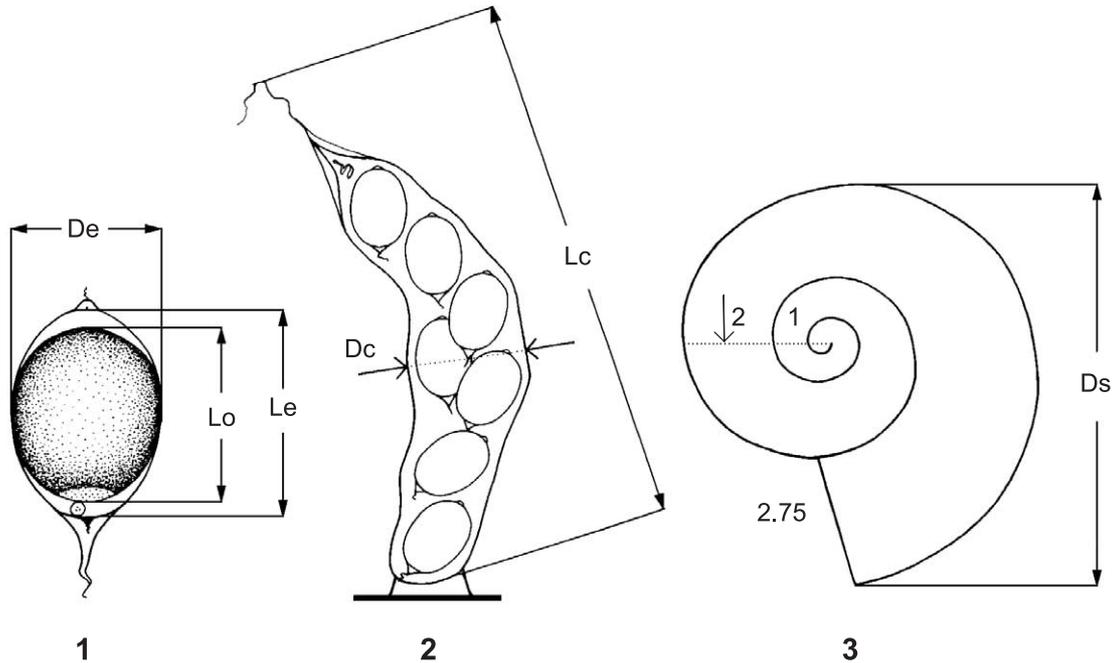KEY WORDS: Gastropoda, Heterobranchia, *Valvata*, life cycle, reproduction

INTRODUCTION

Six members of the genus *Valvata* live in Europe: *cristata* O. F. Müller, 1774, *pulchella* Studer, 1820, *piscinalis* (O. F. Müller, 1774), *naticina* Menke, 1845, *macrostoma* (Mörch, 1864) and *sibirica* (Middendorff, 1851). *Valvata* snails are hermaphroditic. *V. cristata* is the smallest, its shell diameter rarely exceeding 3.5 mm; the shell is usually coiled in one plane. In spite of its wide distribution in Europe and northern Asia, published information on the biology of the species is very scanty (FRETTER & GRAHAM 1962, PIECHOCKI 1979, FALNIOWSKI 1989a); recent papers deal with shell structure and internal anatomy (FALNIOWSKI 1989b, 1990) as well as some biological problems related to systematics (RATH 1986, 1988). Laboratory culture and observations in natural habitats provided new information on the biology of the species, not always confirming the literature data.

MATERIAL AND METHODS

Adult snails were collected in the vicinity of Sąpolno (NE Poland) (lakes, oxbows of the Brda River, drainage ditches) from April 1991 till July 2001, and kept in the laboratory for some time (further called temporary culture) in order to obtain cocoons and observe behaviour. Because of small size of the snails and cocoons, the possibility of observations in the field was very much limited.

The permanent culture included a total of 133 snails hatched in laboratory and eight snails collected as young in the lake Sosnowe. It was started in March 1992 and concluded in July 1998. Fifty nine snails died before reaching maturity, the remaining were kept in pairs (36) or in groups of three specimens (21), or singly without copulation (23, 3 of which reached male maturity only and were excluded from the analyses) or

Figs 1–3. Measurements: 1 – Egg: De – egg chamber diameter, Le – egg chamber length, Lo – egg cell length; 2 – Cocoon: Dc – maximum cocoon diameter in the plane of sutures, Lc – cocoon length in natural position (without the layer of cementing substance); 3 – Shell: Ds – shell diameter, 1, 2, 2.75 – number of shell whorls

after copulation (2, they were later counted together with those kept in pairs/groups). When one snail in a pair died, the other was joined in a new pair or group in the next reproductive season, or placed temporarily with other snails for copulation.

The snails were kept in small closed containers (diameter 25 mm, height 40 mm) filled with water to the level of 15–20 mm; only two of the snails kept singly were placed in larger containers (diameter 60 mm, height 60 mm) filled with water 40 mm deep. Fragments of leaves collected at margins of water bodies were added as food – initially these were randomly collected leaves of various trees and aquatic plants, later mainly of black alder (*Alnus glutinosa*) and oak (*Quercus* sp.), from places where the species was abundant. Initially, the leaves were replaced at irregular intervals of a few weeks, then they were supplied with increasing frequency and in larger quantities. At the end of the experiment food was supplied regularly every 10 days, and the total surface of leaves in each container was ca. 40 cm^2.

The temperature in the culture varied seasonally – from May to September it was close to the temperature of lake littoral i.e. 15–25°C, in the remaining months it was usually 8–18°C. Sporadically, the temperature decreased below 5°C (winter) or increased above 27°C (summer).

Measurements of eggs, cocoons and shells are illustrated in Figs 1–3. The measurements were taken with a calibrated eye-piece to the nearest 0.01 mm (range 0.1–1 mm) or 0.05 mm (range 2.3–5 mm). Whorls were counted with the accuracy of 1/8 [=0.125].

In order to facilitate the description of embryonic development, the whole development period was divided into three stages: "egg" – cf. Figs 43–47, "larva" – cf. Figs 48, 49 and "snail" – cf. Figs 50, 51.

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

RESULTS AND DISCUSSION

COPULATION

The smallest copulating snails had shells of 2.05 mm diameter, with 2.250 whorls. Some of the copulating individuals were still immature. During the breeding season copulation was repeated, being especially frequent at the beginning of the season (March to May). Sporadic copulation took place also in winter if the temperature exceeded 11°C.

Though *Valvata* is hermaphroditic, during copulation partners played roles of different sexes. The snail initiating the copulation always played a male part while the "female" most often kept feeding. After climbing the "female's" shell, the "male" placed its foot next to the partner's aperture margin, and tilted its head strongly to the left (right tentacle almost touching the "female's" shell). The basal (pigmented) part of penis was relatively stiff, while the apical part (milky white) made worm-like movements in various directions and gradually entered the partner's mantle cavity. During the penis insertion the "female" was most often feeding, from time to time violently contracting and relaxing its body. The initial phase lasted from slightly over ten minutes to over 1 hour, then the "female" contracted its head and remained motionless during 5–10 minutes; sometimes slight vibrations of the bodies of both partners could be observed. Then the "female" relaxed its body and resumed feeding while the "male" remained on its shell for another 25–75 minutes. Having retracted its penis, the "male" climbed down from the partner's shell and started to feed. The total time of copulation ranged from 45 to 155 minutes (mean ca. 90 min.). During the rather long post-copulation phase, the "female" sometimes made shaking-off movements or rasped the "male's" foot with the radula.

Soon after copulation, the snails very often changed their roles and copulated again. Sometimes they changed roles twice and copulated three times in a sequence. Though usually only two snails were involved in copulation (irrespective from their number in the container), copulation of three individuals was also observed, the middle snail being at the same time male and female. In some cases during copulation the "female" tried to insert its penis into the "male's" mantle cavity, but no mutual copulation was observed. When the "male's" position on the partner's shell differed from that described, no copulation took place and usually, after several attempts, the "male" changed its position to the typical one.

Besides the typical behaviour, some individuals protruded their penis when alone, stopped feeding and became indifferent to the presence of other snails. Later some of them were observed to have the penis inserted in their own mantle cavity, on the right side of the contracted pallial tentacle. Besides attempts at or a real autocopulation, such snails copulated also with other individuals.
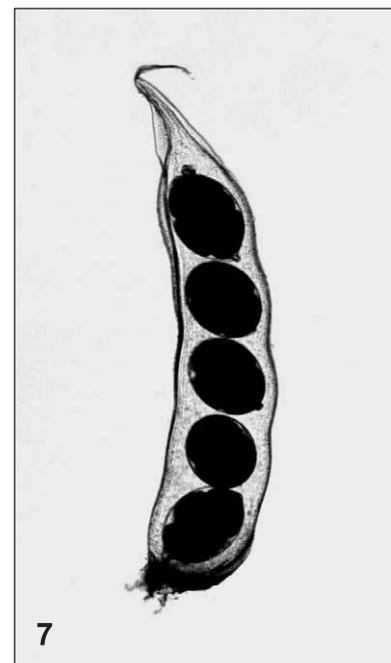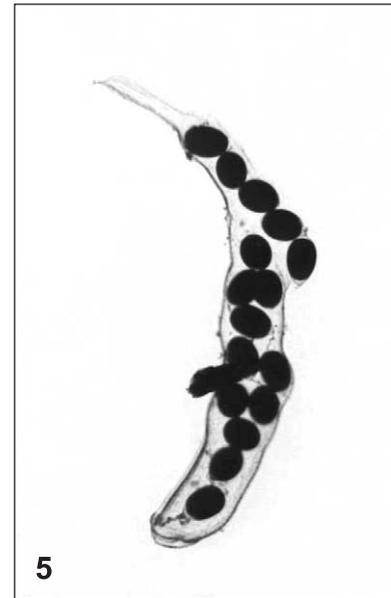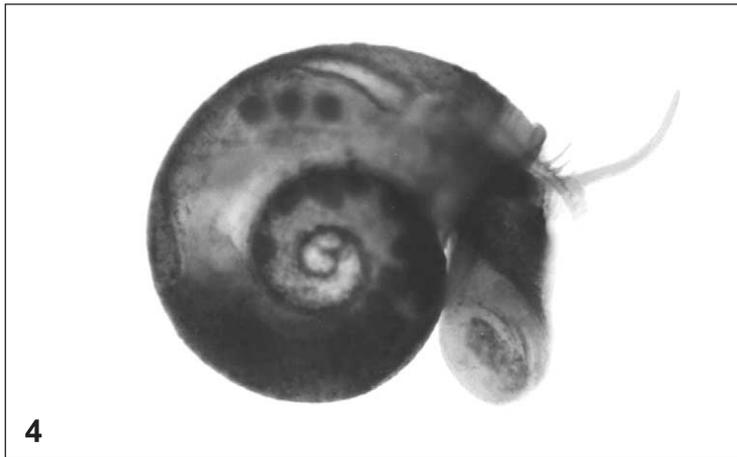
COCOON FORMATION

In mature snails, yolk-containing oocytes were usually well visible through the shell, both in the gonad and during cocoon formation (Figs 4, 8). The oocytes in the gonad were most often spherical or oval, and when more numerous, they had an irregular form. The oocytes leaving the gonad and moving in oviduct

sections I–II (see Fig. 8 for the position of sections of the reproductive system) were strongly elongated, of a length of 0.40–0.80 mm and width 0.05–0.15 mm. The mean time necessary to pass through this oviduct section was 12 min. (7–20). From point II the oocytes started a backward movement, which lasted on an average 8.5 min. (5–12), and gradually changed their shape from sausage-like to ovate. At point III the oocytes approached closely the columellar wall of the whorl, then moved away from it somewhat and proceeded to travel towards the shell aperture. Sometimes their rotation (sinistral) could be observed, and about half length of section III-IV also the external membrane of the eggs could be seen. The maximum number of oocytes observed simultaneously in that section was four. After a mean time of 18 min. (11–28) the eggs entered the shell gland (point IV). During the next 5–22 min. (mean 9) the eggs were poorly visible and one by one moved toward the apical part of the shell gland (point V). Then they became well visible again and moved towards the aperture. At about half length of the shell gland the outline of the cocoon capsule could be observed. The time of movement from point V to the gonopore depended on the position of the egg in the cocoon, being 25–57 min. (mean 38.5) for the first egg, and gradually shorter for each consecutive egg. Sporadically, e.g. during formation of strongly elongated or deformed cocoons, the time spent by the eggs in the shell gland increased even up to 5 hrs. During formation of cocoons with many eggs, consecutive oocytes were usually released from the gonad and appeared in successive points every 4–5 min. Too short an interval between the eggs within the albumen gland resulted in several oocytes being surrounded by a common egg membrane (cf. Figs 42, 56, 62, 66, 67) or being connected by a thick cord. Too long an interval between the eggs in the shell gland caused formation of constricted cocoons (cf. Figs 9, 12).

COCOON DEPOSITION

When the base of the forming cocoon neared the outlet of the shell gland, the snail stopped feeding and searched for a place for cocoon deposition. During ca. 10 min. before deposition it scraped the substratum with its radula. Then it bent the anterior part of its foot to the left, at the same time contracting its mid part, and contracted the head, so that only the apical part of proboscis and tips of cephalic tentacles protruded out of the shell. Some individuals contracted their gills, in other snails the gill was completely protruded. The pallial tentacle was usually straight, in its normal position. Soon after the head contraction, the base of the cocoon with a drop of cementing substance appeared (Fig. 38) while the snail tilted its shell strongly to the right (perpendicular to the substratum) and pressed the cocoon to the sub-

Figs 4–7. Light microscope: 4 – Snail preparing to deposit a cocoon with three eggs, 20 ×; 5 – The largest deposited cocoon, with 16 eggs, 20 ×; 6 – Cocoons deposited by the same snail, 44 ×; 7 – Cocoon deposited by another snail, 44 ×

stratum with its whole body. During the next 1–2 min. it remained motionless (gluing the cocoon to the substratum). Then the shell slowly returned to its normal position, while the apical part of the cocoon was released from the reproductive ducts. Sometimes at this stage the snail moved the right anterior lobe of the foot as if trying to push the cocoon away. During the cocoon deposition the pallial section of the female reproductive organs became much elongated and almost always the gonopore was visible outside the shell. Usually, the total time of cocoon deposition ranged from 6 to 10 min. (sporadically up to 15 min.

or even longer). After the deposition, the snail rested during 1–6 min. and slowly contracted the right side of its body. Then it protruded its head and moved away (to the left) from the cocoon.

Literature data on cocoon deposition include descriptions of fixing them with the foot sole, so that the broader end is glued to the substratum (FALNIOWSKI 1989a) or fixing them vertically to aquatic plants (PIECHOCKI 1979).

Cocoons were deposited in the daytime and in the night, in all the volume of the containers: on the walls, bottom, leaves, on the surface film, sometimes
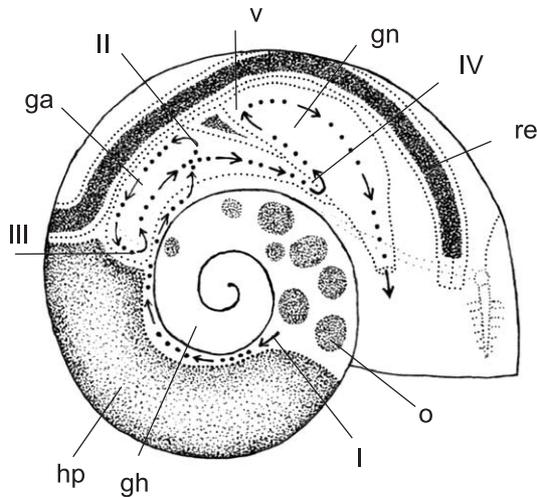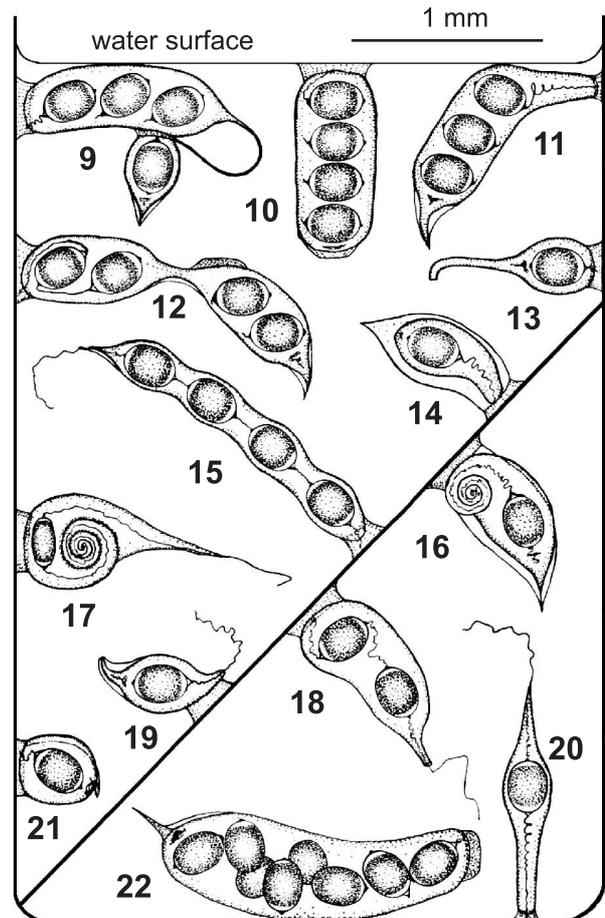
Fig. 8. Cocoon deposition. Arrows and coarse points denote the way of oocytes from the outlet of the gonad, visible through the shell: ga – albumen gland, gh – hermaphrodite gland, gn – shell gland, hp – hepatopancreas, re – rectum, o – yolk-containing oocytes, I, II, III, IV, V – characteristic points on the way of oocyte movement

on shells of other snails or on earlier deposited cocoons (Figs 9–22). The preferred place was a narrow zone (ca. 1 mm wide) just below the water table, both on the walls of the containers and on leaves (9.1% deposited cocoons). A part of the cocoons (6.4%) rested on the bottom and were not fixed. In the field, cocoons were the most numerous just below the water table, on floating leaves, twigs etc.

