A STUDY OF THE FILTER-FEEDING BEHAVIOUR OF SIMULIUM LARVAE (DIPTERA:SIMULIIDAE)

by

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ABSTRACT

The structure of the mouthparts and distribution of cephalic sense organs of larval simuliids was investigated using the scanning electron microscope and methylene blue staining. The effect of water velocity, temperature and quality on larval feeding behaviour was studied in an artificial stream. Short, controlled, pulses of physical and chemical stimulants were injected into the water to observe their effect on larval feeding behaviour. Too rapid for the unaided eye, movements were described frame by frame from video recordings.

Food is filtered from the water by the open cephalic fans. In alternation the fans are rapidly closed , swept by the mandible to remove food particles and opened again. The frequency of this endogenous behaviour pattern was modified by environmental factors that appeared to act mainly on the interval between fan beats.

The interval between fan beats was found to be inversely related to water temperature and velocity and was also affected by water borne stimulants, being significantly shorter in unfiltered natural water than particle-free distilled water. Consequently fan cleaning frequency rose as water velocity and temperature were increased and when natural food was available.

Larvae responded to pulses of a wide variety of chemical compounds with bursts of mandible and maxilla movements. Fan cleaning was inhibited when these mouthpart movements occurred but filtering continued. Short pulses of inert particles at a relatively high concentration caused a similar response but when a series of pulses was delivered bursts of mouthpart movements lengthened and the fans were often closed for longer than normal, inhibiting filtering. It is suggested that overstimulation of peripheral sense organs, responding to the physical and chemical qualities of food particles, initiates the inhibition of filtering.

The temporary inhibition of feeding may regulate the rate of ingestion. A simple model of larval behaviour is proposed, recognising "food gathering" (filtering) and "food ingestion" (mouthpart movements) as its main components.

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CHAPTER 1 INTRODUCTION

1.1 Filter feeding in insects

Filter-feeding is of widespread occurrence amongst the larval and nymphal stages of aquatic insects. True filter-feeding is restricted entirely to juvenile stages and occurs in the Ephemeroptera, Trichoptera and Diptera (Wallace and Merrit, 1980). There are no adult insects that filter water to obtain food as far as is known. Of the two insect orders that have adult aquatic representatives, the Hemiptera and the Coleoptera, those that feed on fine particles do not do so by filtering, but tend to collect them from the substratum (Hynes, 1973, Pennak, 1953). In general, surprisingly little is known about the behaviour of filter-feeding insects, although the larval stages of some of the most important pest organisms, including mosquitos and blackflies, are filter-feeders.

Of the three insect orders in which filter feeding occurs the most important, in terms of the number of species that filter, are the Trichoptera and the Diptera (the latter including the Simuliidae). There are relatively few Ephemeropterans that are known to filter-feed, although this may reflect lack of knowledge rather than the true state of affairs (Wallace and Merrit, 1980).

Filter-feeders often have highly specialised mouthparts (Pucat, 1969, Chance, 1977) while those that do not show morphological specialisations may have characteristic behaviour patterns associated with feeding (Edington and Hildrew, 1981, Hickin, 1967, Wallace, 1975). In general the mayflies that do filter-feed rely on hairy

appendages to capture particles. The Holarctic genus Isonychia, for example, contains species that have fine setae on the forelegs with which to filter the water, using their mouthparts to clean the setae and ingest particles. Among members of the African genus Tricorythus the outside borders of the labrum and mandibles are fringed with fine hairs that funnel particles towards the mouth (Hynes, 1973).

In contrast to the mayflies many of the caddis flies, particularly those in the families Hydropsychidae, Philopotamidae and Psychomiidae have larval stages that filter-feed (Wallace and Merrit, 1980). Filter-feeding trichopterans feed mainly by trapping particles in silk nets, many having extremely fine-meshed nets for the capture of particles (Wallace and Merrit, 1980). Unlike the other groups of filter-feeding insects there are no trichopterans known to feed using setae. However, although the Trichoptera have been studied intensively by many authors, little is known about the processes that control their feeding behaviour.

Among the filter-feeding dipterans the Simuliidae and the Culicidae have specialised mouthpart structures for filtering (Chance, 1970, Pucat, 1965). Culicids are found exclusively in still or very sluggish water and lack the structural and behavioural adaptations of the Simuliids to running water (Wallace and Merrit, 1980). The Chironomidae probably have the greatest diversity of filtering methods (although the majority are not filter-feeders) but they are perhaps the least well known group of aquatic insects and it is likely that the feeding behaviour of many species remains to be interpreted correctly as the group is very diverse. Both structural and net filtering occurs amongst the chironomids and larvae may filter whilst dwelling in leaf mines or in tubes constructed on

stones. Feeding currents may be maintained by body undulations or the larvae may rely on the flow of water to bring food to them (Walshe, 1950). The feeding structures and behaviour of simuliids is reviewed in Section 1.4.

1.2 Blackfly biology: distribution, taxonomy and feeding ecology

Blackflies are associated with running water in all the zoogeographical realms. In tropical West Africa and Central South America they are vectors of the parasitic nematode *Onchocerca volvulus*, which causes the human disease onchocerciasis or River Blindness, although in S. America the parasite is confined to a few, relatively small, foci (Crosskey, 1973). In temperate and Arctic latitudes blackflies may be a great nuisance, because of their abundance (J. Claricoates, personal communication, Ladle and Hansford, 1981) even though they are not thought to transmit any serious human diseases in these areas. They are, however, known to be vectors of a number of animal diseases (Jamnback, 1973).

The eggs, larvae and pupae of simuliids may be found in almost any clean running water with a firm substrate for attachment. Although commonly associated with fast flowing water they may be found in the smallest of trickles (personal observation). Larvae may be extremely abundant; Ladle *et al*, (1972) estimated that there were 8×10^6 larvae in a 200m reach of a southern England chalk stream, while Carlsson *et al* (1977) found 13500 individuals m-2 in a Swedish lake outlet. Consequently simuliids often make an important contribution to the productivity of rivers (Merrit *et al*, 1982).

Progress in understanding the biology of blackflies, at all stages of the life-cycle has, until very recently, been hampered by the difficulty of maintaining breeding colonies in the laboratory (Muirhead-Thompson, 1966) and also by the confused state of the taxonomy of the family (Crosskey, 1973).

The identification of blackflies, using morphological criteria, is generally difficult and, as taxonomic methods have become more refined, it has been discovered that many so-called 'species' of blackfly are complexes of morphologically very similar, or identical, species. Among these groups of sibling species are Simulium damnosum 'the' African vector of onchocerciasis (Rothfels, 1979) and S. ornatum (R. Post, pers. comm. in Rothfels, 1979), the main subject of this thesis.

In general, sibling species can only be separated reliably using cytological methods, particularly the analysis of larval polytene chromosomes. Rothfels et al (1977) showed how these methods were used in the separation of siblings in the S. verecundum-S. veneustum complex. Conventional taxonomic methods had defined no more than three of the seven species found after cytological analysis. Rothfels (1979) states that S. ornatum sl is a group of four sibling species which, for the moment, are known only by the letters A to D. Unpublished work by R. Post (pers. comm.) has shown that all members of the complex so far investigated in Britain belong to species 'A'. Bedo (1979a) has further suggested that the Australian S. ornatipes may be represented by two homosequential sibling species where there are no overt chromosome differences and no morphological differences, yet two reproductively isolated groups exist.

Identifications of the larvae in this study were made using the Freshwater Biological Association key to the British Simuliids (Davies, 1968). Reliable morphological identifications are best made by rearing larvae at least to the pupal stage as the pupae have some of the most easily interpreted characters. Identification of the larvae alone requires the preparation of slides of the mouthparts (particularly the mandibles, maxillary palp and hypostomium) and the anal sclerite (Davies, 1968), although with experience the 'jizz' of the species is learnt. In general only final instar larvae (the sixth or seventh) can be used for identifications because most characters are too variable, in earlier instars, to be relied on. The final instar, actually a pharate larva, is easily recognised by the presence of the pupal gill filaments.

Harrod (1964) stated that S. ornatum var nitidifrons had six larval instars and that the first, fourth, fifth and sixth were identifiable on morphological grounds. However, in practice, only the first (which has an egg burster) and the final instars can be recognised with complete certainty. Fredeen (1976) was able to distinguish the first and seventh instars of S. arcticum; intervening instars were only recognised as size classes based on the length of the post- gena. Granett (1978) and Brenner et al (1981) also separated instars using head capsule measurements. Because large numbers of larvae must be measured before the size classes become apparent there has been a tendency for most authors to use only the final instar in experimental studies or alternatively to group the larvae into arbitrary size classes, not related to instars. (Chance, 1970, Ladle and Hansford, 1981).

In addition, larvae of multi-voltine species such as S. ornatum, which has up to four generations a year (Ladle et al , 1977), reach a different maximum size depending on the time of year at which they are maturing. In southern England Ladle et al (1972) found that the larvae of S. lineatum and S.ornatum that pupated in the spring after overwintering as larvae were about 25% larger than summer generations. An experimental study by Colbo and Porter (1981) of the larvae of S. vittatum and S. verecundum showed that the larvae reared at lower temperatures were larger irrespective of the food supply.

Although field collected eggs and larvae have been reared in the laboratory for many years (Muirhead-Thompson, 1966, Raybould and Grunewald, 1975) it is only very recently that successful breeding colonies have been maintained in the laboratory through many generations. Two problems have dogged attempts to breed blackflies successfully. Firstly, there was often a great deal of larval mortality owing to the sensitivity of the larvae to waste products in the water (Fredeen, 1959, Hall and Harrod, 1963, Tarshis, 1968). Secondly, it proved difficult to achieve large numbers of matings without very time-consuming manipulations of the adult flies (Muirhead-Thompson, 1966).

The first problem was overcome by Brenner and Cupp (1980) who reared between 60% and 94% of the larvae they started with, of four species. With their rearing apparatus they were able to keep up to 20,000 larvae at one time. Simmons and Edman (1978, 1981) were able to solve both problems for S. decorum (an anautogenous species, which avoided the difficulties of having to persuade the females to take a blood meal) and maintained this species through sixteen

generations. The refinements of this system made by Ham and Bianco (1984) suggest that there will soon be more rapid developments in the understanding of blackfly biology at all stages of the lifecycle. Advances in the understanding of adult behaviour were an essential part of the developments in laboratory rearing (see D Davies, 1978, for a review of studies of adult behaviour).

Although there has been an increase in the amount of work done on blackflies recently there are still very many basic gaps in the understanding of their biology. This is especially true of the larvae and little is known of larval physiology, nutrition or behaviour (Laird, 1981). This deficiency in our understanding of larval biology may have important medical consequences larvae being the main target of entomologists attempting to control blackflies, as they are very vulnerable to particles that have been impregnated with pesticides (see Jamnback, 1973, for a review of control measures).

1.3 Insect feeding behaviour: food quality and the control of feeding

Herbivorous insects 'live in a sea of food that is, at best, nutritionally inadequate and, at worst, simply poisonous' (Lawton and McNeil, 1979) and it is widely accepted that plants are a difficult source of food for insects, and probably other arthropods, to exploit (Edwards and Wratten, 1980). In aquatic habitats some blue-green algae (cyanobacteria), such as *Anabaena flos-aquae*, are known to be toxic to filter-feeding cladocerans, as well as being of low nutritional quality, and are rejected more frequently than more acceptable green algae (Porter and Orcutt, 1980). Aquatic vascular plants are rarely eaten by insects (personal observation), while some

terrestrial plants may be actually or effectively inadequate in nitrogen at certain times of the year (Dixon, 1973, Feeny, 1970).

Blackfly larvae feed on a mixture of inorganic and organic detritus and microalgae (see, for example, Kurtak, 1978), and their feeding behaviour may usefully be compared with that of terrestrial herbivores, such as Locusta migratoria, Operophtera brumata and Tyria jacobaeae. The feeding behaviour of these species is, in many ways, related to the qualities of their various food plants (Bernays and Chapman, 1977, Feeny, 1970, Meijden et al 1984) and it is likely that qualities of food will also influence the feeding behaviour of filter-feeders. Unfortunately relatively little is known about the foods of filter-feeding insects, beyond the quantities and types that are available to them, so it is difficult to interpret behaviour pattens in terms of food selection at the moment (see Anderson and Sedell, 1979 and Ladle, 1982 for reviews of the use of detritus as food by stream dwelling aquatic insects).

Detritus is an important source of food for aquatic invertebrates but little is known of its physical and chemical qualities (Ladle, 1982); much of the work done on this subject has been concerned with identifying the role of aquatic micro-organisms which facilitate the use of detritus as food, breaking it down and making nutrients available (Anderson and Sedell, 1979). However, it seems likely that detritus, the principal source of food of simuliids, is no better a source of nutrition than green leaves.

Sugars, amimo acids and organic acids are leached from dead leaves falling into water within two weeks of their being submerged (the rate depends on the species) although the nitrogen content may gradually increase as the leaf is attacked by micro-organisms. These

may raise the nitrogen content from an initial concentration of about 0.75% to 2.0% (ash-free dry weights) for Acer leaves (Willoughby, 1974). This may be compared with oak leaves on the tree which have a total nitrogen content varying from about 2.5% to 5.0%. The lower end of this range was thought to represent a nitrogen shortage for the winter moth (Feeny, 1970) suggesting that detritus may not be a rich source of nitrogen for aquatic insects.

It is possible therefore that, like many terrestrial herbivores, aquatic detritivorous insects may have barely adequate supplies of nitrogen. They may also experience shortages of carbohydrate, although some of these inadequacies may be compensated for by microalgae, which are also an important source of food. There are conflicting accounts of the significance of microalgae to simuliid larvae (Ladle and Hansford, 1981, Merrit et al, 1982) with some authors noting that diatoms in particular seem relatively indigestible (eg Moore, 1977a) On the other hand Ladle and Hansford (1981) noted that S. austeni and S. lineatum attained high assimilation efficiencies on diatoms, whilst the proportion of detrital suspended material that was assimilated was probably low.

With variable, and probably generally low, quality food it is not surprising that many phytophagous insects are forced to be highly selective feeders. It is perhaps for this reason that among these insects there are many elaborate chemosensory mechanisms controlling the ingestion of food (Bernays and Simpson, 1982). Since the food of simuliid larvae seems to be of similar quality it would be reasonable to expect some selectivity in the way that they feed, although, as discussed below, this selectivity does not take the form of the individual selection of more nutritious particles (see section 1.4).

Of central importance to phytophagous insects is the reception, processing and interpretation of sensory information about the plants around them (Chapman, 1982). Starting with the work of Dethier and others in the 1930's on the effect of simple chemicals on the feeding behaviour of blowflies (Dethier, 1976) many studies have gone on to confirm the relationships, often highly specific, between the needs of the insect and the responses of its peripheral nervous system (Schoonhoven, 1973). In the control of feeding it appears that mechanoreception and gustation are the most significant senses once the insect has located its potential source of food. If food must be located from a distance then olfaction and vision also become important (Kaisling, 1971).

Insect feeding is usually considered to be made up of four distinct stages: the initiation of ingestion, the maintenance of ingestion, the termination of feeding when the insect is sated and the time before the next meal (Bernays and Simpson, 1982). In almost all insects that have been studied there is a clear periodicity of meals, provided that food is freely available. Simpson (1981) found an apparently endogenous rhythm, with a medium length periodicity of 12.0 to 16.5 minutes, to which bouts of feeding in L. migratoria seemed to be coupled. Ma (1972) showed that Pieris brassicae larvae had short periods of biting activity of less than 300 seconds with inter-feeds lasting 20 to 30 minutes when provided with food ad libitum. Bernays and Simpson (1982) reported that the same periodicity of feeding behaviour was observed in P. regina. Experiments described by Bernays (1980) suggest that some aspects of this periodicity are due to hormones secreted from the corpora cardiaca in L. migratoria, agreeing with the findings of Green (1964) on P. regina.

The initiation of feeding was the cornerstone of much of the work done by Dethier and his collaborators (described and summarised in Dethier, 1976). Working with the "blowfly-on-a-stick" they obtained a considerable amount of information about what initiates feeding, using proboscis extension as an assay of the effect of stimulants. They provided the idea of acceptance of a chemical stimulant and also developed the concept of aversion and rejection of these compounds when inhibitors, such as salts and acids, were added to solutions of sugars that were otherwise acceptable.

Competition between antagonistic stimulants is probably also at work throughout the feeding processes of most, if not all, herbivorous insects (Bernays and Simpson, 1982). Salt, acids and alcohols were all able to inhibit the blowfly, although it is not entirely clear why the fly should be averse to these. In many cases the same compounds were ingested at lower concentrations, when the fly reacted to them in the same way as to water. Some sugars which were nutritionally valuable, such as melibiose and sorbitol, had no effect on the fly at any concentration (Hassel, Dethier and Gans, 1950) suggesting that the flys' nervous system was not perfectly tuned.

In general for feeding to be initiated a stimulant chemical compound appears to be necessary and many plant compounds are known to act as phagostimulants for insects (Schoonhoven, 1973). In all studies of phytophagous insects to date, sugars, including sucrose, have acted as phagostimulants. In addition, other compounds , such as amino acids (Hatfield et al, 1982), fatty acids and sterols (Hsiao and Frankael, 1968a) and various salts (Ma, 1972) have all been found to stimulate feeding in various species. In most cases the effect

has been demonstrated behaviourally by increased consumption of some relatively inert material such as filter paper, elder pith or agar medium (Hsiao, 1973), often followed by the demonstration of an electrophysiological response to the compound being tested.

Similar behavioural experiments with filter-feeding insects have measured the rate at which food is ingested but have yet to reach the degree of sophistication seen in experiments on terrestrial insects (see Wallace and Merrit, 1980, for numerous references to this technique for simuliids). In addition to nutritive materials acting as phagostimulants many other secondary plant compounds are also phagostimulatory. Hsiao (1972) listed some of the classical examples of this phenomenon, such as the mustard oil glycosides for Pieris brassicae, Plutella maculipennis and Phyllotreta cruciferae and the essential oils of the Umbelliferae for Papilio polyxenes. The effects of both algae and detritus on the chemo-receptors of aquatic insects is completely unknown although this must be of some importance in the control of feeding behaviour in fine particulate filter-feeders.

Amongst oligophagous insects the range of compounds that are phagostimulatory is quite limited compared to the much greater number that inhibit feeding. When Bernays and Chapman (1977) fed 102 species of plants to L. migratoria they found that only grasses and Juncus sp. were eaten. Almost all dicotyledons were rejected and extracts of many of these plants inhibited feeding. They suggested that the failure of L. migratoria to eat these plants was due to the fact that they contained deterrent chemicals, especially alkaloids and monoterpenoids. The signifcance of deterrents to L. migratoria in the field was such that only very rarely were dicotyledons eaten

(Bernays et al, 1976). Woodhead (1983) has demonstrated that surface chemistry may be of importance in mediating rejection of unpalatable compounds in L. migratoria.

The effect of chemical stimulants on lepidopteran larvae may be of greater relevance to studies of blackflies. Larvae such as those of Pieris brassicae have a much smaller population of sense organs than any of the acridids (Ma, 1972) and may be more like blackfly larvae, which also have relatively few external sense organs, in this respect (Craig and Borkent, 1982). Odour discrimination can be achieved by some lepidopteran larvae with only 32 olfactory cells on the antennae (Schoonhoven, 1973). Chapman (1982) noted that lepidopteran larvae achieve the same effect, in terms of food selection, as locusts and grasshoppers that needed 2000 receptors to do the same job. He suggested that the wider diet of oligophagous acridids leads to the need for large numbers of receptors. In contrast to this many specialist feeders, such as may be found among the Hemipteroidea and Endopterygota, need few receptors once these have, in evolutionary terms, become tuned to the particular dietary requirements of the species.

Before ingestion can begin the presence of a phagostimulant appears to be essential for almost all species that have been studied (Bernays and Simpson, 1982). P. regina will not extend its proboscis at any time (irrespective of how long it many have been deprived of food or water for) without the presence of either a sugar or one of the amino acids that stimulates feeding (Dethier, 1976) and initiators are essential if ingestion is to begin in L. migratoria. In this case the initiation of feeding probably depends on the successive simulation of tarsal, palpal and cibarial chemoreceptors

and mechanoreceptors. Although stimulation of a single labellar or tarsal chemoreceptor will stimulate proboscis extension in P. regina (Dethier, 1976) it is likely that a number of sensilla are normally stimulated before ingestion begins. Arab (1959) found that proboscis extension could be elicited with 0.0164M sucrose solution when several receptors were brought into contact with the solution simultaneously, but that a concentration of 0.419M was needed to cause the same response when only a single receptor was stimulated.

For feeding to continue once it has begun it is likely that there must be a continued supply of stimulatory information from the peripheral sense organs. However, once the insect has been stimulated by food its motivational state is often heightened, reducing the threshold concentration of a chemical compound to which a feeding response may be made. Bernays and Chapman (1974a) also found that when L. migratoria lost contact with a source of food once feeding had begun it made more strenuous efforts to relocate the food than were initially made when searching. If the continuing stimulus is from a mild inhibitor the meal size tends to be smaller because the meal is terminated more quickly than normal (Haskell and Schoonhoven, 1969).

Once feeding has begun (and provided there is continued sensory input) there is evidence that it appears to continue automatically suggesting that the behaviour stems from an endogenous rhythm (Bernays and Chapman, 1974a) Feeding then continues until terminated by inhibitory stimuli reaching the brain, generally from stretch receptors in the gut wall. In L. migratoria the stimulataion of stretch receptors by a gut full of food is accompanied by the production of hormones from the corpora cardiaca that leads, among

other things, to the pores of chemoreceptors on the maxillary palps closing for up to one hour (Bernays and Mordue, 1973). In P. regina a similar mechanism operates and distension of the crop also stimulates stretch receptors that feed back to the brain (Gelperin, 1971a). In both cases hyperphagia can be caused by sectioning the posterior pharyngeal and recurrent nerves respectively.

Filter-feeding culicids also show bouts of feeding activity (Dadd et al, 1982), the rate being modified by the type and concentratio of stimulants in the water. Dadd, working with larval Culex pipiens, found that larvae ingested non-nutritious particles, such as diatomaceous earth and kaolin, more rapidly when they were kept in water that had had either yeast extracts, liver extracts or infusions of decayed plant material dissolved in it, than when kept in plain water (Dadd, 1970a and b).

He also showed that larvae were particularly sensitive to yeast adenylic acids and nucleic acids and that a range of sugars and amino-acids were only mildly stimulatory (Dadd, 1982). Like all insects in which feeding behaviour has been studied in detail, larval mosquitos were stimulated by the presence of their normal foods or substances that might be derived from them. However, unlike most other insects, ingestion still occurred even in the absence of these stimulants.

1.4 Blackfly larvae: feeding and food selection

Larval blackflies are amongst the most specialised of filter-feeders morphologically and most species have mouthparts adapted to removing fine particles from running water. A very few species, such as Cnephia crozetensis , have reduced cephalic fans and feed mostly by grazing (Dumbleton, 1962). Blackfly larvae are unable to create feeding currents and consequently cannot live in still water. They can ingest a very wide range of sizes of particles, from sub-micron sized colloids (Wotton, 1976) to algal cells 300-400um long (Chance, 1970).

The most conspicuous parts of the feeding apparatus are the cephalic fans, which are probably derived from labral hairs and are not technically true mouthparts (Craig, 1974). The other appendages are adapted to cleaning the fans. Grazing is often said to be important in larval nutrition but there have not been any critical tests of this contention (Lacey and Lacey, 1983). The 'functional anatomy' of the various mouthparts has been described in detail by Chance (1970) at the level of the light microscope as well as by a number of other authors (such as Craig, 1977 and Davies, 1974). With few exceptions all larvae seem to possess very similar mouthparts, the exceptions including C. crozetensis mentioned above and other island species, like the Hawaiian S. oviceps (Craig, 1977a) both with reduced cephalic fans.

The work of Schroeder (1980a) on the behaviour of individual Odagmia ornata larvae, and Craig and Chance (1982) on S. vittatum is of most relevance to the observations of feeding behaviour reported in this thesis. Schroeder observed larval behaviour closely,

counting the number of fan beats larvae made in one minute in different conditions. He observed behaviour in particle-free water and at a variety of temperatures and water velocities. He also described the pattern of behaviour when different concentrations of algal cells were available and the effect of variations in food concentration and water velocity on the amount of time spent feeding by the larvae. He did not record behaviour in the same detail as Craig and Chance (1982) who described the opening and closing of the cephalic fan using high-speed cine photography.

Movements of the fans and mouthparts are too rapid to be resolved with the unaided eye although still about 100 times less frequent than the most frequent movements of insect wings (Chapman, 1982), larval S. vittatum closing, cleaning and opening its cephalic fans in 0.055s (Craig and Chance, 1982). In a current of particle free water larvae beat their fans with a regular rhythm (Schroeder, 1980a) The frequency of fan cleaning was influenced by the water velocity and temperature and by the age of the larvae. As temperature increased the frequency with which the fans were cleaned also rose, with a maximum being reached at 20°C in particle- free water. At a water temperature of 3°C the fans were cleaned about 35 times min^{-1} rising to 110 times min^{-1} at 15°C in a water velocity of 8 $lcms^{-1}$.

In most cases, as had been observed by other authors (Chance, 1970, Kurtak, 1978), the fans were almost invariably cleaned alternately. Schroeder (1980a) was the first to notice that larvae would beat their fans quite normally in particle-free water and noted that as the velocity of particle free water was increased from 5 cms^{-1} to 20 cms^{-1} the number of fan beats rose from 40 min^{-1} to 80 min^{-1}.

There was a much smaller increase in the frequency of fan cleaning above this velocity. At a water velocity of 60 cms^{-1} and 15°C first instar Odagmia ornata beat their fans about 80 min^{-1} while final instar larvae beat their fans about 130 min^{-1}, both in the absence of food particles.

Schroeder (1980) also described the 'filtrier-rhythmus' finding that periods of active fan cleaning were interrupted by periods of inactivity when the fans were closed and withdrawn into the cibarium. He showed that as the concentration of algal food in the water increased the larvae spent less time cleaning their fans. He did not investigate the effect of the interruption of filtering on the rate of ingestion.

The distribution of sizes of ingested particles in the guts of simuliid larvae does not usually differ significantly from the distribution of particle sizes seen in the water (Wotton, 1977, Kurtak, 1979) showing that feeding is indiscriminate with respect to particle size. However it is possible that the size of microtrichia on the primary filaments of the cephalic fan may influence the efficiency with which particles are ingested but this has not yet been demonstrated conclusively (Kurtak, 1978).

One of the few exceptions to this generalisation was observed by Wenk and Dinkel (1981). They found that the 1st instar larvae of Boopthera erythrocephala, Odagmia ornata and S. damnosum, when fed on latex balls in the size range 1.2-40.3µm selected those of 5-12µm in preference to other sizes (although other sizes were ingested). Larvae of the 2nd instar or older took the entire particle size range indiscriminately. Any particle selection that does occur is assumed to be on the basis of size alone and there is no evidence that

simuliids are able to select the more nutritious particles.

As a result of their indiscriminate feeding larvae ingest large amounts of inorganic material; Kurtak (1979), for example, found that 30% of the gut contents might be mineral particles in S. vittatum. It is not known whether these particles are of any nutritional significance to the larvae, although it is generally assumed that they are not. Small particles, of less than 5µm diameter , are of great significance to a number of species and may often contribute more than 50% of the gut contents despite their small size (Wotton, 1977, Kurtak, 1978). Carlsson et al (1977) found that particles of less than 2µm diameter were essential to the larvae of three species of simuliids in a Swedish lake outlet. The three species, (Metacnephia tredecimatum, Schonbaueria anulitarsis and Simulium truncatum) were far less abundant downstream from the outlet of a lake because of the changes in the nutritive value or abundance of the fine particles on which they appeared to rely for food.

The mechanics of the cephalic fan, and the way in which it catches particles, are rather poorly understood. Craig and Chance (1982) have demonstrated that there is an area of turbulence immediately behind the fan and that water velocity could be reduced by eight to ten times as water passed through the fan; they also suggested that the speed with which the fan was cleaned could influence the amount of food ingested, since the longer it took to clean the fan the less time was spent filtering the water. It appears that the cephalic fans can remove colloids and much larger particles from the water with equal efficiency. It is known that larvae secrete a mucous material onto the fan and this may be important in capturing those particles that have a diameter much

smaller than the distance between fan filaments (Ross and Craig, 1980).

1.5 Blackfly larvae: factors affecting the rate of ingestion

Although little is known of the nutritional value of the food of larval simuliids there have been a number of studies that have measured the rate at which food is ingested (for example, Chance, 1977, Elouard and Elsen ,1977 Mulla and Lacey, 1976). Generally the ingestion rate is affected by water velocity and temperature, particle concentration, age of the larvae and species (although there has not been an unequivocal demonstration that, in the same physical conditions, two species feed at different rates). Because of the transparency of the cuticle it is possible to observe the gut contents through the body wall. There is no peristalsis in the fore and mid-guts so that when larvae are fed with coloured particles, such as powdered chalk, charcoal or particulate fluorescent dyes, a coloured 'plug' forms in the gut within a couple of minutes. The subsequent movement of the plug through the gut can then be used to measure of the rate of ingestion (Ladle et al, 1972).

Using this method it has been found that the feeding rate increases with temperature up to about 15°C for S. vittatum, remaining the same, or declining, above this temperature. Field observations by Mulla and Lacey (1976) showed that the rate of ingestion of S. tescorum was greater at 30°C than at 13°C; at the higher temperature the larvae displaced a fluorescent plug from the gut in 20-30 minutes compared with 30-55 minutes at the lower temperature. Earlier, Ladle et al (1972) had been unable to find any variation in the ingestion rates of S. lineatum and S. ornatum.

Mulla and Lacey (1976) also stated that early instar S. tescorum fed more rapidly because they voided their gut contents more rapidly than older larvae. This is to be expected as early instars have guts that are considerably shorter than the later instars; the rate of feeding, when considered in terms of gut passage times, must be related to the length and diameter of the gut if a valid comparison is to be made. Since the younger larvae, their group C, were almost certainly at least half the length of those larvae in Group A it is likely that the younger larvae were actually ingesting less rapidly. Schroeder (1980a) has shown that early instars clean their cephalic fans less frequently than later instars while Hart and Latta (1985) showed that ingestion rate was positively related to fan cleaning frequency at some particle concentrations, so supporting this interpretation.

In general the rates of ingestion that have been measured are all of the same order, between about 30 and 60 minutes for one complete change of the gut contents, with extremes of 20 minutes and two hours (Ladle et al, 1972, Lacey and Lacey , 1983, Mulla and Lacey, 1976, Elsen and Hebrard, 1979, Wotton, 1978). The exact temperature at which the feeding rate reaches a maximum seems to depend on the species. Growth rates, too, appear to be related to temperature and some species are able to grow rapidly at low temperatures which prevent growth in others (Merrit et al, 1982).

Current velocity also affects the rate of ingestion. There appears to be a general increase in the rate of ingestion up to a water velocity of about 30cm s^{-1} above which larvae ingest less rapidly (Mulla and Lacey, 1976, Kurtak, 1978). This may be due to particles having greater momentum at higher water velocities so

making it more difficult for the fan to stop them. Kurtak (1978) showed that the efficiency of ingestion (the fraction of the particles believed to be passing through the fan that were caught on it) decreased when larvae were fed relatively dense particles, such as glass beads and poly-vinyl alcohol.

Kurtak (1978) also found that the efficiency of ingestion declined as the concentration of particles in the water rose. This decline in the ingestion rate of simuliids with increasing particle concentrations, also noted by Gaugler and Molloy (1982), contrasts with Dadd's (1971a) findings on the ingestion rate of larval Culex pipiens. He showed that the ingestion rate in C. pipiens increased as the concentration of food particles (latex microspheres) rose, up to a concentration of about 600mgl^{-1}, a much higher concentration than those that simuliids are usually exposed to (Baker and Farr, 1977). The incipient limiting concentration (the food concentration above which increasing food availability does not increase the rate of ingestion) for C. pipiens may be as much as 600mgl^{-1} but it is almost certainly much lower in the majority of simuliids (Gaugler and Molloy, 1982), perhaps below 10mgl^{-1}. The reasons for this difference are unclear, but probably reflect the ease with which larval simuliids are inhibited from feeding by high concentrations of particles.

Many of the foods that simuliid larvae may be reared on in the laboratory are able to inhibit them from feeding. This was first observed by Gaugler and Molloy (1982), but a number of authors have reported that larvae might spend as much as 66% of their time not feeding, although this was not recognised as feeding inhibition at the time (Craig and Chance, 1982; Schroeder, 1980a). When not

feeding larvae were either 'browsing', 'cleaning mouthparts' or 'remaining inactive'. Although this suggests that larvae feed periodically, in the sense of terrestrial insects, there is no evidence of a regular rhythm in this inhibition. Gaugler and Molloy (1982) suggested that it was due to the gut becoming packed with food, and that once the gut was full ingestion stopped for a certain time, in the same was as in locusts and blowflies. This seems unlikely, however, because simuliids rely on the continual ingestion of food particles to push through the gut those already ingested (personal observation).

When larvae were fed different concentrations of particles Gaugler and Molloy (1982) found that the time for which larvae were inhibited from feeding increased from 40% to 90% of the time as the concentration of particles was increased from $10mgl^{-1}$ to $200mgl^{-1}$. However even $10mgl^{-1}$ of suspended particles is probably a high concentration for blackfly larvae in the field (Dawson, 1981). As there have not been any observations of feeding inhibition in the field the relevance of this behaviour to the regulation of feeding remains unclear.

Ladle et al (1972) noted that in winter S. ornatum and S. lineatum apparently stopped feeding, showing no detectable ingestion. It is possible that feeding inhibition explains this observation, with high winter particle concentrations preventing ingestion. Kurtak's (1978) observation that larvae ingested more particles at the lower food concentrations and water velocities could also reflect feeding inhibition. Finally, Schroeder (1980a) reported increasing spells spent not feeding by larvae as the concentration of food rose; it is worth noting that, unlike Gaugler and Molloy, he worked with

realistically low food concentrations.

The relationship between feeding inhibition in simuliids and the four stages of feeding in phytophagous insects is unclear. 'Feeding inhibition' may simply occur when larvae are sated or it may be genuinely detrimental to the larvae. This point is considered in the work reported here.

1.6 The external sense organs of aquatic insects

In most cases during the initiation and continuation of feeding insects are supplied with information about their food by chemo- and mechanoreceptors (Bernays and Simpson, 1982). Although relatively little is known about the role of mechanoreceptors during feeding a great deal of time has been devoted to investigating the structure and physiology of chemoreceptors, particularly contact chemoreceptors (see, for instance, Dethier, 1976). Most of this work has involved terrestrial insects, much of it being centred on P. regina. The relevance of studies of receptor physiology to aquatic insects must, therefore, be assumed although Hodgson (1951) did find that the water beetle Laccophilus maculosus responded in behavioural tests of chemical stimulants in much the same way as P. regina. This example, and the work of Dadd (a series of papers up to 1982) mentioned above, are the only investigations of the effect of chemical compounds (other than pesticides) on the behaviour of aquatic insects.

Although the function of a sensillum can only be defined after physiological investigation it is possible to deduce, with some confidence, its likely function following observation of its external form. A general classification of the external structure of chemoreceptors, the most important sensilla in the control of feeding, has been proposed by Zacharuk (1980) and can be applied to aquatic insects.

Zacharuk proposed a revised classification of chemoreceptors based on two main categories that appear to be of general functional significance. Uniporous sensilla, with a single external opening,

are usually concerned with contact chemoreception. Multi-porous sensilla, with many external openings, are most likely to be concerned with olfaction although within these two broad categories there are many different types of sensilla, both in terms of structure and function.

Uniporous sensilla have a variety of forms and may be hairs, pegs, papillae or simple pores in the cuticle. They are most often, though not exclusively, associated with an appendage used to sense by contact but they can also sense by olfaction. Multi-porous sensilla, which Zacharuk divided into two broad categories (those with a pitted surface and those with a grooved surface) are generally found on the antennae although they do occur on other appendages; they are sensitive to odours. It is possible that the differences in the number of pores reflects the greater accessability required of sense organs that must respond to very dilute stimulants.

Although it may be assumed that insects from aquatic environments have sense organs generally similar in function to those of terrestrial forms, only two aquatic species, apart from simuliid larvae, have had any of their external sense organs described, both being mosquito larvae (Jez and McIver, 1980 and Zacharuk et al 1971a and b).

Zacharuk et al (1971) described the fine structure of the sense organs on the antenna of the fourth instar larvae of Aedes aegypti while Jez and McIver (1980) described the antennal sense organs of Toxorhynchites brevipalpis. Although the two species had very different feeding habits, (the former being a filter-feeder, the latter predatory) it was found that the sense organs were very similar in appearance. Both groups of authors considered only the

antennal sense organs and did not investigate the structure of sensilla on the mouthparts which, particularly for the filter feeder, might have been of greater importance.

The antennal sensilla of T. brevipalpis are quite simple. There are five types of sensillum innervated by a total of 26 neurons. At the tip of the antenna were three sensilla: a conical sensillum with a protruberance, a peg and a trichoid sensillum. In the mid-region of the antenna there were two more trichoid sensilla , a branched hair sensillum and a campaniform sensillum. The antennae of fourth instar larval A. aegypti (Zacharuk, 1971a and b) are innervated by 27 to 28 neurons and have six different types of sensilla: a conical sensillum, a peg sensillum, three trichoid sensilla, each innervated by two neurons, a chordotonal organ and a sinusoidal peg sensillum. The differences between the two species seem to be quite small.

The external sense organs, and some parts of the sensory nervous system, of simuliids has been described by Chance (1970), Craig (1977), Craig and Borkent (1980) and Craig and Batz (1982). Chance (1970) found that the larvae of S. vittatum have sense organs on the labrum, mandibles, maxillae, labium and hypostomium, although the numbers involved were small. The membraneous area that joins the labrum to the frons is one area where sense organs are numerous. The sclerite that supports the labrum has teeth at its apex and in an unidentified species Chance (1970) found that the teeth had neural connections. The mandibles of S. vittatum have small sensory hairs scattered over their oral and aboral surfaces. S. tahitiense has a few trichoid sensilla on the dorsal surface of the mandible, which also has a prominent pair of preapical sensilla. The function of all

of these sensilla is unknown (Craig, 1977).

The maxillae have the most diverse population of sense organs although, again, they are few in number. The maxillary lobe has three prominent sensilla: a basiconic sensillum and a trichoid sensillum are paired on a papilla, whilst another basiconic sensillum arises medial to these. S. tahitiense has at least nine sensilla at the apex of the maxillary palp, all of which have the appearance of chemoreceptors, and a smaller number of trichoid sensilla scattered over its surface (Craig, 1977). Chance (1970) stated that six to ten sensilla may be found on the article of the maxillary palp and four to six at it apex, depending on the species. In S. tahitiense there are three cone-shaped sensilla, a single tubular sensillum, an ovoid sensillum, two small nipple-like sensilla and a pair of peg-like sensilla at the apex of the palp.

Craig and Borkent (1982) described the maxillary palpal sensilla of a number of other species of simuliid larvae after investigating their external structure using the SEM. They also described the innervation of the maxilla after staining with methylene blue. They suggested that the palp might be involved with contact chemoreception, although one of the sensilla was multi-porous and therefore more likely to be concerned with 'olfaction'. They did not consider the function of these sense organs physiologically.

The labial lobe has two prominent spherical sensory lobes with several basiconic sensilla, the number depending on the species. The hypostomium also has a variety of sensory hairs (Chance, 1970). A row of trichoid sensilla lies parallel to each margin of the hypostomium, whilst others are scattered over the ventral surface of the head capsule posterior to the hypostomial fold.

Chance compared the sense organs of Twinnia biclavata, a non-filtering species, with those of S. vittatum, a typical filtering species. She did not find any great differences between the two, perhaps suggesting that the external structure of chemoreceptors does not show their function, confirming Lewis' (1970) view. Craig (1977) briefly discussed the possible function of the sense organs of the larvae of S. tahitiense which has fully developed cephalic fans and filter-feeds. He suggested that the adoral basiconic sensillum of the maxillary lobe makes contact with food particles as they are ingested. However, he did not consider that other sense organs, including those of the maxillary palp, played any part in the sensing of food quality. He also compared the sense organs of the typical S. tahitiense with the less typical S. oviceps, which has reduced cephalic fans. Like Chance he did not find any great difference in the number or types of sensilla. Craig (1977) also suggested that the sense organs of the maxillary palp may be used for sensing the quality of the water.

The antennae of S. vittatum were found to have three sense organs by Chance (1970): a terminal conical sensillum and two multi-porous sensilla at the apex of the third article. Craig and Batz (1982) described the fine structure of the antenna and its associated sense organs finding five morphological types, although there were only eight sensilla altogether, innervated by 22 neurons in each antenna.

As has been shown in this review detailed observations of the feeding behaviour of larval simuliids are lacking. The description of larval behaviour was, therefore, the first aim of the work reported in this thesis. In combination with observations of the external structure of various sensilla it was hoped to provide the foundation needed for a detailed study of the control of larval feeding behaviour. Despite the obvious difficulties of working on the sensilla of small aquatic insects such work could ultimately lead to studies of the physiology of larval sensilla.

CHAPTER 2 OBSERVATIONS ON THE STRUCTURE OF THE HEAD CAPSULE OF SIMULIID LARVAE

2.1 Introduction

Preliminary observations showed that larvae spent most of their time feeding and that, while feeding, activity was almost entirely confined to movements of the appendages of the head capsule. To facilitate the interpretation of behaviour patterns, reported later, the external structure of the cephalic fans and mouthparts was described. Observations on the types and distribution of external sensilla on the mouthparts allowed some deductions to be made about their function before neurophysiological investigation.

The scanning electron microscope (SEM) was used to describe the external structure of the appendages and the external structure and distribution of mouthpart sensilla, whilst methylene blue staining demonstrated those structures that had sensory nervous connections.

2.2 Methods: observations using the Scanning Electron Microscope and methylene blue staining

The external structure of the head capsule and mouthparts of *S. ornatum* was investigated using the SEM, with limited observations being made of *S. noelleri* and *S. lineatum*. The gross structure of the sensory nervous system was investigated by staining intra-vitally with reduced with methylene blue. For observation in the SEM pharate larvae were fixed in Pampl's Fluid (Glacial acetic acid, $4cm^3$; distilled water, $30cm^3$; 40% Formaldehyde, $6cm^3$;

95% Ethanol 15cm^3). Larvae were placed in the fixative whole which killed them after a few seconds, following which the thorax and abdomen were cut off and discarded. The time for which they remained in the fixative did not appear to be critical but never exceeded half a day. Fixation in Pampls Fluid was found to give the best results for SEM work, combining the fixation quality of an aldehyde with the slight expansion caused by alcohol that was necessary to display the mouthparts. Other methods of fixation (killing larvae in boiling water and then fixing in alcohol and fixing in glutaraldehyde or formalin) were less successful.

Once fixed the heads were shaken vigorously in a 2% solution of the surface active detergent 'Decon' to remove debris and other particles adhering to the head. In most cases the larvae were then dehydrated in an ethanol series (30%, 50%, 70%, 90% and 100%) spending 30 minutes in each concentration. One change of each alcohol was used in the 30% to 90% range with three changes in 100%. From 100% ethanol the specimens were taken to 100% acetone and then dried in a critical point drier after being infiltrated with liquid CO_2 for two hours. Once mounted on stubs the specimens were coated with 50nm of gold-palladium in a Polaron E5100 Series II 'Cool' Sputter Coater. In some instances acetone replaced ethanol at all stages of the dehydration with no appreciable change in the results. For some micrographs mouthparts were dissected from the head capsule with fine pins after the heads had been fixed. Specimens were observed at various magnifications at an accelerating voltage of 15kV in a Jeol SEM.

Chance (1970) gave an extensive account of the structure and 'functional' morphology of larval mouthparts as seen using the light microscope. This section describes the additional detail that is revealed using the SEM. Where it is not otherwise specified the micrographs refer to S. ornatum. No attempt has been made to determine which member of the 'ornatum' species group was used but all larvae that are described were collected from the same reach of the river Itchen in Hampshire (see Chapter 3.1 for details of collecting sites). Table 1 lists the abbreviations used to label the scanning electron micrographs. On each micrograph the number beside the scale bar gives its length in microns.

The gross structure of the sensory nervous system of the head capsule and mouthparts was observed in whole mounts of the head capsules of pharate larvae after staining intra-vitally with reduced methylene blue. Pharate larvae were anaesthetised by placing them in CO_2 enriched water for one to two minutes. They were attached to double sided 'Sellotape' and reduced methylene blue injected into the body until it became slightly distended. The reduced methylene blue was prepared according to the method of Burgess and Rempell (1966). After injection the larvae were placed in aerated water for up to one hour, when the heads were cut off in 12% ammonium molybdate solution and then refrigerated overnight in this solution. After molybdate fixation the heads were washed in distilled water for five hours, dehydrated in an ethanol series, cleared in xylene and mounted in 'DePex' mounting medium.

TABLE 1. Abbreviations of labels used on scanning electron micrographs.

Abbreviation	Structure
a	antenna
ab	apical brush
at	apical teeth
ats	apical trichoid sensilla
bl	basal lobe
bs	basiconic sensillum
cb	covering brush
cfs	cephalic fan stem
co	central oval brush
db	dorsal brush
feb	first external brush
gs	galeal sensillum
hb	hypopharyngeal bristles
hc	head capsule
hy	hypostomium
hy s	hypopharyngeal setae
la	labrum
lab b	labial brush
lb	labral brush
lbb	large basal brush
l hy com	labio-hypopharyngeal complex
lob	lobed area
l or b	large oral brush
m	microtrichia
mff	medial fan filaments
mb	medial brush
ml	maxillary lobe
mn	mandible
mp	maxillary palp
mps	multiporous sensillum
mx	maxilla
pat	pre-apical teeth
pf	primary cephalic fan
pff	primary fan filaments
sbb	small basal brush
sc	sensory cone
sf	secondary cephalic fan
sob	small oral brush
t	trichoid sensillum
ucs	uniporous cone-shaped sensillum
uds	uniporous dome-shaped sensillum
ups	uniporous peg sensillum
uts	uniporous tubular sensilla
us	uniporous sensillum
cf	cephalic fan
seb	second external brush
uds	uniporous digitiform sensillum

2.3.1 The external structure of the cephalic fans

The SEM reveals the intricate structure of the head capsule and mouthparts of S. ornatum showing the structures used for filter feeding. As the ventral view of the head capsule (hc) in Plate 1.1 shows, the most prominent feature of the head is the pair of cephalic fans (cf). The origin of these organs is slightly uncertain; they do not appear to be derived from other mouthparts but are thought to be modified labral hairs (Craig, 1977). Thus despite being the most typical feature of simuliid larval feeding apparatus they are not true mouthparts.

Plate 1.2 shows the right cephalic fan (cf) more closely. In the case of S. ornatum it is made up of about 40 primary fan filaments (pf) each about 1mm in length, carried on a sclerotised fan stem. The expanded bases of the individual primary fan filaments (pff) can be seen in Plate 1.4. The fan also has two smaller elements, the secondary fan (sf) and the medial fan(mf). Both can be seen in Plate 1.2 whilst the base of the medial fan is shown in more detail in Plate 1.3. It is not known whether either of these two subsidiary structures has a role distinct from that of the primary fan. Both the primary and secondary fan filaments are lined with microtrichia (m) whilst those of the medial fan are smooth.

Plates 2.1 and 2.2 show the microtrichia of S. ornatum while Plate 2.3 shows those of S. noelleri. It should be noted that the microtrichia do not lie at right angles to the water current as it passes between the fan filaments but point straight into it. The length of microtrichia of S. ornatum varies from 2-5µm with groups of eight to ten filaments arranged in order of increasing size, with

the longest at the distal end of each group. S. noelleri shows a different arrangement of microtrichia, with groups of twelve to fifteen, of 2-3μm length, interspersed with longer microtrichia, up to 10μm long. Each group of microtrichia, bounded by the longest and shortest microtrichia in a group, may be recognised as a unit on the cephalic fan filament (see Section 7.12).

The significance of such differences, if any, which have also been reported by Chance (1970), Grenier (1949) and Kurtak (1978) is unknown. Indeed the function of the microtrichia in general is unclear although their size suggest that they may be concerned with trapping fine particles. Alternatively they may increase the surface area of the filaments to which particles may be stuck. The microtrichia do not stretch from one filament to the next, however, except at the very bases of the filaments, and any impression of them creating a net (such as might be suggested by Plate 2.2) is false.

There is no evidence from SEM observations to suggest that the fan has any sense organs associated with its surface, although it is possible that stretch receptors monitor the position of the fan or stresses on it. Plate 2.4 shows the typical position of the fans when they are closed. In this position it is possible for the mandible (mn) to rake down over the closed fan and push food into the cibarium. The micrograph shows both fans closed at the same time, something that normally only occurs when the larvae are inhibited from feeding. Apart from these occasions the fans are always closed for cleaning alternately. Fan movements are described in detail in Chapter 4.

Plate 1. The external structure of the cephalic fans.

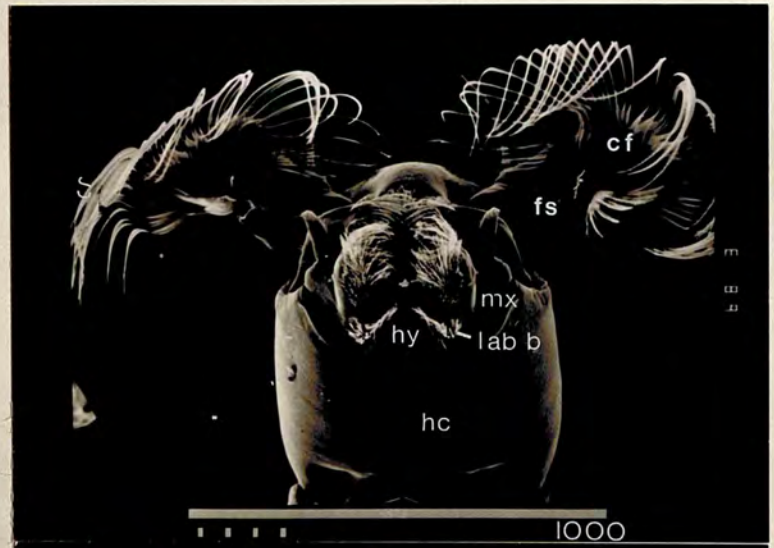
1.1 Ventral view of the head capsule.

1.2 Ventral view of right cephalic fan.

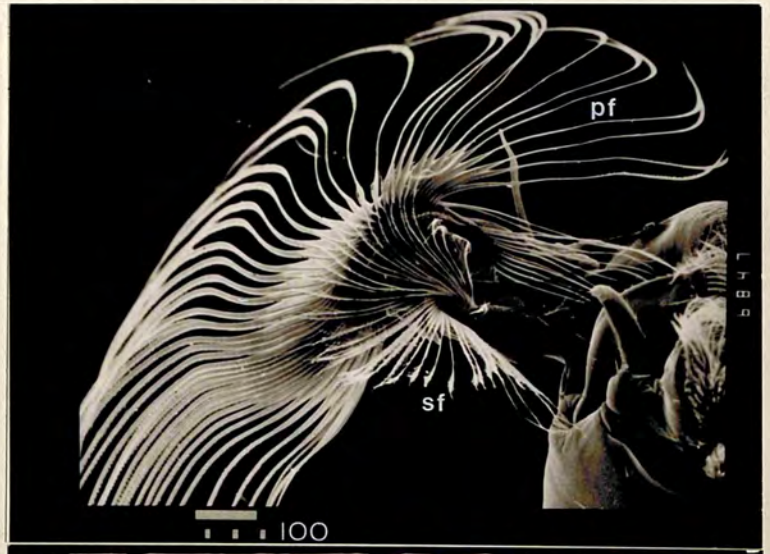
1.3 The base of the medial fan.

1.4 The bases of the primary fan filaments.

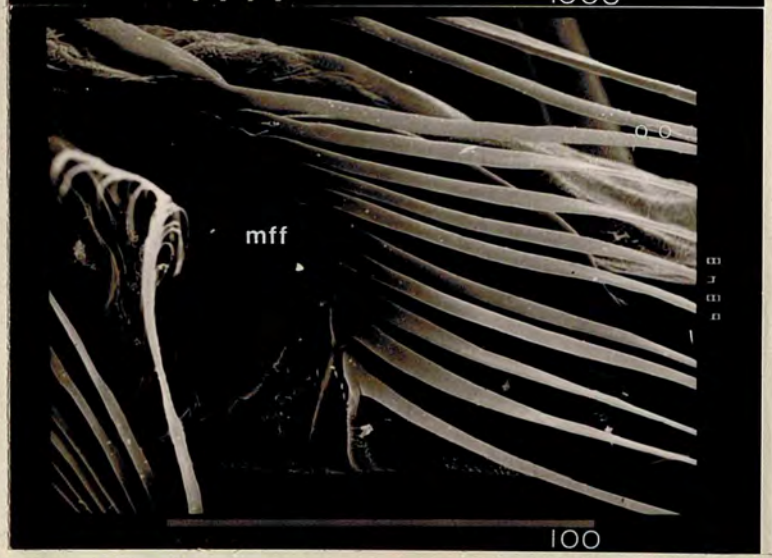
1.1



1.2



1.3



1.4

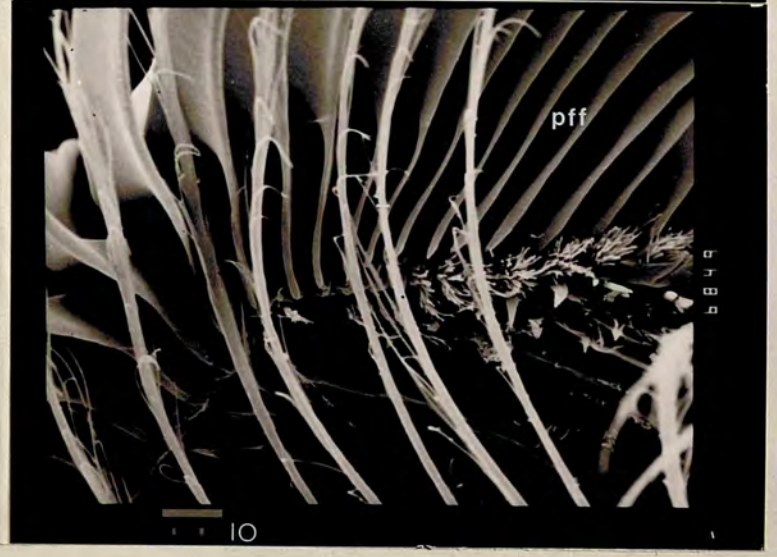


Plate 2 The external structure of the primary cephalic fan filaments.

