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Studies on the immature stages of the *Simulium neavei* Roubaud complex and their associated crabs in the Eastern Usambara Mountains in Tanzania

I.—Investigations in rivers and large streams*

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(*Received for publication April* 18*th*, 1969)

The *Simulium neavei* Roubaud complex is of considerable interest both because the larvae and pupae attach themselves to freshwater crabs (van Someren and McMahon, 1950) and because certain species are important vectors of human onchocerciasis. Two species of the complex occur in the Eastern Usambara Mountains of north-eastern Tanzania, *S. woodi* De Meillon and *S. nyasalandicum* De Meillon (Amani form) (Lewis, 1961). They have been described by Lewis (1960*a*, 1960*b*, 1961) and by Raybould (1967, 1968). *S. woodi* is probably the only important vector of human onchocerciasis in the area. *S.*

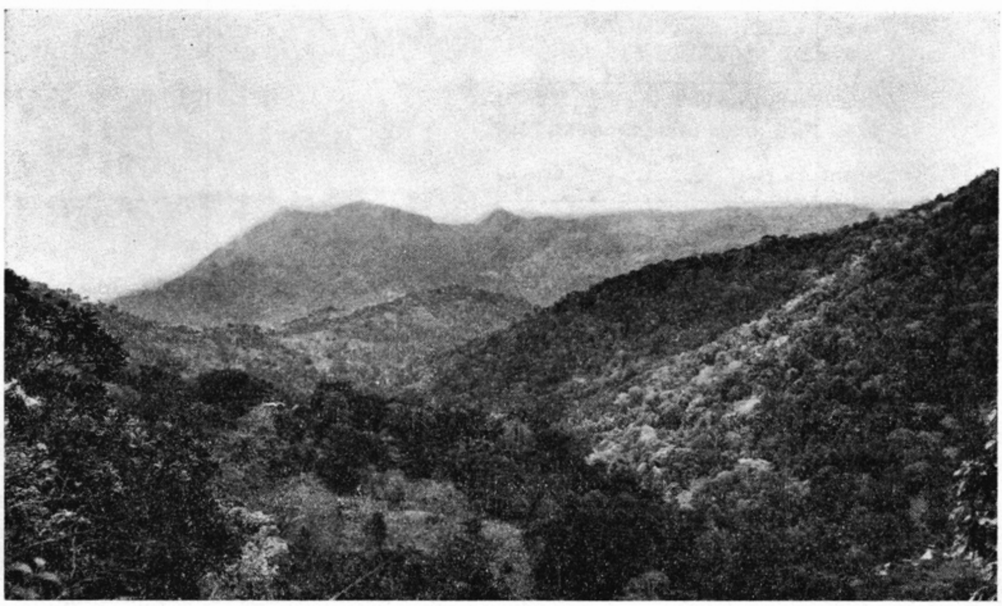
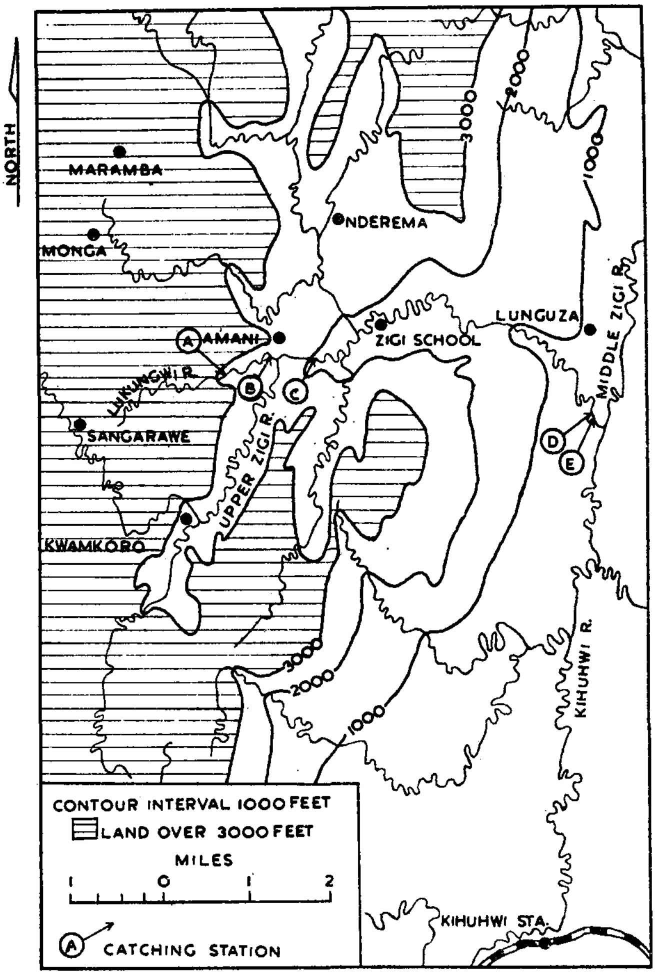


FIG. 1. View of the upper Zigi valley.

*Most of this paper forms part of a thesis accepted for the Ph.D. degree of the University of London in 1968. The thesis is available for consultation in the University Library.

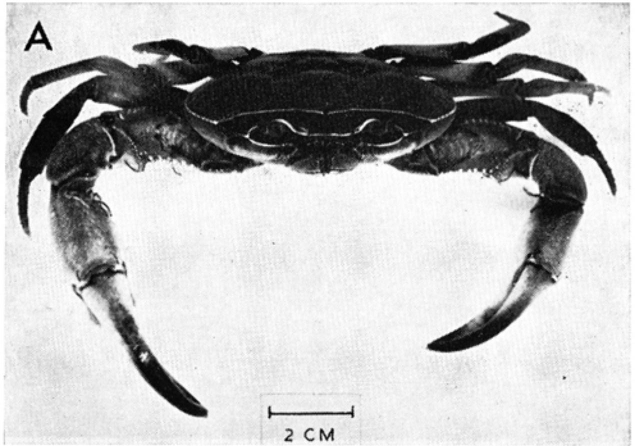


MAP of the River Zigi valley in the Eastern Usambara Mountains, showing the location of the five catching-stations A, B, C, D and E.