TIMING AND DURATION OF REPRODUCTION

Snails kept in pairs or groups and maturing from March to August usually started copulating and depositing cocoons within a short time after maturation. The time between female maturation and deposition of the first cocoon in a container was most often 6–79 days (mean 25 days). In three containers the first cocoons were deposited as late as in the second reproductive season after maturation, and the maximum time was 335 days. Snails reaching maturity from September till February deposited their first cocoons after 60–203 days (mean 140 days). The mean time between maturation and the first cocoon for most snails kept in pairs and groups was 113 days (SD = 97 days, n = 24). Snails kept singly deposited the first cocoons not earlier than 96 days from maturation, and often as late as in the second breeding season, even after 528 days. The mean time between maturation and the first cocoon deposition was 253 days (SD = 115 days, n = 15). Besides, three snails deposited no cocoons during their lifetime, though their gonads contained numerous mature oocytes.

Figs 9–22. Examples of variation of shape and fixing of cocoons. Container with an obliquely positioned leaf diagrammatic. Note the cocoon under the surface film (10), cocoon with a spirally coiled base (16), the whole cocoon spirally coiled (17) and unfixed cocoon resting on the bottom (22)

Depending on the life span, cocoons were deposited during one, two or three breeding seasons. The beginning and the end of the breeding season (i.e. deposition of the first and the last cocoon in particular containers in a given year) are presented in Table 1. The differences in the beginning of the first season were associated with reaching maturity during that season, while death of snails resulted in a variation in termination of the second and third seasons. The duration of the breeding seasons in the laboratory is presented in Fig. 23. The mean duration of the season (single depositions excluded) for snails kept in pairs and groups was ca. 140 days, for snails kept singly ca. 80 days.

Snails kept in pairs and groups usually started cocoon deposition in March or April, less often as early as the second half of February, the earliest deposition date being January 26th (2 cocoons with a total of 3 eggs). The last cocoons of the season were most often deposited in August or September, and sporadically

Table 1. Beginning and end of breeding seasons in the laboratory (the numbers given are numbers of containers with co-
coons)

season	month										total
	I	II	III	IV	V	VI	VII	VIII	IX	X	
				first coccon laid in the season							
				snails kept in pairs or groups							
1st	–	2	5	8	3	1	2	3	–	–	24
2nd	1	3	4	7	1	–	1	–	–	–	17
3rd	–	2	1	2	–	–	–	–	–	–	5
total	1	7	10	17	4	1	3	3	–	–	46
				snails kept singly							
1st	–	–	–	1	1	1	4	4	–	–	11
2nd	–	1	–	3	2	4	–		–	–	10
total	–	1	–	4	3	5	4	4	–	–	21
				last coccon laid in the season							
				snails kept in pairs or groups							
1st	–	–	–	–	–	–	3	7	11	3	24
2nd	–	–	–	1	–	1	5	5	2	3	17
3rd	–	1	–	1	–	1	–	1	1	–	5
total	–	1	–	2	–	2	8	13	14	6	46
				snails kept singly							
1st	–	–	–	–	–	–	1	3	5	2	11
2nd	–	–	–	–	3	2	2	2	–	1	10
total	–	–	–	–	3	2	3	5	5	3	21

till October 11th (Figs 24, 25). Later, in spite of a fairly high temperature (ca. 18–20°C) and abundant food, no cocoons were produced. Yolk-containing oocytes appearing in the gonads after the end of the season (sometimes already in August) were retained there till spring. After the end of the season, the gonad of some snails was strongly shrunken and its wall was detached from the shell. The absence of a permanent partner often resulted in accumulation of ma-ture oocytes in the gonad, and deposition of only few cocoons and eggs.

Irrespective of the season, nearly all adult snails collected in the field started depositing cocoons soon after being brought to the laboratory (1–7 days), if only there was a proper temperature, food, and a partner for copulation.

The breeding season of snails kept singly started much later compared to those kept in pairs and
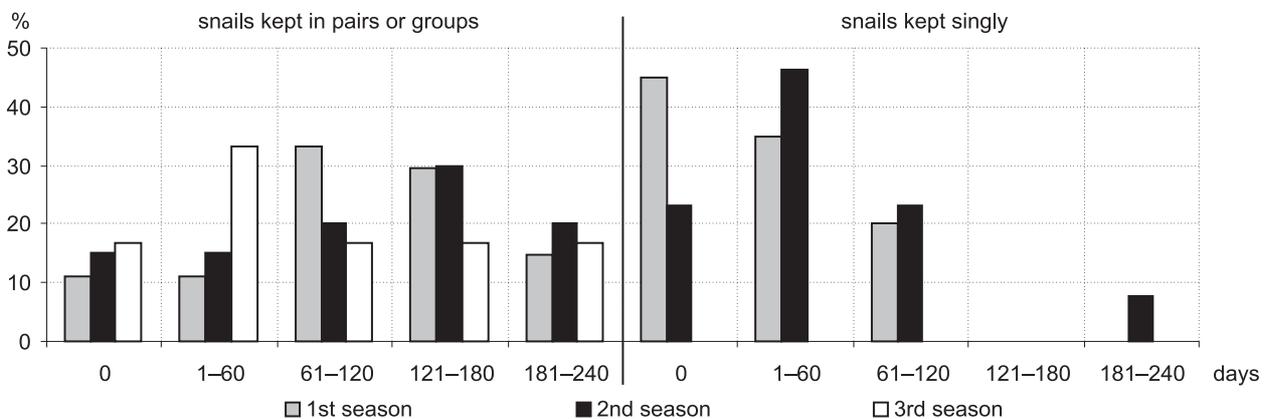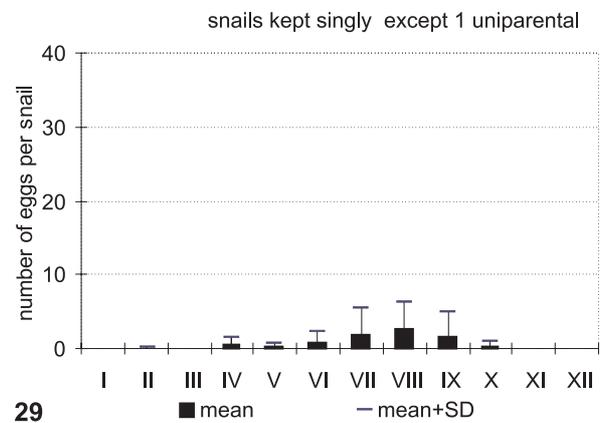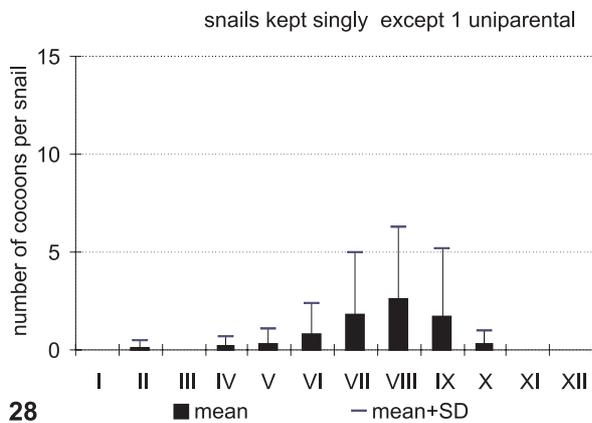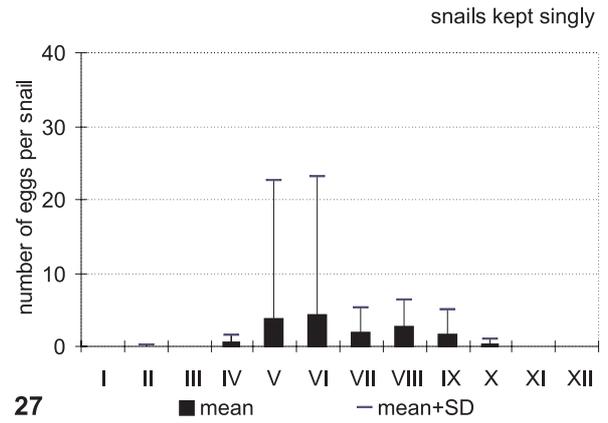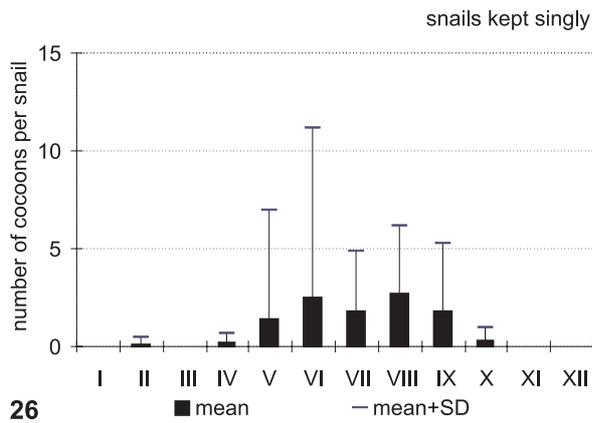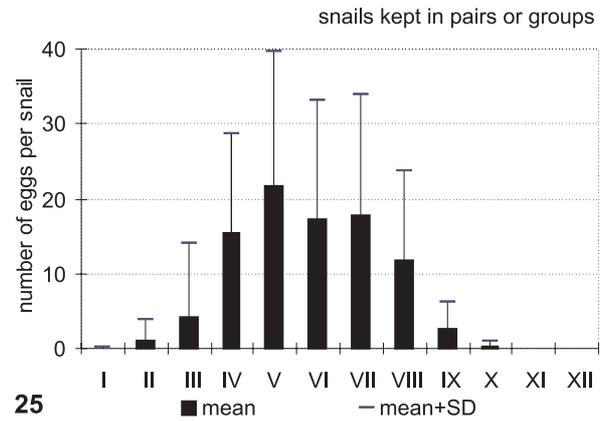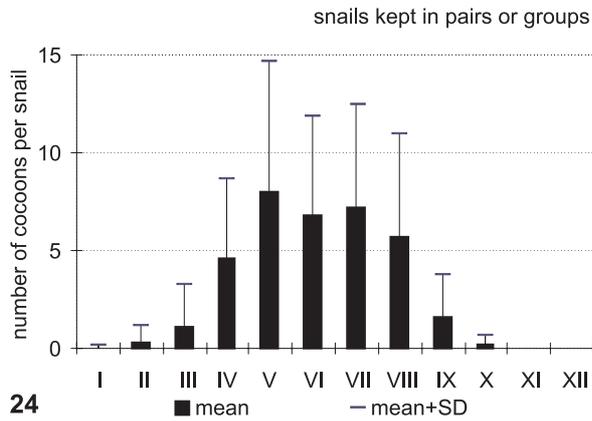

Fig. 23. Duration of reproductive seasons in laboratory. "0" means the percentage of containers in which no cocoons were de-posited in a given season
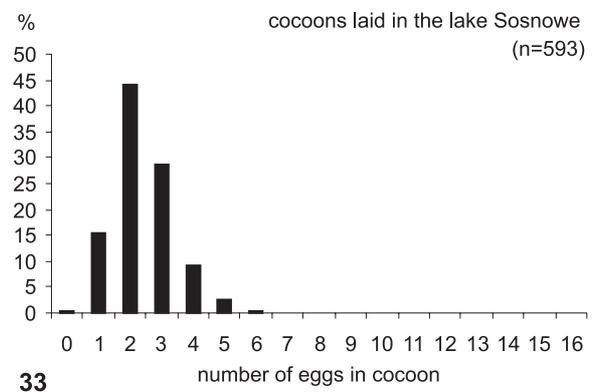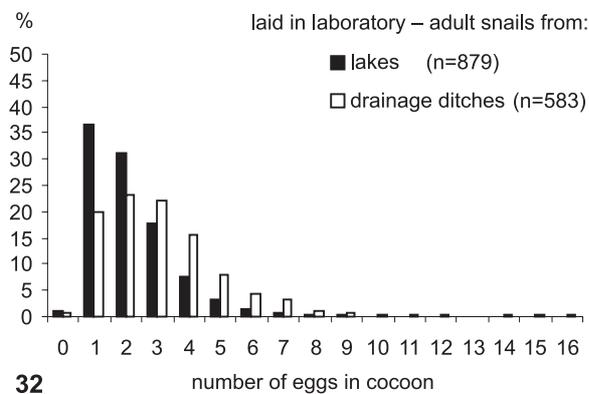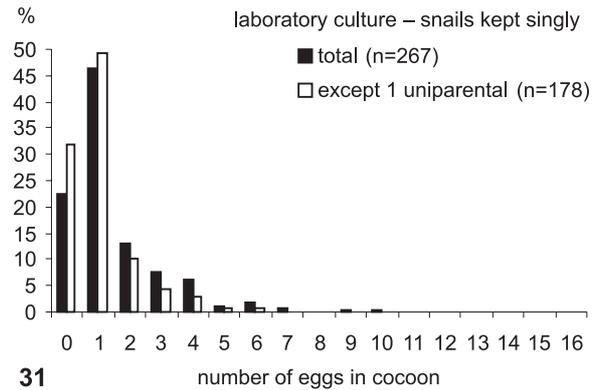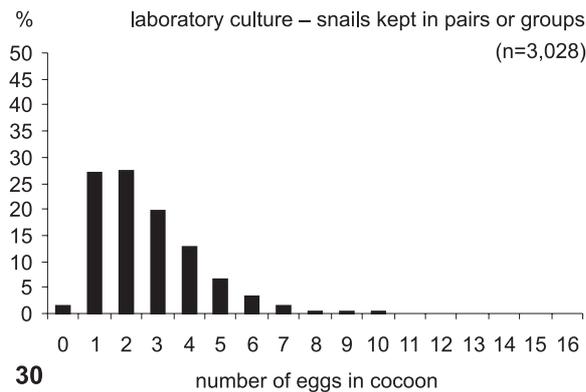
groups, and the number of deposited cocoons and eggs was usually small, in spite of the presence of numerous yolk-containing oocytes in their gonads (Figs 24–31) (see also "Fertility").

In the field, the beginning of the breeding season clearly depended on weather conditions. The first co-

coons were usually found ca. 1 week after the ice cover disappeared completely. Dates of finding the first cocoons in particular years were: March 19th 1992, April 8th 1993, April 8th 1994, April 12th 1995, April 30th 1996, March 18th 1997. The earliest copulation was observed on March 11th 1997.

Figs 24–29. Mean numbers of cocoons and eggs deposited in laboratory in consecutive months (mean of all breeding seasons); data from snails kept in pairs and groups, converted to egg and cocoon number per snail

Figs 30–33. Distribution of egg number in cocoons originating from different snails; number of cocoons is given in parentheses

The last cocoons of the year were usually found in the second half of August or at the beginning of September. In some years, cocoons were deposited even later: on September 28th 1994 a single cocoon was found with two eggs (embryos at egg stage), on September 22nd 1996 one cocoon with three eggs (egg stage) and two cocoons with two eggs each (snail stage).

The reproduction in the laboratory was clearly seasonal, though it usually started earlier than in the field and terminated later. The differences resulted probably from the higher temperature in the culture. The most intense cocoon deposition took place from April till August and was well compatible with the observations in the lake Sosnowe. There were significant differences between the distribution of the egg number in cocoons deposited in the laboratory and in the field (Figs 30–33), though this could result partly from the different number of examined cocoons.

In the field, the female maturation has probably the same course as in the laboratory. The smallest snails with mature oocytes in the gonad had shells of 2.05 mm diameter. In snails of shells of up to 2.40 mm diameter oocytes were found rather rarely, while at the shell diameter of 2.90–3.00 mm the gonad almost always contained oocytes.