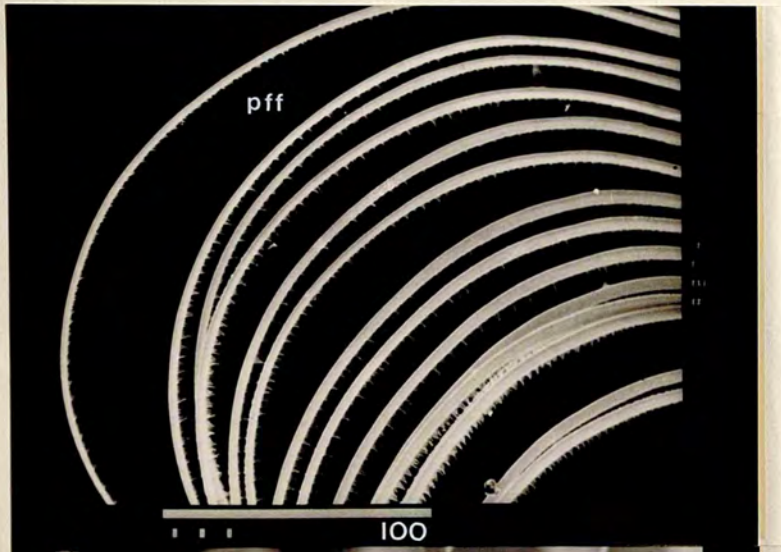
2.1 The primary cephalic fan of S. ornatum showing microtrichia.

2.2 Detail of the microtrichia of the primary cephalic fan of S. ornatum.

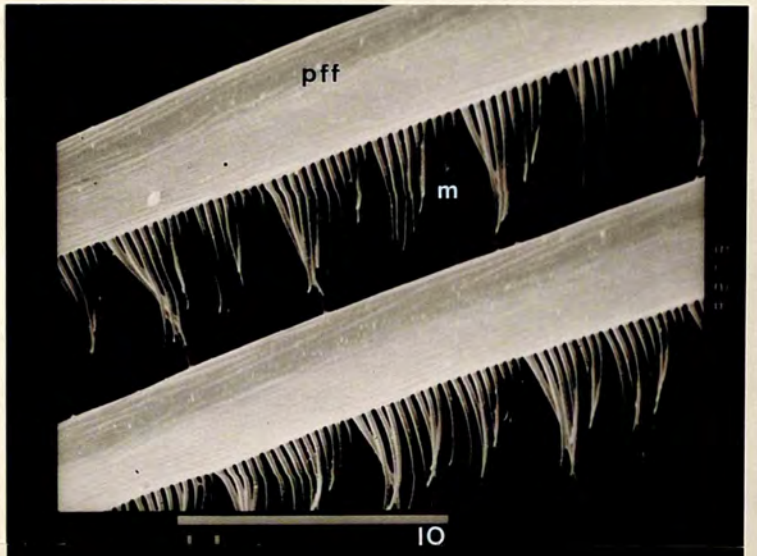
2.3 The primary cephalic fan of S. noelleri showing microtrichia.

2.4 Medial view of the head capsule of S. ornatum.

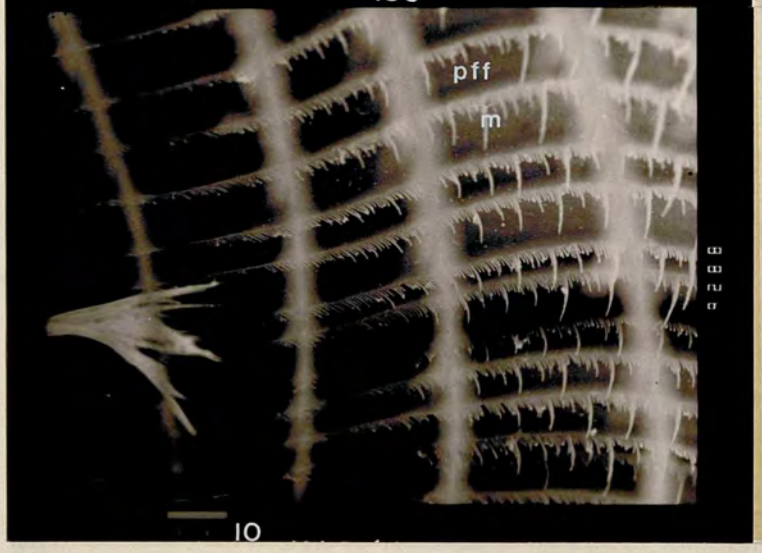
2.1



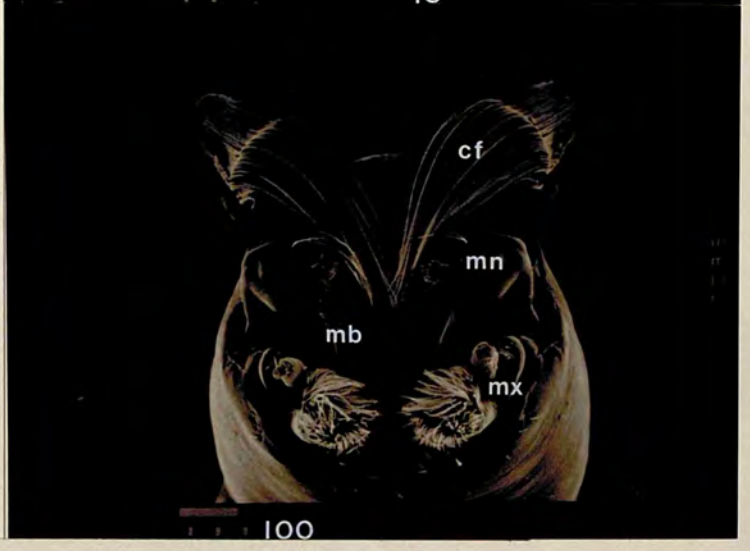
2.2



2.3



2.4



2.3.2 The external structure of the mandibles

The mandibles begin their raking stroke from the shoulder visible two-thirds of the way up the closed fan (see Plate 2.4). It is possible that the labrum (obscured in Plate 2.4) is also involved in removing food from the fans, although perhaps more passively, as it is probably in contact with the inner surface of the closed fan where food particles are concentrated.

The mandibles are the most active of the mouthparts and observations of the extent and frequency of their movements are important in the quantitative analysis of feeding behaviour. Their general shape can be seen from Plates 3.1 and 3.2 which show the aboral and oral surfaces respectively. The plates shows the precise arrangement of the hairs making up the brushes carried by the mandible.

Chance (1970) described seven separate brushes that were recognisable on the basis of differences in the structure of their component hairs. Her groups are retained here, although the largest brush, the first external brush (feb) is composed of three distinct groups of hairs. Almost all the hairs on the mandible appear to be structural and without neural connections and little is known of the functional significance, if any, of the differences in their structure. This is partly because it is difficult to observe the inter-relationships of the brushes with the fans and other mouthparts in the living animal, owing to the rapidity of movement and small size of the mandibles. The relationships apparent on fixed specimens must be treated with some caution as they may not be typical of relationships in the whole animal.

Figures 1a, b and c show the arrangement of the seven different brushes with reference to Plates 3.1, 3.2 and 4.3 respectively. The position of the mandibles in relation to the other mouthparts can be seen in Plate 2.4. The micrographs and figures show that there are two major groups of brushes; those originating on the oral surface of the apical half of the mandible (see Plate 3.2) and those on the oral edge of the medial half of the mandible (see Plate 3.1). Since the most important function of the mandible appears to be the cleaning of food particles from the closed fan this concentration of brushes on its inner surface is to be expected.

Plate 3.3 shows the tip of the mandible from the aboral side, displaying in more detail the apical teeth (at), which are of taxonomic importance, and the chisel-like apical brush (ab). Plate 3.4 shows the apical teeth of S. ornatum whilst Plate 4.1 shows those of S. lineatum. The most obvious difference between the two is in the number of teeth, S. ornatum having many more than S. lineatum while the shape of the largest apical tooth also differs. The thicker hairs behind the apical brush and teeth comprise the covering brush (cb) which probably comes into contact with the fan at the end of the raking stroke. The hairs of the covering brushes, which interdigitate when the mandibles are fully adducted may form a net for gathering and restraining food particles while they are pushed into the cibarium. The apical teeth and brushes may also be concerned with silk manipulation.

In lateral view the arrangement of the brushes at the apex of the mandible presents a confused appearance (see Plates 3.1 and 3.2). However, if the mandible is viewed from the ventral side it becomes clear that the covering brush, which has two distinct parts, lies

orally of the other two groups at the apex of the mandible (see Plate 4.2). In Plates 4.2 and 4.3 the oral surface is towards the top of the picture. Once again the significance of the arrangement is unclear but this is not an artefact of fixation for the SEM, as it can also be seen in unfixed material.

The largest brush on the oral surface of the mandible is the first external brush (feb) which is probably responsible for removing food particles from the closed fan. The SEM reveals its three component types of hair, each with characteristic position and structure. The innermost group are the longest and thickest and have a distinctive kink at the tip. Outside these are a group of shorter, finer hairs which are lined with microtrichia. Below these are a group of smooth, but equally, fine coiled hairs. The position of the mandible on the closed fan makes it difficult to observe the relationships of the various parts of the first external brush with the fan.

It is possible that this brush is also concerned with cleaning the labrum when extra mandible movements are made (see Figure 11 for the definition of extra mandible movements). At the proximal end of the first external brush is a group of longer hairs that Chance (1970) distinguished as the second external brush (seb).

Although it is clear that the brushes of the distal half of the mandible are involved with raking the fan and pushing food into the cibarium, it is more difficult to suggest functions for the brushes on the proximal half of the mandible.

Plates 3.1 and 3.2 show the position of the remaining brushes (and also in Figures 1a, b and c). Plate 4.4 shows the frond like structure of the medial brush which may be seen on the intact head in Plate 2.4 projecting medio-ventrally from the mandible. The small (sbb) and large basal (lbb) brushes occupy the most proximal part of the mandible (see also Plate 5.1) and are strap-like with frayed tips. Like the medial brush their function is unknown. The group of pegs on the proximal oral surface of the mandible does not have any neural connections.

The mandibles carry very few sensilla. Methylene blue staining suggests that, amongst the brushes, only the apical trichoid sensilla and apical and largest preapical teeth are innervated (see Plate 3.3 and 3.4).

Plate 3 The external structure of the mandible.

3.1 General view of the aboral surface of the left mandible.

3.2 General view of the oral surface of the right mandible.

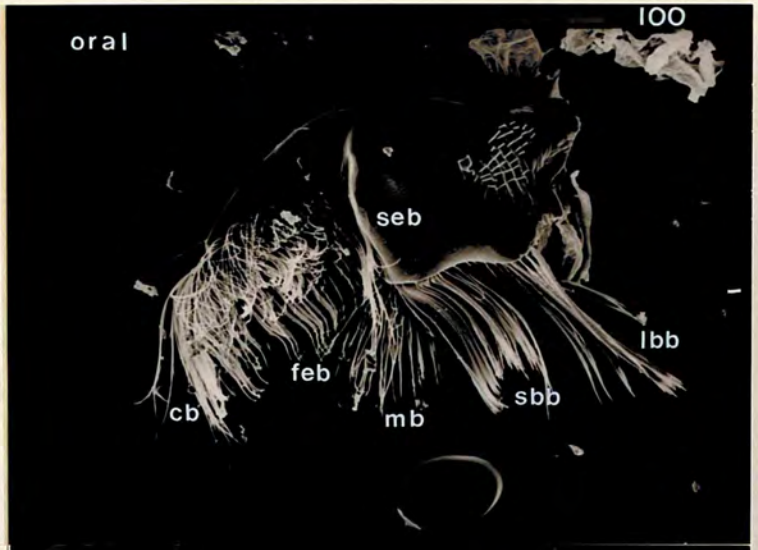
3.3 The apex of the mandible showing brushes and teeth.

3.4 The apex of the mandible showing pre-apical and apical teeth

3.1


aboral
100
cb
ab
mb
lbb

3.2


oral
100
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feb
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3.3

ats
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ab
at
100

3.4

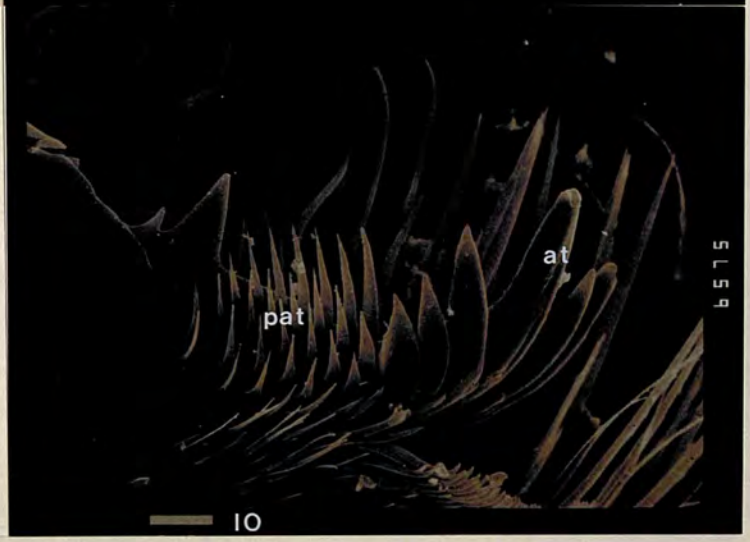

at
pat
10

Plate 4. The distribution of brushes on the mandible.

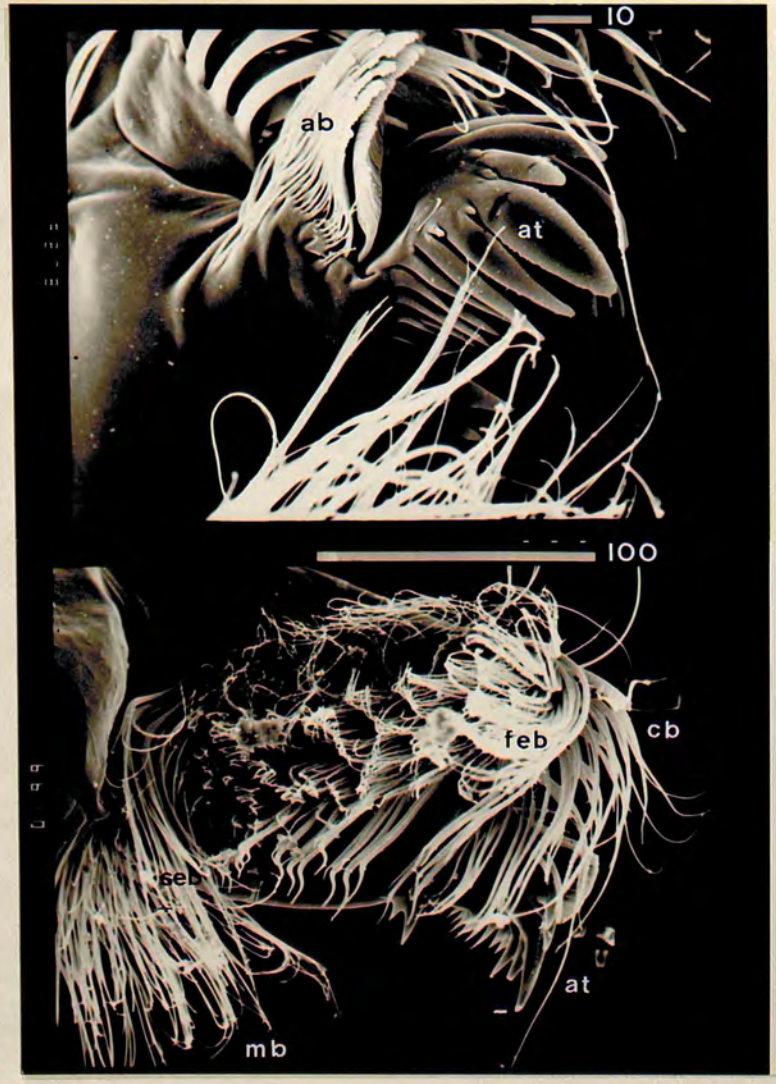
4.1 The apex of the mandible of *S. lineatum*

4.2 Ventral view of the apex of the mandible of *S. ornatum* showing structural brushes.

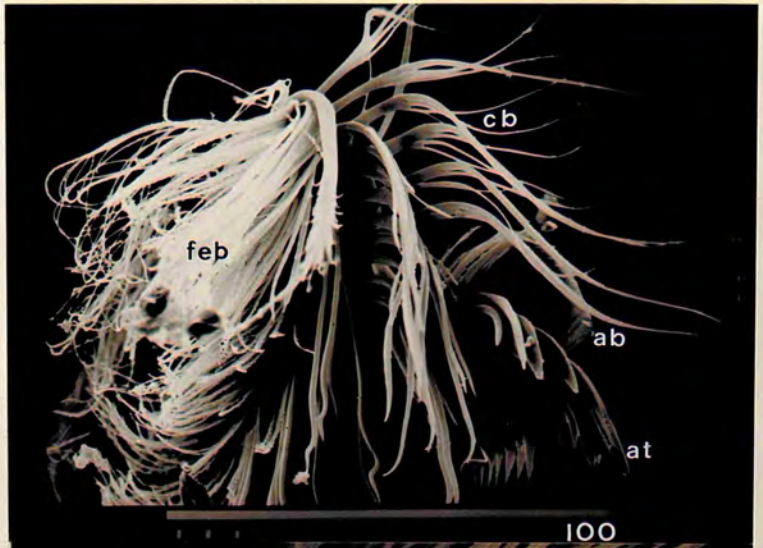
4.3 The structure of the first external brush.

4.4 The structure of the medial brush.

4.1



4.2



4.3

4.4

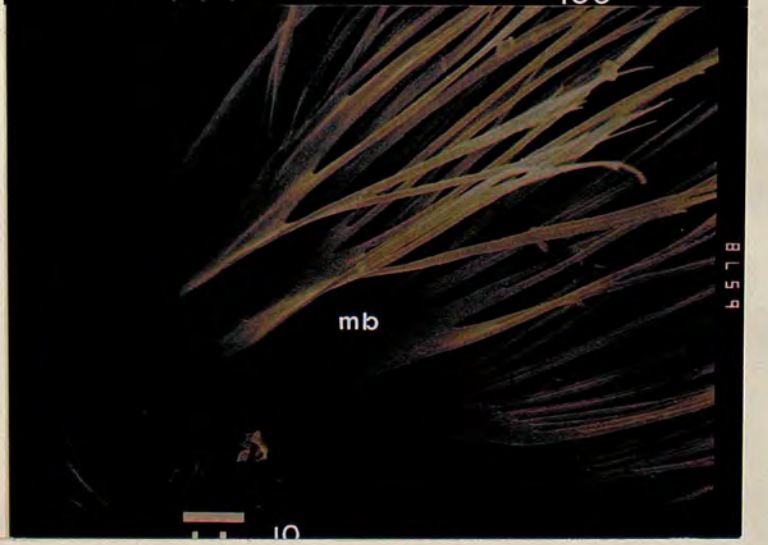


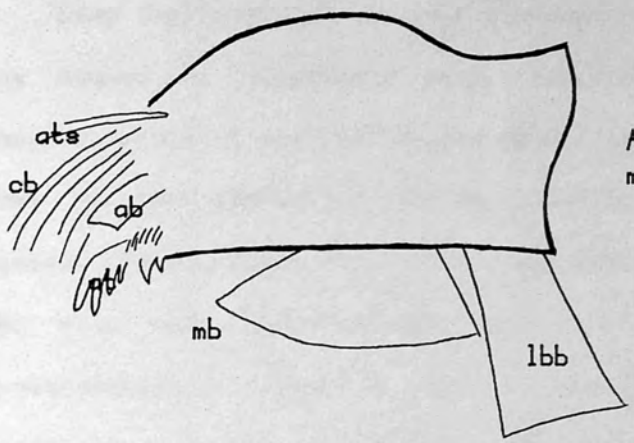
Figure 1. The external structure of the mandible

a) Aboral view of the left mandible.

b) Oral view of the right mandible.

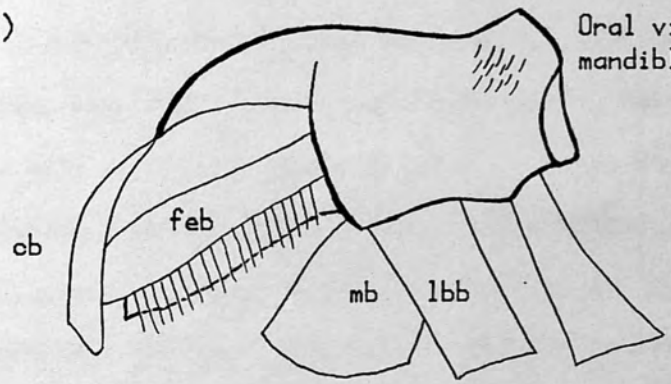
c) Ventral view of the apex of the left mandible.

(a)



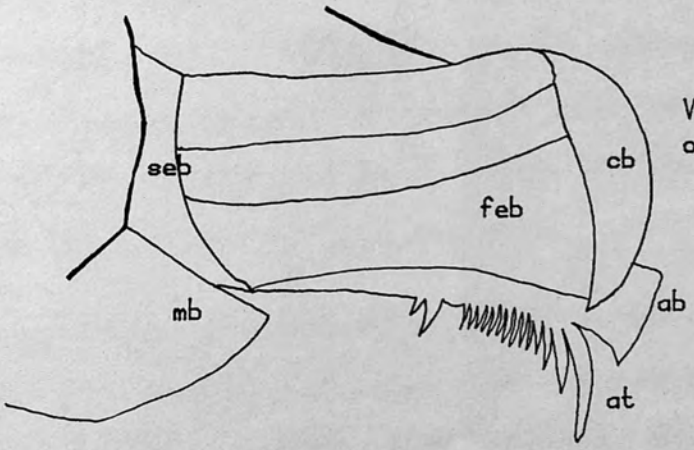
Aboral view of the left mandible

(b)



Oral view of the right mandible

(c)



Ventral view of apex of left mandible

Figure 1

2.3.3 The external structure of the maxillae

Like the mandibles the most prominent feature of the maxillae is the number of structural hairs that they carry (see Plate 5.2). However, while it was possible to make inferences about the role of some of the mandibular brushes, particularly as the relationship between the mandibles and the fans was relatively clear, this was not the case with the the maxillae. Like the mandible there is a concentration of brushes on the oral surface of the maxilla (see Plates 5.3 and 6.1) suggesting interactions with other mouthparts. Unlike those of the mandible, however, the five brushes are quite uniform in appearance (Figures 2a and b).

The medial (mb), apical (ab), dorsal (db) and large oral (lob) brushes are all composed of fine, smooth, hairs although the first three also have some compound hairs. This does not suggest any particular specialisation among the brushes. The structure of the small oral brush (sob) differs in that it is composed of compound frond-like hairs (see Plate 6.4). All the brushes are carried on the lobe of the maxilla (ml in Plates 5.3 and 6.3) whereas the maxillary palp (mp), to the right in Plate 5.3, carries a number of sense organs. There are also a number of probable chemoreceptors on the lobe of the maxilla but, whereas the maxilla appears to have more sense organs than the mandible, the number is still quite small. It is likely that the medial and apical brushes are concerned with cleaning either the mandibles or the labrum (la).

The function of the lobed area (lob) on the oral surface of the maxilla is unknown (see Plate 6.1 and 6.4) although it could be concerned with the manipulation of particles. Plate 6.4 shows the

position of the single uniporous peg sensillum in the lobed area, adjacent to the small oral brush. None of the other lobes seem to have any neural connections (see section 2.4.3). The large oral brush (lob) on the aboral surface may be concerned with silk manipulation or grazing since it probably only comes into contact with the substratum and not other mouthparts.

As well as there being a concentration of structural brushes on the oral surface of the lobe there is also a concentration of trichoid sensilla (ts) on the medio-oral surface of the palpal article (see Plate 5.3, with the oral surface of the maxilla towards the camera) further suggesting interactions between the maxilla and other mouthparts. Apart from this group of sense organs, which could be sensitive to either mechanical or chemical stimulation, most of the sense organs of the maxilla are probably chemoreceptors. Like the brushes of the mandible those of the maxilla have few sensilla amongst the structural hairs. The chemoreceptors of the palp, shown in Plates 7.1 and 7.3, are similar to those described by Craig and Borkent (1980) for the tribe Simuliini. Behavioural observations suggest that they are probably contact chemoreceptors that sense the substratum (personal observation) frequently making contact with the substratum when the larvae are spinning new silk pads; they seem less likely to be responsive to water quality or the presence of food. There is no suggestion that this group of sense organs regularly comes into contact with trapped food particles.

The aboral surface of the maxillary lobe carries a group of six sensilla; there is a single trichoid sensillum (ts) with the remainder resembling the uniporous peg sensillum (ups) described by Craig and Batz (1982) in association with the antenna. The group is

shown in Plate 6.2, lateral to the large oral brush, while Plate 7.4 shows one of the uniporous sensilla in detail. On the medial surface of the lobe are three more sensilla. The paired sensilla, one basiconic (bs) and one trichoid (ts), can be seen in Plate 6.3 and in more detail in Plate 7.2. The function of these sense organs is unknown although their external structure suggests that they are contact chemorecptors and mechanoreceptors. The sensilla of the maxilla and mandibles are listed in Table 3.

Plate 5 The external structure of the maxilla.

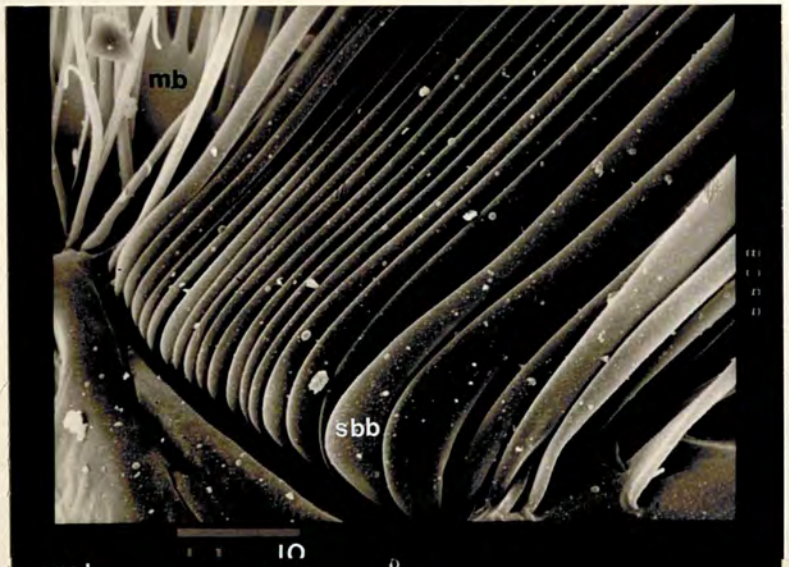
5.1 The structure of the small basal brush.

5.2 Medial view of the head capsule showing mandibles fully adducted.

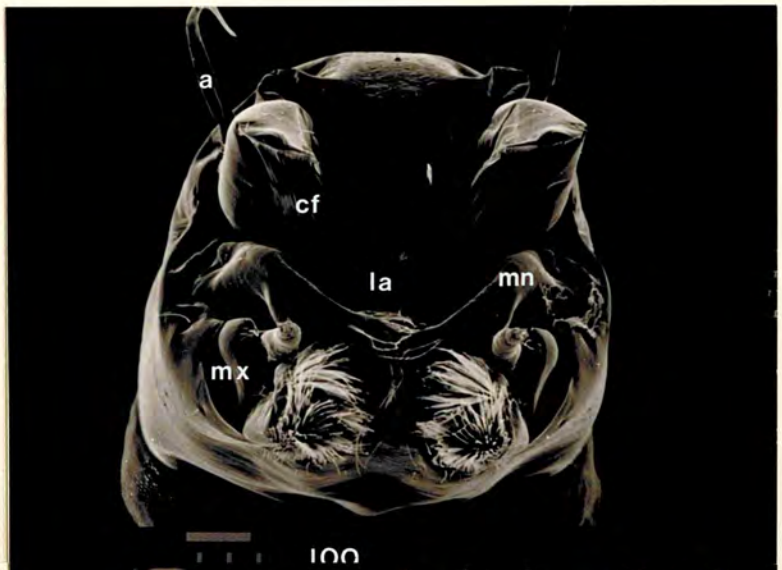
5.3 The oral surface of the right maxilla.

5.4 The aboral surface of the right maxilla.

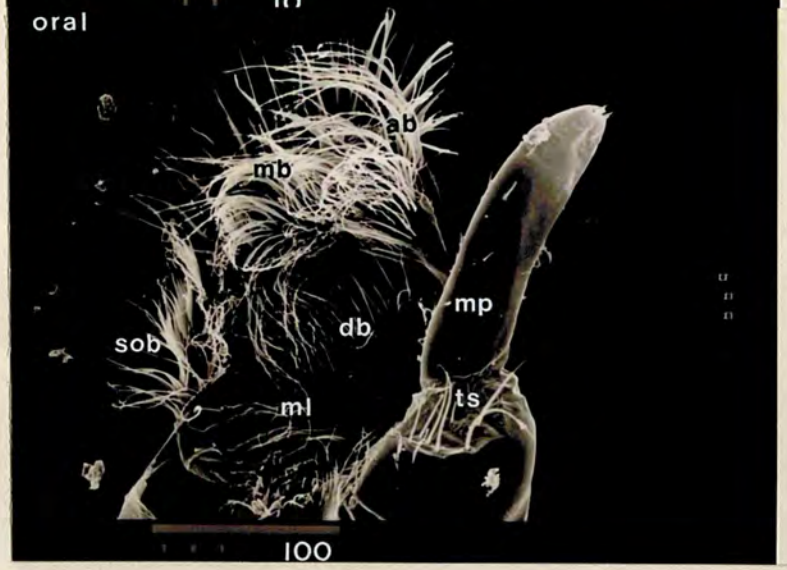
5.1


mb
sbb
10

5.2


a
cf
la
mn
mx
100

5.3


oral
ab
mb
mp
db
sob
ml
ts
100

5.4

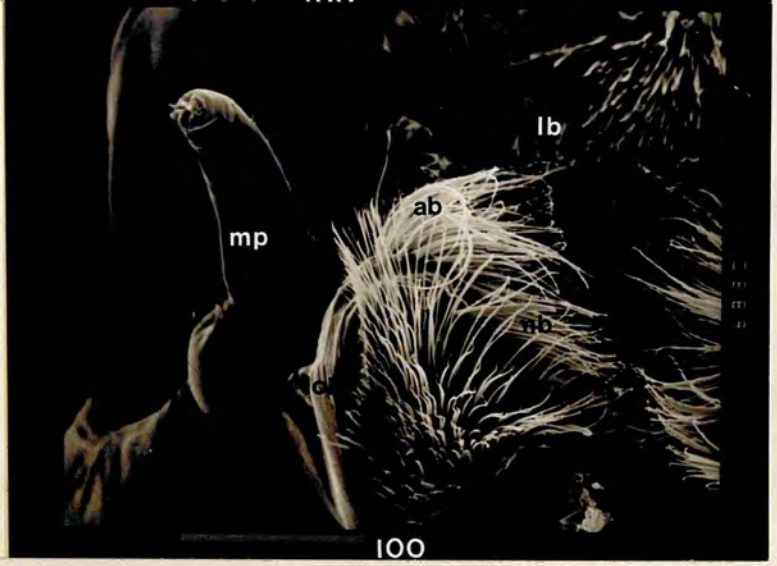

lb
ab
mp
mb
100

Plate 6 The external structure of the maxilla.

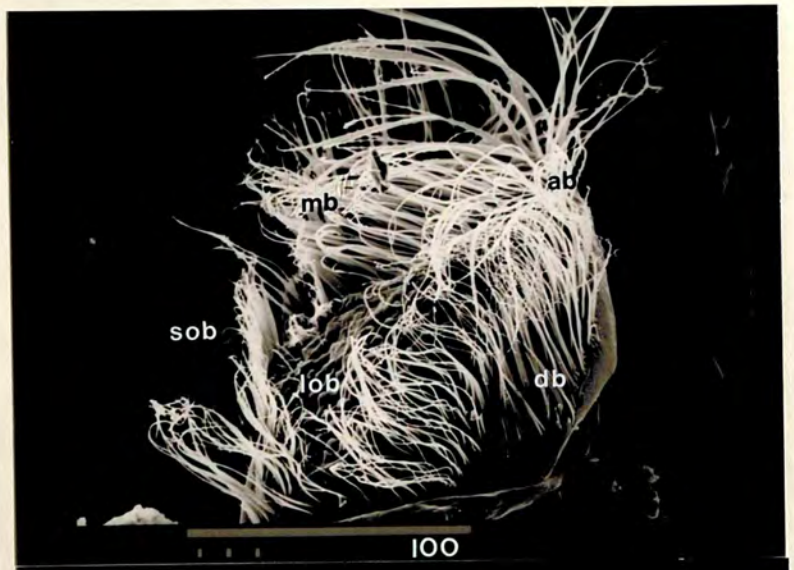
6.1 The structural brushes of the maxilla: oral surface.

6.2 The structure of the large oral brush.

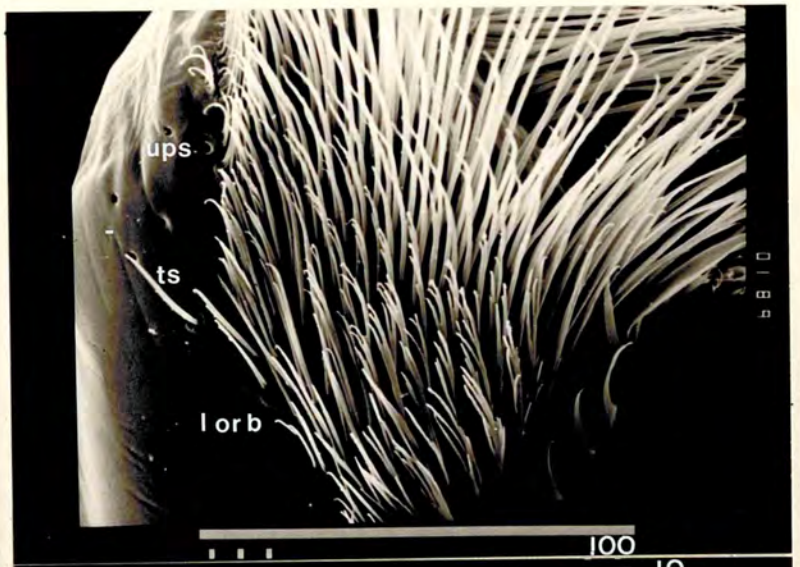
6.3 The medial surface of the maxilla.

6.4 The structure of the small oral brush and lobed area.

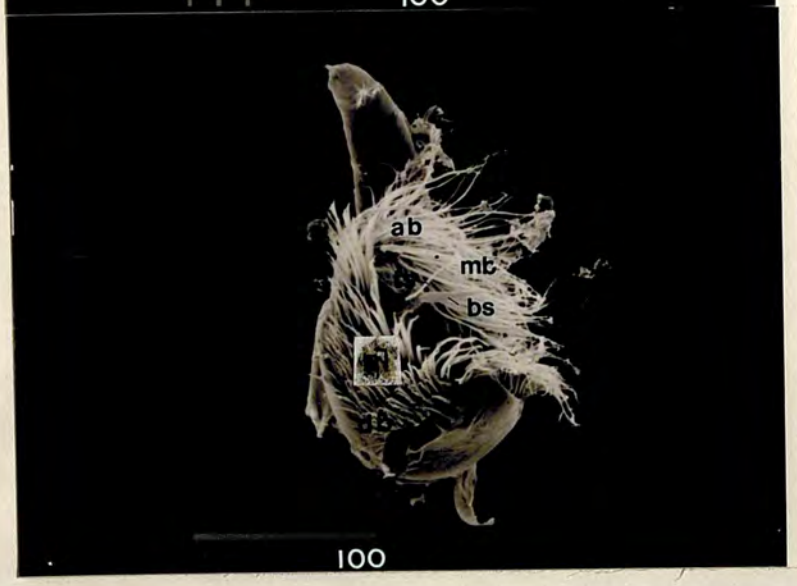
6.1



6.2



6.3



6.4

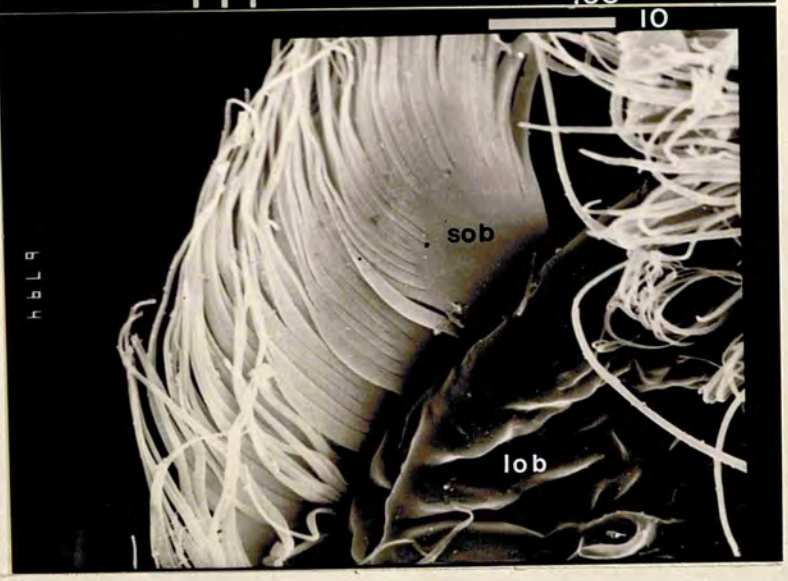


Plate 7 The external structure of the maxilla

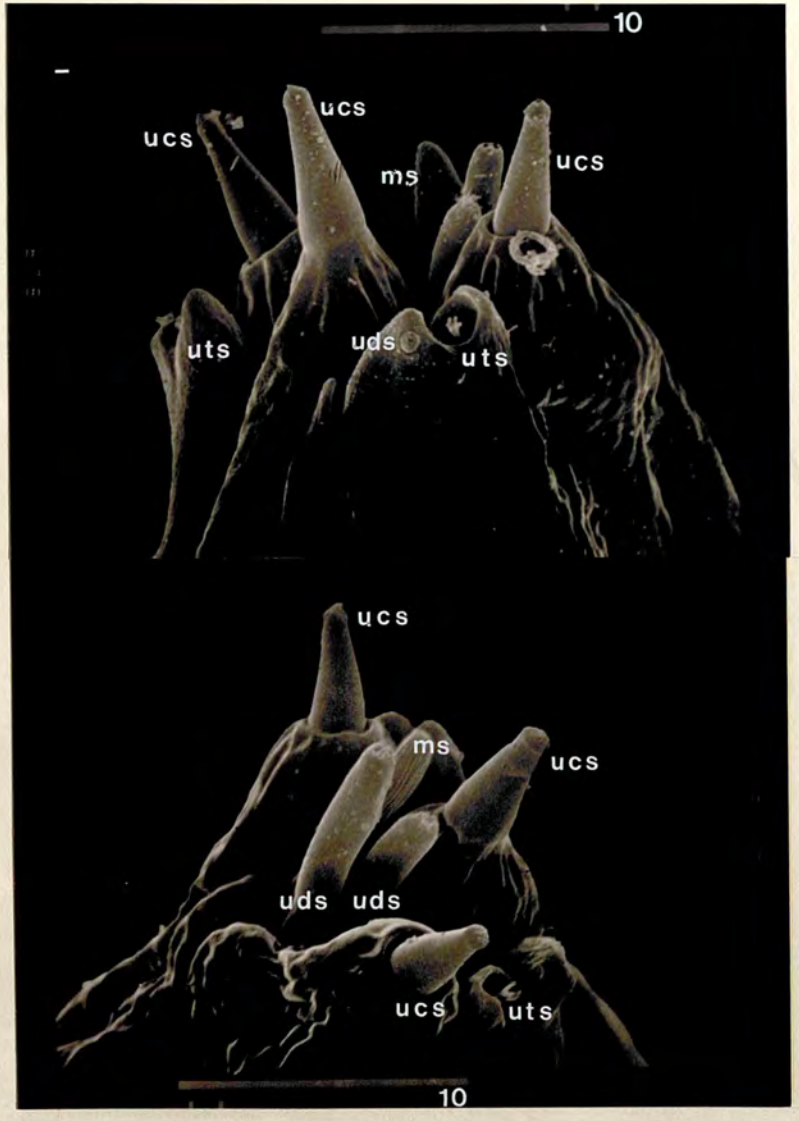
7.1 The tip of the maxillary palp: oral view

7.2 Medial sensilla of the maxillary lobe.

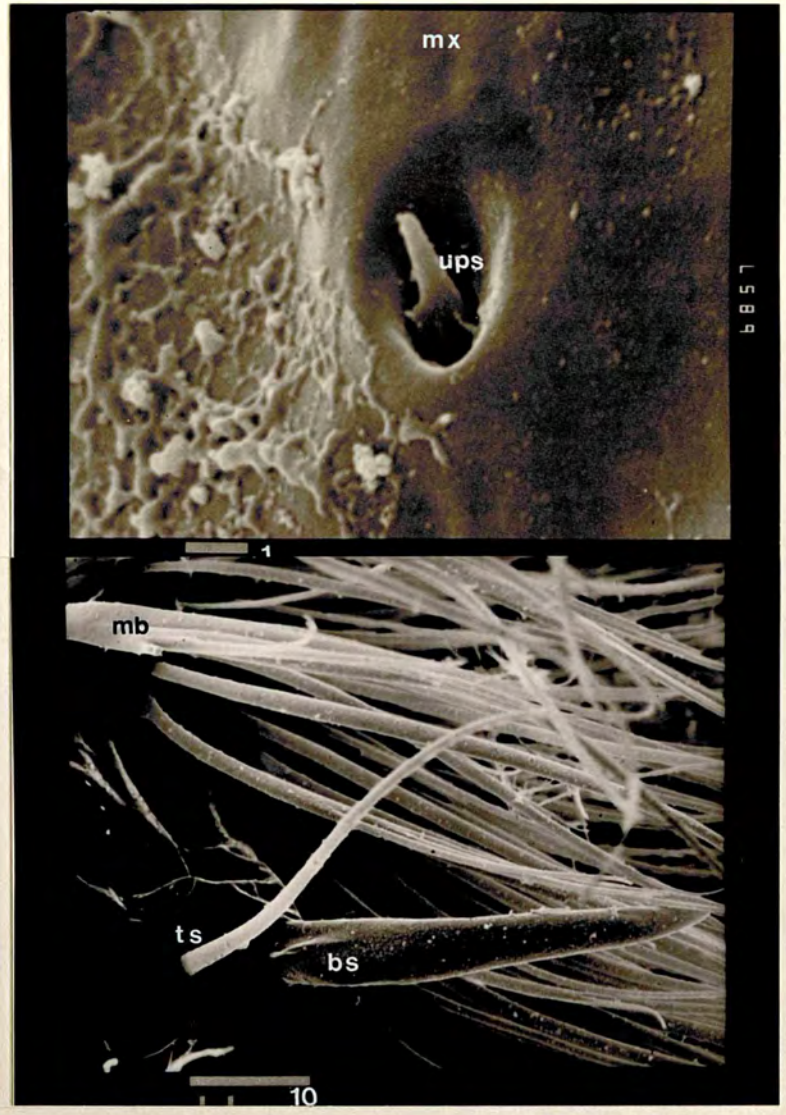
7.3 The tip of the maxillary palp: aboral view.

7.4 Uniporous sensillum on the aboral surface of the maxillary lobe.

7.1



7.2



7.3

7.4

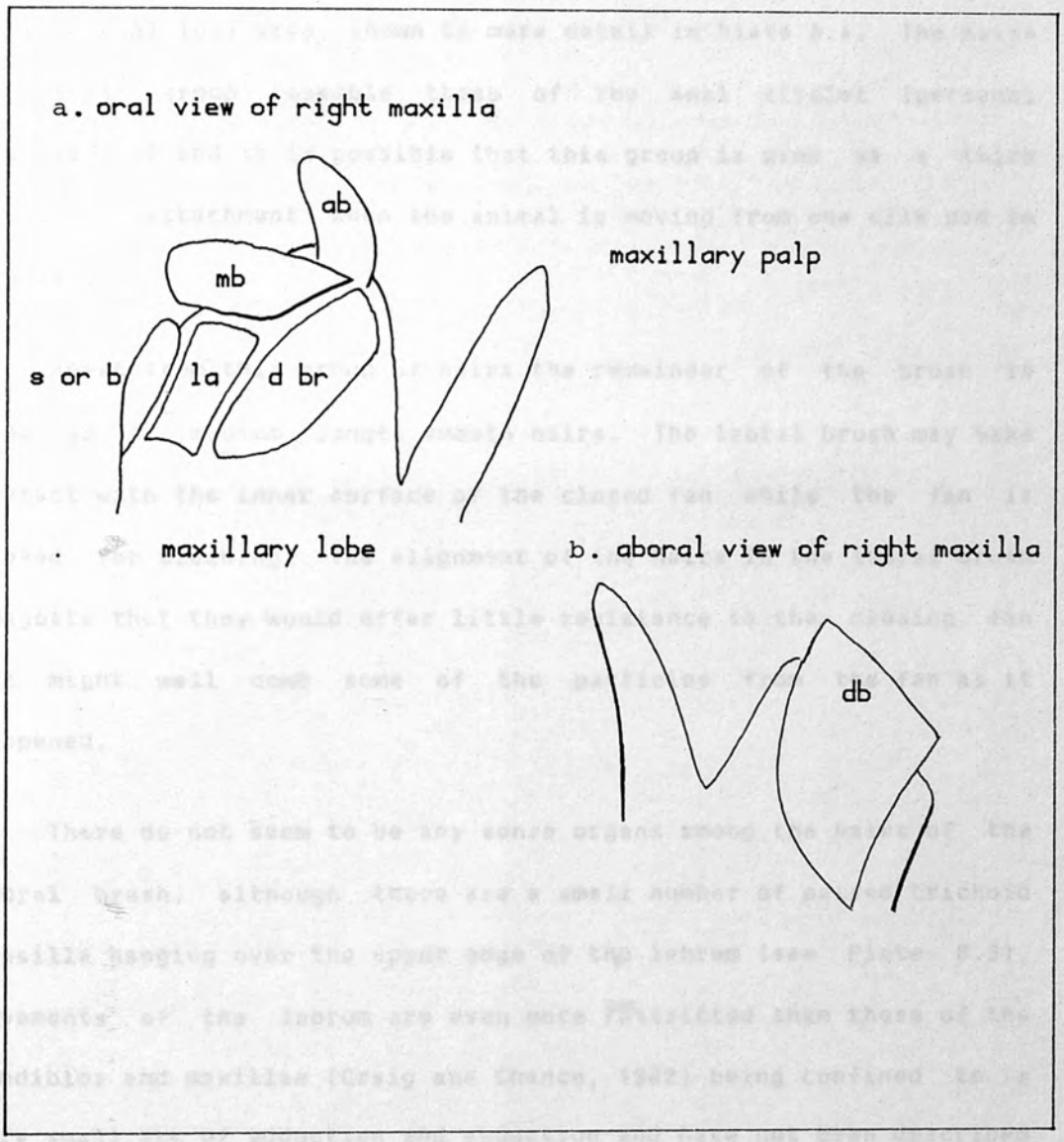


Figure 2 The distribution of brushes on the maxilla. a) oral view of right maxilla. b) aboral view of right maxilla.

2.3.4 The external structure of the labrum

The labrum of S. ornatum closely resembles that of S. vittatum described by Chance (1970), its surface being entirely concealed by the hairs of the labral brush (1a b). Plate 8.1 shows a general view of the upper half of the labrum, with both mandibles fully abducted, whilst Plate 8.2 shows the lower half. The hairs comprising the brush are all of the same type with the exception of those of the central oval (co) area, shown in more detail in Plate 8.4. The hairs of this group resemble those of the anal circlet (personal observation) and it is possible that this group is used as a third point of attachment when the animal is moving from one silk pad to another.

Apart from this group of hairs the remainder of the brush is made up of medium length smooth hairs. The labral brush may make contact with the inner surface of the closed fan while the fan is closed for cleaning. The alignment of the hairs in the labral brush suggests that they would offer little resistance to the closing fan but might well comb some of the particles from the fan as it reopened.

There do not seem to be any sense organs among the hairs of the labral brush, although there are a small number of paired trichoid sensilla hanging over the upper edge of the labrum (see Plate 8.3). Movements of the labrum are even more restricted than those of the mandibles and maxillae (Craig and Chance, 1982) being confined to a very small arc of adduction and abduction and have not been described further here.

Plate 8 The external structure of the labrum.

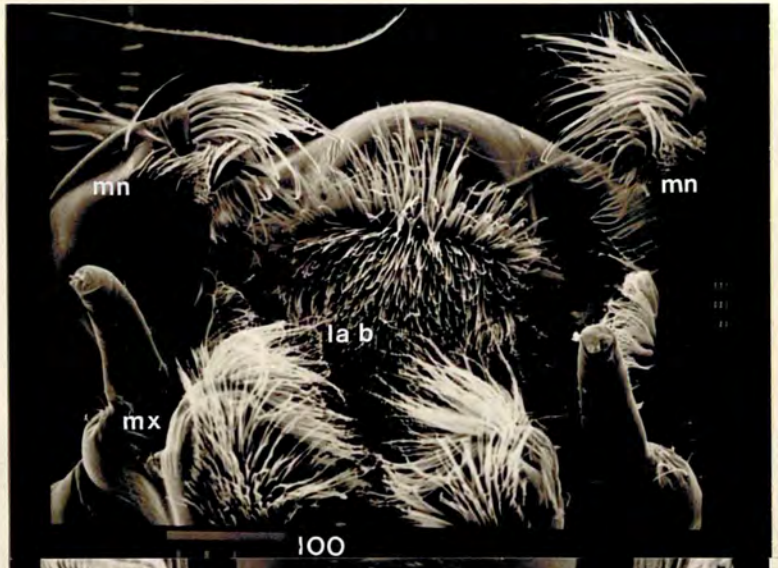
8.1 A general view of the upper half of the labral brush.

8.2 A general view of the lower half of the labral brush.

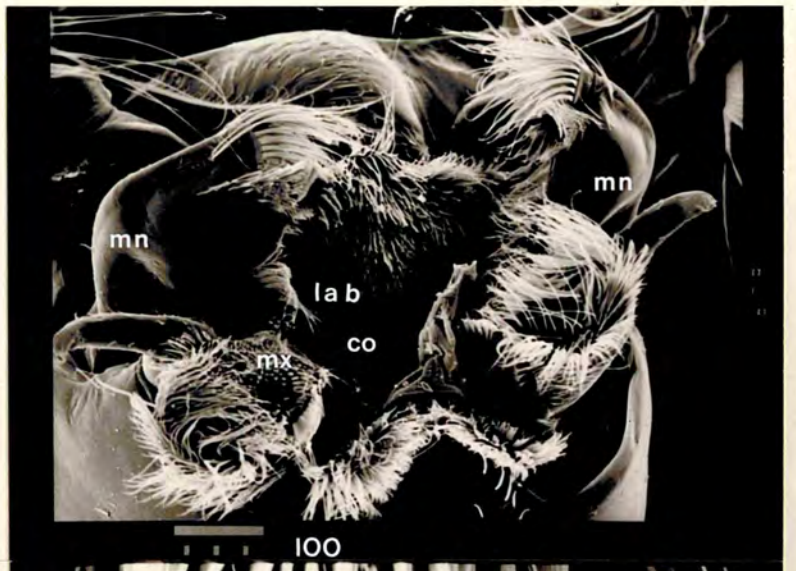
8.3 Trichoid sensilla at the dorsal edge of the labrum.

