Acta Adriat., 31 (1/2): 139—151 (1990)

YU ISSN: 0001-5113
AADRAY

UDC 594:612.6 (262.3)
Original scientific paper

SEXUAL CYCLE AND BIOMETRY OF DATE SHELL, *LITHOPHAGA LITHOPHAGA* LINNAEUS (MYTILIDAE)

SPOLNI CIKLUS I BIOMETRIJA PRSTACA, *LITHOPHAGA LITHOPHAGA* LINNAEUS (MYTILIDAE)

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Sexual cycle and biometry of date shell *Lithophaga lithophaga* were studied on samples collected from the area of Split (middle Adriatic) during 1987 and 1988. The results show that these shellfish are ripe and deposit their sexual products during the warmest part of the year (July, August and September) at sea water temperature over 22°C.

Variations in weight of the flesh and shell are due to the changes in the relationship between shellfish growth, growth of somatic and gonad tissue, and loss of the material during spawning.

INTRODUCTION

The date shell *Lithophaga lithophaga* are distributed in the Mediterranean and Red Sea and in the warm Atlantic Ocean waters (Nordsieck, 1969). Along the eastern Adriatic coast they are most abundant in the endolition of the medio and infralittoral zones, down to 8 m depths (Horvath, 1963; Stjepčević, 1967; Peres and Gamulin-Brida, 1973; Legac and Hrs-Brenko, 1982 and others). They bore holes in coastal rocks where they are attached by byssus threads.

The literature on the species of genus *Lithophaga* mainly present the data on the mechanism of their boring the substrate they live in (Pierotti *et al.*, 1965; Agius, 1976; Bolognani, 1979; Barthel, 1981; Lee-shing Fang and Pouyan Shen, 1988).

The papers on the Adriatic date shell are rather scarce. They are mainly concerned with the burrowing rate in different kinds of limestone (Kleemann, 1973), space competition with other rock-hole living species (Kleemann, 1974) and biochemical composition (Tudor, 1987). Data on their

reproductive cycle have not been reported to date. Due to their highly appreciated flesh date shell are harvested so that in some areas their populations have seriously declined and coastal rocks damaged. In some areas along the Adriatic coast the local authorities prohibited their collection to allow *Lithophaga* population to recover. Despite these regulations their harvesting was continued and therefore comprehensive studies of their general biology, particularly their reproduction, recruitment intensity and growth rate are called for.

This study is the first report on the reproductive cycle of date shell. The timeing of gametogenesis, spawning and resting, effects of main environmental factors (temperature and salinity) as well as the variations of some biometrical parameters (length, wet flesh and shell weight) during reproductive cycle were established.

MATERIAL AND METHODS

The samples of date shell *Lithophaga lithophaga* were collected from about 2 m of the littoral zone in the Split area (middle Adriatic Sea) between March 1987 and September 1988. The water temperature was recorded at the time of date shell collecting at monthly intervals and water was also sampled for salinity determination.

Sex ratio and gonadal cycle

In the laboratory, individuals were opened and the position of gonads and sex ratio determined.

The stages of gonadal cycle were determined by a bit modified Lubet (1959) gonad classification for mussels, using standard histological techniques for gonad sections, so:

stage 0 — resting gonads

stage I — proliferation of the gonad and diferentiation of gametes

stage II — advanced gametogenesis

stage III — ripening of gametes
- III a — maturation and spawning
- III b — spent stage

Microscopic records of histological gonad cross-sections were made under 100 times magnification.

Biometrical relationships

Samples for determination of the total shellfish wet weight (with intervalvular water), shell and flesh wet weight were classified by shell length within the range of 30 to 100 mm. Length of the shell was taken along the longest shell axis.

The length of each date shell was measured by vernier caliper, and weight by electronic balance.

