**Zoology.** — *The Oestrus Cycle of Rhodeus Amarus Bloch* ♀. By J. MELTZER. (Communication No. 20 of the Workcommunity for Endocrinology from the Laboratory of Comparative Physiology and the Laboratory of General Zoology, University Utrecht.) (Communicated by Prof. CHR. P. RAVEN.)

(Communicated at the meeting of May 31, 1947.)

## 1. Introduction.

*Rhodeus Amarus* is often kept in aquaria. It is a gratifying object as it is easily brought to spawn. Since the investigations of NOLL (1869, 1877) the female is known to lay her eggs in mussels of the genera *Anodonta* and *Unio*. When ovulating the female inserts the ovipositor into the gills of the mussel. Out of the spawning season the ovipositor is hardly existing or not at all; only a slight elevation immediately behind the anus, the urogenital papilla, indicates the place where the excretory ducts emerge. In springtime this papilla starts developing into an ovipositor which may reach beyond the tailfin. Figure 1 shows the position of the ovipositor and the internal organs.

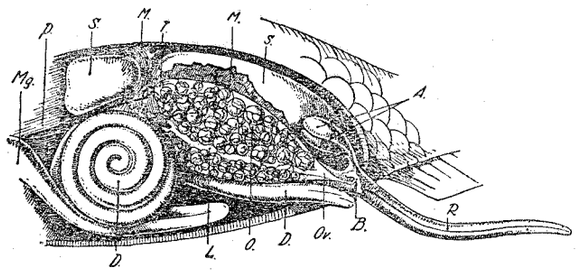



Fig. 1. Position of the ovary and the internal organs during the spawning season. The liver has been removed. $O$ = ovary; $OV$ = internal oviduct; $A$ = bladders; $R$ = ovipositor; $S$ = swimming bladder; $P$ = ductus pneumaticus; $D$ = intestine. After *OLT* (1893).

It is remarkable that the ovipositor only grows if both mussel and male are present, though WUNDER (1933, 1934) reports that the mere presence of the mussel is sufficient. Reports of experiments as to that will be issued elsewhere. Here a short summary of the mating-biology in so far as necessary for a good understanding of the oestrus cycle, may suffice.

When in early spring a couple of *Rhodeus* is brought together with an *Unio*, and then observed, one can trace in what manner the ovipositor develops. On a given day both fishes begin to show an interest in the mussel. And it is not long before the mussel is being guarded by the male only. All congenera, male as well as female are chased away from the territory of the mussel. In the mean time we see how the ovipositor of the female is gradually beginning to grow. When the ovipositor has reached its full length, we can observe that all of a sudden the behaviour of the male towards the female entirely changes. Instead of chasing her he lures the female with shuddering movements of his entire body towards the mussel. Once near the mussel the male sheds his milt-liquid above the siphon. Under the influence of the trembling male the female is thrown into extasy; with her head directed towards the discharge aperture of the mussel and her tail pointing upward she awaits the right moment for shooting downward. Quick as lightning the base of the ovipositor is now brought above the siphon of the mussel and at the same moment an egg slips through the ovipositor, driven forward by a liquid (urine?). Thus a wateraxis is formed, by which the ovipositor becomes rigid and disappears into the mussel (c.f. DUYVENÉ DE WIT and BRETSCHNEIDER 1940). In a fraction of a second the egg is laid and the female swims away.

Without going further into the behaviour of the roach, it must be remarked that the cause of the change in the behaviour of the male towards the female must be sought in the typical movement of the female when about to ovulate. When ripe for ovulating the female assumes an oblique pose whereby the head is directed downward and the tail upward. I should like to call this position the inclination-pose. It goes without saying that this inclination-pose is extremely important for the external recognition of the condition of oestrus of the female. It is interesting that JASKI (1939) discovered a similar pose with *Lebistes reticulatus*. With *Lebistes* the females that are ripe for copulation assume the so-called elevation-pose. There are important differences, however, between the elevation-pose of *Lebistes* and the inclination-pose of *Rhodeus*. With the first the head is directed upward, whereas with the last it is directed downward. From this different behaviour it is evident, however, that the position cannot be merely the result of the physical conditions caused by the heavy ovary. Rather a hormonal and psychical regulation should be considered here. Another difference is to be found in the way in which the elevation or inclination-pose is brought about. With *Lebistes* the elevation takes place gradually during several days, i.e. there is a gradual increase of the gradient. With *Rhodeus*, on the other hand, the inclination can be seen to take place suddenly and frequently during about 24 hours, whenever an egg is to be laid. For the eggs are laid one by one, with intervals of two minutes to even one or more hours. A non-interrupted inclination-pose would moreover result in the female being continually importuned.