8.4 The central oval brush of the labrum.

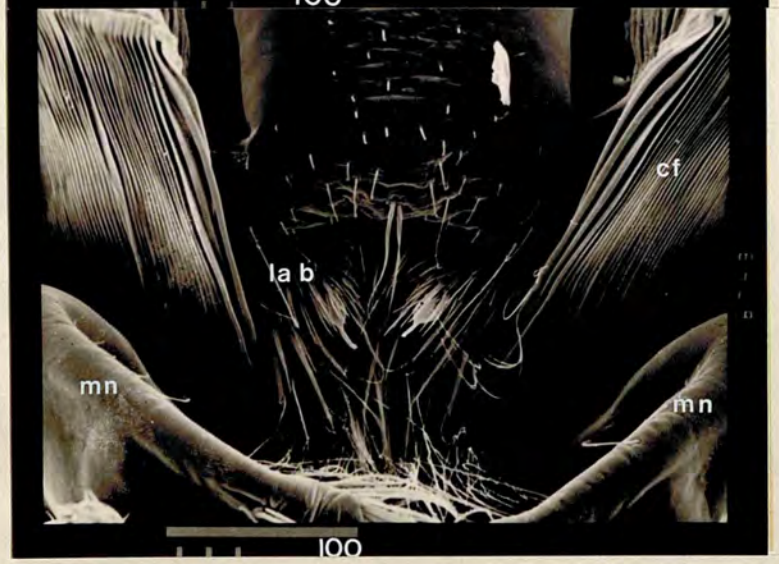
8.1



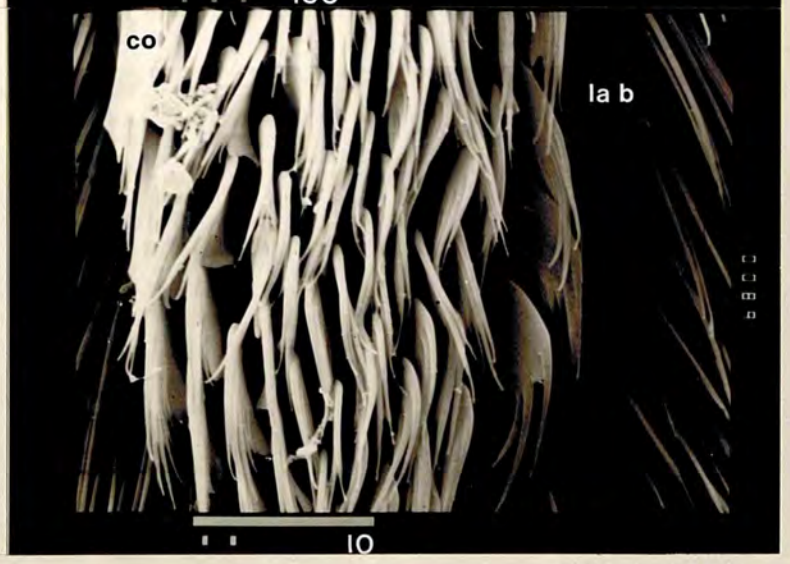
8.2



8.3



8.4



2.3.5 The external structure of the labio-hypopharyngeal complex, hypostomium and antennae

The ventral surface of the head capsule is bounded by the least conspicuous group of appendages, those of labio-hypopharyngeal complex (l. hy. com), that can only be seen clearly in dissected specimens. In Plate 1.1, for example, it is possible only to see the tips of the labial brushes (lab b) beneath the maxillae and above the hypostomium. Part of the complex, dissected from the head capsule and viewed from above, is shown in Plate 9.2.

Plate 9.2 shows that the principal groups of brushes ventro-laterally in the complex are the labial brushes which are made up of fine, smooth hairs. Chance (1970) states that there is a pair of brushes medial to these although they cannot be distinguished on the micrographs of S. ornatum. The stouter, hypostomial bristles (hb) along the margin of the hypopharynx and labium were not named by Chance. Antero-ventral to the labial brushes is the hypostomium (see Plate 11.1); some of its teeth are innervated (Chance, 1970). The function of all of these structures is unknown although it has been suggested that the hypostomium may be used to scrape algae from the substratum when larvae are grazing (Chance, 1970).

A group of uniporous basiconic sensilla are found on each basal lobe (bl). Plate 9.1, which shows a lateral view of the left basal lobe in which two of the four sensilla are prominent, shows that they are very close to the point at which silk is extruded, suggesting that they could be involved in controlling its use (the mass of cellular material above the lobes in this figure is fixed silk).

There do not appear to be any trichoid sensilla associated with the labio-hypopharyngeal complex, although the hypostomium (hy) does have a number of sensilla of this type (see Plate 11.1).

Further back in the hypopharynx (not shown on the dissected specimens) are at least five rows of very fine setae (hy s), shown in Plates 9.3 and 9.4. The function of these is unknown and they have not been reported previously. The small distance between each hair suggests that they might be able to function as filters, further contributing to the ability of the mouthparts to trap fine particles.

The antennae of S. ornatum and S. lineatum are characteristically simple and carry only a small number of sense organs. Previous reports of antennal structure in S. vittatum have been reviewed in Section 1.6 and observations made here did not show any external differences in structure compared to that species. The position and size of the antenna (a) can be seen in Plate 10.1. At its tip is a single cone-shaped sensillum, also known as a sensory cone (sc), (see Plate 10.2) and at the distal end of the third segment there is a pair of multiporous sensilla, shown in Plate 10.4. The sensilla described by Craig and Batz (1982) at the base of the antenna may be seen in Plate 11.2. Here the 'bacteria-covered' multiporous sensillum as well as a number of trichoid sensilla that they described, may be seen on S. ornatum. There is no evidence that the hairs on the cephalic fan stem, which are visible in this plate and appear to be articulated, are innervated.

Plate 9 The external structure of the labio-hypopharyngeal complex.

9.1 A medial view of the sensilla of the basal lobes.

9.2 Dorsal view of the anterior edge of the labio-

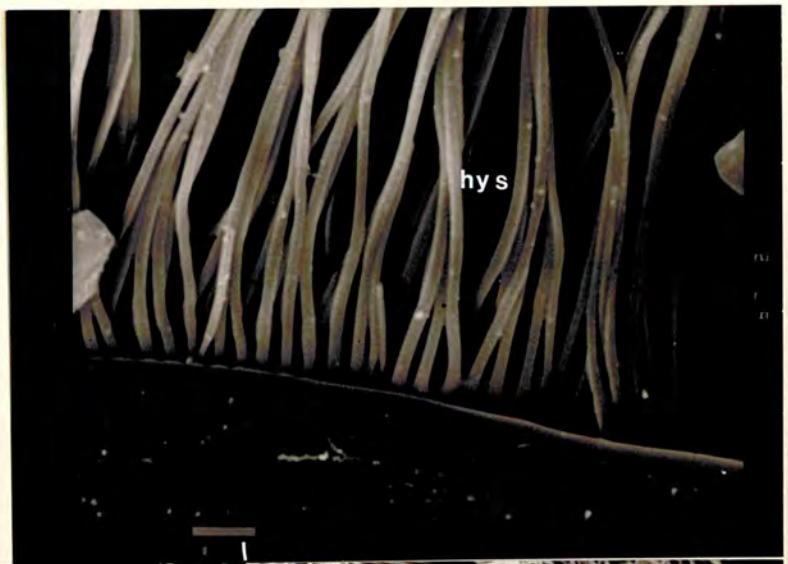
hypopharyngeal complex

9.3 General view of the groups of hypopharyngeal setae.

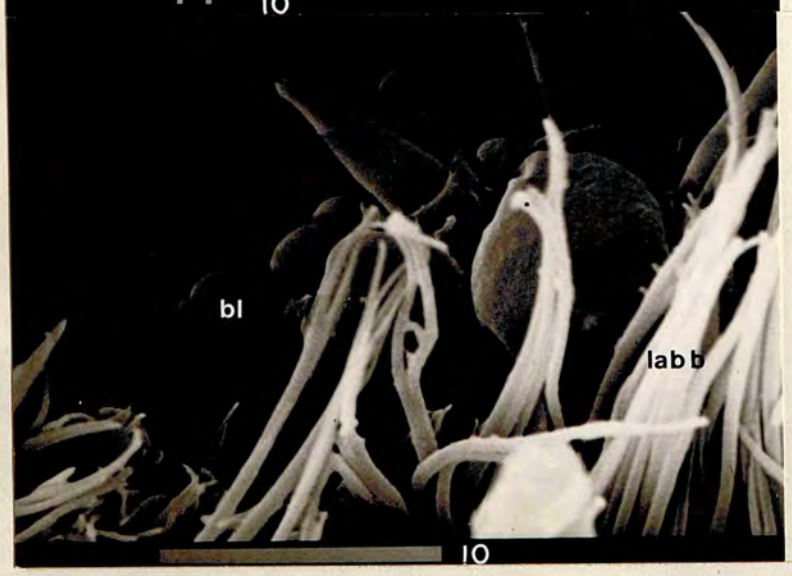
9.4 Detail of hypophayngeal setae.

9.3

9.4



9.1



9.2

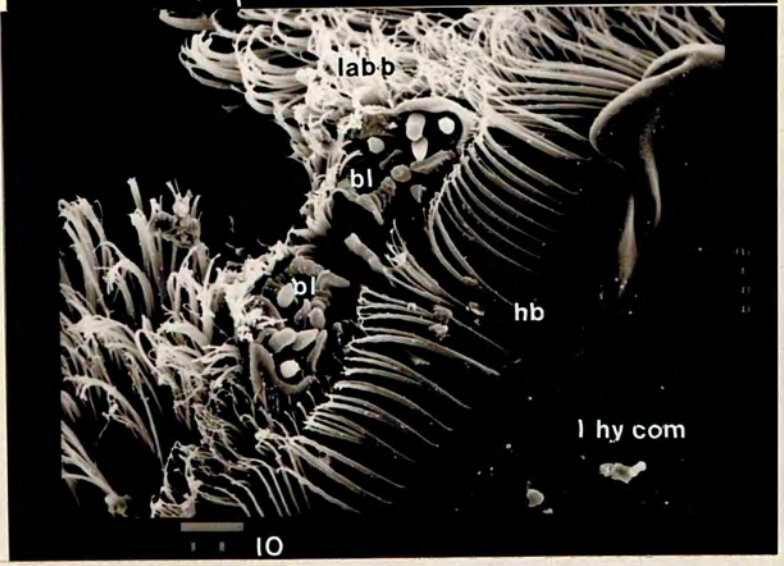


Plate 10. The external structure of the antenna.

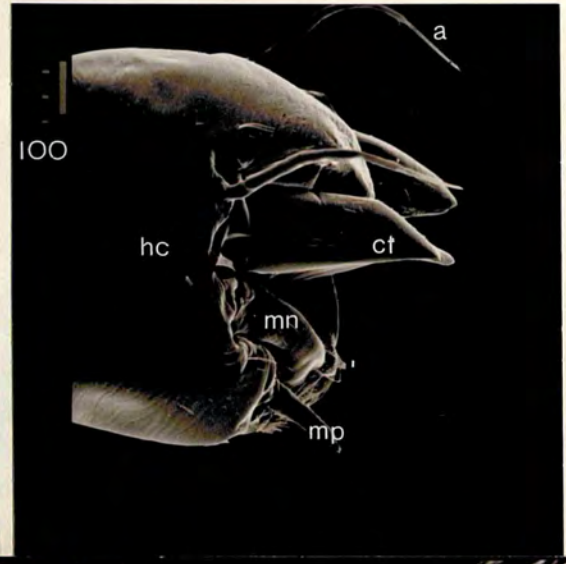
10.1 A lateral view of the head capsule showing antennae, cephalic fans and mouthparts.

10.2 The tip of the antenna.

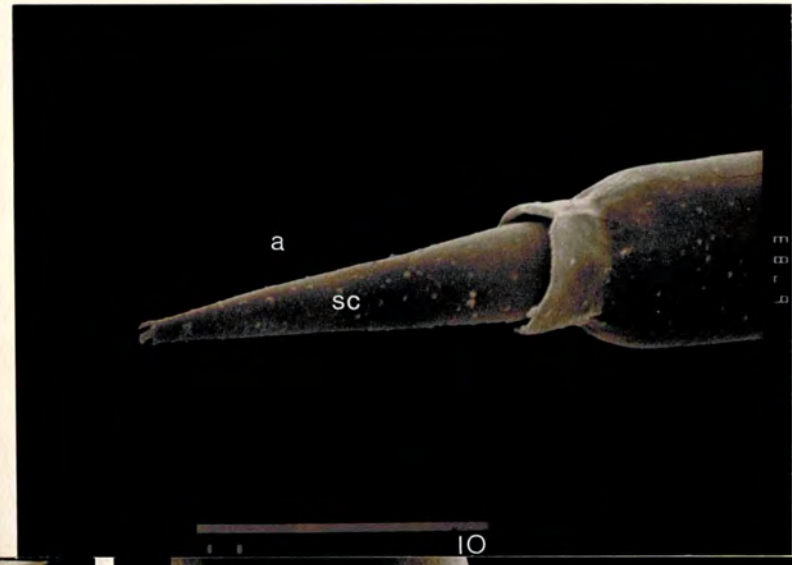
10.3 Medial view of the labio-hypopharyngeal complex.

10.4 Multi-porous sensilla on the 4th segment of the antenna.

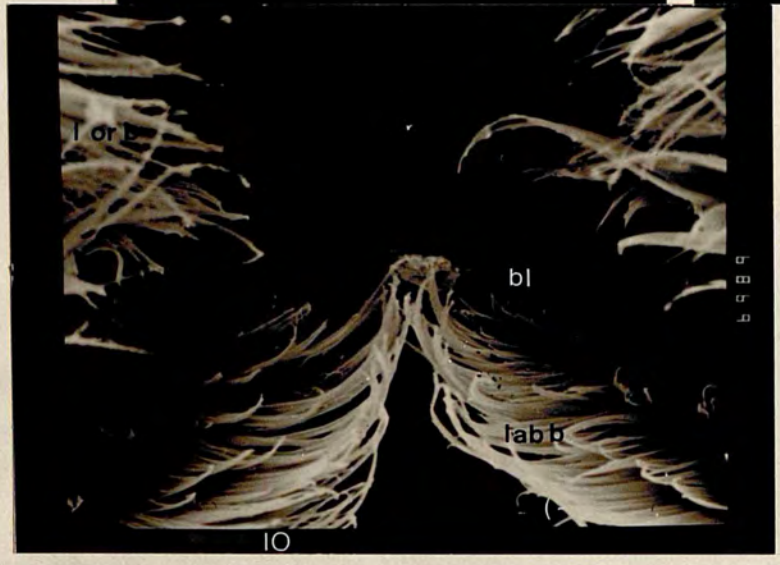
10.1

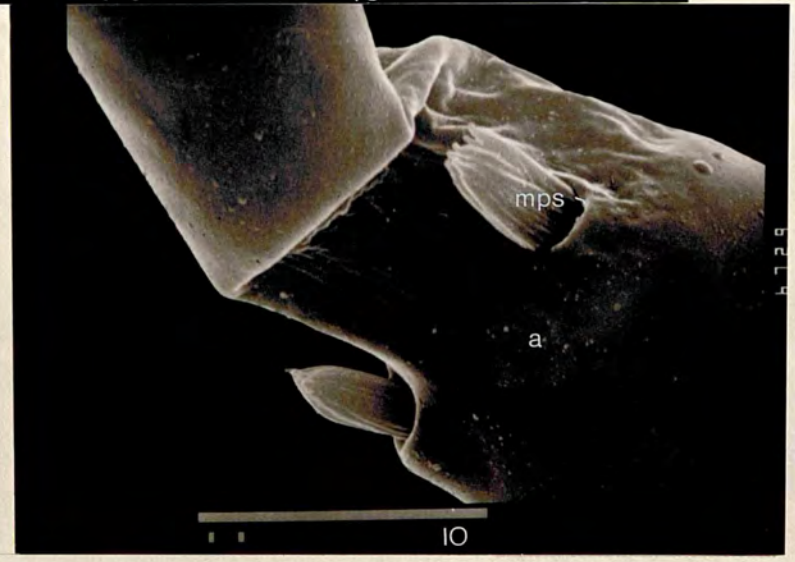


10.2



10.3





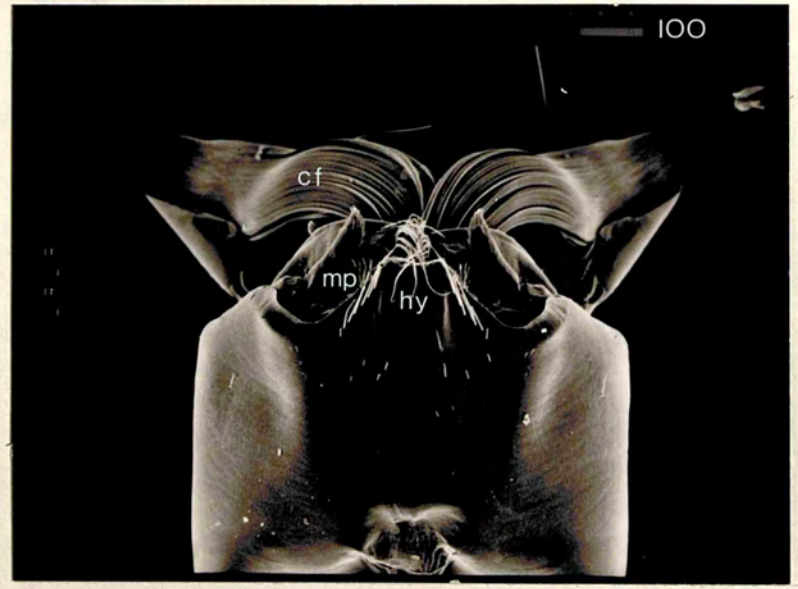
10.3

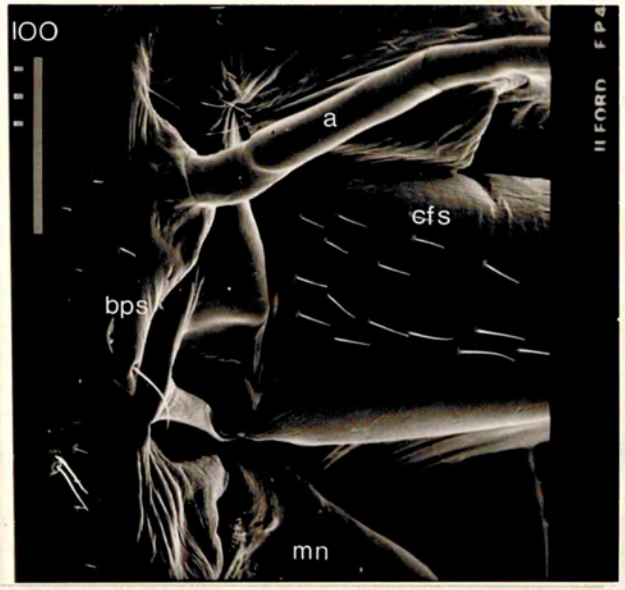
Plate 11. The distribution of sensilla on the hypostomium and base of the cephalic fan stem.

11.1 Ventral view of the head capsule showing trichoid sensilla on the hypostomium.

11.2 Sensilla the base of the cephalic fan and antenna.

11.1





11.2

2.4.1 Methylene blue staining of the sensory nervous system of the head capsule and its appendages: results

The results of staining approximately seventy heads intra-vitally with methylene blue are summarised in Figures 3 to 8. The figures show the positions of the major sensory nerves and some of the more detailed aspects of sensory innervation. Table 2 lists the abbreviations used in the figures. As a result of the small size and translucency, when cleared, of the head capsule some of the staining produced very clear results.

The structures that have the external appearance of sense organs and have a neural connection as shown by methylene blue staining are listed in Table 3, although the table does not include the groups of small sensilla (possibly mechanoreceptors) distributed generally over the surface of the head capsule. Some structures that appear to be sensilla, but which were not stained, are also listed in paraentheses.

2.4.2 The sensory innervation of the ventral surface of the head capsule

Figure 3a shows the main sensory nerves leading to the sub-oesophageal ganglion (SOG), which receives sensory nerves from the maxillary palp and lobe, the mandibles and the labio-hypopharyngeal complex, as well as a number of minor inputs. The positions of the mandibular nerve (MN), maxillary lobe and palpal nerves (MLN and MPN) and basal lobe nerves I and II (BLN I and II) are shown before their terminal ramifications in Figure 3b, an enlargement of 3a. Figures 4a and b show more detailed illustrations

of the sensory nerves associated with the basal lobes (4a) and with the ventral surface of the head capsule and the hypostomium (4b). There are two branches of the basal lobe nerve which join shortly before the nerve enters the ganglion (see Figure 3b). Basal lobe nerve II appears to lead from the most medial sensilla on the lobe, while the remaining three sensilla in each lobe all appear to be innervated by basal lobe nerve I. The presence of more bipolar cells connected to these sensilla than the number of sensilla themselves further suggests that they are concerned with chemoreception.

Figure 4b shows the distribution of sensory cell bodies in the ventral body wall in the region below the sub-oesophageal ganglion, showing how these are linked to the basal lobe nerve by a prominent tract (NL) in the dorso-ventral plane. The axons leading away at the top right of the figure probably innervate the lateral trichoid sensilla of the hypostomium which can be seen in Plate 11.1. That the staining has been taken up by most cell bodies in this superficial group is suggested by Figures 5a and 5b which show the same area as Figure 4b but on two different specimens in which the apparent density of bipolar cell bodies is similar. Also shown in Figure 5a is a group of cell bodies characteristically linked to the maxillary palpal nerve.

TABLE 2 Abbreviations used on diagrams showing methylene blue preparations.

Abbreviation	Structure
A	Antenna
AN	Apical nerve
ANE	Antennal nerve
ATS	Apical trichoid sensilla
BL	Basal lob
BLN I	Basal lobe nerve (I)
BLN II	Basal lobe nerve (II)
CF	Cephalic fan
FG	Frontal group (of cell bodies)
FN I	Frontal nerve (I)
FN II	Frontal nerve (II
GS	Galeal sensilla
HT	Hypostomial teeth
M	Mandible
MMN	Mandibular and maxillary nerves
MLN	Maxillary lobe nerve
MP	Maxillary palp
MPN I	Maxillary palp nerve (I)
MPN II	Maxillary palp nerve (II)
NL	Neural link
P	Pharynx
PA	Pigmented area
PAN	Pre-apical nerve
PON	Post-apical nerve
SC	Superficial cell
SOG	Sub-oesophageal ganglion
SOGA	Supra-oesophageal ganglion

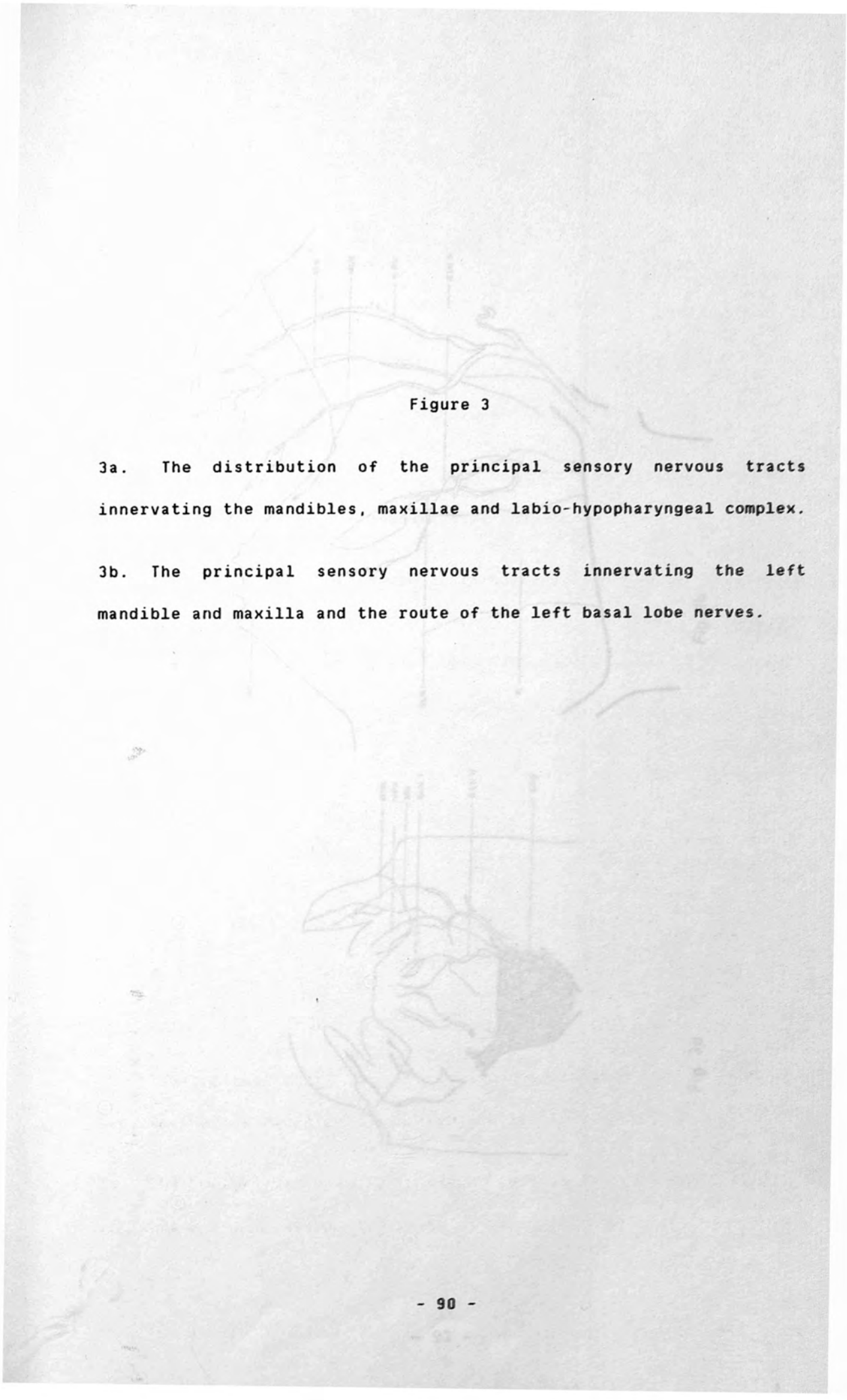


Figure 3

3a. The distribution of the principal sensory nervous tracts innervating the mandibles, maxillae and labio-hypopharyngeal complex.

3b. The principal sensory nervous tracts innervating the left mandible and maxilla and the route of the left basal lobe nerves.

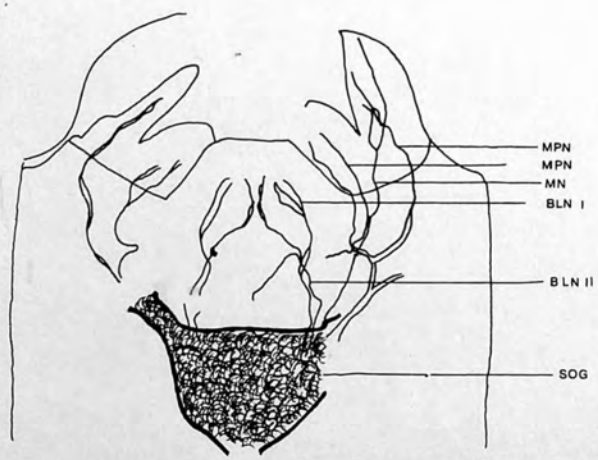


Fig. 3a

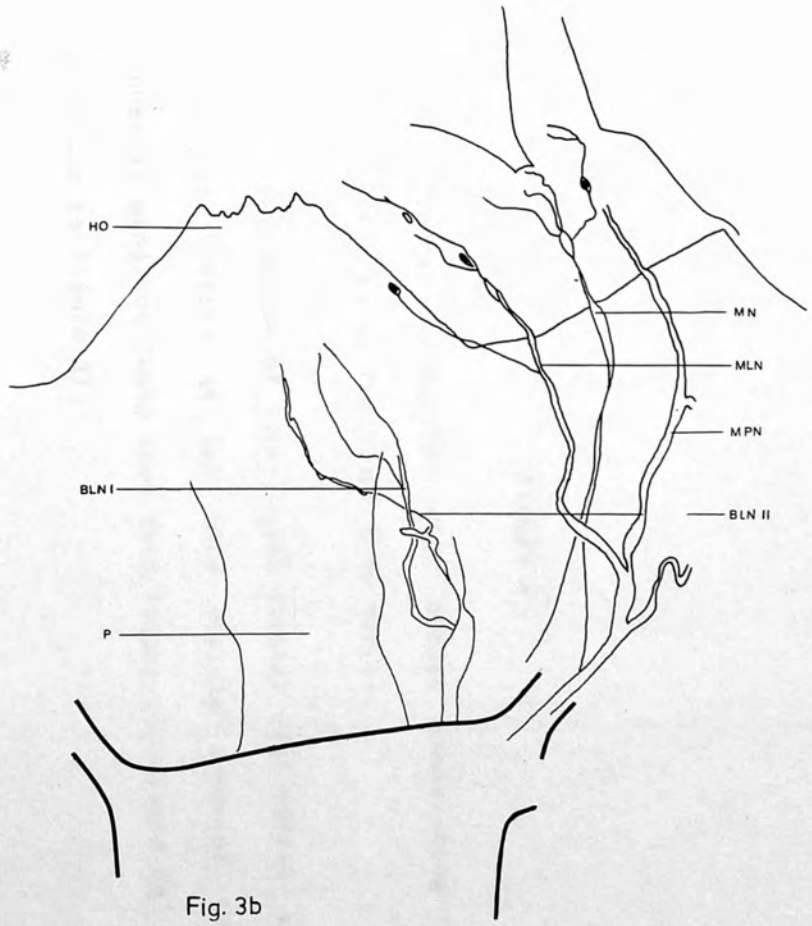


Fig. 3b

Figure 4

4a The distribution of bipolar cell bodies innervating the basal lobes in the tracts of the basal lobe nerves.

4b. The distribution of superficial bipolar cell bodies innervating the ventral surface of the head capsule anterior to the sub-oesophageal ganglion (note that this Figure is rotated 45° clockwise in relation to Figure 3).

Fig. 4a

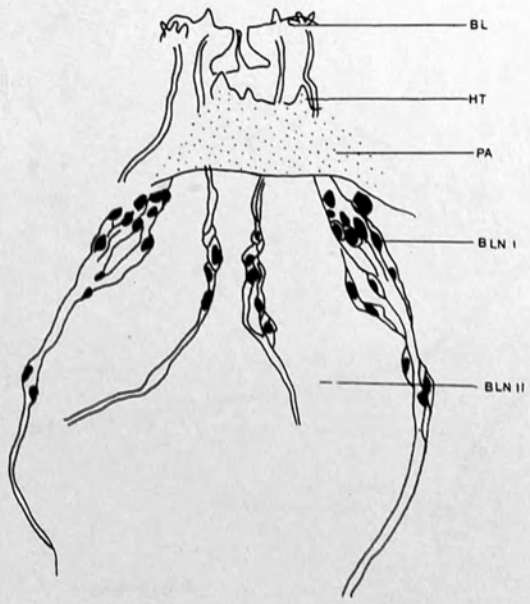


Fig. 4b

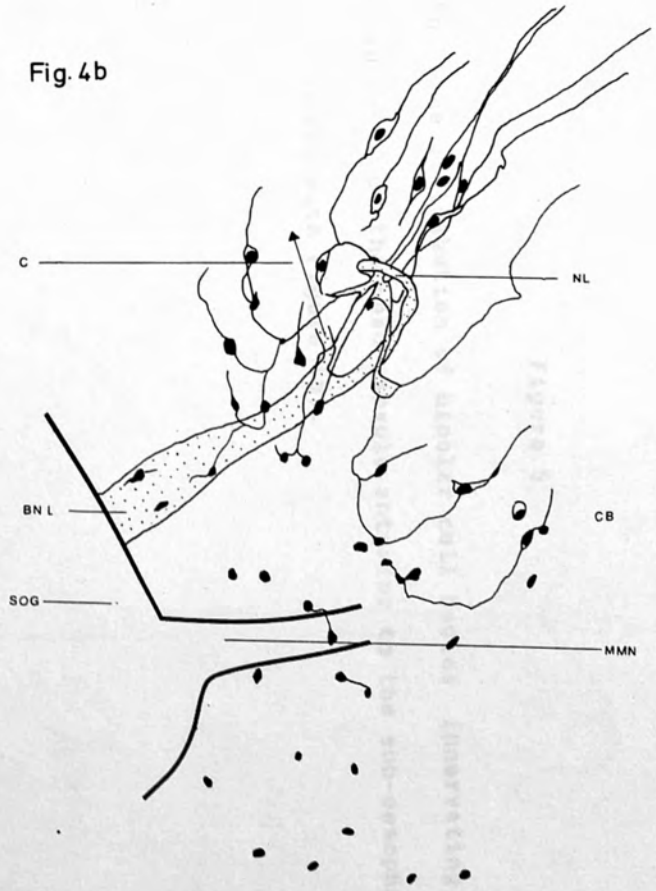


Figure 5

5a and 5b. The distribution of bipolar cell bodies innervating the ventral surface of the head capsule anterior to the sub-oesophageal ganglion (compare with Figure 4b).

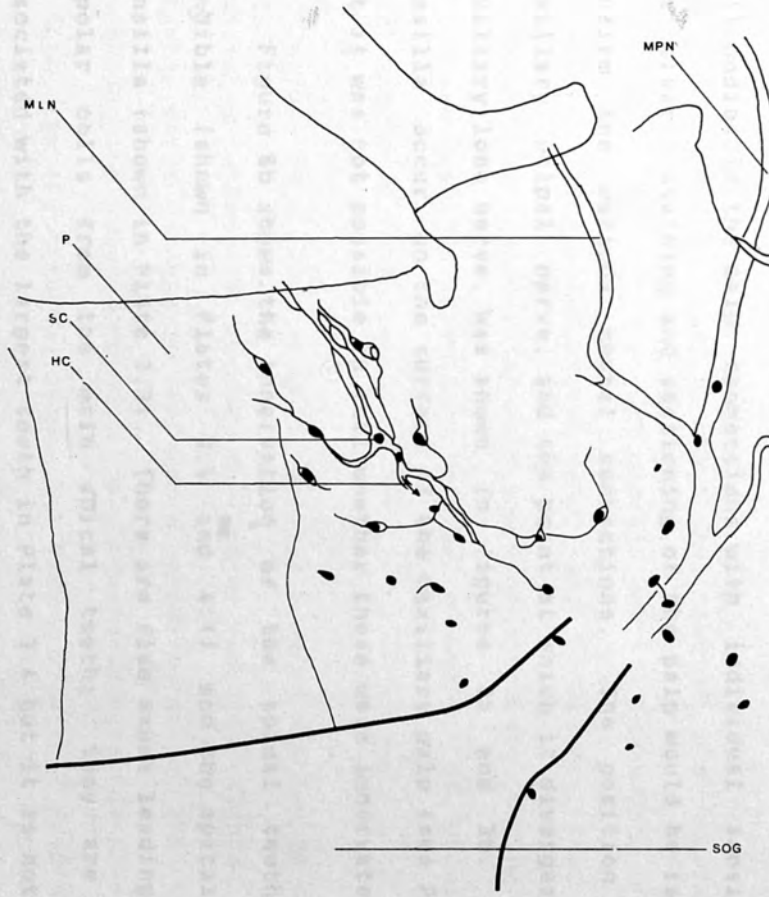


Figure 5a

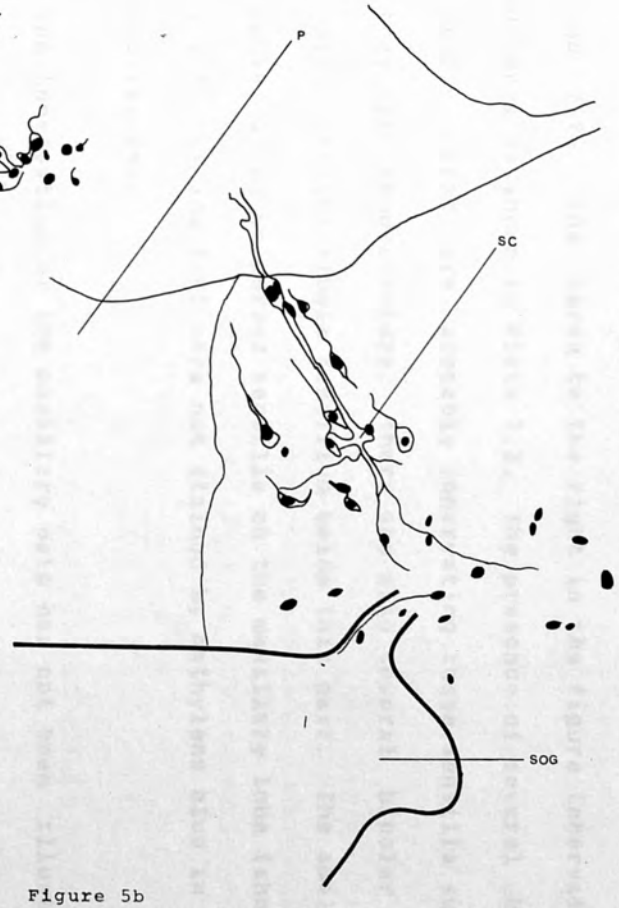


Figure 5b

2.4.3 The sensory innervation of the mandibles and maxillae

Figures 6a and 6b show the right maxilla and left mandible respectively. Figure 6a shows the nerves from the three prominent sensilla on the medial face of the maxillary lobe, shown in Plates 6.3 and 7.2. The nerve to the right in the figure innervates the paired sensilla shown in Plate 7.2. The presence of several bipolar cell bodies that are probably innervating these sensilla suggests that they are chemoreceptors. There are also several bipolar cells associated with the single sensillum below this pair. The small pegs that appear to be uniporous sensilla on the maxillary lobe (shown in Plates 6.2, 6.4 and 7.4) were not stained by methylene blue in any of the preparations.

The innervation of the maxillary palp has not been illustrated. Although methylene blue was taken up by neural tracts and bipolar cell bodies in the palp, connections with individual sensilla were not clear. Staining and sectioning of the palp would be required to confirm the various neural connections. The position of the maxillary palpal nerve, and the point at which it diverges from the maxillary lobe nerve, was shown in figures 3a and 3b. Trichoid sensilla occur on the surface of the maxillary palp (see Plate 5.3) but it was not possible to tell whether these were innervated.

Figure 6b shows the innervation of the apical teeth of the mandible (shown in Plates 3.4 and 4.1) and the apical trichoid sensilla (shown in Plate 3.3). There are five axons leading to five bipolar cells from the main apical teeth; they are probably associated with the largest teeth in Plate 3.4 but it is not possible to see the origins of the axons as the teeth are heavily sclerotised.

Several specimens were observed with a similar small number of bipolar cells in this group suggesting that the teeth may be sensitive to mechanical stimulation alone. A single axon leads to the sub-apical teeth (which were shown in Plate 3.4). The apical trichoid sensilla are innervated by a nerve derived from the mandibular nerve as it enters the mandible. Only three bipolar cells innervate this pair of sensilla suggesting that they may be mechano-receptors.

Although a small number of bipolar cells were seen in other parts of the mandible they were not observed consistently and could not be associated with any external sensilla. Consequently they have not been illustrated in this figure.

2.4.4 The sensory innervation of the antennae and dorsal surface of the head capsule

Figures 7a and b and 8a and b show some of the innervation of the dorsal surface of the head capsule and its appendages. There are no external sensory structures (nor any structures that take up methylene blue) associated with the cephalic fans, but the antennae, labrum and frons do have nerves and a number of bipolar cells associated with them.

Figure 7a shows a slightly oblique view of the two major nerves associated with the dorsal surface of the head capsule. Both the antennal nerve and the frontal nerve appear to enter the commissure connecting the sub-oesophageal and supra-oesophageal ganglia. Figure 7a shows the two antennal nerves but only one pair of frontal nerves. The frontal nerve has two distal tracts which appear to innervate the dorsal surface of the head capsule. Their position is also

illustrated in Figure 8. Figure 7b shows an enlargement of 7a; the number of bipolar cells associated with the antennal nerve is small, presumably reflecting the insignificance of the antennae as sense organs (Craig and Batz, 1982).

The group of bipolar cells innervated by frontal nerve I is considerably larger and may be associated with the numerous small articulated hairs on the frons and labrum (see Plate 9.3). However there were no specimens showing clear connections between these cell bodies and any of the hairs in this part of the head capsule. Frontal nerve II is probably associated with the large trichoid sensilla medial to the antennae (see Plate 11.2). Figures 8a and b show the same part of the sensory nervous system in dorsal view to demonstrate further the arrangement of the nerves. The dorsal surface of the head capsule has a moderate density of bipolar cell bodies in the region above the supra-oesophageal ganglion and the frontal nerves, although the main concentration is at the distal end of frontal nerve I. These are shown in Figure 8b.

A complete list of external sensilla with neural connections, p105
shown by methylene blue staining, is given in Table 3; structures with the appearance of sensilla but which did not take up methylene blue are listed in parentheses.

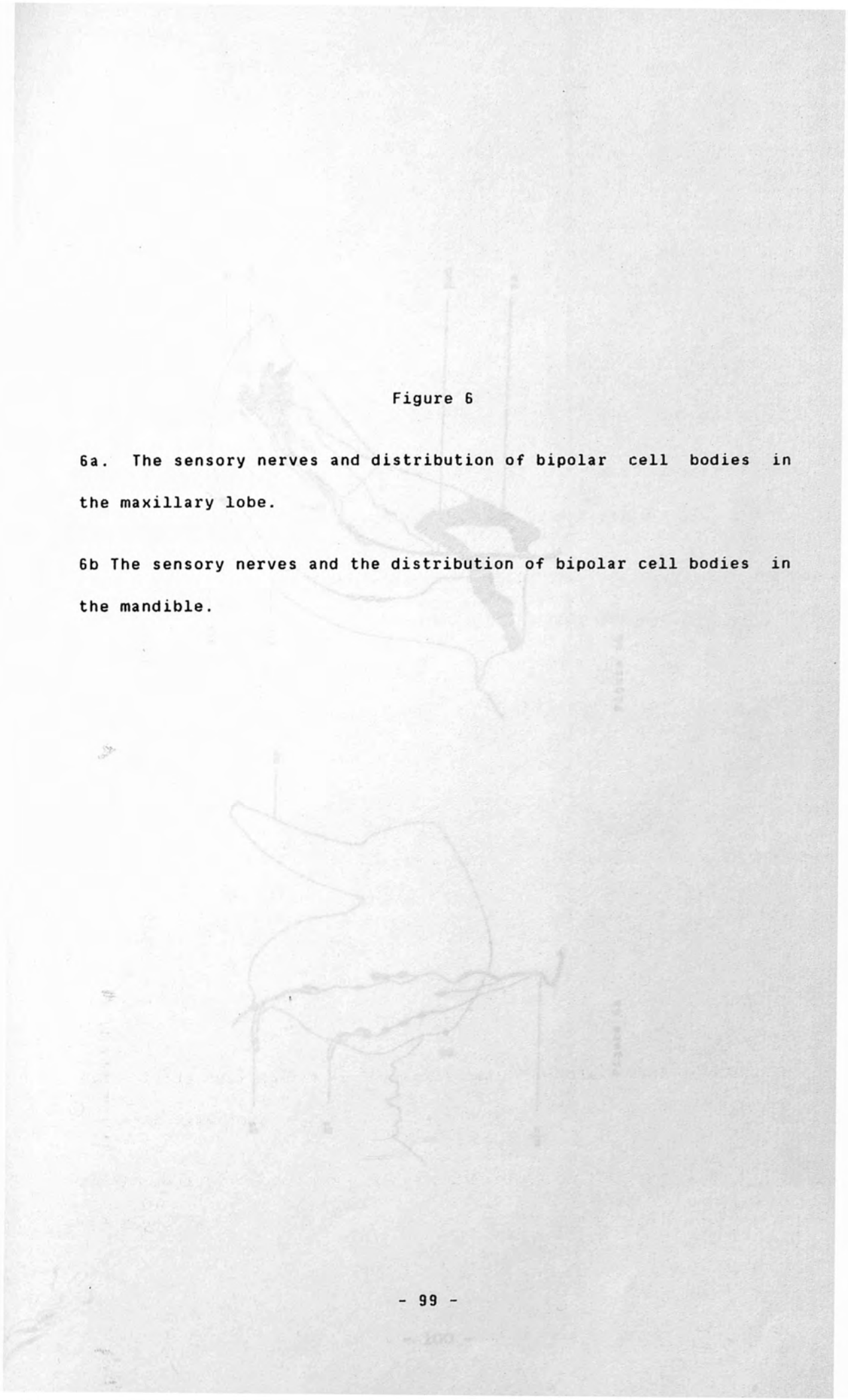


Figure 6

6a. The sensory nerves and distribution of bipolar cell bodies in the maxillary lobe.

6b The sensory nerves and the distribution of bipolar cell bodies in the mandible.

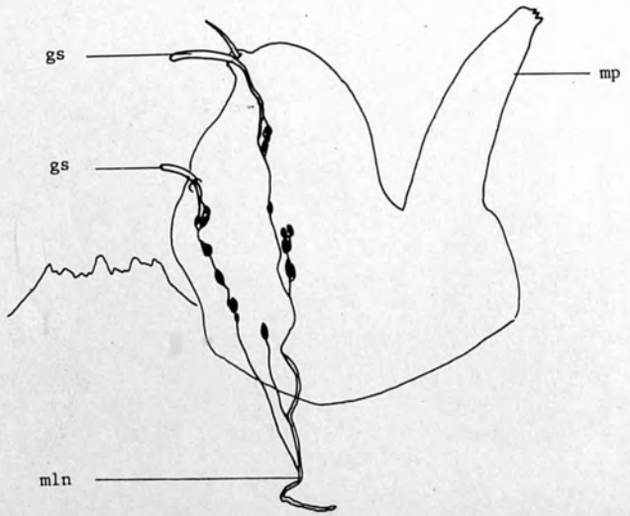


Figure 6a

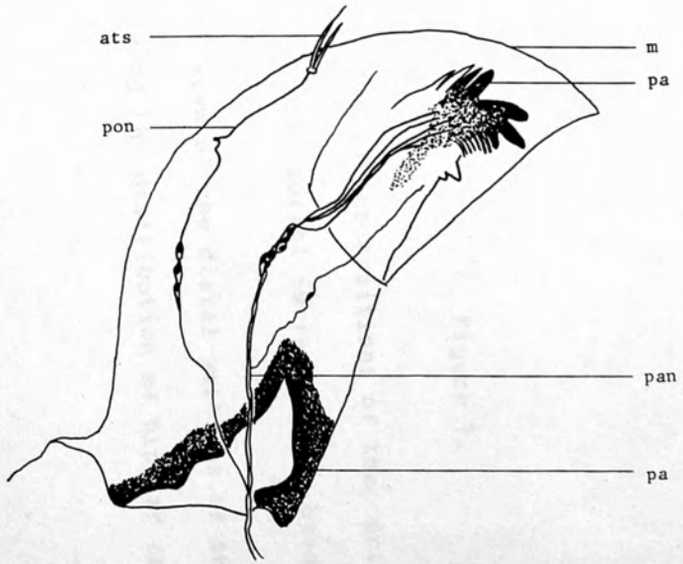


Figure 6b

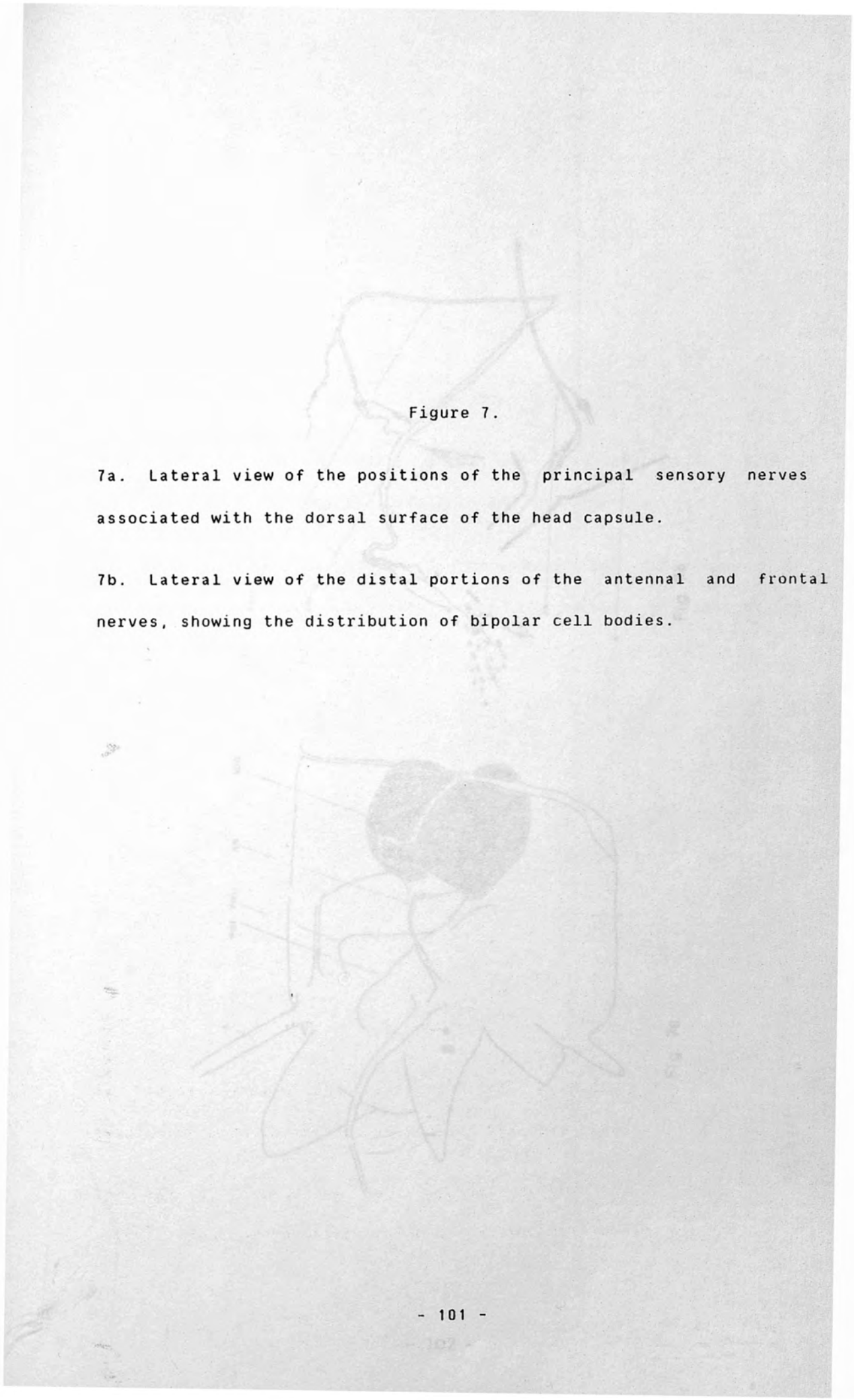


Figure 7.

7a. Lateral view of the positions of the principal sensory nerves associated with the dorsal surface of the head capsule.

7b. Lateral view of the distal portions of the antennal and frontal nerves, showing the distribution of bipolar cell bodies.

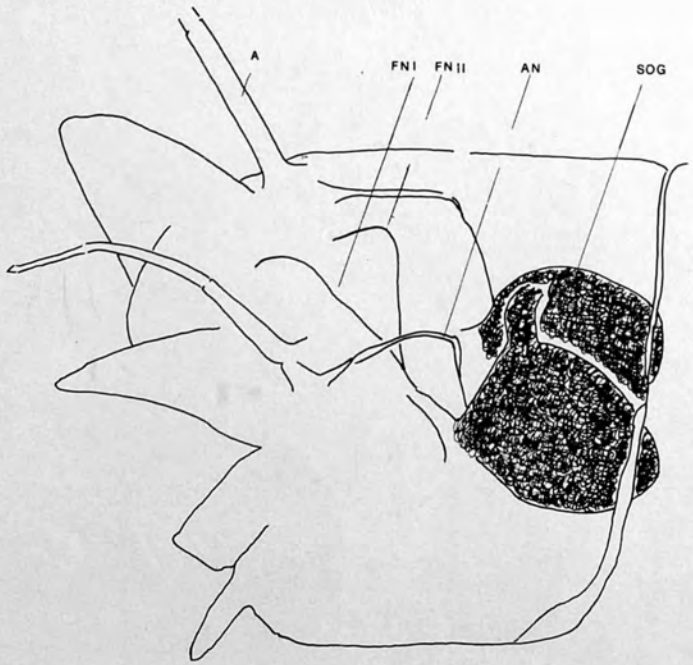


Fig. 7a

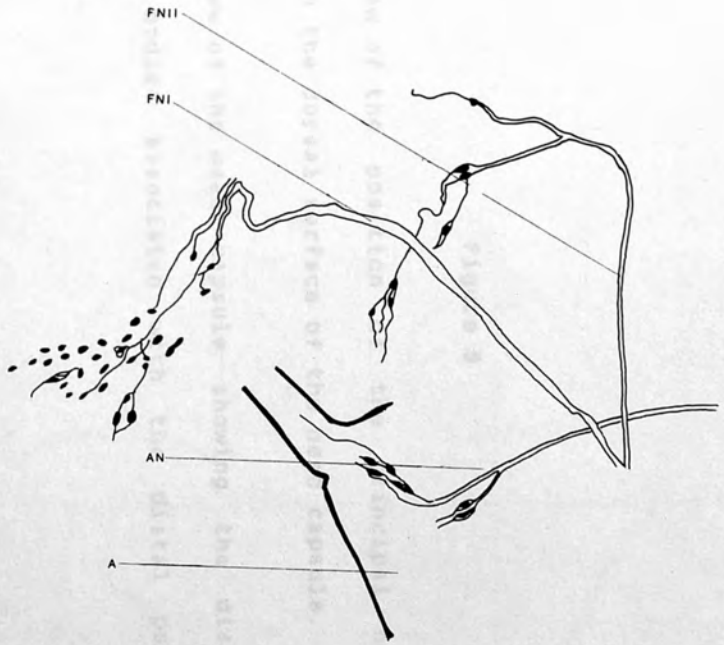


Fig. 7b

Figure 8

8a. Dorsal view of the position of the principal neural tracts associated with the dorsal surface of the head capsule.

8b. Dorsal view of the head capsule showing the distribution of bipolar cell bodies associated with the distal portions of the frontal nerves.

