

A COMPLEX VIEW OF BREEDING STRATEGY AND LIFE-HISTORY IN ONE POPULATION OF *SPHAERIUM CORNEUM* LINNAEUS 1758 (BIVALVIA: SPHAERIIDAE)

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Abstract A population of the viviparous freshwater bivalve *Sphaerium corneum* L. from an artificial mill race subjected to irregular changes of water level was investigated using quantitative monthly sampling, paraffin histological sections and chromosome preparations. Unlike most of the previously studied populations of the genera *Sphaerium* and *Musculium*, that presented in this paper exhibits less synchronised life-spans. Despite two main birth periods, many specimens also release their broods individually during the season. An adult usually breeds at least twice in a season. The life span is more than one, usually one and a half years. All age and length classes are able to overwinter. Spermatogenesis and oogenesis apparently proceeds simultaneously from spring to autumn and they occur also in fully developed larvae which are still retained inside their parents' gills. Relationships between the observed breeding strategy and fluctuations of the environment are discussed, as well as the intraspecific and interspecific variation in life-history traits within the Sphaeriidae.

Key words *Sphaerium*, life-history, gametogenesis, precocious maturation

INTRODUCTION

Hermaphroditic, primarily freshwater clams of the family Sphaeriidae are unique among bivalves in exhibiting true viviparity. This is why the reproductive biology of the genera *Sphaerium*, *Musculium* and *Pisidium* has been attracting attention for more than 100 years – see Table 1 for summarized data on the first two genera and Heard (1965, 1977), Mouthon (2005a, b), Mouthon & Daufresne (2008), Pettinelli & Bicchierai (2009) for information on *Pisidium*. Most studies on sphaeriid reproduction and ecology have been carried out between the 1960s and the early 1980s.

Viviparity in these organisms is achieved by the development of larval stages in the cavities of their parent's inner demibranches, where special nutritive and protective structures are present. Fully developed young individuals, the so-called extra-marsupial larvae (Okada, 1935b), which are already self-sustaining (Beekey *et al.*, 2000), are retained until conditions are favourable for their release. Two general breeding strategies can be distinguished with respect to the individual life-span: semelparity (only one brood per year or per entire life cycle – see Heard, 1977) and iteroparity (more than 1 brood in the individual life span). The former is typical for some species

of the genus *Pisidium* s. l. (Heard, 1965; Mouthon & Daufresne, 2008; Pettinelli & Bicchierai, 2009; but see Mouthon, 2005a for an exception) and the latter for *Sphaerium* s. l. (Heard, 1977). The reproduction mode of the population depends both on individual breeding strategies and environmental conditions, as demonstrated for example by Mackie *et al.* (1976), Mackie (1979) or Mackie & Flippance (1983). Although the reproduction of both the semelparous and iteroparous individuals can occur continuously from spring to late autumn, the gonad activity of the individuals, and accordingly the hatching of young clams, is to a large extent synchronised and confined usually to two short periods of the season, in spring and autumn. As shown by field and laboratory experiments (Thomas, 1963; Gale, 1977), newborn individuals of some species (particularly genus *Musculium*) can grow very fast and complete the whole life span within 1 or 2 months but, on the other hand, they can also cease to grow for few months during periods of hibernation or aestivation (Thomas, 1963; Mackie *et al.*, 1976; Mackie, 1979; Hornbach *et al.*, 1982). Individuals born in spring usually reach maturity, reproduce in autumn and either die after first reproduction leaving overwintering generation of the offspring, or hibernate as adults and reproduce again the next year. Individuals born in autumn usually overwinter and reproduce in the following year. Individual life spans can range

Table 1 Overview of studies on life-histories of *Sphaerium* and *Musculium* species: hib. – hibernating stages; est. – aestivating stages; A – adult; J – juvenile (=postlarval stage short after release from the parent's body); N – newborn. Months of the year indicated by Roman numerals. Arabic numerals indicate duration of the life-span in months.

