THE SEASONAL PRODUCTION OF SPERMATOZOA AND OTHER NOTES ON THE BIOLOGY OF *ANODONTA CYGNEA* (L.)

By H. H. Bloomer

(*Read* 8 *March* 1946)

In my last paper [1] I discussed various aspects of sperm-morulae and spermatozoa in time and location in the gonads of *Anodonta cygnea* from the river Frome area, and in a paragraph treating of the ova stated that after spawning apparently the surplus products of the gonads were but slowly absorbed: it should now be made plain that this applies equally to the male products; that between the spawning seasons it seems the frequency of male products varies whether the individuals are carrying larvae or not: in some they may be relatively plentiful and in others nearly if not quite absent; that in some individuals there was probably a completion of the ripening of the sperm-morulae and the liberation of spermatozoa long after the normal spawning season; that generally towards midwinter, even beyond, in those individuals where sperm-morulae and spermatozoa were present they were mostly confined to the dorsal part of the gonads: this subsequently transpires would be more applicable to the antero-dorsal region; that it was suggestive that in some individuals the testicular parts may not have produced spermatozoa at the last spawning season, their development being delayed until long afterwards; that whereas some of these spermatozoa seemed to be normal in appearance others showed signs of degeneration; and that it was also inferred there is a possibility the spermatozoa of the post-spawning period may eventually degenerate and would not be those functioning at the next spawning season [2].

[1] *Proc. Malac. Soc.* 25, 192–200 (1943).

[2] There is reason for believing that towards the spawning season and the advance of warmer and sunnier weather the mussels migrate from deeper to shallower water for the maturing of the gonads and for spawning. Although the view may be held that they go into deeper water to seek protection from the severity of wintry weather (*J. Conchol.* 19, 14, 1930), I think the behaviour has a wider and more significant meaning, that is, the migration to the shallower water is composed chiefly, and probably forming a large proportion of the total population, of individuals intent on spawning eventually, and those remaining in the deeper water may not spawn that season. Unfortunately, this problem was unforeseen when collecting myself and since investigating the river Frome area *cygnea* I have been unable to attend for a number of years and see them gathered. This has been and is still a difficulty, and if my theory is correct it may alter some of the quantities of the products of the gonads at present and in my previous papers given, yet in the main it will not detract from the matter under discussion because in the colder months the samples would be taken from the normal habitat at that time, so that both categories would be represented. In shallow waters where there is a good depth of mud the migration may not be far, but where the bottom of the lake or stream very gradually slopes from the bank to deeper water, especially further north than the river Frome and in exposed positions, the migration may be a much greater distance. The small lake under investigation is one not exceeding about 2 feet in the deepest part and has a moderate depth of mud with a growth of flags, etc., thus the animals are able to get a certain degree of protection by burying themselves deeper in the mud without travelling far. There are also indications of the large mussels to segregate.

Both these problems require more research in different waters at all times during the year.

Since then the examination of samples gathered in 1944 and 1945 and the re-examination of the smears obtained in former years, I believe, makes it possible to pursue with advantage the subject further.

A scrutiny of the quantity of spermatozoa in the smears of the gonads between October and March shows with some exceptions [3] that though the spermatozoa are present in a moderate but varying number, yet more frequently the number of them is relatively not considerable when compared with the large number seen in July or August at the approach of the spawning season and after the testicular parts have ripened over a larger area of the gonads.

A reference to the table [4] shows the variation in quantity during that period. The unpublished tables for 1944 and former years disclose a similar course.

If a detailed examination of the smears prepared from January onwards and including those taken in the same period of previous years is made it seems there is a fluctuation in the quantity of sperm-morulae and spermatozoa, which on analysis, and omitting the so-called exceptional cases, would appear to imply that usually prior to May the trend is for a moderate to a much less quantity to be present in the gonads: that in

[3] The animals discharge their glochidia sometime from March to May presumably according to the weather conditions that have prevailed since the entry of the eggs into the outer gills. The comparatively large quantity of male products in the gonads of some individuals in January and not carrying glochidia, also from the state of the outer gills, suggests that these animals did not spawn at the last spawning season, but regarding those that had the outer gills filled with glochidia the cause of the presence of a large quantity of male products in the gonads in January can only be surmised. Yet this condition of the gonads, apart from any question of instability that may arise in consequence of some possible change from one sex-phase to another, is apparently not altogether unusual in the Unionidae, as from the examination of smears made some years ago of such a supposed stable dioecious species as *Unio pictorum* I found a similar abundance of spermatozoa, with a lesser quantity of sperm-morulae, in the months of September, March and July. So far as I am aware the normal spawning time for this species is in the summer months. Incidentally, a smear of *Unio tumidus* made in March also revealed a similar state.