FERTILITY

The mean numbers of cocoons and eggs per snail in particular breeding seasons and per lifetime are presented in Table 2. Snails kept in pairs and groups deposited from 3 to 105 cocoons (mean 51.3, SD = 21.3, n = 59) containing a total of 10–353 eggs (mean 133.6, SD = 68.5, n = 59) during their lifetime. Snails kept singly deposited 0–89 cocoons (mean 13.3, SD = 21.3, n = 20), containing a total of 0–217 eggs (mean 19.8, SD = 46.9, n = 20). The variation in the number of eggs and cocoons deposited within a lifetime (per one snail, snails in pairs and groups) and in the mean number of eggs per cocoon is presented in Figures 34–36.

Maximum numbers of cocoons deposited by one snail during 24 hrs were:
– shell diameter 4.35 mm (3.500 whorls), April 30th 1991: 4 cocoons with a total of 39 eggs; May 2nd 1991: 3 cocoons with a total of 28 eggs;
– shell diameter 3.00 mm (2.875 whorls), March 26th 1992: 4 cocoons with a total of 16 eggs; March 27th 1992: 4 cocoons with a total of 12 eggs;
– shell diameter 3.00 mm (2.875 whorls), March 23rd 1993: 4 cocoons with a total of 16 eggs.

Table 2. Mean number of eggs and cocoons deposited by laboratory snails; n – number of live individuals in a given period, 1st, 2nd, 3rd – consecutive breeding seasons after maturation, Σ – total per lifetime, * – cocoons and eggs deposited by the uniparental snail

period	snails		number of cocoons per snail			number of eggs per snail			number of eggs per cocoon		deformed eggs in %
	n	%	mean	SD	max.	mean	SD	max.	mean	SD	
snails kept in pairs or groups											
1st	59	100	28.5	19.8	63	70.5	53.3	194	2.5	1.5	1.9
2nd	37	64	32.3	22.2	74	86.2	66.3	188	2.7	1.6	1.9
3rd	10	17	15.2	15.9	36	53.1	63.3	147	3.5	2.4	9.6
Σ	59	x	51.3	21.3	105	133.6	68.5	353	2.6	1.6	2.5
snails kept singly											
1st	20	100	6.1	8.7	36	6.5	9.9	42	1.1	1.0	50.4
2nd	13	65	11.1	22.7	82*	20.4	55.3	210*	1.8	1.8	23.8
Σ	20	x	13.3	21.3	89*	19.8	46.9	217*	1.5	1.6	32.6
snails kept singly except 1 uniparental											
1st	19	100	6.1	8.9	36	6.5	10.2	42	1.1	1.0	49.2
2nd	12	63	5.2	10.1	38	4.6	8.1	30	0.9	1.1	69.1
Σ	19	x	9.4	12.7	48	9.4	12.7	42	1.0	1.0	55.3

In the temporary cultures, the maximum numbers of cocoons and eggs deposited by one snail (collected in the field as adult) were: from the lake near the village of Korne 83 cocoons/194 eggs, from the lake Sosnowe 71 cocoons/107 eggs, from an oxbow of the Brda River 21 cocoons/138 eggs, from drainage ditches 63 cocoons/111 eggs.

Cocoons deposited by snails kept in pairs and groups contained from 0 to 10 eggs (Fig. 30), the mean number of eggs per cocoon being 2.60 (SD = 1.58, n = 3,028). The mean number per lifetime for particular pairs (groups) ranged from 1.38 to 3.84 egg per cocoon. Clear differences in the mean number of eggs per cocoon were observed also between months (Fig. 37) and breeding seasons (Table 2).

Cocoons produced by snails kept singly contained 0–6 eggs (in the uniparental snail to 10 eggs) (Fig. 31). The mean number of eggs per cocoon was 1.48 (SD = 1.56, n = 267), and excluding the uniparental snail 1.01 (SD = 1.04, n = 178). For the uniparental snail in the first season the mean was 1.0, and in the second 2.56 eggs per cocoon.

Though *V. cristata* is hermaphroditic, and sometimes autocopulation may take place, copulation seems to be necessary for normal reproduction. Both the quantitative distribution of eggs in deposited cocoons (Figs 30, 31) and the number of cocoons and eggs in particular months (Figs 24–29) show significant differences between the snails kept in pairs or groups and those kept singly. Snails kept singly laid low numbers of eggs, while their gonads were most often completely filled with mature or nearly mature oocytes. The embryos in their eggs sometimes devel-

oped, but most often they died at early stages. In the second season, out of 20 snails kept singly, only one (termed uniparental in the further text and figures) laid eggs from which young hatched. In the first season, it deposited 7 cocoons containing a total of 7 eggs (5 of them deformed), in the second season 82 cocoons with a total of 210 eggs (25 of them deformed). Sixty seven young hatched from these eggs, but most of them showed smaller or greater developmental defects (defective shells, kidneys, etc.) and died soon after hatching. Out of 17, which survived and were kept in the laboratory, only one reached maturity and, kept singly, deposited 4 cocoons with a total of 6 eggs (3 deformed).

Snails temporarily devoid of partner laid increasingly fewer eggs with increasing time elapsed from copulation, while oocytes accumulated in the gonad and the percentage of defective eggs increased clearly.

Snails kept in pairs or groups showed individual periods of activity (feeding, reproduction) and shorter or longer motionless periods (from a few days to a few weeks, rarely longer). Some pairs did not reproduce for the whole season. Only 50–70% (mean 64%) snails collected in the field had their alimentary tracts filled at the moment of collecting, which indicates activity and inactivity periods similar to those observed in the laboratory. A possible explanation for the breaks in activity and reproduction is avoidance of inbred: during longer periods of time there is a greater chance for dispersal of snails being progeny of the same parents, and thus for meeting an unrelated partner.
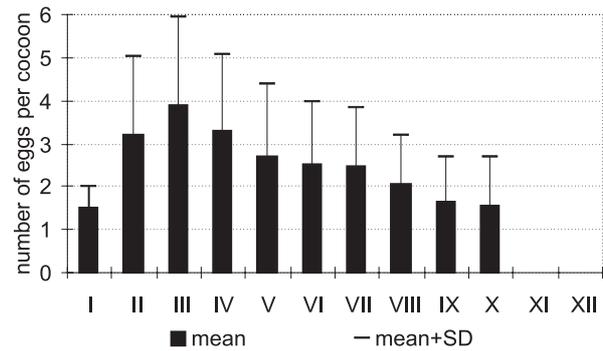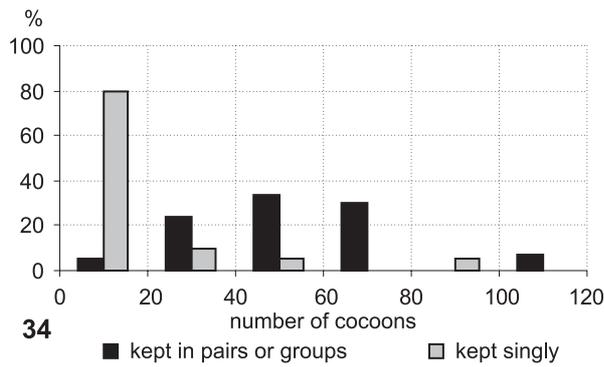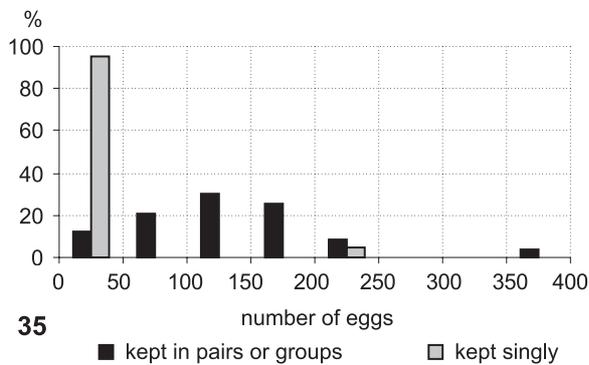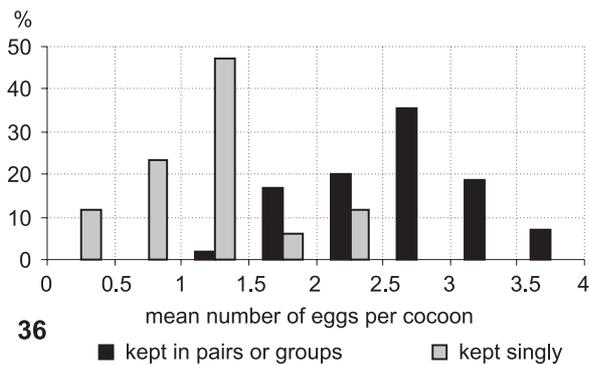
34
■ kept in pairs or groups ■ kept singly

Fig. 37. Variation in the mean number of eggs per cocoon in particular months (mean of all breeding seasons, snails kept in pairs or groups)

35
■ kept in pairs or groups □ kept singly

36
■ kept in pairs or groups □ kept singly

Figs 34–36. Variation in the number of cocoons and eggs deposited during lifetime, and in the mean number of eggs per cocoon

The limited observations on the effects of inbred (crossing siblings of the same generation) indicate a negative effect of such a reproduction on the condition of the progeny. The mean number of cocoons and eggs decreased in consecutive generations (parents: 105 cocoons/242 eggs, generation I: 56 cocoons/162 eggs, generation II: 46 cocoons/119 eggs, generation III: 19 cocoons/57 eggs). Besides, the whole lineage was susceptible to trematode larvae in-

fection. One of the parents was infected at the end of its life, while individuals of generations I and III were infected as juveniles. Outside this lineage only one snail in the culture was infected. The shortening of life span in consecutive generations resulted probably from improved food conditions.
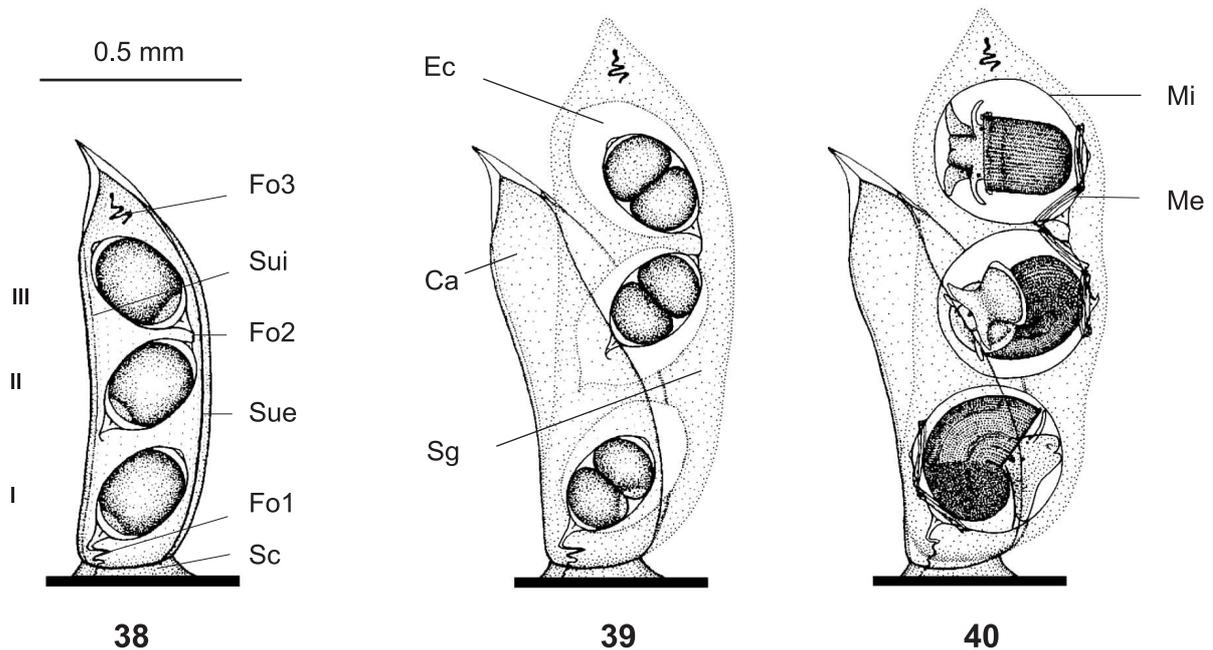
COCOON STRUCTURE

The structure of a typical cocoon is presented in Figures 38–40. The cocoons were usually elongated, cylindrical, slightly bent, with a narrowed apical part. Less frequently they were mace-like, spindle-shaped or of a different form. Some snails deposited cocoons with spirally coiled bases (Figs 16, 17) or with narrow, elongated bases containing no eggs (Figs 11, 20). Sporadically, a very thin egg cord protruded from the base (Fig. 19). The eggs were most often arranged in one row, only in some cocoons, containing at least 5 eggs, a more or less distinct second row was visible. Ca. 1% cocoons contained only a twisted egg cord (cf. Fig. 41) of variable length and structure. The capsule of the cocoon (Ca) consisted of two parts united by sutures (Fig. 38). Both parts were more or less equal, or the part formed on the ventral side of the shell gland was slightly larger. The sutures were located on the internal, concave (Sui) and the external, convex (Sue) margin of the cocoon, and almost always both were clearly visible. In long cocoons the plane of the sutures near the apex was slightly twisted to the right relative to the base (Figs 6, 7). Next to the apex, along the sutures, very often there were membranous wings. In some cocoons they extended from the base to the apex (Figs 14, 16). The cocoon capsule (Ca) was very thin (thickness ca. 0.001 mm or less), relatively translucent and most often colourless. More or less distinct longitudinal striae (and also transverse striae near the base) were visible on it, and its external surface was finely granular. Rather seldom cocoons were found

Figs 38–40. Cocoon structure: 38 – Newly deposited cocoon: Fo – egg cord, Fo1, Fo2, Fo3 – sections of the cord located in various places in the cocoon, Sc – cementing substance gluing the cocoon to the substratum, Su – sutures on capsule, Sue – suture located on the external (convex) margin of the cocoon, Sui – suture located on the internal (concave) margin of the cocoon, I, II, III – sequence of release of oocytes from the gonad; 39 – 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; 40 – Cocoon on the last day of incubation, embryos ready to hatch: Me – egg external membrane, Mi – egg internal membrane

without capsules or with capsules only partly formed (e.g. without apex, with apertures).

The cementing substance (Sc) gluing the cocoon to the substratum was most often located centrally on the base. However, in cocoons with rather narrow base or in strongly elongated constricted cocoons, it was always located on the convex side of the cocoon (Figs 12, 19), being of 0.10 mm, to ca. 0.20–0.30 mm in diameter. Cocoons without cementing substance on their bases were very rare.
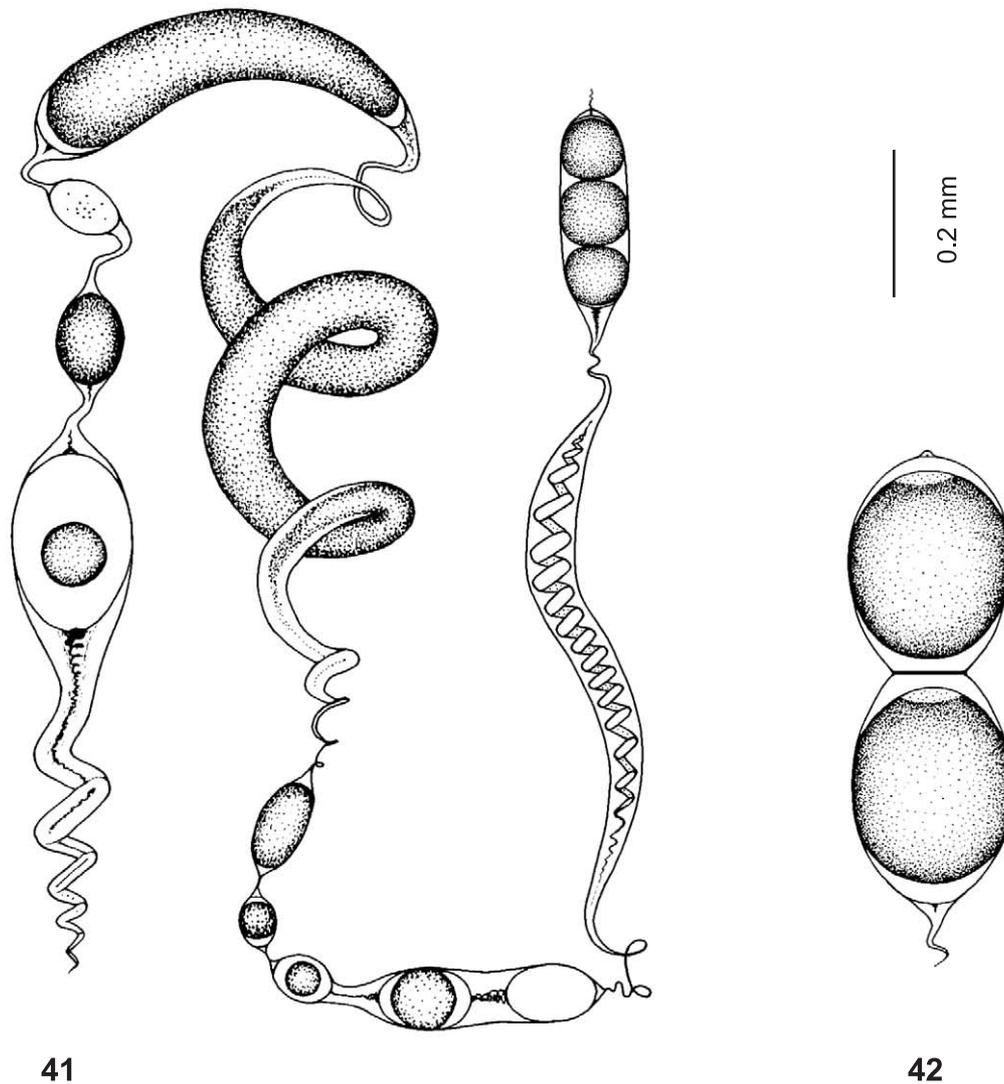
The apical part of the cocoon varied in its shape and length. On the internal side of the cocoon, it formed a clearly delimited chamber (Figs 10, 14, 21, 22) or gradually narrowed towards the apex (Figs 9, 11–13, 15–20). Externally it most often had a shape of a dagger or a funnel with wings on sides, less often it was tube-shaped (Figs 13, 18) or resembled a short, wide lamella (Fig. 10). Sometimes the apex of the capsule was extended into a long, narrow filament (Fig. 17), and in some cocoons a long section of egg cord protruded outside the capsule (Figs 15, 18, 20).