## 2. The periodicity of the growth of the ovipositor.

DUYVENÉ DE WIT (1939) already supposes that the phenomenon of the

growth of the ovipositor under normal conditions probably would appear periodically. Herewith corresponds the observation of NOLL (1877) that with some couples of *Rhodeus* ovulations took place several times in one season.

From the 15th of April till the 10th of June 1942 in the aggregate some 40 females were being watched daily. The length of the ovipositor was measured daily in anal units (A.U.) and set out in a diagram.

One anal unit is ⅛th part of the anal. The relation between the length of the fish and the length of the anal appears to be almost constant. An exact measurement in mm would of course be no criterion, considering the great variation in size of the fishes. Moreover the fishes would be too much upset when being fetched out of the water for each measurement. After some excercise the estimation of the length of the ovipositor is done with fair accuracy. The average fault amounts to about 0,5 A.U.

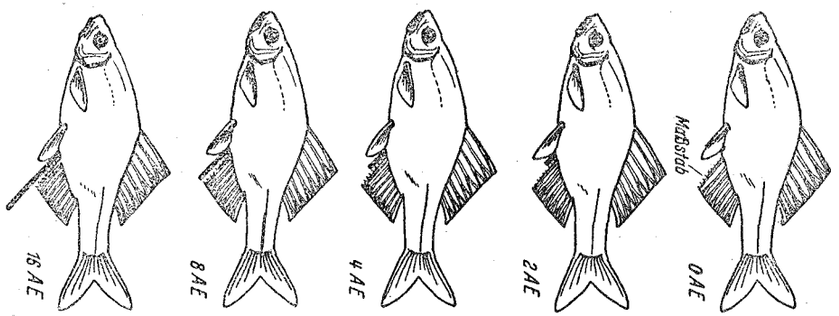


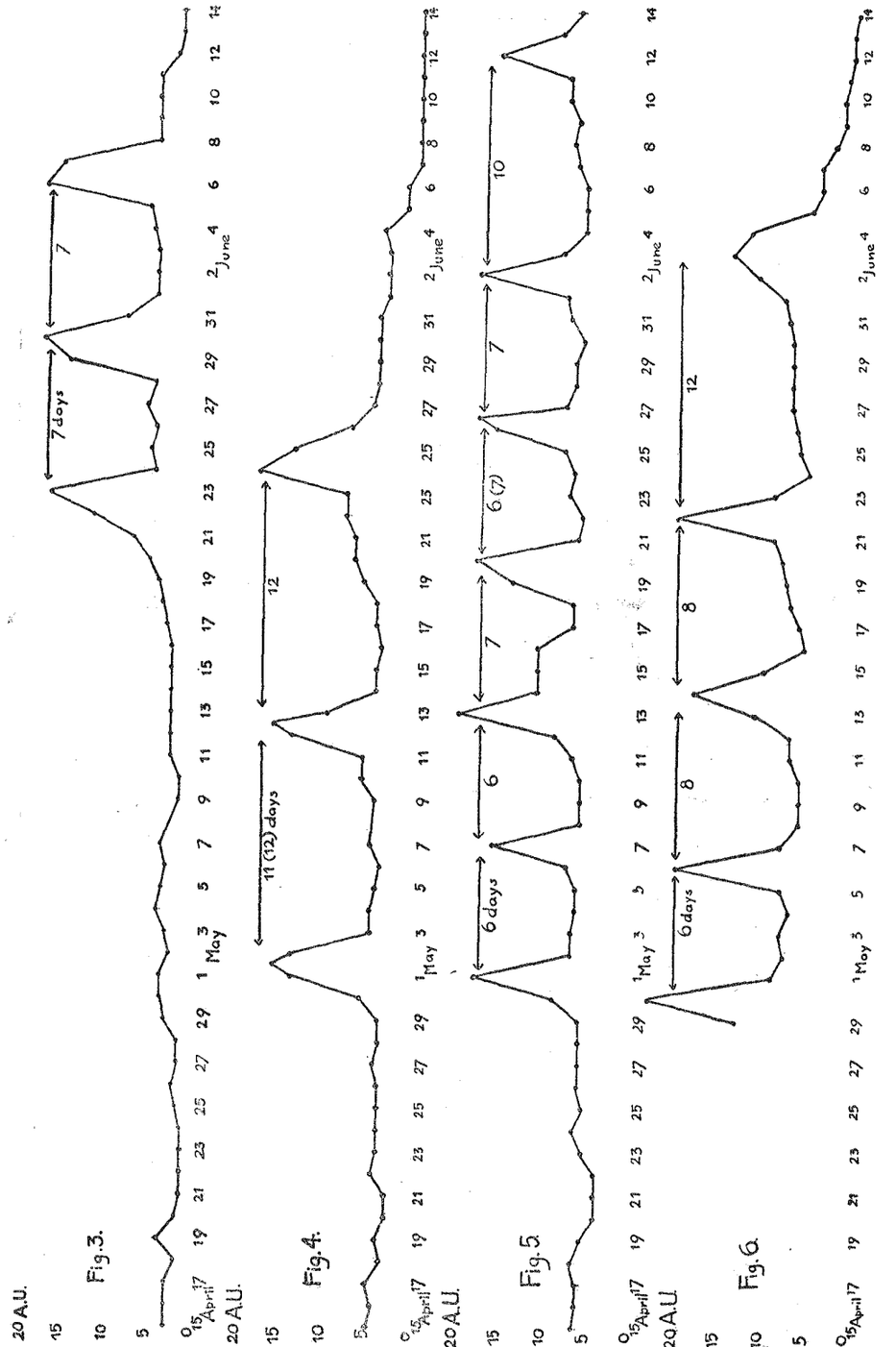

Fig. 2. The foremost radius of the anal has been divided into 8 parts and serves as a measure for the length of the ovipositor. ⅛th part is called 1 anal unit (A.U.). With the above fishes the ovipositors are resp. 0, 2, 4, 8, and 16 A.U.

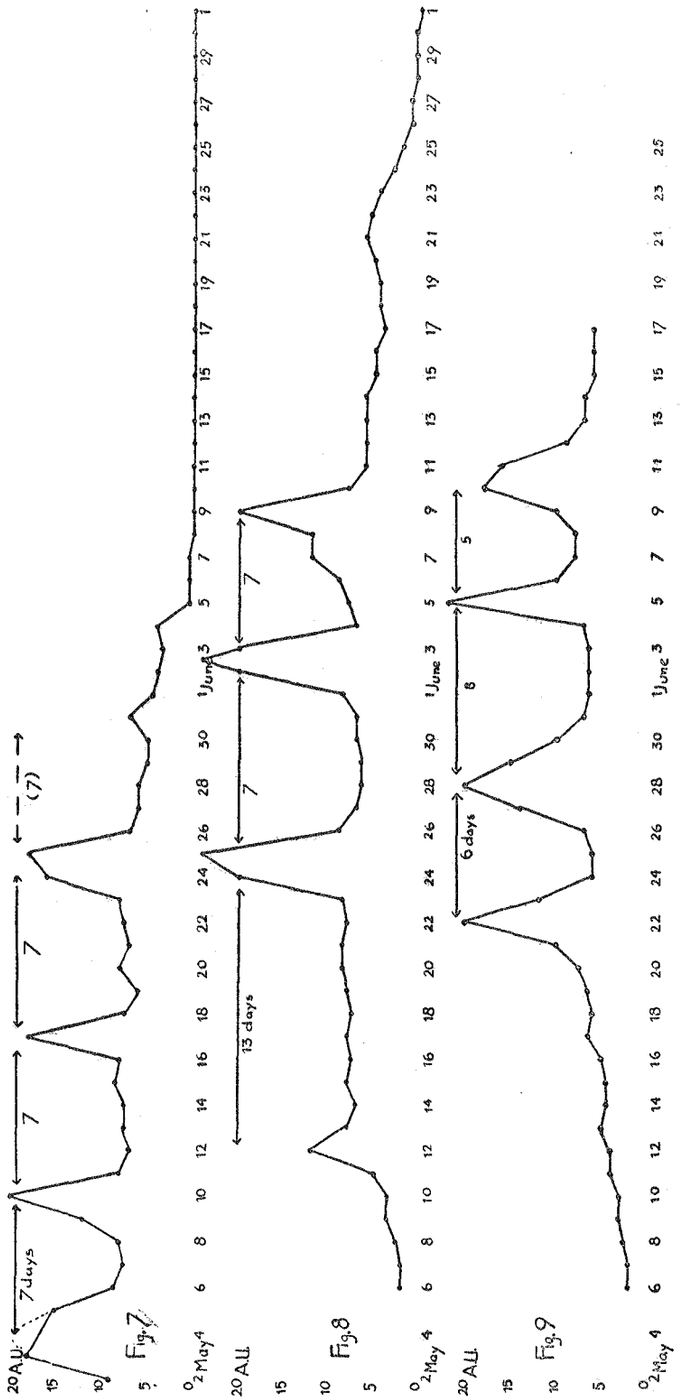
Of those fourty females one or two did not ovulate at all. A few others ovulated once or twice, but most of them three or four times. One female even came to ovulating seven times in this one season. It appears from the diagrams (Fig. 3—9) that the ovipositor develops in periods that slightly vary with the several individuals. Mostly a period was 6 to 8 days, but with some also periods of 11 till 13 days occurred. It is quite possible, however, that these animals simply passed over an ovulation-phase.