No attempt has been made to see if there is any correlation of age of the animal, represented by size of shell, with the testicular area of the gonads. A much larger number of individuals would be required than has been available.

[4] Three smears of the gonads were taken from each individual, one from the antero-dorsal, the second from the central and the third from the ventral region. They were fixed in Bouin's fluid, passed through alcohol, stained and mounted. After examination the quantities of sperm-morulae and spermatozoa were recorded as shown in Table 1.

In assessing the quantities of products in the smears the scope of the terms used is somewhat arbitrary, especially in the one 'moderate number', which probably covers a wider range than the others and includes some that may be termed 'fairly numerous'. It should also be borne in mind that in taking a smear the puncture of the gonads, though fairly large, may by chance, be at a part where maleness or femaleness is more extensive than in the surrounding tissue; this refers more particularly to the central and ventral parts.

The number of individuals in each sample is small and it would have been more satisfactory to have made it larger, but the gatherings were carried out under extreme difficulty. When began the density of the lake's population was unknown, and as it was necessary to secure samples regularly until the time the eggs had entered the outer gills before exhausting the source of supply, the number in each sample was limited to what was then considered a minimum.

TABLE of the number of sperm-morulae and spermatozoa in the smears of the gonads of *A. cygnea* from the small lake connected with the river Frome.

samples	size	glochidia in outer gills	antero-dorsal region		central region		ventral region		monthly summary: abs.		few		mod.		num.		abun.	
1945	cm.		sm	*sz*	sm	*sz*	sm	*sz*	sm	*sz*	sm	*sz*	sm	*sz*	sm	*sz*	sm	*sz*
Jan.	11·0	.	m	a	m	a	m	a										
	12·0	.	m	a	o	o	o	o										
	10·7	+	f	f	o	o	f	f										
	11·9	+	m	m	f	m	m	m										
	11·4	.	m	m	o	f	o	o										
	12·3	.	f	m	m	m	n	a	5	*4*	4	*3*	8	*6*	1	*0*	9	*5*
March	10·2	+	m	m	f	m	f	m										
	7·8	+	m	a	m	m	f	f										
	12·6	+	f	a	f	a	f	m										
	12·4	.	n	a	m	a	f	f										
	7·2	.	m	m	m	a	m	m	0	*0*	7	*2*	7	7	1	*0*	0	6
May	12·4	+	a	a	m	m	m	a										
	9·8	.	m	m	f	f	m	m										
	8·7	.	m	m	o	o	o	o										
	8·8	.	m	m	m	m	m	m										
	13·8	.	f	f	o	f	o	o										
	9·7	.	a	m	m	m	m	m										
	14·0	.	m	m	f	f	o	o	5	*4*	3	*4*	11	*11*	0	*0*	2	*2*
June	8·3	.	a	m	a	m	m	m										
	9·7	.	a	m	m	m	m	m										
	12·8	.	m	f	m	f	f	f										
	9·7	.	m	f	f	f	o	o										
	7·8	.	m	f	m	f	f	f										
	7·5	.	m	f	n	m	n	f	1	*1*	3	*10*	9	*7*	2	*0*	3	0
July	9·7	.	m	m	m	m	m	a										
	10·6	.	m	m	m	m	m	m										
	12·1	.	f	a	f	a	f	a										
	9·9	.	f	f	f	m	m	m										
	10·1	.	m	a	m	a	m	n										
	9·5	.	m	a	m	a	m	a	0	*0*	5	*1*	13	*7*	0	*1*	0	9
Aug.	10·0	+	m	m	m	m	f	f										
	8·3	+	m	a	m	m	m	m										
	10·2	+	m	m	m	m	m	m										
	9·2	+	m	n	f	f	f	f										
	10·4	.	m	a	f	m	f	m										
	7·8	+	m	m	f	f	f	f	0	*0*	7	*5*	11	*10*	0	*1*	0	2

The size in column 2 is the antero-posterior measurement.

sm, sperm-morulae
sz, spermatozoa
+, glochidia present
o, abs., absent or not detected
f, few
m, mod., moderate number
n, num., numerous
a, abun., abundant

May and June there is an indication of a decline in the number of spermatozoa: that in June and July the development of the sperm-morulae takes place, followed by a steady and large increase in the number of spermatozoa in July and August, when a great quantity may be amassed shortly preceding the new spawning season. Thus generally before the resumed development of the gonads the quantity of spermatozoa although fluctuating is relatively not large in comparison with the great quantity afterwards available for fertilization.