The cocoon was filled with a gelatinous substance (Sg – Fig. 39) which, when the capsule was broken, swelled and increased its volume by ca. 50%. Just after the capsule was broken, the substance very easily

stuck to leaves or other submerged objects. Within it there were eggs and the egg cord (fila ovi) being a prolongation of the egg membranes (Fig. 41). Sometimes it also contained scattered single yolk granules or, very rarely, spermatozoa. The egg cord was located in several characteristic places in cocoons: Fo1 – connecting the first egg with the base of the cocoon (twisted to the right or, less often, straight), Fo2 – extending from the anterior pole of the egg and sometimes connected with the preceding egg, Fo3 – the section of the cord present in all cocoons, behind the last egg, strongly coiled. The cord connecting eggs (Fo2) was present in 10–20% cocoons, strongly deformed eggs being almost always connected.

The number of eggs in the cocoon ranged from 0 (only cord) to 16 (most often 1–5) (Figs 5–7, 9–22). A cocoon with 16 eggs was found on April 21st 1995 in a container with three snails of shell diameters of 3.20, 3.35 and 3.45 mm. The distribution of the number of eggs per cocoon approximated Poisson's distribution (Figs 30–33).

Since most cocoons had no distinct border of the internal chamber, their length was measured from the base (without the cementing substance) to the very apex, along the chord of the arc (Fig. 2). The

0.2 mm

41   **42**

Figs 41–42. Variation of the egg cord: 41 – Long cord with various structures inside; 42 – Eggs directly connected

measurements are presented in Table 3. The length of egg-containing cocoons was strongly positively correlated with the number of eggs (r = 0.87, n = 2,688); the cocoon diameter showed a very poor correlation with the number of eggs (r = 0.35, n = 2,688). Cocoons containing no eggs and only an egg cord were the most variable with respect to length and diameter. The smallest were 0.30 mm long and 0.07 mm in diameter, the largest were 5.40 mm long. The shortest cocoon with one egg was 0.45 mm long (Fig. 21), the longest typically shaped cocoon, with 15 eggs, was 3.20 mm in length (measured as shown in Fig. 2). The largest diameter (0.60 mm) was that of the cocoon with egg located perpendicular to the long axis of the

cocoon, and a cocoon which was completely spirally coiled (length 0.60 mm, diameter 0.65 mm), with two deformed, strongly elongated eggs.

Cocoons deposited in natural conditions usually contained 2 or 3 eggs (Fig. 33). The largest cocoon, found in an oxbow of the Brda River, contained seven eggs, and in the lake Sosnowe two cocoons were found with six eggs each.

The literature data on cocoons are scanty and do not reflect the variation. The reported number of eggs per cocoon is 4 (FRETTER & GRAHAM 1962), 1–4 (PIECHOCKI 1979) or 2–4 (FALNIOWSKI 1989a); the cocoon length ca. 1 mm (PIECHOCKI 1979, FALNIOWSKI 1989a).

Table 3. Length and diameter of cocoons

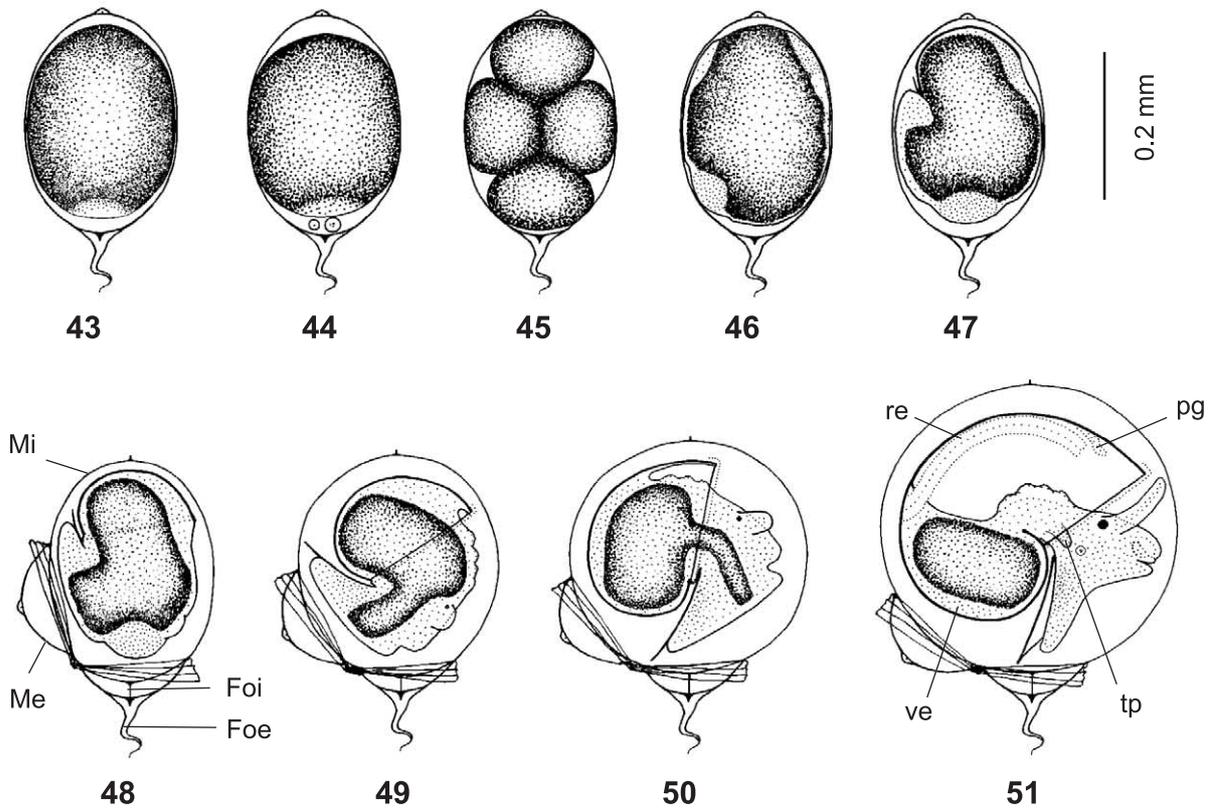| number of eggs in cocoon	number of measured cocoons	length of cocoons in mm				diameter of cocoons in mm			
		mean	SD	minimum	maximum	mean	SD	minimum	maximum
0	34	1.49	1.10	0.30	5.40	0.23	0.07	0.07	0.44
1	667	0.78	0.18	0.45	1.85	0.33	0.04	0.20	0.60
2	617	1.00	0.18	0.65	2.30	0.35	0.04	0.25	0.58
3	533	1.21	0.18	0.80	2.55	0.36	0.04	0.28	0.55
4	405	1.42	0.19	0.95	2.45	0.37	0.04	0.27	0.54
5	232	1.59	0.20	1.05	2.40	0.37	0.04	0.28	0.52
6	128	1.76	0.22	1.15	2.55	0.37	0.03	0.29	0.52
7	57	1.90	0.20	1.45	2.65	0.39	0.05	0.31	0.56
8	18	1.91	0.22	1.55	2.30	0.41	0.05	0.29	0.56
9	14	2.24	0.24	1.90	2.70	0.41	0.05	0.34	0.51
10	7	2.36	0.33	1.95	3.00	0.39	0.04	0.34	0.47
11	3	2.40	0.08	2.30	2.50	0.40	0.05	0.36	0.48
12	1	2.50	–	2.50	2.50	0.37	–	0.37	0.37
13	–	–	–	–	–	–	–	–	–
14	2	2.95	0.05	2.90	3.00	0.42	–	0.42	0.42
15	3	2.87	0.40	2.30	3.20	0.45	0.04	0.42	0.51
16	1	3.15	–	3.15	3.15	0.46	–	0.46	0.46

EGG STRUCTURE (Figs 43–67)

Each egg was covered by two, closely adjoining, colourless, translucent membranes: an external, rather stiff membrane (membrana externa) and an internal, membranous (membrana interna) (Figs 40, 48). The presence of both membranes was necessary for normal development of the embryos. The yolk-rich egg cells were surrounded by their own, very thin, membrane (membrana vitellina). The egg cell was most often located centrally, adjoining the membranes in the region of the egg equator, and occupied 65–96% (on an average 81.5%) egg volume. Some eggs, besides the egg cell, contained from one (Fig. 56) to several yolk granules which were broken and dispersed during the development of the embryo and did not affect it. However, the presence of 2–3 egg cells in one external membrane almost always resulted in the death of embryos (only two eggs with a strong constriction hatched – Figs 66, 67). Examples of variation in the egg shape and size are presented in Figures 52–67.

The yolk contained in the egg cells ranged in colour from yellow (yellow-white, light or dark yellow), through yellow-green of various intensity, to green (light green, bluish green). Sporadically, bi-coloured eggs were found, e.g. one pole yellow, the other yellow-green. The most frequent colour was yellow, but a slight addition of "green" blue-green algae in the food (e.g. *Oscillatoria*) resulted in the newly formed oocytes being yellow-green or green. In cocoons collected in the lake Sosnowe most eggs were coloured yellow (79.5%), much less often they were yellow-green (12.3%) or green (8.2%).

In the region of equator the external membrane of the egg was very thin, becoming thicker towards the poles, passing gradually into the egg cord. From the anterior pole of the egg (pointing downwards in the figures), there almost always extended a rather thick cord, whereas on the posterior pole only a small, tubercular thickening was visible. The internal membrane on both poles also passed into a cord, which was located inside the cord extending from the external membrane. Next to the anterior pole of the egg, both cords were coiled dextrally or irregularly (Figs 57, 61, 62). Some cocoons contained very long sections of coiled cord of varying diameter and containing numerous chambers (empty, with single yolk granules, completely filled with yolk etc.). Examples of the variation of the cord are shown in Figure 41. The smallest egg from which a snail hatched was 0.24 mm in chamber length, 0.20 mm in egg cell length, and 0.19 mm in diameter (Fig. 52). Figures 53 and 54 show the largest eggs from which snails hatched.

The mean egg measurements were: chamber length 0.307 mm (SD = 0.020 mm), egg cell length 0.251 mm (SD = 0.026 mm), chamber diameter 0.205 mm (SD = 0.011 mm, n = 662). Correlation coefficients: chamber length/chamber diameter r = 0.30, egg cell length/egg cell diameter r = 0.34, chamber length/egg cell length r = 0.84, n = 662. The size variation of normal eggs is presented in Figures 68–70.

Figs 43–51. Diagram of embryonic development at a temperature of ca. 19°C: 43 – Newly laid egg; 44 – Egg after 5 hrs (polocytes I and II visible); 45 – Egg after 10 hrs (2 divisions, viewed from vegetal pole); 46 – Fifth day of incubation; 47 – Sixth day of incubation: larval shell and operculum visible; 48 – Seventh day of incubation; 49 – Eight day of incubation: eyes, hearbeat and short mantle cavity visible; 50 – Ninth day of incubation; the embryo can contract its body and hide in the shell; 51 – Twelfth day of incubation, embryo just before hatching. Lettering: Me – egg external membrane, Mi – egg internal membrane, Foe – egg cord extending from external membrane, Foi – cord extending from internal membrane, pg – incipient gill, re – rectum, tp – pallial tentacle, ve – stomach
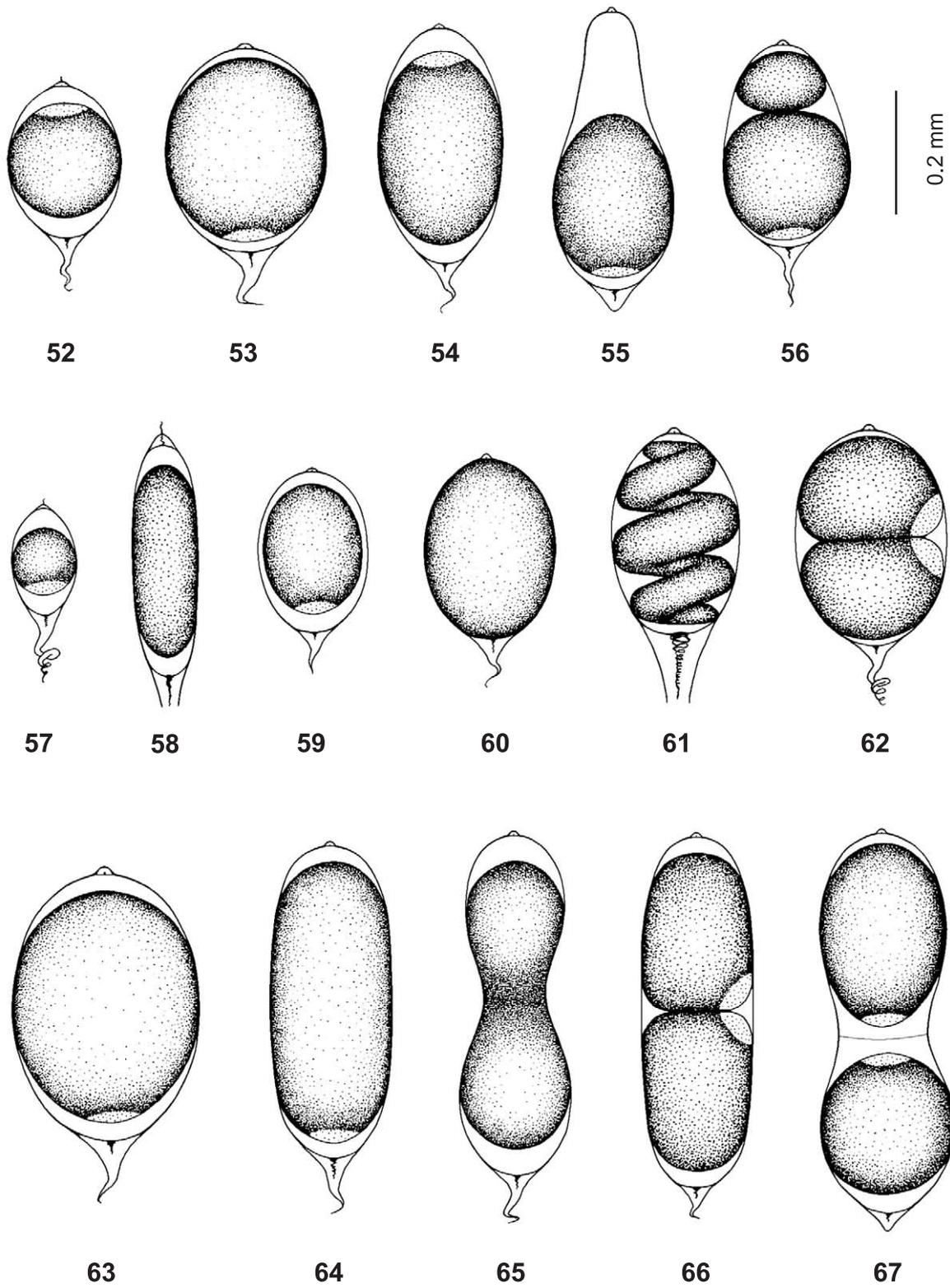
The remaining eggs, of smaller or larger size, with 2 or 3 egg cells, broken egg membranes (external or internal), lack of vitelline membrane (yolk scattered over all the chamber) and other presented in Figures 57–67 were regarded as deformed. No young hatched from the eggs shown in Figures 57–65. Two snails accreted with their posterior parts of feet hatched from the egg presented in Figure 66, and two snails (one with normal and one with tubular shell) hatched from the egg shown in Figure 67. Deformed eggs constituted ca. 2.5% of the total number of eggs laid by snails kept in pairs or groups, and 32.5% eggs laid by snails kept singly. The largest egg containing 1 egg cell was: chamber length 0.44 mm, egg cell length 0.37 mm, diameter 0.29 mm (Fig. 63). The longest egg (with a constriction similar to that presented in Figure 67) was: chamber length 0.68 mm, maximum diameter 0.20 mm and contained 2 egg cells. Since there was no objective lower limit of the egg size, those with egg cells which had diameter larger than 0.10 mm were regarded as the smallest (their cleavage was observed sporadically, Fig. 57). Smaller yolk granules (spheres, rolls etc.) were regarded as variations of the egg cord.
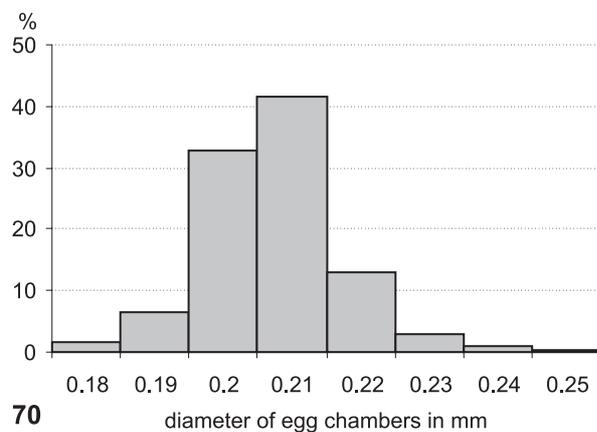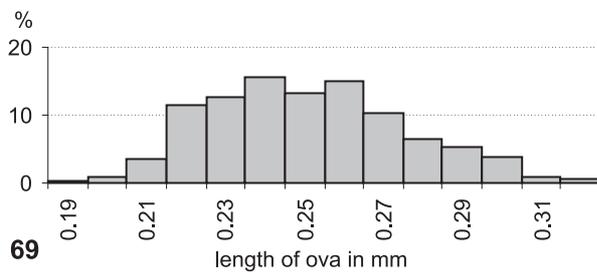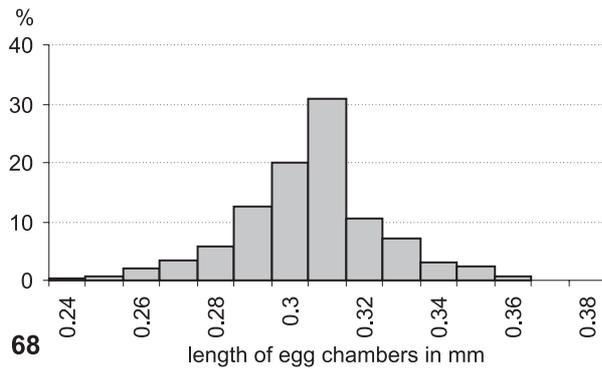
EMBRYONIC DEVELOPMENT