Species	postlarval life-span (months)	birth periods	hib.	est.	broods per life	Reference
<i>Musculium lacustre</i>	6 months to 1 year	summer, autumn	J		1	Mackie, 1979
<i>Musculium partumeium</i>	13 (late spring–late spring)	late spring	J	J		Way <i>et al.</i> , 1980
<i>Musculium partumeium</i>	13 (spring/next summer)	VI/VII, IX/XI				Way <i>et al.</i> , 1980
<i>Musculium partumeium</i>	12–13 (summer/summer)	summer before dry period	J	J		Thomas, 1963
<i>Musculium securis</i>	6 (spring–fall, fall–spring) to 15 months	early summer, autumn	J	J	1–2	Mackie, 1979
<i>Musculium securis</i>	VII–early VIII of next year, more than 1 year	late VII	A	J		Mackie <i>et al.</i> , 1976
<i>Musculium securis</i>	1 year	VII–VIII, IX				“
<i>Musculium securis</i>	1 year	continuous, mainly fall–winter and VI				“
<i>Sphaerium corneum</i>	4–8 (spring–fall, fall–spring)	IV, VIII/IX	J			Thiel, 1924
<i>Sphaerium corneum</i>	1–2 years	continuous, one or two peaks	A, J			Dussart, 1979
<i>Sphaerium fabale</i>	12 (summer–summer, fall–fall), 15 (summer–next fall)	early summer, late fall	A, J		1	Mackie, 1979
<i>Sphaerium rhomboideum</i>	14 (spring–next summer, summer–next fall)	spring–fall, peak in VII	A, N,		2	Mackie & Flippance, 1983
<i>Sphaerium rhomboideum</i>	12 (fall–fall)	whole season, with 3 maxima	A, J		1–2	Mackie & Flippance, 1983
<i>Sphaerium simile</i>	18–24	whole season, mainly summer and winter	A, J			Avolizi, 1971 cit in Zumoff, 1973; Zumoff, 1973
<i>Sphaerium solidulum*</i>	12	winter, VIII	J, A			Foster, 1932
<i>Sphaerium striatinum</i>	12 (spring–spring, autumn–autumn)	IV/VII, VIII/X	A, J			Hornbach <i>et al.</i> , 1982

* redetermined as *S. striatinum* by Hornbach *et al.* 1982

from a few months, usually with one period of reproduction (born in spring and reproducing in autumn or born in autumn and reproducing in spring), to more than 1 year (undergoing at least 2, but usually more, reproductive phases in 2 subsequent seasons). An interesting shift of this general pattern of reproduction was noted

by Foster (1932) in a population of *S. striatinum* (Lamarck), where the main period of maximum reproduction is from November to January and the second peak takes place in August.

The reproduction and breeding strategies of the most common European fingernail clam, *S. corneum*, has been studied already by Thiel

(1924; 1926) and revised by Heard (1977). Dussart (1979) has compared the seasonal size-frequency distribution of several *S. corneum* populations from different habitats. However, none of the authors has applied a complex approach combining regular sampling and evaluation of population structure over longer periods of time with histological and cytogenetical investigations. The aim of the present study was thus a detailed investigation of one population of *S. corneum* chosen as a model, using a combination of the above mentioned methods.

MATERIAL AND METHODS

Clams were sampled from an old mill-race connected to Radotínský brook, near Maškův mlýn ca. 2 km SW of Praha-Radotín, Český kras Protected Landscape Area. The water was running, with short periods of stagnation in the summer months and with irregular fluctuations of the water level caused by opening/closing of the lock gate. The bottom contained silt, leaves and organic material. Width of the channel was 1 m, max. depth 0.5 m. The characteristics as measured with YSI 556 MPS multiprobe (YSI Inc., Yellow Springs, USA) in September 2004 and April 2005 were as follows: water temperature 7.1°C (autumn)/13.1°C (spring), conductivity 1012–1043 $\mu\text{s}/\text{cm}^2$, pH 7.9 (autumn) –9 (spring), saturation by oxygen ranged from 63% after summer stagnation to 138% in spring.

From February 2004 to July 2005 (except for those periods when the site was covered with ice and snow), monthly samples containing usually 50 to 100 specimens were obtained by dredging the bottom within a randomly chosen square 0.5×0.5 m, using a bowl-shaped sieve. The specimens (further referred to as “adults” to be distinguished from larvae) were transported to the laboratory. They were killed by a short immersion in carbonated water, fixed in 70% ethanol, measured with a slide calliper to the nearest 0.1 mm and dissected under a stereomicroscope (magnification 16 to 25 times). The following characters were measured: adult shell length; number of brood pouches; number; shell length; and ontogenetic stage of the larvae in each brood pouch. Larvae, which attained earlier developmental stages but reached less than 50% (arbitrary chosen limit) of the shell length of

their kin in the same brood pouch were classified retarded. In further analyses, both adults and larvae were divided into length classes (to the nearest mm for adults and to 0.2 mm for larvae). The data were processed using MS Excel and Statistica 6.0 (Statsoft Inc.) to obtain the following descriptive statistics: average and median shell length of the adults, number and percentage of adults in the respective length classes, percentage of gravid (i.e. bearing larvae of any developmental stage) adults in each monthly sample, number of larvae within the respective length classes (counted per 100 adults).

From each sample, 5 adults of different shell length (range 5–10 mm) were examined karyologically: gonads were dissected out and hypotonised for 30 min. in deionised water; then fixed in 3 changes (5 min. each) of methanol:acetic acid (3:1 v:v); next dissociated with the aid of fine tungsten needles on a microscope slide in a drop of 60% acetic acid to make a suspension. Finally, the slide was placed on a hot plate (40°C) and the cell suspension was smeared on the slide, using a pair of fine tungsten needles.