Again the table for 1945 will assist in following the discussion. The 1944 one and those made in previous years from the river Frome area, although not taken at such regular intervals, in the main support it. The fluctuation, however, in these varies somewhat in length and time of the different stages, presumably due to weather conditions. The 1945 table does not contain the quantities between the end of the spawning season and the month of January, but from the results derived from the smears taken in former years there are only a few cases in which either of the male products just exceed a moderate number.

There is one point which is not altogether clear and that is, what becomes of the spermatozoa during the time of reduction? This seems not to be wholly accounted for by the process of slow absorption.

Moreover, it will be seen that the subject dealt with analytically does not appear to support the suggestion of the storage of sperm.

EXAMINATION OF FURTHER SAMPLES

After the examination of samples as recorded in my last paper [5] 11 more samples of *Anodonta cygnea* have been obtained with great difficulty during 1944 and 1945 from the river Frome area, but the source of the former supply having become nearly exhausted they were gathered from a small lake—it is really an enlarged arm of the river—connected at both ends with the river, about five miles above Holme bridge.

The quantities of male products in the gonads (for 1945) are incorporated in the table, and other features are contained in the body of this paper; therefore they are not referred to here, but the female characteristics, which agree with those already given, may be briefly mentioned.

In all 63 individuals were examined and no case was found in which the outer gills were not of a marsupial character. Ova were always present in the gonads, and prior to April they are usually from small to moderate size; that formerly ripe ones showing signs of disintegration were constantly met with. It was not before April that there was general activity in the gonads in the development of the ova, and this continued with increasing vigour until August when ripe ones were plentiful, although embryos were not found in the outer gills until September in 1944 and August in 1945. In April 1944, at the time of this renewed activity, the animals were still discharging their glochidia.

CONTENTS OF OUTER GILLS

In September 1944 a smear was taken of the contents of the outer gill of one individual carrying embryos, which had the glochidial shell

[5] *Proc. Malac. Soc.* **25**, 192–200 (1943).

beginning to be formed, and it was observed that in the contents was a number of spermatozoa. Their presence had not been seen before, but then the smears used for a different purpose had only been permanently mounted when the embryos were much more developed. Four more gravid individuals examined shortly afterwards disclosed a somewhat similar condition but had a much less number of spermatozoa. Sections of the outer gills of two of the individuals showed the spermatozoa in the interlamellar spaces with the embryos.

The investigation was continued during 1945 with the object of eliciting the time when the spermatozoa enter the gills. In January and March smears of three individuals had a few spermatozoa with the glochidia, whilst they were absent from the gills of the other gravid ones. The discharge of the glochidia happened between April and May. From then until August (the time of the beginning of the new spawning season) smears of the outer gill contents were taken and in no instance were spermatozoa found in them. Five newly gravid individuals were examined in August and the smears of the outer gills revealed spermatozoa in varying numbers with the embryos and in one instance a few sperm-morulae as well. One individual of the same sample which had not yet spawned exhibited in the smears of the gonads a large number of ripe or nearly ripe ova and more than moderate number of spermatozoa, while the condition of the outer gills [6] showed that they were ready for the reception of the eggs, yet in the smears of their contents were no spermatozoa.[7] On the whole of the evidence it therefore appears safe to assume that the spermatozoa do not enter the outer gills before the eggs.

For some time I have been uneasy in accepting the view that the ova of the river Frome *A. cygnea* are fertilized in the supra-branchial chambers [8] and have had the impression rightly or wrongly that the process of self-fertilization takes place in the genital ducts or ductules, but the finding of spermatozoa in the interlamellar spaces of the outer gill-plate during the early developmental stages of the embryos suggests that fertilization may also occur there and that my assumption may have to be modified.[9]

The passage of the spermatozoa then would seem to be in the same direction as the ova and possibly at the same time.