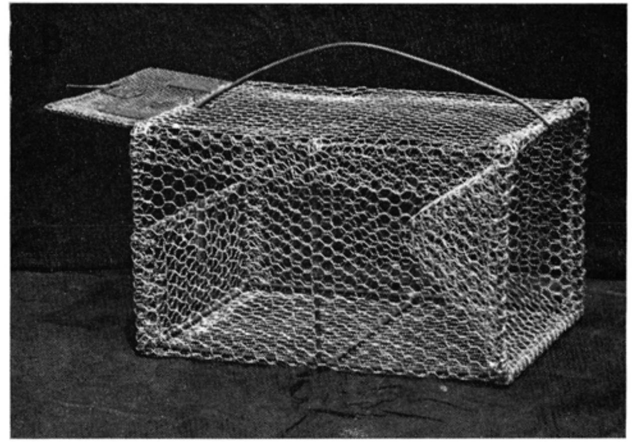
nyasalandicum (Amani form) feeds on cows and occasionally on man; whether or not it plays any part in the transmission of human onchocerciasis is not yet known.

The present paper is concerned with the larvae and pupae of these two *S. neavei*-complex species and with the freshwater crabs with which they are associated. The investigations were carried out in the heavily wooded valley of the River Zigi and its tributaries which descend the eastern slopes of the Eastern Usambaras (see map and fig. 1).

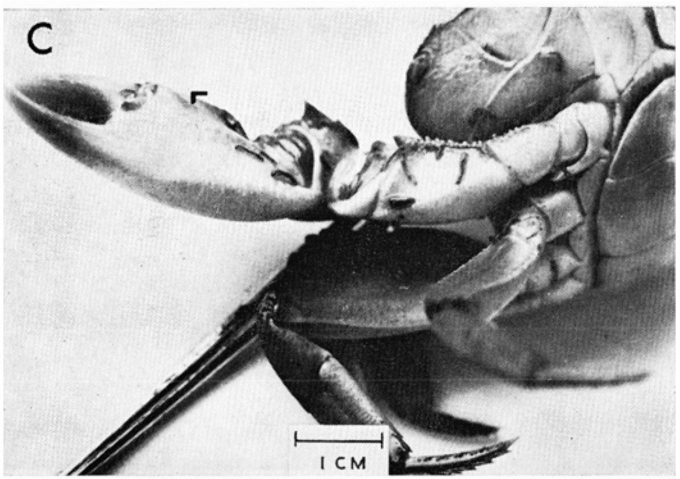
A detailed account of the ecology of the Usambara Mountains is given by Moreau (1933, 1935), and Lewis (1960*b*) draws attention to various features of the area which doubtless affect the distribution of the *S. neavei* complex and the crabs with which they are associated. The evergreen forests of the Usambaras are widely separated from other forests.

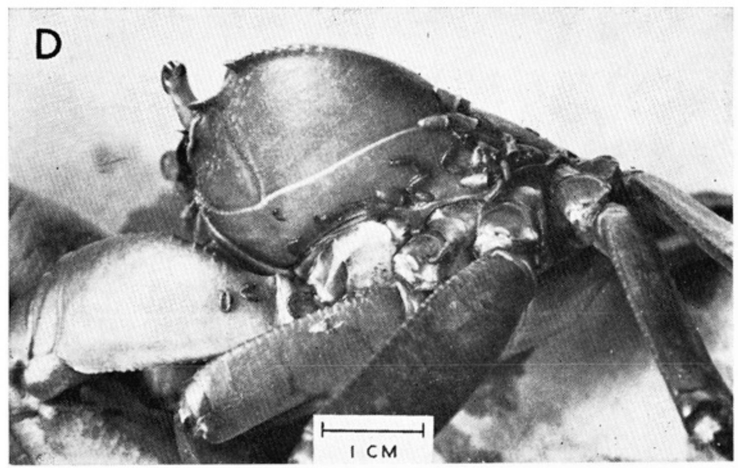


A. The large brown crab.



B. Funnel-entrance trap used for catching crabs.





C–D. *S. nyasalandicum* (Amani form) larvae and pupae attached to the large brown crab.

Because of their topography and proximity to the coast, the Usambara range and two other mountain ranges to the south, the Ngurus and the Ulugurus, have an annual rainfall of about 80 inches. The result is a magnificent forest-formation at a comparatively low altitude. There is very little closed forest anywhere else in East Africa below 3,000 ft., and the greater part is above 5,000 ft.

Moreau points out that throughout Usambara nearly half the year's rain comes in the long rains of March, April and May, and much of the remainder in the short rains in or around November. Although the rainfall on the Eastern Usambara Mountains and in the Zigi valley shows the same two peaks, it is exceptionally well distributed. Consequently, the eastern face of the Eastern Usambara Mountains is the only place in East Africa where a practically unbroken stretch of forest is present down to within 500 ft. of sea-level. Although considerable areas of the eastern foothills have recently been cleared for cultivation, much of the forest still remains.

Moreau remarks that temperatures in the Usambara Mountains are exceptionally low for their altitude and latitude, and that the Zigi falls so steeply—nearly 1,000 ft. in five miles near Amani—that it, as well as its neighbouring streams, retains its mountain character at a lower altitude than any river elsewhere in East Africa.

Three species of freshwater crab occur in the River Zigi and its tributaries, and all three are associated with the larvae and pupae of *S. woodi* and *S. nyasalandicum* (Amani form). Their presence was recorded by Lewis (1960*b*), and Williams *et al.* (1964) have made some observations on their ecology. Williams (*personal communication*) has since informed me that all three crabs were formerly incorrectly identified. The largest species is mostly brown in colour and reaches a size of up to about 9·0 cm. across the carapace (Plate XVIII, A). It has been referred to as *Potamonautes lirrangensis* (Rathbun) (Lewis, 1960*b*; Williams *et al.*, 1964) and as *Potamon lirrangensis* (Raybould, 1966). As its correct name has not yet been decided, it will be referred to in the present paper as the large brown crab.*

The large brown crab has been found at all altitudes in the Eastern Usambaras at which collections have been made, i.e., from 500 to 3,000 ft. It occurs in nearly all streams and rivers in the area, from tiny rivulets in thick forest to large rivers at the forest edge, in both fast and slow-flowing water. The immature crabs are found under stones in the river bed, while mature individuals normally shelter under larger more permanent rocks.