Besides the quantitative samples, 2 to 5 additional specimens were sampled each month for histological sections. These animals were killed and fixed in 70% ethanol for a few days and the thicker shells were removed by hand. Thin and fragile shells were dissolved by immersion in Bouin’s fixative for 24–48 hours and the specimens were then rinsed in 70% ethanol to remove the residue of picric acid.

In both cases, the soft tissues were then dehydrated by successive immersion in 96% ethanol (6–12 hrs. according to the size of the animal), propanol (2×6–12 hrs.), propanol-methyl benzoate (3–6 hrs.), methyl benzoate (6–12 hrs.), benzene (12–24 hrs.), benzene-paraffin (12–24 hrs.) and finally paraffin. After stiffening, the blocks of paraffin were sectioned at 1–10 μm (average 5 μm) using a Leica microtome, stained (after removal of the paraffin using xylene and series of 96%–80%–60%–40% alcohol) in Masson’s triple stain and, after dehydration in propane and xylene, immersed in Canada Balsam.

RESULTS

Population size and composition In 2004, periods of population decline (by 50%) (estimated from the sample size) were recorded, in March, May

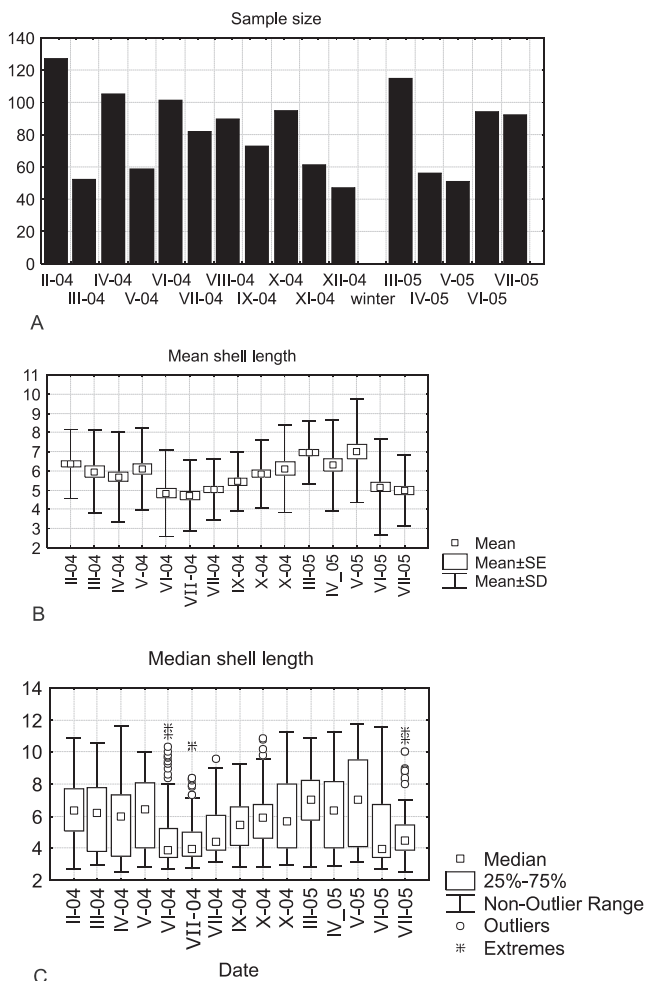


Figure 1 Breeding strategy in *Sphaerium corneum*. Average and median shell sizes. A – Sample size, B, C – mean (B) and median (C) shell length in monthly samples.

and December (Fig. 1). In March and May, the declines were followed by an immediate increase and changes in the distribution of length classes in favour of the smallest post-larval individuals.

The shell length of free-living animals ranged from 2.5 (smaller newborn clams) to 12 mm. For average and median shell lengths and distribution of the length classes in monthly samples see Figs 1 and 2, respectively. All length classes in the range 3–9 mm were present throughout the year. Shell length of most newborn clams ranged from 3 to 4 mm, newborns of the length class 2–2.9 mm were less numerous. The length class 3–3.9 mm was the most frequent one from March to August 2004 and also in June and July of the following season. Its maximum frequency was observed in June, when, together with the 2–2.9 length class, it made up more than 60% of the population. A

less prominent peak in the occurrence of smallest clams occurred in March–April. Adults with shell length between 6 and 9 mm were present in all monthly samples; their smaller percentual proportion in June–August was an effect of the rapid increase in population size after the release of newborns rather than of the dying out of the parental generation. In autumn, the proportion of these length classes again increased, as the generation born in summer grew larger.

