was an increase in the number of *A. scottae* larvae on the stones-in-current. Although the numbers of *A. scottae* were lower then than the numbers of *C. thomasseti* in the preceding months, the temporal separation of these two species allowed for an extended period of predation on Simuliidae by Hydropsychidae.

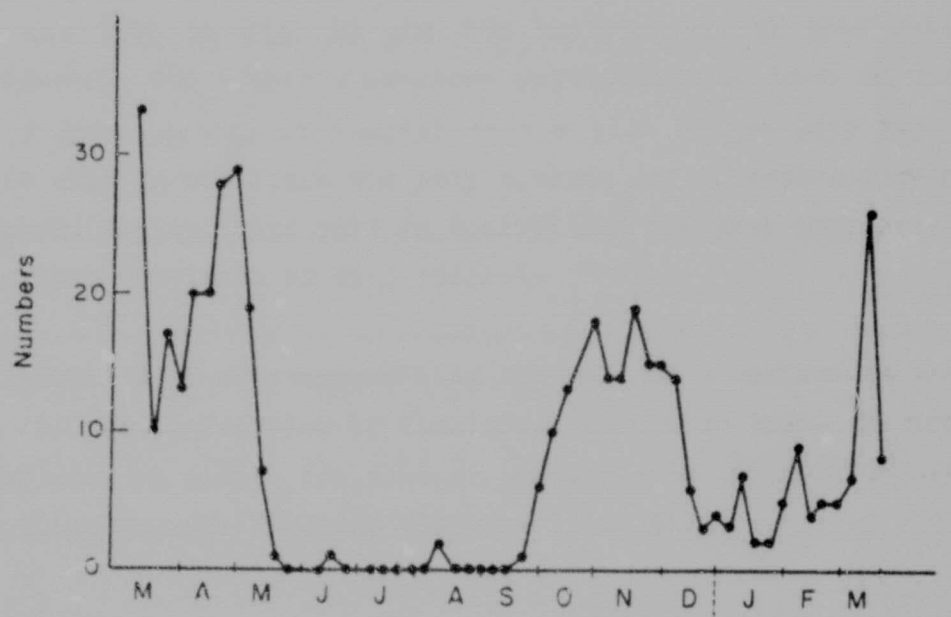

FIGURE 10: The number of small hydropsychid larvae encountered in 1 000 litres of drifting river water. Samples collected upstream of the study rapid at weekly intervals from 10 March 1979 to 20 March 1980

It could thus be hypothesised generally that a decrease in large *C. thomasseti* larvae in the late winter (Fig. 9) was associated with the emergence of adults (Ch. 7, Pg. 135) and a subsequent start of egg laying in September (Appendix 2.8, Table 2.27). Small hydropsychid larvae now entered the drift in increasing numbers from late September onwards (Fig. 10) and started colonizing stones-in-the-current during October (Fig. 9). While this was happening there was a massive increase in the population of Simuliidae (Fig. 9). By November 1978 and 1979 and by October 1980 there was a distinct increase in the number of larger *C. thomasseti* larvae on stone samples and an associated decrease of simuliid larvae (Fig. 9).

It would thus appear that reduced predation on Simuliidae in August and September led to population increases of these animals and when larger hydropsychid predators again appeared in appreciable numbers in October and November, decreases in the population of blackflies followed.

The population of *C. thomasseti* was considerably higher when levels of Simuliidae were also higher (see population levels for 1978, 1979 and 1980 in Fig. 9) and the implications of this should be considered. The effect a predator population can have on controlling a prey species is limited by the size of the prey population and if this is too large the prey species may effectively swamp the predators making their role in controlling the prey population size negligible (Carlsson *et al.*, 1977).

The inference of over-emphasizing the role of predators in controlling the population size of Simuliidae should be borne in mind and it is best to assess the role of predators in conjunction with the many other factors involved (See Chs 7 and 8).

g) Competition

Owing to differential usage of available resources by the different species encountered on the stones in the rapids at Witrand, interspecific competition between any of the Simuliidae and other species seemed unlikely (see Ch. 7, Pg. 108 ff. for discussion). Other species which occurred in sufficiently high numbers to possibly influence the availability of some of the resources utilised by Simuliidae were however considered.

The highest population density of chironomid larvae on stones from the current over the three years occurred in October 1978 (Fig. 11a). This was also when the largest density of Simuliidae was seen (Fig. 5). Further large increases in chironomid larval numbers occurred in September 1979 and August 1980. These were consecutively one month prior to and one month after the largest population of Simuliidae was observed.