Just after cocoon deposition, the egg cells were most often at the stage of oocyte I. Their animal poles were usually located at the anterior pole of the egg. When the cocoon spent a long time within the snail body, the development was more advanced – the egg membranes contained polocytes, or even the first cleavage division took place short after deposition.

Falling out of the gelatinous substance had no effect on the embryonic development. Also incubation in boiled and cooled water (decreased oxygen content) did not affect the development negatively, and at low temperatures it was even favourable. Attempts at staining water revealed that both the cocoon capsules and the egg envelopes were permeable to dissolved substances.

The normal embryonic development at a temperature of ca. 19°C is presented in Figures 43–51. The first cleavage division took place in ca. 7.5 hrs from cocoon deposition, the second division after 10 hrs. A position of the blastomeres different from that shown in Figure 45 implied serious disturbances of

0.2 mm

52 53 54 55 56

57 58 59 60 61 62

63 64 65 66 67

Figs 52–67. Examples of egg size and shape variation: 52–56 – Eggs from which snails hatched; 57–67 – Deformed eggs with no cleavage or with embryos dead at early stages (2 exceptions, see text: Figs 66 and 67); 57 – The smallest egg in which cleavage was observed; 63 – The largest egg deposited; 62 and 66 – Eggs containing two egg cells each, in a common internal membrane; 67 – Egg containing two egg cells separated by internal membrane

Figs 68–70. Variation of size of eggs containing one egg cell (n = 662)

development, and most often the embryo died after a few divisions. On the 6th day of incubation a bowl-like larval shell could be observed and an operculum, while the embryo rotated slowly (always to the right, irrespective from the position of the body). On the next day the external membrane of the egg broke, while the internal membrane gradually swelled. On the 8th day of incubation eyes, a small mantle cavity, pulsation of the dorsal side and then heartbeat were visible. On the 9th day the em-

bryo resembled a small snail, could contract its body and retract into the shell. Its further development resulted in formation of rectum, stomach and the pallial part of kidney. On the last, 12th day of incubation, incipient gill (from a tubercular swelling to an elongate tubercle) was visible, as well as a short pallial tentacle and radula. The shell was colourless and the body contained no pigment. The diameter of the swollen internal membrane of the egg was usually 0.35–0.45 mm, and in eggs fallen out of the gelatinous substance it reached even 0.80 mm. Before hatching the young snail moved vigorously inside the membrane and scraped its surface with the radula. Having made a small hole, it retracted into the shell, while the membrane shrunk and finally came to adhere to the shell. Then the snail extended its body and crawled on the internal surface of the membrane which resulted in its twist and then breaking and hatching. Usually during further few hours (sporadically up to two days) the snail remained within the gelatinous substance. Eating egg membranes, other parts of cocoons, or less advanced embryos was not observed.

The cocoon capsule was usually broken on the 8th–10th day of development, along the suture on the convex side of the cocoon. A fairly late breaking of the capsule often resulted in deformation of shells (cf. Fig. 79) caused by their mutual pressure. Sporadically this led to snails being retracted in their shells and the growth being stopped. The moment of breaking the capsule was usually marked on the shells as a more or less distinct radial stria.

The mean duration of the stages of egg, larva and snail depended on the temperature of incubation, like the total duration of development. The mean relative duration of each development stage was: egg 51.2% (SD = 7.9%), larva 14.9% (SD = 5.7%), snail 33.9% (SD = 9.8%, n = 1,037, deviations of normal distribution). When the snail stage was shorter than the mean stage duration, the shells of the young snails were clearly smaller, but prolongation beyond the mean of this stage usually did not affect the shell size. In case of development disturbances (osmotic disorders, shell underdeveloped), the border between the larva and snail stages was usually difficult to define. Sometimes the whole development took place within the unbroken external membrane of the egg, and no hatching followed.

A total of 1,544 eggs obtained from snails collected in natural water bodies, 847 eggs from laboratory snails kept in pairs or groups, and all the eggs laid by snails kept singly were incubated. The duration of embryonic development was strongly negatively correlated with the mean temperature (r = –0.87, n = 1,077). At the temperature of 12°C, the time from cocoon deposition till hatching was 21–36 days (mean 24.9 days), at ca. 26°C it was only 5–10 days (mean 7.4 days). At temperatures above 26°C the percentage of
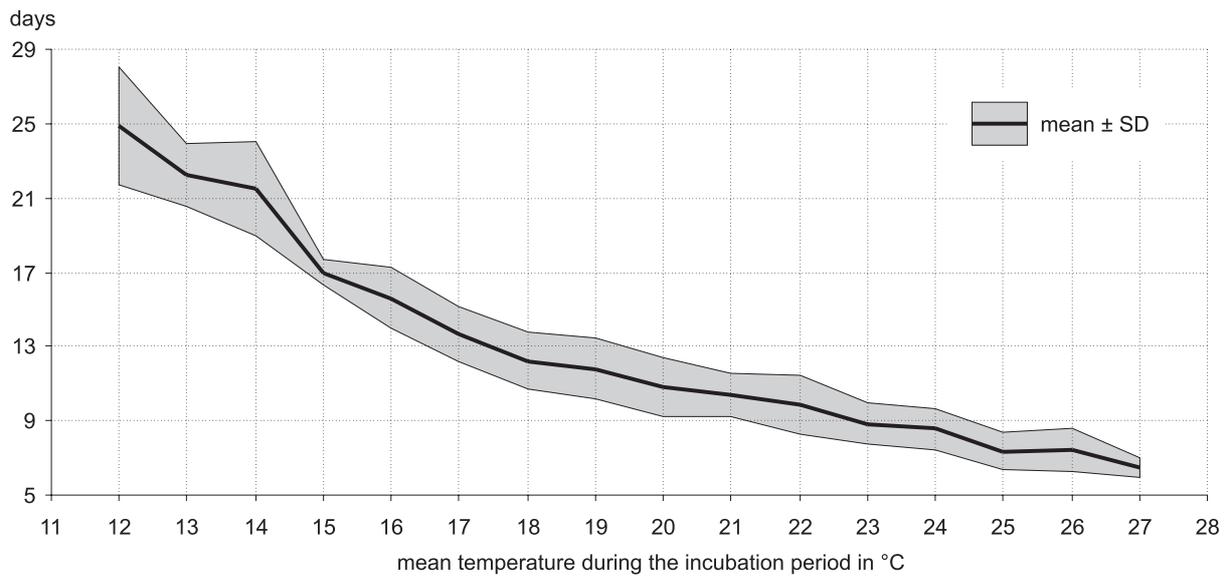
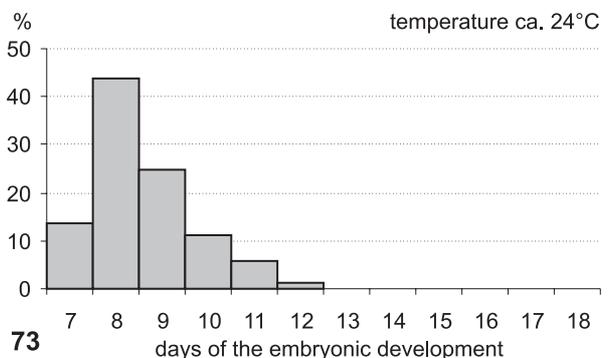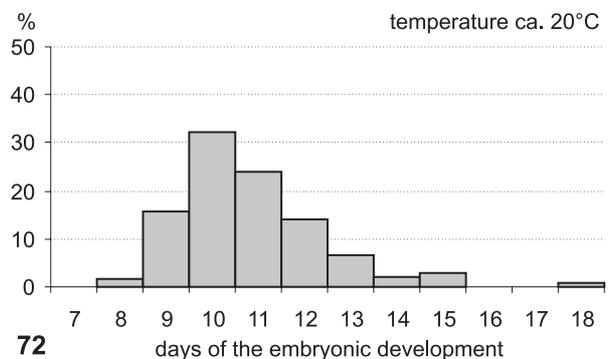Fig. 71. Dependence between the mean temperature and the mean duration of development

development disturbances increased, especially when prior to cocoon deposition the snails were also kept in such temperatures. Fig. 71 illustrates the dependence between the mean duration of embryonic development and the mean temperature. Examples of variation in the duration of incubation period at a constant temperature are shown in Figures 72 and 73.

The duration of embryonic development reported in literature is 30–40 days (FRETTER & GRAHAM 1962, PIECHOCKI 1979, FALNIOWSKI 1989a).

The percentage of hatched snails (without visible developmental defects) varied considerably, ranging from 0 to nearly 100% depending on the origin of eggs (parent snails). Most often it was ca. 30% to over 60%. No dependence between the percentage and the age of the snails was found, while there was a clear effect of incubation temperature (Fig. 74). Within the range of 16–25°C the percentage of hatched snails was the highest, the mean being 48.5% (31.9% for the uniparental snail). Below 16°C or above 25°C the percentage of hatching was clearly lower, though a short-lasting increase in the temperature even up to 30°C did not cause death of the embryos. The embryos died at various development stages, but at the stage of snail it was rather rare (ca. 0.4% incubated eggs).

The embryonic shells (Figs 75–81), from the apex to the aperture margin, were almost always covered with dense, evenly spaced spiral striae (thickenings of shell wall). On the outer margin of shell at ca. 0.250 whorl the striae were 0.007–0.010 mm wide, with interspaces narrower than the striae. Towards the aperture the striae became somewhat narrower and the smooth interspaces gradually wider. Towards the columellar margin of the whorl the striae became gradually wider spaced. On shells with detached

whorls (Figs 77 and 80), the columellar walls of the whorls bore no striae. Very rarely, on the apex the spiral striae were poorly marked, while next to the aperture they became distinct or they appeared only on post-embryonic whorls. The apex (diameter ca. 0.10

72 days of the embryonic development

73 days of the embryonic development

Figs 72–73. Variation in incubation time at constant temperature (20°C and 24°C) – percentage of snails hatched on consecutive days
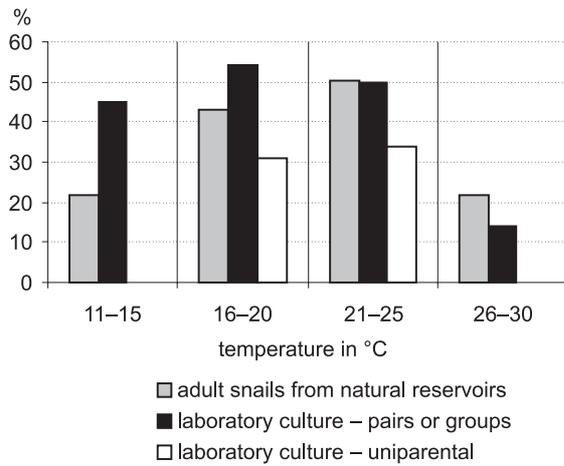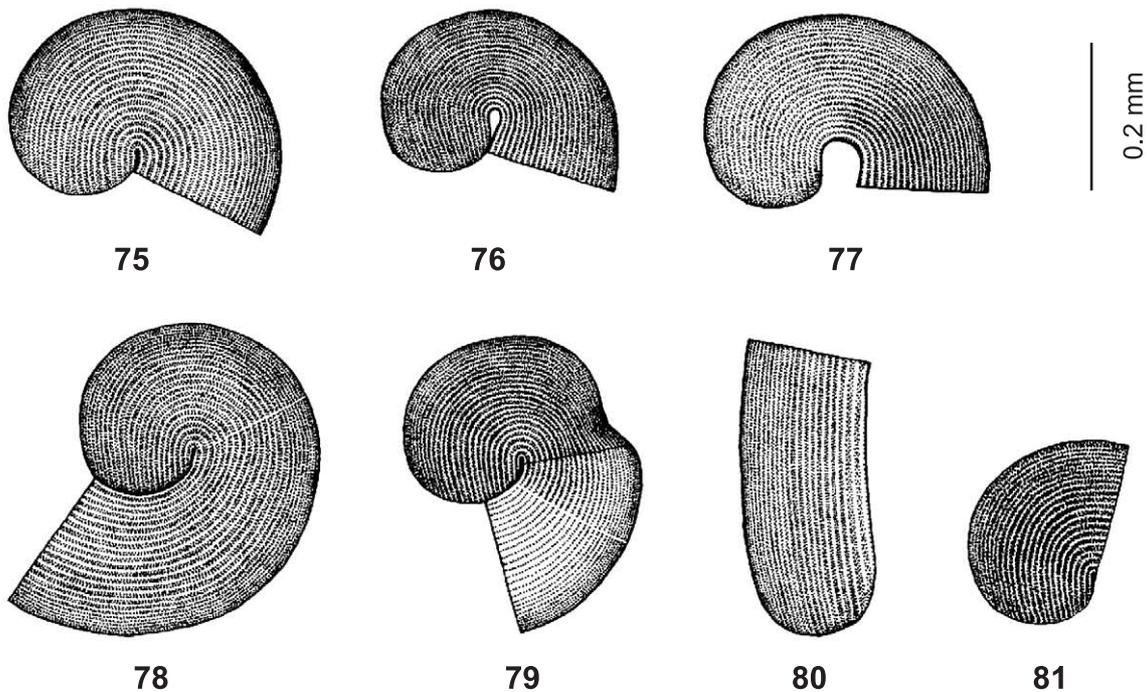
Fig. 74. Variation in the percentage of hatched snails (with normal shells) depending on the mean incubation temperature and origin of eggs
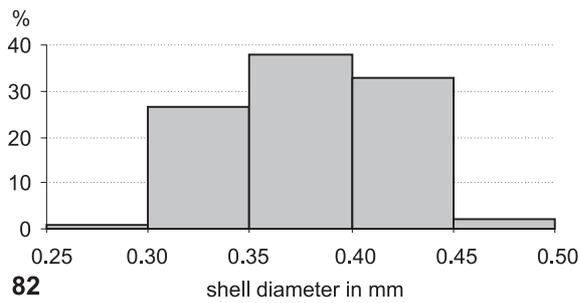
mm, later covered by increasing whorls) was covered by irregular granularities passing on sides into spiral striae. On some shells radial striae were also visible: light – narrow lines without thickenings and dark – thickening of the aperture margin during growth inhibition (e.g. prior to breaking the cocoon capsule). Sporadically, there was a distinct radial stria just next to the aperture margin, and a slight increment identi-

cal with a postembryonic increment. In typical shells the upper margin of aperture was slightly produced relative to the lower margin, while the aperture was slightly higher than wide (by ca. 0.02 mm – width of operculum increment). The opercula had 1.5–2.5 whorls, their major diameter being 0.19–0.28 mm, and corresponding to the aperture size. The embryonic whorl (its height corresponding to the shell height) was usually the highest at the aperture, sometimes however the whorl was the highest at ca. 0.25–0.5 and then became lower. Examples of shape and size variation of embryonic shells are presented in Figures 75–81. The mean size of typical embryonic shells was: diameter 0.38 mm (SD = 0.04 mm, n = 565), height 0.22 mm (SD = 0.02 mm), number of whorls 0.57 (SD = 0.14). The smallest shell was 0.28 mm in diameter, had slightly over 0.25 whorl (Fig. 81) and could hardly accommodate the contracted body. The largest shell was 0.49 mm and 0.875 whorl (Fig. 78). Size variation of embryonic shells is presented in Figures 82–84. Correlation coefficients between shell parameters were: diameter/height r = 0.52, diameter/number of whorls r = 0.84, height/number of whorls r = 0.42, n = 565.
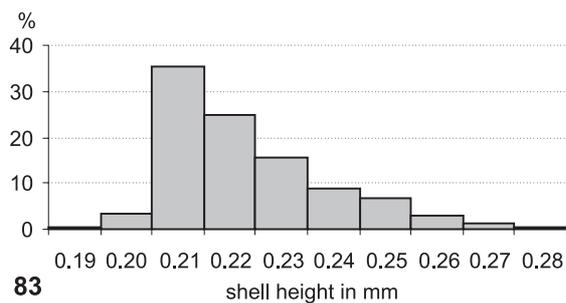
Some data on the structure and size of embryonic shells in *V. cristata*, though with little information on variation, are given in BINDER (1967), PIECHOCKI (1979) and FALNIOWSKI (1989a, b).
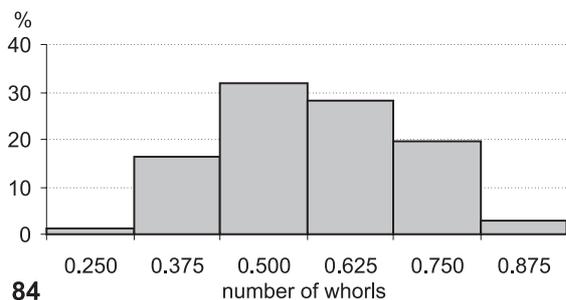