The largest individuals (shell length > 9 mm) were also the least numerous during the whole period, except for May 2005, when the 3 largest length classes together made up more than 30% of the population. However, both this maximum and those reached in June and November 2004 were followed by immediate declines in number of these large individuals. In October, the percentage of the largest individuals started increasing again and remained unchanged (6–7% of the population) until the following spring. Most empty shells found at the collection site had lengths under 5 mm or above 9 mm, which implies that these size groups exhibit the highest mortality.

Gravidity and fecundity The percentage of all gravid adults (bearing larvae of any developmental stage) in the monthly samples ranged from 24% (June 2004) to 95% (March 2005) (Fig. 3). Brood pouches with developing F1 embryos were already present in clams of the length class 3–3.9 mm, with the average percentage of gravid individuals reaching 13.5% and the average number of F1 per adult low, at 0.07. Gravid individuals of this length class occurred scattered throughout the whole season. Individuals larger than 7 mm (including those examined shortly after death, the tissues of which had not yet decayed) were all gravid and the average number of larvae per adult increased to 17.7 in the length class 10–10.9. The maximum number of brood pouches found in one adult was 12, with a total of 45 F1 embryos and larvae.

Embryos and larvae of different developmental stages were usually found within the demibranches of the adult. During the autumn and winter months the total number and percentage of the youngest developmental stages increased substantially (Fig. 4).

Shell length of the extramarsupial larvae (EML) ranged from 2.4 mm to 3.6 mm. They ranged in