Statistical analysis

Statistical analysis was applied to determine the weight-length relationship of shellfish. The relationship between weight and length was examined by the allometric model

$$y = ax^b$$

where y — the dependent variable, x — independent variable, a — constant and b — parameter denoting the rate at which the variable y — changes in dependence of x. Upon logarithmic transformation of y and x ($\log_{10}$), the equation parameters a and b were determined by linear regression analysis. The examination of differences between parameters of several regression lines was performed by the covariance analysis (S o k a l and R o h l f, 1969).

RESULTS

Sex ratio

In *Lithophaga lithophaga,* like in the majority of shellfish, sexes are separate. Gonads are in the dorsal part of the body having the shape of a pair, symmetric and elongated glands above the gut.

Sexes may be macroscopically distinguished by colour. Female gonads are orange-redish while male gonads are greyish-white. Hermaphroditism has never been reported.

The sex ratio was determined on the basis of gonad colour in a total of 613 analyzed individuals. It was found to be 1.3 : 1 in favour of males (56.3 % ♂, 42.4 % ♀ and 1.3 % of undifferentiated gonadal tissue).

Gonads were white in individuals smaller than 30 mm. The smallest individual with orange gonads was 32 mm long and sexually active.

Gonadal cycle

Histological analysis of gonad tissue showed that sexual activity has a normal annual cycle as follows:

Stage 0 — resting gonad

During the cold season, from December to early April, at sea water temperature lower than 14°C, shellfish are in a resting stage. Gonad sections showed only a few shrunken follicles between proliferated connective cells. At some sections of follicle walls primordial cells may be found (Fig. 1a, b).

In this gonad stage the sex was not easily identifiable by histological analysis of tissue but could be distinguished by colour.

Stage I — proliferation of the gonad and differentiation of the gametes

During April, May and partially June, when the sea water temperature ranged from 14 to 20°C, primordial cells at walls of shrunken follicles were observed to begin to multiply. Oogonia could be distinguished from spermatogonia. The size of the follicle tended to become larger (Figs. 2a, b; 3a, b).

Gonad tissue could be distinguished by colour like in the preceding stage.

Stage II — advances gametogenesis

By the temperature increase (over 20°) gametogenesis advanced (from June to beginning of July). Follicle became larger and connective tissue was reduced. Meiotic cleavage of oogonia and spermatogonia was recorded while a part of cells remained as an important stock for later spawnings. Pear-shaped oocytes attached to the follicle walls by stalks spread to the follicle lumen (Fig. 4a, b).

Stage III — ripening of the gametes

IIIa — maturation and spawning

During the warmest part of the year (early July, August and late September) when temperature ranged from 22 to 27°C, follicles contained ripe or almost ripe gametes. Oocytes were of more regular shape and polygonal. Ripe oocytes tore off the walls almost filling the follicle (Fig. 5a, 6a, 7a). Ripe spermatozoa radiate in with flagellae towards the follicle centres (Figs. 5b, 6b, 7b). Connective tissue was almost completely absent and thickened between follicles. A number of subsequent maturing stages during which ripe cells are released followed.

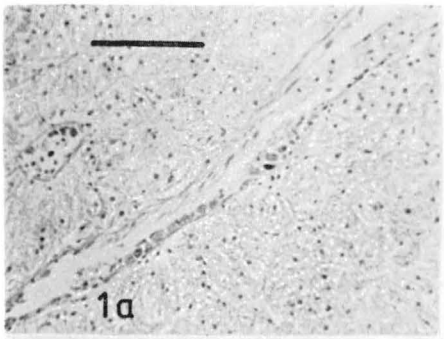
IIIb — spent stage

With the decrease of water temperature in autumn the gametogenetic activity was also reduced. In November when the sea water temperature was lower than 20°C sexual activity completely stopped and the process of breaking down and resorption of unreleased sex cells began (Fig. 8a, b). Resting period occurred in December (Fig. 1a, b). Further observations of gonads tissue state as affected by temperature showed that the gonads are ready to be recycled.