Figs 75–81. Examples of variation of embryonic shells: 75 – Typical embryonic shell; 76, 77 – Scalariform shells; 78 – The largest shell found; 79 – Shell concave on external side; 80 – Uncoiled, tubular shell, found sporadically; 81 – The smallest shell that could accommodate contracted snail

82

shell diameter in mm

83

shell height in mm

84

number of whorls

Figs 82–84. Size variation of embryonic shells (n = 565)

The shell measurements of newly hatched snails showed a weak positive correlation with some of the egg measurements, the correlation coefficients being: shell diameter/egg chamber length r = 0.56 (n = 41), shell diameter/egg cell length r = 0.56 (n = 36), shell height/egg diameter r = 0.56 (n = 41). The shell diameter and egg diameter, and the shell height and egg cell length (egg chamber length) showed no correlation.

POST-EMBRYONIC DEVELOPMENT AND MATURATION

Hatching snails were provided with yolk sufficient usually for the first few days of life. In intensely feeding snails the yolk disappeared within 3–7 days. In less active snails single yolk granules were still visible even 20 days after hatching. The snails fed in the daytime and in the night, with irregular breaks of a few days to

several weeks. Initially during crawling the shell was positioned almost perpendicularly to the substratum, and with growth it tilted gradually to the left. Under favourable conditions the young snails grew very fast, e.g. the shell of 0.42 mm diameter at hatching reached 0.76 mm in 10 days, 1.17 mm in 20 days, 1.66 mm in 30 days, 1.98 mm in 40 days and 2.20 mm in 50 days. However, most often the growth was much slower, with irregular periods during which no growth was observed.

The border between the embryonic shell and the later increments was usually marked as a clear radial stria. At the same time the appearance of spiral striae changed – they became more delicate and crossed by weak radial striae. Very rarely post-embryonic increments did not differ and there was no noticeable border. Outside the embryonic shell the spiral striae disappeared gradually and most often at about 1–1.250 whorl they were no longer visible, only very rarely reaching 1.750 whorl.
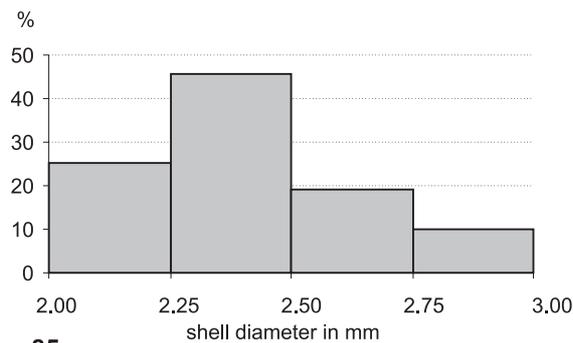
Mortality of young snails from cocoons laid by parents kept in pairs and groups ranged from 11.1 to 57.1%, depending on a year (mean 37.1%); of these snails 75% died during the first 60 days from hatching, and few snails lived as long as till the age of 290 days not reaching maturity. Almost all the progeny of isolated individuals (16 out of 17) died within 79 days from hatching.

At a shell diameter of 0.50–0.60 mm (sometimes more) single granules of black pigment appeared on heads of young snails, while tubercular lamellae could be observed on the elongated axis of the gill. At a diameter of 0.92–1.20 mm, rarely more (mean 1.08 mm, SD = 0.11 mm, n = 36) and 1.500–1.875 whorl (mean 1.64, SD = 0.12) a colourless swelling – penis – appeared below the right cephalic tentacle. Roughly at the same time, next to the shell apex, a lighter spot (gonad) appeared, and in the mantle cavity, near the columellar margin of the whorl, pallial section of female reproductive organs became visible. The time from hatching till the appearance of penis was most often 20–60 days, but sporadically it was as long as 190 days. When the penis reached a length of 0.50 mm, single granules of pigment started appearing on its base. With the body growth (shell) there was a rather quick growth of reproductive organs, though there was a considerable individual variation. In some snails at a shell diameter of 1.9 mm the penis was already almost completely formed and pigmented like in adults (base black pigmented, tip milky white) while in others it was still relatively short (length ca. 0.30 mm). Also the gonad was rather often already not much smaller than in adult individuals (Fig. 8), only the albumen and shell glands were usually rather small.
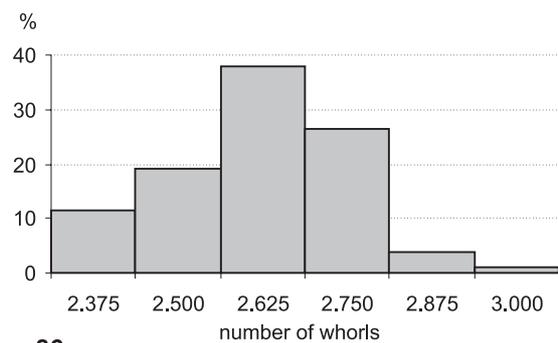
In adult snails the gonad filled all the initial whorls except the last section of the body whorl (ca. 0.125 whorl), irrespective of the number of whorls. The penis length depended, among others, on the

Table 4. Comparison of some data on snails kept in pairs/groups or singly in different periods (variable food conditions – initially the worst and then gradually improving)
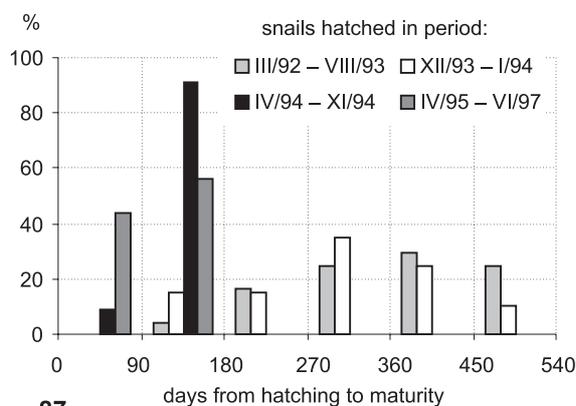
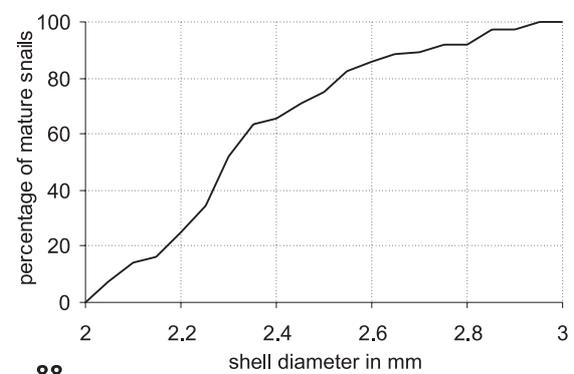| period of hatchings	kept in pairs or groups			kept singly			total		
	n	mean	SD	n	mean	SD	n	mean	SD
time elapsing between hatching and maturity (in days)									
III/92 – VIII/93	18	393.9	77.6	6	292.2	115.9	24	368.5	99.0
XII/93 – I/94	18	316.8	99.0	2	316.5	166.5	20	316.7	107.7
IV/94 – XI/94	10	146.9	31.7	1	157.0	–	11	147.8	30.3
IV/95 – VI/97	7	96.9	9.5	9	82.3	16.7	16	88.7	15.8
total	53	281.9	134.9	18	182.4	137.0	71	256.7	142.2
shell diameter at female maturity (in mm)									
III/92 – VIII/93	24	2.26	0.17	8	2.22	0.10	32	2.25	0.15
XII/93 – I/94	18	2.41	0.16	2	2.32	0.02	20	2.40	0.15
IV/94 – XI/94	10	2.28	0.11	1	2.35	–	11	2.29	0.11
IV/95 – VI/97	7	2.56	0.14	9	2,72	0.26	16	2.65	0.23
total	59	2.34	0.19	20	2.46	0.30	79	2.37	0.23
number of whorls at female maturity									
III/92 – VIII/93	24	2.57	0.14	8	2.53	0.12	32	2.56	0.14
XII/93 – I/94	18	2.64	0.09	2	2.62	0.00	20	2.64	0.09
IV/94 – XI/94	10	2.60	0.12	1	2.62	–	11	2.60	0.12
IV/95 – VI/97	7	2.68	0.06	9	2.78	0.13	16	2.73	0.12
total	59	2.61	0.12	20	2.66	0.16	79	2.62	0.14
life span (in days)									
III/92 – VIII/93	18	896.3	146.9	6	792.2	30.1	24	870.3	135.8
XII/93 – I/94	18	794.0	180.3	3	713.0	157.7	21	782.4	179.5
IV/94 – XI/94	10	620.0	213.1	2	778.0	2.0	12	646.3	203.3
IV/95 – VI/97	7	454.4	213.5	10	377.5	55.6	17	409.2	148.4
total	53	751.1	235.8	21	582.0	209.6	74	703.1	241.1
shell diameter after death (in mm)									
III/92 – VIII/93	19	3.23	0.36	7	3.74	0.29	26	3.37	0.41
XII/93 – I/94	18	3.31	0.39	2	3.70	0.05	20	3.35	0.39
IV/94 – XI/94	10	3.28	0.27	1	4.05	–	11	3.35	0.34
IV/95 – VI/97	7	3.35	0.38	9	4.12	0.28	16	3.78	0.50
total	54	3.28	0.36	19	3.93	0.33	73	3.45	0.45
number of whorls after death									
III/92 – VIII/93	19	3.08	0.19	7	3.36	0.18	26	3.15	0.22
XII/93 – I/94	18	3.08	0.18	2	3.44	0.06	20	3.11	0.20
IV/94 – XI/94	10	3.12	0.08	1	3.50	–	11	3.16	0.13
IV/95 – VI/97	7	3.02	0.17	9	3.40	0.15	16	3.23	0.25
total	54	3.08	0.17	19	3.39	0.16	73	3.16	0.22

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Figs 85–88. Attainment of female maturity: 85 – Shell diameter of newly female matured snails; 86 – Number of whorls of their shells; 87 – Variation in the time of female maturation depending on food conditions (initially the worst, then gradually improving); 88 – Cumulative, with death of adult snails considered

body size, for example at a shell diameter of 2.05 mm it was 1.25 mm, at 2.60 mm – 1.75 mm, at 3.15 mm – 2.10 mm.

The female maturity here is defined as appearance of first yolk-containing oocytes in the gonad. Usually the first oocytes appeared in the distal part of the gonad, on the external surface of the whorl. The maturity was reached at a shell diameter of 2.05–2.95 mm and the number of whorls was 2.375–3 (Figs 85, 86).
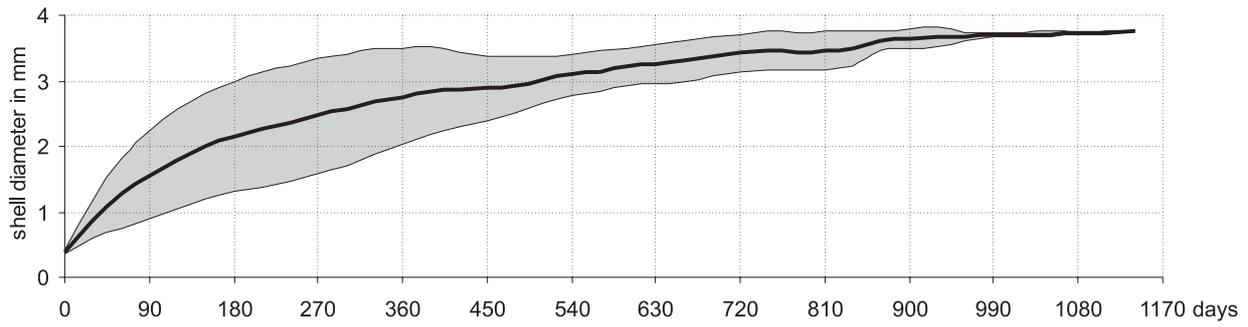
The mean shell measurements were: diameter 2.37 (SD = 0.23 mm, n = 79), number of whorls 2.62 (SD = 0.14). The time from hatching varied from 55 to 533 days and was the longest at the beginning of the culture (179–533 days), and the shortest at the end (55–111 days) (Fig. 87, Table 4). It was observed that in some snails copulation could speed up the appearance of mature oocytes in the gonad.

The smallest snail with mature oocytes in its gonad started depositing cocoons having a shell of 2.05 mm diameter and 2.375 whorls.
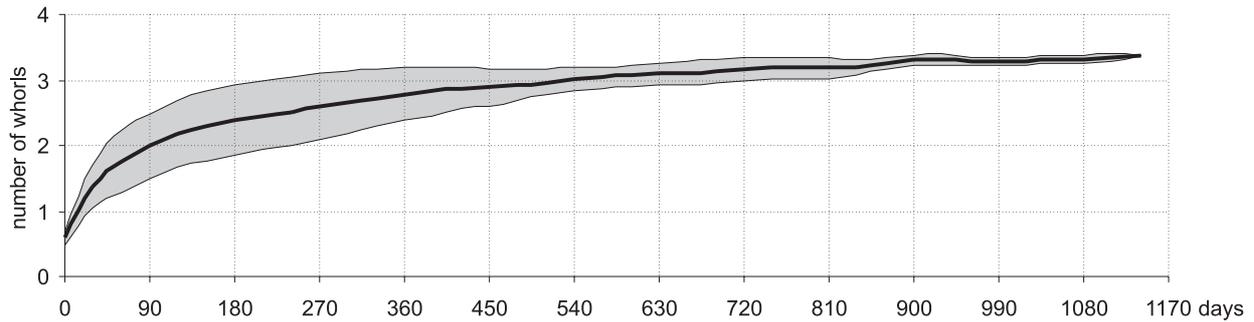
ADULT GROWTH AND DEATH

The shells grew during the lifetime, with irregular periods of growth inhibition e.g. during intense reproduction or scarcity of food. The mean curves of increase in the shell diameter and the number of whorls for most snails from the culture are presented in Figures 89 and 90 (parabolic curves). The mean growth curves differed not only between the snails kept in pairs/groups and those kept singly, but also varied depending on the life span (Figs 91, 92). The dependence of the mean growth curves on feeding conditions (which were the worst at the beginning of the experiment and then gradually improved) for snails kept in pairs or groups is presented in Figure 93. In the initial period of life there were very large differences in the shell size reached by particular individuals (coefficient of variability 25–30%). After reaching maturity the growth was more uniform, and the variability coefficient decreased gradually to 4–5%. Individual growth curves showed also differences independent from feeding conditions (Fig. 94).