Genital products in the rectum

When examining a section of the gonads of an individual fixed in early July 1944 it was noticed that the section cut across the rectum apparently near its junction with the intestine, which lies in the visceral mass and is surrounded by the gonads. Inside the rectum and on one side of the

[6] After the expulsion of the glochidia the outer gills gradually revert to a normal state and the interlamellar septa become contracted. Then before spawning the gills again become expanded and the interlamellar septa much widened so that the interlamellar spaces are much larger.

[7] Whether the animal would have had the outer gills so fully charged with embryos as the others is uncertain as below the antero-dorsal part of the gonads the testicular areas were much more restricted.

[8] *Proc. Malac. Soc.* **25**, 196–197 (1943).

[9] The possibility of the occurrence of transition of one sex-phase to another (*Proc. Malac. Soc.* **23**, 294, 1939) has to be considered, and during this transition indications of greater instability of the gonads may be encountered.

typhlosole was an elongated mass consisting of sperm-morulae, a few spermatozoa and some ova; and in places they were mixed with the faeces. In another individual killed two days before the preceding one, the section also passed through the rectum near the same place and in its lumen was a number of sperm-morulae, besides a few spermatozoa. Further search among later and older microscopic slides has produced examples of a similar nature, including those of *A. cygnea* from Epping Forest, and *A. anatina* from the Newport and Brecon canal; in all 12 individuals.

On consideration it seems improbable that their presence is due to an accident or faulty manipulation in the preparation of the tissue for sectioning, hence if this is a natural process the questions arise (*a*) how have these products of the gonads entered the alimentary canal? and (*b*) is it possible that the animal has some means of disposing of these products with the faeces? [10]

The only reference I am able to trace relating to the subject is that quoted by Lang [11], who states regarding the rectal gland of Dentalium 'Eggs and spermatozoa have been met with in the lumen of this gland and it is supposed that they have been accidently drawn out of the mantle cavity by the swallowing-like action of the hind gut'.

Professor J. H. Orton has drawn my attention to the able and very instructive account of feeding and digestion in *Ostrea edulis* by Professor C. M. Yonge [12] wherein he reviews the work of previous investigators on this species and other Lamellibranchs including Nelson's on *Anodonta*, and after an exhaustive research he describes the selective mechanisms of the gills and labial palps of the particles taken in at the mouth, their passage along the alimentary tract and the ejection of undigested material at the anus. Then in his general discussion says 'Sherwood (statement in Savage's paper) and Nelson (1921) have both noted the presence of living oyster larvae in the faeces of the adults'. I have so far failed to gain access to these papers, but Professor Yonge in his description does not mention having seen sperm-morulae, spermatozoa or ova in the alimentary tract so the question of their inclusion at times in the rectum of *A. cygnea* remains unsettled until it is ascertained how and where they enter it.

Experiments

An attempt was made to keep three isolated individuals from April 1942 to September 1943, a period extending over two successive spawning seasons, but owing to the restrictions on cereals curtailing the use of oatmeal during the latter and greater part of the time the experiment cannot be regarded as satisfactory or a fair test. One individual died in July 1943. The other two when examined had no larvae in the outer gills and the smears of the gonads disclosed only a few imperfect ova but no perceptible evidence of male elements.

[10] The investigation is still being continued: furthermore this question might be borne in mind in connection with the reduction of spermatozoa referred to earlier in the paper.

[11] *Text-book of Comparative Anatomy*, part 2, p. 196 (1896).

[12] Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. Mar. Biol. Ass.* **14**, 298–386 (1926).

Professor Orton suggested that a shorter experiment might be tried covering one maturing and spawning period alone and allowing the individuals to derive what nutriment they could arising from any vegetative growth that may occur in the vessels and so excluding artificial feeding.

The experiment commenced on 12 April 1944 and the animals were examined between 2 and 9 October. Four individuals were employed and each one was placed in a separate vessel. In two vessels soil from a lily pond and garden was added. In addition, two individuals in separate vessels were fed on crushed puppy biscuits. The result was the same in all six cases. None carried larvae in the outer gills. All of them were more or less in a poor condition and the smears of the gonads showed few signs of either male or female products. It is only right to add that this experiment was begun two months earlier than the successful ones alluded to in my last two papers [13] when the mussels were fed on oatmeal (Quaker Oats).