Seasonal variation of wet weight and growth rates

Results of calculations of parameters of allometric equations giving the relationship between weight and length of date shell are presented in Table 1. The analysis of covariance showed intercepts and exponents of equations to be homogeneous that is showed no significant differences ($P>0.05$). This is indicative of the fact that there is no difference in total shellfish weight (with intervalvular water) when the length is fixed. Common exponent of allometric equations was 2.806 showing no significant difference ($P>0.05$) from the exponent of isometric growth ($b = 3$) in total weight in relation to shell length.

Figs. 1—8. *Lithophaga lithophaga*. Light micrograps of the gonads sections at various stages in the annual cycle (scale bars — 100 μm). 1. resting stage, a-female and b-male (28. 12. 1987); 2. proliferation, a-female and b-male (14. 4. 1987); 3. proliferation, a-female and b-male (17. 6. 1987); 4. gametogenesis, a-female and b-male (5. 7. 1987); 5. sexual maturity and spawning, a-female and b-male (31. 7. 1987); 6. sexual maturity and spawning, a-female and b-male (12. 8. 1987); 7. sexual maturity and spawning. a-female and b-male (19. 10. 1987); 8. spent stage, a-female and b-male (5. 11. 1987).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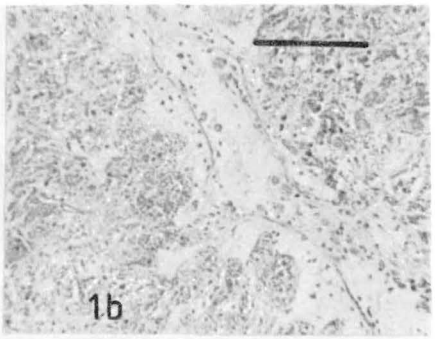
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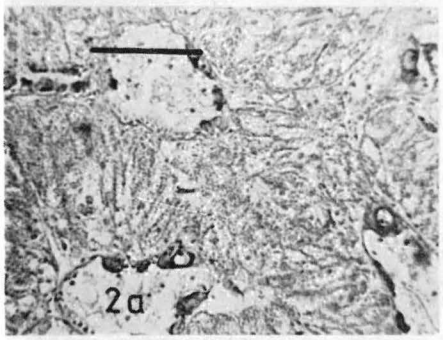
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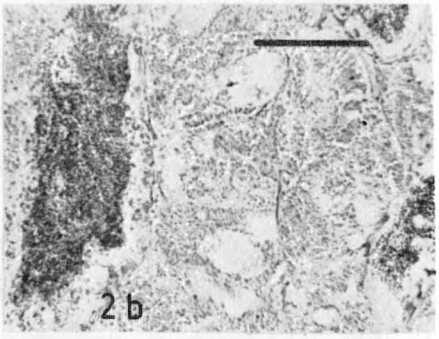
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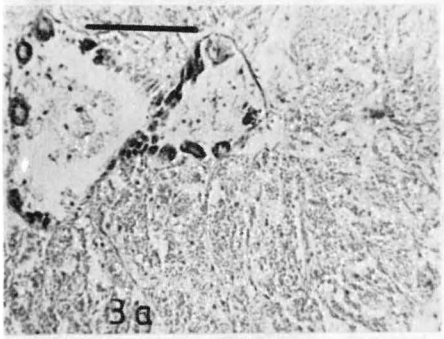
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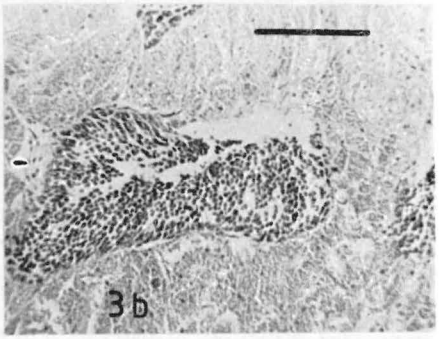
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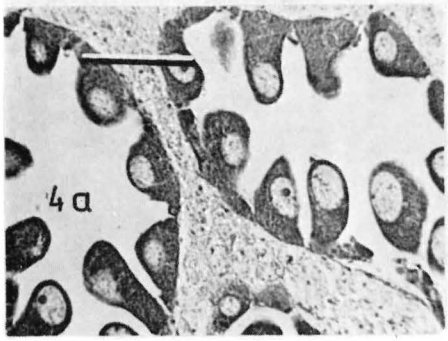
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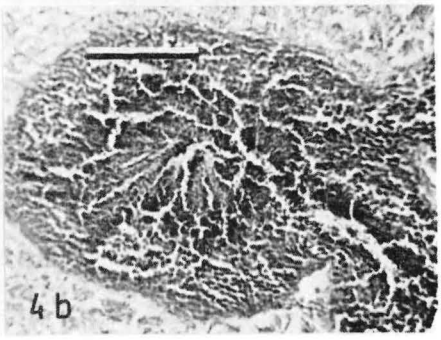
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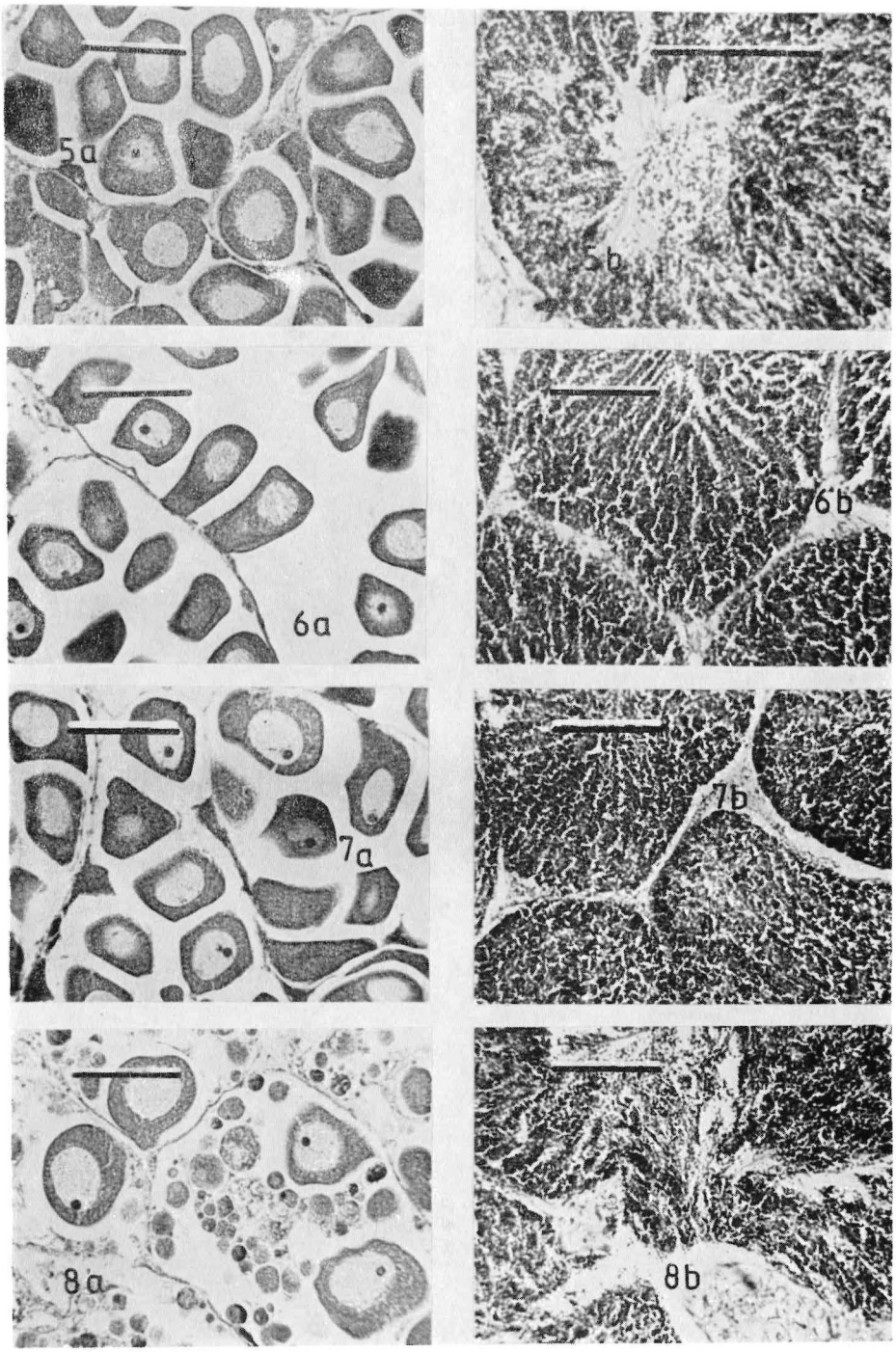
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Table 1. *Lithophaga lithophaga*. Parameters a and b for allometric equation $W = aL^b$, describing relationship between total weight (W, g) and shell length (L, cm)