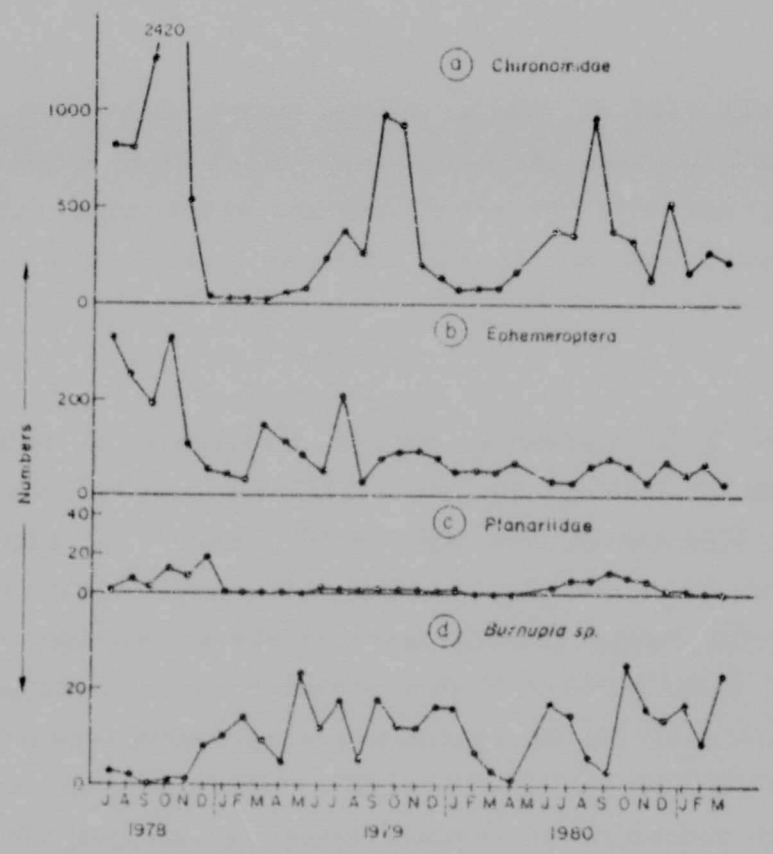

FIGURE 11: Numbers of fauna other than Simuliidae encountered on stone samples collected at monthly intervals from the study rapid from July 1978 to March 1981. Counts are Sichel mean numbers per 1 000 cm^2 of stone surface area

Ephemeroptera nymphs (Fig. 11b) were evenly represented on stones in the current throughout the three year period except when population levels reached peaks in October 1978, and March and July 1979. No seasonal pattern was observable but this could be due to the fact that Ephemeroptera in Fig. 11b were represented by a number of species which showed differing seasonal variations in occurrence and abundance (Appendix 5, Table 5.3).

Planariidae (Fig. 11c) showed an increase in numbers on stones after a series of 'shut downs' had been effected in December 1978 and September 1980. No other seasonal increase in the abundance of this group of animals was detected.

A *Burnupia* sp. was present in low numbers throughout 1978, but appeared in appreciable numbers from December 1978 onwards, peak densities being attained in January, June and October 1980 (Fig. 11d).

None of the above four faunal groups appear to have played any significant role in influencing the population size of Simuliidae and it appears that their varying modes of life and resource utilization in no way interfered with that of the Simuliidae.

h) Parasitism

The relationship of a decrease in the percentage of *S. chutteri* pupae as a total of all Simuliidae and an increase in mermithid parasitism found by Chutter (1968) was not discernible in the present study (Fig. 12). The highest percentage of mermithid parasitized *S. chutteri* larvae occurred between August and October 1979 when simuliid population levels were increasing (Figs 3 and 5) and the *S. chutteri* pupae as a percentage of all Simuliidae (to make this data set comparable to Chutter's (1968) study) was also increasing. The decrease of pupal numbers in September 1979 was due to vandalism described in Appendix 2.8, and is indicated by the arrow in Fig. 12a. A note of caution could be added at this stage, in that there are seasonal fluctuations in pupae (Chs 6, 7 and 8) and when expressed as a percentage of the total Simuliidae the role parasitism by Mermithidae plays in controlling pupal numbers may be erroneously correlated. The discovery of a live mermithid worm in an adult female *S. chutteri* by Mr G.J. Begemann in September 1977 indicated that infection of *S. chutteri* larvae by Mermithidae did not necessarily prevent pupation, confirming Anderson and Dicke's (1960) observation on North American species. However reproduction would be prevented because the ovaries of this female were atrophied.

A higher percentage of parasitism in Simuliidae was observed by Carlsson (1967) to occur only when certain minimum population densities were attained. The population density of *S. chutteri* in 1979 and 1980 was apparently low enough to prevent intense para-

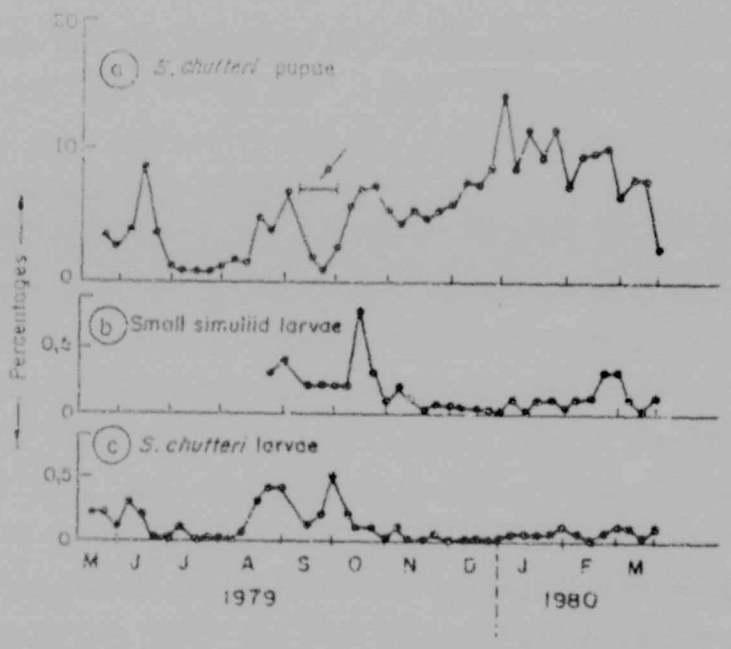

FIGURE 12: a) *Simulium chutteri* pupae as a percentage of the total number of Simuliidae. b) The percentage of small simuliid larvae parasitized by Mermithidae. c) The percentage of *S. chutteri* larvae parasitized by Mermithidae. Samples of Simuliidae examined collected from artificial substrata at weekly intervals from 10 March 1979 to 20 March 1980

sitism by Mermithidae and observed parasitism remained below one per cent throughout that period (Fig. 12b and c). This indicated that parasitism played a minor role in controlling the population size of *S. chutteri* during the period discussed above.