The other two species of crab found in the Zigi river system are a small purple or reddish species with pink claws, frequently carrying young, and a larger species (not so large as the brown crab) with black coloration on most of the dorsal surface of the carapace, orange legs, and a lateral orange strip at the edge of the carapace on each side. This crab will be referred to as the orange-legged crab.

The orange-legged crab is a member of the *Potamonautes hilgendorfi* (Pfeffer) species-group. Its taxonomic status is at present uncertain, but from the ecological differences which distinguish it from *P. hilgendorfi* it seems probable that it is a good species (Williams, *personal communication*). The small purple crab is an undescribed species possibly related to *Potamonautes infravallatus* (Hilgendorf) (Williams, *personal communication*). As has been pointed out by Williams *et al.* (1964), both these crabs are confined to small shallow side-

*Since this paper was written, Mr. T. R. Williams has informed me that he has received the type specimen of *Potamonautes pseudoperlatus* (Hilgendorf) on loan from the Zoologisches Museum, Berlin, and that the large brown crab is undoubtedly of that species.

streams. They are usually found under stones, either in the soft soil of the river bank or at the water's edge, and rarely occur in deep water. The orange-legged crab is seen out of water more frequently than the other two species.

Two other species of crab have been found in the Zigi valley, but neither is associated with the immature stages of the *S. neavei* complex. *P. infravallatus* (Hilgendorf) occurs in wet soil in seepages, and an undescribed species inhabits tree-holes in the forest.

MATERIAL AND METHODS

Crabs bearing larvae and pupae of the *S. neavei* complex were collected with baited traps. Basket-traps were found suitable for the purpose (Raybould, 1966) but they proved to be too fragile for long-term investigations. More permanent traps were therefore constructed from ½-inch-mesh (about 12·5 mm.) wire supported by a steel frame and fitted with a funnel entrance (Plate XVIII, B). From February 1966 until May 1967 crabs were trapped at fortnightly intervals in five catching-stations situated in the Lukungwi and Zigi rivers (see map). Four traps baited with the ripe fruit of *Artocarpus integrifolia* (jack-fruit*) were placed in each catching-station. The traps were positioned with the funnel entrance facing downstream. The range of temperature of the water, during the 24-hour period in which the traps were in position, was measured with a maximum-and-minimum thermometer attached to one of the traps.

The numbers were recorded of the *Simulium* pupae and small, medium-sized and large larvae found on each of the crabs trapped. A record was also kept of the species, sex and largest transverse measurement across the carapace of each crab. The crabs were marked with the name of the catching-station and the date of capture, and were then released. This information was scratched on the carapace of the crab, and was also stamped on a metal tag wired around the meropodite of the right cheliped. In the case of recaptured crabs, the dates on which they had previously been captured and released were recorded.

Fifty larvae (or, if fewer were found, as many as were available) were removed from the crabs at each catching-station during each collection, and were taken to the laboratory for identification. The species composition of the sample was used in estimating the numbers of *S. woodi* and *S. nyasalandicum* larvae and pupae present in the various collections. The shape of the larval hypostomium (Lewis, 1960*a*, 1961) was used as the major criterion for distinguishing the two species.

The Catching-Stations

The catching-stations were located at two points in the Lukungwi and at three points in the Zigi (see map).

Catching-station A (fig. 2) was situated at an altitude of approximately 2,850 ft., at a point where the Lukungwi is a small, shallow, fast-flowing, heavily shaded stream in thick forest. The forest canopy is continuous above the stream, and the stream bed is rocky with stones and boulders.

The Lukungwi increases in volume slightly before reaching the site of catching-station B (fig. 3). This catching-station was situated near the forest edge at an altitude of

*The merits of jack-fruit as a bait for crabs are noted by Raybould (1966).

FIG. 2. Catching-station A.

FIG. 3. Catching-station B.

FIG. 4. Catching-station C.

FIG. 5. Catching-station D.

about 2,350 ft., in an area which has been cleared for cultivation. Scattered trees and ground vegetation provide relatively little shade. The stream bed is rocky with stones and boulders, and the water is shallow in most places, although the catching-station includes a pool at the base of a waterfall.

Catching-station C (fig. 4) was situated in the Zigi at about 1,725 ft. above sea-level, near the village of Chemka, at a point where the Zigi is much larger than the Lukungwi. Although passing through forest, the river itself is not overarched by trees and receives little shade. Here the Zigi is very fast-flowing and passes between large rocks and over stones and boulders. Owing to the presence of deep rock-pools, the depth of the water at this catching-station ranges from a few inches to several feet.

FIG. 6. Catching-station E.

Catching-station D (fig. 5) was situated in the Zigi near the edge of the lowland forest at just over 500 ft. above sea-level. The Zigi at this point is fairly heavily shaded and in most places is less than two feet deep; the river bed is covered with stones and boulders.

Catching-station E was situated a few hundred yards downstream from catching-station D, at about 500 ft. above sea-level. As can be seen from fig. 6, the Zigi at this catching-station has lost the characteristics of a rapidly descending mountain river and flows over a sandy river bed. It is several feet deep, completely exposed, and devoid of stones and boulders.

RESULTS

Collections from the Various Catching-Stations

The large brown crab was the only species trapped in the five catching-stations. The traps sampled mostly medium-sized and large crabs. The males ranged in size from 2·0 cm. to 8·5 cm. in maximum width across the carapace, with a median size of 6·0 cm.; the females ranged from 2·0 cm. to 9·0 cm., with a median width of 6·5 cm. Only 0·9 per cent. of the females trapped and 4·0 per cent. of the males had a maximum carapace width of 3·5 cm. or less. It can be seen, therefore, that small crabs did not normally enter the traps. No soft crabs that had recently undergone ecdysis were trapped.

The number of crabs trapped during individual collections was very variable in all the catching-stations (fig. 7). When heavy rain resulted in a substantial rise in the water level while the traps were in operation, a small collection frequently resulted. This was often the case in catching-station E, where there were no rocks or boulders to provide shelter for the crabs.