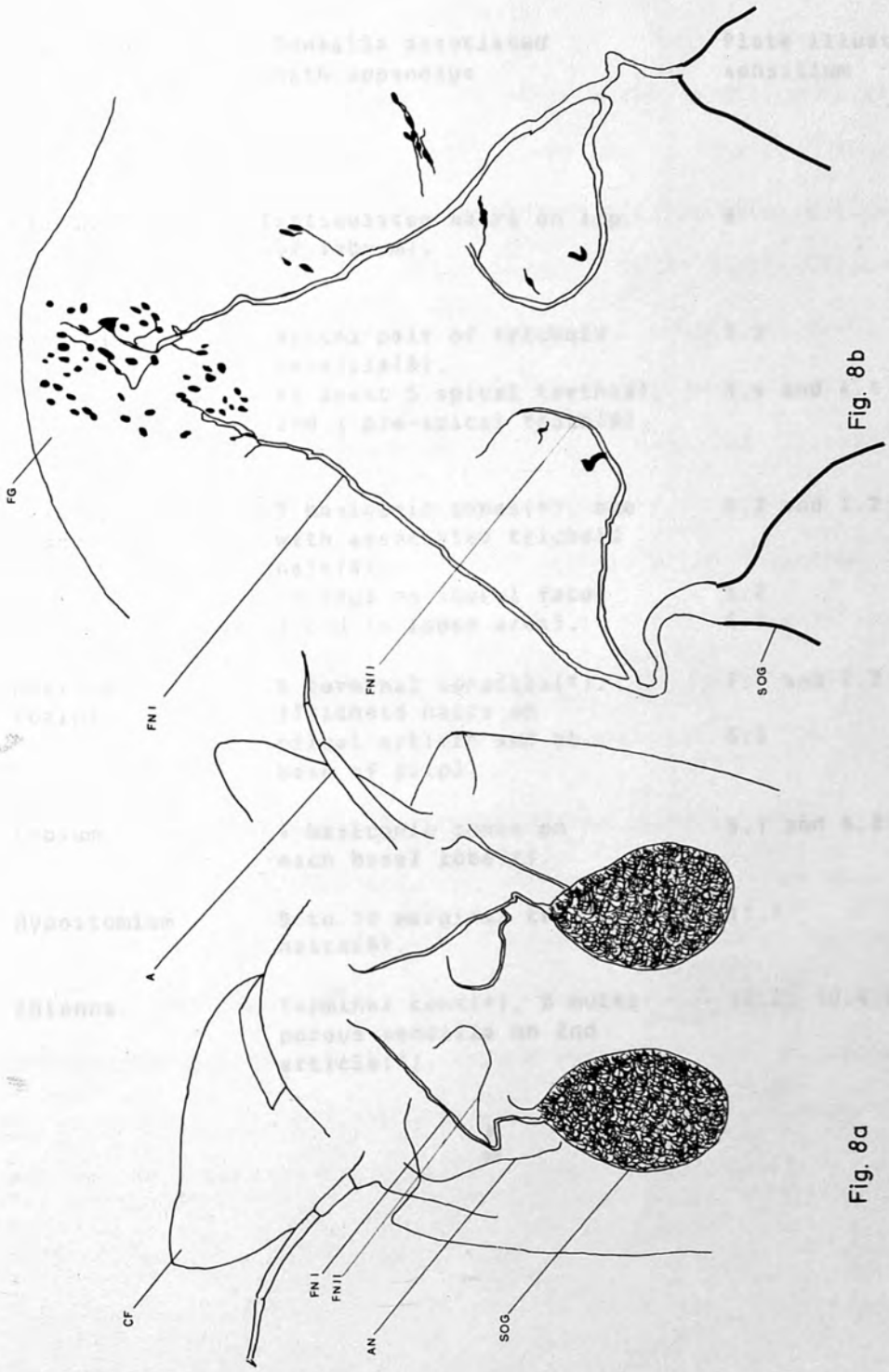


Fig. 8b

Fig. 8a

TABLE 3 A list of external sensilla on the appendages of the head capsule that have been shown to be innervated by sensory nerves. (Sensilla for which external structure suggests sensory function but for which neural connections have not been demonstrated are given in parentheses). * indicates a sensillum that is probably a chemoreceptor, # a sensillum that is probably a mechanoreceptor.

Appendage	Sensilla associated with appendage	Plate illustrating sensillum
Labrum	(articulated hairs on lip of labrum).	9
Mandible	Apical pair of trichoid sensilla(#).	3.3
	At least 5 apical teeth(#), and 1 pre-apical tooth(#).	3.4 and 4.1
Maxilla (lobe)	2 basiconic cones(*), one with associated trichoid hair(#).	6.3 and 7.2
	(6 pegs on aboral face;	6.2
	1 peg in lobed area).	6.4
Maxilla (palp)	9 terminal sensilla(*).	7.1 and 7.3
	(Trichoid hairs on palpal article and at base of palp).	5.3
Labium	4 basiconic cones on each basal lobe(*).	9.1 and 9.2
Hypostomium	8 to 10 marginal trichoid hairs(#).	11.1
Antenna	Terminal cone(*), 2 multi-porous sensilla on 2nd article(*).	10.2, 10.4 and 11.2

CHAPTER 3 THE DESCRIPTION OF LARVAL BEHAVIOUR PATTERNS: MATERIALS AND METHODS

3.1 The species of larvae used in experiments

Observations were made of the behaviour of Simulium ornatum and S. lineatum, two species which are among the most abundant in southern England. Larvae were collected from the River Wey in Surrey (mostly at Tilford; Grid reference SU 873435) and the River Itchen in Hampshire. All S. ornatum larvae were collected from a 400m reach of the Itchen near Alresford (Grid reference SU 564318), whereas S. lineatum came from the River Wey. Only the final instar, pharate, larvae were used in experiments as this was the only late instar that could be recognised with certainty, and without lengthy manipulations of the larvae, owing to the presence of pupal gill filaments. Use of one instar standardised the physiological condition of the larvae as much as possible.

Larvae were mostly collected from the aquatic plant Ranunculus fluitans on which they were often very abundant. They were brought back to the laboratory on slightly dampened vegetation (not in water) and kept in tanks of river water that was vigorously aerated at a constant temperature of 10°C. Larvae and water were collected from the same site at the same time. Larvae were not used in experiments if they had been kept in the tanks for more than one week.

3.2 The observation and recording of larval behaviour

The experimental apparatus, designed to allow close observation of the larvae in purified or natural water under closely controlled conditions, is shown diagrammatically in Figure 9. The behaviour of larvae was observed in a variety of conditions, inside the observation cell shown in Plate 12, usually being recorded on video-tape for subsequent analysis. Some observations, reported in Sections 4.6 and 6.3, were made whilst viewing the larvae directly with a Wild M5 binocular microscope at 25x magnification, without video recording. Filming larval behaviour proved to be straightforward because larvae are largely sessile and close observations could be made without restraining the larvae unnaturally.

Larvae were selected for experiments rather than being taken at random, only those that were actively cleaning their fans while still in the holding tank being used in experiments. Just before pupation larvae stop feeding so this procedure was adopted to avoid wasting time establishing larvae in the observation cell that had finished feeding. On a number of occasions larvae that initially appeared moribund in the observation cell, and did not feed, spun their pupal cocoon and pupated.

It was felt that the results were not biased by this selection as larvae would always be excluded from the analysis of feeding behaviour once they had stopped feeding. The point at which this exclusion occurred (before or during an experiment) should not affect the results.

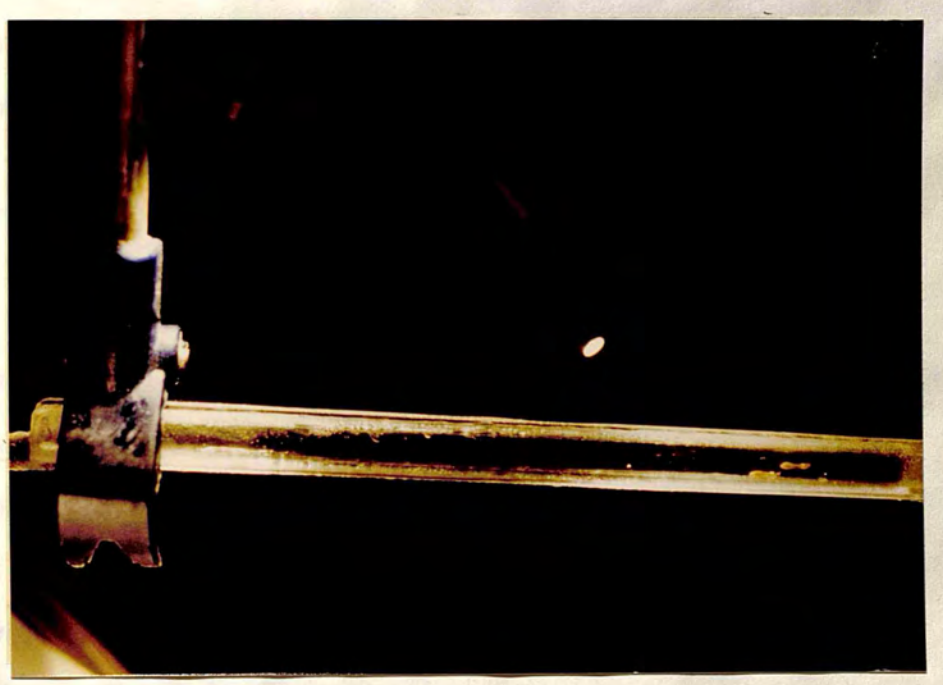


PLATE 12

The observation cell with larvae

At the beginning of each experiment a number of larvae, usually about 10 and never more than 20, were introduced into the upstream end of the observation cell. Once all the larvae had been placed inside the cell, using fine forceps, the water velocity was raised to the velocity to be used in the experiment. A 'standard' water velocity of $17cms^{-1}$ was used which was equivalent to a volume of $100cm^3\ min^{-1}$. While the quantity of water passing over the larvae was measured as a volume, the water velocity inside the flow cell could be calculated from the relationship,

$$velocity = v/(t\pi r^2)$$

where the r = the radius of the observation cell, t = time and v = the volume of water pumped. Larvae usually spun a new silk pad on the glass walls of the observation cell, and attached themselves to it, within a few seconds. Once attached they could be exposed immediately to any water velocity within the range used in these experiments ($0-70cms^{-1}$).

The observation cell was made from standard 1mm wall, 5mm bore Pyrex glass tubing cut in half to make a semi-circular trough. A length of microscope slide glass was attached to the top of the trough with silicon rubber cement to give a good optical surface through which the larvae could be viewed. After each series of observations the trough was opened to clean out the silk left by the larvae. Any silk left in the cell made it very difficult to establish a further group of larvae as they were often trapped in the old silk, greatly reducing the number able to attach themselves.

The cell was connected to the rest of the system using silicon rubber tubing and held in a moveable clamp beneath the microscope. Larvae were filmed through a Watson Microsystem 70 Compound microscope fitted with a 2" (x6) objective lens (the microscope has been omitted from Figure 9 for clarity). Recordings were made on a Panasonic-National NV-8200 VHS video tape recorder (VTR) with a black and white television camera attached to the microscope. This gave an effective magnification of about 300x when recording were replayed on a television screen. To "freeze" the movements of the larvae, some of which were very rapid, they were illuminated with a stroboscope (Dawe Instrumants, Strobosun Type 1203B). This was also necessary to create distinct frames (in the sense of a cine film) on the video tape .

The 'frame advance' facility of the VTR provided twenty-five advances of the tape for each second of recording time. By adjusting the stroboscope so that it flashed twenty-five times a second it was possible to create one 'frame' on the video tape every 0.04s. Since each flash from the strobe had a duration, according to the manufacturers handbook, of 1us all movements were frozen. The recordings show the larvae for 1us every 0.04s. The combination of strobe lighting and video tape provided better resolution of rapid movements and greater ease of handling than high speed cine film (the duration of each frame used by Craig and Chance, 1982, was 3.3ms), although the picture quality was generally poorer than photographic emulsion. In these experiments all illumination was from below so that larvae were mostly seen in silhouette. More information would be obtained if the light from the stroboscope could be carried by fibre optics to illuminate the larvae from above as well.

Water was pumped through the observation cell by a peristaltic pump which gave water velocities between 0cms^{-1} and 70cms^{-1}. The pulses created by the rotor of the pump were smoothed using two compression flasks in series whilst the quantity of water pumped was measured using a 'Flowbits' flowmeter. This had a range of 0 to 200cm^3 min^{-1}; greater volumes (70cms^{-1} = 400cm^3 min^{-1}) were measured by recording the time taken to fill a graduated cylinder. The pump was driven by an induction motor, rather than a moving coil motor, so that fluctuations in the mains frequency did not affect its speed. All connecting tubing was silicon rubber to reduce the chance of the larvae being exposed to chemical compounds leached from the tubing.

Temperature control was initially provided by two constant temperature tanks. However, because of the length of relatively small bore tubing used to carry the water from the storage vessels in the CT tanks to the observation cell, high summer temperatures (up to 25°C) in the room where experiments were done often cancelled the effect of the CT tanks. To overcome this problem the water was passed through a cooling coil immediately before the cell, giving closer control over the temperature.

The water supply for all experiments using particle-free distilled water was single distilled tap water, deionised in an 'Elga' cartridge. Water was stored in Pyrex glassware when cooling and then filtered through a 0.22µm 'Millipore' filter before being used. pH was not controlled but was measured continuously and remained in the range 4.5 to 5.5 in experiments using distilled water alone. As Figure 9 shows both temperature and pH were measured immediately downstream of the observation cell. Temperature was recorded with a Grant thermistor probe and pH was recorded with a

silver calomel electrode connected to an Electronic Instruments Ltd Model 7060 pH meter. Both the pH electrode and the thermistor projected into the flow of water.

Despite the possible drawbacks of using highly purified water for the majority of experiments (particularly the risk of stressing the larvae osmotically) it was essential when investigating the effects of water-borne stimulants to have a closely controlled physical and chemical environment, free of potential stimulants, against which the effect of stimulants applied experimentally could be judged. Throughout the experiments larvae freely attached themselves inside the observation cell, and 'fed', in particle free distilled water.

3.3 The application of stimulant pulses

Physical and chemical stimulants, including potential food items, were added to the water to investigate their influence on larval behaviour. Pulses of stimulants were injected into the water using apparatus designed by Professor CT Lewis, but adapted for this work. Although designed for the injection of gases into a stream of air (during investigations of olfaction in terrestrial insects) few modifications were required to inject solutions and suspensions into water.

The potential stimulant was injected automatically from a glass syringe into the stream of particle-free distilled water 50cm upstream of the observation cell. The syringe was closed by a variable-speed electric motor driving a piston that pushed against the syringe plunger. The electric motor was controlled electro-mechanically to close the syringe in short bursts to give

pulses of the injectant. The pulses could be of any duration from 0 to 100s with from 1s to 5 mins between each pulse. The syringe was connected to the water stream through 1mm bore silicon rubber tubing. A solenoid clamped this tube to ensure that the pulse was of the right duration; if the tube was not clamped liquid was drawn out of the syringe into the stream of water, presumably as a result of the Venturi effect.

Solutions of chemical compounds to be injected as stimulants were prepared using 'Analar' grade chemical whenever possible and were always made up in particle-free distilled water. All glassware was cleaned in the surface active detergent 'Decon 90' before the preparation of stimulant solutions or suspensions.

Suspensions were stirred continuously in the syringe with a magnetic follower to prevent them from settling out and thereby altering the concentration. The temperature of stimulants in the syringe was not regulated and followed room temperature. In most experiments each pulse was of one second duration ($1cm^3$ or less of stimulant) so that this lack of control was not considered significant. Responses to stimulation, described in Chapter 6, were measured using the behaviour of the larvae as a bioasssay.

The concentration of a stimulant passing over the larvae was calculated, not measured directly because the dose was diluted on injection into the water. To calculate this concentration the volume injected, its concentration and the volume to which it was added must be known. The volume injected in each pulse was estimated by calibrating the injection system. To calibrate the system fifteen consecutive pulses of water, of one second duration, were collected in pre-weighed, dry, plastic vials. Each vial was re-weighed to

measure the volume of water ejected in the pulse. The mean volume of a pulse was calculated from these measurements and was taken as the volume injected at each pulse in that days experiments.

To calculate the final concentration of the injected compound or suspension in the water it was assumed that the pulse was diluted, as it was injected, by the volume of water flowing through the cell in the same time as the length of the pulse (in most cases one second). At a flow rate of $100cm^3\ min^{-1}$ $1.66cm^3$ water flowed through the cell every second. Therefore, when the pulse was injected the original concentration was diluted by $1.66cm^3$ + the estimated volume of the pulse (in cm^3) assuming that they were perfectly mixed. To ensure that mixing was as complete as possible the pulse was injected 50cm upstream of the observation cell. Thus an original concentration of 0.5M, when injected as a pulse of $1cm^3$ volume, gave an estimated concentration inside the cell of 0.18M.

With the apparatus described it was not possible to determine, independently of the responses of the larvae, when a stimulant reached the larvae. However this could be estimated, knowing that the point of injection was 50cm upstream of the observation cell. At a water velocity of $45cms^{-1}$ (the velocity in the narrow connecting tubing when pumping $100cm^3\ min^{-1}$) it took just over one second for the stimulant to reach the larvae. The injection of a pulse was signalled on the video tape either by a synchronised sound signal or by observing a characteristic passive movement of the body of the larva caused by the brief change in pressure as the pulse was injected.

In a number of experiments observations were made on the behaviour of larvae in unfiltered natural water. Water for these experiments was either collected on the day of the experiment, or in the evening the day before the experiment, in 20l plastic containers. If water was kept overnight it was vigorously aerated at 10°C in the dark. During the experiment vigorous aeration was maintained to ensure that particles in the water remained suspended. Temperature control of this water was provided by a cooling coil.

Filtered natural water was prepared from natural water collected in the way described in the paragraph above. It was filtered through a 0.22µm 'Millipore' filter and stored in 10l 'Pyrex' glass jars. It was used on the same day that it was filtered.

The majority of observations in all water qualities were made at the relatively low water velocity of 17cms^{-1} (100cm^3 min^{-1}). At this velocity preliminary observations showed that larvae were almost unaffected by turbulence, but that above this velocity were buffeted more and more, making the interpretation of the video recordings more difficult. Larvae were observed on a number of occasions at velocities up to 70cms^{-1}, when the effect of changing water velocity on behaviour was investigated, and there appeared to be no qualitative changes in behaviour at these higher velocities. It was felt, therefore, justifiable to work at this far more convenient, but rather low, water velocity.

Before recording larval behaviour on video-tape, larvae inside the cell were assigned a number so allowing individuals to be recognised throughout an experiment (which could last up to four hours; see Section 4.5). When the behaviour of a group of larvae was recorded larvae were observed in sequence from the downstream to

the upstream end of the observation cell.

3.4 The analysis of video-tape recordings

Because of the highly repetitive nature of larval behaviour it was found that a short extract of behaviour could be taken as typical of behaviour over a much longer time. In the experiments reported here results were based on ten second extracts of behaviour from recordings that usually lasted several minutes. Justification for the use of short extracts is presented statistically in section 4.5 (p. 166).

Figure 10 shows the record sheet used for transcribing the activities of the larvae from the video-tape recordings. The analysis of behaviour began with the playing back, frame by frame, of ten seconds of a recording, each frame being assigned to one of the stages in the cycle of behaviour shown on the flow chart in Figure 11. The stage to which each frame belonged was recognised according to the position of the fans and mandibles. Each line on the recording sheet was used to represent one of the stages of the fan cycle and the number of frames showing that particular stage scored on the sheet (see Figure 10). The columns should be read from top to bottom and show a continuous sequence of movements, from left to right. In the example shown in Figure 10 the larva cleaned ('beat') its fans ten times, with two extra mandible movements, during the ten seconds. Ten seconds of behaviour is described by 250 'frames'. From this raw data it was possible to calculate frequencies and durations of various activities.

As can be seen from the specimen recording sheet (Figure 10) and from Figure 11 feeding behaviour the fan cycle was divided into ten stages for analysis, known as the 'fan cleaning cycle'. The flow chart in Figure 11 shows the classification that was used in the analysis of video-tapes and defines each stage of the fan cycle. It was necessary to distinguish between the fan nearest the observer and the fan further from the observer in this analysis because the movements of the two were not equally visible when the larvae were viewed closely. Mandible movements made without any movements of the fan were treated as a separate activity (and based on methods in Sokal and Rohlf, 1969 and Steel and Torrie, 1981).

A number of statistical tests have been used to analyse the results of behavioural observations. All were available on the Elementary Statistics Package designed by Dr A Wroot and available at Royal Holloway College Computer Centre.

The influences operating on larval behaviour have been investigated by comparing the patterns of behaviour in 'unstimulatory' conditions (those provided by particle-free distilled water) with those in a variety of 'stimulatory' conditions (provided by unfiltered natural water and particle-free distilled water with the controlled addition of potential stimulants). With this approach it is necessary first to describe behaviour in particle-free distilled water.

Figure 9

A diagrammatic representation of the apparatus used to observe larval behaviour. Arrows indicate the path of water (and stimulants), through the apparatus. Water was drawn from a storage jar in a constant temperature bath (at the top left) by a peristaltic pump, flowed over the larvae in the observation cell and passed to waste.

Figure 10

The record sheet used to transcribe larval behaviour from video tapes, showing a short extract of behaviour with ten fan beats and two extra mandible movements. Each mark represents one frame from the recording.

Figure 11

The stages of the fan cleaning cycle. Numbered boxes represent larval head capsules, showing the sequence of movements when the fans are cleaned. It is assumed that larva is seen in lateral view, so only the ipsi-lateral appendages are represented. Thin lines on the 'head capsule' represent cephalic fans (above horizontal, fan open; below horizontal, fan closed) and broad bars represent mandibles (angle above horizontal indicates degree of abduction; angle below horizontal indicates degree of adduction). Other mouthparts not shown.

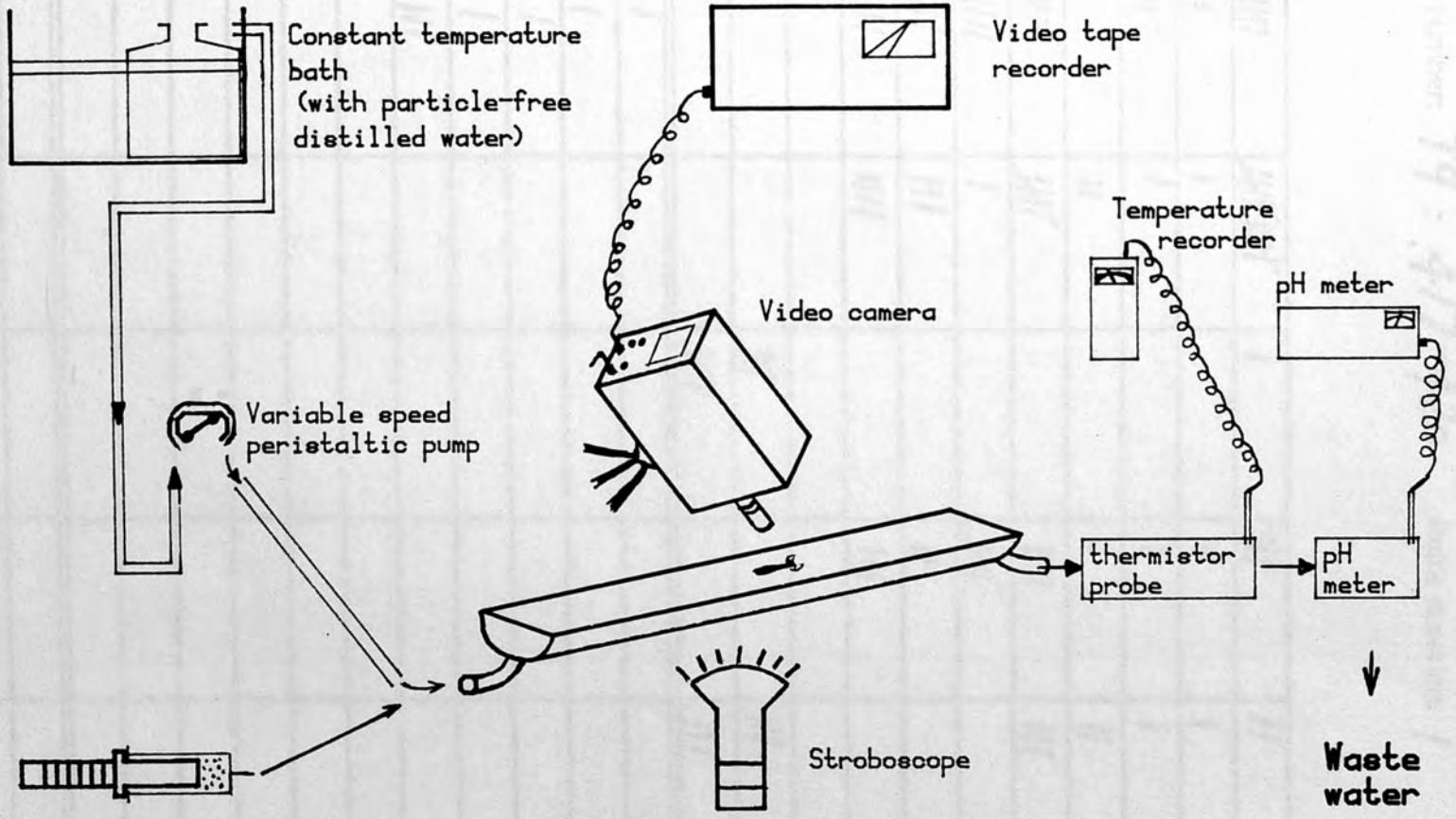

Constant temperature bath (with particle-free distilled water)
Video tape recorder
Temperature recorder
pH meter
Video camera
Variable speed peristaltic pump
thermistor probe
pH meter
Stroboscope
Waste water
Electro-mechanical pulse controller/timer driving glass syringe (with stimulant)
Larvae observed in glass cell (shown in centre of diagram)

Figure 9

Figure 10

Date: 21.6.83
Specimen: 6
Video tape number: T9 : 417-420

Treatment: Pre-stim. 100cm^3 PFDW 0.01M NaCl
Pulse Interval: 30
Pulse duration: 1

1	𝍸	𝍸 𝍸	1	111	11	𝍸
2	1	1		1	1	1
3	11	1		1	1	1
4		11		1	11	1
5	𝍸	𝍸		1111	111	𝍸
6	1111	1		𝍸		1
7	111	111		11		111
8	111	1111		111		𝍸
A			111		111	
B			𝍸		111	
1	1					
2	1					
3	1					
4	1					
5	111					
6						
7						
8						
A						
B						

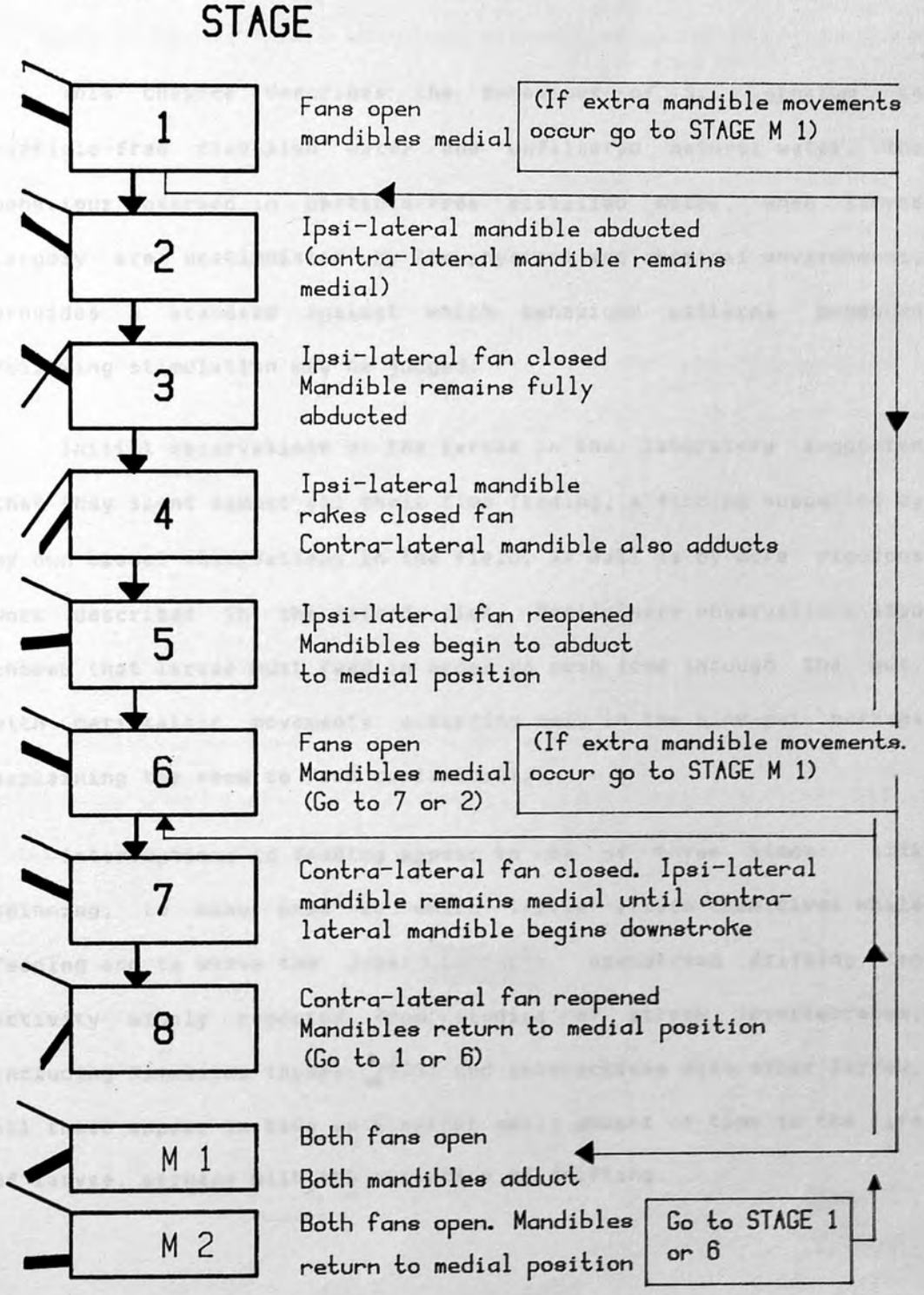

STAGE
1
Fans open
mandibles medial
(If extra mandible movements
occur go to STAGE M 1)
2
Ipsi-lateral mandible abducted
(contra-lateral mandible remains
medial)
3
Ipsi-lateral fan closed
Mandible remains fully
abducted
4
Ipsi-lateral mandible
rakes closed fan
Contra-lateral mandible also adducts
5
Ipsi-lateral fan reopened
Mandibles begin to abduct
to medial position
6
Fans open
Mandibles medial
(Go to 7 or 2)
(If extra mandible movements.
occur go to STAGE M 1)
7
Contra-lateral fan closed. Ipsi-lateral
mandible remains medial until contra-
lateral mandible begins downstroke
8
Contra-lateral fan reopened
Mandibles return to medial position
(Go to 1 or 6)
M 1
Both fans open
Both mandibles adduct
M 2
Both fans open. Mandibles
return to medial position
Go to STAGE 1
or 6

Figure 11

CHAPTER 4 THE FEEDING BEHAVIOUR OF LARVAE IN PARTICLE FREE DISTILLED WATER AND UNFILTERED NATURAL WATER

4.1 Introduction

This chapter describes the behaviour of *S. ornatum* in particle-free distilled water and unfiltered natural water. The behaviour observed in particle-free distilled water, when larvae largely are unstimulated by the physical and chemical environment, provides a standard against which behaviour patterns produced following stimulation may be judged.

Initial observations of the larvae in the laboratory suggested that they spent almost all their time feeding, a finding supported by my own casual observations in the field, as well as by more rigorous work described in the introduction. Preliminary observations also showed that larvae must feed in order to push food through the gut, with peristaltic movements occurring only in the hind-gut, perhaps explaining the need to feed continuously.

Interruptions to feeding appear to be of three kinds: silk spinning, to make pads to which larvae attach themselves while feeding and to weave the pupal cocoon; downstream drifting, an activity widely reported from studies of stream invertebrates, including Simuliids (Hynes, 1973) and interactions with other larvae. All these appear to take up a rather small amount of time in the life of larvae, perhaps with the exception of drifting.

The factors that cause larvae to drift are largely unknown. It is unlikely that it is simply due to the larvae being dislodged by strong currents since they are able to prevent themselves being washed away, even in the strongest currents, by attaching silk threads to the substrate (personal observation). Drifting could be an escape response to predators as larvae are otherwise very vulnerable to predation as a result of their tendency to occupy exposed positions. The behaviour of simuliid larvae at the approach of predatory Rhyacophilid caddis larvae suggests that releasing themselves from their silk pad is their first reaction, a behaviour pattern with obvious survival potential (personal observation).

It is legitimate, then, to consider larval behaviour as largely synonymous with larval feeding behaviour, feeding being the main activity of these sessile filterers. With this justification other aspects of larval behaviour have not been considered in the work reported here.

Preliminary observations of the larvae of S. ornatum quickly confirmed the highly stereotyped behaviour pattern. Immediately clear is the almost constant alternate beating of the cephalic fans and the associated movements of the other mouthparts. The patterns of behaviour are both simple and highly repetitive and lend themselves to quantitative analysis. In the rest of this chapter the 'beating' of the fans, when they are closed to be cleaned, will be referred to as the "fan cleaning cycle".

4.2 Larval behaviour: the stages of the fan cleaning cycle

The cleaning of the cephalic fans, the dominant activity of the larvae, has been divided into a sequence of movements, called stages, which correspond to natural subdivisions in the fan cleaning cycle. Behaviour was divided into these stages, not only for ease of understanding but also because it seemed likely that neurophysiological analysis of feeding behaviour by subsequent workers would be facilitated if definite stages in behaviour were recognised. When larvae are viewed at very short range, movements of the two fans must be described separately, one being designated ipsi-lateral and the other contra-lateral for this purpose.

The designation is a necessary, but artificial, distinction as there is no real difference in the activity of the two fans. The designation depends on the position of the larva and it changes if the larva moves and the fan that was nearest the camera becomes the one further from it. The movements of the ipsi-lateral fan are described by STAGES 1 to 6 and those of the contra-lateral by STAGES 7 and 8.

The need for a division into STAGES 1 to 6 and 7 to 8 is best understood by looking at Plate 14 and Figure 11. The plate shows a typical side view of a larva; only the mandible and maxilla nearest the camera are visible, whilst the outline of the nearer (ipsi-lateral) cephalic fan dominates that of the fan further from the camera (it can be seen more easily when the tapes are running). Because of the angle of the head capsule it is not possible to see all the movements in the fan cycle of the contra-lateral mandible, maxilla and fan. Consequently this always appears slightly shorter

than the fan cycle of the ipsi-lateral group, so that the two cannot be compared directly and have not been used together in statistical tests.

The majority of larvae were observed in one or other of the two lateral views shown in Plates 14 and 15 and 16 to 20, respectively. A much smaller number were seen in dorsal and ventral view (such as in Plate 22) where the distinction between ipsi-lateral and contra-lateral fan cycles is no longer needed because both fans can be seen equally clearly (but as the plate shows other mouthparts were less clearly seen). Plates 23 to 25 show an angle intermediate between 14 to 15 and 22. A description of the different angles from which the larvae were observed is presented here because each of the four main angles gives different, and complementary, information.

By counting the number of frames that each stage of behaviour occupied the duration of each stage was calculated by multiplying the number of frames by 0.04s, the effective length of each frame. In this way a description of the behaviour patterns of the larvae was built up.

4.3.1 The stages of the fan cleaning cycle during uninhibited feeding

Once freed from disturbance larvae beat their fans spontaneously, some even doing so in still water, showing that the force of moving water is not needed to open the fan . The movements described remove food particles from the cephalic fans for ingestion and are described with reference to Figure 11 and the Plates 14 to 25. All of these plates are photographs of video stills and, except where specified, they show groups of consecutive frames from the video tapes.

4.3.2 The closing and cleaning of the ipsi-lateral fan

The movements described in this section are illustrated in Plates 14 and 15 which show seven consecutive frames from a video recording. The fan cycle begins with STAGE 1 when both fans are open and filtering the water. The mandible is in the 'medial' position, and is stationary; in particle-free distilled water the mandible may remain in this position for up to half a second between consecutive fan beats. The positions of the various mouthparts may be seen more clearly in the scanning electron micrograph shown in Plate 13.2, which shows a ventral view of the head capsule. Both cephalic fans are open (in life the filaments would be a little further apart as there is some contraction during fixation) and the mandibles (mn) are in the medial position, lying roughly at right angles to the stem of the cephalic fan.

STAGE 2 begins as the ipsi-lateral mandible moves away from the mouth (abduction) to make room for the fan to close. This movement is recorded in Plate 14.2 and occurs before any movement of the fan.

At this stage there is no movement of the maxilla. In Plate 14.2 the mandible has been recorded halfway to maximum abduction, as can be seen from 14.3 where the mandible has abducted further. The fan filaments have also begun to move together on Plate 14.3 which shows the beginning of STAGE 3, when the fan starts to close. In many cases STAGE 2 occurs too quickly (that is, takes less than 0.04s) to be recorded.

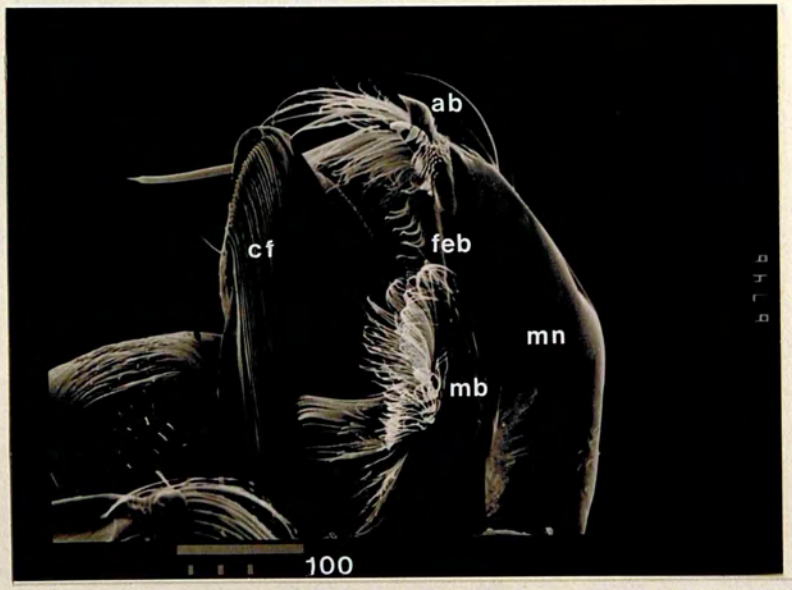
STAGE 3 is represented in the micrograph shown in Plate 13.1 showing the mandible fully abducted beside the closed fan. Plate 14.4 shows the outline of the closed fan with the filament tips in the cibarium. The filaments of the contra-lateral fan are visible as a blur below the closed fan and above the head capsule. As soon as the fan is closed the distribution of water pressure on the head changes and the head moves down about 0.1mm. This movement can be used to decide when the fan is closed, even when the fan cannot be seen.

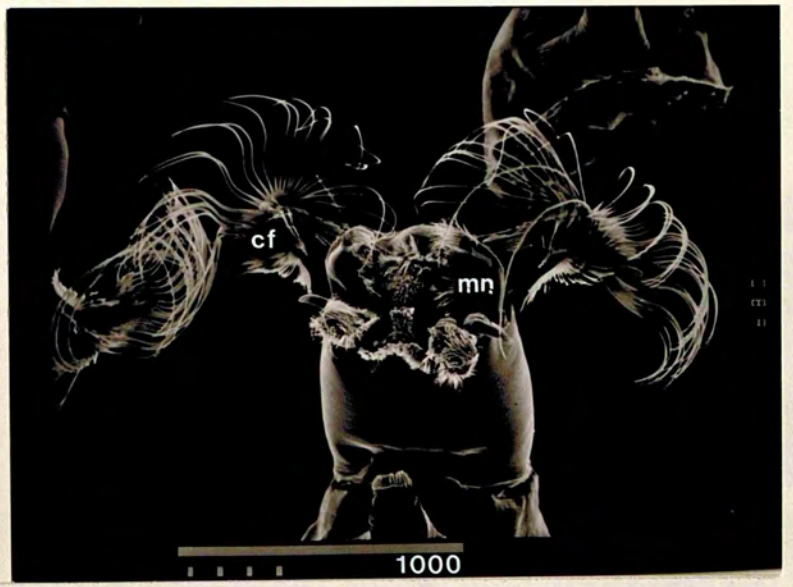
STAGE 4 of the fan cycle begins as soon as the mandible starts to rake down over the fan filaments and continues for as long as the fan is closed or opening. In Plate 14.4 the tip of the mandible may be seen halfway through the raking stroke. Of the stages of the fan cycle during which the fan is closed this is usually the longest. In this example there are three frames showing the closed fan. Plate 15.2 shows how the filaments move away from the cibarium while still bunched together before fanning out again.

Plate 13.1 The approximate position of the mandible in relation to the closed cephalic fan when mandible fully abducted at the beginning of the fan cleaning cycle.

13.2 Ventral view of the head capsule showing the mandibles in the medial position.

13.1





13.2

Plate 14 A lateral view of the larval head capsule showing

STAGES 1, 2 and 3 of the fan cycle.

14.1 Stage 1. 14.2 Stage 2. 14.3 and 14.4 Stage 3.

PLATE 14

14.1

14.2

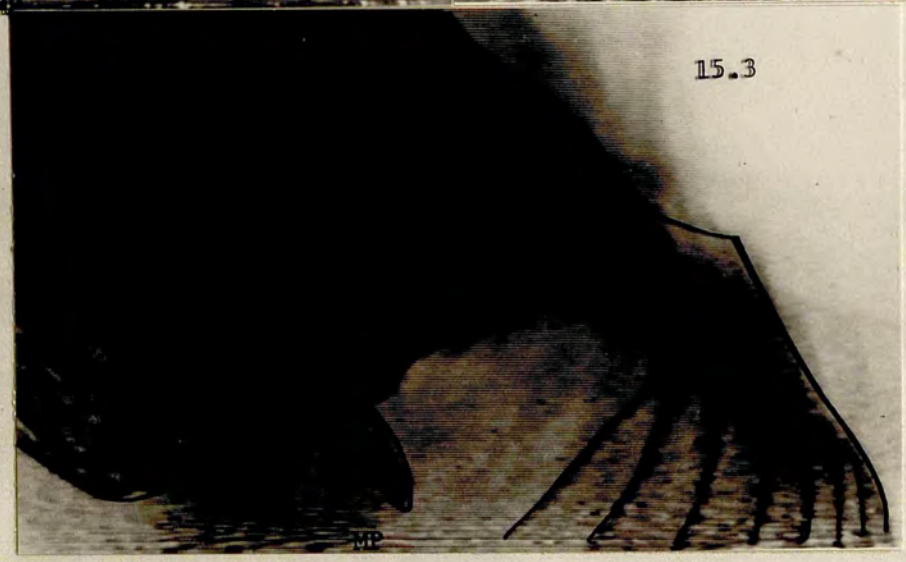
14.3

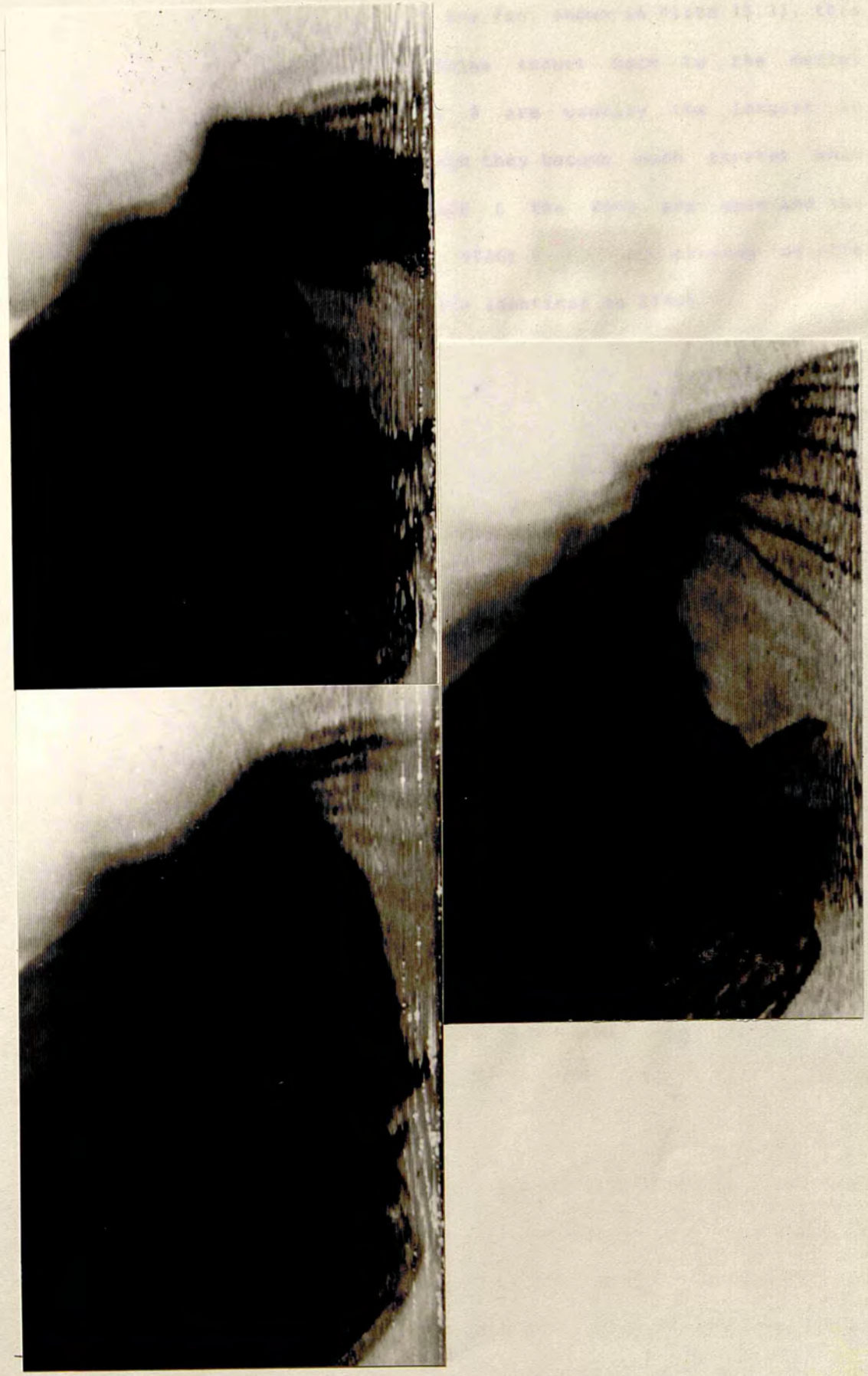
14.4

Plate 15. A lateral view of the larval head capsule showing STAGES 4 and 5 of the fan cycle.

15.1 and 15.2 Stage 4. 15.3 Stage 5.

PLATE 15





As soon as the fan is open STAGE 5 begins (another of the stages where food is being gathered on the fan, shown in Plate 15.3), this being the stage when the mandibles abduct back to the medial position. Stages 1, 5, 6 and 8 are usually the longest in particle-free distilled water though they become much shorter when food is available. During STAGE 6 the fans are open and the mandibles in the medial position. STAGE 6 precedes closing of the contra-lateral fan, but is otherwise identical to STAGE 1.

As soon as the mandible has returned to the medial position, shown in Plate 14.1, the next fan cycle may begin. Usually this involves the contra-lateral fan as the fans are almost always cleaned alternately. In the lateral views shown in Plates 14 and 15 the movements of the contra-lateral mandible could not be seen. Clearly it would not be possible to subdivide the contra-lateral fan cycle into three stages based on the position of the mandibles so all movements of the contra-lateral fan and mandibles, during fan cleaning, are lumped together as STAGE 7.

4.32 The cleaning of the contra-lateral fan

Plate 16, an alternative lateral view of the head capsule, shows the contra-lateral fan closing. Plate 16.1 shows the medial position, STAGE 6, before the fan begins to close.

Since not all the details of mandible and fan movements needed to describe STAGES 2, 3 and 4 can be seen STAGE 7 appears to be slightly shorter than the sums of STAGES 2, 3 and 4, mainly because it does not include the initial abduction of the mandible. Plate 16.2 shows the fan closing while the mandible is still medial on the ipsi-lateral side; the mandible on the opposite side to the fan that is closing does not need to move to make room for the fan and so remains in the medial position until the mandible cleaning the fan begins its downstroke. The contra-lateral fan remains closed or opening in Plates 16.3, 16.4 and 17.1; none of the detail visible in the closing of the ipsi-lateral fan can be seen now. The ipsi-lateral mandible continues to adduct assisting the invisible contra-lateral mandible in pushing food into the cibarium.

In Plate 17.2 both mandibles are fully adducted and now out of sight whilst the fan has reopened. This represents the beginning of STAGE 8, when the mandible recovers to the medial position again following the cleaning of the contra-lateral fan. STAGE 8 is distinguished from STAGE 5 because it follows the cleaning of the contra-lateral fan (see Figure 11).

Plate 18 shows the ipsi-lateral fan closing from the same angle and it is evident that more detail is visible. Plate 18.1 shows the fully abducted mandible (STAGE 2, cf Plate 16.1), Plate 18.2 the closing fan (STAGE 3) and Plate 18.3 the closed fan just before the

raking stroke begins (STAGE 3), where the tip of the mandible is clearly visible. In Plate 18.4 the raking stroke has nearly been completed (STAGE 4) with the mandible down at the tips of the fan filaments. Plate 19.1 shows the final frame of STAGE 4 with the fan nearly fully open. The difference between the fan being fully open and the fan opening is seen by comparing Plate 18.1 with 19.1. Finally Plate 19.2 shows the first frame in STAGE 5 where the fan is open again, the mandible fully adducted and the maxilla fully abducted.

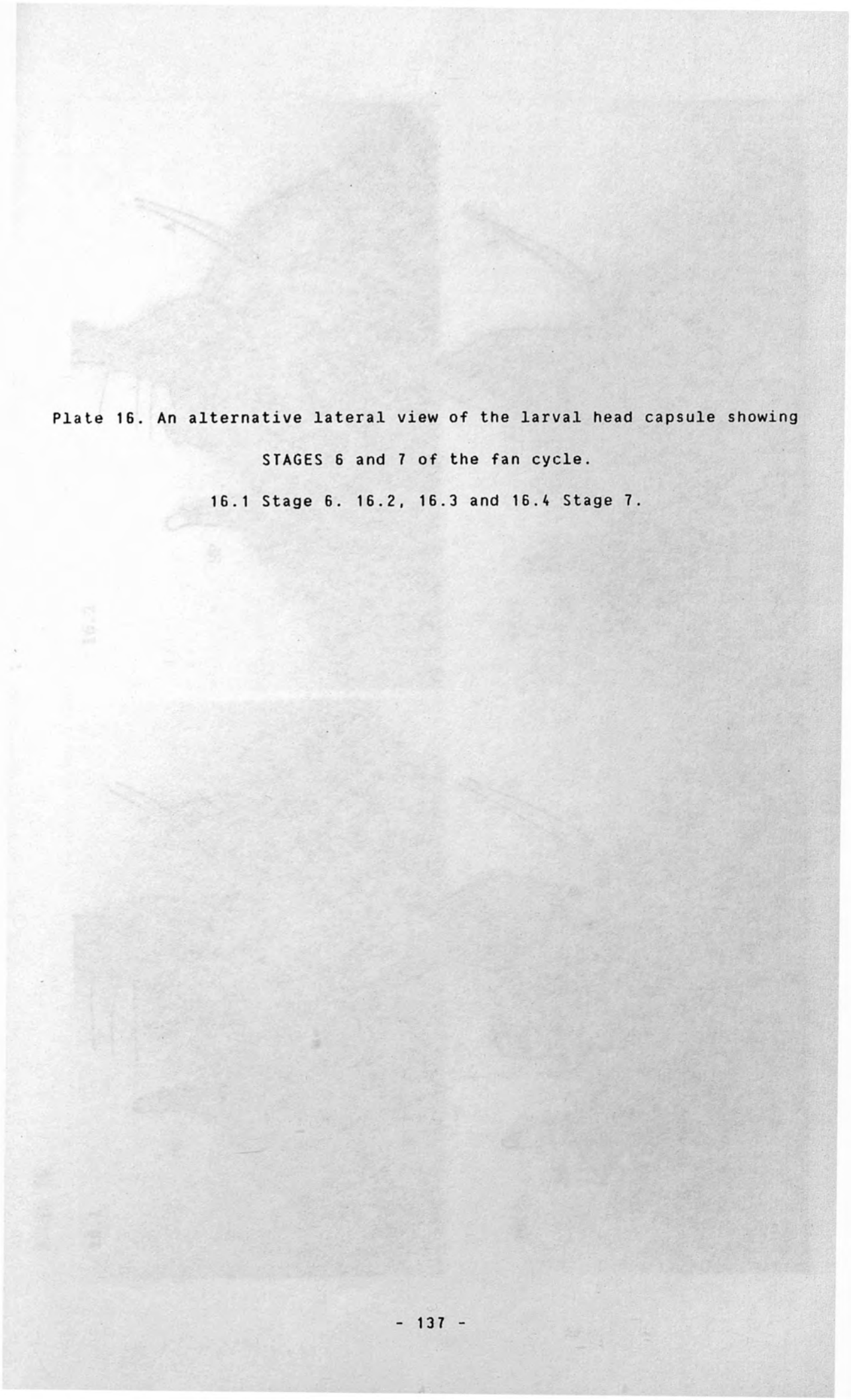
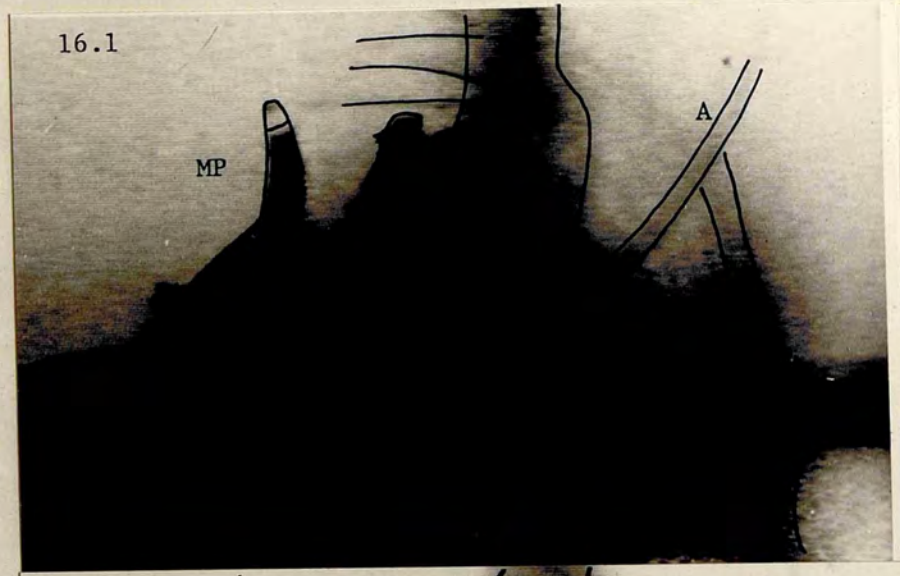
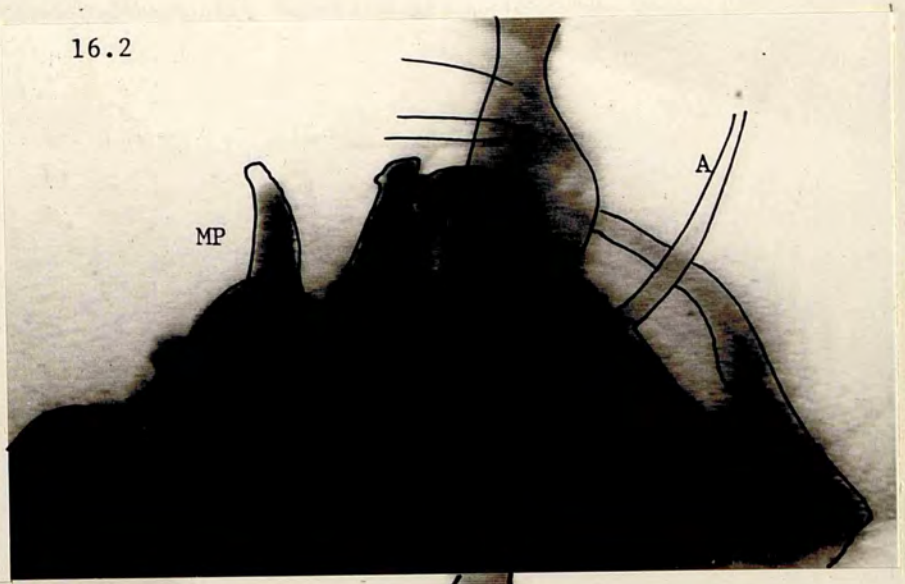


Plate 16. An alternative lateral view of the larval head capsule showing STAGES 6 and 7 of the fan cycle.