It should be noted however, that parasitism by Nematodes was only discernible when large worms were seen in the gut and body cavity of *Simulium* larvae, and many larvae infested by small nematodes, as well as those that may have perished due to nematode parasitism may have been missed in the survey for parasitism. The standarized way of observing parasitism however made levels of parasitism comparable throughout and this was then a useful comparative method.

Simulium adersi was parasitized by Mermithidae and Microsporidia (Fig. 13). The percentage of larvae parasitized by Mermithidae was again low (Fig. 13a) but increased as the population size of *S. adersi* larvae grew (Fig. 3e). Mermithidae thus appear to have

played a minor role in controlling the population size of *S. adersi*. Parasitism by Microsporidia (Fig. 13b) may have influenced the decline of the larval *S. adersi* population in June 1979 (Fig. 3e) and also the low number of *S. adersi* pupae from November 1979 through to March 1980 (Fig. 5e).

It was apparent that *S. adersi*, even though present in the rapids at Witrand in lower numbers than *S. chutteri* during 1979 and 1980, was more severly infested by parasites, both Mermithidae and Microsporidia, than the latter species. This could be due to *S. adersi* occupying a habitat not optimally suited to the average individuals of that species and a broadening of its niche then reduced the fitness of individuals in the population which occupied this new habitat (see also Ch. 7.4 for discussion on coexistence of *Simulium* species).

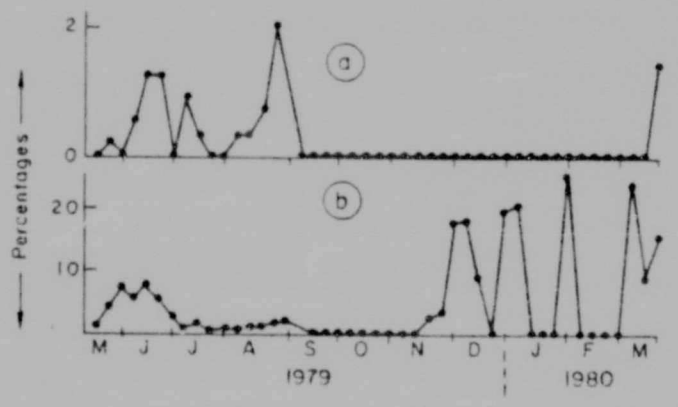

FIGURE 13: The percentage of parasitized *S. adersi* larvae on artificial substrates collected at weekly intervals from 10 March 1979 to 20 March 1980. a) Parasitized by Mermithidae. b) Parasitized by Microsporidia

4.4 CONCLUSIONS

Seasonal fluctuations in simuliiid population densities were brought about by a number of naturally occurring events and artificially induced water flow changes.

An increase in particulate food matter closely followed a population increase of Simuliidae. Water and air temperature as well as wind

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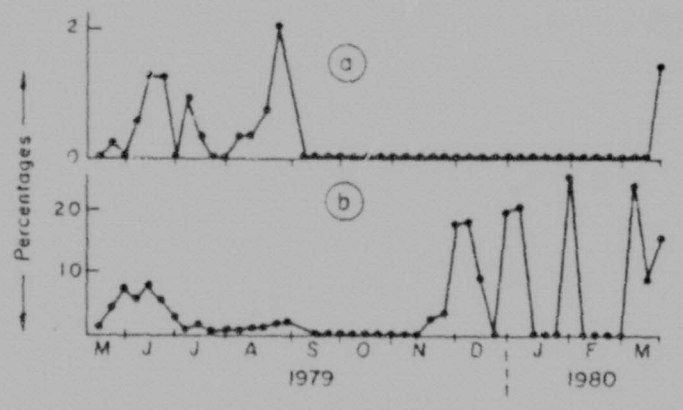

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influenced egg laying activity. Rainfall influenced air and water temperature, food availability and also the flow regime of the river. The flow rate of water in the river was important in making larger or smaller areas suitable for colonization by Simuliidae. Water flow manipulation considerably increased the mortality of the aquatic stages of Simuliidae and was thus an important factor in controlling the population size of blackflies. Hydropsychidae as natural invertebrate predators also showed seasonal abundances that were followed by a decline in simuliid numbers. Various species considered as competitors did not have any obvious effect on simuliid numbers and parasites of Simuliidae were also of minor importance in affecting the population levels of their hosts at the densities encountered in 1979 and 1980.

A discussion and conclusions on the significance of the seasonal occurrence and abundance of some of the simuliid species encountered in the rapid in the Vaal River at Witrand is given in Chapter 7.

5. THE RELATIVE ABUNDANCE OF SPECIES IN A SMALL STREAM FLOWING INTO THE VAAL RIVER

5.1 INTRODUCTION

From the detailed study carried out in the rapid on the farm Witrand (Ch. 4), and at the four other stations along the Vaal River (Ch. 3), it was apparent that some distinct seasonal or habitat preferences were shown by the various species of *Simulium* s.l. encountered in the Vaal River.

A small permanently flowing stream was created by a leakage from the main irrigation canal about seven hundred metres below the Vaalhartz Diversion Weir. This stream flowed underneath a small bridge before running on back into the Vaal River (Plate 7).

As conditions in this stream were very different from those in the main river, it was considered worthwhile to examine the composition of the benthic fauna. A comparison of the stream fauna with that from the main river would add valuable information as regards the ecological preferences of the various aquatic species encountered in the nearby Vaal River.

5.2 MATERIALS AND METHODS

At monthly intervals from June 1977 to January 1980 samples were collected upstream of the bridge (Plate 7). On each occasion a number of small stones and bits of trailing vegetation, when present, were collected randomly along a five metre stretch of stream by hand net with netting of pore size 287 µm . The fauna on these stones and vegetation were scraped off and preserved with formalin for later laboratory analysis.