Sampling date	Intercept a	Exponent b	No. animals n	Coefficient of determination r^2
1987				
28 Mart	0.096	2.681	34	0.972
14 Apr.	0.049	2.989	33	0.96
18 May	0.057	2.91	31	0.958
17 June	0.76	2.743	30	0.974
31 July	0.057	2.948	33	0.972
19 Oct.	0.062	2.888	38	0.966
5 Nov.	0.097	2.635	35	0.963
28 Dec.	0.063	2.843	32	0.966
1988				
8 Feb.	0.083	2.694	31	0.963
5 Apr.	0.072	2.792	36	0.963
22 Apr.	0.077	2.747	34	0.98
5 July	0.069	2.823	42	0.973
12 Aug.	0.065	2.844	40	0.985
11 Sep.	0.059	2.883	38	0.978

Table 2. *Lithophaga lithophaga*. Parameters c and d for allometric equation $F = cL^d$, describing relationship between wet flesh weight (F, g) and shell length (L, cm)

Sampling date	Intercept c	Exponent d	No. animals n	Coefficient of determination r^2
1987				
28 Mart	0.028	2.825	34	0.95
14 Apr.	0.01	3.326	33	0.961
18 May	0.012	3.267	31	0.952
17 June	0.02	2.904	30	0.948
31 July	0.009	3.376	33	0.958
19 Oct.	0.012	3.202	38	0.929
5 Nov.	0.031	2.657	35	0.915
28 Dec.	0.016	2.986	32	0.957
1988				
8 Feb.	0.021	2.883	31	0.942
5 Apr.	0.028	2.735	36	0.92
22 Apr.	0.021	2.845	34	0.967
5 July	0.021	2.868	42	0.942
12 Aug.	0.018	2.969	40	0.966
11 Sep.	0.015	3.045	38	0.953

Table 3. *Lithophaga lithophaga*. Parameters e and h for allometric equation $S = eh$, describing relationship between shell weight (S, g) and shell length (L, cm)

Sampling date	Intercept e	Exponent h	No. animals n	Coefficient of determination r^2
1987				
28 Mart	0.047	2.447	34	0.927
14 Apr.	0.018	2.913	33	0.927
18 May	0.022	2.82	31	0.923
17 June	0.033	2.581	30	0.94
31 July	0.018	2.956	33	0.949
19 Oct.	0.023	2.809	38	0.932
5 Nov.	0.048	2.421	35	0.939
28 Dec.	0.031	2.671	32	0.93
1988				
8 Feb.	0.035	2.549	31	0.943
5 Apr.	0.029	2.673	36	0.947
22 Apr.	0.027	2.707	34	0.961
5 July	0.027	2.732	42	0.944
12 Aug.	0.023	2.794	40	0.973
11 Sep.	0.024	2.769	38	0.964

Parameters of allometric equations showing the relationship between flesh weight and shell length at the moment of sampling are presented in Table 2. The equations are significantly heterogeneous ($P<0.05$) as to the intercepts and exponents.