According to WUNDER there are two stages of growth of the ovipositor to be distinguished: In the first stage the ovipositor reaches a length of a few mm, whereas in the second stage the ovipositor develops into the complete length of some cm within a week. The diagrams however give a totally different aspect of the process. Fig. 3 e.g. shows that the ovipositor is oscillating round 3 A.U. for a long time, until on the 16th of May a continuous growth sets in, culminating in a top on the 23th of May.

As a matter of fact the growth of the ovipositor takes place in two stages, but different from the way WUNDER reports. The increase from 0 to 5 A.U. may take weeks. The increase from 5 A.U. to the complete










length on the other hand takes place very rapidly, generally within 24 hours. The decrease following the top has a quick course too; its original length is mostly regained within twenty-four hours. The ovipositor, however, is never reduced to zero during the spawning season. During this period the ovipositor is oscillating between 5 and 8 A.U., reaching a top with intervals of about a week. In the diagrams three stages of the ovipositor are to be discerned, i.e.:

**1st stage:** Length 0—5 A.U. The ovipositor is in the period of growth. Histologically this period is characterised by the appearance of several mitoses (c.f. BRETSCHNEIDER and DUYVENÉ DE WIT 1941).

**2nd stage:** Length 5—8 A.U. The ovipositor is in the period of disposition, i.e. it is periodically able to quickly attain a toplength, so that ovulation is possible. The length of 5—8 A.U. is characteristic only for the phase of rest. According to the processes inacting themselves in and round the ovipositor, the following phases may be distinguished:

a. Phase of rest, length 5—8 A.U.

b. Phase of ovulation, i.e. the period during which the ovipositor after a quick increase as quickly decreases. In this phase we may discern again:

*α.* *Phase of infiltration.* The ovipositor is lengthened by infiltration with lymphe and by swelling of the tissue until a length of 16—20 A.U. (individual differences).

*β.* *Phase of inclination.* During this phase the female ovulates. The ovipositor may lengthen here, but the extension amounts to 3 A.U. at most. With several individuals the length varies from 16—24 A.U. Characteristic for this phase is the inclination-pose of the female.

*γ.* *Phase of defiltration.* The ovipositor is reduced to the length of the phase of rest.

**3rd stage:** Period of reduction. After the spawning season the ovipositor is reduced again to zero.

In the first stage the growth of the ovipositor is very slow. The artificial lengthening of the ovipositor as described by DUYVENÉ DE WIT (1939) belongs to this part of the growth curve of the ovipositor. With the artificial growth of the ovipositor the rate of growth however is accelerated. Under natural conditions the mitoses will not appear as quickly and numerously, and probably the actual growth of the cells will take place directly after the division.