In spite of the close proximity of catching-stations D and E, the largest numbers of crabs were trapped in station D and the smallest numbers in station E (Table I). Males and females were present in approximately equal numbers in collections from catching-stations C, D and E, but in collections from catching-stations A and B approximately two-thirds of the crabs were female.

A small fish, *Amphilius krefftii* (Siluroidae), was occasionally trapped; this fish is a predator of *Simulium* larvae (Lewis, 1960*b*). A *Garra* (*Discognathus*) sp. (Cyprinidae) was also collected in the Zigi at Chemka, but this fish because of its very small size was not normally retained by the traps. A species of prawn, *Macrobrachium lepidactylus* Hilgendorf, was frequently trapped in catching-station E but only very rarely in the other catching stations. No *Simulium* larvae were found attached to these prawns.

Recaptured Crabs

In catching-stations A and B respectively 22 per cent. and 30 per cent. of the crabs originally collected were subsequently recaptured. A much smaller proportion of marked crabs were recaptured in catching-stations C, D and E (Table I and fig. 7). A number of crabs were recaptured two or more times, particularly in catching-stations A and B. Male and female crabs were recaptured in approximately the same proportions as in the original collections. One crab marked in catching-station D was recaptured two weeks later in catching-station E. This was the only individual recaptured in a different catching-station.

The numbers of crabs recaptured at various periods after marking and release are recorded in Table II. It can be seen that nearly 100 crabs were recaptured 10 or more weeks

TABLE I
Showing the numbers of crabs collected and the numbers recaptured at each catching-station

Catching-station	No. of crabs collected*			No. of crabs recaptured				No. of crabs recaptured more than once				Mean no. of crabs captured per collection†
	Males	Females	Total	Males	Females	Total	Percentage of no. collected	Recaptured twice	Recaptured 3 times	Recaptured 4 times	Recaptured 5 times	
A	116 (35·0%)	215 (65·0%)	331	27 (36·5%)	47(63·5%)	74	22·4	21	3	1	1	12·0
B	88 (32·2%)	185 (67·8%)	273	27 (33·3%)	54(66·7%)	81	29·7	30	5	3	—	11·5
C	145 (52·7%)	130 (47·3%)	275	14 (43·8%)	18(56·3%)	32	11·7	9	1	—	—	9·3
D	181 (46·5%)	208 (53·5%)	389	13 (44·8%)	16(55·2%)	29	7·5	3	1	—	—	12·4
E	109 (47·0%)	123 (53·0%)	232	10 (38·5%)	16(61·5%)	26	11·2	4	1	—	—	7·7
Totals...	639 (42·6%)	861 (57·4%)	1,500	91 (37·6%)	151(62·4%)	242	16·1	67	11	4	1	53·7

*Excluding recaptures. †Including recaptures.

TABLE II
Showing the numbers of crabs recaptured at various periods after marking and release in each catching-station during 34 fortnightly collections

Catching-station	No. of weeks between marking, release and recapture																							
	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
A: no. of crabs recaptured	24	23	13	13	6	6	6	3	2	2	2	1												1
B: ,,	33	25	15	13	10	5	5	2	3		1	4	3				1				1		1	1
C: ,,	13	10	4	3	4		1	2								1	1		2					
D: ,,	12	4	6	1	5	1		2			1	1												
E: ,,	11	6	2	2	2	4	1									1					1	1		
Totals...	93	68	40	32	27	16	13	9	5	2	4	6	3			2	2		2		2	1	1	2

after being marked and released, and that more than 50 were recaptured after 14 or more weeks. Two crabs were recaptured after 48 weeks, indicating that they had not undergone ecdysis during that period.

Distribution of Larvae and Pupae on the Crabs

Larvae and pupae tended to be concentrated on the sides of the crab, the chelipeds, the basal segments of the walking legs, and in the eye sockets (Plate XVIII, C-D). Of those attached to the chelipeds, the majority were present on the inner surface of the various segments and were therefore well protected when the chelipeds were folded in their normal position in front of the body. Small early-stage larvae appeared to be less specific in their choice of attachment sites than large larvae and pupae.

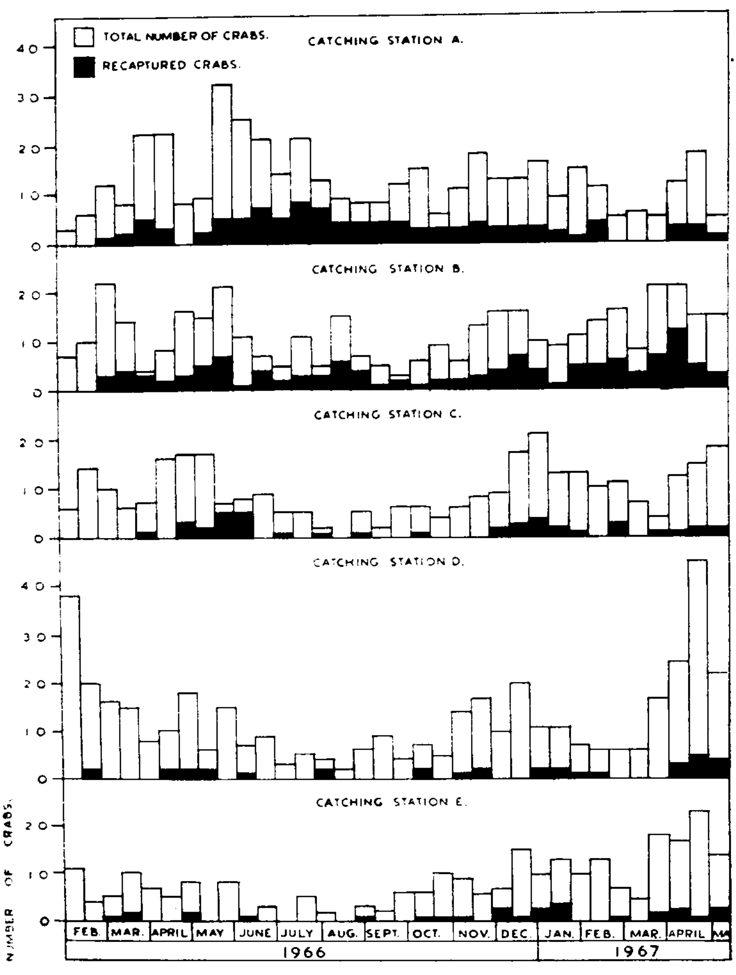


FIG. 7. Showing the numbers of large brown crabs collected at each of the five catching-stations.