Hermaphroditism in North American Naiades

In a previous paper [14] reference was made to the occurrence of hermaphroditism in certain North American Naiades. Since then H. van der Schalie and F. Locke [15] have investigated the sex of *Anodonta grandis*, 'one of the most common and widespread North American Naiades' and have found that of 'fourteen sexually mature specimens of *A. grandis*' examined 'nine were males, three were females, and two were hermaphrodites'. W. C. Tepe [16] has re-investigated *Carunculina parva* and confirms the implication of Sterki that this species is hermaphroditic; and it is interesting to notice that he says 'in four out of five individuals of *C. parva* under observation some follicles were found containing products of both sexes'.

I am again indebted to Professor J. H. Orton for his kindness in perusing this paper and for his valuable suggestions.

NOTES ON THE ANATOMY OF SOME AFRICAN NAIADES
PART III

By H. H. Bloomer

(*Read* 12 *April* 1946)

I am indebted to Major M. Connolly for the opportunity of examining the following examples of *Cacatura* from the Nile Valley and for kindly giving information concerning them.

Unfortunately and probably owing to unavoidable causes the animals are not in a good state of preservation; the tissues of all of them are hard

[13] *Proc. Malac. Soc.* **24**, 113–121 (1940), and **25**, 192–200 (1943).
[14] *Proc. Malac. Soc.* **21**, 306–308 (1935).
[15] Hermaphroditism in *Anodonta grandis*, a fresh-water mussel. *Occ. papers Mus. Zool., Univ. Michigan*, no. 432 (May 1941).
[16] Hermaphroditism in *Carunculina parva*, a fresh-water mussel. *American Midland Naturalist*, **29**, no. 3, 621–623 (May 1943).

and very contracted, and it has not been practicable to cut satisfactory sections of the gills.

In each subspecies the chief features have been drawn and described mainly from one individual, but some of the details are supplied by the examination of the other specimens, and it is possible the drawings, judging from the material available, may trend towards extreme forms.

Caelatura aegyptiaca figdiana new subspecies

Locality: Wad Figda, canal between Khartoum and Sennaar, Soudan. Collected by Dr F. W. Andrews.

Measurements of shells: 5·2 × 3·0, 4·9 × 3·0 (holotype), 3·8 × 2·3 and 3·7 × 2·3 cm.

Shell suboval, narrowing anteriorly and broadening posteriorly, moderately thin, a little inflated. Colour of periostracum dull olive-brown, merging into bands of a blackish shade. Umbones eroded, about one-third of the whole length distant from anterior end: apices a little prominent. Sculpture, mostly confined to the umbonal region where the ridges tend to run in a ventral direction and are more nodular in its posterior part: slight ridges along the dorso-posterior slope of the shell, and anteriorly near the dorsal margin infrequent and slight zigzag ridges. Lines of growth prominent. Teeth: left valve, pseudocardinal long, moderately large, somewhat compressed and curved outwardly with a fine denticulate edge; a second pseudocardinal relatively a little less compressed, long, triangular with its apex under the umbonal apex: it has a denticulate edge; laterals long, moderately thin, the lower larger than the upper one: right valve, pseudocardinal double, both parts rather compressed, moderately high and curved outwardly with wavy and slightly denticulate edge; one lateral, long, thin and narrow: a somewhat indistinct second one. Ligament long, exposed and rather weak. Lunule broadly subtriangular. Inner side of valves a pale slaty blue tinge. Muscular impressions deep in anterior and shallow in posterior part.

These shells vary in all respects; some (measuring dorso-ventrally) are narrower at the anterior end than others; the one from which the animal is drawn is one of the narrow forms.

The animal is much broader, dorso-ventrally, in the posterior than in the anterior part. Mantle is rather thin with a rather weak circumpallial muscular band. Branchial aperture is much larger than the anal one and there is no concrescence of the mantle lobes between the two apertures. It is bordered by a number, 30 to 50, of short, conical papillae on each side. Anal aperture is a little wrinkled but has no papillae. Colour of both apertures is a dark brown, except the ventral part of the branchial. Supra-anal aperture is about the same length as the anal, is smooth and separated from it by the concrescence of the mantle lobes which is nearly of the same length as the supra-anal.

The labial palps are very conspicuous: posterior parts being produced into long and broad lobes extending in a postero-ventral direction, and the distal ends are curved with the tips pointing posteriorly; there is posteriorly a coalescence with the mantle for about two-fifths of their length. One specimen has lobes 1 cm. long and the animal measures 4·3 cm. In two specimens the lobes tend to lie more posteriorly.