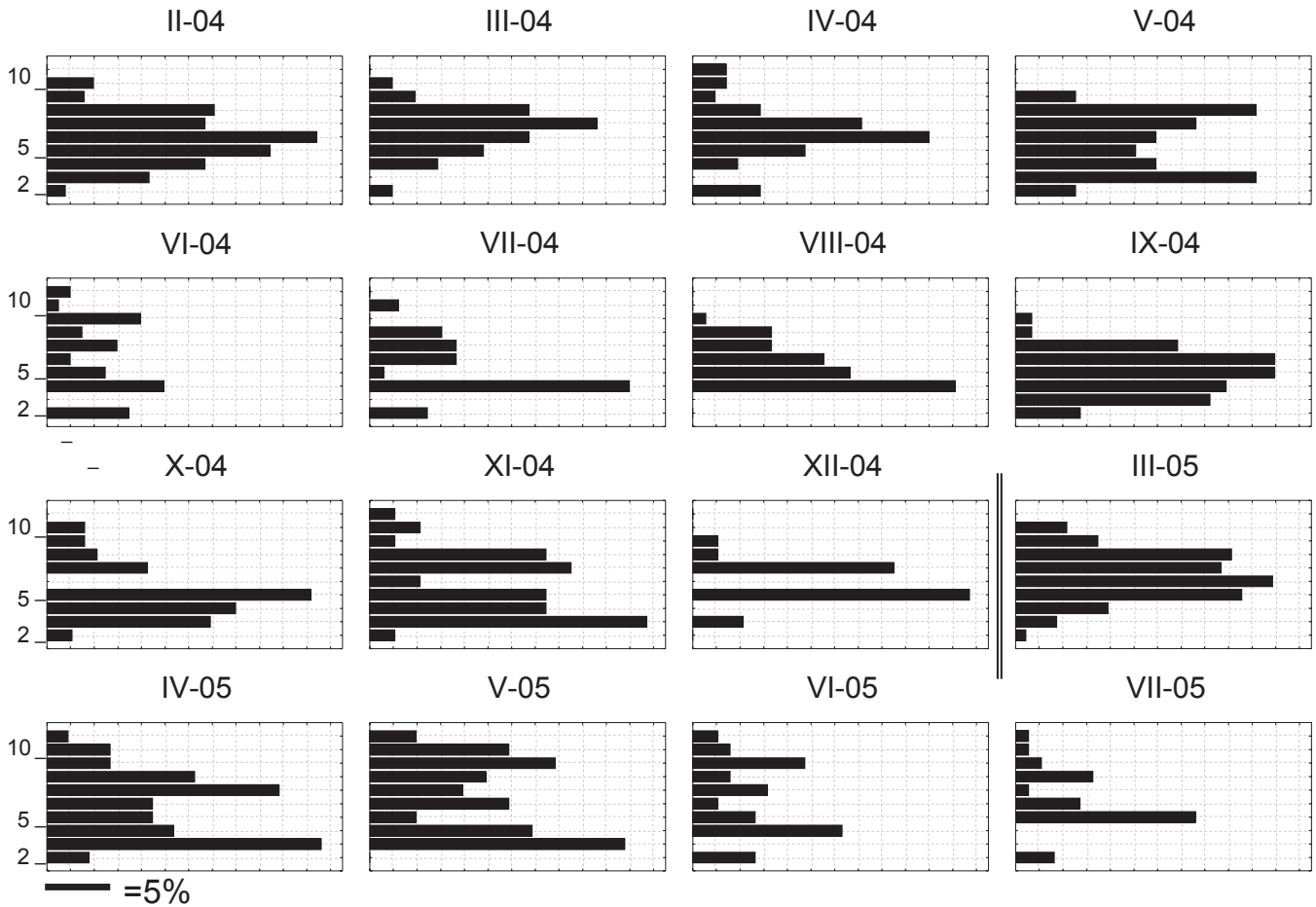


Figure 2 Breeding strategy in *Sphaerium corneum*. Size-frequency histograms of the adult length classes in monthly samples. Each length class ranges 1 mm. The frequencies are given in percent. A vertical line separates the seasons 2004 and 2005.

number from 1 to 7 per parent, and occurred only from April to August in individuals larger than 7 mm. The maximum percentage of individuals with EML was reached in May (22% in 2004 and 29% in 2005), followed by rapid decline (Fig. 3).

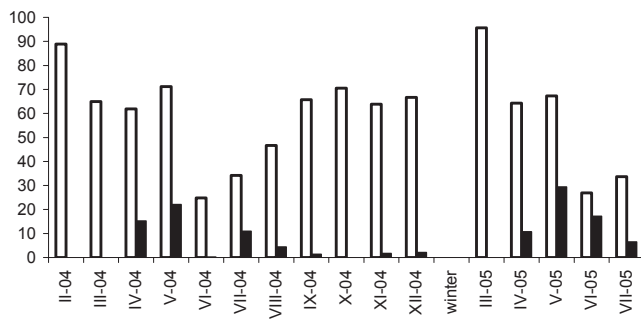


Figure 3 Breeding strategy in *Sphaerium corneum*. Total percentage of gravid adults (bearing larvae of any developmental stage, white columns) and percentage of adults bearing EML (black columns) in monthly samples.

In July and August 2004, few (12 and 6.7 per 100 adults, respectively) EML were still present in the demibranches of the parental generation, and in the autumn they were absent, which implies either completion of their development and release from their parents' organisms, or death of the large parental organisms together with their descendants.

The percentage of retarded larvae was under 5% in the most monthly samples (Fig. 3).

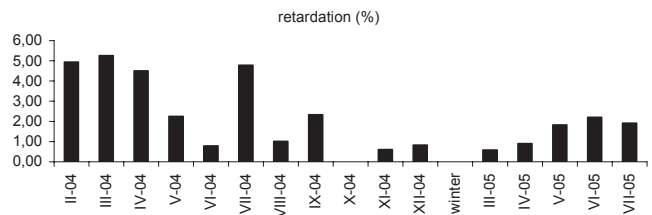


Figure 4 Breeding strategy in *Sphaerium corneum*. Percentage of retarded larvae in monthly samples.