On each occasion water temperature in the flowing water was taken and current velocities were measured with an OTT current meter at several points along the stream. The fauna collected was analysed according to species and abundance. Faunal counts were not related back to surface area of substrate sampled, and were used only as a qualitative estimate of the abundance of different species relative to each other.

PLATE 7: The small stream formed by leakage from the Vaalhartz Irrigation Canal showing small service bridge. Samples were collected in the stretch of water shown in foreground.

5.3 RESULTS AND DISCUSSION

Water current velocities in the small stream were usually low, between 30 to 40 cm/s when samples were collected over the three years (Appendix 3, Table 3.1), the pH and temperatures of the water in this stream were similar to those measured in the Vaal River (Appendix 2.7, Table 2.24).

A detailed analysis of the percentage occurrence of all the more prevalent faunal groups identified, and the total number of individuals collected on each sampling occasion are presented in Appendix 3, Table 3.1. All the rarer fauna encountered are also recorded (Appendix 3, Table 3.2). Figure 14 is compiled from selected data in Appendix 3.

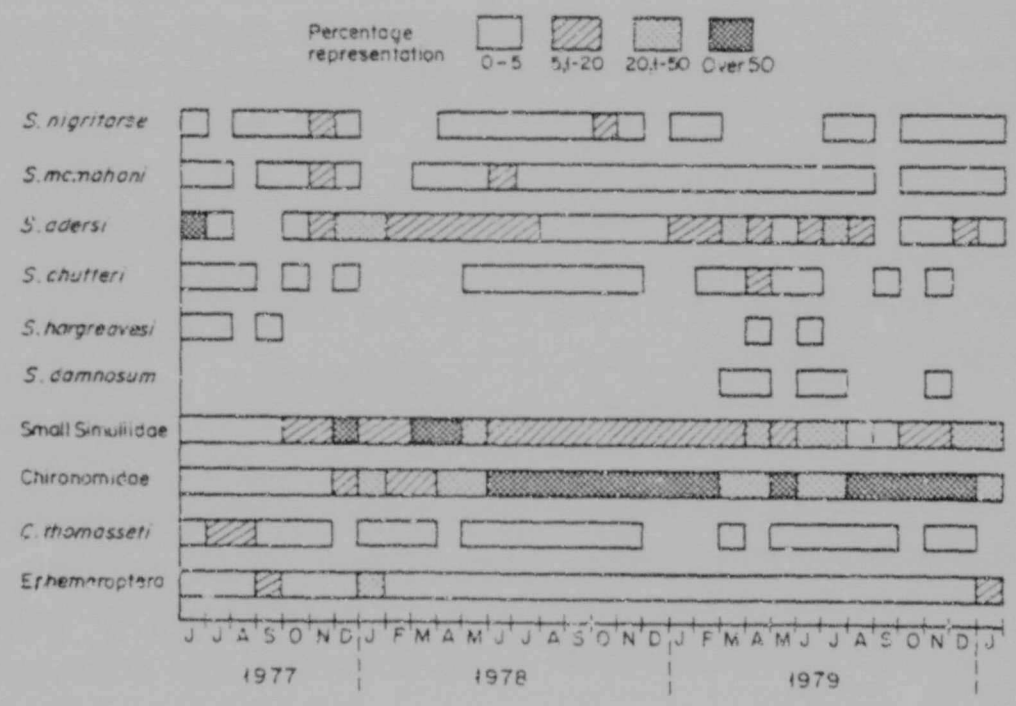

FIGURE 14: The relative abundance of selected fauna from the small stream as a percentage of the total number of animals collected on each occasion. Information obtained from Appendix 3, Table 3.1

The slower water velocities in the stream were more suited to *S. adersi*, *S. mcmahoni* and *S. nigritarse* than to *S. chutteri* or *S. damnosum* and besides other ecological factors like ovipositing sites and larval behaviour (Ch. 7) water current velocity must have been the major factor that led to only sporadic appearances of these latter two species (Fig. 14).

Simulium adersi was the most abundant of the six simuliid species encountered in the stream during the thirty-two months samples were collected. It apparently had two seasonal cycles when peak population levels were attained, one in summer (December 1977 to January 1978, March and December 1979) and a second one in winter (June 1977 and July 1979).

Simulium nigritarse was also found in most of the samples collected from the small stream, but this species had only one seasonal peak of abundance which occurred in spring (October to November 1977, October 1978 and October 1979).

The percentage representation of *S. nomahoni* was low throughout the period and only reached over 5 per cent on two occasions (Fig. 14). Appendix 3, Table 3.1 however shows that relative to its occurrence throughout the period, peak abundances were attained during summer (November 1977 and January 1979) and winter (June 1978 and June 1979).

If the sporadic appearance of *S. chutteri*, in the stream (Fig. 14) with a maximum occurring in April 1979, is compared with the abundance of this species in the Vaal River (Fig. 15), where usual increases in numbers were in spring (Ch. 4) it would seem that this species is not well adapted to the small stream environment.

The appearance of the other three simuliid species in the small stream, *S. hargreavesi*, *S. damnosum* and *S. ruficorne* Macquart (see Appendix 3, Table 3.2), was also of a sporadic nature and did not give any indication of a seasonal cycle of abundance.