The shell weight-length relationship at the moment of sampling is shown by the parameters of allometric equations in Table 3. Segments and exponents are significantly heterogeneous ($P<0.05$).

Flesh weight and shell weight for a hypothetical date shell of 7cm shell length (taken as standard) were calculated from the regression equations (Tables 2 and 3) of each sampling. Seasonal variations in flesh weight and shell length of standard date shell are given in Fig. 9.

Flesh weight varied from 5.3 to 6.9 g or from 31 to 41% of the total weight of standard date shell in the period between March and December 1987, while it varied from 5.3 to 5.8 g or from 31 to 34% of the total weight from February to September 1988. Shell weight ranged from 5 to 5.7 g or from 30 to 34% of the total weight of standard date shell for the entire study interval. Results show no difference that is almost insignificant variations of flesh mass during the years of observations.

DISCUSSION

A seasonal reproductive pattern is the characteristic of most bivalve molluscs that live in temperate latitudes. Timing of gametogenesis and spawning of individual species depends on the seasonal changes of environ-

mental factors, and is regulated by neuroendocrine secretion controlling factors (Orton, 1920; Lubet, 1959, 1980/1981a, b; Sastry, 1979; Seed, 1976 and others). Since *Lithophaga lithophaga* are distributed in the European warm seas, their reproduction was expected to tatke place in summer. The histological examinations of date shell gonad sections and seasonal changes of flesh weights showed one annual reproductive cycle with several spawnings from late June to mid October at water temperature over 22°C. On the contrary, the reproduction of *Mytilus galloprovincialis,* close relative species of date shell, extends all year round with two conspicuous spawnings in December and March (Hrs-Brenko, 1971) or only one in January-February (Da Ros *et al.*, 1985) in the northern Adriatic.

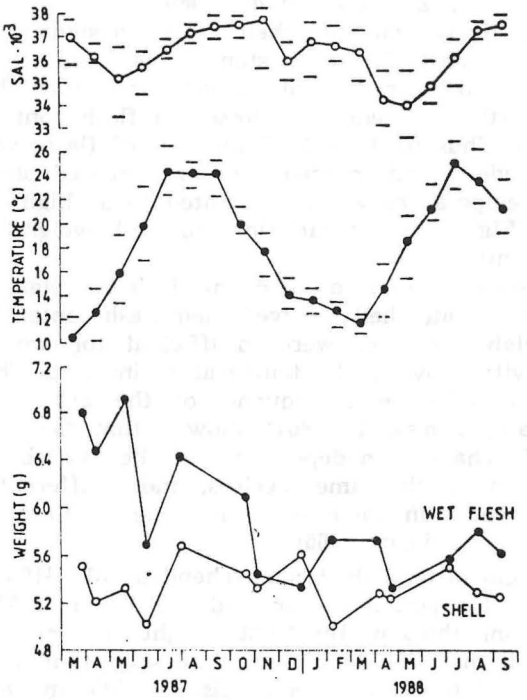

Fig. 9. *Lithophaga lithophaga.* Seasonal variations of monthly mean salinity, temperature (horizontal lines mark minimum and maximum), wet flesh weight and shell weight for standard date shell of 7 cm length.

While the mussel gonads are in resting stage with the higher content of flesh in summer (Hrs-Brenko, 1973) the gonad sections of date shell indicate a resting period in the cold season. It seems possible that date shell use different suspended particles, particularly phytoplankton algae, during autumn and spring blooms (Pucher-Petković and Mara-

sović, 1980) to store nutrient reserves for utilisation during the next cycle. Bayne and Worrall (1980) showed that in various bivalves gametogenesis initiated at certain temperature level provided by sufficient nutrient reserves within the animal or food from the environment. The proliferation of gametes in date shell coincides with spring increase of water temperature and amount of seston. In June, rapid gametogenetic processes in date shell result with the first release of sex cells in late June or early July. The gonad sections showed the renewed gametogenesis with several spawnings during summer extending by mid October, as well as resorption of unreleased gametes in early November at the beginning of new resting period in winter.

