

016400-1. 1937

Div. Insects
U. S. Nat Mus

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ATROPARVUS IN PORTUGAL**

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*Reprinted from the Transactions of the Third International Congress
of Tropical Medicine and Malaria, 1938, Vol. II, pp. 178-184*

PRINTED IN HOLLAND BY C. A. SPIN & ZOON N.V.

THE BIOLOGY OF ANOPHELES MACULIPENNIS, VAR.
ATROPARVUS IN PORTUGAL¹

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The *atroparvus* variety of *Anopheles maculipennis* is biologically somewhat different from the other recognised varieties. Within the variety itself one finds morphological variations resulting from changes in environment, while there are certain apparent differences in behavior in different regions. Thus in certain countries of Europe, *atroparvus* is a malaria vector, while in others it has not been incriminated. In Portugal, it is responsible for hyperendemic malaria in some areas, it produces a certain amount of sporadic malaria in others, while in still other sections where it is present, no malaria occurs. On account of this and other apparent differences in behavior, we have given special attention to its biology under the different environmental conditions. Since our observations may have more than a local interest, we wish to summarize here certain of our findings of the past five years, with special reference to the hyperendemic area of Aguas de Moura.

The morphology of this variety has been described by various authors and need not be repeated here. Although we find appreciable variations in the same locality and even in the same egg batch, the egg pattern and type of float are essentially that described by van Thiel, Swellengrebel, Hackett, Martini and Missiroli, and others. The length and width of egg, length of float, and relation of float to egg length vary considerably even in the same egg batch. Thus in one batch from a wild-caught female, the float length varied from 19 to 41 per cent. of total egg length, and in another batch from 22 to 45 per cent. These are of course extreme examples. In the majority of batches approximately 10 per cent. variation is found. On the average the float length is approximately 34 per cent. of total egg length. The larval and adult characters are similar to those described by other authors, but here again considerable variations are found in each of the characters commonly used for classification.

¹ The studies and observations reported in this paper were carried out under the auspices and with the support of the International Health Division of the Rockefeller Foundation, in collaboration with the General Direction of Health of Portugal.

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Larval habitat

Atroparvus breeds in practically all clean waters with a negative bio-chemical oxygen demand, and pH between 6.0 and 8.0, when sun, some vegetation, and but little or no current are present. These waters include swamps, canals, irrigation and drainage ditches, and other semi-permanent collections of water, and especially, rice fields. In the latter, larval concentration is much greater than anywhere else, and on account of their extent, the rice fields produce at least 9/10 of all anopheles in Portugal. They are flooded from May to September, and hence constitute a continued source of anopheles during the whole malaria season.

The larvae can withstand, and appear to suffer no harm or even to be favored, by relatively high temperatures. In the summer months, three years observation shows that the water temperature in the rice fields at 4 o'clock varies from 24° to 32° C., with a mean of 27°, 29° and 26° C., for June, July and August, respectively.

In spite of prolific breeding near tide water, where many of the rice fields are located, *atroparvus* is a purely fresh water breeder. Larvae are found in water with total chlorides varying between 50 and 130 milligrams per liter. When salinity rises, as in some salty rice plots, *atroparvus* is replaced by *Aedes*, principally *A. caspius*. Conversely, when salinity is reduced to 170 mg., *atroparvus* again appears, replacing the *Aedes*. Under laboratory conditions, adults may be obtained in water containing up to 0.8 per cent. of sodium chloride, but larva mortality is high and the cycle is prolonged.

Oviposition

In the spring and summer months, at room temperatures ranging from 6° to 30°, and from 10° to 35° respectively, about 80 per cent. of wild-caught females with developed eggs will oviposit in small vials. In September, this percentage begins to diminish, and from November to January, inclusive, only about 3 per cent. of selected females will oviposit. At this time room temperature varies from an occasional night temperature of 2° C., to an average maximum of 16° C. during the day. This reluctance to lay eggs can be overcome in a certain proportion by continued warmth. The large majority, however, will not deposit their eggs until the middle of February, when the percentage ovipositing suddenly rises to approximately 70 per cent. This indicates a tendency to a cyclical development, which however is not absolute.

A similar occurrence is found in nature. In January, a few scattered eggs and small larvae may be found in the shallower waters, but eggs and larvae do not appear in any quantity until the middle of February. The first males appear in the first half of March, and the last ones die in December.