The varying length of the ovipositor also during this period proves, however, that the infiltration with lymphe is not exclusively restricted to the

infiltration-phase of the second stage. Moreover BRETSCHNEIDER and DUYVENÉ DE WIT (1941) pointed out that also in the period of mitoses the infiltration with lymphe plays an important part. Under natural conditions the part of the lymphe-infiltration during the growing stage is certain to be less important, whereas the cells originating by division have sufficient time to grow. Hyperaemia and lymphe-infiltration are much important quantitatively in the second stage of the development of the ovipositor.

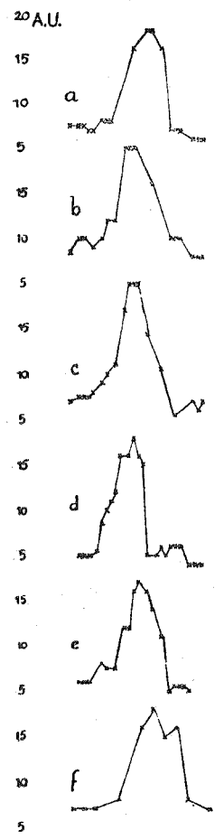



Fig. 10. Different types of tops occurring. The phase of infiltration varies from 10—24 hours. The inclination phase lasts about 24 hours. Only at *f* another top occurs. The defiltration-phase varies from 12—24 hours.

The disosition period is the actual spawning season of the female concerned; only during this time ovulation can take place. According to the diagrams the spawning season of the test animals fell between the end of April and the middle of June. Without artificial interference the females could not be brought to ovulate before the 29th of April. After the 15th of June only those females ovulated who were kept in water of 13° C.

The phase of infiltration often lasts twelve hours, but may extend to twenty-four hours (Fig. 10). So within twenty-four hours the greatest lengthening of the ovipositor takes place. The phase of inclination as a rule lasts about twenty-four hours. Between 16 and 24 A.U. ovulation takes place, i.e. some females already begin at a length of 16 A.U., whereas others ovulate not until 22 A.U. Only once an ovulation at a length of 12 A.U. occurred. During the phase of ovulation the ovipositor mostly gets another slight increase. Once the curve even shows two tops (Fig. 10). This is another illustration of the fact that infiltration in itself is not a characteristic of the infiltration-phase.

During the inclination-phase the inclination-pose is assumed by the female, with interruptions as remarked before. The defiltration-phase finally lasts again 12—24 hours. A schematical curve of the oestrus cycle, only two ovulation-phases being drawn, may elucidate facts mentioned (Fig. 11).



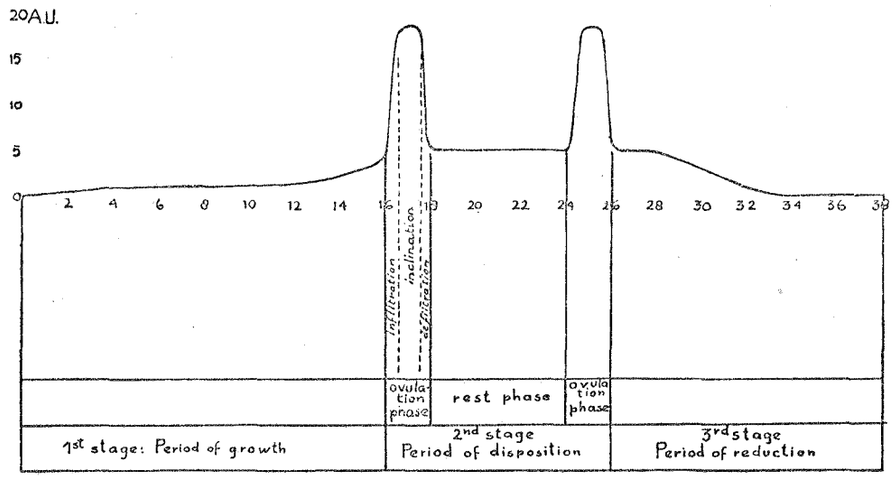



Fig. 11. Diagram of the development of the ovipositor during the spawning season. The first stage requires as a rule a long time. In the second stage the duration depends on the number of ovulation phases of the individual concerned. The third stage is finished as a rule within a week.

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