Typically formed shells of *V. cristata* were usually coiled in one plane, with initial whorls slightly de-
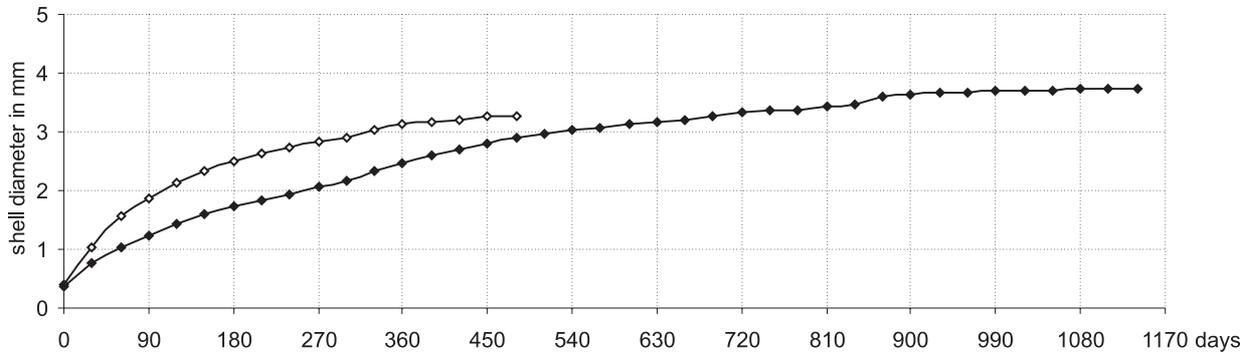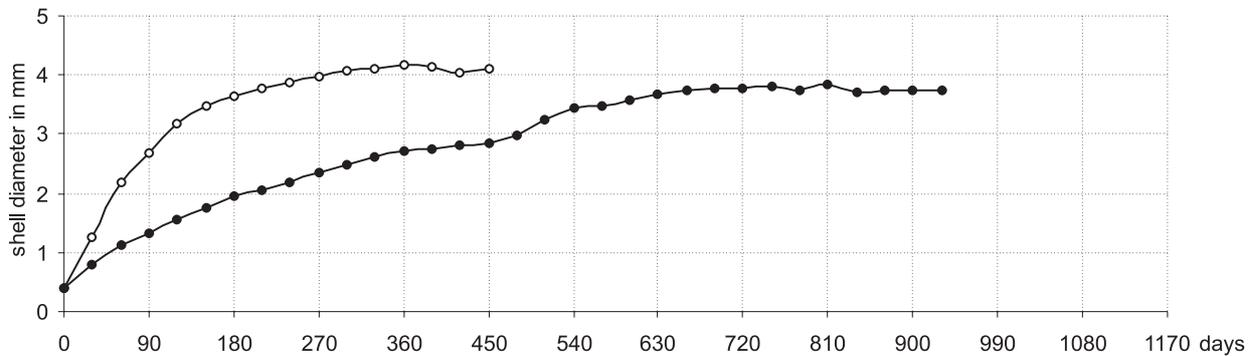
Figs 89–90. Mean growth curves for the shell diameter (89) and number of whorls (90) – total graph for laboratory culture (initial number n = 66)

Figs 91–92. Variation in mean growth curves for shell diameter depending on life span for snails kept in pairs or groups (91) and singly (92). Mean growth curves for snails of life spans of ca. 2 years and ca. 3 years were nearly identical and hence were presented jointly
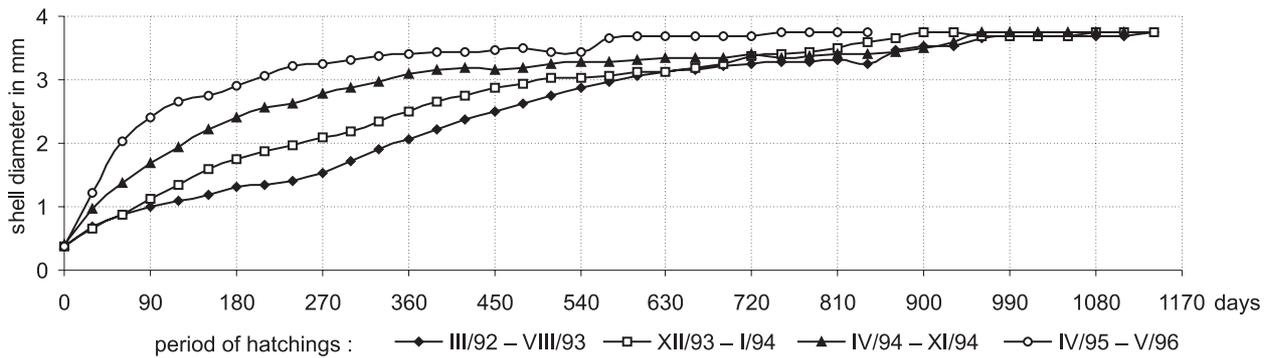
Fig. 93. Variation in mean growth curves of shell diameter of snails kept in pairs and groups depending on food conditions (initially the worst, then gradually improving)
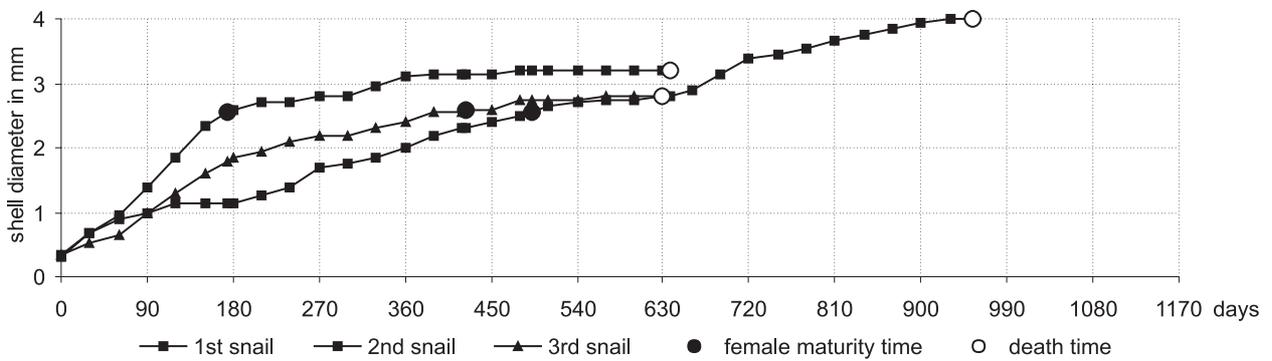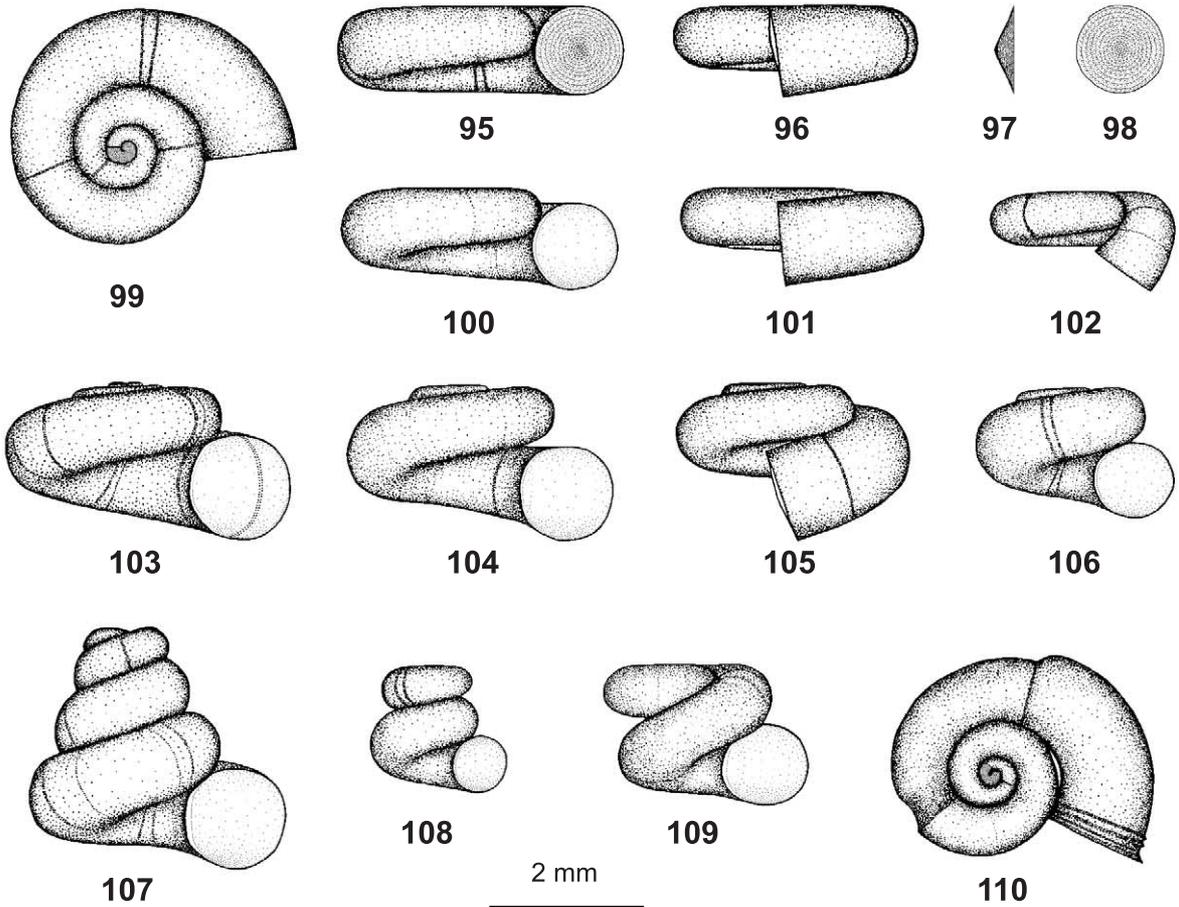

Fig. 94. Example of individual growth curves of shell diameter of three snails kept in the same container

pressed. In young snails, the body whorl was usually slightly raised, in older individuals it was more or less descending (Figs 95, 96, 99–101, 103–105). Scalariform shells and detached body whorl were observed in both young and older snails (Figs 104, 105, 110). Wide radial striae on shells were formed when food was scarce or of poor quality for a longer time (Figs 99, 103, 107, 110), while a complete absence of food in the containers was marked at most as a weak stria. Sometimes a long starvation caused a decrease in the body size and consequent decrease in the diameter of the whorl formed immediately afterwards (Fig. 110). Some shells had their aperture margins slightly reflected (Fig. 110), and, when further growth followed, there was almost always a lamellate growth line (stria). A sudden improvement of feeding conditions resulted sometimes in a rapid increase in the whorl diameter (Fig. 110).

The dependence between the mean shell diameter (with no strongly descending whorls) and the number of whorls in the culture snails is presented in Figure 111. Since the variability coefficient was fairly low (5.5–6.5%), only maximum and minimum values of the shell diameter for particular numbers of whorls are marked in the figure. Shells of snails kept in pairs or groups reached 2.60–3.75 mm in diameter, and those of snails that were still alive in the next season

after their partners had died – grew up to 4.00 mm (mean 3.28 mm, SD = 0.36 mm, n = 53), the number of whorls being 2.750–3.375 (mean 3.08, SD = 0.17, n = 53). Snails kept singly reached 3.30–4.20 mm in shell diameter, and, when kept in large containers, 4.50 and 4.70 mm (mean 3.93 mm, SD = 0.33, n = 19), the number of whorls being 3.125–3.750 (mean 3.39, SD = 0.16, n = 19). Differences between the respective values were significant. The variation in the shell size among the dying snails is presented in Figures 112–113.

The shell height depended mainly on its diameter, arrangement of whorls and aperture size. In typical shells there was no spire or it was relatively low, constituting not more than 10–20% their height. Rarely shells were found with higher spires, forming even up to 45% height. In snails infected with trematode larvae the spire height was most often 45–55% shell height. When the spire was very low, the height/diameter ratio was ca. 1:1.7 (embryonic shells) to 1:2.9 (the largest shells from the culture – large shells were relatively more flattened than small ones). Examples: I. shell diameter 2.60 mm, height 0.94 mm (spire 0, aperture 0.94 mm); II. shell diameter 3.84 mm, height 1.54 mm (spire 0.18 mm, aperture 1.36 mm); III. (the largest snail found in the field) shell diameter 4.35 mm, height 1.52 mm (spire 0.12 mm, aperture 1.40

Figs 95–110. Examples of shell variation: 95, 96, 99–101 – Typical shells; 102, 106–109 – Shells of trematode-infected snails; 103 – Shell of uniparental snail; 110 – Examples of shell deformations (narrowing of whorl, sudden widening of whorl, whorls detached, thick radial striae, reflected apertural margin); 97 and 98 – Opercula
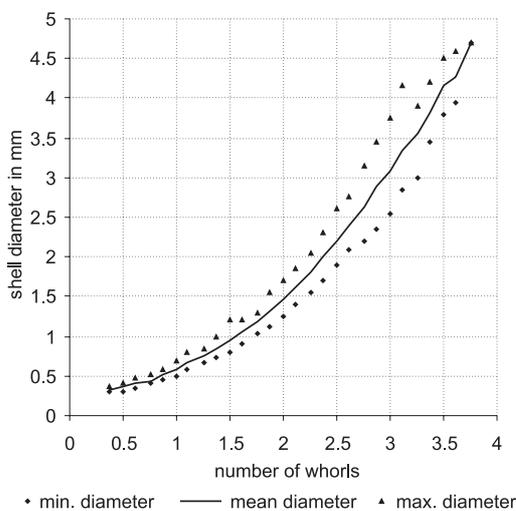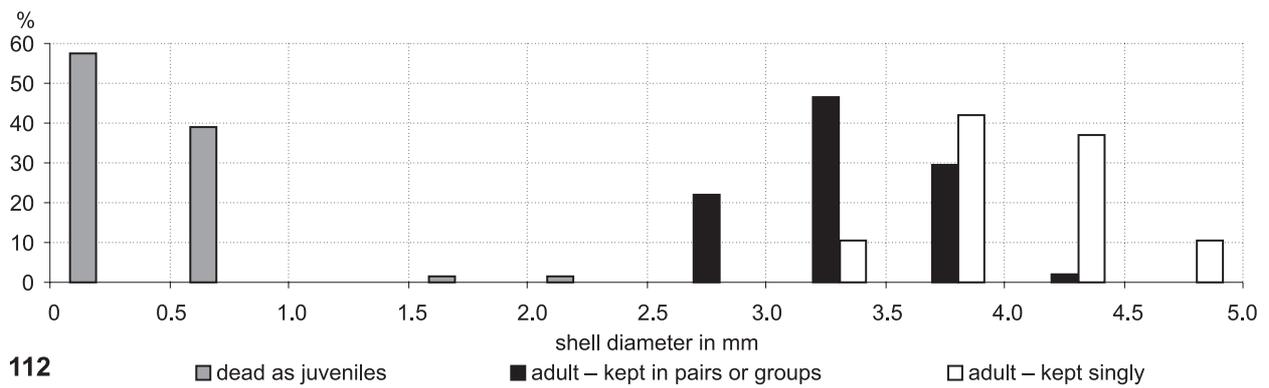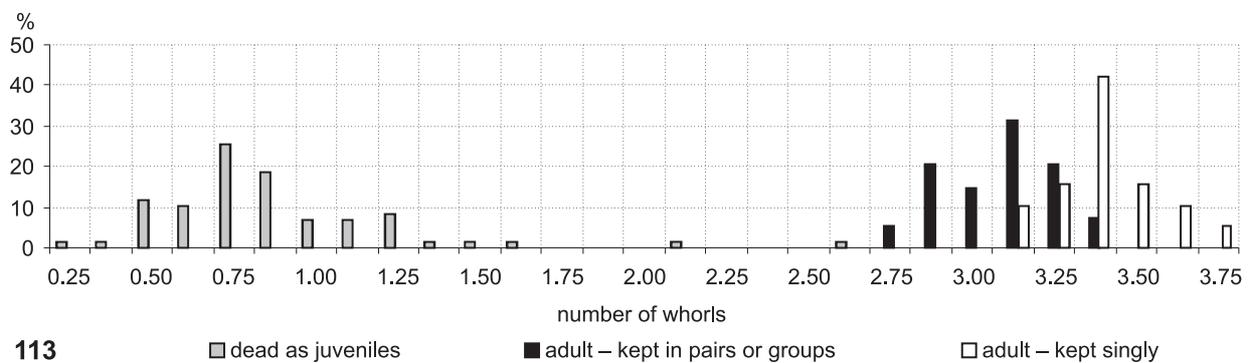

Fig. 111. Dependence between number of whorls and shell diameter in laboratory snails

mm); IV. shell diameter 4.50 mm, height 1.54 mm (spire 0, aperture 1.54 mm); V. (the largest snail in the culture) shell diameter 4.70 mm, height 1.82 mm (spire 0.21 mm, aperture 1.61 mm). The height of shells with convex spires reached 2.14 mm (Figs 103–105), and in snails infected with trematode larvae even 3.25 mm (Fig. 107).