Hibernation

In this relatively mild climate, where the thermometer only momentarily reaches 0° C., *atroparvus* does not usually hibernate. During the second half of September, the females begin to develop fat-bodies, and egg-laying is suspended in October. Approximately 4 per cent. have both fat bodies and partially developed eggs at this time, but ovarian development does not advance.

The more exposed shelters are gradually abandoned and refuge is sought in the well-built and relatively warm inhabited stables and rabbit hutches. Here there is a gradual increase in numbers until the end of January. Anopheles are not found in houses or attics during the autumn, winter or even in the spring until their numbers increase with the new generations.

During the relatively warm winter days, when the temperature may rise to 20° C., feeding is stimulated and a number of anopheles may be seen with fresh blood. At the same time there is a movement into the open, due to the same stimulus. They may return to the same or a different shelter, quite by chance. Thus in two rabbit pens where all anopheles were caught weekly during three years, the number was renewed each week winter and summer.

Females either caught in nature about the middle of October, or bred out in the laboratory — the latter either infected with *Plasmodia* or not — can pass the winter in small cages, both when given a weekly blood meal and when kept without food of any kind. The result is the same whether the cages are placed in the sheltered rabbit hutches where the anopheles usually congregate, or in the protected but open space under the laboratory, where the temperature remains low throughout the winter. In both situations, the majority suddenly die off during the latter half of January, but a small percentage survive until the last week in May. Thus some live for over 7 months.

Under natural conditions, we also note a sudden falling off in the number of anopheles in the shelters the last of January. Many survive, however, and those captured at this time live as long as

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those which have been caged through the winter. Due to the emergence of the first anopheles of the new generation in March, it is impossible to tell when the last of the old ones die off, but the length of life is probably not much different from those held captive.

It should also be noted that at the beginning of the cold weather, several hundred of anopheles usually find their way to the space under the laboratory, where the large majority seem to pass the winter without feeding. It is probable that after having chosen the spot, they are unable to quit it, since the temperature does not rise sufficiently for them to move, as it does in the shelters more exposed to the action of the sun. It thus seems that under certain conditions the local *atroparvus* can pass the winter in a more or less complete state of hibernation.

Reproduction and Longevity

The over-wintering females, after having taken a blood meal in the spring, lay from 2 to 4 batches of fertile eggs, in spite of insemination having taken place the previous autumn. The number of eggs varies from 100 to 350, the average being about 250. Usually only one blood meal is necessary for maturation of the ova, though more may be taken if opportunity is given. On one occasion, a female which had never taken blood, laid a small number of eggs of normal appearance, which however did not develop.

In the spring, the cycle from egg to adult lasts approximately 30 days, while in the summer it is shortened to from 15 to 20 days, with an average of 18 days in a natural environment. Six to seven generations are produced in the season.

In the rice fields, the number of larvae increases up to June, when a mean of 400 larvae per square meter are found. In July this falls to 100, and in August the number is further reduced to 70 per square meter. Adults in catching stations show a similar decrease as summer advances.

The length of life of the adult varies with the season, from a maximum of 7 months in the winter, to approximately 2 months in the summer. This holds true both for infected and non-infected anopheles. A few even survived for 3 months during the summer, in our old laboratory which faithfully reflected the outdoor temperatures. Occasionally the temperature rose to 35° C., and once reached 39° C.

The adult *atroparvus* is exceedingly resistant to high tempera-

tures. In one series of experiments batches of females were exposed in small gauze cages to direct sunlight during the entire day, without access to food or water. Temperatures ranged from 27° to 33° C., and relative humidity from 40 to 80 per cent. The majority survived for 4 days, and a few for 5 days. In another experiment, where the temperature rose to 40° C., and relative humidity varied from 27 to 55 per cent., they survived two days without liquids.

Mating

Both in the laboratory and in nature during the summer months, anopheles begin to be very active when the evening light intensity reaches 1.4 foot candles. The male is very aggressive, and mating takes place in small cages (20 x 15 x 15) without swarming. In the open air, swarms of males can be observed at a height of about 3 meters. The swarm usually chooses the same spot day after day. Swarming had been observed both in the vicinity of houses, and at the edge of the rice fields, as far as two kilometers from the nearest village.

Sheltering and feeding

The Portuguese *atroparvus* is without doubt one of the most domestic of anopheles. It shelters almost exclusively in inhabited quarters. If one clears a shed of animals, the number of anopheles immediately drops about 75 per cent. Animal quarters are preferred, but when anopheles production is great, all shelters are invaded, even to some extent unoccupied buildings. In this way some anopheles find their way into houses.