A decrease of *C. thomasseti* as a percentage of the total number of individuals occurred each year from 1977 to 1979 between August and September (Fig. 16). This supports the hypothesis (Ch. 7) that *C. thomasseti* emerge as adults from September through to October leading to reduced numbers of this species in the aquatic habitat. Egg laying and recruitment of small larvae replenished the aquatic population of this species again from October onwards (Chs 4 and 7). Unfortunately small hydropsychid larvae were not comparable with the remainder of each sample collected because the coarse netting used for collecting samples

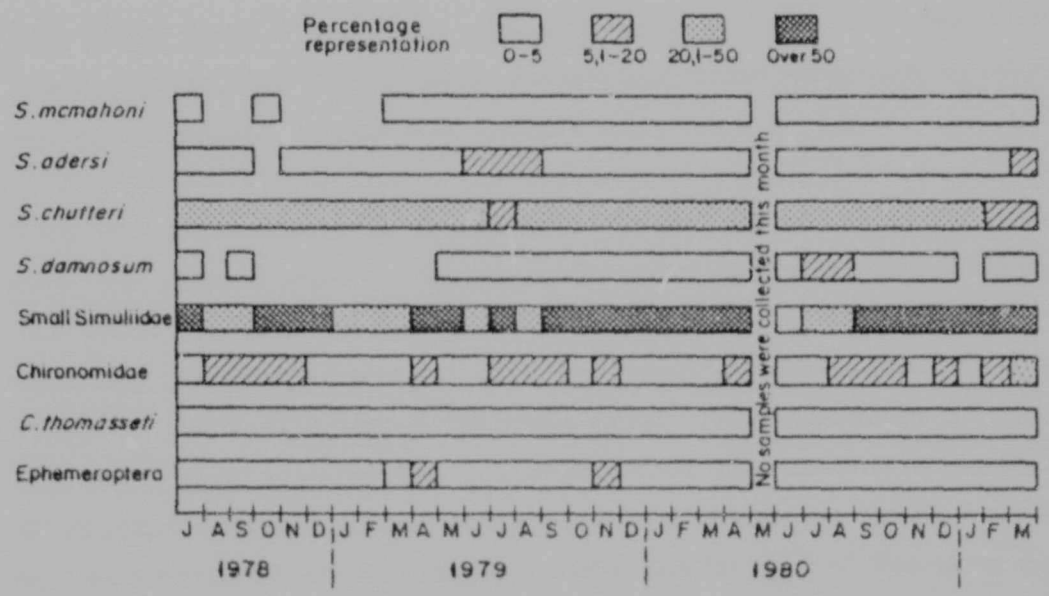

FIGURE 15: The relative abundance of selected fauna from the Vaal River at Witrand as a percentage of the total number of animals involved. Information obtained from data in Appendix 5

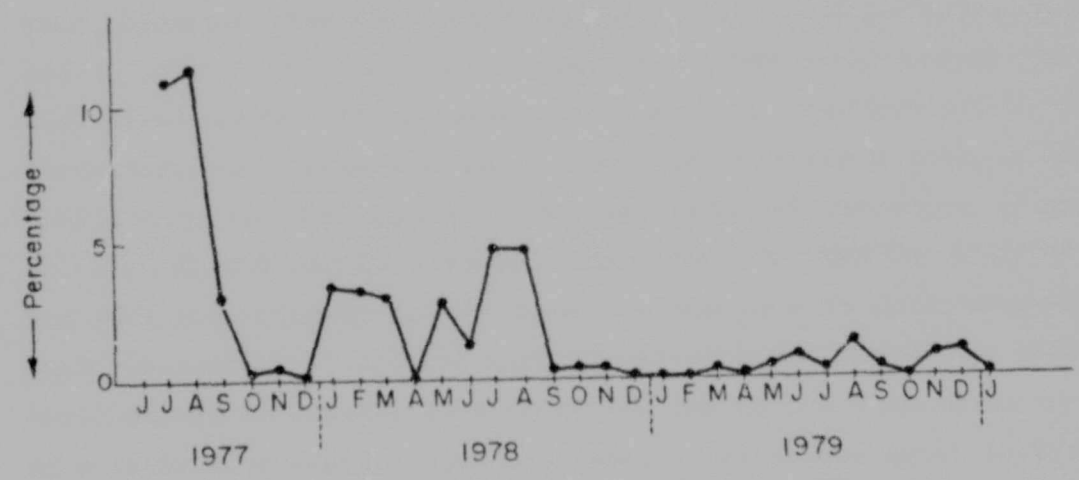

FIGURE 16: *Cheumatopsyche thomasseti* larvae as a percentage of all the animals collected from a small stream running into the Vaal River

in this study would have allowed a large number of smaller animals to pass through. The reduced percentage of most Simuliidae in August 1977 may have been influenced by *C. thomasseti* predation, but as no quantitative data were collected this is conjecture.

When the Vaal River was in flood (January and February 1978) backwash of the river into the stream was followed by a severe depletion of the fauna leading to a very high percentage representation of *Austrocaenis capensis* Barnard during that period (Appendix 3, Table 3.1).

With two exceptions, in December 1977 and March 1978, when Simuliidae were the most abundant, Chironomidae were the dominant faunal group in the small stream throughout the three year period covered (Fig. 14). This was in sharp contrast with the simuliid dominated faunal community found in the Vaal River at Witrand (Fig. 15). Although *S. chutteri* was the most abundant species collected from the stones-in-current habitat at Witrand throughout the study period *S. damnosum* became more numerous in late winter (July to August 1980) and *S. adersi* was more abundant in winter (June to August 1979) and late summer (March 1981) than at other times of the year. *Simulium mcmahoni* occurred in low numbers throughout the period in samples collected from the Vaal River.

Summarising the comparison of fauna from the small stream (Fig. 14) with that collected from the Vaal River (Fig. 15), *S. nigritarse, S. mcmahoni* and *S. adersi* were all more abundant in the small stream than in the Vaal River whereas the reverse was true for *S. chutteri* and *S. damnosum*. *Cheumatopsyche thomasseti* was present in both lotic water biotopes all year round, but had distinct seasonal peaks of abundance (Fig. 16 and Ch. 4). Ephemeroptera were not separated into species (Figs 14 and 15) and were also present at all times of the year in both biotopes. They were, percentage-wise, most numerous after flood waters had inudated the small stream in January 1978 (Fig. 14) and in the Vaal River at the end of a river flow manipulation programme in March and April 1979 (Fig. 15). Two possible reasons that could account for this are, firstly that Ephemeroptera could be more successful early colonizers of disturbed habitats than other fauna, and secondly that they may not have been as severely affected by water flow disturbances as the other fauna.