The pupae showed no obvious orientation in any particular direction. On crabs with a high pupal density, the orientation of individual pupae appeared to be partly determined by the space available.

Numbers of Larvae and Pupae on Crabs of Different Sizes

It may be seen from Table III that, on crabs measuring up to about 5 cm. across the carapace, the number of attached larvae and pupae increased progressively with the size of the crab. On crabs above that size, no further increase in the number of attached larvae and pupae was apparent.

TABLE III

Showing the numbers of *S. neavei*-complex larvae and pupae found on large brown crabs of various sizes collected at catching-stations A, B, C, D and E

Maximum width of carapace, in cm.	No. of crabs	No. of larvae and pupae	No. of larvae and pupae per crab
2·0	3	7	2·3
2·5	3	7	2·3
3·0	9	65	7·2
3·5	22	152	6·9
4·0	63	912	14·5
4·5	100	1,626	16·3
5·0	156	3,134	20·0
5·5	212	5,435	25·6
6·0	273	6,914	25·3
6·5	299	7,799	26·1
7·0	270	7,149	26·4
7·5	230	5,037	21·9
8·0	126	2,418	19·2
8·5	53	1,368	25·8
9·0	7	163	23·3

S. vorax *Larvae Attached to Crabs*

Four larvae of *S. vorax* Pomeroy were found attached to crabs at catching-station C. Three of these larvae had well-developed pupal respiratory histoblasts, and probably belonged to the final instar; the fourth larva was at a slightly earlier stage. The two crabs on which the larvae were found were both collected on the same day, when the Zigi was swollen after heavy rain.

Numbers of S. nyasalandicum *and* S. woodi *Larvae and Pupae Collected from Crabs at the Various Catching-Stations*

From Table IV and fig. 8 it can be seen that large numbers of *S. nyasalandicum* larvae and pupae were present in all the catching-stations and that they were most numerous in catching-stations C and D. The immature stages of *S. woodi*, on the other hand, were almost wholly confined to catching-stations A and B (Table IV and fig. 10); only two larvae of this species were collected from other catching-stations during the investigation.

These results suggest differences in the distribution of the breeding-sites of the two species. Whereas *S. nyasalandicum* was more numerous in two of the catching-stations situated in the Zigi, a relatively large river, than in those located in Lukungwi, a small tributary, *S. woodi* was virtually confined to the Lukungwi. The only exception to this general pattern of distribution was in catching-station E, where fewer *S. nyasalandicum* larvae and pupae were present per crab than in catching-stations B, C and D. At catching-station E, however, the Zigi had lost the characteristics of a steeply descending mountain river and represented a habitat quite different from the other catching-stations.

A second difference between the two species was that *S. woodi* larvae and pupae were present in only very small numbers, whereas those of *S. nyasalandicum* were present in abundance. Even in catching-stations A and B *S. woodi* represented only 1·0 per cent. and 0·5 per cent. respectively of the total number of *S. neavei*-complex larvae and pupae collected. While mean numbers of *S. woodi* were always much less than one larva or pupa per crab, the mean number of *S. nyasalandicum* larvae and pupae per crab in catching-station C, for example, was 38. The largest number of the immature stages of *S. nyasalandicum* found on a single crab during the investigation was 275.

TABLE IV

Showing the numbers of *S. nyasalandicum* and *S. woodi* larvae and pupae collected at each catching-station during 34 fortnightly collections*, together with the mean water temperature† at each catching-station

Catching-station	Mean water temperature, in °C.	*S. nyasalandicum* larvae and pupae			*S. woodi* larvae and pupae		
		Total no. collected	Mean no. per collection	Mean no. per crab	Total no. collected	Mean no. per collection	Mean no. per crab
A	19·0	5,120	150·6	11·9	48	1·4	0·1
B	20·0	7,030	206·8	17·9	35	1·0	0·09
C	21·0	11,982	352·4	37·9	—	—	—
D	23·0	14,205	417·8	33·7	1	0·03	0·002
E	24·0	4,010	117·9	15·2	1	0·03	0·004
	Totals	42,347	1,245·5	23·2	85	2·5	0·05

*Estimated from the percentage species composition of 50 larvae sampled at each collection.
†The means of 24-hour maximum and minimum temperatures taken at fortnightly intervals.

Relative Numbers of S. nyasalandicum *Pupae and Small, Medium-Sized and Large Larvae Collected from Crabs at the Various Catching-Stations*

It can be seen from Table V that the relative numbers of pupae and of small, medium-sized and large larvae were remarkably similar at all the catching-stations. Large larvae, which represented about 55 per cent. of the total number of larvae and pupae collected, were by far the most numerous while small and medium-sized larvae represented about 21 per cent. and 16 per cent. respectively of the total collections. Pupae were present in the smallest numbers and represented only about 7 per cent. of the total. Larvae, at all except the very early stages of development, and pupae were present on the same crabs.

Seasonal Changes in the Numbers of S. nyasalandicum *and* S. woodi *Larvae and Pupae in the Five Catching-Stations*

Changes in the mean numbers of *S. nyasalandicum* larvae and pupae per crab at various stages in the investigation followed a rather similar pattern in most catching-stations (fig. 8). A fairly representative picture of the over-all situation, therefore, may be obtained by reference to the combined results from all five catching-stations (fig. 9).