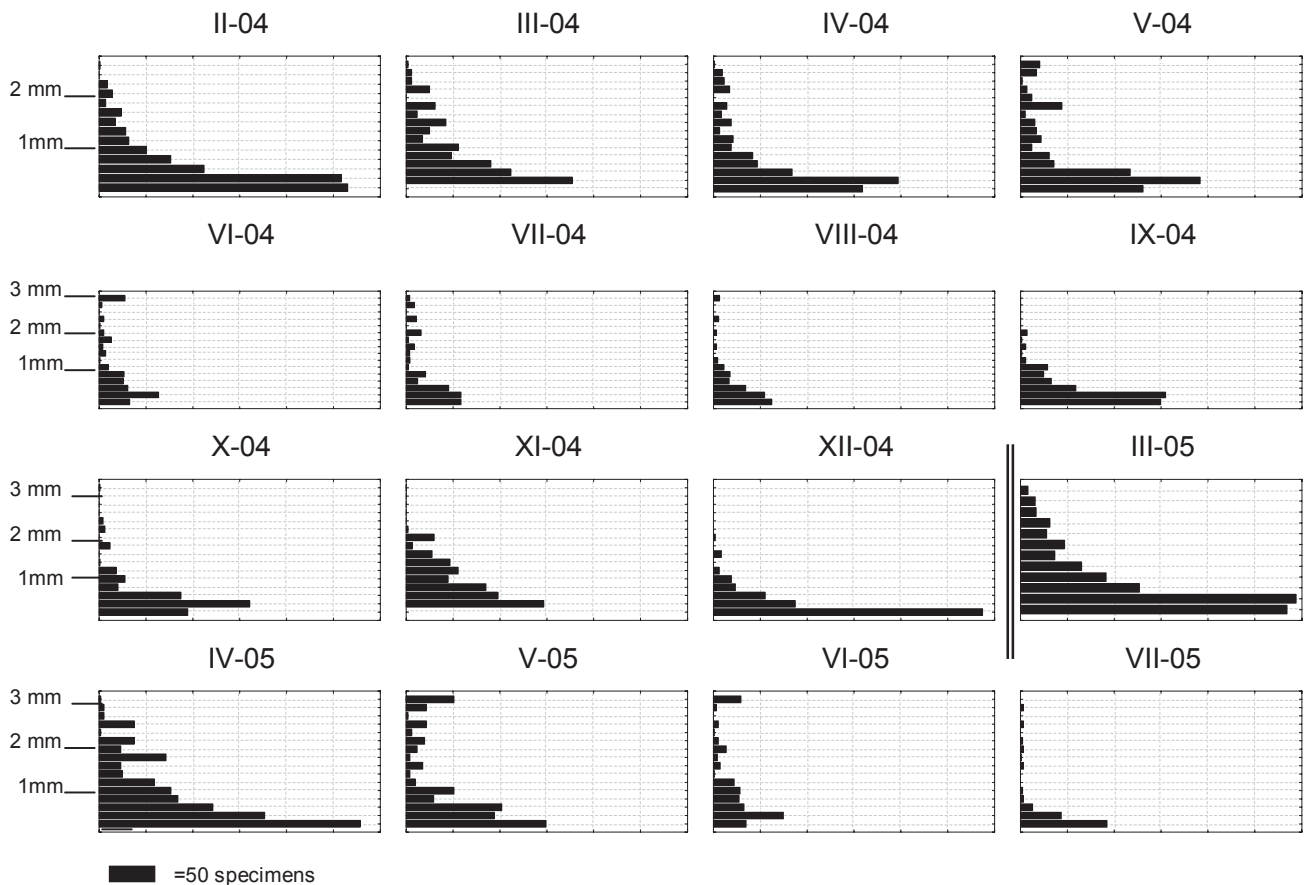


Figure 5 Breeding strategy in *Sphaerium corneum*. Size-frequency histograms of larval length classes. Each length class range is 0.2 mm. The frequencies are expressed as number of larvae per 100 adults. The vertical line separates the seasons 2004 and 2005.

Gametogenesis The course of meiosis is achiasmatic (Keyl, 1956; Kořínková & Král, 2011) with a diffuse stage occurring after pachytene and followed by postpachytene stage (Kořínková & Král, 2011). Chromosome preparations containing meiotic divisions were obtained from animals collected between May and July. In May, mainly pachytene and diffuse stages were found, whereas postpachytene and metaphase I stages predominated in material from June and July. In July, sporadic occurrences of meiosis II plates (prophase and metaphase II) were also noticed. A few pachytene stages were obtained also from July samples and few postpachytene plates from November samples, which implies a possible second, smaller, peak of gametogenesis in the autumn. As the ovarian and testicular part of the gonad were usually smashed together on preparation of the chromosome plates, it was not possible to distinguish between spermatogenetic and oogenetic meiotic divisions.

Histological sections revealed in most of the specimens collected throughout the season

(February–December) simultaneous occurrence of oogenesis and spermatogenesis. The oogonia and primary oocytes in the ovarian part were present in all monthly samples, but most abundant from February to April and again from September to December. Analogically, spermatogonia, spermatocytes and spermatids appeared in all monthly samples, but with spermatozoa being most numerous in February and September–October. All of the adults undergoing gametogenesis were at the same time bearing larvae of various developmental stages. In one EML found inside a parent in April, the ovarian part of the gonad was found, though no oocytes were probably present at that stage.

DISCUSSION

The study brings forward valuable data on the breeding and life-histories of a thoroughly sampled population of *S. corneum*.