Conclusions as to the occurrence and abundance of Simuliidae with respect to seasonality and habitat selection are drawn at the end of Chapter 7.

6. LIFE CYCLE STUDIES OF SIMULIIDAE FROM THE VAAL RIVER NEAR WARRENTON

6.1 INTRODUCTION

Simulium chutteri was the most abundant species in the regions of the Vaal River where population studies were carried out (Ch. 4) and detailed studies on the number of instars and duration of the life cycle were directed mostly [redacted] studies on the ecology and control of population size of this species (Chutter, 1968; Howell and Holmes, 1969) were carried out without any knowledge of the duration of the life cycle, the number of instars and methods for separating the various instars from each other. Merritt *et al.* (1978) believe that such information is a prerequisite for further studies on larval feeding biology, resource partitioning and the planning of environmentally acceptable population control programmes

Numerous studies on the number of larval instars in Simuliidae have been made and four (Puri, 1925; Smart, 1934), six (Cameron, 1922; Terterjan, 1957; Harrod, 1964; Stone and Snoddy, 1969; Reisen, 1975; Mokry, 1976; Ross, 1979), seven (Grenier and Feraud, 1960; Johnson and Pengelly, 1969; Halgos and Jedlicka, 1973; Mansingh and Steel, 1973; Rühm and Sander, 1975; Fredeen, 1976; Elouard, 1978; Ross and Craig, 1979; Ross, 1979), eight (Smith, 1969) and nine (Crosby, 1974; Craig, 1975) instars have been reported in the literature. Ross and Merritt (1978) recorded a decrease from seven to six larval instars with an increase in stream temperature in *Prosimulium mixtum* Syme and Davies, and *P. fuscum* Syme and Davies.

Dyar (1890) showed that there was a geometric increase in the size of sclerotized structures in lepidopteran larvae as they moulted from one instar to the next. Most of the abovementioned studies used morphometric data of selected sclerotized parts of the head or body, measurements and counts of antennal segments, number of primary cephalic fan rays and numbers of hooks present in anal circlets as criteria for determining the number of larval instars which occurred in simuliid larvae prior to pupation.

Many studies on the duration of the pre-imaginal stages of the life cycle of Simuliidae under both natural and laboratory simulated conditions have been carried out, and some of these studies are summarized in Table 4.

From Table 4 it is clear that even within a single species a wide range of variation in the time taken for individuals to develop from one stage to the next is encountered. Experiments carried out under laboratory conditions in particular show an extended period of time for the aquatic stages of Simuliidae to reach eclosion. Hynes (1972) mentioned that for studies on the duration of life cycles, a combination of field and laboratory work yields much more information than would either of the two approaches alone. Field work leaves many gaps in the knowledge as regards fate of small eggs and the life span of terrestrial adults, whereas laboratory results always suffer from the uncertainty as to how they can be applied to the natural situation. Variation in the duration of larval and pupal stages of the life cycle of Simuliidae is strongly affected by temperature, nutrition, oxygen concentration, water velocity, pH and a host of other factors pertaining to the chemistry of the water in which the pre-imaginal stages live (WHO report, 1966). As it is difficult to determine which of these factors are the more important in controlling the duration of the life cycle of individuals, laboratory studies on the life cycle duration of the aquatic stages of Simuliidae should be viewed with caution.

Variation in the duration of the life cycle of *S. damnosum* s.l. recorded before 1972 could be due to various sibling species being used in these earlier observations. Raybould and White (1979) reported that a total of twenty-six cytotaxonomic entities had been recognized in the *S. damnosum* s.l. complex.

Studies on the duration of the egg stage from laying to hatching were often carried out from eggs collected, and reared in the laboratory (Hocking and Pickering, 1954; Wright, 1957; Raybould and Grunewald, 1975). Direct observations of eggs in their natural habitat were also undertaken (Wu, 1931; Chutter, 1972b). For determination of the duration of the larval stage until pupation, laboratory-reared larvae were used (Wright, 1957; Fredeen, 1959; Grunewald, 1973; Mokry, 1976) or