16.1 Stage 6. 16.2, 16.3 and 16.4 Stage 7.

PLATE 16





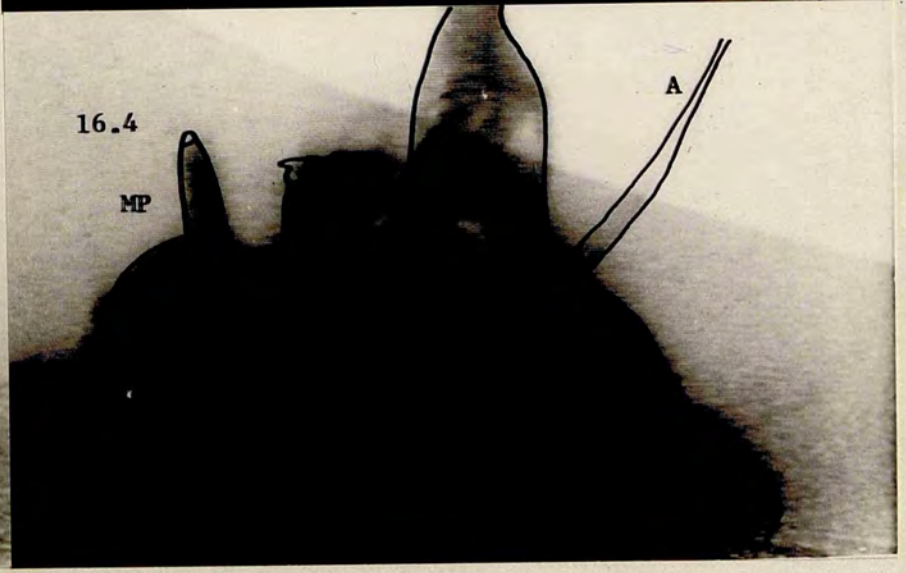
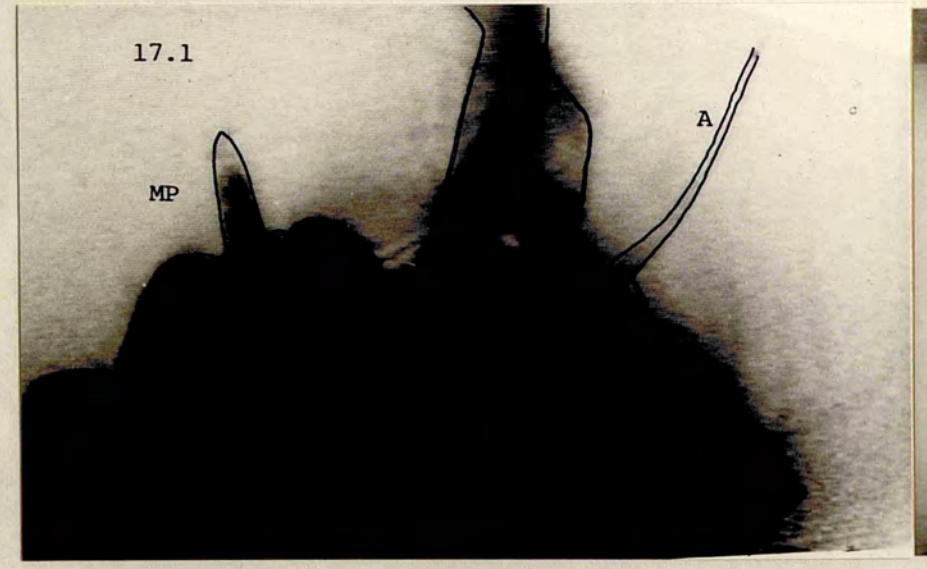
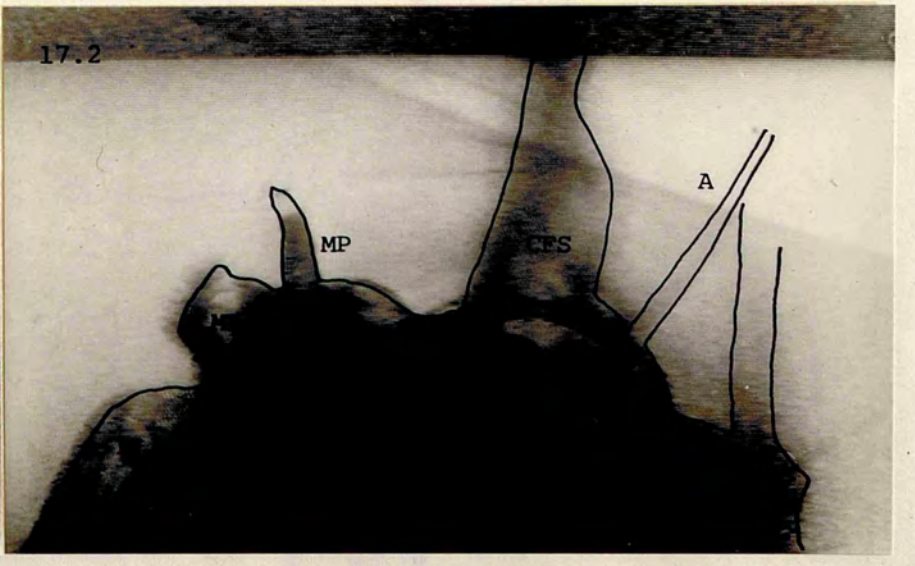


Plate 17. An alternative lateral view of the larval head capsule showing STAGES 7 and 8 of the fan cycle.

17.1 Stage 7. 17.2 Stage 8.

PLATE 17





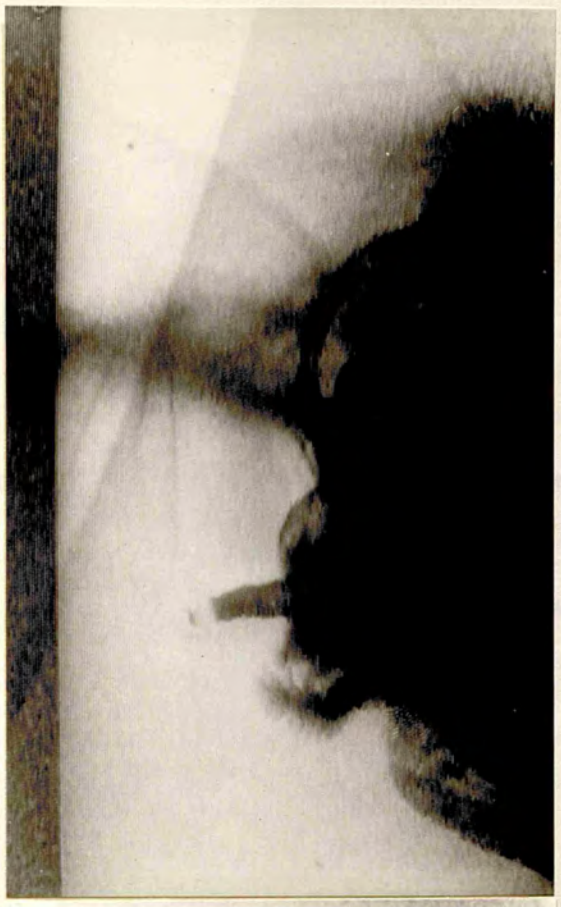
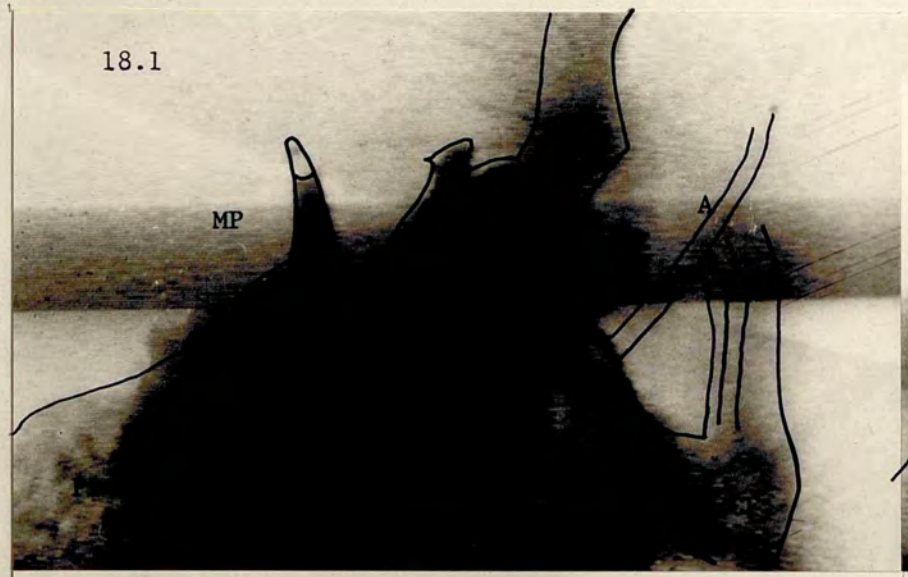


Plate 18. An alternative lateral view of the larval head capsule showing STAGES 2,3 and 4 of the fan cycle.

18.1 Stage 2. 18.2 and 18.3 Stage 3. 18.4 Stage 4.

PLATE 18

18.1



18.2

18.3

18.4

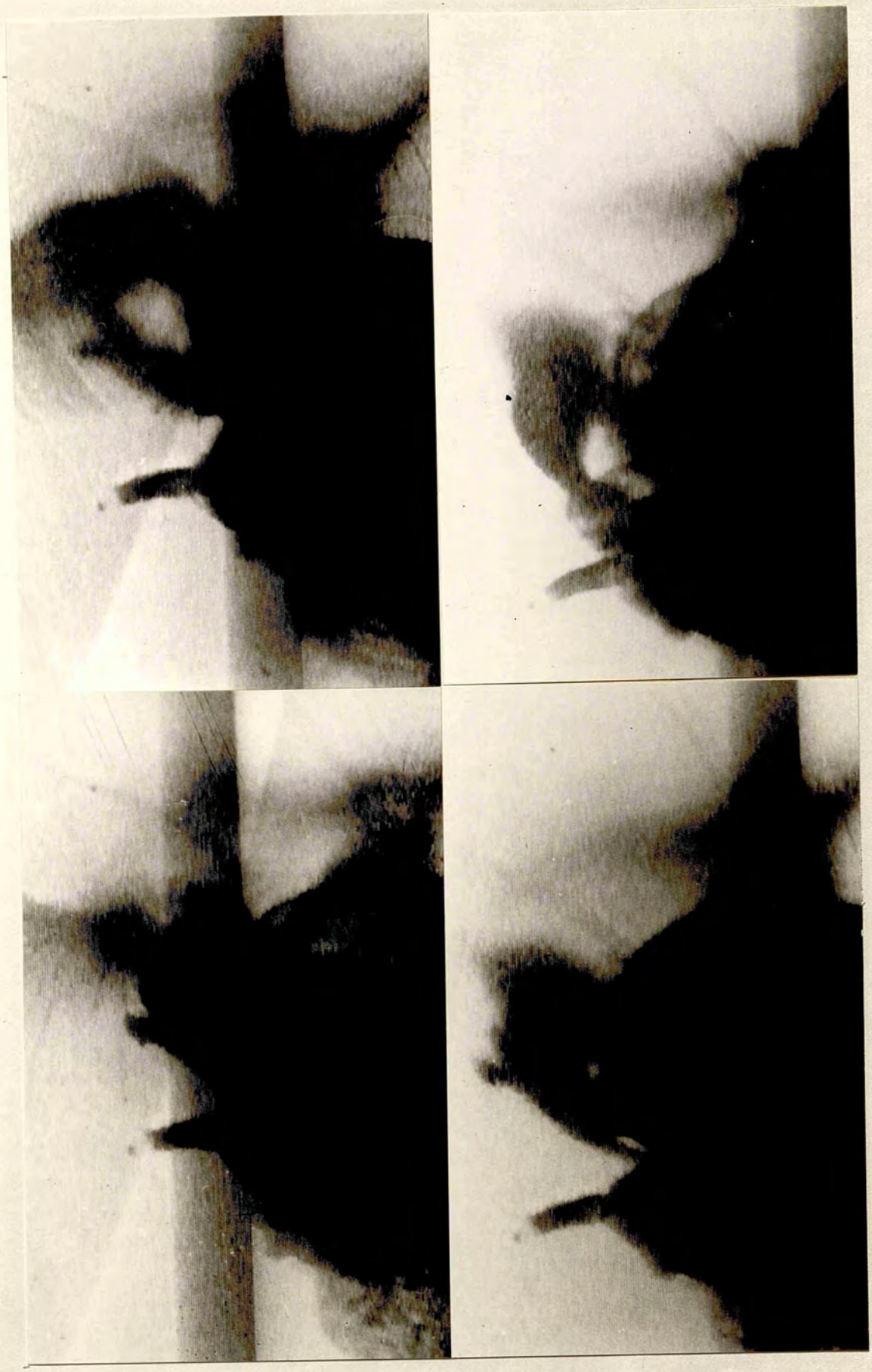
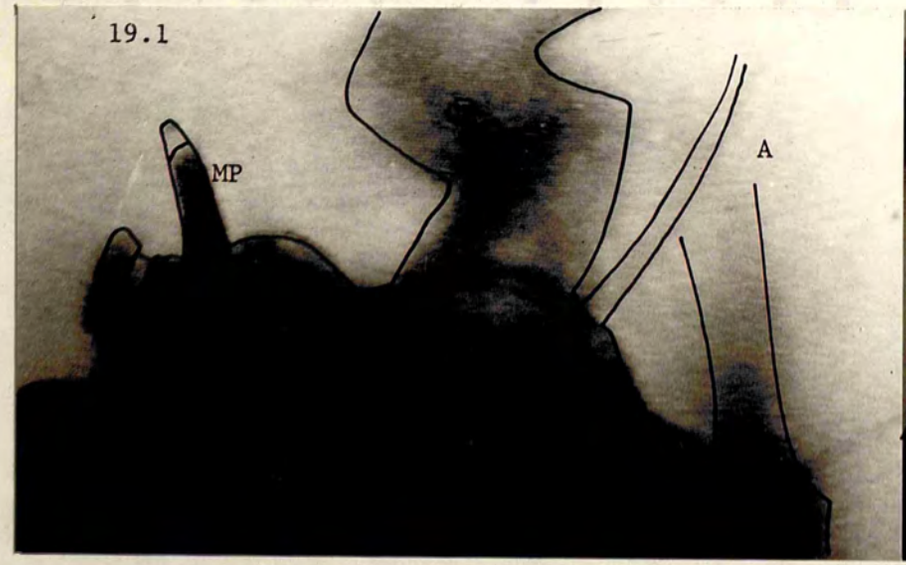


Plate 19. An alternative lateral view of the larval head capsule showing STAGES 4 and 5.

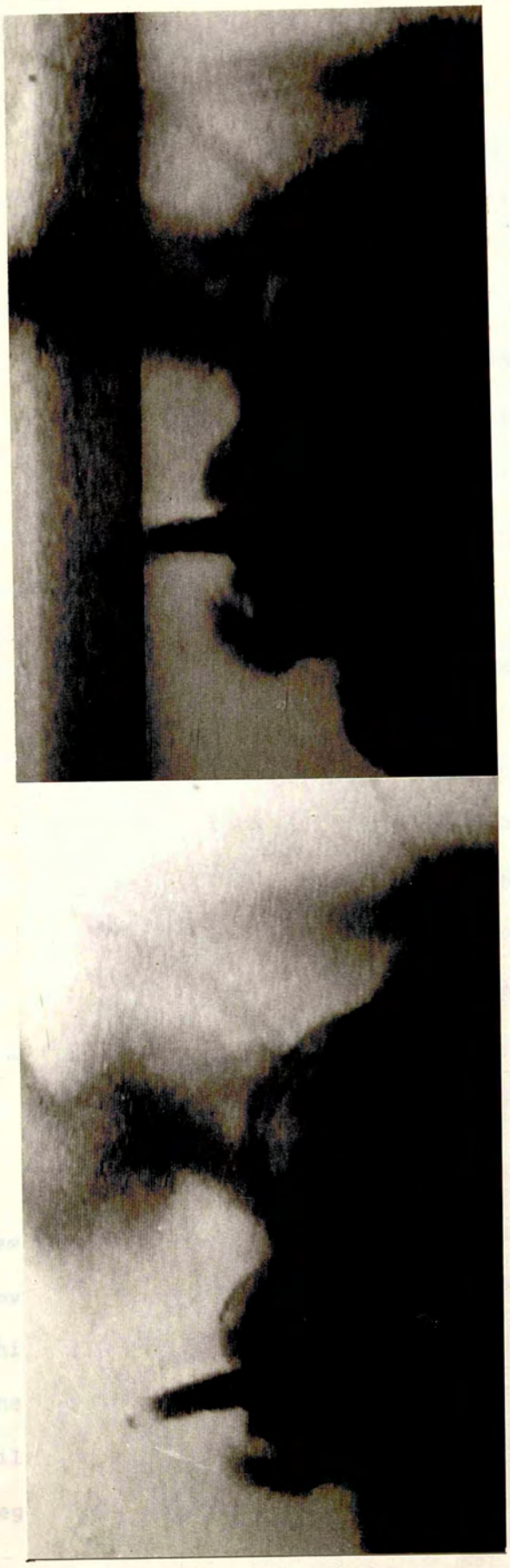
19.1 Stage 4. 19.2 Stage 5.

PLATE 19

19.1



19.2



4.33 The recovery of the mandible to the medial position

Plate 20 shows the recovery of the mandible from STAGES 5 or 8 to the medial position, with 20.4 showing the medial position (STAGES 6 OR 1). This lateral view also demonstrates some of the movements of the maxilla during fan cleaning. On Plates 16 and 17 it can be seen that the maxilla is fully adducted when the mandible is in the medial position and that as the mandible moves towards the cibarium the maxilla abducts progressively showing maximum abduction in Plate 17.2. On Plate 20 the return of the maxilla to full adduction, from abduction in Plate 20.1, occurs as the mandible returns to its medial position.

4.35 The remaining angles from which larvae were viewed

Plates 21 to 25 show the remaining angles from which larvae were observed (resulting from the positions they adopted inside the observation cell). Plate 21 shows a ventral view where both fans are equally visible so that the fan cycles of the two fans are observed to be of equal length. Plates 21.1 and 21.3 show the mandible moving from an angle of 90o degrees to the fan stem (see also Plate 13.1) to an angle of 45o before the fan closes (Plate 21.3).

Plate 21.2 shows STAGE 2 of the fan cycle. Plate 21.3 further demonstrates how the fan filaments come together in a group before moving down into the cibarium (STAGE 3). Following directly from this frame Plate 22.1 shows the final stage of the fan reopening where the fan stem has moved away from the head capsule but the fan filaments have yet to spread fully (STAGE 4). Plate 22.2 shows the beginning of STAGE 5. At this angle it is difficult to see the

mandibles until they are almost in the medial position, while the movements of the maxillae cannot be detected.

Plates 23 to 25 show the head capsule at an angle intermediate between the ventral view of Plates 21 and 22 and the lateral view of Plates 14 and 15. This is the last of the four common angles from which the larvae were observed. Plate 23 shows an ipsi-lateral fan cycle; plate 23.1 shows the mandible in the medial position and the fan open (STAGE 1). Plate 23.2 shows the start of the fan cleaning cycle as the mandible abducts to lie almost parallel to the fan stem (STAGE 2). Plate 23.3 shows the fan tightly closed (STAGE 4), and also demonstrates how the head moves down as the fan closes, obscuring some detail of the mandible movements in this case.

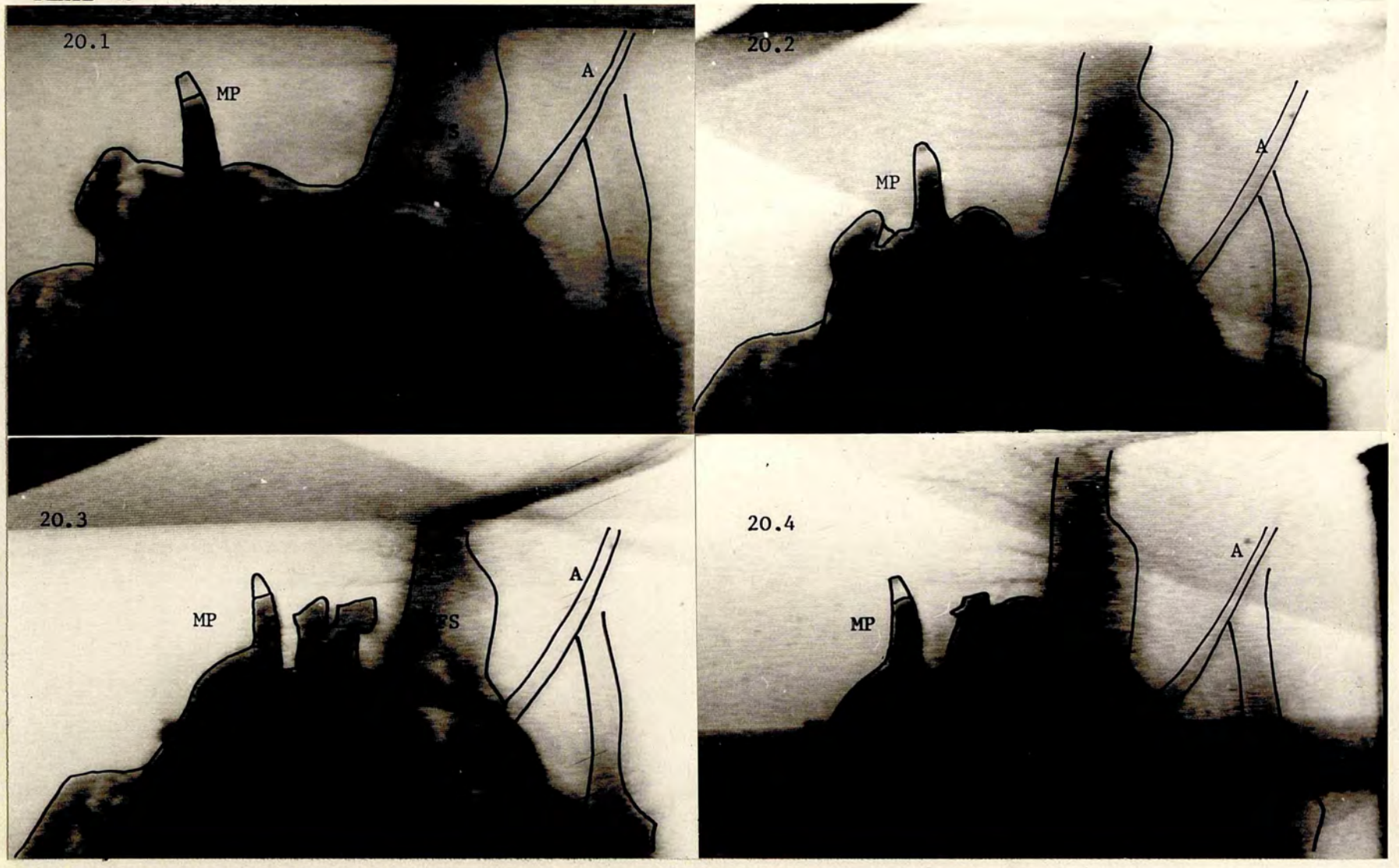
Plate 24 shows the recovery of the mandible to the medial position after the fan has reopened; only two frames of a long STAGE 5 are included (24.1 and 24.2) and the mandible is in the medial position again in 24.3.

Plate 25 shows a contra-lateral fan cycle from the same angle as Plate 24. Again, the movements of the contra-lateral mandible are invisible and all the movements of the fan and mandibles during cleaning are grouped together as STAGE 7. Plate 25.1 shows the fan filaments just starting to bunch together at the top of the plate, while Plates 25.2 and 25.3 show the fan tightly closed. STAGE 8 begins on the final frame showing the reopened fan.

Plate 20. An alternative lateral view of the larval head capsule showing STAGES 5 and 6. (Can also represent STAGES 8 and 1).

20.1, 20.2 and 20.3 Stage 5 (or 8). 20.4 Stage 6 (or 1).

PLATE 20



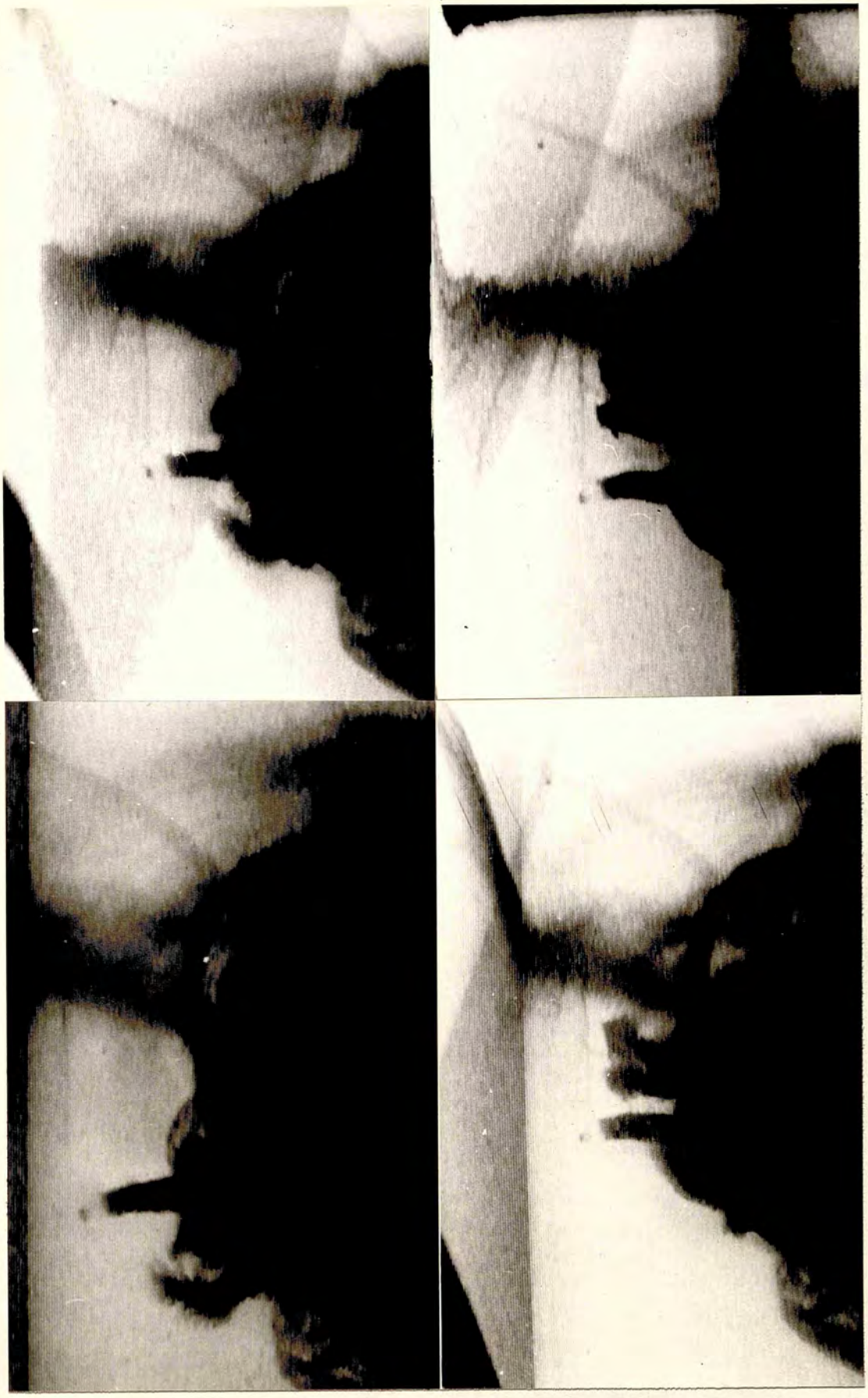


Plate 21. A ventral view of the larval head capsule in which both fans are equally visible (STAGES 1, 2, 3 and 4 of the fan cycle).

21.1 Stage 1. 21.2 Stage 2. 21.3 Stage 3. 21.4 Stage 4.

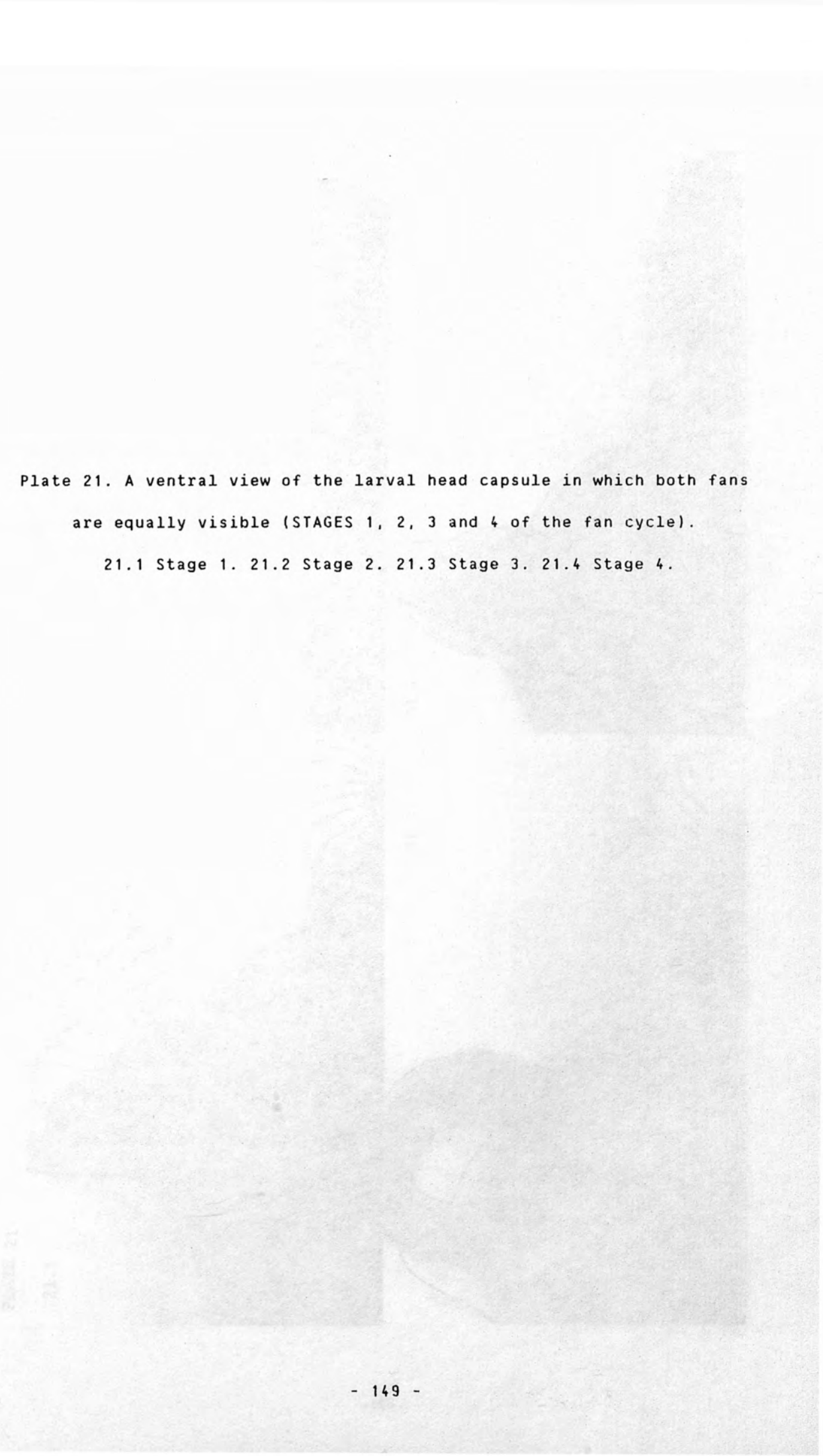
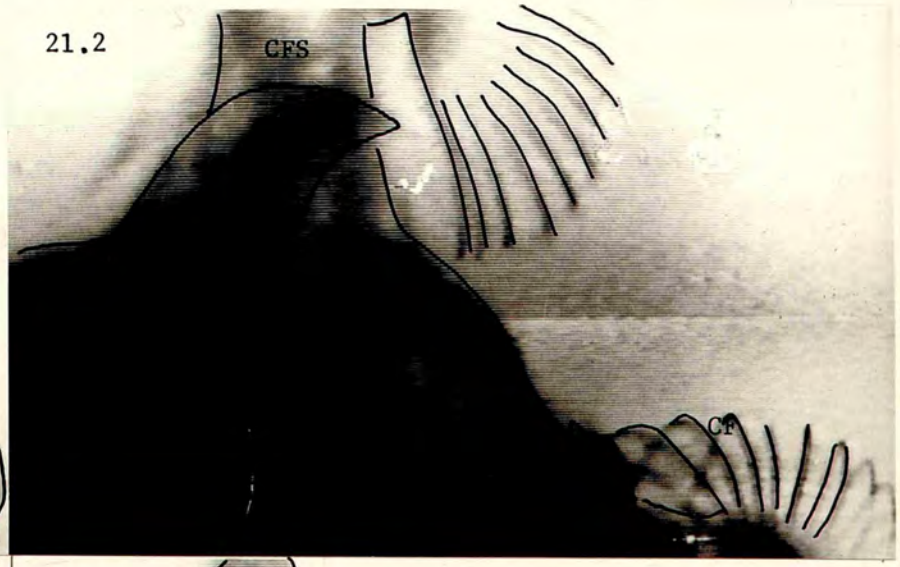


PLATE 21



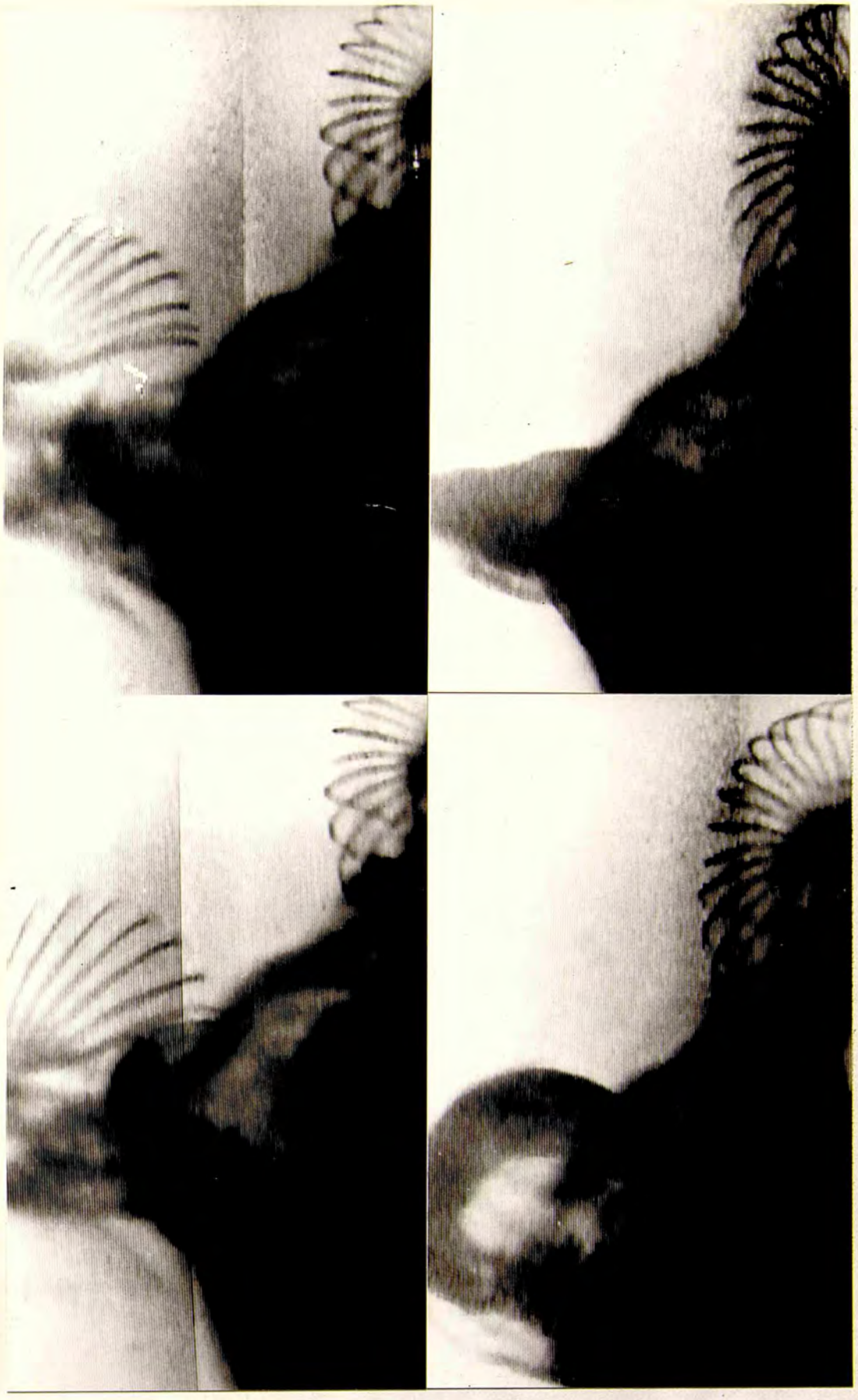
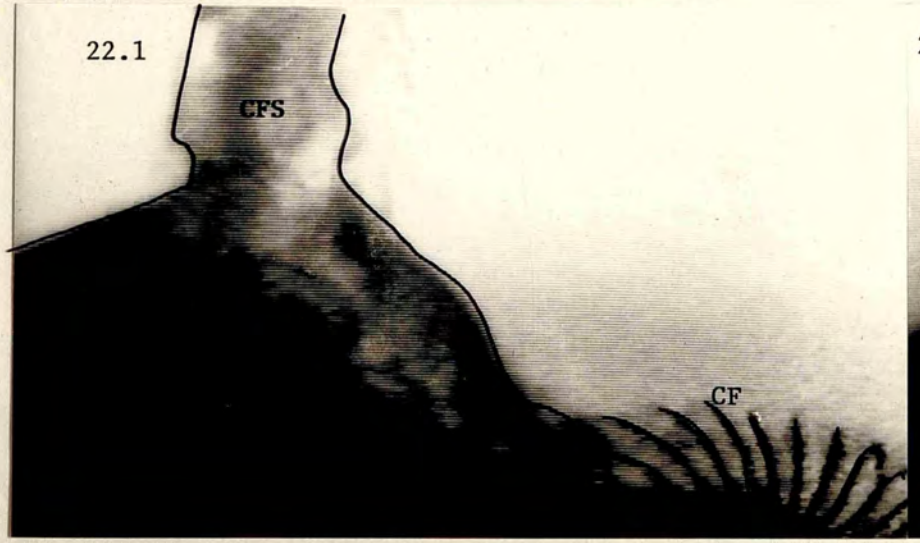


Plate 22. A ventral view of the larval head capsule in which both fans are equally visible (STAGES 4 and 5 of the fan cycle).

22.1 Stage 4. 22.2 Stage 5.

PLATE 22



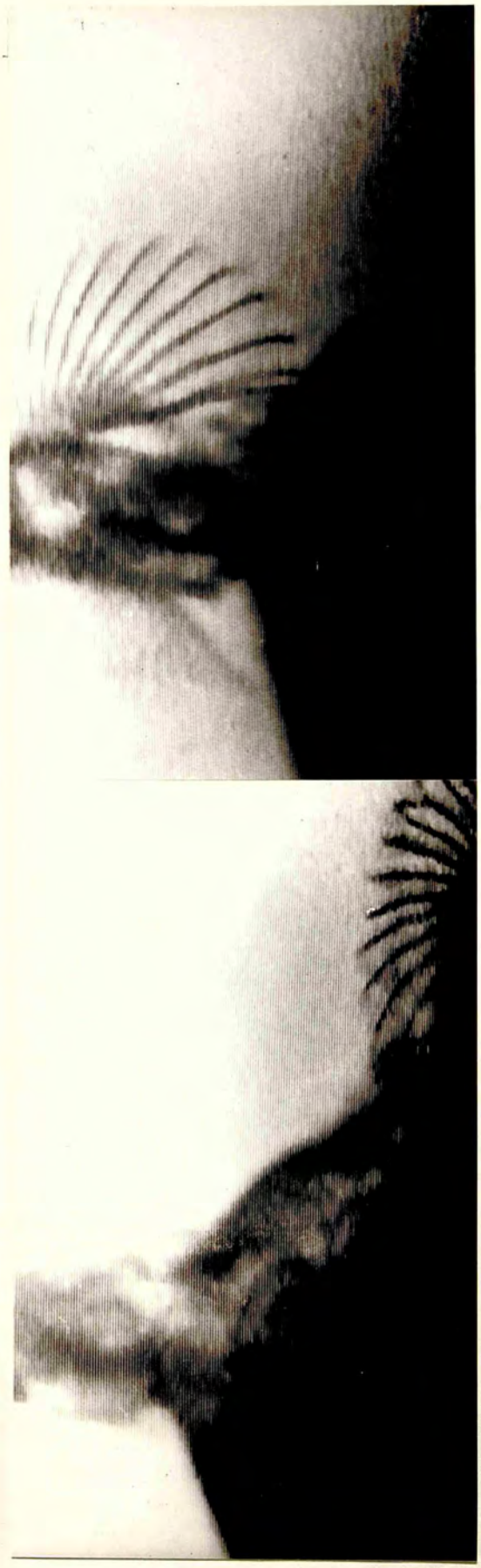
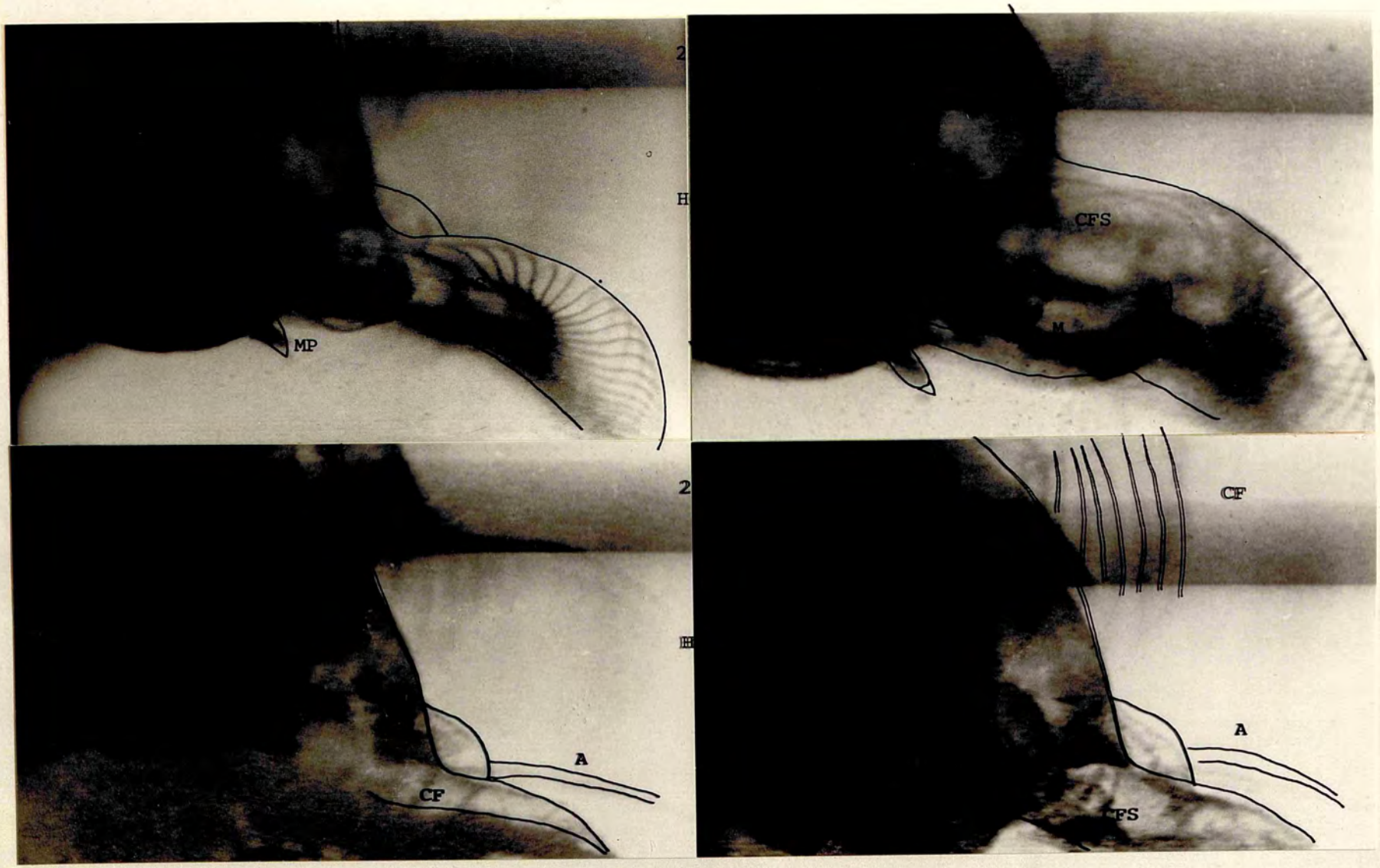


Plate 23. An intermediate lateral view of the larval head capsule showing STAGES 1, 3 and 4 of the fan cycle.

23.1 Stage 1. 23.2 Stage 3. 23.3 and 23.4 Stage 4.

PLATE 23



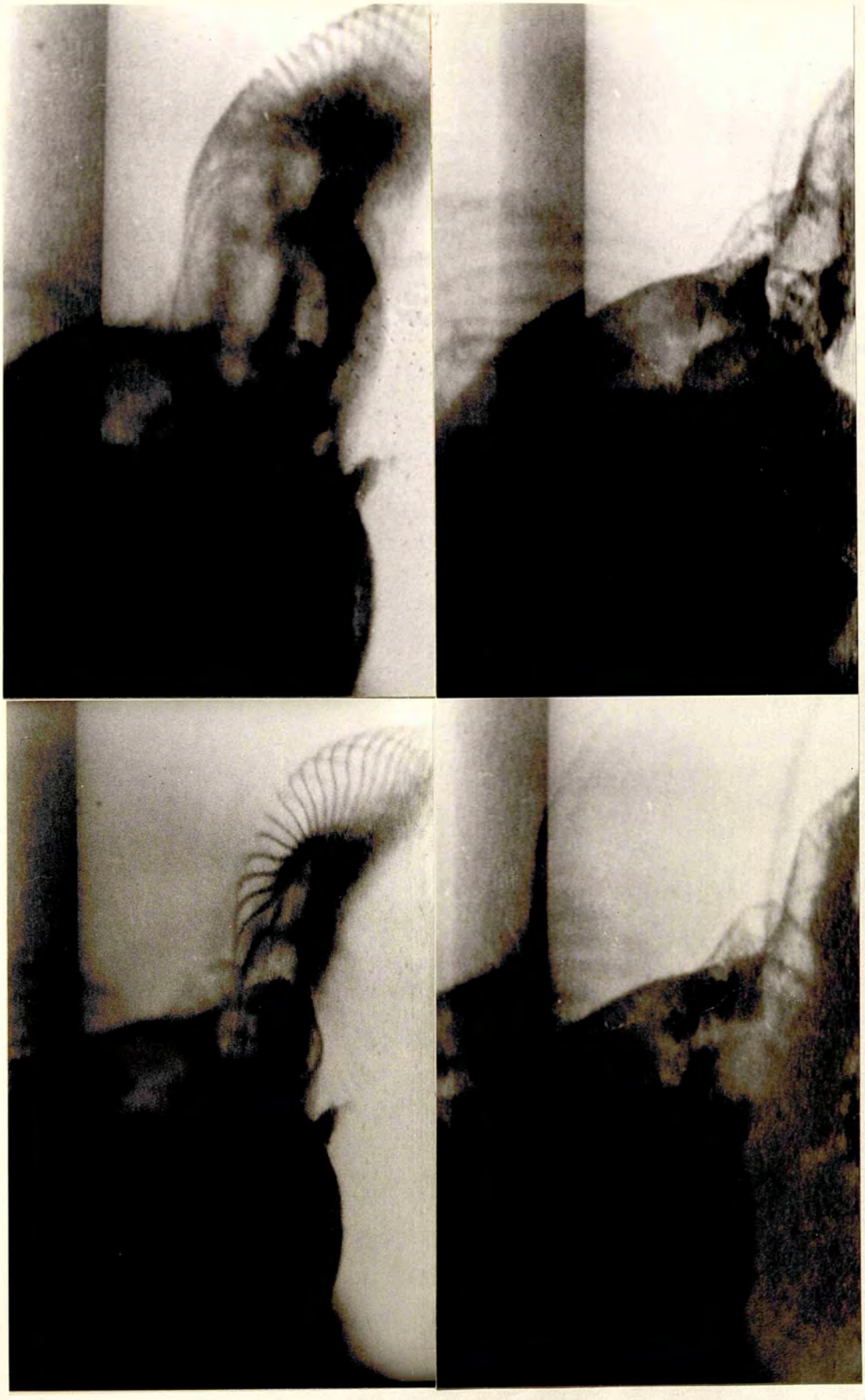
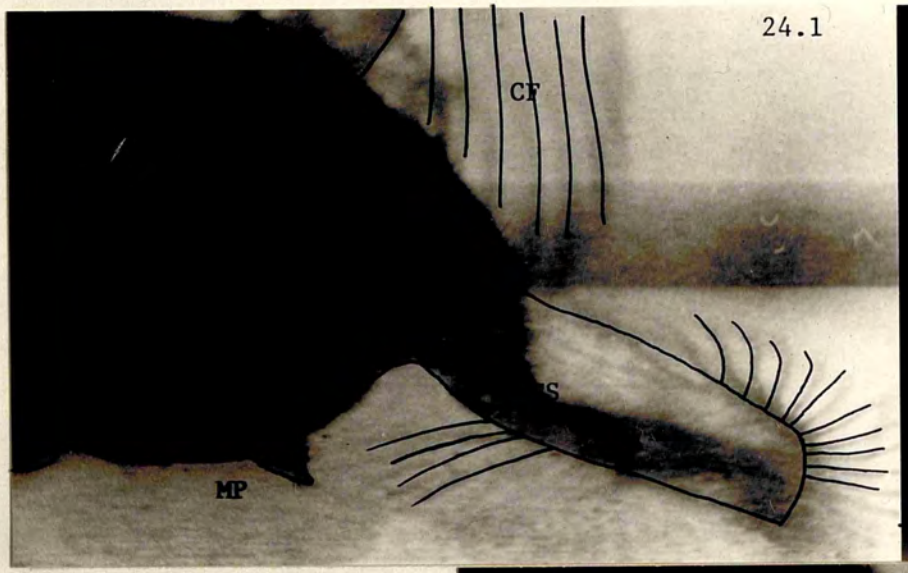
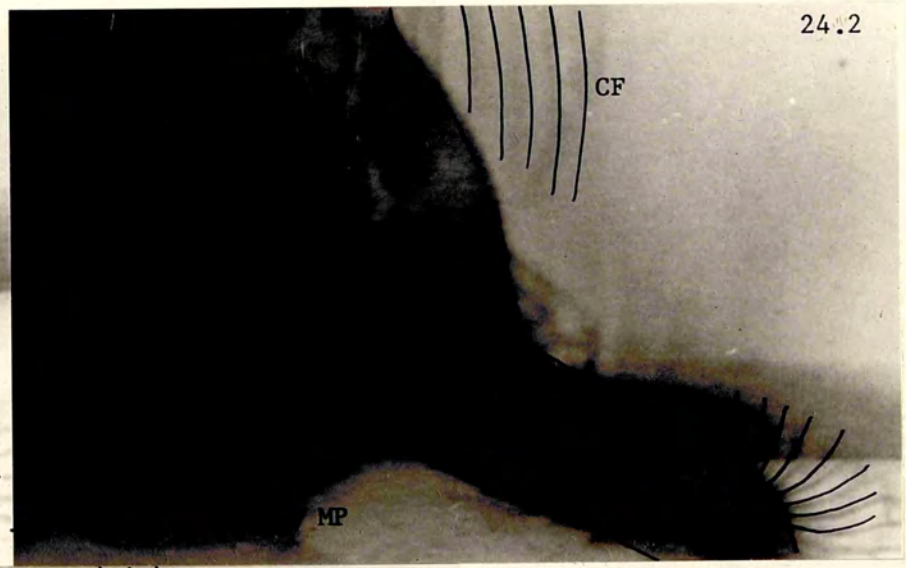


Plate 24. An intermediate lateral view of the larval head capsule showing STAGES 5 and 6 of the fan cycle.