Biting occurs under cover, or in the immediate vicinity of shelter. Biting never occurs in the open. Repeated attempts to catch *atroparvus* with human or animal bait in the open fields or at the edge of the rice plots have invariably failed.

The effective flight range of the hungry female is at least 3 kilometers, but after feeding the insect is very heavy, and can fly with difficulty only a few meters. If undisturbed, the insect feeds until gorged, and one or two drops of blood are extruded from the anus. This at least partially explains the choice of feeding and resting places.

While the majority feed after dusk, *atroparvus* in this region is not necessarily a night feeder. When competition is great, as in the Aguas de Moura region with its 500 hectares of rice, the insect

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can be observed feeding in rabbit pens, open on 3 sides, at any time of the day. They frequently venture into the direct sunlight to attack the rabbits. In the more subdued light of large barns, where enormous numbers collect, they will also attack man or animals at any time of the day.

Although preferring rabbit, horse, and pig blood in the order named, all the usual domestic animals, and man; are attacked when there is a relative scarcity of hosts. It is this tendency to take any available food, united with the chance selection of houses for shelter when numbers of anopheles are great, which we believe is largely responsible for the continuance of hyperendemic malaria. Thus in a combined rabbit pen and stable in Aguas de Moura, we have estimated the anopheles population at almost 100,000 at the height of the season. In one of our standard rabbit pen catching stations at the same time, the number reaches 5,000, while in the most favored houses approximately 100 anopheles can be found.

Aside from the press of competition for food, which is the principal factor, there are others which influence the biting habits. In the laboratory, in summer the female will feed every 2—3 days. Both here and in the open rabbit pens many of the attacking anopheles still have blood in visible quantities when they feed again. Since one blood meal is usually sufficient for maturation of the eggs, they probably feed at shorter intervals both to provide food and to replace lost liquids.

It is also to be noted that when the temperature is low at night, especially during the spring months, large numbers of anopheles begin to feed as the morning temperature rises to 20° C.

Hence there are probably a number of factors which influence our local anopheles to feed during the day. Among these are :

1. Enormous production of anopheles and relative scarcity of animals under shelter.
2. Cold nights preventing night feeding, and making morning feeding necessary for those anopheles which did not feed the previous evening.
3. Hot dry days which both harden biting parts of newly emerged anopheles, and cause rapid evaporation of liquids in older insects.

Furthermore, we cannot neglect the stimulus for biting that occurs on a humid sultry night in summer. On such occasions, it is

common experience that the mosquitoes are more aggressive. A similar phenomenon has been observed by Shute in the laboratory.

Atroparvus as a Malaria Vector

Malaria is present to any extent in Portugal, only in the rice growing regions, where there is a tremendous anopheles production. *Atroparvus*, as we have shown, has a strong inclination to rest only in inhabited quarters, and to bite only under shelter. Though rabbits are its preferred host, it feeds on any animal, including man, that is accessible. Given these characteristics, we have here an explanation for the presence of endemic malaria in regions of high *atroparvus* density. If there are few anopheles, there is no malaria. But when density is high, some anopheles overflow into houses, partly by chance, partly perhaps because sufficient animal quarters are not to be found nor sufficient animals available on which to feed. Some of them bite man. The relative number is small, but the absolute number feeding on human beings is high.

Atroparvus then is a malaria transmitter in Portugal largely by force of numbers. When anopheles density is high, malaria is endemic. When density is low, the number attacking man is insufficient to make it an efficient vector. Any measures which reduce the number of anopheles, tend to reduce the malaria incidence to an even greater degree.

Summary.

The *atroparvus* variety of *Anopheles maculipennis* in Portugal is similar in morphology to that described in other parts of Europe, although these characters are variable. In its behavior it shows certain constant characteristics which make it a vector in certain regions but not in others.

It breeds prolifically in all fresh waters, finding optimal conditions in rice fields, where larval density is enormous.

Elsewhere malaria is rare or absent, but in the rice areas it is hyperendemic. A peri-domestic mosquito, *atroparvus* rests almost entirely in inhabited quarters, preferring those of animals but overflowing into houses when large numbers are present.

It feeds only under shelter. While preferring the rabbit, *atroparvus* will feed on any animal, including man, that is readily available. Extremely resistant to high temperatures and hence long-lived, feeding frequently, even in the day-time, *atroparvus* is a malaria vector largely in proportion to its density.