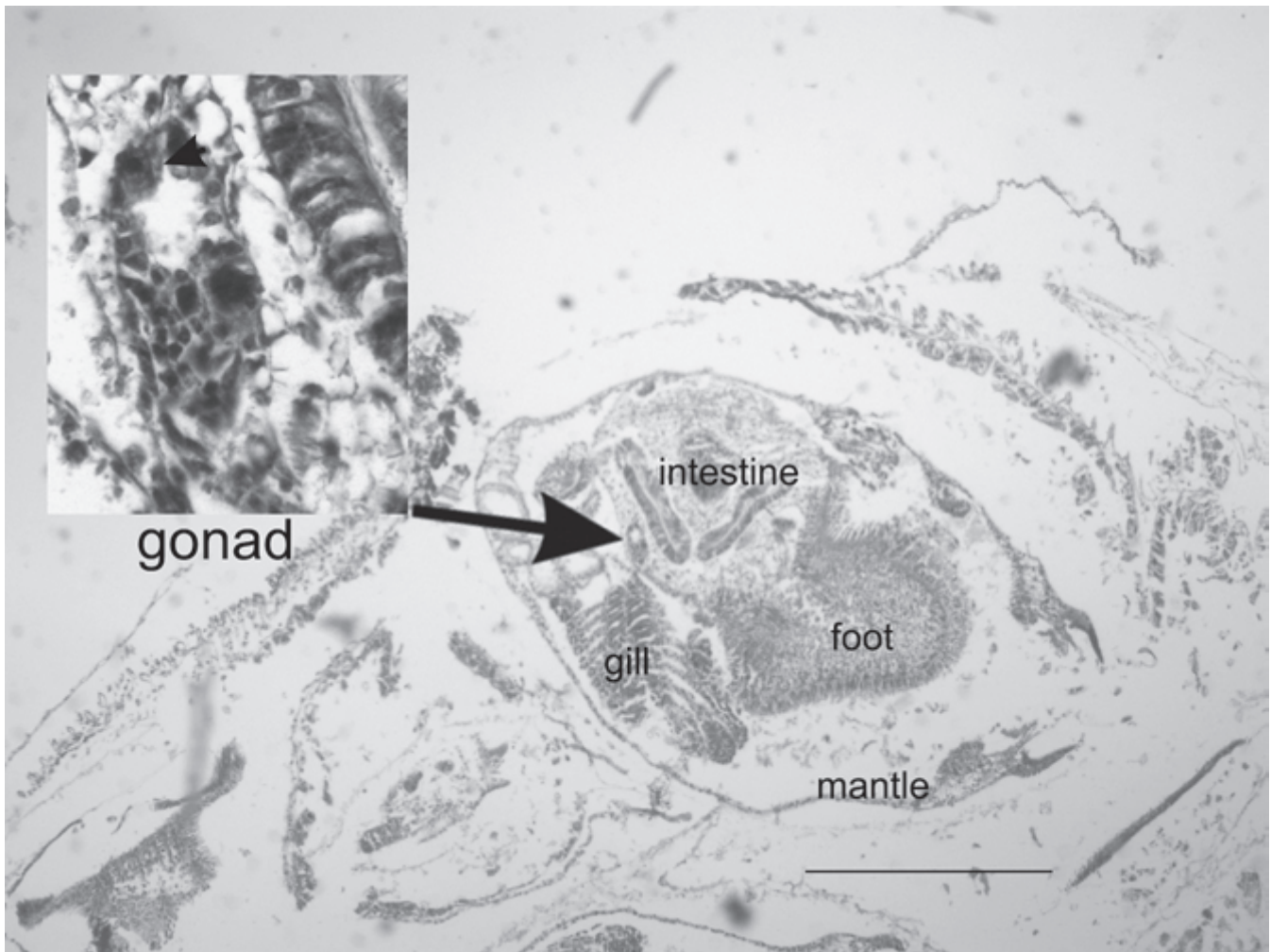


Figure 6 Part of a paraffin section through adult *Sphaerium corneum* (shell length 8 mm, sampled in May 2004), showing well-developed extramarsupial larva with oogonia (arrowhead) in the gonad (detailed view in the left upper corner). Stained with Masson's triple stain. Scale bar = 1 mm.

Adult animals release their first brood at the shell length of 7 mm and the young postlarval individuals are born at a length of 2–4 mm. This corresponds to the previous findings from other populations by Thiel (1924) and Dussart (1979). The present results also corroborate the previous authors' estimate of the life span duration (8 months to more than one year) as well as their finding that the reproduction of *S. corneum* occurs through most of the season with one or two peaks. As is evident from a comparison of recent and previously published data, the timing of reproduction peaks in particular populations apparently differs according to environmental factors.

In the population examined here, the distribution of shell lengths throughout the season also implies two birth periods (April and June),

more or less coincident with the death of older animals. Nevertheless, the parental generation is never fully replaced by the offspring. Part of the population of young adults with shell length 7–8 mm probably continues growing after their first brood to produce at least one more litter either in the same season or after overwintering. Secondly, reproduction of a small proportion of individuals is probably not synchronised with the rest of the population. This results in the overwintering of animals of all sizes and ages and in a broad overlap of generations. A similar pattern was revealed e.g. by Zumoff (1973) in a population of *S. simile* (Say), on the basis of histological sections and dissections of small monthly samples. Also *S. corneum* populations from England investigated by Dussart (1979) exhibited a generation overlap which, as tenta-

tively suggested by the author, might be a common feature of *Sphaerium* from habitats that are not periodic.

On the contrary, in other previously examined sphaeriid species, mostly from temporary waters of North America, the parental generation often completely dies out within a few days of giving birth to offspring (Foster, 1932; Thomas, 1963; Mackie, 1979; Way *et al.*, 1980). The collection site under investigation was an artificial mill-race. Unlike in natural, flooding or drought-prone habitats, the fluctuations of water current, water level and other characteristics caused by economical exploitation must be to a large extent unpredictable. The heterogeneity of breeding strategies in the population might be a sort of "bet-hedging" as a response to the varying conditions.