24.1 and 24.2 Stage 5. 24.3 Stage 6.

PLATE 24





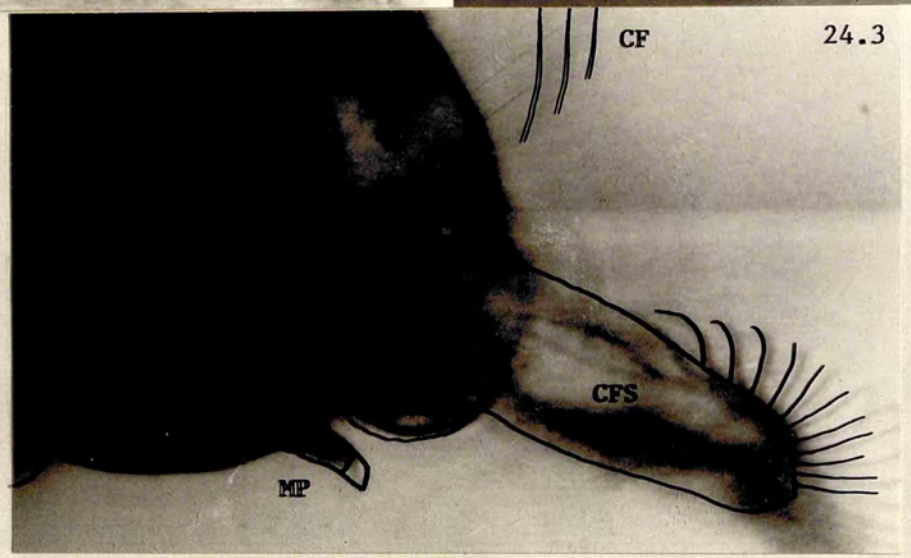
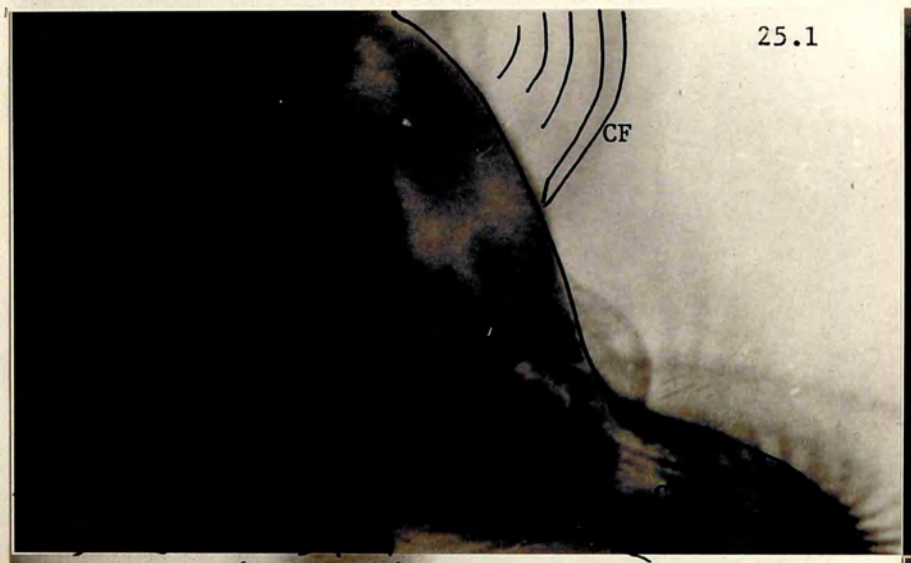
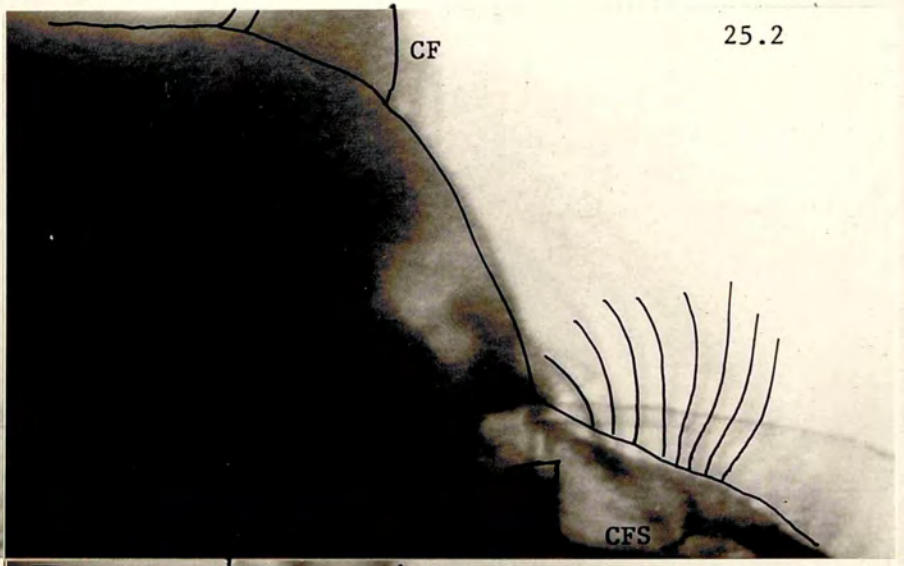


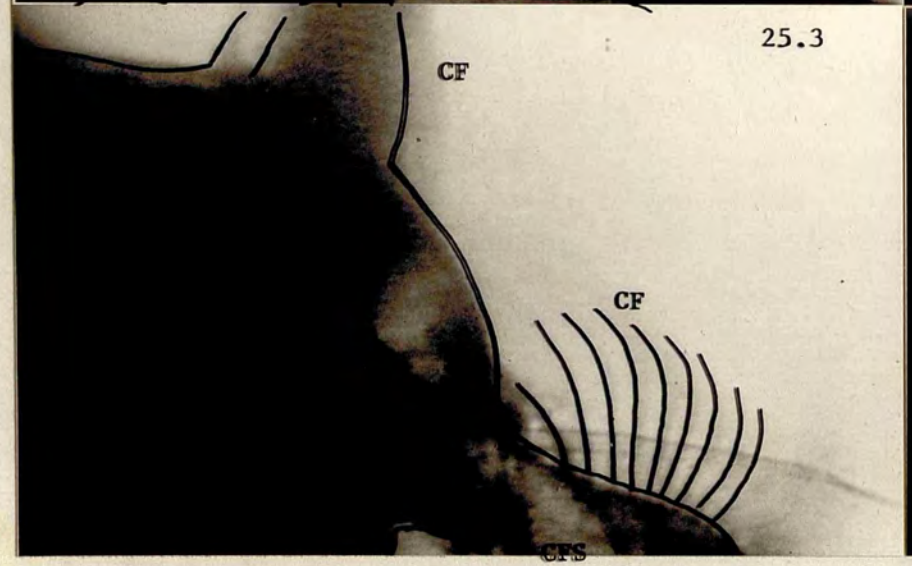
Plate 25. An intermediate lateral view of the larval head capsule showing STAGES 7 and 8 of the fan cycle.

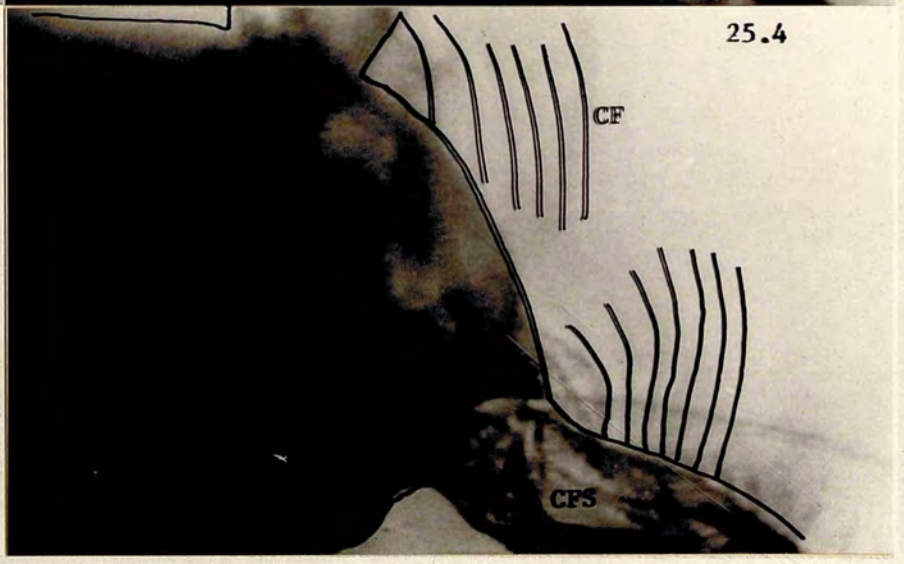
25.1, 25.2 and 25.3 Stage 7. 25.4 Stage 8.

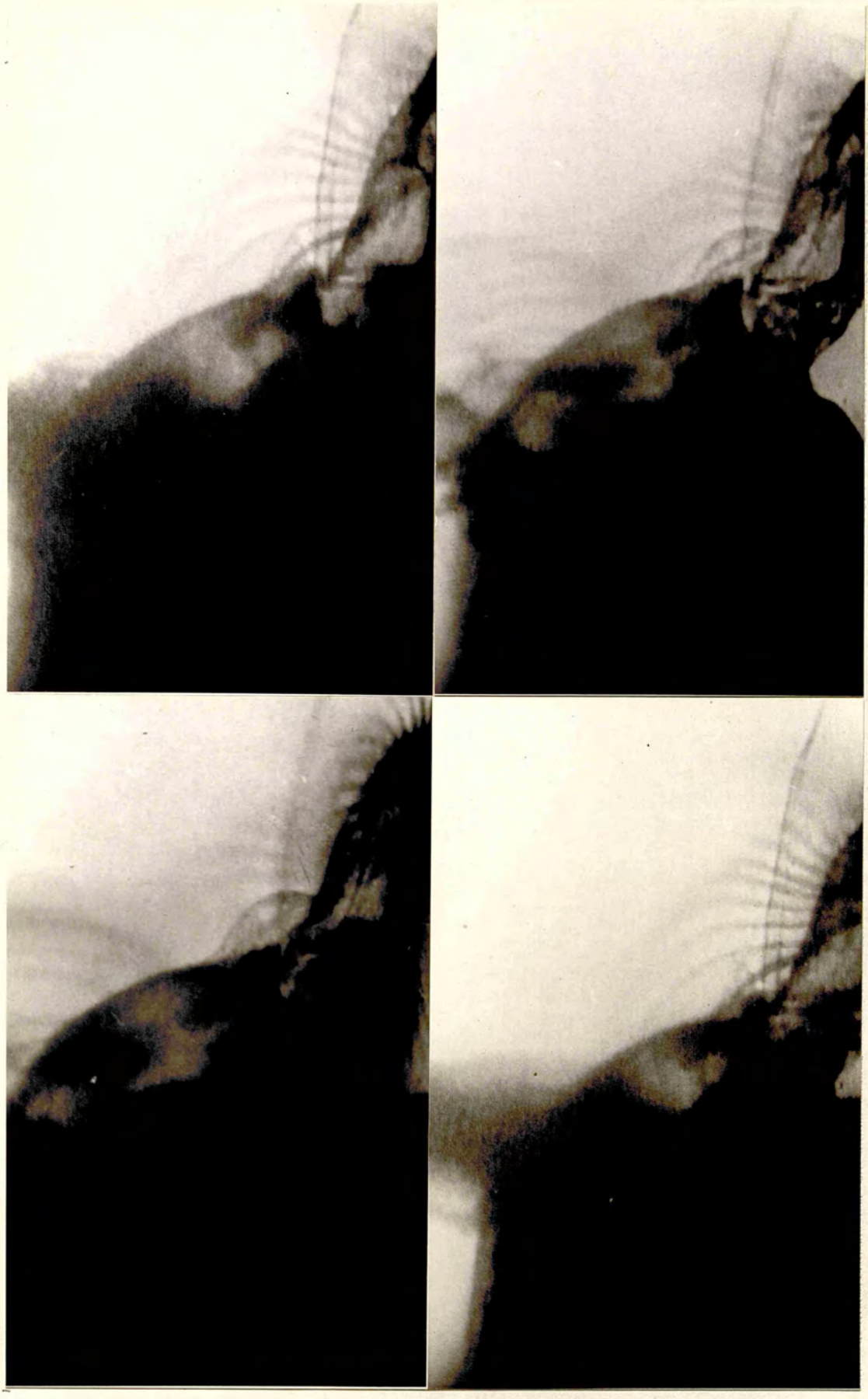
PLATE 25











The mouthpart movements shown in Plates 2 to 8 represent the complete range of movements made during uninhibited feeding behaviour. Significant changes in the range of movements made occurred only when larvae were stimulated with chemical compounds or suspensions of particles, the effects of which will be described in Chapter 6.

4.4 The effect of natural water on larval behaviour

As a result of the simplicity of uninhibited feeding behaviour the behaviour of the larvae can be represented clearly on bar diagrams (see Figures 12 and 13). Using diagrams such as these it is possible to compare the behaviour of larvae in the least stimulating conditions (particle-free distilled water) with that in highly stimulatory conditions (unfiltered natural water) showing the influence of food and chemical compounds in natural water on behaviour.

Each bar on the diagram shows the behaviour pattern of one larva for ten seconds. The diagram shows the duration and frequency of fan cleaning movements and any isolated mandible movements. The upper half of each bar represents the ipsi-lateral fan and mandibles and the lower half, the contra-lateral fan and mandibles. The time when the fan is closed for cleaning is shown by the black bars; movements of the mandibles independent of the fans are shown by the hatched bars. Intervening, unshaded areas show where the fans were open and filtering the water. In terms of Figure 11, the upper black bars represent the sum of stages 2, 3 and 4; the lower black bars, stage 7 alone. The hatched bars show the sum of stages M1 and M2 while the unshaded areas represent stages 1, 5, 6, and 8.

Table 4a describes the symbols used in these diagrams, which are used in the remaining chapters of this thesis.

Although the types of movements made by the larvae were very stereotyped there was considerable variation in the frequency of movements, with less variation in their durations. The two extremes of behaviour may be seen in the block diagrams of Figures 12 and 13. Figure 12 shows examples of the feeding behaviour of eight larvae in particle-free distilled water and Figure 13 shows examples of behaviour in unfiltered natural water, both at water velocities of $17cms^{-1}$ and temperatures of 12° ± 1°C. In unfiltered natural water larvae clean their fans considerably more frequently than in particle-free distilled water. Table 5 shows that on average there were 105 and 170 fan beats min^{-1} in particle-free distilled water and unfiltered natural water. This was a consequence of a reduction in the mean interval between each fan beat from 0.45s in particle-free distilled water to 0.25s in unfiltered natural water.

As the block diagrams show there was comparatively little difference in the time taken to clean the fans in the two different qualities of water and the change in frequency of fan cleaning was almost entirely due to a reduction in the interval between each fan beat.

It is suggested that the interval between fan beats is a more fundamental parameter of larval behaviour than the frequency of fan beats and it has been used in several of the analyses of larval feeding behaviour reported here in preference to fan beat frequency. It is likely that neural mechanisms operate mainly on this quantity rather than on fan beat frequency which is a composite of mean interval between fan beats, the time taken to clean the fan and the

duration of any extra mandible movements (see Figure 11). The interval between fan beats is also likely to be an important quantity because it is the time for which the fans are open and filtering the water (it is synonymous with the sums of stages 5 and 6 and 8 and 1). The time for which fans are open in any given time may influence how much food they can gather.

The factors responsible for the reduction in the interval between each fan beat in natural water, when compared to purified water, are considered in the following chapters.

TABLE 4a A key to symbols used in bar charts (Figures 12, 13, 15 16, 26, 27, 30, 31, 33, 34a, b, c and d and Appendix Figures 1a-d).

Length of bar: most bars show a ten second extract of behaviour.

Vertical subdivision of bar: upper half represents ipsi-lateral fan and mandible; lower half represents contra-lateral fan and mandible. Maxilla and other mouthparts are not represented separately from the mandibles.

Solid shading: duration of each fan cleaning cycle (sum of STAGES 2, 3 and 4 for ipsi-lateral fan, STAGE 7 for contra-lateral fan).

Hatched shading: duration of mandible cycle (when fans remain open and filtering water; sum of STAGES M1 and M2)

Unshaded areas: fan open and filtering water. Mandible abducting to medial position or in the medial position (STAGES 5, 6, 8 and 1).

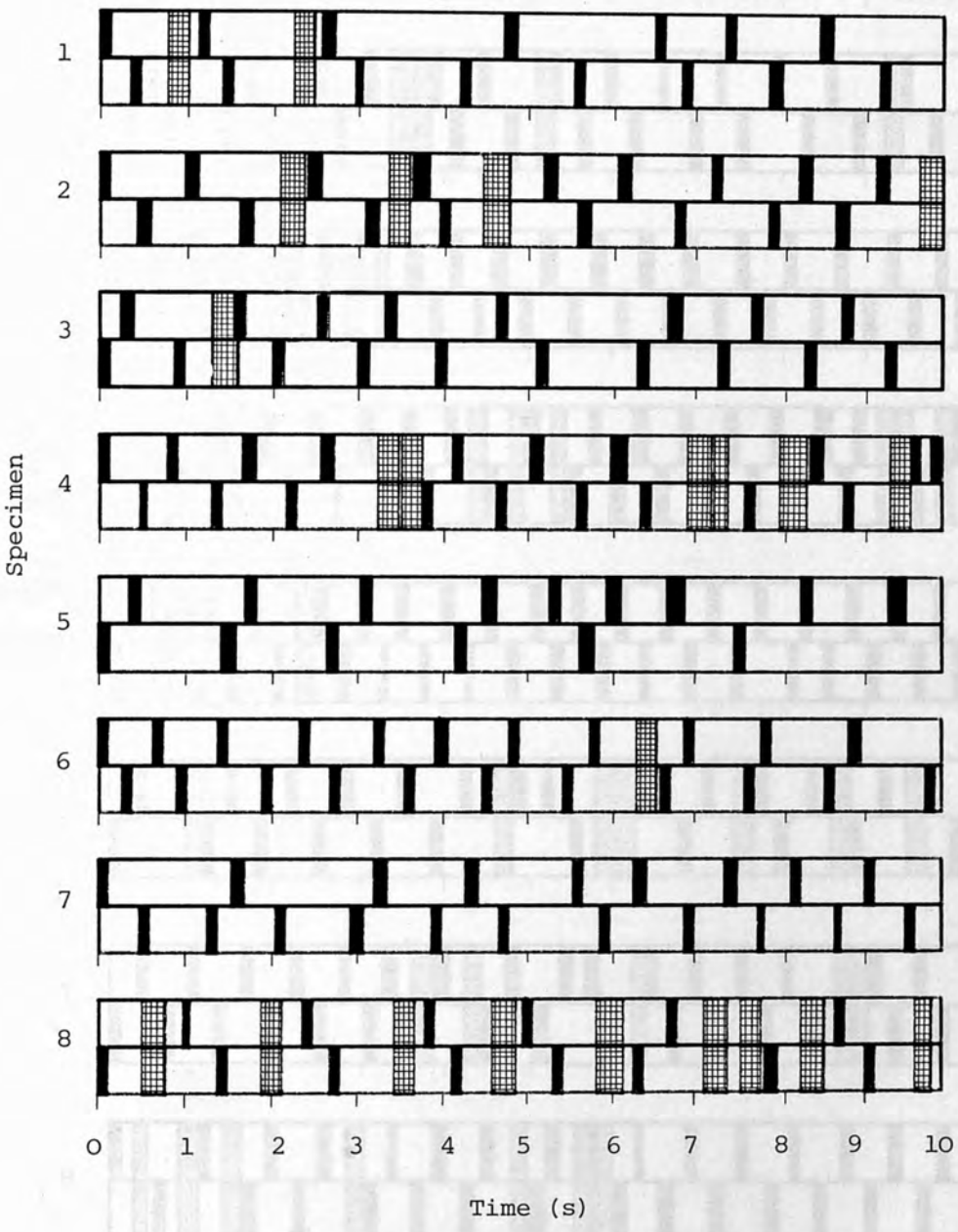


Figure 12 Examples of the pattern of larval behaviour in particle-free distilled water. Water velocity 17cms^{-1}; water temperature 12^{o} $\pm$ 1^{o}C. See Table 4a for key to symbols.

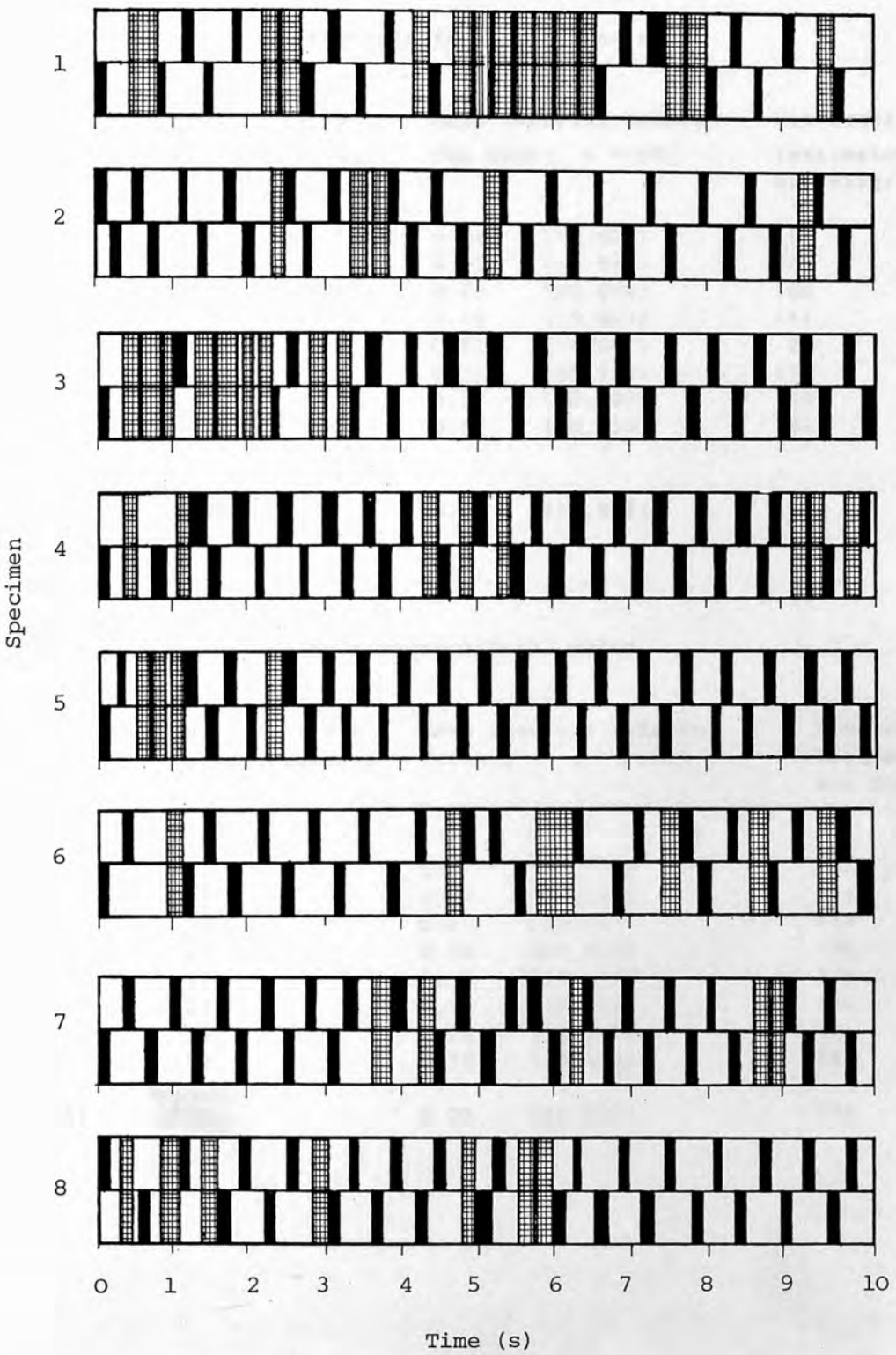


Figure 13 Examples of the pattern of larval behaviour in unfiltered natural water. Water velocity 17cms^{-1}; water temperature 12^{o} $\pm$ 1^{o}C. See Table 4a for key to symbols.

TABLE 5 The mean intervals between fan beats and number of fan beats in the extracts of larval behavioural depicted in Figures 12 and 13.

(a) Particle free distilled water

Specimen	Number of fan beat intervals	Mean interval between fan beats, s (±SE)		Fan beats min^{-1} (estimated from bar diagrams)
1	14	0.54	(±0.028)	90
2	16	0.43	(±0.013)	102
3	17	0.43	(±0.010)	108
4	19	0.40	(±0.013)	114
5	14	0.53	(±0.027)	90
6	21	0.34	(±0.009)	132
7	20	0.37	(±0.007)	120
8	14	0.59	(±0.030)	84
Total	135	0.45	(±0.002)	105

(b) Unfiltered natural water

Specimen	Number of fan beat intervals	Mean interval between fan beats, s (±SE)		Fan beats min^{-1} (estimated from bar diagrams)	
1	17	0.47	(±0.113)	102	
2	29	0.23	(±0.020)	174	
3	29	0.22	(±0.041)	174	
4	31	0.18	(±0.020)	192	
5	37	0.16	(±0.010)	210	
6	23	0.29	(±0.034)	150	
7	29	0.22	(±0.022)	174	
8	31	0.19	(±0.019)	186	
Total	226	0.25	(±0.034)	170	(±11.5)

4.5 The absence of variation in larval behaviour

In order to validate an experimental method where a short extract of behaviour was used to represent the typical behaviour of the larvae it was necessary to investigate whether or not their behaviour in constant conditions changed with time. Larvae kept in particle-free distilled water might initially be expected to deteriorate in condition quite rapidly without food. If experiments were to last long enough for larvae to become acclimated, and for a variety of procedures to be carried out on a number of animals, it was necessary to know whether such a deterioration occurred.

Larvae were observed in controlled conditions in particle-free distilled water, filtered natural water and unfiltered natural water for three and a half to four hours, after one hour acclimation, to determine whether their condition deteriorated. Behaviour was recorded at approximately hourly intervals and the differences in the behaviour pattern throughout this time tested statistically using non-parametric* analysis of variance. This led to the testing of the hypotheses that there were no significant changes in the mean interval between fan beats with time, in the time taken to clean the cephalic fan or in the duration of individual extra mandible movements.

The results of the observations on mean intervals between fan beats are shown graphically in Figure 14 while the statistical tests of variations in behaviour in each water quality are shown in Tables 6 and 7. The values plotted on the graphs are the individual mean intervals between fan beats; this quantity is a convenient measure of activity, accurately summing up larval behaviour in a single

* because data were collected sequentially

value. Individual mean intervals between fan beats were plotted to indicate the range that occurred. The individual fan cleaning times are shown in Appendix 1 whilst the duration and number of extra mandible movements are shown in Appendix 2.

There were no significant changes in the mean intervals between fan beats in 3.5 hours in any of the water qualities. Similarly there were no changes in the time taken to clean the cephalic fans or in the number and duration of extra mandible movements. Although there was a wide range of mean intervals in particle-free distilled water (from 0.8s to 0.171s) and filtered natural water (0.91s to 0.22s) the results show that there was no significant deterioration in the condition of the larvae, measured in terms of the behaviour pattern, in this time.

When the mean intervals between fan beats in particle-free distilled water and filtered natural water [illegible] was compared there was no significant difference suggesting that the chemical quality of the water had rather little effect on behaviour. In contrast to this the mean interval between fan beats in unfiltered natural water was about half that in the two other water qualities and there was a smaller range among individual means (see Figure 14c and Table 6d). Clearly the presence of food in the water stimulated fan cleaning.

While Table 7 shows that there was no significant change in the time taken to clean the fans (the sum of STAGES 2, 3 and 4; STAGE 7 was not considered in this analysis) throughout the 3.5 hours in any water quality, there was a difference in the time taken to clean the fans between water qualities. In unfiltered natural water it took 30ms less than in particle-free distilled water.

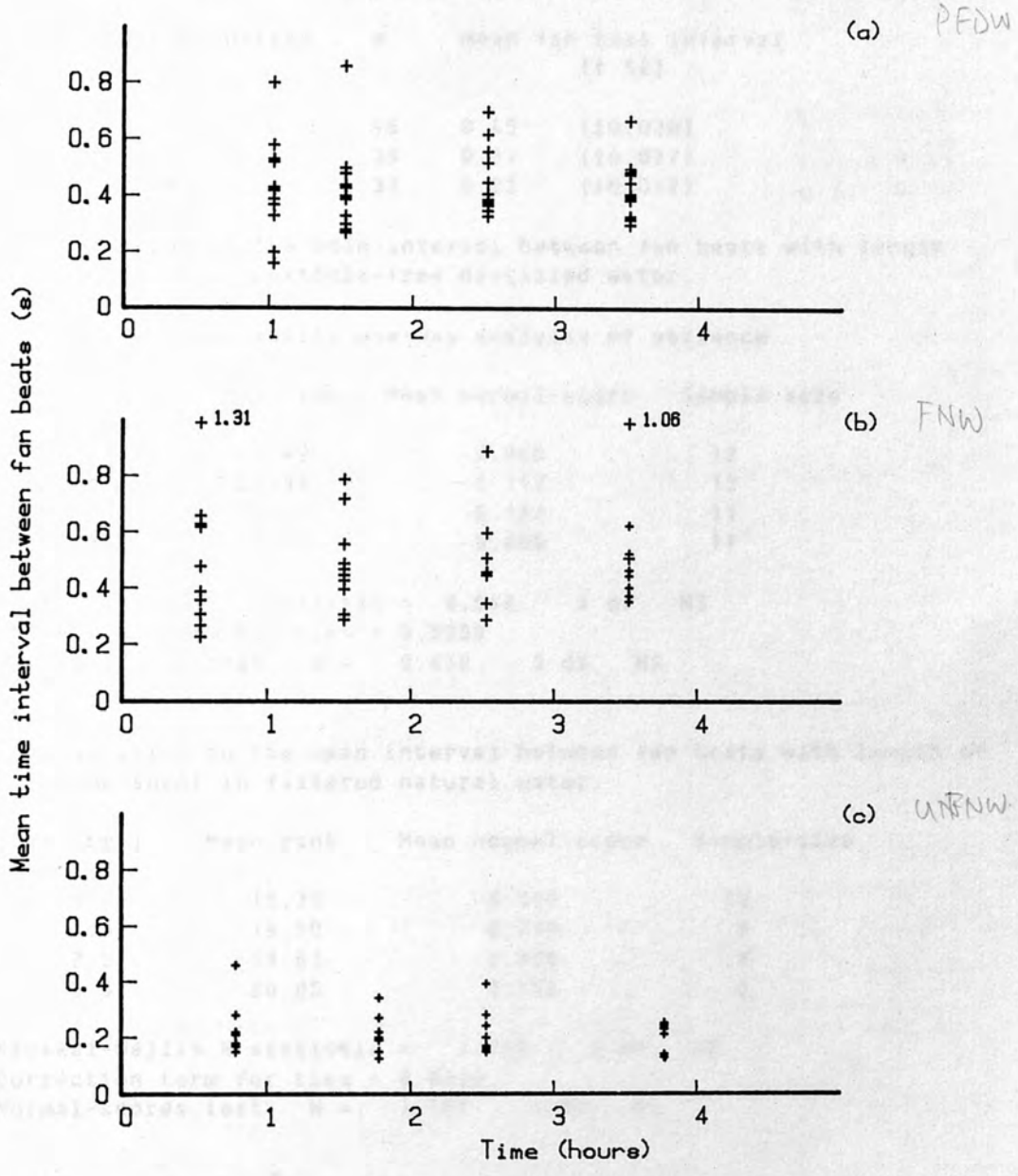


Figure 14 Variation in the mean interval between fan beats in three water qualities over four hours. (a) particle-free distilled water (b) filtered natural water (c) unfiltered natural water. All observations at a water velocity of 17cms^{-1}. Temperature: (a) 12^{o} $\pm$ 1^{o}C (b) 11^{o} $\pm$ 1^{o}C (c) 12^{o} $\pm$ 1^{o}C.

n = no. of Simulium-observation
= no 10 sec intervals

TABLE 6. The mean duration of the interval between fan beats in particle-free distilled water, filtered natural water and unfiltered natural water: the absence of variation in mean intervals with time (see Figure 14). n=no. of larvae observed

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Water quality	n	Mean fan beat interval (± SE)	
PFDW	46	0.45	(±0.020)
FNW	36	0.51	(±0.037)
UFNW	33	0.23	(±0.012)

0.41 - 0.49
0.44 - 0.58
0.20 - 0.25

(a) Variation in the mean interval between fan beats with length of time spent in particle-free distilled water.

Kruskal-Wallis one-way analysis of variance

Time (hrs)	Mean rank	Mean normal-score	Sample size
1	23.42	-0.046	12
1.5	21.58	-0.117	12
2.5	25.73	0.187	11
3.5	23.45	-0.009	11

Kruskal-Wallis H Statistic = 0.548 3 df NS
Correction term for ties = 0.9998
Normal scores test W = 0.656 3 df NS

(b) Variation in the mean interval between fan beats with length of time spent in filtered natural water.

Time (hrs)	Mean rank	Mean normal-score	Sample-size
1	15.35	-0.290	10
1.5	19.50	0.079	9
2.5	19.63	0.098	8
3.5	20.00	0.156	9

Kruskal-Wallis H statistic = 1.249 3 df NS
Correction term for ties = 0.9999
Normal-scores test W = 1.387 3 df NS

Kruskal-Wallis one-way analysis of variance

(c) Variation in the mean interval between fan beats with length of time spent in unfiltered natural water.

Time (hrs)	Mean rank	Mean normal-score	Sample size
0.75	17.69	0.135	8
1.75	17.11	-0.020	9
2.5	15.94	-0.049	8
3.75	17.25	-0.063	8

Kruskal-Wallis H Statistic = 0.144 3 df NS
Correction term for ties = 0.9992
Normal-scores test W = 0.236 3 df NS

(d) The effect of water quality on the mean intervals between fan beats.

Water quality	Mean rank	Mean normal-score	Sample size
PFDW	69.86	0.317	46
FNW	76.11	0.542	36
UFNW	21.71	-1.033	33

Kruskal-Wallis H Statistic = 55.536 2DF P<0.001
Correction term for ties = 0.999
Normal-scores test W = 53.69 2 DF P<0.001

Pairwise contrasts Based on ranks

Contrast	lower confidence limit	Upper confidence limit
PFDW with FNW	-24.41	11.90
PFDW with UFNW	29.52	66.76*
FNW with UFNW	34.73	74.06*

Any confidence interval that does not include zero is significant at the 5% level (*).

TABLE 7 The time taken to clean the fans in particle-free distilled water and unfiltered natural water: the absence of variation with time. n=no. of larvae observed

Water quality	n	Mean duration of fan cleaning (sum stages 2, 3 and 4), seconds	±SE
PFDW	45	0.14	0.005
UFNW	33	0.12	0.002

(a) Variation in the time taken to clean the cephalic fan with length of time spent in particle-free distilled water.

Time (hrs)	Mean rank	Mean normal-score	Sample size
1	25.46	0.128	12
1.5	20.96	-0.116	13
2.5	21.10	-0.098	10
3.5	24.60	0.100	10

Kruskal-Wallis H Statistic = 1.094 3 df NS
Correction term for ties = 0.9974
Normal-scores test W = 0.653 3 df NS

(b) Variation in the time taken to clean the cephalic fan with length of time spent in unfiltered natural water.

Time (hrs)	Mean rank	Mean normal-score	Sample size
0.75	19.56	0.282	8
1.75	18.06	0.078	9
2.5	18.06	0.107	8
3.75	12.19	-0.478	8

Kruskal=wallis H Statistic = 2.760 3 df NS
Correction term for ties = 0.9953
Normal-scores test W = 3.077 3 df NS

Table 7 continued

(c) The effect of water quality on the time taken to clean the fans

Group	Mean rank	Mean normal-score	Sample size
PFDW	50.72	0.475	45
UFNW	24.20	-0.647	33

Kruskal-Wallis H Statistic = 26.131 1 DF P<0.001
Correction term for ties = 0.9983
Normal-scores test W = 26.108 1 DF P<0.001

Appendix 2 shows that there was no change in the time taken for individual extra mandible movements over four hours in these observations, confirming the stability of the duration and frequency of the components of larval feeding behaviour. As a result of these observations further experiments in particle-free distilled water were restricted to a length of four hours.

The behaviour patterns of two individual larvae in particle-free distilled water are shown in the block diagrams in Figures 15 and 16, demonstrating graphically the regularity of behaviour over three and a half hours. The short extracts appear to be representative of behaviour both with, and without, food.

It should be noted that these block diagrams are concerned with a shorter time than those of Schroeder (1980) who also displayed larval behaviour on block diagrams. He did not describe separate mouthpart movements but simply the times when larvae were and were not feeding. Such observations, being less precise, are not directly comparable to those described here although Schroeder was also concerned with the effects of food on larval feeding behaviour (see Section 1.4).

It is important to note that there appears to be little, if any, difference in the type of movements seen in particle-free distilled water and unfiltered natural water. Clearly, conclusions drawn from experiments in particle-free distilled water would be of little significance if the behaviour patterns were unpredictably different from those seen in natural water. Differences in behaviour in the two water qualities are differences in the frequency and duration of various movements rather than in the types of movement observed.

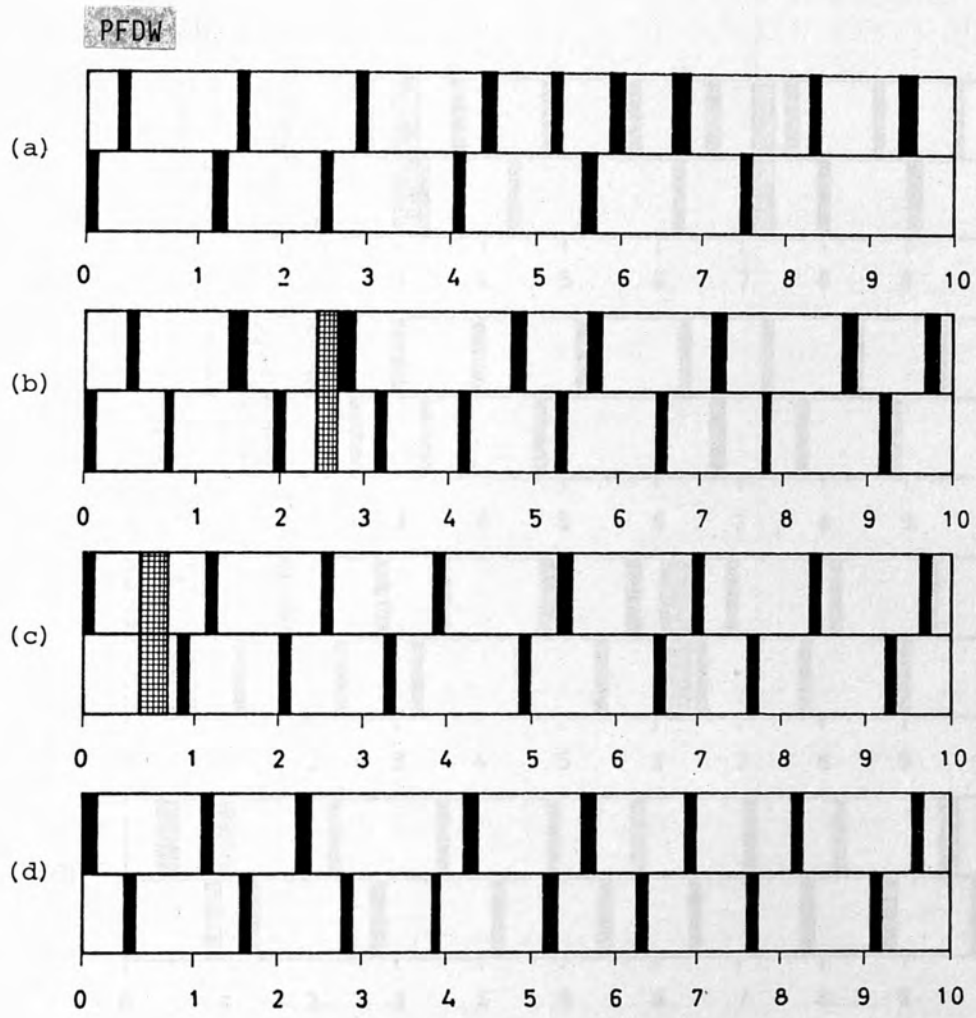


Figure 15. Ten second extracts of the behaviour of one larva at (a) 1hr (b) 1.5hr (c) 2.5hr (d) 3.5hr showing the absence if variation in individual behaviour with time. Water velocity 17cms^{-1}; water temperature 11^{o} $\pm$ 1^{o}C. See Table 4a for key to symbols.

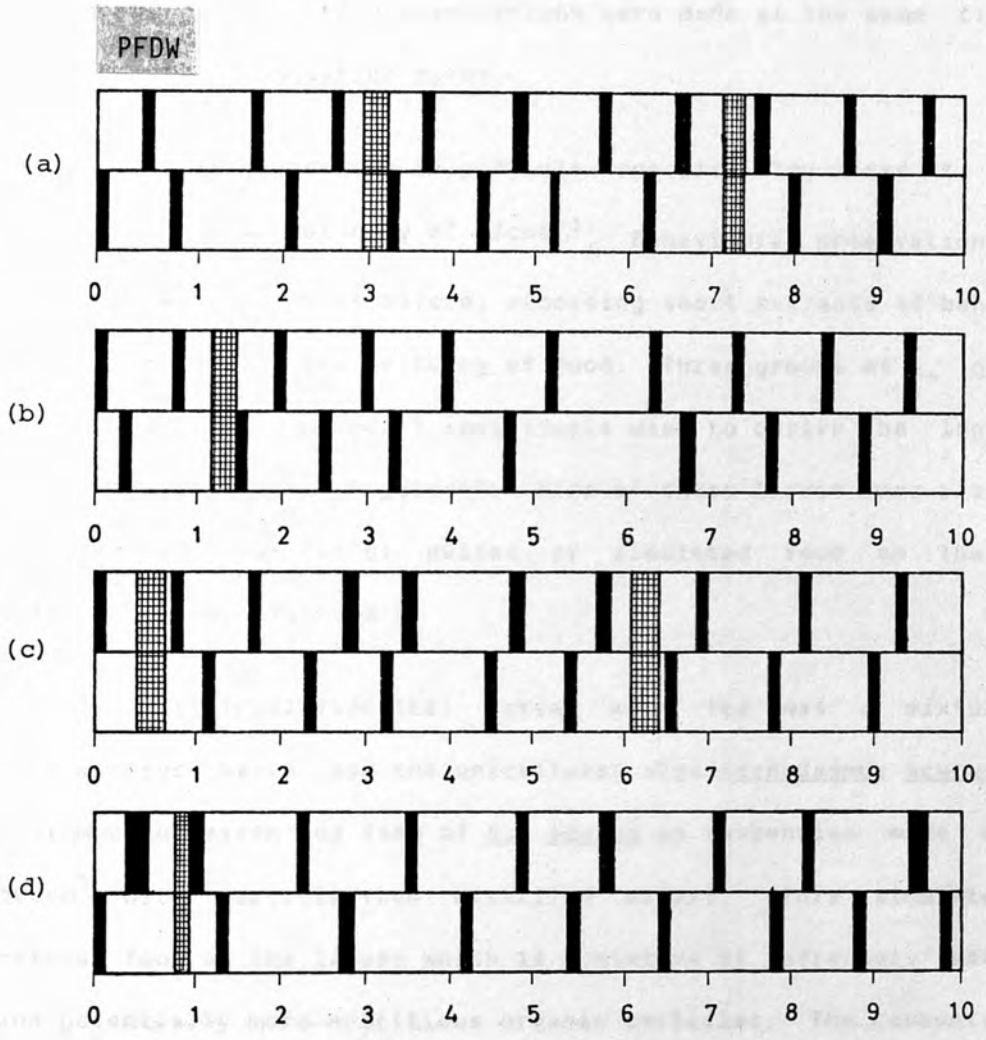


Figure 16. Ten second extracts of the behaviour of one larva at (a) 1hr (b) 1.5hr (c) 2.5hr (d) 3.5hr showing the absence of variation in individual behaviour with time. Water velocity 17cms^{-1}; water temperature 11^{o} $\pm$ 1^{o}C. See Table 4a for key to symbols.

4.6 Larval ingestion rates and the effect of pulses of simulated food on larval feeding behaviour

Having described the effect of natural water on the pattern of larval behaviour the effect of pulses of simulated food on behaviour was investigated. These observations were made at the same time as measurements of ingestion rates.

Larvae were observed in particle-free distilled water at 11° ± 1°C at a water velocity of $17cms^{-1}$. Behavioural observations were made in the same way as before, recording short extracts of behaviour before and during the delivery of food. Three groups of *S. ornatum* larvae were observed and 12 individuals used to derive the ingestion rate data plotted in Figure 17. Nine of these larvae were also used to describe the effect of pulses of simulated food on the mean interval between fan beats.

The artificial food that larvae were fed was a mixture of diatomaceous earth and the unicellular alga *Scenedesmus acutus* (5mg diatomaceous earth and 15mg of *S. acutus* in suspension made up to $500cm^3$ with particle-free distilled water). This simulated the natural food of the larvae which is a mixture of refractory material and potentially more nutritious organic particles. The concentration that was injected into the water was approximately $30mgl^{-1}$ giving an estimated concentration in the water of slightly more than $10mgl^{-1}$ (when pulses of one second duration were injected into 1.66 cm^3 of particle-free distilled water at 30s intervals). This concentration was about five times greater than the average concentration of suspended material that would be found in a chalk stream such as that from which the larvae came (Dawson, 1981). However, because the food

was injected in short pulses it appeared to cause little inhibition of feeding (Gaugler and Molloy, 1982).

Before the injection of pulses of food a small quantity of powdered charcoal was injected into the water. This was ingested by all the larvae and formed a short black band in the gut which was used as a marker to measure the rate of progress of food through the gut. The behaviour of the larvae was recorded before the injection of either food or charcoal and then during the injection of food. It was possible to observe directly the movement of gut contents through the gut with a stereomicroscope.

The position of the gut marker in relation to the thoracic segments was plotted against time for each larva; Appendix 3 gives the raw data used to to plot Figure 17 where the data for all larvae are pooled showing that, at least in the short term, feeding proceeded at a constant rate. In the conditions of the experiment larvae took between 20 and 40 minutes to displace the plug from the beginning of the fore gut to the first loop of the hind gut. This ingestion rate is within the range of values commonly observed in the field. Larvae appeared to fill their guts at a constant rate suggesting a correlation with the constancy of the behavioural pattern already described.

The behaviour of larvae, measured as the mean interval between fan beats, before and during the provision of short pulses of food particles, is shown in Table 8.

TABLE 8 The effect of simulated food (a mixture of diatomaceous earth and algae) on the mean interval between fan beats in *S. ornatum*.

Specimen	Mean interval between fan beats (s)	
	PFDW (no food)	PFDW (with food)
1	0.72	0.47
2	1.26	0.55
3	0.68	0.37
4	0.52	0.36
5	0.55	0.36
6	0.55	0.30
7	0.65	0.32
8	0.68	0.27
9	0.54	0.68
x	0.68	0.41

ANOVA showed that the two groups differed significantly in their means. $F = 9.679$, df: 1,6, $0.01>P>0.001$

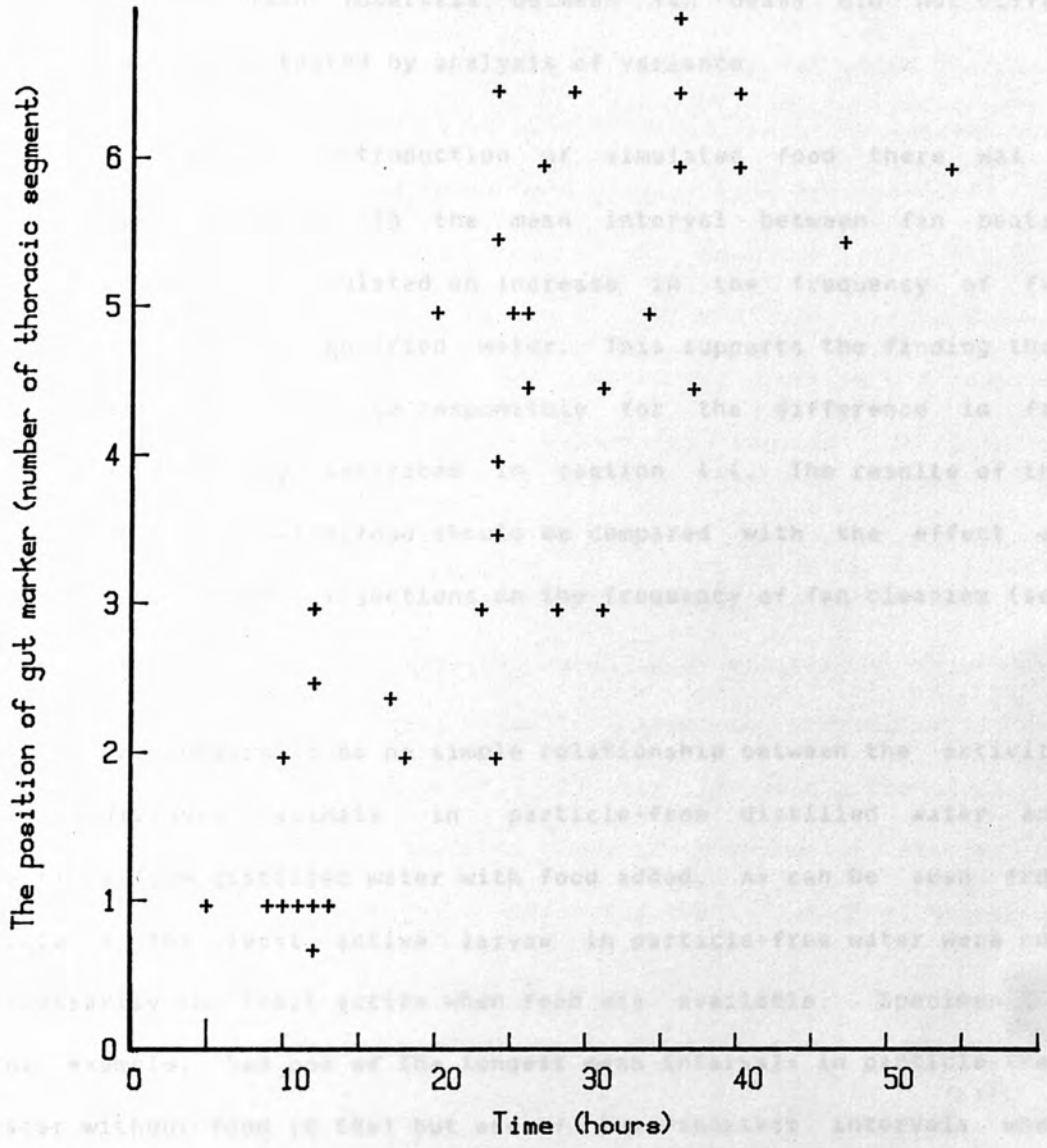


Figure 17 The rate of ingestion of final instar S. ornatum larvae feeding on a mixture of Scenedesmus acutus and diatomaceous earth at 10°C and a water velocity of 17cms^{-1}. Values are individual observations of position of gut marker (see Appendix 3 for list of values).

The mean intervals between fan beats when the larvae were not being fed are shown in column 1 and column 2 shows the mean intervals between fan beats when artificial food was provided. The hypothesis that the two mean intervals between fan beats did not differ significantly was tested by analysis of variance.

Following the introduction of simulated food there was a significant reduction in the mean interval between fan beats. showing that food stimulated an increase in the frequency of fan cleaning, even in purified water. This supports the finding that food quality appears to be responsible for the difference in fan cleaning frequency described in section 4.4. The results of the injection of simulated food should be compared with the effect of diatomaceous earth injections on the frequency of fan cleaning (see Chapter 6).

There appears to be no simple relationship between the activity of individual animals in particle-free distilled water and particle-free distilled water with food added. As can be seen from Table 8 the least active larvae in particle-free water were not necessarily the least active when food was available. Specimen 8, for example, had one of the longest mean intervals in particle-free water without food (0.68s) but one of the shortest intervals when food particles were added. In contrast, Specimen 9, with one of the shortest intervals in particle-free water (0.536s), had the longest mean interval when food was added to the water. However, all but specimen 9 cleaned their fans more frequently when food was available than when it was not.

These observations suggest that food availability had a considerable influence on the behaviour of the larvae. As might be expected when food was available the larvae were more active and cleaned their fans more frequently. This increase in activity was inevitably accompanied by a reduction in the time for which the fans were open and filtering so it cannot automatically be assumed that more frequent fan cleaning leads to a greater rate of ingestion. The possible effects on ingestion rates of this increase in fan cleaning frequency and the associated decline in filtering time are discussed in Chapter 7. The relative importance of the physical and chemical qualities of food particles in influencing feeding behaviour is investigated further in Chapter 6.