TABLE 4: Summarized data on the duration of developmental stages of Simuliidae

Species and country where found	Duration in days of the various life cycle stages				Temperature range	Reference
	Egg hatching to adult	Egg	Larval stage	Pupal stage		
Simulium vittatum Zetterstedt (USA) Wisconsin	17-22	4-5 F	13-17 F	3½-5 L	18-23°C	Wu 1931 L* F**
S. vittatum (Canada)	14	?	11	3	20-33°C	Fredeen 1959 L
	21-41	?	19-37	2-4		
S. vittatum (Wisconsin)	187-194	?	overwintering 180	7-14	0,2-5,0°C	Anderson and Dicke 1960 F
	56-63	?	-	-	11,1°C	
	24-25	?	21	3-4	15,6°C	
S. venustum Say (Canada)	20-26	?	15-18	5-8	13-16°C	Peterson & Wolfe 1958 F
S. venustum (Canada)	13	-	8	5	20-32°C	Fredeen 1959 L
S. venustum (Manitoba, Canada)	-	8-9 L	-	4-9 F	-	Hocking & Pickering 1954 L F
S. venustum (Canada)	-	-	36	-	15°C	Mokry 1976 L
	-	-	29	-	18°C	
	-	-	22	-	22°C	
	-	-	20	-	24°C	
Boophthora erythrocephala de Geer (Germany)	23	-	18	5	18-19°C	Grunewald 1973 L
Austrosimulium pestilens Mackerras & Mackerras (Australia)	7½-8½	-	6-7 summer	1½-2	23-29°C	Hunter 1978 F
	12-13	-	9-10 winter	3	13-16°C	
S. damnosum s.l. Theobald (Leopoldville Zaire) +	9	-	5	4	-	Wanson & Henrard 1945 F
S. damnosum s.l. (Uganda)	-	2-3	6-8	-	26,5°C	Barnley 1957 F
S. damnosum s.l. (Ghana)	-	1½	10-13	-	26,0°C	Crisp 1956 F
S. damnosum s.l. (Ghana)	min 16-20	1½	14-17	-	21,1-31,1°C	Wright 1957 L
	-	-	14-40	-		
S. damnosum s.l. (Ghana)	10-13	1½-2	8-9	2-4	20,0-28,5°C	Marr 1962 F
S. damnosum s.l. (Ghana)	-	2	9-10	-	28,5-34,0°C	Thompson, Walsh & Walsh 1972 F
S. damnosum s.l. 'Sanje' 'Kibwezi' (Tanzania)	18-50	4	-	-	20°C	Raybould & Grunewald 1975 L
S. damnosum 'Kibwezi'	18-38	-	14-34	4	23,8-25,3°C	Grunewald & Grunewald
'Kilelwani' (Tanzania)	16-38	-	12-34	4	24,3-25,4°C	1978 L
S. damnosum s.l.(6 types) (Ghana)	-	-	10-14	-	-	Raybould 1979 L
S. nigritarse Coquillett (South Africa)	24	<7	-	-	-	Chutter 1972b F
S. nigritarse (South Africa)	-	3-13	-	2-5	25°C	Begemann 1980a L
	27-87	-	20-69	3½-5	20°C	
	-	-	-	3-11	15°C	
	-	-	-	11-15	10°C	
	-	-	-	12-23	6°C	
S. adersi Pomeroy (Ghana)	-	-	-	2-4	20,0-28,5°C	Marr 1962 F
S. adersi (South Africa)	-	3-13	-	-	25°C	Begemann 1980a L
	-	-	17-?46	-	20°C	

L* = Studies done in laboratory
F** = Studies done in the field
\+ = Leopoldville = Kinshasha

direct observations on developing larvae in their natural environs, noting the first appearance of pupae (Wu, 1931; Hocking and Pickering, 1954; Peterson and Wolfe, 1958; Anderson and Dicke, 1960, Hunter, 1978) or else measuring and dividing up larvae into various size classes and plotting the abundance of each larval stage periodically on histograms. The cohorts of larval size groups could then be followed and the appearance of pupae would indicate the total time taken for small larvae to develop through to pupation. The advantage of this last method was that the duration of individual stages could be more closely followed and local climatic effects could also be more closely monitored (Chutter, 1972b; Ross and Merritt; 1978).

It should be emphasized that the determination of the duration of the various aquatic stages of Simuliidae from field studies in permanently flowing rivers is more difficult in a warmer climate like South Africa than in a more temperate climate as found in Canada or Europe. Although multivoltine species of Simuliidae do occur in Canada (Hynes, 1972) their growth and development is usually stopped by the winter freeze. A distinct summer - winter cycle thus occurs in these temperate climates.

In *S. chutteri* individuals at all stages of development were found to be present throughout the year, and an overlap of generations occurred at all times (see Ch. 4).

The duration of the pupal stage to eclosion was studied either by observation on laboratory reared pupae (Wu, 1931; Fredeen, 1959; Grunewald, 1973; Grunewald and Grunewald, 1978) or else direct observations on pupae in their natural environs (Wanson and Henrard, 1945; Hocking and Pickering, 1954; Peterson and Wolfe, 1958; Anderson and Dicke, 1960; Marr, 1962; Hunter, 1978).

A decrease in the duration of the aquatic stages has been observed with an increase in water temperature (Davies and Smith, 1958; Davies, 1961; Zwick, 1974; Mokry, 1976; Ross and Merritt, 1978). 'Semaphoronts' (individuals at a specific given stage of development - Craig, 1974) show seasonal and geographical variation in size and this has been attributed to a change in food availability (Colbo and Porter, 1979) or seasonal fluctuation of water temperature (Chutter, 1970b; Neveu, 1973;

Rühm and Sander, 1975; Hechler and Rühm, 1976). An increase in water temperature inhibited the action of the moulting hormone in final instar *S. damnosum* s.l. larvae and led to a two to three day delay in commencement of pupation (Thompson *et al.*, 1972). Water temperatures during that study were exceptionally high (30 to 34 °C).

Sexual size differences in a population of Simuliidae were suggested by Craig (1975) and illustrated for the last three larval instars of *S. damnosum* s.l. and for the last two instars of *S. adersi* by Elouard (1978).

6.2 MATERIALS AND METHODS

6.2.1 Determination of the number of larval instars and variation in sizes of larvae and pupae through an annual cycle

For this study the aquatic stages of *S. chutteri* were collected from their natural stones-in-current habitat. Morphometric studies to determine the number of larval instars and to see if there was any seasonal variation in the number and size of instars, were carried out at various times over a three year period. Pupae were examined for variation in size associated with season and sex.