Comparison between the two seasons of investigation suggests that the shift of the coldest period to February in the year 2005 could have partly delayed both the growth, production of the early-spring litter, and death of the largest individuals. In 2004, January was the most severe month (see the website of the Czech Hydrometeorological Institute, <http://www.chmi.cz/meteo/ok/infklim.html> for maximum, minimum and mean temperatures), whereas in February the temperatures were usually above zero and most clams were active. In 2005, a relatively mild (though with snow and ice cover) January was followed by a cold February, which made a monthly collection impossible. The data from March 2005 did not indicate such an important increase in number of the youngest clams, as that in March 2004. An even more dramatic change of population size and life-history pattern has been observed by Mouthon & Daufresne (2008) in a population of *Pisidium amnicum* as a consequence of a summer heatwave.

The meiotic divisions, which culminate in May–July, followed by substantial increases in the number of the earliest embryonic stages in September–October, imply gamete production and fertilization in autumn. As reported by Okada (1935a) for *S. heterodon*, the gonads of *S. corneum* contain immature gametes throughout the whole season, though there are certain peaks in production of primary oocytes and spermatozoa. Primary oocytes probably undergo division after fertilization, as suggested by Okada (1935c) and Woods (1931).

The large proportion of embryos in brood pouches in February and March 2004 can be explained either by a continuation of fertilization until late in the season, or by interrupted development of the F1 due to hibernation. It seems probable that most individuals underwent multiple fertilisations during one autumn-winter period. This corresponds to the formation of more brood pouches of a differential developmental stage and to the production of two (exceptionally even more?) litters in the following season. In some animals the gametogenesis can also have different timing, as evident from the occurrence of meiotic divisions in chromosome preparations from November. The small (3–4 mm) clams, which are already gravid shortly after hatching probably undergo gametogenesis during the last stages of larval development. This phenomenon of the so-called precocious maturation in *S. corneum* has already been described by Thiel (1928) and Heard (1977), who found mature gametes in histological sections of EML. Though no meiotic chromosome plates were found in larvae from this locality, they were scarcely present in chromosome preparations from other populations (Kořínková, unpublished), and evidence for well-developed gonads in the EML was found in the course of this study. Fertilization of such precociously mature individuals supposedly occurs immediately after birth, if not within the parent's demibranches by means of sperm from their own siblings. Self-fertilization might also take place, as evident from the arrangement of the genital tract (Woods, 1931; Okada, 1935a) and from the simultaneous occurrence of mature ova and sperm (Woods, 1931; Heard, 1965; Pettinelli & Bicchierai, 2009; this study). Precocious maturation is thus another aspect contributing to the heterogeneity of breeding strategies in the population – individuals born in spring and becoming gravid immediately can produce their first litter in the summer of the same season.

Differences in growth and developmental stage might be encountered among individuals of the same age within one brood pouch. This so-called larval retardation has already been found by Foster (1932), who noticed that in brood pouches containing higher number of embryos, some can be considerably smaller. Mackie & Flippance (1983) suggested that the suppressed larvae not only reach smaller size, but are in an earlier developmental stage, and most of them probably

die or never mature. The authors supposed the suppressed larvae to be "sacrificed" for their kin to grow larger.

Hetzel (1993) opined that growth suppression of some larvae in comparison with their kin in the brood pouch also represents one strategy to increase temporal heterogeneity of reproduction: in favourable conditions, retarded individuals may continue growth and development up to the extramarsupial stage. The population examined in this study exhibited quite a low percentage of retarded larvae (at most 5%), the frequency of which increases as the growth and development of the brood pouch and of most enclosed larvae proceeds. Considerable size variation (by ca. 50%) was not exceptional even among extramarsupial larvae. This would support the hypothesis of Hetzel (1993).

The present study gave a detailed view of breeding strategy of one population inhabiting a small drain with seasonal fluctuation in some environmental conditions. The population differs from most of the previously studied populations of 8 species of the genera *Sphaerium* and *Musculium* by its considerable variation in individual breeding strategies. This is accomplished by a life span of up to one and a half years (compare with other population of *S. corneum*; Thiel, 1924), combined with overwintering at any stage, a capability for precocious maturation and probably also self-fertilization, and ability to produce more broods at various times of the season. Sphaeriids exhibit variation in life-history tactics on both the specific (Heard, 1965; Mackie *et al.*, 1976; Way *et al.*, 1980) and generic levels (Heard, 1965, 1977). Thus, in the case of such a euryvalent species as *S. corneum*, examination of greater range of populations by a combination of quantitative sampling with the preparation of histological sections and chromosome preparations is desirable to describe more fully the variation in its life-history strategies.

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