A number of authors have demonstrated that both temperature and water velocity affect the rate of ingestion in the field. In general higher temperatures and water velocities lead to greater rates of ingestion (see Section 1.4). It was important therefore to investigate the effect of the both temperature and water velocity on larval behaviour to explore indirectly the influence of behaviour over ingestion rates.

CHAPTER 5 THE EFFECT OF TEMPERATURE AND WATER VELOCITY ON LARVAL FEEDING BEHAVIOUR.

5.1 Introduction

The observations described in this Chapter quantify the effect of water temperature and velocity on larval feeding behaviour, refining the work of Schroeder (1980) described in section 1.4. Two facets of larval feeding behaviour, the mean interval between fan beats and the time taken to clean the cephalic fan, were chosen for investigation since, as was shown in Chapter 4, these are the main components of uninhibited feeding behaviour.

5.2 Methods

The effect of temperature on larval behaviour was investigated in the range 7.8°C to 18°C. These extremes were chosen to represent the range of values observed over some years by Crisp et al (1980) in two chalk streams in Southern England (see Table 9). Observations on the effect of water velocity on behaviour were made at 17cms^{-1} 34cms^{-1}, 51cms^{-1} and 68cms^{-1} These values were chosen arbitrarily to encompass the range of water velocities to which larvae are probably exposed.

Observations were made in both unfiltered natural water and particle-free distilled water. In experiments using unfiltered natural water no attempt was made to measure the concentration of particles in the water, nor to define its chemical quality (beyond routine measurement of pH). Although such observations would be essential in more critical work, they were not necessary in these experiments where the main aim was to observe how changes in water

velocity and temperature modified the influence of natural water on behaviour (described in Chapter 4).

Observations of the effect of temperature on the behaviour of S. ornatum were made on groups of larvae held at a single temperature with comparisons of temperature being made on different groups of larvae over six months. Larvae were observed in groups of up to 15 individuals using the apparatus described in Chapter 3. As larvae were kept in tanks at a constant temperature of 10°C before experiments they could be exposed to an increase in temperature of as much as 11°C at the beginning of an experiment, a change that they would never normally experience. In the R. Frome, for example, monthly temperature ranges never exceeded 3.2°C (Crisp et al, 1980). Although such large changes in temperature may have unknown detrimental effects on larvae none were apparent here.

Observations were also made on the effect of temperature on the behaviour of S. lineatum. As it was more difficult to collect enough final instar larvae of this species for experiments a few individuals were exposed to more than one temperature, although most though were treated in the same way as S. ornatum larvae.

TABLE 9 A summary of the temperatures of two chalk streams in Southern England (from Crisp *et al*, 1980).

	R. Frome, East Stoke Mill Stream		Bere Stream	
	1962-1968		1967	1968
	Mean	Range		
January	6.5	7.1- 5.7*	9.0	9.4
February	6.8	8.4- 5.7*	8.8	8.0
March	7.4	9.0- 5.8	10.0	9.6
April	10.6	10.8-10.2	11.0	11.2
May	13.3	14.0-12.6	12.8	12.3
June	16.7	18.1-16.2	14.7	15.2
July	17.4	18.5-16.6	16.2	15.2
August	17.0	16.9-16.1	15.1	14.2
September	14.6	16.5-13.6	16.2	15.2
October	11.9	12.5-10.0	11.8	12.0
November	8.3	9.4- 7.4	9.6	9.4
December	6.7	7.7- 5.3	9.0	7.7
Amplitude of monthly means	10.9		7.4	7.5

* Omitting values for the 1962-1963 winter

5.3 The effect of water temperature on larval feeding behaviour

The effect of temperature on the following aspects of the behaviour of *S. ornatum* was investigated: mean interval between fan beats in particle-free distilled water (Figure 18); the mean interval between fan beats in unfiltered natural water (Figure 20); the time taken to clean the fans in particle-free distilled water and unfiltered natural water (Figure 21). The effect of temperature on the mean interval between fan beats was investigated in *S. lineatum* (Figure 19).

The mean interval between fan beats, and not the frequency of fan cleaning, was again chosen to represent the activity of larvae for the reasons given earlier. Clearly, as the interval between fan beats is reduced the frequency with which the fans are cleaned rises. On all graphs each value refers to one individual and is the mean interval between all the fan beats (or the mean duration of all fan beats) in an extract of behaviour lasting ten seconds.

Figure 18 shows the effect of temperature on the interval between fan beats for *S. ornatum* in particle-free distilled water. The relationship suggests that for every 1°C increase in temperature there was a reduction in the mean interval between fan beats of 26(±9) ms (note that the slope of the regression is calculated in seconds). $Q_{10} = 1.7$

Figure 19 shows the effect of temperature on the mean interval between fan beats in *S. lineatum*. Unfortunately, few measurements could be made at higher temperatures as a result of difficulties in obtaining larvae. Consequently, although the slope of this regression is twice that of Figure 18, implying some difference in $Q_{10} = 3.19$.

the response to temperature in the two species, covariance analysis showed that this difference was slightly outside statistical significance at the 5% level (see Appendix 6).

In both cases there was considerable individual variation in the interval between fan beats at all temperatures. In the case of S. ornatum the mean interval between fan beats fell by about 33% within the range of temperatures naturally experienced by the larvae (at 8°C the mean interval between fan beats, calculated from the regression equation, is about 0.6s; at 18°C, about 0.4s). This change was the basis of an increase in the frequency of fan cleaning of about 25%. Assuming that it takes 0.1s to clean the fan at both temperatures there are 85 fan beats min^{-1} at 8°C, since 60/(0.6+0.1) = 85 and 120 fan beats min^{-1} at 18°C, since 60/(0.4+0.1) = 120.

Figure 20 shows the effect of temperature on the mean interval between fan beats in S. ornatum in unfiltered natural water. Comparison of the slopes of the regressions of mean interval between fan beats on temperature in the two water qualities (particle-free distilled water and unfiltered natural water) for S. ornatum showed that there was no significant difference between them (Table 11 and Appendix 5a). This shows that temperature, as would be expected, influences feeding behaviour in some way independent of water quality.

However, comparison of the adjusted means of these two regressions showed a highly significant difference (see Table 11 and Appendix 5a) indicating that in unfiltered natural water, at all temperatures, larvae had shorter mean intervals between fan beats than in particle-free distilled water. At the higher temperatures this resulted in much higher rates of fan cleaning in unfiltered

natural water. At 18°C the mean interval between fan beats in natural water was about 0.2s. Assuming the fan is cleaned in 0.1s (a slight over estimate) this gives a fan cleaning frequency of at least 200 beats min . (60/[0.2 + 0.1]s = 200).

Although of much shorter duration than the interval between fan beats changes in the time taken to clean the fan could be resolved with video-tape analysis. Figure 21a shows the effect of temperature on the time taken to clean the fan in S. ornatum in particle-free distilled water. The figure shows that as temperature increased there was a marked reduction in the time taken to clean the fan. For each 1°C increase in temperature there was, on average, a reduction in the time taken to clean the fan of 9(±0.7) ms, so that while it took 176 ms to clean the fan at 8°C it took only 96 ms at 18°C, a 45% reduction. Figure 21b shows the effect of temperature on the duration of fan cleaning in unfiltered natural water. There was a similar significant reduction in the time taken to clean the fans in this water quality.

The cleaning of the fan took less time in unfiltered natural water than in particle-free distilled water as demonstrated by the analysis of covariance shown in Table 11(b) and Appendix 5b. At all temperatures tested fan cleaning was 20 ms quicker in unfiltered natural water than in particle-free distilled water (as Figure 21 shows the slopes of the two regressions are the same, only the intercepts differing). The equations describing the relationships shown in Figures 18, 19, 20 and 21 are shown in Table 10.

Clearly temperature has a considerable influence on the behaviour pattern throughout the range of temperatures found in the typical chalk stream. It should be noted that normal activity continued at all the temperatures investigated whereas Ladle et al (1972) recorded that there was no feeding in winter, by larvae inhabiting a chalk stream, as measured in terms of the progress of gut markers. This observation and others relevant to a discussion of the effect of water temperature on behaviour will be considered in Chapter 7.

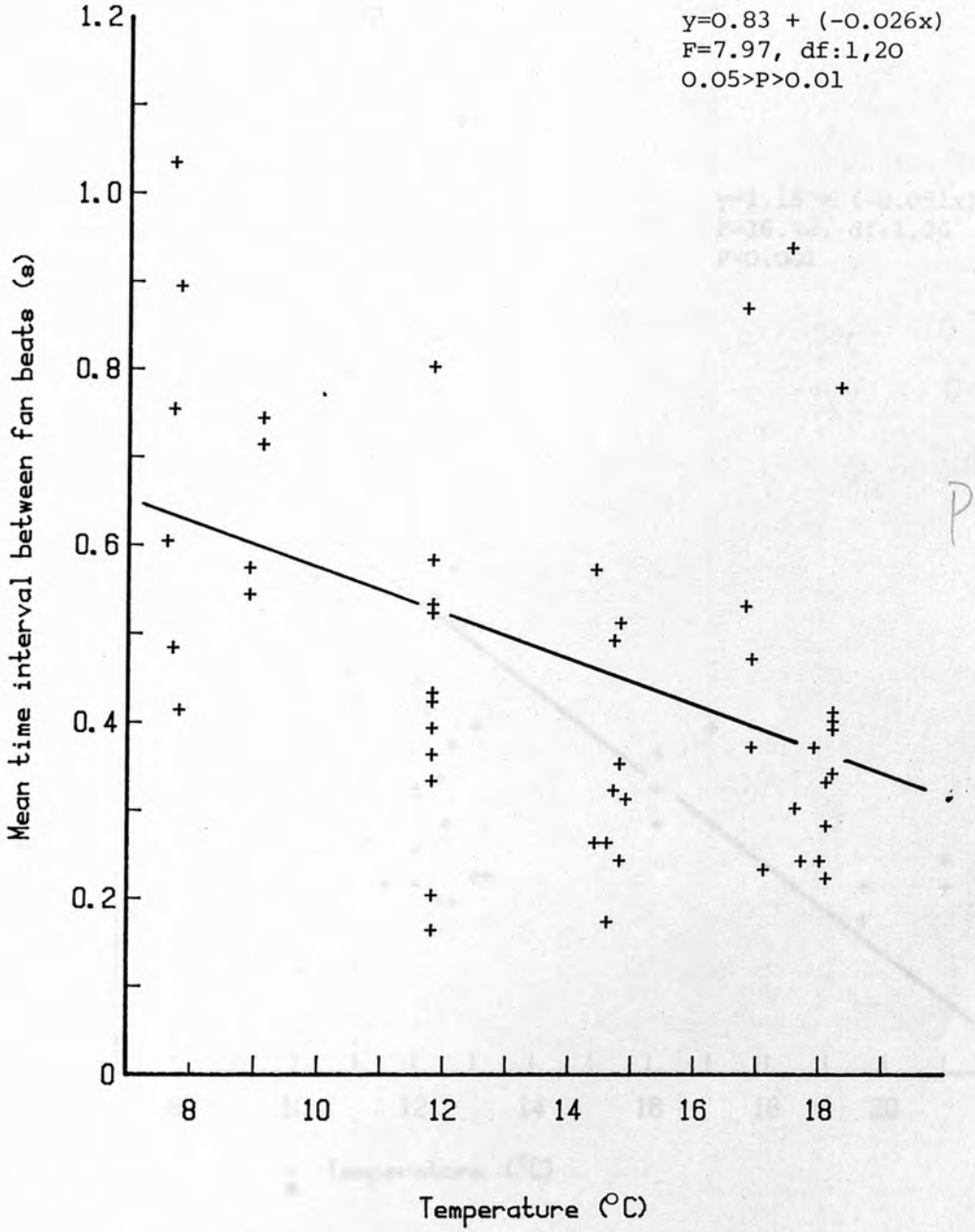


Figure 18 The relationship between mean interval between fan beats and water temperature in S. ornatum in particle-free distilled water at a water velocity of 17cms^{-1}. Values are mean intervals between fan beats calculated from ten second extracts of behaviour.

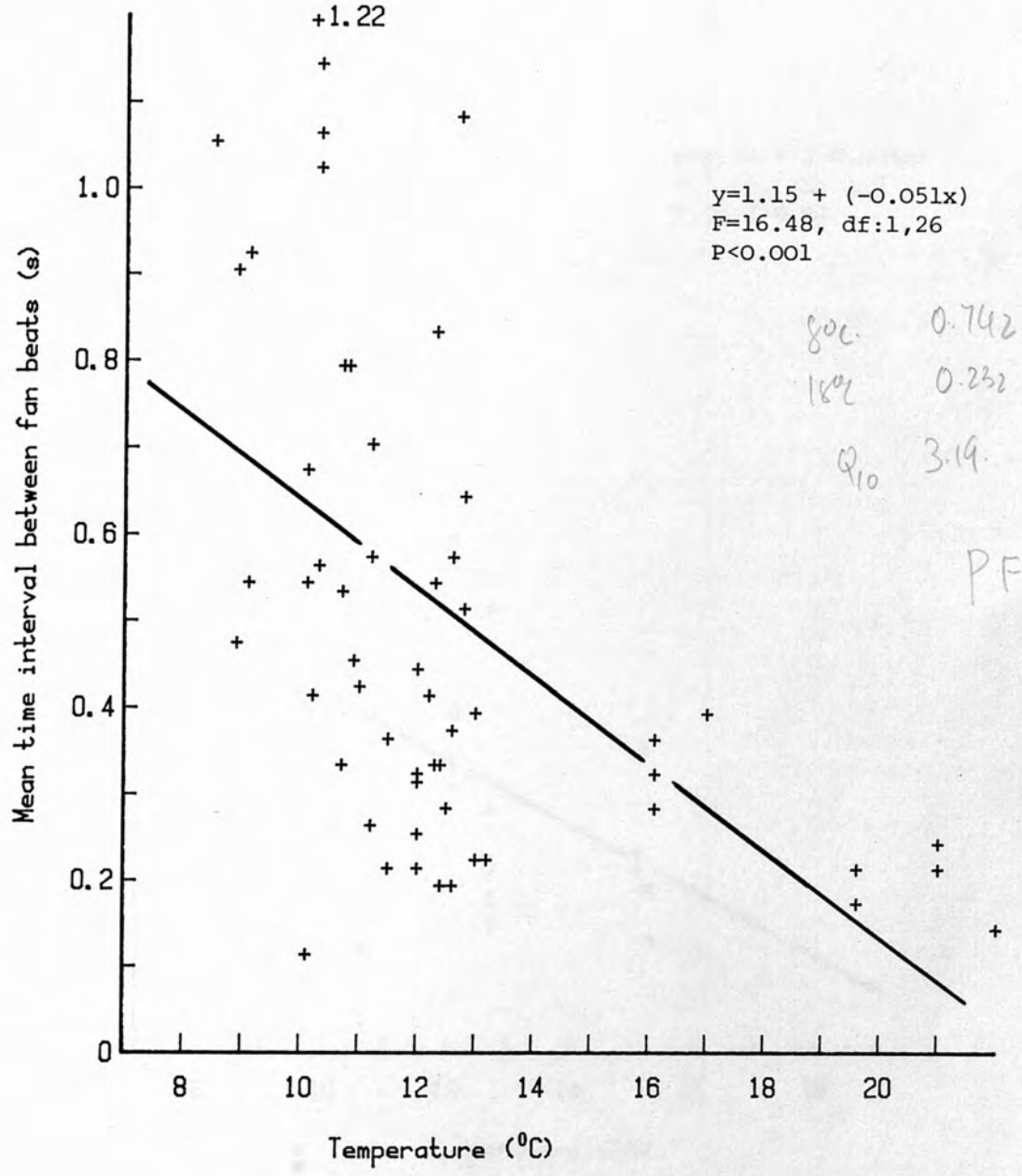


Figure 19 The relationship between mean interval between fan beats and water temperature in particle-free distilled water in S. lineatum. Water velocity 17cms^{-1}. Values are mean intervals between fan beats calculated from ten second extracts of behaviour.

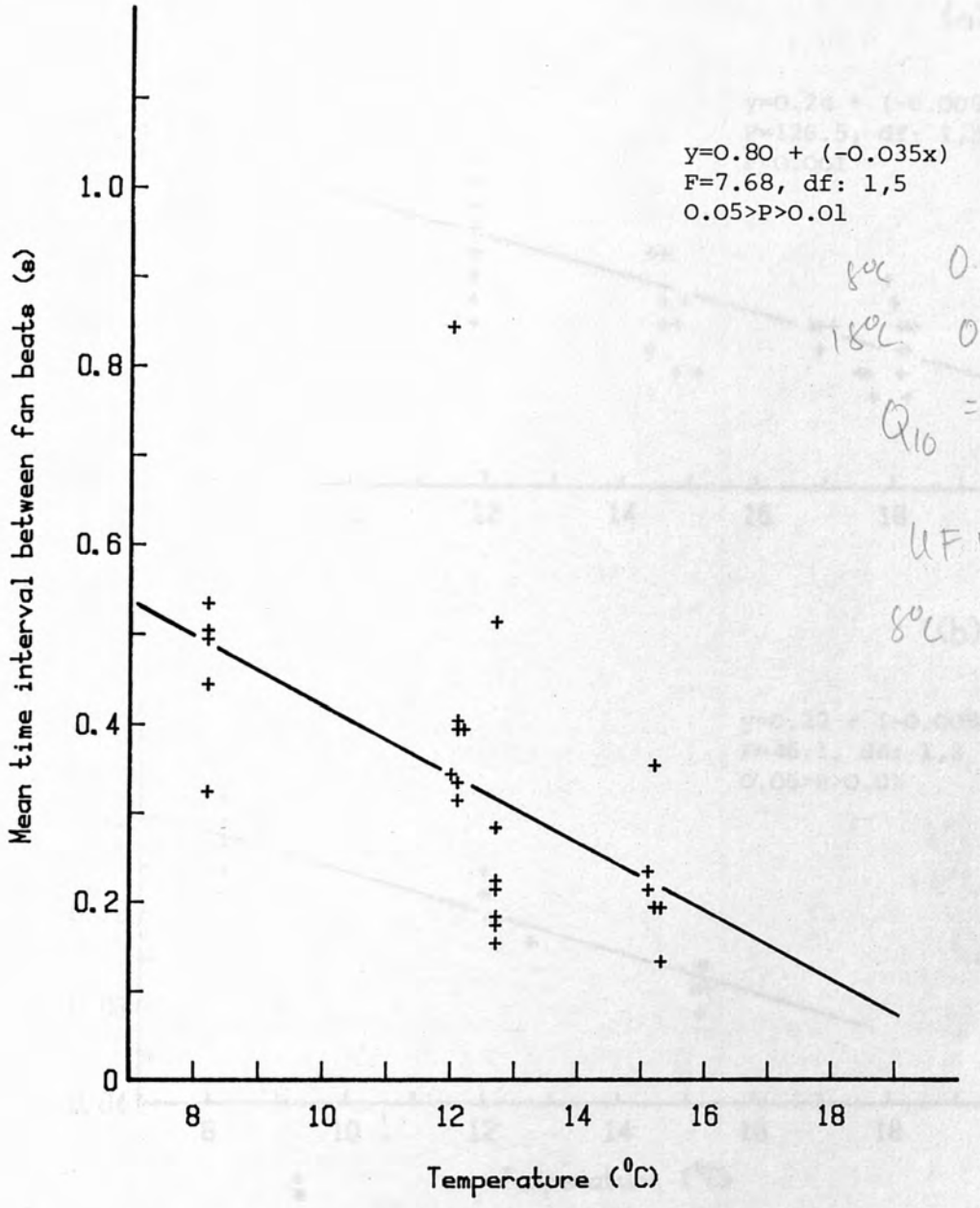


Figure 20 The relationship between mean interval between fan beats and water temperature in unfiltered natural water at a water velocity of 17cms^{-1}. Values are mean intervals between fan beats calculated from ten second extracts of behaviour of S. ornatum.

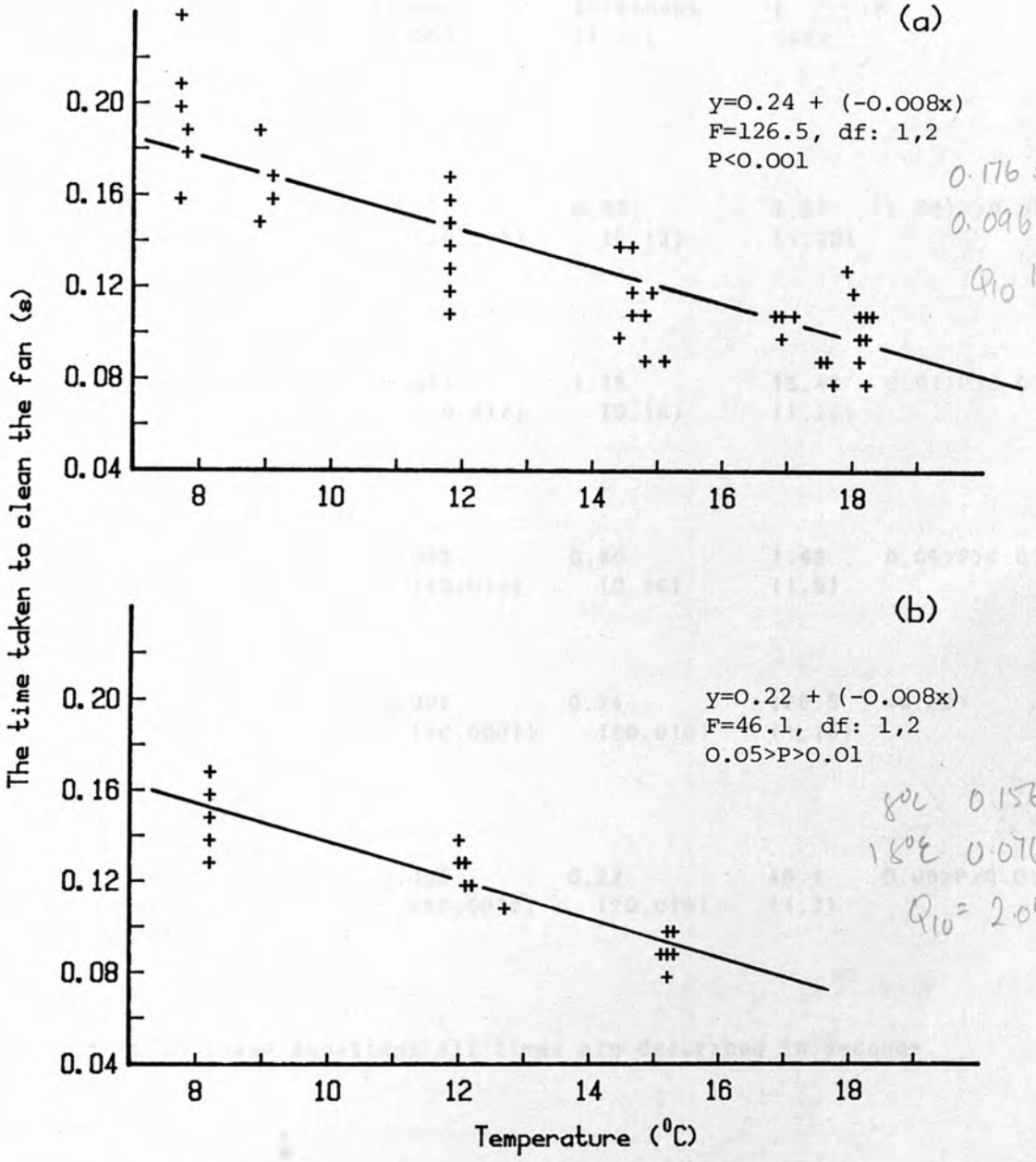


Figure 21 The relationship between the time taken to clean the cephalic fans and water temperature in S. ornatum. Water velocity 17cms^{-1}; water quality (a) particle-free distilled water (b) unfiltered natural water. Values are means calculated from ten second extracts of behaviour.

TABLE 10. Equations describing the regressions presented in Figures 18, 19, 20 and 21. The effect of temperature on larval behaviour. All equations refer to *S. ornatum*, except Equation 2 (Figure 19) which refers to *S. lineatum*.

Parameter of behaviour and water quality	n	Slope (± SE)	Intercept (± SE)	F (df)	P
(1)Mean interval between fan beats; PFDW (Fig. 18)	52	-0.026 (±0.009)	0.83 (0.13)	7.97 (1,20)	0.05>P>0.01
(2)Mean interval between fan beats; PFDW (Fig. 19)	56	-0.051 (±0.012)	1.15 (0.16)	16.48 (1,26)	0.01>P>0.001
(3)Mean interval between fan beats; UFNW (Fig. 20)	20	-0.035 (±0.012)	0.80 (0.16)	7.68 (1,5)	0.05>P>0.01
(4)Mean time taken to clean fans PFDW (Fig. 21a)	48	-0.008 (±0.0007)	0.24 (±0.010)	126.5 (1,18)	<0.001
(5)Mean time taken to clean fans UFNW (Fig. 21b)	28	-0.008 (±0.0012)	0.22 (±0.015)	46.1 (1,2)	0.05>P>0.01

Note that in these equations all times are described in seconds.

TABLE 11 Analysis of covariance of (a) the regressions of mean interval between fan beats in particle-free distilled water and unfiltered natural water and (b) the regressions of the time taken to clean the fan in particle-free distilled water and unfiltered natural water in S. ornatum

(a) covariance analysis of mean intervals between fan beats

Water quality	Adjusted mean interval between fan beats (± SE)	Analysis of Covariance F	P
PFDW	0.49 (±0.125)	9.743	0.01>P>0.001
UFNW	0.31 (±0.125)		

Water quality	Regression coefficient (± SE)	Analysis of Covariance F	P
PFDW	-0.026 (±0.007)	0.380	NS
UFNW	-0.035 (±0.01)		

(b) covariance analysis of the time taken to clean the fans

Water quality	Adjusted mean time taken to clean the fan (± SE)	Analysis of covariance F	P
PFDW	0.134 (0.011)	26.29	<0.001
UFNW	0.117 (0.011)		

Water quality	Regression coefficient (± SE)	Analysis of covariance F	P
PFDW	-0.0082 (±0.005)	0.016	NS
UFNW	-0.0084 (±0.004)		

5.4 The effect of water velocity on larval feeding behaviour

The effect of water velocity on larval feeding behaviour was investigated at four temperatures in particle-free distilled water and three temperatures in unfiltered natural water. Groups of larvae were exposed to four different water velocities with 15 minutes acclimation between each velocity. At the end of three of the four experiments using particle-free distilled water the water velocity was returned to its starting value (always $17cms^{-1}$) to check that the responsiveness of the larvae had not changed during the course of the experiment. The mean intervals between fan beats for each specimen at $17cms^{-1}$, at the beginning and end of each experiment, are given in Appendix 4.

The larvae used in these experiments showed mean intervals between fan beats which varied between 0.5s and 0.74s at the start of an experiment (note that there was no control for 10°C in particle-free distilled). At the end of an experiment the mean intervals varied between 0.44s and 0.6s, a difference that was not statistically significant from starting values. Similar control observations were not made in unfiltered natural water since it was thought unlikely that the condition of the larvae would deteriorate in river water (see Section 4.4).

The results of the experiments are presented graphically in Figures 22 a to d (for particle-free distilled water) and Figures 23a to c (for unfiltered natural water). The graphs show the mean interval between fan beats plotted against water velocity. All values are mean intervals between fan beats for an individual larva, each value being derived from a ten second extract of behaviour. In

Figures 22 and 23 the range of variation of the individual mean intervals is demonstrated. Figure 24 shows the means and standard deviations of the mean intervals at all temperatures, and both water qualities, plotted on the same scale, the overlay showing the values in unfiltered natural water.

Figures 22 and 23 show that while at most temperatures the mean interval between fan beats was reduced as the water velocity increased there was a range of individual values at each water velocity. The significance of the reduction in mean intervals with increasing water velocity was tested using a non-parametric analysis of variance because the individual observations were not independent each larva being observed at four water velocities.

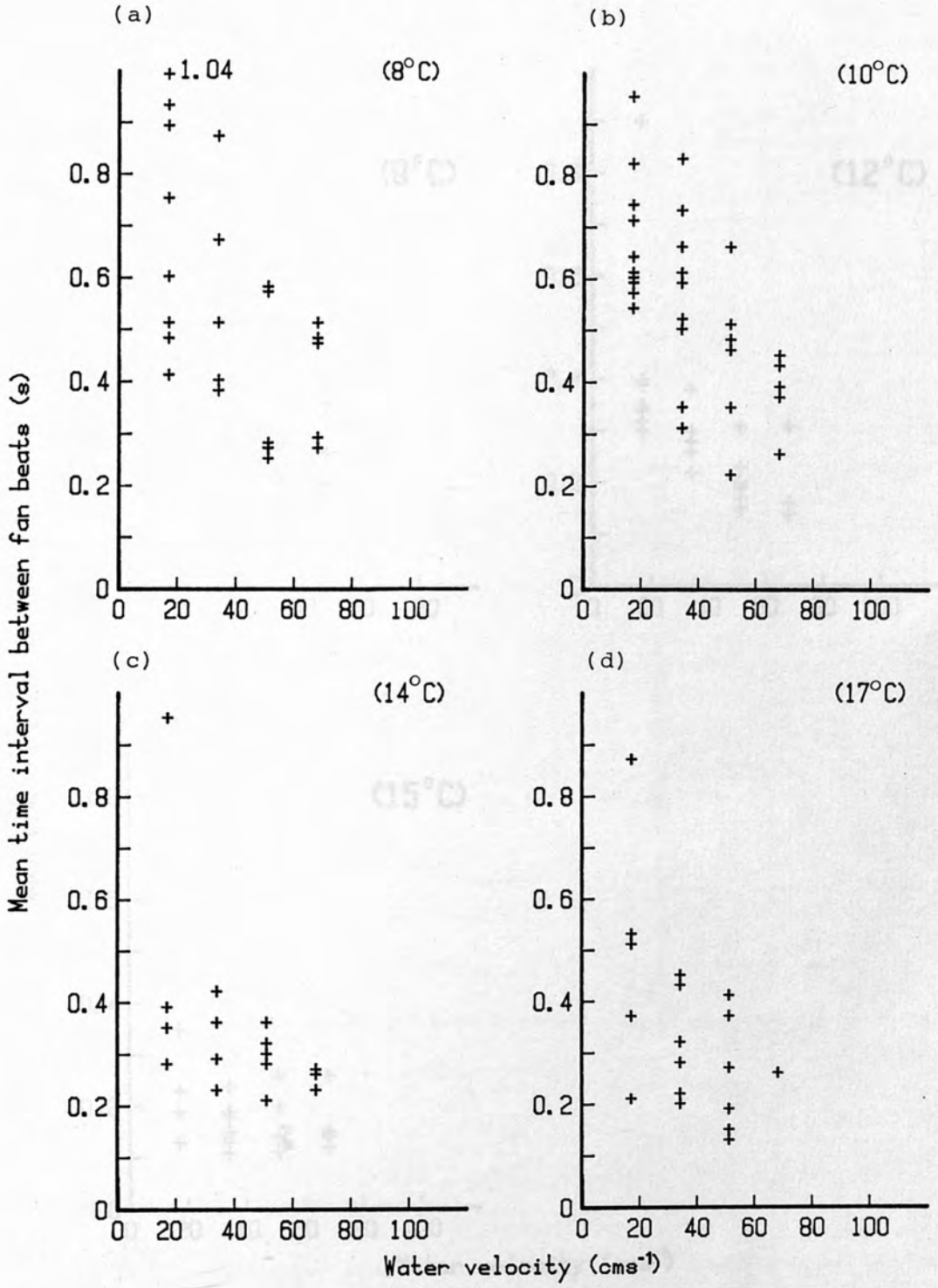
Table 12 shows the results of Kruskal-Wallis one-way ANOVA of the effect of water velocity on the mean intervals between fan beats at each temperature (see Appendix 7 for intermediate calculation in these statistical tests). Only at 10°C in particle-free distilled water and at 9°C and 12°C in unfiltered natural water was the reduction in the interval between fan beats statistically significant (noting that at 8°C in particle-free distilled water the result was very close to statistical significance). In both water qualities at the higher temperatures there was no significant change in the mean intervals as the water velocity increased, a trend that was particularly clear at 15°C in unfiltered natural water (Figure 23c). Figures 22 and 23 also show that the range of mean intervals in unfiltered natural water at each water velocity was smaller than the range of mean intervals in particle-free distilled water.

These results suggest that water velocity, particularly at the higher temperatures, may have little effect on the frequency of fan cleaning in comparison to the effect of temperature. Table 9 shows that in summer in a typical chalk stream the temperature did not fall below 10°C; it is possible that throughout the summer in such a stream water velocity would have little direct effect on larval behaviour (although it may still influence behaviour as a result of variations in the amount of food delivered to the larvae at different water velocities).

Figure 24 confirms (see sections 4.4 and 4.5) that larvae were more active in unfiltered natural water, cleaning their fans more frequently (with smaller intervals between fan beats) than at equivalent temperatures in particle-free distilled water. Table 13 shows that there was a statistically significant difference between the mean intervals between fan beats in unfiltered natural water and those in particle-free distilled water.

In general it appears that the effect of water velocity on behaviour is most marked at the lowest water velocities and the relationship between the mean intervals between fan beats and water velocity appears to be a curvelinear one. At water velocities greater than about 40cms^{-1} there was little evidence of change in the mean intervals between fan beats.

Increasing water velocity appeared to have little effect on the time taken to clean the fan (see Appendix 7) and only at the lowest temperature in particle-free distilled water was there any suggestion that the time taken to clean the fan might increase with increasing water velocity.



S. ornatum?

Figures 22a to d. The effect of water velocity on the mean interval between fan beats, at four temperatures, in particle-free distilled water.

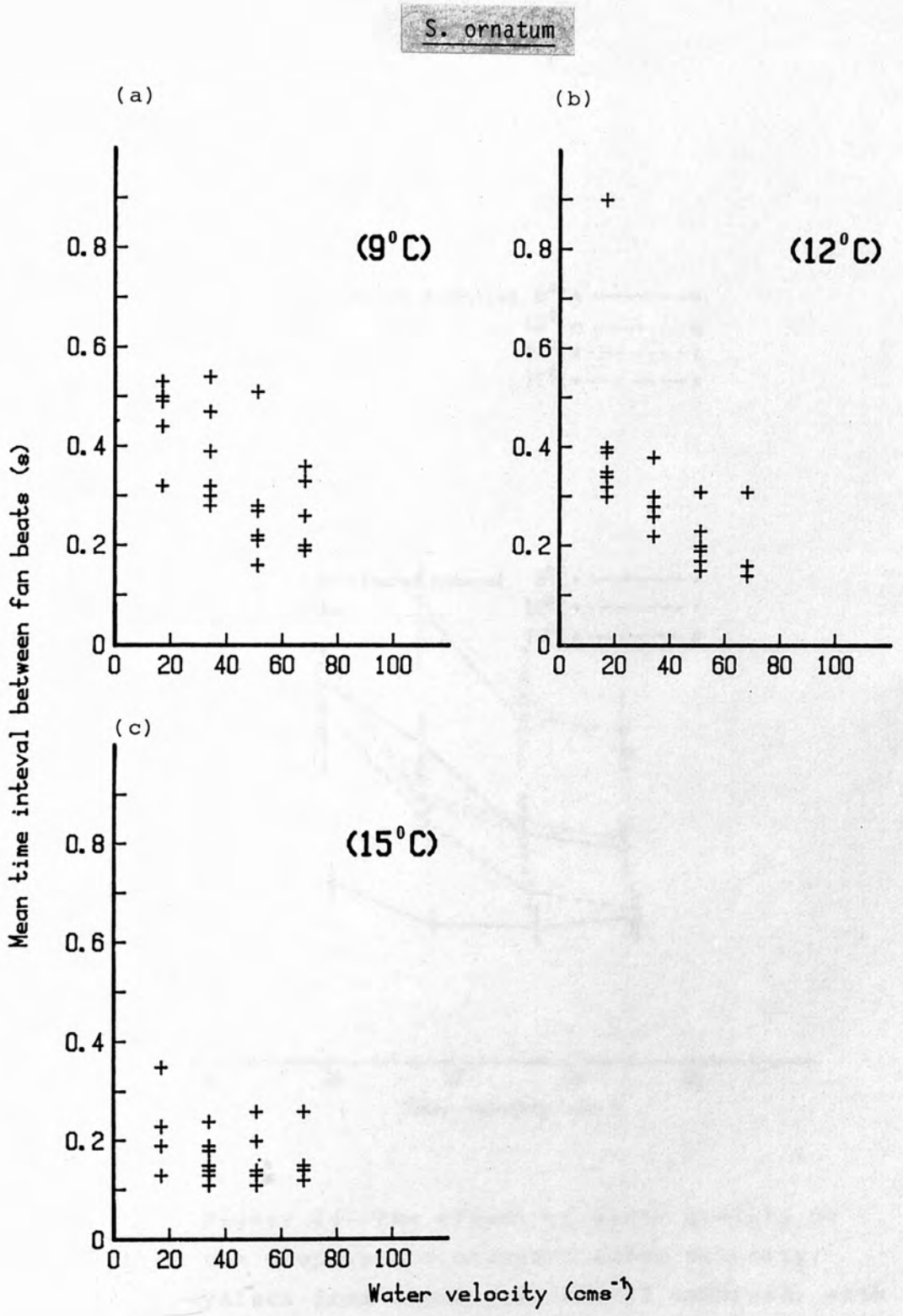


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Figure 23a to c. The effect of water velocity on the mean interval between fan beats, at three temperatures, in unfiltered natural water.

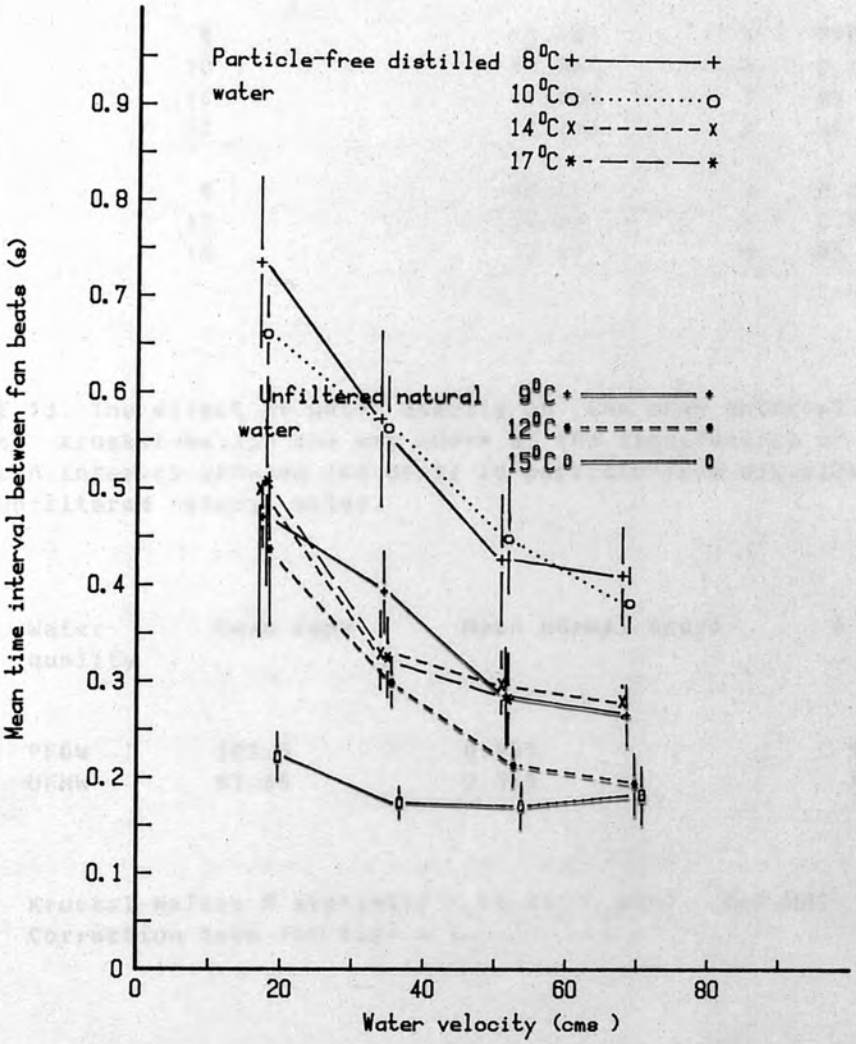


Figure 24. The effect of water quality on the response to changing water velocity; values from Figures 22 and 23 combined, with unfiltered natural water shown on the overlay. Values are means (with standard errors).

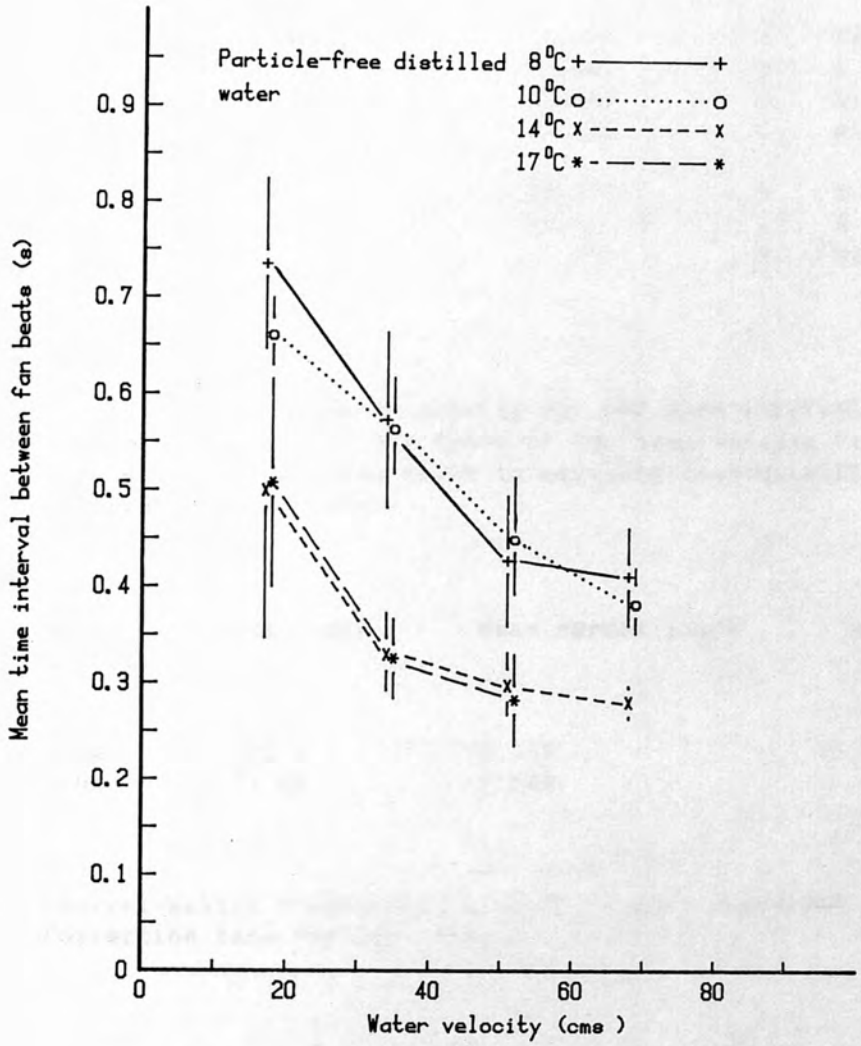


Figure 24. The effect of water quality on the response to changing water velocity; values from Figures 22 and 23 combined, with unfiltered natural water shown on the overlay. Values are means (with standard errors).

TABLE 12 Summary of Kruskal-Wallis ANOVA of the effect of water velocity on the mean intervals between fan beats at several temperatures in two water qualities. In all cases water velocities compared were 17cms^{-1}, 34cms^{-1}, 51cms^{-1} and 68cms^{-1}.

Water quality	Water temperature (°C)	Kruskal-Wallis H Statistic	df	P
PFDW	8	7.487	3	P=0.057
PFDW	10	13.861	3	0.01>P>0.001
PFDW	14	4.302	3	NS
PFDW	17	4.788	3	NS
UFNW	9	10.447	3	0.05>P>0.01
UFNW	12	14.82	3	0.01>P>0.001
UFNW	15	3.47	3	NS

TABLE 13. The effect of water quality on the mean interval between fan beats. Kruskal-Wallis one way ANOVA of the significance of differences in mean interval between fan beats in particle-free distilled water and and unfiltered natural water.

Water quality	Mean rank	Mean normal score	n
PFDW	103.6	0.455	90
UFNW	53.88	-0.569	72

Kruskal-Wallis H statistic = 44.94 df=1 P<0.001
Correction term for ties = 1.

The results described in this chapter have shown that, even though larvae were more active in unfiltered natural water than in particle-free distilled water, the behaviour patterns in both water qualities were predictable and dominated by the physical environment.

However, little has been said of the disruption of these behaviour patterns which occurs when extra mandible movements are made (mandible movements without fan cleaning movements). As has been shown in Chapter 4, these movements are rare during uninhibited feeding but they may be of considerable significance in the control of ingestion as a result of the way in which they alter the pattern of uninhibited feeding behaviour. These modifications, their significance and the factors that initiate them are considered in Chapter 6.

All feeding behaviour described so far has been 'uninhibited', with continuous fan cleaning and few extra mandible movements. Factors influencing this behaviour pattern only modify it, causing changes in frequency and duration of movements. In the next Chapter factors that disrupt uninhibited feeding behaviour are considered. Disruption must be distinguished from modification because it causes behaviour patterns that generally inhibit fan cleaning.

CHAPTER 6 THE EFFECT OF FOOD PARTICLES AND CHEMICAL STIMULANTS ON LARVAL FEEDING BEHAVIOUR

6.1 Introduction

In Chapters 4 and 5 uninhibited feeding behaviour, and the effect of unfiltered natural water on this behaviour pattern, was described. Modifications of uninhibited feeding by the physical environment and natural food were both demonstrated. This suggests that fan cleaning frequency is not only modified by water velocity and temperature but also by the physical and chemical qualities of food particles. This conclusion is supported by the results of section 4.6, where pulses of 'artificial' food were found to reduce the mean interval between fan beats. It appears that dissolved compounds in natural water do not affect the frequency of fan cleaning in S.ornatum (see Figure 14).

Larvae may make occasional extra mandible movements, without fan cleaning movements, at any time (see Figure 11), but large numbers of these movements appear to have specific causes. In this chapter investigations of the relative importance of particles and chemical compounds in stimulating these movements are described

6.2 The relationship between the numbers of fan movements and extra mandible movements

Extra mandible movements have not previously been recognised as an important part of the behaviour of larval simuliids. These movements may occur at any time when larvae are feeding, the number made probably depending on the type of stimulation to which larvae

are exposed (see section 6.3 and 6.4). In any given time the number of fan and mandible movements made is inversely proportional, as is shown in Figure 25. The number of fan beats and extra mandible movements made, in two water qualities, are plotted against each other. Each value refers to the number of movements recorded in a 5s extract of behaviour. Movements made in unfiltered natural water are shown as solid circles and movements in particle-free distilled water as crosses.

The graph shows, as already demonstrated, that there were significantly more fan beats made in unfiltered natural water than in particle-free distilled water, although there was no significant difference in the number of extra mandible movements in the two water qualities. More importantly the figure also shows the negative correlation between the number of mandible movements and fan movements in both water qualities. As might be expected, the more time that was spent cleaning the fans the less time that there was available for mandible movements. This relationship suggests that the initiation of large numbers of extra mandible movements could lead to a temporary cessation of fan cleaning. Tables 14 and 15 summarise statistically the arguments presented in this figure.

TABLE 14. Kolmogorov-Smirnov test of the significance of differences in the numer of fan and mandible movements in unfiltered natural water and particle free distilled water.

Water quality	n	Mean number of fan movements in five seconds (± SE)	n	Mean number of mandible movements in five seconds (± SE)
Unfiltered natural water	48	13.6 (± 1.6)	49	2.7 (± 0.86)
Particle-free distilled water	95	9.1 (± 0.6)	93	2.1 (± 0.48)

Comparison of number of fan movements: $D_{max} = 0.58$, $P<0.01$
Comparison of number of mandible movements: $D_{max} = 0.17$ NS.

TABLE 15 Correlation analysis of the relationship between the number of fan movements and mandible movements in particle-free distilled water and unfiltered natural water.

Particle-free distilled water

n = 92
Correlation coefficient = -0.24
r squared = 0.058
0.05>P>0.01

Unfiltered natural water

n = 50
Correlation coefficient = -0.42
r squared = 0.182
P<0.001

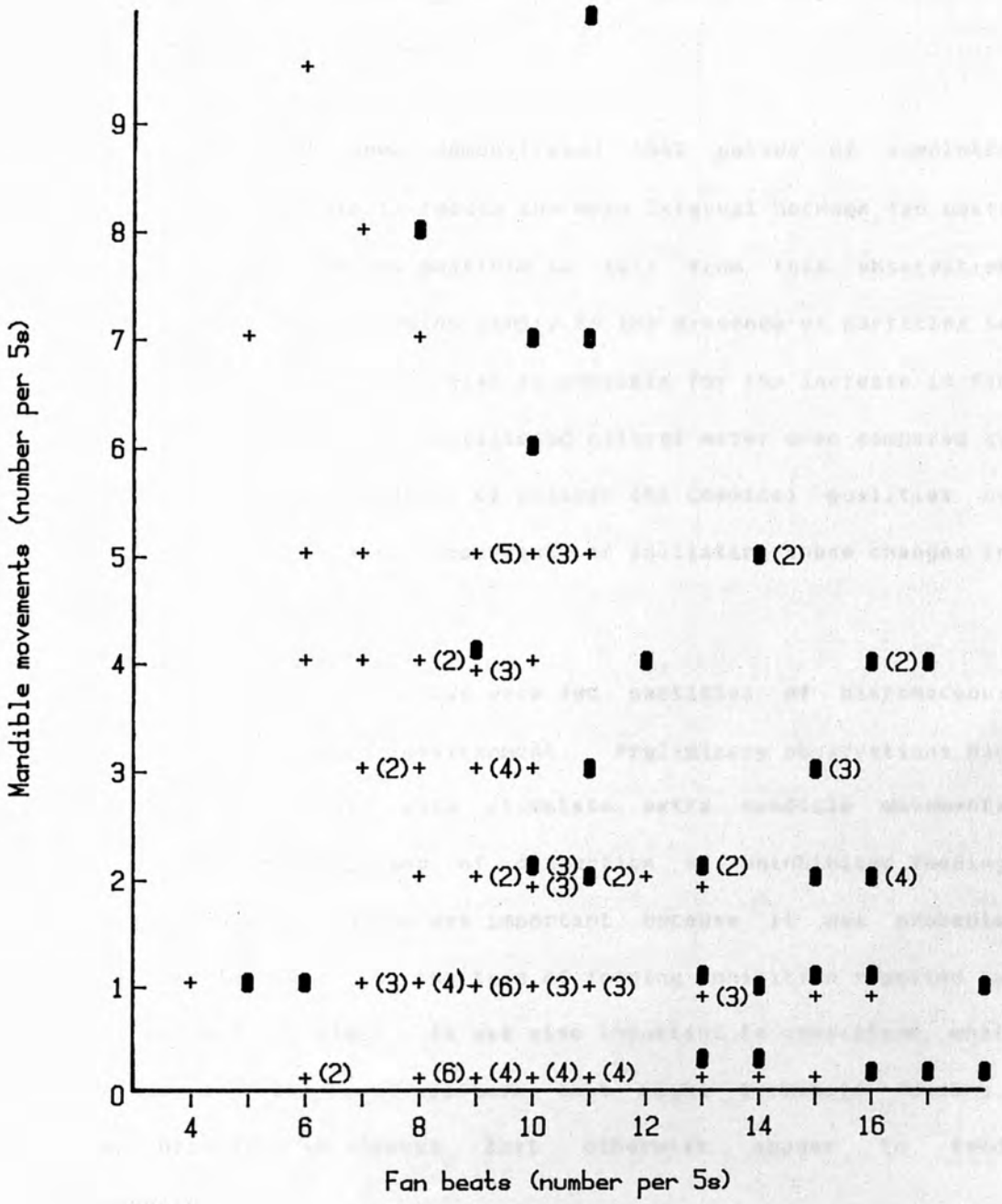


Figure 25 The correlation between the number of fan beats and the number of mandible movements in particle-free distilled water (+) and unfiltered natural water (●). Water temperature, $10^{o} \pm 1^{o}C$; water velocity, $17cms^{-1}$. Correlation coefficients: particle-free distilled water, r= -0.24, 0.05>P>0.01; unfiltered natural water, r= -0.42, 0.01>P>0.001. Values are derived from 5s extracts of behaviour.

6.3 The effect of pulses of diatomaceous earth on larval feeding behaviour

It has already been demonstrated that pulses of simulated natural food were able to reduce the mean interval between fan beats (section 4.6). It was not possible to tell from this observation whether larvae were responding simply to the presence of particles in the water (and that this was also responsible for the increase in fan cleaning frequency seen in unfiltered natural water when compared to particle-free distilled water) or whether the chemical qualities of the particles were also important in initiating these changes in behaviour.

To investigate this larvae were fed particles of diatomaceous earth in a controlled environment. Preliminary observations had shown that this would also stimulate extra mandible movements allowing the investigation of disruption of uninhibited feeding behaviour. Such disruption was important because it was probable that it would lead to the type of feeding inhibition reported by Gaugler and Molloy (1982). It was also important to understand what controlled any behaviour pattern that might terminate feeding, however briefly, in larvae that otherwise appear to feed continuously.

Diatomaceous earth is a geological deposit of the frustules of diatoms which, following processing, is 80-90% silicon dioxide. It is chemically inert, is widely used for fine filtration, and was chosen to represent the refractory, inorganic particles that larvae ingest naturally on the assumption that it would be of no nutritional value to them. The range of sizes of particles of diatomaceous earth

is similar to that ingested by simuliid larvae (Kurtak, 1978). Larvae often feed on live diatoms so diatomaceous earth seemed to be an appropriate source of 'non-stimulatory' food.