The opercula were almost circular or slightly oval and rather thin. Their central part was somewhat concave, and the mean apical angle was 136° (SD = 5.2°, n = 35) (Fig. 97). The operculum margins were very thin and even in live snails almost always somewhat damaged. The increments (whorls) were sinistral (dextral shells!) and their margins slightly overlapped. The whorl width increased gradually from ca. 0.02 mm on embryonic operculum to 0.04–0.10 mm at an operculum diameter of 1–1.5 mm. The last increment on some opercula was wide and clearly marked, on others its width increased gradually almost on the entire perimeter of the operculum (Figs

Figs 112–113. Variation in shell size reached in laboratory; measured after death of snails

114, 115). Periods of growth inhibition were sometimes marked as oblique lines crossing the whorl. The number of whorls in the operculum was most often ca. 3 times higher than the number of shell whorls. Though on most opercula the borders between the whorls were poorly marked, on some opercula up to 11 whorls (including embryonic whorls) could be distinguished, while the number of shell whorls was 3.5. On a few last increments there were rather often fine spiral lines, their number being 15–20 on each whorl.

Figs 114–115. Light microscope: 114 – Operculum with gradually increasing last whorl, 44 ×; 115 – Operculum with wide last whorl, 44 ×

Usually the operculum size was equal to or somewhat smaller than the aperture size, but some snails produced larger opercula which could not be retracted inside the shell. The ratio operculum major diameter/shell diameter in adult snails was 1:2.5 – 1:3.4 (mean 1:2.9). The largest operculum, 1.54 × 1.50 mm in size, belonged to a snail of shell diameter 4.70 mm.

The variation observed in snails from natural water bodies (parasite-infected snails excluded) involved:

– a slight descent of the body whorl, especially in snails with shells exceeding 3.00 mm in diameter;
– scalariform whorls or only the body whorl detached near the aperture;
– local narrowing or widening of the whorl (sometimes repeated);
– in some populations irregular and distinct radial striae (usually 2–3, sometimes even up to 10);
– shell colour most often from light yellow to dark brown, less often white or greyish-white.

The literature contains information on basic shell measurements and on operculum (FRETTER & GRAHAM 1962, PIECHOCKI 1979, FALNIOWSKI 1989a, b), which is compatible with the results presented above, and on shell variation. A rather frequent anomaly consists in detachment of the last part of the body whorl, which is often descending (associated most probably with water chemistry) (PIECHOCKI 1979, FALNIOWSKI 1989a, b); besides some shells are scalariform to various degree, or have irregular and pronounced growth lines (FALNIOWSKI 1989a, b).

The time between the deposition of the last cocoon and death ranged from 1 to 326 days and did not differ significantly between the snails kept in pairs/groups and those kept singly. Most snails (71.4%) died within 60 days after the last cocoon deposition, but some survived winter and died only in the next breeding season. After death the gonad still contained yolk-containing oocytes of various size –

from large and mature to rather small, of 0.15 mm or less in diameter. In snails kept in pairs or groups, there were most often 2–30 such oocytes, and in snails that lived for a long time after their partners died – even up to ca. 60 oocytes. In snails kept singly the oocytes usually filled the whole gonad (except initial 0.5–0.75 whorl), and their number reached even ca. 150 (at the shell diameter of 4.70 mm).

The life span of adult snails ranged from 166 to 1,170 days, the snails at the beginning of the laboratory culture (the poorest feeding conditions) lived the longest (cf. Table 4). The mean life span of snails kept in pairs or groups was 751.1 days (SD = 235.8 days, n = 53), of those kept singly 582 days (SD = 209.6 days, n = 21). Figure 116 illustrates dying of cultured snails with a division into those kept in pairs/groups, kept singly and dead as juveniles. Both snails kept in pairs/groups and those kept singly died much more often in certain age classes (the "stairs" in the graph). The mortality showed also clear seasonal fluctuations, with a maximum in July/August and a much smaller peak in February (Table 5), though in laboratory young snails hatched in various months. The life span of snails kept singly showed a strong positive correlation with the duration of juvenile period (till female maturity): r = 0.82 (n = 18), and in those kept in pairs or groups there was only a weak correlation r = 0.46 (n = 53).

The life span depended clearly on food conditions. In the worst conditions at the beginning of the culture it ranged from 716 to 1,170 days (mean 896 days), while in the best conditions for snails kept singly it was 290–460 days (mean 377 days) (Table 4).

DEVELOPMENTAL ANOMALIES

The most frequent anomaly in late stages of embryonic development was underdeveloped shell or operculum. This was often associated with simulta-
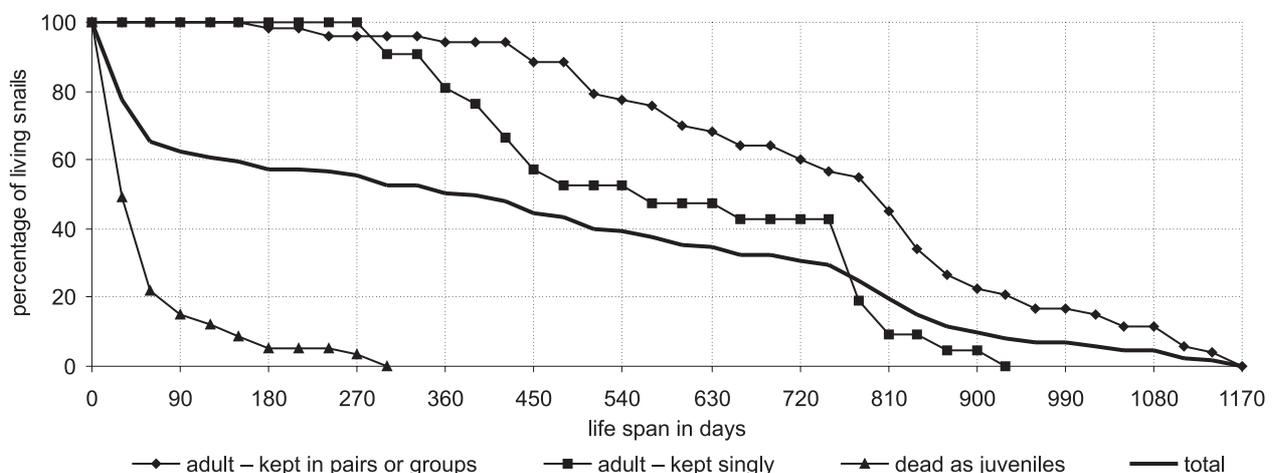

Fig. 116. Death of snails in laboratory

Table 5. Death 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	4	7	1	4	–	–	19
2	2	1	–	–	3	5	12	10	9	1	4	–	47
3	–	2	2	1	2	4	2	1	–	–	–	–	14
Σ	2	4	2	1	6	10	18	18	10	6	5	–	82

neous osmotic disturbances manifest as swelling of the whole body or of its particular organs (head, foot, mantle). The shells formed during such osmotic disturbances were clearly deformed (bowl-like or strongly dilated at the aperture) and most often relatively small (0.250–0.375 whorl, rarely more). The aperture was most often nearly circular or broad oval, less often kidney-shaped, and its height was up to 0.33 mm. Sometimes no shell was formed or there was only an irregular lump at the apex, formed by a backward--drawn mantle. Anomalies of operculum development were also observed, consisting in its lack or growth inhibited completely at a diameter of 0.10–0.17 mm. In some embryos without body swelling the shell at the moment of hatching was relatively small and did not cover the back, or the mantle was drawn backward and, instead of a shell, only an irregular lump occurred. Sometimes the shell was normally developed but there was no operculum, or the operculum was small, not covering the aperture. Examples: I. whole body much swollen, bowl-like shell 0.29 mm in diameter, aperture height and width 0.29 mm, number of whorls 0.375, no operculum; II. head and foot swollen, shell of 0.40 mm diameter, aperture height 0.33 mm, 0.625 whorl, operculum 0.21 mm in diameter; III. strongly swollen head, shell of 0.48 mm diameter, aperture height 0.33 mm, whorl funnel-like expanded towards aperture, 0.750 whorl, operculum diameter 0.22 mm; IV. body normal, shell diameter 0.28 mm, aperture height 0.20 mm, 0.375 whorl, operculum diameter 0.11 mm; V. body normal but mantle drawn backwards, instead of shell an irregular lump, operculum diameter 0.21 mm.

Most embryos with osmotic disturbances or only with underdeveloped shells died when still within the egg membranes, but some hatched and lived for a short time. Most (ca. 60%) died within 4 days after hatching, few lived longer, even up to 25 days, using the yolk resources (feeding was very rarely observed). The incubation period of such embryos was most often longer (even by half) than of those developing normally. In some snails slight osmotic disturbances ceased, leaving a trace on their shells in the form of increased whorl diameter or its decrease with a simultaneous height increase. Sporadically, double increments of shells were observed – an external one of a larger whorl diameter, formed during the disturbances, and an internal one of smaller diameter after the disturbance ceased.

Sometimes deformed shells got detached from the body and were found separately inside the egg membranes or in the container after hatching. Normally developed shells and opercula were detached sporadically in juvenile stages. In several cases a gradual protrusion of adult body from the shell and finally its detachment was observed. Such shell-less snails were usually active during 2–3 days, then the body swelled and the snail died.

Even a slight damage to initial whorls of the shell almost always resulted in osmotic disturbances (e.g. gonad swelling) and caused death. In adult snails sometimes there appeared osmotic disturbances without any visible reason, and sometimes they later ceased.

PREDATORS AND PARASITES

Egg cells, embryos at various development stages and hatched snails were fairly often damaged by parasitic fungi (probably Saprolegniales). The fungus development was very quick and most often within a few days its hyphae penetrated the whole incubated egg batches or the whole culture. Also within bodies of some living adult snails fungus hyphae were visible, and such snails could not retract into their shells and close them. Short after death the body became surrounded by radiating hyphae.

Out of 82 adult snails in the culture, four had trematode sporocysts in their gonads (trematodes brought probably with leaves). In three snails the sporocysts were noticed already at the juvenile stage, in one in the third breeding season. In early infected snails the penis, pallial parts of female reproductive organs and the gonad reached their normal size, but no yolk-containing oocytes appeared in their gonads (no female maturity). After infection of the adult snail, the number of eggs laid in the container decreased very considerably, though the partner had its gonad filled with oocytes. The sporocysts located in the gonads most often remained there till the snail's death and only sporadically they were expelled. No infection of other snails in the same container was ob-

served. Following infection further growth of the shell was much changed, and initially the aperture was most often directed downwards instead of laterally. The whorls formed were much more tightly coiled, descending and often scalariform (Figs 102, 106, 107). The largest regularly built shell in Figure 107 was 3.25 mm in height, 3.25 mm in diameter and had 4.875 whorls. The life span of infected snails did not differ from that of uninfected ones and ranged from 290 to 1,170 days.

In the studied lakes (Sosnowe near Sąpolno and a nameless lake near Korne) snails infected with tematode larvae were found, with characteristically deformed shells (Figs 108, 109). No infected snails were found in drainage ditches and oxbows of the Brda River.

In the culture, cocoons of *V. cristata* were wholly eaten by e.g. planorbids of the genus *Anisus* and other larger snails. Also dipteran larvae often damaged the cocoon capsules and consumed eggs.

POPULATION DENSITY AND STRUCTURE

V. cristata was the most numerous in the littoral of lakes and in some drainage ditches. In natural conditions, only locally dense populations were observed, in which other snail species were present only rarely. For example in the lake Sosnowe such populations formed during several years just next to the shore, in a layer of alder leaves with an admixture of oak. The surface area of such accumulations was most often ca. 1–2 m^2 (rarely more) and they changed their location from year to year. Examples: on November 30th 1991, 147 snails were collected from ca. 300 cm^2 leaves; November 2nd 1993 – 264 snails from ca. 300 cm^2 leaves (4,900 and 8,800 per 1 m^2, respectively). In August 1994, in a thick layer of duckweed covering a drainage ditch the density was 1,800 indiv./m^2.

In the laboratory, snails kept in pairs or groups laid a mean of 74.4 eggs per year. Considering the mean percentage of hatching (47.9%) and the percentage that reached maturity (62.9%), the population size may, within a year, increase by a factor of 22.4. In favourable circumstances (abundant food, absence of predators, e.g. *Anisus*) in two years (the mean life span) the number of snails could increase over 500 times; this is probably the reason for locally very dense populations.

According to FALNIOWSKI (1989a) populations of *V. cristata* never reach high densities.

The snails in such accumulations had their shells reaching 3.15 mm in diameter (rarely 3.50 mm) while the snails outside them attained a much larger size: in a neglected drainage ditch 3.70 mm and 3.125 whorls, in depressions at the shore of the lake Sosonowe 3.86 mm and 3.250 whorls, in an oxbow of the Brda River 4.35 mm and 3.500 whorls.

Snails collected from leaves floating near the shore of the lake Sosnowe (from November 1991 till September 1994), occurring in large densities, showed a fairly constant population structure between months. From November till April the most numerous (43–46%) were snails with shells of 1.50–1.95 mm diameter, though also smaller (0.50–0.95 mm) snails were found (0.6–1.7%), as well as much larger individuals of 3.00–3.45 mm (1.4–3.4 %). In May, the proportion of snails with shells of 2.00–2.95 mm diameter increased from 21 to 46%, while the percentage of shells up to 1.45 mm decreased (newly hatched snails were not collected). In August, the most numerous were snails with shells of 1.00–1.45 mm diameter, while individuals with shells exceeding 2.50 mm were rather few (2.2%). Till the end of September, the percentage of the latter snails increased slightly (7.4%) and the population structure was already close to that observed in winter.

In other places (water-filled depressions at the shore of the lake, oxbows, drainage ditches) at a lower density the population structure was much variable. Large snails of shell diameter ca. 3.50 mm were found sporadically throughout the year. Because of the time-consuming character of observations and disappearance of high-density populations these observations were not continued.

FOOD, FEEDING AND THEIR EFFECT ON GROWTH, REPRODUCTION AND LIFE SPAN

Aquatic plants, detritus and snail faeces are mentioned as food sources of *V. cristata* (FRETTER & GRAHAM 1962, PIECHOCKI 1979); a possibility of filtration was also considered (PIECHOCKI 1979).

My observations did not allow to establish the food composition precisely. No filtration was observed; the only way of feeding was scraping fine organisms off the substratum (leaves, bottom and walls of containers, surface film). Sometimes small holes were made in parenchymal tissues of tree leaves. A major part of faeces was almost always constituted by a much pulverised brown mass containing among others damaged diatom shells, parenchymal cells of leaves, living organisms (e.g. diatoms) and, seasonally, pine pollen, spores of fungi, sand grains. In the culture, the quickest shell growth occurred after placing in the containers black alder leaves (less often oak or poplar leaves) collected from places where the species occurred in natural habitats. The leaves were covered with a thin film of periphyton and had their natural colour (e.g. alder leaves from green-brown to dark brown). On the other hand, the shell growth was very slow when the only food was fallen leaves collected at a distance from water (e.g. from a garden). Small quantities of green-blue algae (Cyanophyta) on the leaves were consumed, but a

thicker layer (like Chlorophyta) caused avoidance of such places by feeding snails.

The food in stomachs was formed into pellets of a length depending on the size of the snail (ca. 0.3–0.4 shell diameter), and a roughly even diameter, most often 5–6 × smaller than the length. During digestion the food pellet rotated dextrally, most often 20–70 × per minute. Its rotation was sometimes stopped suddenly and was as suddenly resumed.

Faeces had most often a form of short, mucuscemented pellets of a diameter depending on the snail size (at a shell diameter of ca. 3 mm the faecal pellets were 0.12–0.18 mm in diameter and 0.30–0.50 mm in length). Sometimes the faeces were not divided into pellets but formed spirals of several whorls and total length of over 20 mm.

The feeding conditions had a significant effect on the growth rate, maturity and life span (Fig. 93, Table 4). While in unfavourable conditions the female maturity was attained as late as 1–1.5 year from hatching, in good conditions snails were able to reproduce in 2 months. The feeding conditions had only a slight effect on the shell structure (e.g. ratio diameter/number of whorls), shell size at maturity and – in snails kept in pairs or groups – ultimate size of shell at the moment of death. However, attaining a much larger shell size by snails kept singly was associated with a lower energy expenditure for reproduction (a similar dependence was observed in snails temporarily having no possibility to copulate and reproduce).

The number of eggs laid by snails kept in pairs or groups within a given period depended mainly on the feeding conditions. However, in poorer conditions the mean life span became much longer and the reproduction took place not only in one, but during two or three breeding seasons, thus increasing the total number of eggs produced.

OTHER INFORMATION

The heartbeat of *V. cristata* showed a considerable variation. During activity periods in juvenile snails it was 90–100/min., during rest and in older individuals it dropped to a single contraction every few minutes.

The maximum speed of movement was 0.35–0.37 mm/sec. (ca. 1.3 m/hr).

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