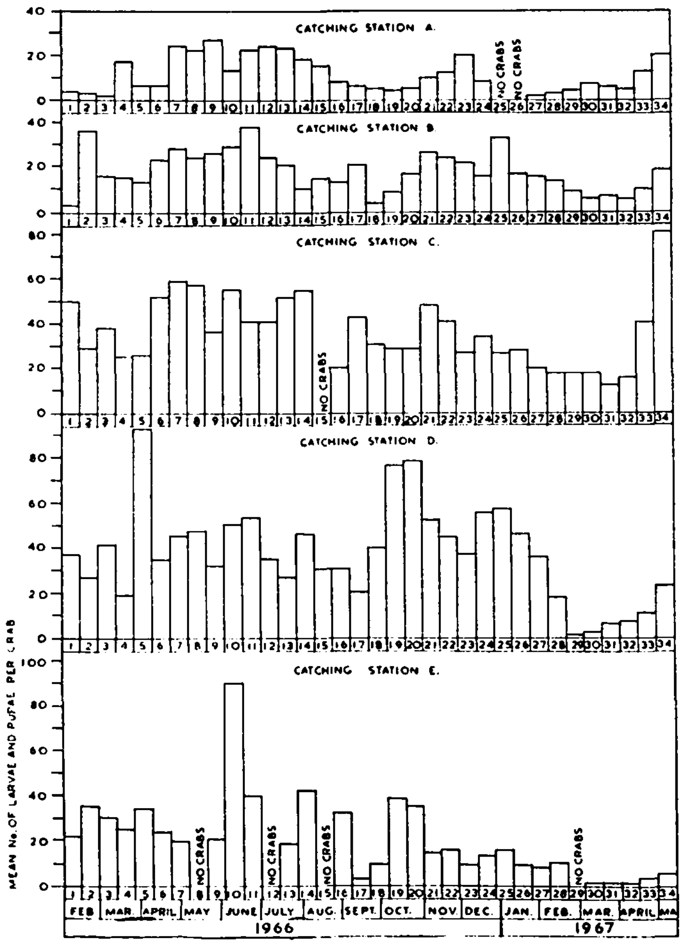


FIG. 8. Showing the mean numbers of *S. nyasalandicum* larvae and pupae found per crab in each of 34 collections at each collecting-station.

These combined results indicate that the immature stages reached peak densities from April to July 1966 and from October 1966 to January 1967. Smaller numbers were collected in February and March 1966 and in August and September 1966. Numbers were smallest in February, March and April 1967, following a period of unusually low rainfall in November and December 1966 and January, February and the early part of March 1967. Much smaller numbers were present during that period than in the corresponding period of the previous year. A marked increase in the numbers of larvae and pupae collected occurred during the latter part of April and in May 1967. These results appear to show a correlation with rainfall (fig. 9).

The mean numbers of *S. woodi* larvae and pupae present per crab in individual collections in catching-stations A and B are shown in fig. 10. Numbers of this species were too small for seasonal changes to become apparent.

TABLE V

Showing the numbers of *S. nyasalandicum* larvae (small, medium-sized and large) and pupae collected at the five catching-stations, together with each total expressed as a percentage of the total number of larvae and pupae collected

Catching-station	Larvae						Pupae		Total
	Small		Medium		Large				
	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	
A	1,003	19·4	860	16·6	2,869	55·5	436	8·4	5,168
B	1,593	22·5	1,138	16·1	3,951	55·9	383	5·4	7,065
C	2,621	21·9	2,088	17·4	6,598	55·1	675	5·6	11,982
D	2,955	20·8	2,225	15·7	7,781	54·8	1,245	8·8	14,206
E	792	19·7	569	14·2	2,339	58·3	311	7·8	4,011
Totals	8,964	21·1	6,880	16·2	23,538	55·5	3,050	7·2	42,432

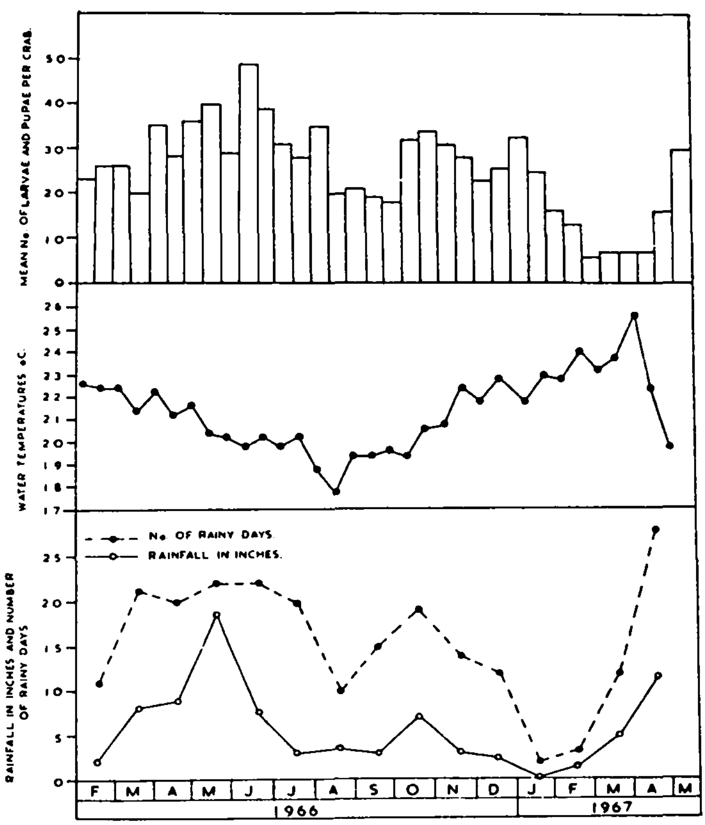


FIG. 9. Showing the mean numbers of *S. nyasalandicum* larvae and pupae found per crab at all five catching-stations combined, together with mean water temperatures (means of 24-hour maximum and minimum temperatures taken at fortnightly intervals in each catching-station) and mean monthly rainfall recorded at Amani.