It is well known that energy coming from directly accepted environmental food and nutrient reserves is used for somatic growth and gamete production (Gabbot, 1976; Zandee *et al.*, 1980; Hawkins *et al.*, 1985 and others). It is very likely that date shell expend a significant part of energy for movement and boring of holes in stones. The energy losses in most bivalves seem to be due to the release of sex cells and other physiological activities which are further evidenced as losses in flesh content (Bayne and Worrall, 1980). Thus in June 1987 the loss of flesh content in standard date shell was obviously the consequence of increased physiological activity caused by high temperature which amounted to as high as 23°C or on the average to 20°C (Fig. 9). Later variations in flesh weight during 1987 were due to sexual activity.

In 1988, however, there is quite changed flesh weight of standard date shell. During winter, date shell renewed their flesh content to a certain level so that the available reserves were insufficient for any further intensive physiological activity provoked by temperature increase. This weight decrease in 1988 may also be the consequence of the lack of appropriate food. Trevallion and Ansell (1967) showed that flesh weight of *Tellina tenuis* was rapidly changed in dependence of the available food in environment. The specimens of the same speciees, under different ecological conditions, show differences in their seasonal cycle of flesh and shell weight (Ansell and Trevallion, 1965).

The flesh weight of date shell comprehend up 31—41% of the total shellfish weight during the period of our study. Tudor (1987) also found that flesh constituted one third of the total weight of various sized date shell. This ratio is much more favourable in date shell than in mussel *(Mytilus galloprovincialis)* and oyster *(Ostrea edulis)* (15.33% and 8.62% of the total weight respectively) (Marinković-Roje, 1968).

Parameters of allometric growth equations, the exponent and intercept, vary with seasonal variations of environmental factors and sexual cycle of the animal (Wilbur and Owen, 1964). In fact the exponent of allometric equation is the ratio of relative growth rates of dependent variable to that of independent variable. The exponent of equations showing the relationship between flesh weight and shell length as well as that between shell weight and shell length showed differences in values and modes of temporal changes between observations in 1987 and 1988. Sea water temperature and salinity were some of the factors controlling the exponents of allometric equations for the period between December 1987 and September 1988. The activity of date shell in March, April and May 1987 was very likely

reduced due to the lower temperature (10—16°C) in comparison to same months of 1988 (12—18.5°C). The difference in the growth rate of *L. lithophaga* shell between these two years may be due to the same reason. On the other hand the differences between the equation exponents are also affected by feeding conditions of the environment.

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Mr. Mladen Alajbeg, the associate of the Institute of Oceanography and Fisheries in Split, for microphotographs.

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Accepted: February 8, 1990

SPOLNI CIKLUS I BIOMETRIJA PRSTACA, *LITHOPHAGA LITHOPHAGA* LINNAEUS (MYTILIDAE)

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KRATKI SADRŽAJ

U ovom radu prvi put se iznose podaci o spolnom ciklusu prstaca kao i njegovi biometrijski odnosi.

Spolni ciklus i biometrija prstaca istraživani su na uzorcima sakupljenim kod Splita (srednji Jadran) tokom 1987. i 1988. godine. Period spolne zrelosti i emisija spolnih produkata odvija se u najtoplijem dijelu godine (srpanj, kolovoz i rujan) pri temperaturi mora iznad 22°C.

Fluktuacije težine mesa i školjke su rezultat promjena ravnoteže između rasta školjke, rasta somatskog i gonadnog tkiva te gubitka materijala tokom mrijesta.