Larvae and pupae used for morphometric studies were taken from samples collected on 8 June 1977, 11 September 1977, 7 December 1978, 8 March 1979, 7 June 1979, 21 June 1979, 5 July 1979, 13 September 1979, 9 October 1979 and 6 December 1979. The 9 October 1979 sample was collected on the farm Sydney's Hope which is approximately 6 km upstream of the farm Witrand where all the other samples were collected. In the laboratory larval head capsules of a selected number of individuals (Appendix 2.4.1) were measured along the postgena from the centre of the darkened hypostomal margin of the mandibular phragma to the centre of the darkened post occipital margin (Freedeen, 1976). Larval body length was measured from the post occipital margin to the lateral distal margin of the posterior circlet. The dorsal aspects of the head capsules of the recognized larval instars were drawn to scale (Fig. 17) and measurements were made of the size of the dorsal gap in the postocciput. Variation in pigmentation of the head capsules was noted and specimens

consisting of one instar, selected by postgenal head capsule measurements, were divided up into three colour phases namely white, yellow or brown depending on the degree of pigmentation. Body length measurements of these larvae were also recorded. Pupae were measured from the base of the respiratory histoblast to the tip of the wing along a straight line. The sex of pupae was also determined by examining the eyes of the developing adults where there are two sizes of facets present in the males and only one in females (Crosskey, 1973). All measurements were made with the aid of an occular micrometer on a Zeiss stereomicroscope model IVb. Larval head capsules were measured at 50 x magnification (1 unit = 20,5 µm) and larval body length at 12,5 x magnification (1 unit = 77,5 µm). Pupae were measured at 25 x magnification (1 unit = 40 µm). All measurements were made to the nearest 0,5 micrometer unit. Size frequency histograms based on postgenal lengths were constructed. The size range for each instar was defined from sizes where frequencies were low. These points are indicated by arrows in Figures 18, 19, 20 and 21.

Mean sizes of each graphically delimited larval instar were tested for equality of variances (variance ratio test) and means (Student's *t* test) (Elliott, 1977). These tests were used to verify that the mean sizes of instars were statistically different from each other. Calculated mean sizes of instars were then subjected to least squares regression analyses and an exponential curve, usually associated with regular growth rates (Simpson *et al.*, 1963), was found to be the closest fit to the data in all cases (Tenzer, 1978). The natural log ($\log_e$) of the mean postgenal lengths of each instar was calculated and subjected to a least squares linear regression analysis to check the closeness of fit of the data points to a straight line. Any deviations would indicate that an instar might have been overlooked (Wigglesworth, 1965). Crosby's growth rule (Craig, 1975) which states that if there is a difference greater than 10 per cent between two consecutive Brook's ratio (obtained by dividing the mean size of an earlier instar's head capsule length into the mean head capsule length of the next larval instar) there is a possibility that an instar is missing, was also applied to the data to check the accuracy of the instar groupings. The geometric growth rate (*Kg*) between instars was also calculated (Simpson *et al.*, 1963).

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Calculated mean sizes of male and female pupae from seven samples collected at various dates were compared to test statistically for differences in size between the sexes throughout a year.

A series of ten samples of final instar larvae and pupae in their cocoons, were oven dried at 60 °C for 64 hours and then weighed on a Mettler H16 chemical balance. The biomass figures obtained were calculated to give weight estimates of one hundred *S. chutteri* final instar larvae or one hundred pupae. Comparisons of biomass loss between larvae and pupae on the same dates and within larvae and pupae during various times of the year were made using the paired t statistic or the t statistic for two means (Hewlett-Packard, 1976) respectively. The variation in head pigmentation in the last five instars (collected on 13 September 1979) was also tested for variation in means of total body length measurements within single instars using Student's t test (Elliott, 1977).

6.2.2 The duration of the aquatic life cycle stages of some of the Simuliidae encountered

To determine the duration of the life cycle of the various aquatic stages of *S. chutteri* a number of experiments were carried out.

a) Eggs

Simuliid eggs collected from drift samples were taken to the laboratory on several occasions and observations were made on samples of eggs kept in aerated and non-aerated aquaria over several days. The number of eggs hatching into first instar larvae was noted. These were almost certainly *S. chutteri* eggs (refer Chs 4 and 7).

Larvae developing from eggs in drift samples could not be successfully reared through to pupation. Data obtained from drift at daily intervals over sixteen days was examined to give an indication of the duration of the egg stage (Appendix 2.2.7, Fig 2.3).

Eggs belonging to *Simulium* species other than *S. chutteri* were often found in patches on partially submerged stones in the river

and also on submerged sedges and reeds (*Phragmites communis* Trin.) upstream of the study rapid. These eggs were collected and taken to the laboratory on several occasions where they were kept in aquaria and observed for the hatching and development of larvae. Measurements of the size of individual eggs were made along three planes - the greatest length, the height when viewed from the side showing the characteristic triangular shape of simuliid eggs (Obeng, 1967) and the width when viewed at right angles to the measurements taken for height. Occasionally the eggs found in individual patches were also counted and some idea was thus obtained of the fecundity of the various species of *Simulium* s.l. encountered.

b) Larvae

For the determination of the duration of the larval stage, larvae which had hatched from eggs in the laboratory were kept in aerated aquaria and fed on finely powdered Tetra Min staple food or Liquifry No. 1 food for egglaying fish. The water in the aquaria was often partially replaced with river water which was passed through 92 µm pore sized netting to prevent contamination of the sample with other simuliid eggs and larvae. Regular observations were made and when larvae were large enough to be identified, some were examined alive under the microscope. The first appearance of pupae and the emergence of adults were also noted.

The duration of developmental stages of the larvae of *S. chutteri* was also ascertained by monitoring natural populations. Initially a sample of five stones was taken from the natural habitat and the fauna on each stone was separately collected and preserved in formalin. In the laboratory each sample was individually analysed for size frequency distribution of larvae and also the number of pupae (Appendix 2.3). On advice from Miss Ten Krooden, of the National Research Institute for Mathematical Sciences (NRIMS) at the CSIR in Pretoria, a chi-squared statistic analysis was done on the percentage distribution of the size classes of larvae and pupae on individual stones.