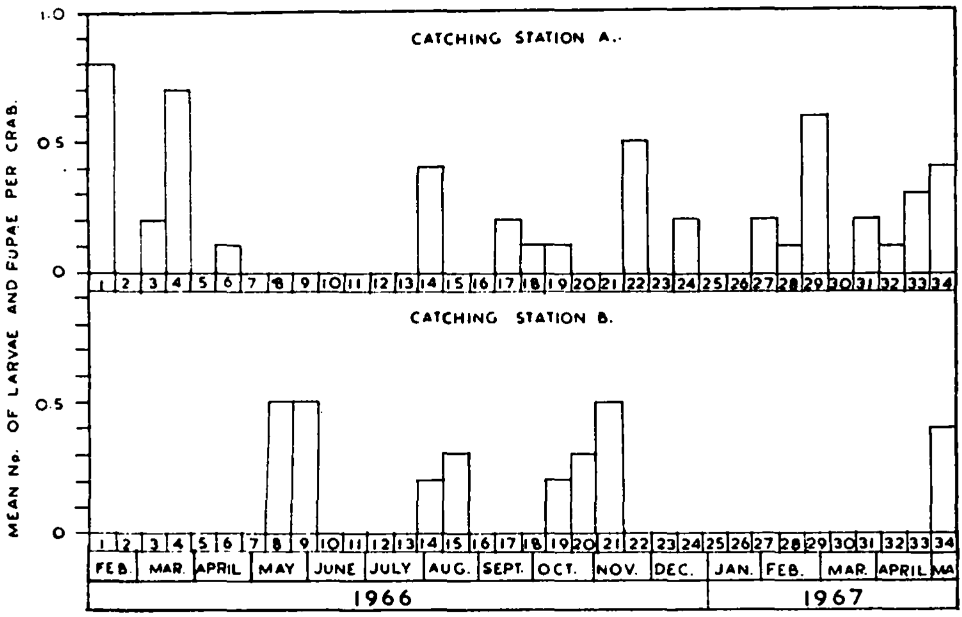


FIG. 10. Showing the mean numbers of *S. woodi* larvae and pupae found per crab in each of 34 collections at catching-stations A and B.

DISCUSSION

The Distribution of the Breeding-Sites of S. woodi *and* S. nyasalandicum

Two of the most significant findings of this investigation were that the breeding-sites of the two *S. neavei*-complex species appeared to differ in their distribution, and that the immature stages of *S. woodi* were apparently confined to small tributaries throughout the year.

The fact that larvae and pupae of *S. woodi* were present only in very small numbers even in the Lukungwi suggested, however, that none of the five catching-stations were located in places particularly favourable for the breeding of the species. It seemed likely, therefore, that the main breeding-sites of *S. woodi* were located in even smaller streams. This hypothesis has since been substantiated. The results of subsequent investigations on the breeding-sites of this vector species are described in the second of these papers (Raybould and Yagunga, 1969).

The Efficiency of Funnel-Entrance Traps for Sampling Crabs

The number of crabs trapped during individual collections was very variable in all the catching-stations. More consistent results would probably be obtained by using larger numbers of traps. Even with more traps, however, the effect of changing conditions, such as a rise in water level after heavy rainfall, would probably not be overcome.

The funnel-entrance traps gave a biased sample in that they cannot be used in shallow streams and small crabs were not normally collected. Similar observations were made by Turnbull-Kemp (1960), who noted that 'Trapping is selective in that small specimens having a maximum carapace width of less than 17·0 mm. are not attracted to baits so far tested.' Hand collections, however, are difficult when the streams are swollen after heavy rainfall, and they cannot be carried out in places like catching-station E where the water is too deep. Because mature crabs tend to shelter under large permanent rocks, while smaller individuals are found under stones in the river bed, hand collections of the large brown crab are biased in favour of small crabs.

Further studies are required to obtain more information on the efficiency of the various sampling techniques in different habitats. In particular, the traps described by McMahon, Highton and Goiny (1958) should be tried out in the Eastern Usambaras.

Numbers of Crabs Collected in the Various Catching-Stations, and the Significance of Crab Recaptures

The fact that only the large brown crab was trapped supports earlier observations that both the orange-legged and the small purple crab are confined to small side-streams at the water's edge.

No explanation can be given for the preponderance of female crabs in catching-stations A and B.

The fact that much smaller numbers of crabs were collected from catching-station E than from catching-station D, despite their close proximity, was probably due to major differences between the two habitats. The absence of rocks and boulders in catching-station E to provide shelter for the crabs was probably significant in this respect.

Scratching information on the carapace of the crab was found to be a more satisfactory method of marking than wiring a metal tag around the cheliped, for the wire eventually rusted and some of the metal tags were lost. Also, the presence of the wire may have caused difficulties during ecdysis and could possibly have resulted in the death of some of the crabs. This could have affected the results.

A much larger proportion of the large brown crabs collected at catching-stations in the Lukungwi were subsequently recaptured than was the case at catching-stations in the Zigi (Table I). This suggests that in the Zigi the crab population was larger and able to disperse more freely.

Considering that only four traps were used per catching-station, the number of recaptured crabs was high, especially in catching-stations A and B. A considerable number of crabs were recaptured twice, and a few were recaptured three or more times.

The large number of recaptured crabs, some of which were collected many weeks or even months after being marked and released (Table II), suggests that dispersal by the crabs is limited. It is not known how much these results were affected by marked crabs undergoing ecdysis, after which they would no longer be distinguishable. The results indicate, however, that at least a proportion of the population of larger crabs (very small crabs were not sampled by the traps) undergo ecdysis very infrequently, and that the large brown crab probably has a life-span of a number of years.

Further investigations are required on the size of the crab populations. Turnbull-Kemp (1960) made quantitative estimations of crab populations in Rhodesian trout-streams. Similar information for the Eastern Usambaras would make possible a crude estimate of the total numbers of *S. neavei*-complex larvae and pupae per stream or river.

Numbers of Larvae and Pupae on Crabs of Different Sizes

The results of the present investigation are somewhat similar to those obtained by Browne (1960) with *S. neavei* in the Congo. One difference between the two sets of data is that Browne's results suggest that the numbers of attached larvae and pupae increased with the size of the crab, even with crabs measuring more than 5·0 cm. across the carapace. The two sets of results, however, are not strictly comparable, because Browne recorded the percentage of crabs in each size-category found carrying larvae or pupae, but did not count the actual number of larvae and pupae present.

Distribution of Larvae and Pupae on the Crabs

Both the larvae and the pupae appeared to be located in positions on the crab which would probably afford good protection from both damage and predators, and where the water current would be expected to be turbulent and of reduced velocity. They were almost never found on the exposed dorsal surface of the carapace, where a strong laminar flow would be expected, or on the underside of the crab, on the abdomen or the sterna of the thorax. Both these positions would probably be disadvantageous in that the larvae and pupae might be crushed when the crab squeezes beneath rocks. The dorsal surface of the carapace would also presumably be more exposed to the attack of predators, such as larva-eating fish, than the positions actually occupied by the larvae.

These observations are of particular interest in connection with the recent findings of Maitland and Penney (1967), who carried out detailed studies on the ecology of the Simuliidae in a Scottish river. They observed that 'On boulders, larvae occur mainly on the upper surface near the front where the current is strong and laminar, whilst pupae are found mainly on the back surfaces where the current is weaker and turbulent in nature.' They point out that during the actual process of spinning a cocoon and pupation the larvae are probably more likely to be swept away by the current, and that if a pupa is swept off a boulder it has no means of regaining a suitable position. It is obviously advantageous, therefore, if a sheltered position can be found.

These considerations may help to explain the distribution of the pupae on the crabs. They fail, however, to explain why the larvae have a similar distribution, though it is possible that being swept away is a greater hazard for larvae of the *S. neavei* complex than for those of free-living species. Whereas the larvae of most species have a good chance of finding another suitable anchorage-site, the chances of finding another crab are probably remote.

Lewis (1960*b*) has pointed out that the slender and delicate gills of the *S. neavei* complex suggest that these species are derived from a form which bred in small, usually slow, streams, and that by associating themselves with crabs they have been able to breed in both slow and fast streams, and thus to extend their range. If this is in fact the case, then perhaps it is not surprising that both the larvae and the pupae appear to be confined to positions on the crab where the current would be expected to be weakest. However, when considering the possible effect of water current on the distribution of larvae and pupae on the crabs, it is important to remember that the crabs spend the greatest part of their lives in rather still water underneath boulders.

Much more information is required on the nature of the water current that passes over the different parts of the crab's body, and on the mode of attack of predators such as fish, in order to assess the possible significance of these and other environmental factors in determining the distribution of the larvae and pupae on the crab. Such information might help to elucidate the advantages that the larvae and pupae gain from the association.

The Orientation of Pupae on the Crabs

The fact that the pupae showed no obvious orientation in any particular direction does not appear to support the hypothesis of Corbet (1961) that the crabs give 'protection against disorientation of the pupae with respect to the current'. The absence of any dominant orientation of the pupae on the crabs is probably related to the type of water current to which they are subjected. Interesting observations on the effect of water current on the orientation of pupal Simuliidae have been made by Maitland and Penney (1967). They point out that when pupae occur in laminar flow they are normally orientated, as noted by Grenier (1949), with the posterior end towards the current and the postero-anterior axis in line with the current flow. On the back surfaces of boulders and in crevices and ledges, where the flow is turbulent, the orientation is much more varied, and sometimes appears to be partly determined by the degree of crowding. Their description of pupal orientation under crowded conditions in turbulent flow, based on observations made in a Scottish river, could equally well apply to the situation observed on crabs during the present investigations.

Relative Numbers of Pupae and of Small, Medium-Sized and Large Larvae on the Crabs

The results obtained are no doubt partly a reflection of the duration of the various stages of development. The relatively small number of pupae, for example, is probably largely due to the short duration of the pupal stage.

The fact that large larvae were more numerous than small and medium-sized larvae combined is of particular interest, in that it suggests that some larvae do not attach until the later stages of development. This apparent preponderance of large larvae would appear to support the hypothesis put forward by Corbet (1961) 'that the biological significance of the association is to be found in some adaptive requirement peculiar to *older larvae* or to *pupae*, since otherwise it is presumably the *smallest* larvae that would be the most numerous'. No definite conclusions, however, can be drawn until more is known about the duration of the various stages of development. A slower growth rate in the later instars, for instance, might possibly account for the apparent preponderance of large larvae. In any case, the present results are of doubtful validity, because the larvae were counted in the field with the naked eye and large numbers of very small larvae may have been overlooked.

Although the majority of larvae observed were large, very small larvae were also present in considerable numbers. This finding differs from the observations of Browne (1960), who found no very small *S. neavei* larvae on the crabs which he examined in the Congo. He also observed that 'On individual crabs, the larvae were usually of the same length; rarely, a single crab harboured larvae of differing lengths'. In the present investigation larvae at various stages of development were present on the same crabs.

More detailed investigations are required on this subject. If the relative numbers of attached larvae of each instar (or size-category, if the different instars are found to be indistinguishable) could be accurately determined, the information would be of considerable interest and might shed further light on the possible biological significance of the association.

Seasonal Changes in the Numbers of S. nyasalandicum *Larvae and Pupae*

The number of crabs collected during individual collections was too variable for minor apparent fluctuations in the number of larvae and pupae per crab to be regarded as significant. A marked reduction, however, in the numbers of *S. nyasalandicum* larvae and pupae on crabs collected during February, March and April 1967, following a period of unusually low rainfall, was noted in all the catching-stations, and would seem to be significant. Although there appears to be a correlation with rainfall, such correlation does not necessarily imply a direct effect on the larvae and pupae themselves; changes in their numbers are no doubt also influenced by factors affecting the adults and the eggs.

SUMMARY

1. Two species of the *Simulium neavei* complex occur in the Eastern Usambara Mountains in Tanzania, *S. woodi* and *S. nyasalandicum* (Amani form). Studies on the larvae and pupae of these two simuliids, and on the three species of crab with which they are associated, were carried out in the Zigi River and in one of its tributaries.

2. At fortnightly intervals for 16 months, crabs were collected with baited traps in five catching-stations. Relevant information on the crabs and their attached *Simulium* larvae and pupae was recorded, and larvae were removed for identification. The crabs were marked with the place and date of capture and were then released.

3. Only one species of crab was trapped. The number of crabs collected was very variable and small crabs rarely entered the traps. The need is stressed for further study on methods of sampling crab populations. The results suggest that the crabs are long-lived and that their dispersal is limited.

4. The distribution of larvae and pupae on the crabs is described, and its possible significance is discussed. The pupae showed no dominant orientation. Data are provided on the numbers of larvae and pupae attached to crabs of various sizes. Attached larvae were much more numerous than pupae. The significance of the size distribution is considered.

5. Larvae and pupae of *S. woodi* were present only in very small numbers; those of *S. nyasalandicum* were much more numerous. Numbers of *S. nyasalandicum* larvae were smallest after a period of unusually low rainfall.

6. The results indicate that the breeding-sites of the two *S. neavei*-complex species differ in their distribution, and that the immature stages of *S. woodi* are confined to small tributaries throughout the year.

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