Larvae were exposed to a series of one second pulses of diatomaceous earth in particle-free distilled water, pulses being used in an attempt to simulate natural variation in the intensity of stimulation, an approach suggested by CT Lewis (personal communication). This method, adding particles to otherwise stimulant free water, is preferable to observing larval behaviour in water where particles are always present because it allows changes in behaviour, due to transient stimulation, to be recognised.

Preliminary observations had shown that after approximately twenty pulses, with a twenty second interval between each pulse, larvae often became partially or fully inhibited from feeding. If the addition of particles to the water was stopped larvae quickly began feeding again. Once larvae had become inhibited, further pulses of food appeared to maintain the inhibition.

Fifteen larvae were exposed to up to twenty-five consecutive pulses of diatomaceous earth, of one second duration and an estimated concentration of $19mgl^{-1}$, with twenty seconds between each pulse. $19mgl^{-1}$ is a relatively high concentration, similar to the maxima reported from chalk streams by Dawson (1981). The responses to diatomaceous earth were observed and recorded using the methods already described. The responses of two representative larvae to pulses of diatomaceous earth are shown in Figures 26 and 27, whilst examples of the behaviour of a further four larvae are shown in Appendix Figures 1a to d (see Appendix 9). The bar diagrams depict behaviour according to the conventions given in Table 4a.

In each figure the uppermost bar shows the unstimulated behaviour of the larva before being exposed to any diatomaceous earth. Each of the remaining bars show the responses of the larvae for 10s after the injection of a pulse of diatomaceous earth; each extract of behaviour begins at the end of the injection of a pulse so that there is usually a one to two second gap on the diagrams before any response is apparent (the time when particles travelled from the point of injection to the larvae in the observation cell, a distance of about 50cm). Only four responses of up to twenty in the series are shown as not all pulses were analysed in each series. The responses of all the larvae that were observed are shown graphically in Figures 28a to d where the numbers of fan and mandible movements made in response to pulses of diatomaceous earth are shown. The effect of different particle concentrations on the behaviour of the larvae has not been considered in these experiments, the main aim of which was to describe the type of response that was made to particles.

The statistical significance of differences in the numbers of fan and mandible movements before and after stimulation with diatomaceous earth were tested using Kruskal-Wallis one-way analysis of variance. In these tests all values during stimulation were pooled and compared with with the pooled values before stimulation, testing the hypotheses that (a) the number of fan movements and (b) the number of mandible movements before stimulation did not differ significantly from the number made following stimulation in a ten second extract of behaviour immediately after the addition of diatomaceous earth to the water.

As the uppermost bars in Figures 26 and 27 show, before stimulation larvae were 'feeding' in a typically uninhibited fashion, making very few extra mandible movements in the ten second extracts of behaviour (see also Appendix Figures 1a-d). The addition of diatomaceous earth to the water caused a highly significant increase ($P<0.001$, see Appendix 9) in the number of mandible movements in the ten seconds following stimulation when compared to the number of mandible movements before stimulation.

There were very slight increases in the number of fan beats as the pulses of diatomaceous earth were added to the water. These usually occurred, if at all, as the first pulses were introduced (see Specimens 1, 4 and 5, Figure 28a; Specimens 7 and 8, Figure 28b; Specimen 13, Figure 28c; Specimens 15 and 16, Figure 28d). However, following these slight increases there was a more general reduction in the number of fan beats, which was statistically significant (see Appendix 9). The decline may be seen in the graphs depicting the behaviour of the following specimens: Specimens 4 and 6, Figure 28a; Specimen 7, Figure 28b; Specimens 15 and 16, Figure 28d),

A reduction in the number of fan cleaning movements made is to be expected if diatomaceous earth stimulated mandible movements in preference to fan cleaning movements, remembering the relationship between the number of mandible movements and fan cleaning movements shown in Figure 25.

Repeated raking movements made by the mandibles without fan cleaning movements probably represents the first stage of feeding inhibition, when uninhibited feeding behaviour is disrupted. Figure 26, pulse 7, illustrates the next stage of this process when one fan is closed permanently (where the black bar is continuous) and

therefore no longer filtering the water to collect food. Although both mandibles continued to move in synchrony over the closed fans, the movements of only one can be shown in the figure.

If the pulses of diatomaceous earth continue to be delivered both fans may be completely withdrawn into the cibarium, while the mandibles continue to make their raking strokes. At this stage larvae may 'shelter' from the excess of food. This response to high particle concentrations was also observed by Kurtak (1978), larvae turning back from the typical feeding posture (in which the head and anterior thorax are rotated through up to 180o, so that the ventral surface is uppermost) and attaching themselves by the thoracic proleg as well as by the hooks of the anal circlet. The time for which larvae remained in this fully inhibited position was very variable and was not investigated here.

The results of stimulation with diatomaceous earth at a relatively high concentration suggest that a physical stimulant alone would be unlikely to cause an increase in the frequency of fan cleaning and would not cause the greater amount of activity seen in unfiltered natural water as compared to particle-free distilled water. However, it is possible that lower concentrations of chemically inert particles could stimulate fan cleaning, although work described in the introduction does not support this (Schroeder, 1980, Gaugler and Molloy, 1982). In addition a concentration of diatomaceous earth and algae of $10mgl^{-1}$ caused an increase in fan cleaning frequency (section 4.6) suggesting that high particle concentrations alone do not necessarily cause feeding inhibition. This point is discussed further in section 7.2.3.

The stimulation of mandible movements could be an important disruptive influence on uninhibited feeding, as fan cleaning was always inhibited when extra mandible movements were made. Initially fans remained open, but uncleaned, during diatomaceous earth stimulation but with more intense stimulation they could be closed for long periods.

Since this chemically inert particle seemed only to interfere with fan cleaning the influence of pure chemical compounds on behaviour was next considered as a source of stimulation that might cause the high frequency of fan cleaning seen in unfiltered natural water. Chemical stimulants were also of general interest because there have been few observations of the sensitivity of aquatic insects to chemical compounds (see Section 1.6).

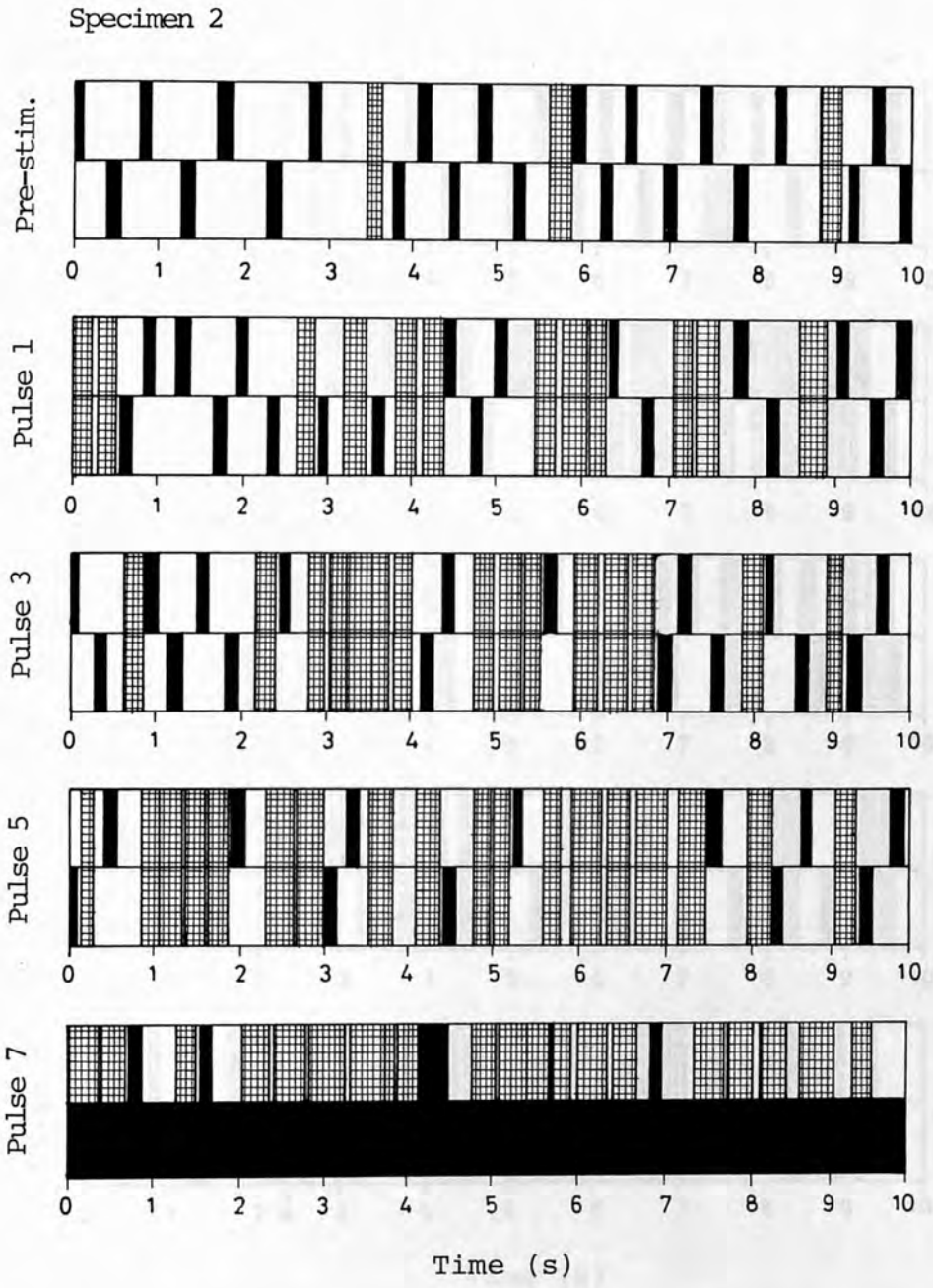


Figure 26 The response of an individual larva to a succession of one second pulses of diatomaceous earth at an estimated concentration of 19mgl^{-1}. Water temperature 11^{o} $\pm$ 1^{o}C; water velocity 17cms^{-1}. See Table 4a for key to symbols.

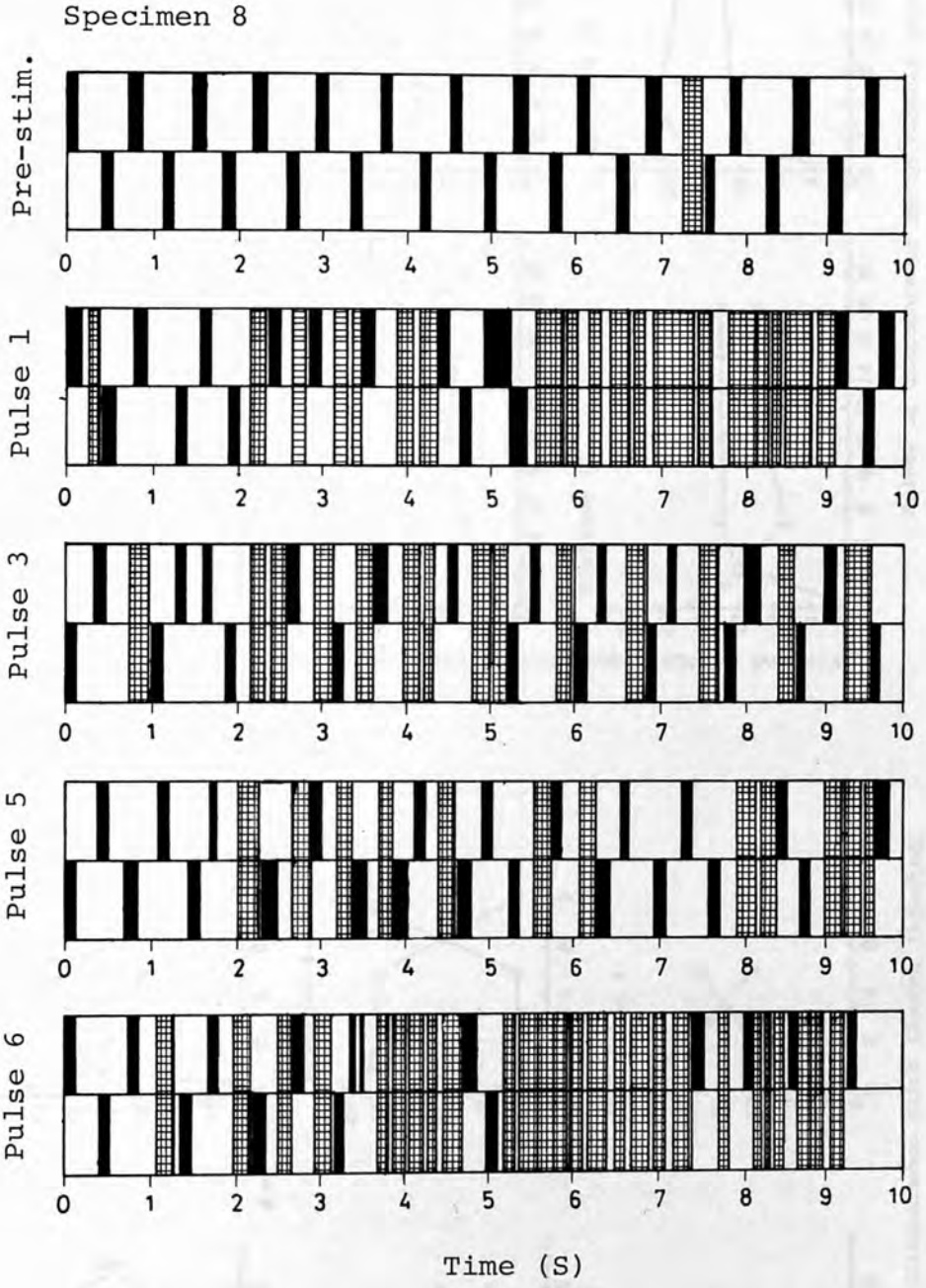


Figure 27 The response of an individual larva to a succession of one second pulses of diatomaceous earth at an estimated concentration of 19mgl^{-1}. Water temperature 11.5^{o} $\pm$ 1^{o}C; water velocity 17cms^{-1}. See Table 4a for key to symbols.

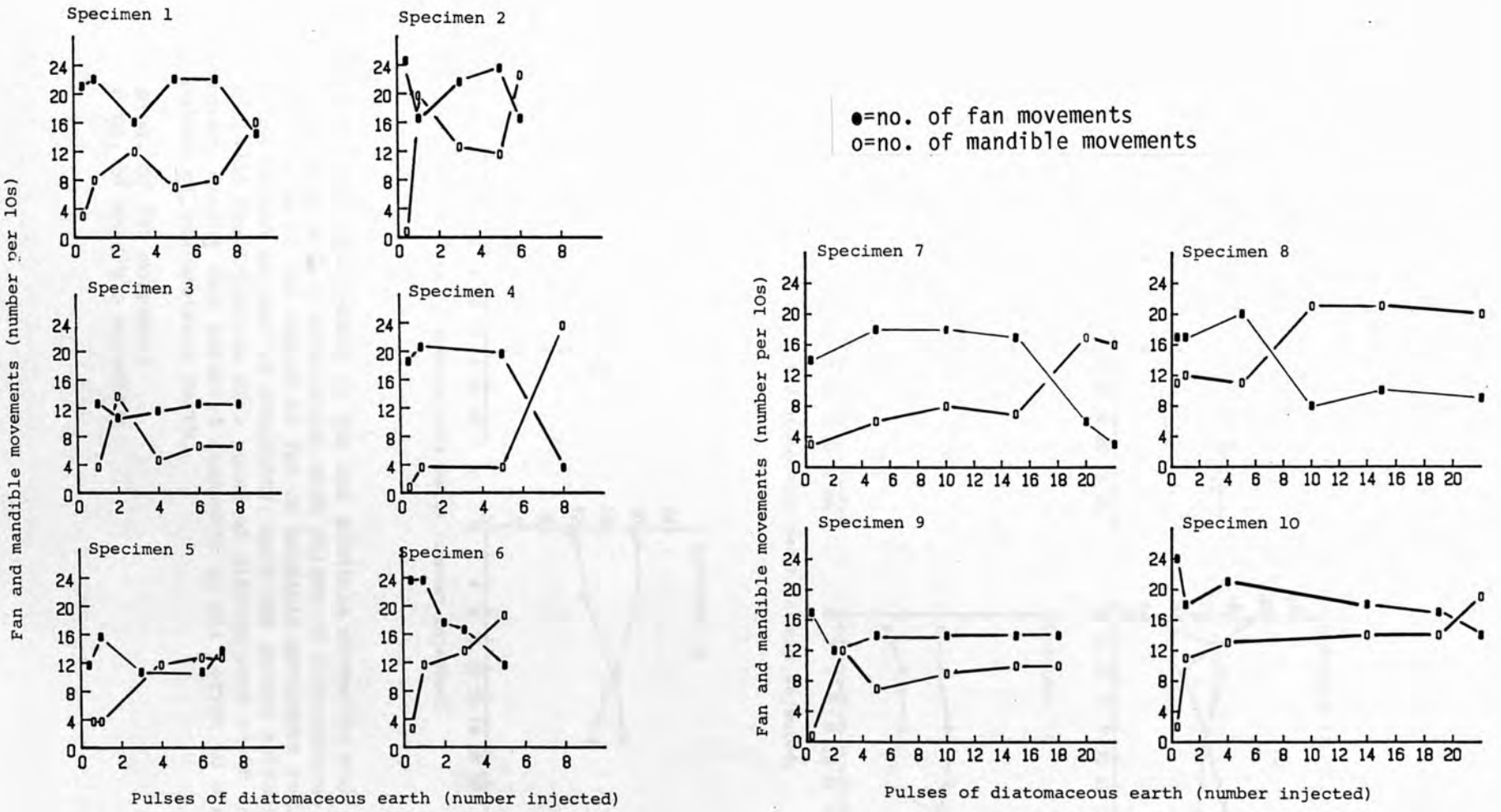


Figure 28a. The numbers of fan and mandible movements made by larval S. ornatum during stimulation with pulses of diatomaceous earth. Each value is the number of fan or mandible movements recorded during a ten second extract of behaviour; each ten second extract immediately followed the injection of a pulse of diatomaceous earth into the water. Graphs show selected responses of all larvae to a series of pulses of diatomaceous earth.

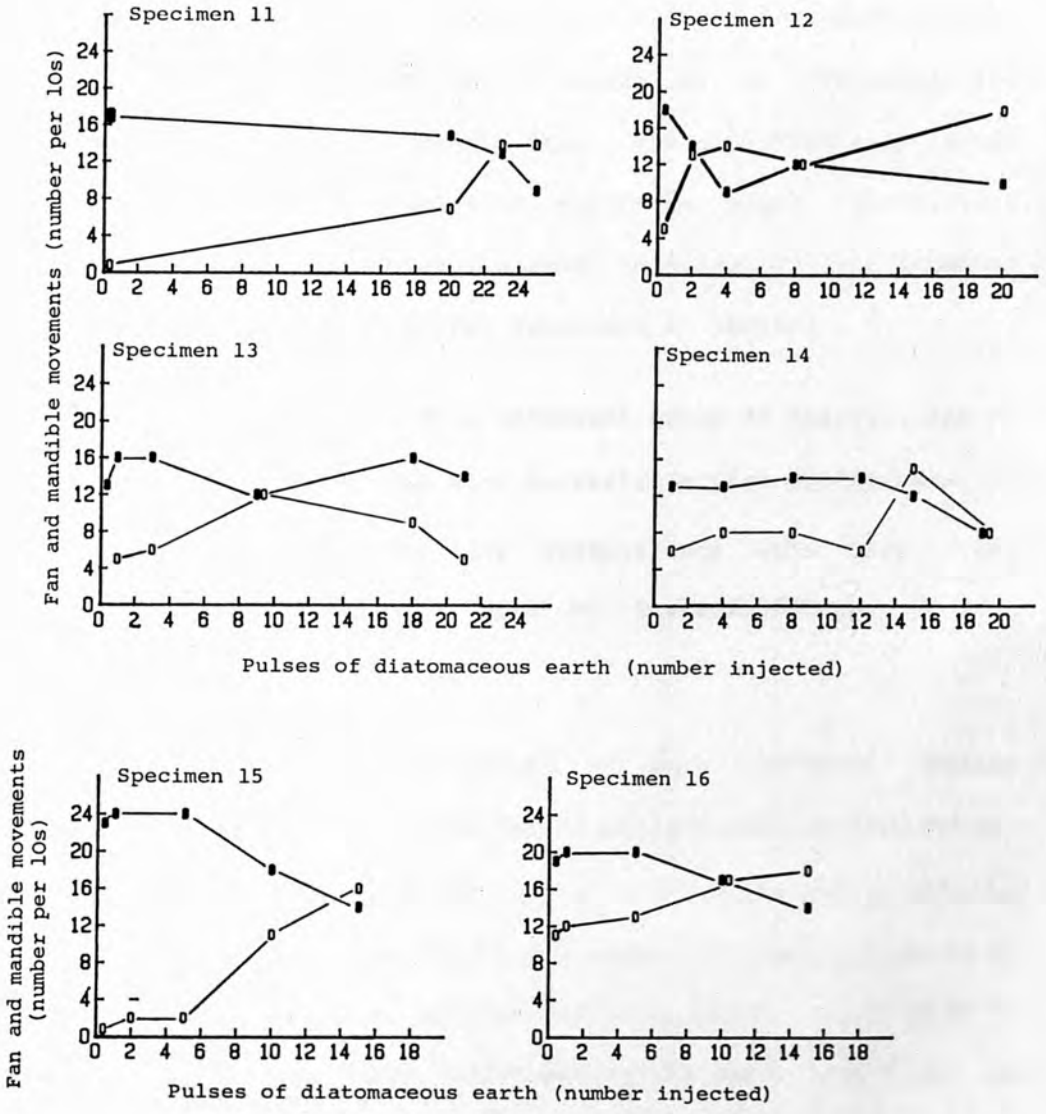


Figure 28b. The number of fan and mandible movements made by larval S. ornatum during stimulation with pulses of diatomaceous earth. Each value is the number of fan or mandible movements recorded during a ten second extract of behaviour; each ten second extract immediately followed the injection of a pulse of diatomaceous earth into the water. Graphs show selected responses of all larvae to a series of pulses of diatomaceous earth.

●=no. of fan movements
o=no. of mandible movements

6.4 The sensitivity of larvae to chemical stimulants

As nothing was known of the range of chemical compounds to which larvae might be sensitive they were first exposed to a wide variety of chemical compounds in a qualitative, bioasssay to determine the type of compounds able to stimulate them. The sensitivity of larvae was tested in particle-free distilled water to avoid interference from natural solutes. Larvae were exposed to pulses of each compound using the pulse injection apparatus described in section 3.3.

Each compound was tested on a different group of larvae , all of which were acclimated for one hour in particle-free distilled water at $17cms^{-1}$ and 10° ± 1°C before any observations were made. The larvae were observed with a Wild M5 Stereomicroscope at 25x magnification.

Larvae were exposed to five pulses of each compound; pulses were of one second duration, with thirty seconds between each pulse. Responses to compounds were judged 'by eye'. If, after five pulses, a larva had made at least one noticeable response it was judged to be sensitive to that compound at the concentration given. If it made no response during the first five pulses it was judged to be unresponsive. This method, while less precise than the analysis of behaviour patterns using video tape recordings , did allow a number of compounds to be tested quickly.

Preliminary observations showed that when exposed to pulses of chemical compounds larvae usually responded with a short burst of mandible and associated mouthpart movements (and whole body movements in the case of more extreme stimulation). Fan cleaning movements were affected only in as much as they were inhibited while the other

movements were made. Differences in the types of mouthpart movements made could be recognised and the responses observed have been placed in the classification shown in Table 16.

The types of response made appeared to be related to the intensity of stimulation. Response type 1 followed relatively mild stimulation, while response type 6 followed intense stimulation (Categories 5 and 6 were rarely seen and have not been observed in animals feeding in unfiltered natural water, whereas all other categories may be observed in natural water). There were no sharp boundaries between the categories and one frequently developed into the next. Response types 1 and 2, which are described in more detail below (see Section 6.5), were the responses most commonly observed.

The compounds that were tested, and the number and percentage of animals responding positively, are shown in Table 17.

TABLE 16. A classification of the responses made by larvae to pulses of chemical stimulants.

Response	Description of response
0	No apparent behavioural response
1	Rapid adduction and abduction of mandibles; short strokes not reaching up to the fan stem.
2	Slow adduction and abduction of mandibles; long strokes reaching up to the fan stem.
3	Combination of 1 and 2, the latter usually developing from the former.
4	Head movements in addition to mouthpart movements.
5	Writhing of body in addition to any mandibular or head movements (excessive stimulation and occasionally in final stages of feeding inhibition).
6	Detachment and floating downstream (only seen with noxious stimulants).

The compounds tested fell into the following groups: primary alcohols, diols, amino-acids, salts of alkaline and alkaline earth metals, organic acids, esters and aldehydes, phenols, amines and sugars. They were selected to represent a wide range of chemical structures, with particular emphasis on those that might be the products of decomposition, as larvae are exposed to large amounts of detrital material while feeding. Of these, all diols were non-stimulatory, as were four of the sugars and six of the amino acids, at the concentrations tested. Sugars appeared to be the least effective among the stimulants causing mandible movements only at relatively high concentrations. Non-stimulatory compounds, which were in the minority, are underlined in Table 17.

Larvae were most sensitive to phenols, amines, esters, aldehydes and acids, including carboxylic acids. In many cases the concentrations used, in the range 10^{-2}M to 10^{-3}M, elicited responses in all the larvae tested suggesting that some would still respond to these compounds at a concentration one order of magnitude lower (see also Figure 29). Some of the compounds in the groups to which larvae were particularly sensitive, are known to be associated with decomposition. Phenol and its derivatives, for example, are produced during the microbial breakdown of lignin, while acids, such as malic and citric, are released from Fraxinus leaves decomposing in fresh-water (Willougby, 1976). It is not surprising, therefore, to find that larvae that feed on a very heterogeneous mixture of 'detritus', as well as living material, are sensitive to chemical compounds associated with decay.

TABLE 17. A survey of the sensitivity of S. ornatum larvae to a variety of chemical compounds.

Compound	Estimated concentration (molar)	Number responding to stimulant (%)		Response type (see Table 16)
Methanol	1.61	4/22	(18)	2,4
Ethanol	1.6	21/21	(100)	3
Propanol	1.61	13/13	(100)	2, 5/6
Butanol	See Figure 29			
Pentanol	0.003	20/21	(95)	1
Hexanol	0.003	20/21	(95)	3
Heptanol	0.003	21/23	(91)	3
Octan-2-ol	0.003	10/10	(100)	2
Glycine	0.31	9/19	(47)	-
Arginine	0.03	17/25	(68)	5
Tyrosine	0.05	0/10	(0)	0
Cysteine	0.027	1/18	(5)	-
Histidine	0.035	3/17	(18)	-
L-lysine	0.05	10/10	(100)	2
Alanine	0.085	0/11	(0)	0
Isoleucine	0.023	6/9	(67)	2,3,4
Methionine	0.03	0/8	(0)	0
Serine	0.055	0/10	(0)	0
Threanine	0.013	0/10	(0)	0
Cystine	0.02	7/9	(78)	2
DL-aspartic acid	0.04	7/9	(78)	1
Hydroxyproline	0.035	10/10	(100)	2
Valine	0.04	4/10	(40)	1
Phenylalanine	0.035	9/10	(90)	1
Asparagine	0.035	4/10	(40)	-
DL-lysine mono-HCl	0.03	18/18	(100)	-
LiCl	0.03	23/25	(92)	1
KCl	0.03	17/20	(85)	1
$MgCl_2.6H_2O$	0.03	24/26	(92)	1
$CaCl_2.2H_2O$	0.03	11/23	(48)	1
NaCl	See Figure 29			
NaF	0.03	17/24	(71)	1
NaBr	0.03	16/18	(89)	1
NaI	0.03	14/26	(54)	1
Sodium acetate	0.03	1/11	(9)	1

TABLE 17 Continued

Compound	Estimated concentration (molar)	Number responding to stimulant (%)		Type of response
$NaNO_2$	0.037	10/10	(100)	2,4
Na_3PO_4	0.035	10/10	(100)	2,4
$NaHCO_3$	0.035	0/10	(0)	0
NaOH	0.035	5/5	(100)	2,4
$Na_2SO_4.10H_2O$	0.015	0/10	(0)	0
$Na_2CO_3.10H_2O$	0.013	9/10	(90)	2
$FeCl_3.6H_2O$	0.035	0/4	(0)	0
$Pb(NO_3)_2$	0.035	3/7	(43)	1
Phenol	0.0017	1/11	(9)	1
m-Cresol	0.003	10/10	(100)	3
m-Chlorophenol	0.0035	6/6	(100)	2,6
m-Nitrophenol	0.0017	10/10	(100)	3
Aniline	0.004	10/10	(100)	2,4
Methylamine	0.003			2,4,5
Ethylamine	0.004	9/9	(100)	2,4,5
Dimethylamine	0.01	10/10	(100)	2
Propylamine	0.03	10/10	(100)	2,4,5
sec-Butylamine	0.03	9/9	(100)	3,4
Glycerol	0.025	0/15	(0)	0
Ethylene glycol	0.035	0/10	(0)	0
Propane 1,2-diol	0.035	0/10	(0)	0
Digol	0.035	0/10	(0)	0
Methyl formate	0.035	4/10	(40)	-
Methyl acetate	0.035	10/10	(100)	2
Ethyl acetate	0.035	10/10	(100)	2
Amyl acetate	0.035	10/10	(100)	2
Ethyl formate	0.035	10/10	(100)	2
n-propyl acetate	0.04	10/10	(100)	2
n-buytl acetate	0.005	10/10	(100)	2
Vanillin	0.0035	10/10	(100)	3
Piperonal	0.0035	10/10	(100)	2,4
Acetaldehyde	0.033	8/10	(80)	2
Propionaldehyde	0.035	9/9	(100)	3
Paraldehyde	0.004	6/10	(60)	2
Hydroquinone	0.0045	3/10	(30)	2
HCL	See Figure 29			
Formic acid	0.0035	10/10	(100)	1
Ascorbic acid	0.035	10/10	(100)	2
Malic acid	0.004	10/10	(100)	1
Butyric acid	0.004	10/10	(100)	3

TABLE 17 continued

Compound	Estimated concentration (molar)	Number responding to stimulant (%)		Type of response
Salicyclic acid	0.004	10/10	(100)	2
Acetic acid	0.025	16/17	(94)	1,4
Urea	0.04	0/10	(0)	0
Tartaric acid	0.04	6/6	(100)	4,5,6
Oxalic acid	0.045	4/7	(57)	3
Citric acid	0.05	1/7	(14)	1
Benzoic acid	0.0045	10/10	(100)	1
Chloroacetic acid	0.0035	10/10	(100)	1
Trichloro-acetic acid	0.0025	10/10	(100)	2,4
D-xylose	0.01	1/26	(4)	-
L-rhamnose	0.06	0/25	(0)	0
L-arabinose	0.095	6/27	(22)	1
D-galactose	0.132	5/22	(23)	1
D-fructose	0.10	0/17	(0)	0
Mannose	0.077	2/21	(10)	-
Maltose	0.13	5/18	(28)	1
D-glucose	0.11	5/24	(21)	1,2
Melibiose	0.075	3/19	(16)	1
Sucrose	See Figure 29			

As little is known of the sensitivity of aquatic insects to chemical compounds in general some of the results presented in Table 17 are compared with literature values for the sensitivity to chemical compounds of Phormia regina, which has been tested very extensively, and Laccophilus maculosus, an aquatic beetle, which appears to be the only other aquatic insect for which such observations have been made.

Table 18 shows the molar concentrations at the 50% acceptance threshold for some sugars for the blowfly P. regina (Dethier, 1976) compared with the responses made by S. ornatum to the same sugars. By the criterion of 50% responding representing a positive response only one of the sugars (sucrose) stimulated S. ornatum at the concentrations given. However, this does not prevent comparisons being made since the 50% limit is arbitrary. With the exceptions of maltose, fructose and sucrose, which were highly stimulatory to P. regina and far less so to S. ornatum , the other sugars tested were similar in their effects on the two species (D-galactose, L-arabinose, L-galactose, D-xylose and mannose). The latter two of this group were effectively non-stimulatory for P. regina.

There was also a close similarity between the range of amino-acids that stimulated the labellar chemoreceptors of P. regina, as reported by Shiraishi and Kuwabara (1970), and those that initiated a response in S. ornatum. The results of Shiraishi and Kuwabara are compared with those found here in Table 19. All but one of the amino acids were tested in the concentration range 0.1M to 0.5M in both studies, the one exception being alanine, which was tested at 0.08M on S. ornatum.

Of the amino acids tested by Shiraishi and Kuwabara only three, Glycine, Cystine and Methionine, did not affect S. ornatum in a broadly similar way. Glycine and Cystine elicited mandibular responses in S. ornatum but were non-stimulatory to P. regina, whilst the converse was true of Methionine. Although several of the amino acids that stimulated P. regina affected the sugar receptor cell, most of these acids also stimulated S. ornatum, even though the larvae appeared generally rather insensitive to sugars.

When exposed to an ascending group of homologous primary alcohols S. ornatum responded to concentrations in a range similar to the threshold stimulatory concentrations for both Laccophilus maculosus (Hodgson, 1956) and P. regina (stimulated on tarsal chemoreceptors (Dethier, 1951). The results of Dethier and Hodgson are compared to those reported here for S. ornatum in Table 20. Although each compound was tested on S. ornatum at only one concentration (with the exception of butanol) the results appear to be very similar. The concentrations to which S. ornatum exposed elicited, with the exception of methanol, responses from 91% to 100% of the larvae, suggesting that S. ornatum was more sensitive to alcohols than L. maculosus and of similar sensitivity to alcohols as P. regina.

Table 20 also compares the sensitivity of S. ornatum and L. maculosus to various salts, HCl and NaOH. All compounds, except HCl, were tested at a concentration of 0.03M and at these concentrations at least 54% of the larvae were stimulated. S. ornatum appears to be slightly more sensitive than L. maculosus to all the salts but not to NaOH. It is at least ten times more sensitive to HCl than L. maculosus, although the significance of such sensitivity to the

larvae is unclear.

It is possible that the differences in sensitivity observed in the responses of S. ornatum and L. maculosus may reflect differences in the behavioural assay used to measure the response to stimulation. Hodgson (1956) recorded the movement of adult L. maculosus away from a resting place when they were exposed to a stimulating solution. As has been reported, a much more subtle response was observed with the simuliids and it is possible that lower concentrations were needed to elicit this response than would be needed to produce a movement of the whole animal.

Gillary (1966) reported on the sensitivity of the labellar hairs of P. regina to LiCl, KCl, NaCl, RbCl and CsCl. His results suggest that the blowfly was of similar sensitivity to salts as S. ornatum larvae. No responses to any of these compounds were detected in the labellar chemoreceptors of P. regina at concentrations below 0.1M, but as responses were measured electrophysiologically on single receptors it is likely that simultaneous stimulation of a number of receptors would show P. regina to be as sensitive as S. ornatum (cf Arab, 1959, who found that 0.0164M sucrose, when applied to the entire labellum of P. regina, stimulated proboscis extension, but that 0.419M sucrose was needed to achieve the same effect when a single receptor was stimulated).

Although most compounds were assayed in this study at only one concentration four were tested in more detail as representatives of major groups of compounds. Concentration response curves were found for NaCl, HCl, Butanol and Sucrose and are shown in Figure 29. The probit value of the percentage of larvae stimulated was plotted against the common logarithm of the molar concentrations of each

compound tested. The regressions of the lines fitted to these points are shown in Appendix 10. Larvae were exposed to concentrations of the compounds in random order, one group of larvae being exposed to all concentrations of each compound. Only final instar larvae were used in these experiments and they were acclimated to the apparatus (the observation cell already described) for one hour before any observations were made, with the one exception described below.

Figure 29 shows that for three of the four compounds tested there was approximately one order of magnitude difference in the concentration that stimulated no larvae and that which stimulated all larvae; it seems likely that the other compounds in each of these groups would elicit similar responses. However, the responses to sucrose do not appear to show a clear linear relationship between concentration and probit percentage responding positively. Two curves were plotted for HCl; curve 1 shows the number of larvae responding after being kept in flowing particle-free distilled water overnight and curve 2 shows the sensitivity of larvae acclimated in particle-free distilled water for one hour. Prolonged exposure reduced the sensitivity of larvae to HCl by about one order of magnitude. The concentrations of these compounds stimulating 50% of the larvae are shown in Appendix 10.

Considering the small number of 'olfactory' sensilla possessed by the larvae it is possible that the responses observed here followed the stimulation of uniporous, presumably contact, chemoreceptors. Consequently, the interpretation of the sensitivity of simuliids to chemical compounds probably awaits descriptions of the surface chemistry of algal cells and detritus, although it is also possible that larvae are able to sense compounds by 'olfaction'

from the background of dissolved compounds in the water. In the Bere Stream, a small chalk stream in Dorset, Crisp (1970) found the following concentrations of the principal elements (which I have converted from parts per million to molarity for ease of comparison): Na, $5x10^{-4}$; K, $3x10^{-5}$; Ca, $2x10^{-3}$; P, 10^{-6} and N, $2x10^{-4}$. Dawson (1981) estimated that the maximum dissolved organic matter concentration in the nearby R. Piddle never exceeded 24mgl-1.

As many compounds in the concentration range 10^{-2}M to 10^{-3}M stimulated 100% of the larvae tested it is possible that larvae could sense the more concentrated of these elements. However it is of more immediate concern to describe the movements made by larvae in response to chemical compounds as this provides further evidence for the role of mandible movements in the behavioural repertoire of the larvae.

TABLE 18. A comparison of the sensitivity of *S. ornatum* larvae and *P. regina* to various sugars. Values for *P. regina* from Dethier (1976).

Sugar	Concentration tested on *S. ornatum* (molar)	% positive	50% threshold concentration (molar) for *P. regina*
D-maltose	0.14	28	0.0043
D-fructose	0.18	0	0.0058
Sucrose	0.33	50	0.0098
D-glucose	0.11	21	0.132
D-galactose	0.13	23	0.5
L-arabinose	0.095	23	0.144
D-mannose	0.077	NS	7.59
D-xylose	0.01	NS	42.57
Melibiose	0.075	16	NS
L-rhamnose	0.06	NS	-

NS = non-stimulatory

TABLE 19. A comparison of the sensitivity of *S. ornatum* larvae and *P. regina* to various amino acids. Values for *P. regina* from Shiraishi and Kuwabara (1970)

Amino aicd	*S. ornatum*	*P. regina*
Glycine	*	NS
Alanine	NS	NS
Serine	NS	NS
Threonine	NS	NS
Cystine	*	NS
Tyrosine	NS	NS
Proline	*	SS
Hydroxyproline	*	SS
Valine	*	SGS
Leucine	*	SGS
Isoleucine	*	SGS
Methionine	NS	SGS
Phenylalanine	*	SGS
Tryptophan	*	SGS
Aspartic acid	*	NSI
Glutamic acid	*	NSI
Histidine	*	NSI
Arginine	*	NSI
Lysine	*	NSI
Asparagine	*	-
DL-lysine mono-HCl	*	-
Cysteine	*	-

* Stimulated *S. ornatum*

Effect on *P. regina*:

NS Non-stimulatory
SS Salt cell stimulator
SGS Sugar cell stimulator
NSI Non-specific inhibitor
\- Not tested

TABLE 20. The sensitivity of S. ornatum larvae, L. maculosus and P. regina to alcohols and salts. Values for L. maculosus from Hodgson (1956) and from P. regina from Dethier, (1976).

Compound	Concentration delivered to S. ornatum (molar) and (% +ve)		Concentration stimulating 50% of L. maculosus (Hodgson, 1956)	Concentration stimulating 50% of P. regina (Dethier, 1976)
Methanol	1.6	(18)	3.6	0.782
Ethanol	1.6	(100)	4.3	0.377
Propanol	1.6	(100)	3.2	0.077
Butanol	0.033	(95)	0.046	-
Pentanol	0.003	(95)	0.0073	0.0753
Hexanol	0.003	(95)	0.0011	0.0061
Heptanol	0.003	(91)	-	0.0011
Octanol	0.003	(100)	-	0.0001
LiCl	0.03	(92)	0.46	-
KCl	0.03	(85)	0.078	-
HCl	0.0003	(89)	0.0044	-
NaBr	0.03	(89)	0.14	-
NaI	0.03	(54)	0.098	-
NaOH	0.035	(100)	0.01	-

- Compounds not tested

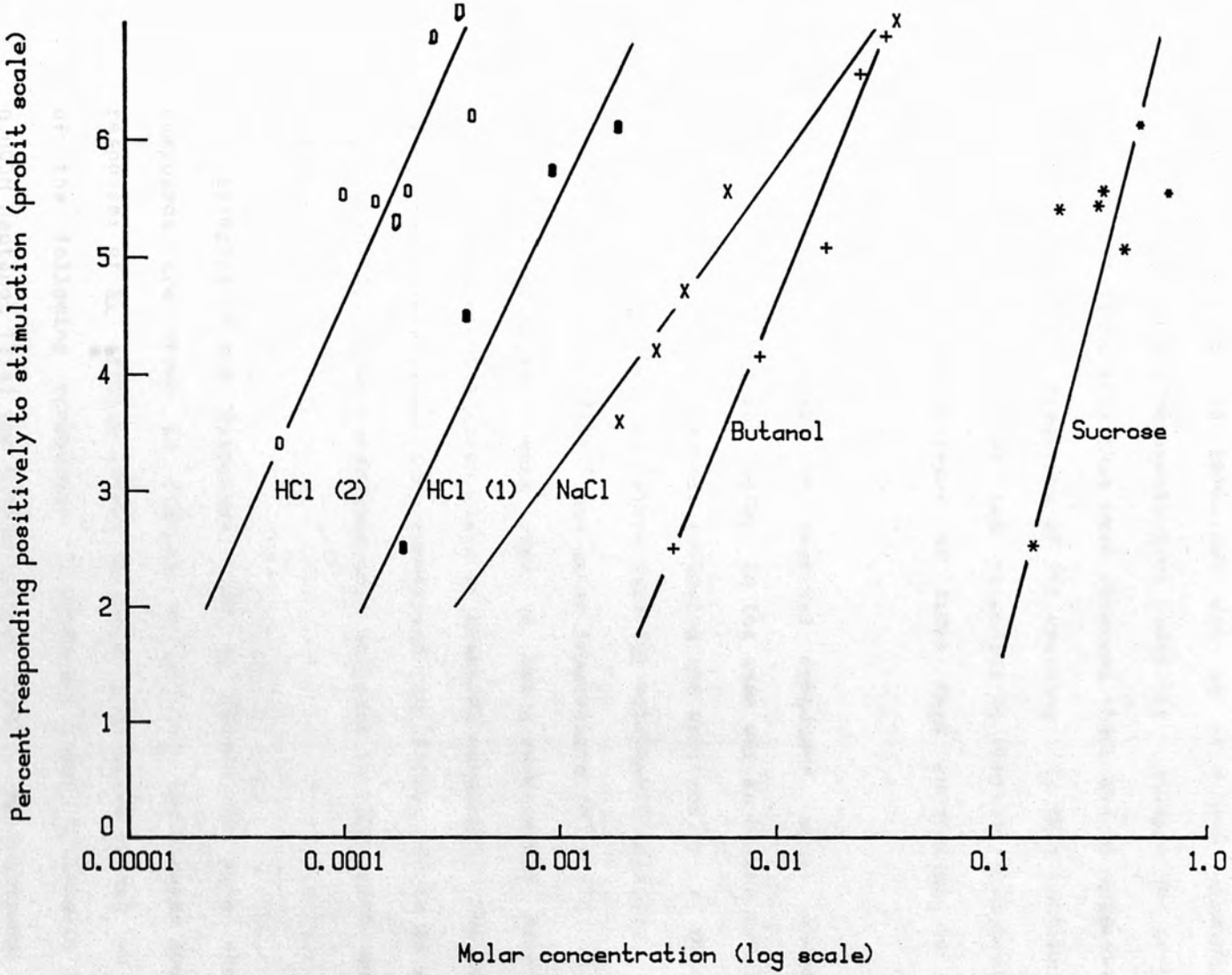


Figure 29 The sensitivity of S. ornatum to NaCl, HCl, Butanol and sucrose

6.5 The effect of chemical stimulants on larval feeding behaviour

It was shown in Section 6.4 that the commonest effect of pure chemical compounds on behaviour was to stimulate mandible and associated mouthpart movements (see Table 16). Amongst the responses to chemical stimulants that were observed there was no suggestion of any increase in the frequency of fan cleaning. In this section more detailed observations of the responses to chemical stimulants are described based on analyses of video tape recordings of these responses.

One second pulses of chemical compounds were added to particle-free distilled water in the same way as before and larval responses, in the ten seconds following the addition of a pulse to the water, recorded on video tape for subsequent analysis. Water velocity was always $17cms^{-1}$ and water temperature 10° ± 1°C. A small number of stimulants were used in these experiments, each being chosen to represent a large class of chemical compounds. The results of these observations are summarised in Tables 21 to 24 and in Figures 30-33. Video recordings were analysed in the same way as before.

Examples of the responses made by larvae to pure chemical compounds are shown in Figures 30 and 31. The figures show the responses of *S. ornatum* larvae to three consecutive pulses of each of the following compounds: 0.0035M HCl (30a), 0.05M NaCl (30b), 0.003M heptanol (31a) and 0.003M butanol (31b). The responses shown represent the responses that were described in Table 16 as response type 1 or type 2. The main feature of these figures is the occurrence of extra mandible movements (represented by the vertical, hatched, bars). The bars show the time taken for the mandible to adduct from

the medial position down to the cibarium and then abduct back to the medial position (and sometimes beyond the medial position). Most of the bars are close together because the mandibles were often constantly in motion during the response to chemical stimulation. The end of each mandible movement and the beginning of the next is shown by the sightly heavier, vertical, subdividing lines if there was no stationary phase between mandible movements. A key to all bar charts is given in Table 4a.

This simple sequence of movements, and its relationship with the fan cleaning cycle, was described in Figure 11. In all cases extra mandible movements were made without the fans being closed; the movements described in response to chemical stimulants did not involve fan cleaning. Although it has not been investigated experimentally it is assumed that these mandible movements would normally push food into the cibarium.

The figures show the distinct bursts of mandible movements, uninterrupted by fan cleaning, that immediately follow the injection of a stimulant pulse. After this first burst, when there were no intervening fan cleaning movements, fan cleaning began again with occasional extra mandible movements occurring between fan beats, as is typical of uninhibited feeding behaviour. In these figures uninhibited feeding behaviour, before the injection of a stimulant, is not shown on a separate bar. In most cases, however, normal uninhibited feeding resumed within ten seconds of the stimulus pulse and can be seen in the second half of each bar in the responses to HCl, heptanol and butanol (this was less clear in the responses to NaCl depicted in Figure 30, but can be seen incidentally in the responses to NaCl shown in Figure 33).

It was assumed that the duration of a response was the duration of the first uninterrupted burst of mandible movements following the injection of a stimulus pulse. Such bursts may be seen in Figure 30, HCl pulses 2 and 3, NaCl pulse 1 and Figure 31, heptanol pulses 2 and 3, butanol pulses 2 and 3. In HCl pulse 3, for example, the first group of seven mandible movements is regarded as the response and any remaining isolated movements are assumed not to have been caused by the stimulant. This probably underestimated the duration of a response, but it was not possible to make a more accurate measurement of its duration without electrophysiological measurements.

It might be expected that increasing the concentration of a stimulant would cause an increase in the number of mandible movements in each burst made in response to stimulation. To test this hypothesis larvae were exposed to pulses of three concentrations of NaCl (estimated to be 0.004M, 0.008M and 0.02M) and their responses recorded. Higher concentrations were not used because preliminary observations had shown that larvae reacted with response type 4 movements, which could not be used in this analysis, at concentrations greater than 0.02M. In this case the total number of mandible movements made in the first five seconds following a stimulant pulse was measured rather than the number in the first burst of movements. This was done so that larvae that were not exposed to NaCl, and which did not make bursts of mandible movements, could be included in the analysis.

The results of these observations are shown in Figure 32 and in Table 21. The values plotted in Figure 32 are mean numbers of mandible movements made in the first five seconds following the injection of a stimulus pulse. n is the number of responses to

stimulant pulses that were analysed, each original value among the n being derived from the first five seconds of extracts of behaviour such as are shown in Figures 30 and 31. The first value on the graph shows the mean number of mandible movements made in five seconds of unstimulated behaviour; standard errors are in parentheses (all standard errors were inside the dimensions of the characters at this scale).

There were significantly more mandible movements made in the first five seconds after a pulse of stimulant, at an estimated concentration of 0.008M or greater, than in any five seconds without a stimulant (see Table 21). At an estimated concentration of 0.004M there was no significant difference in the number of mandible movements made in response to one second pulses of NaCl, when compared to unstimulated larvae (Student-Neuman-Keuls comparison of means test).

The number of mandible movements in a burst in response to pulses of NaCl (as opposed to the number of mandible movements in the five seconds following stimulation) showed no significant difference at the two concentrations at which a response was made with between three and four movements per burst (see Table 22).

Table 22 shows that the other compounds that larvae were exposed to did caused up to seven mandible movements in each burst. Although there were differences in the number of mandible movements caused by different compounds, a pairwise comparison of ranked values from a Kruskal-Wallis ANOVA (see Appendix 11) demonstrated that the statistical significance of this result was due mainly to differences between the unstimulated Group 1 (0.004M NaCl) and all remaining groups. Among the positive responses only that caused by 0.008M NaCl

differed significantly from any other stimulus response (0.035M butanol and 0.0035M heptanol).

These observations suggest that the number of mandible movements in a burst did not respond to changes in stimulant concentration. Rather, as the concentration rose it was more likely that the type of response made would alter, though this has not yet been demonstrated conclusively. However, whilst the number of extra mandible movements in each burst did not differ significanly, their durations did.

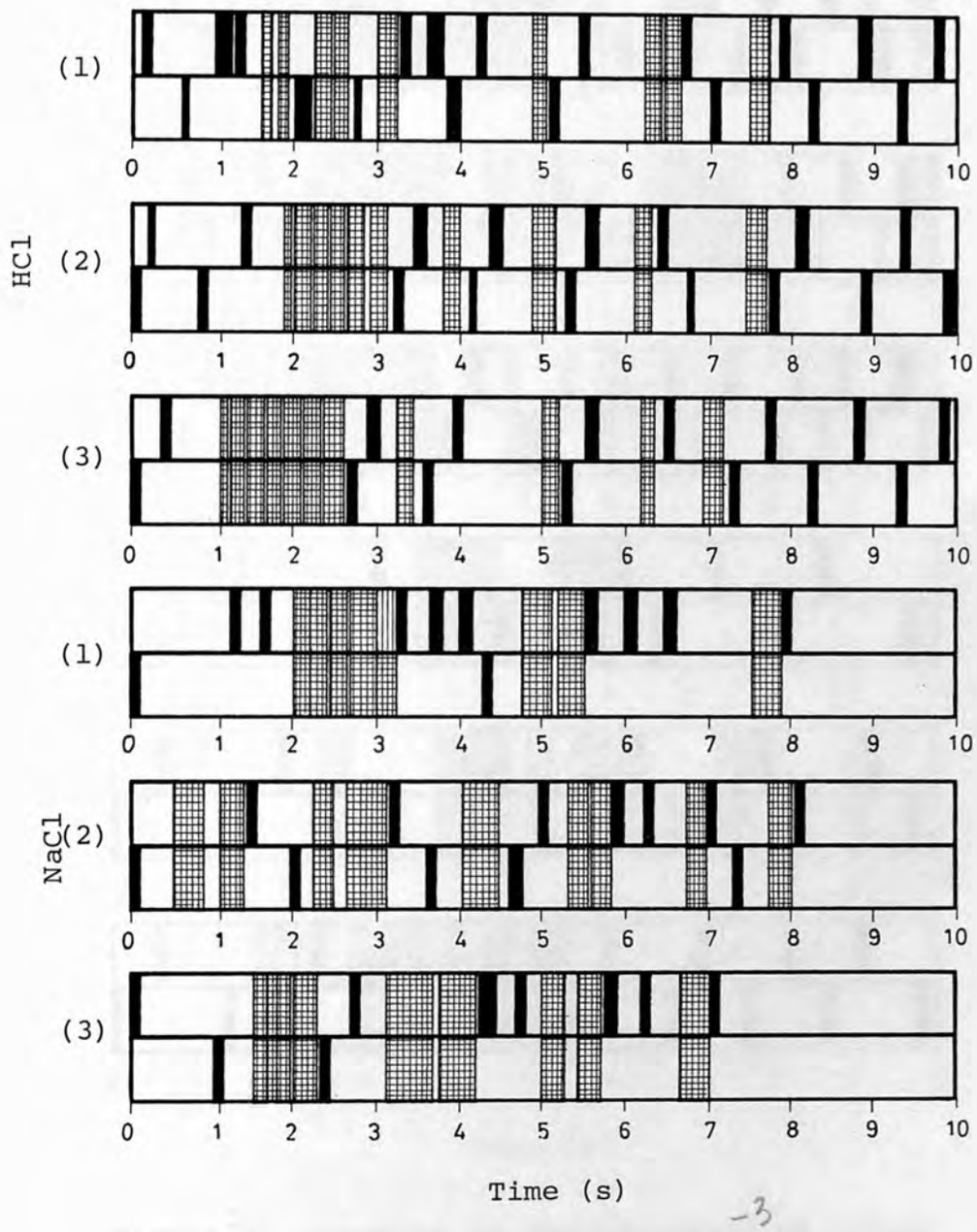


Figure 30 Examples of responses of larvae to consecutive pulses of HCl and NaCl, at an estimated concentration of 3.6×10^{-3}M and 5×10^{-2}M respectively. Water velocity 17cms^{-1}; water temperature 11.5^{o} $\pm$ 1^{o}C. See Table 4a for key to symbols.

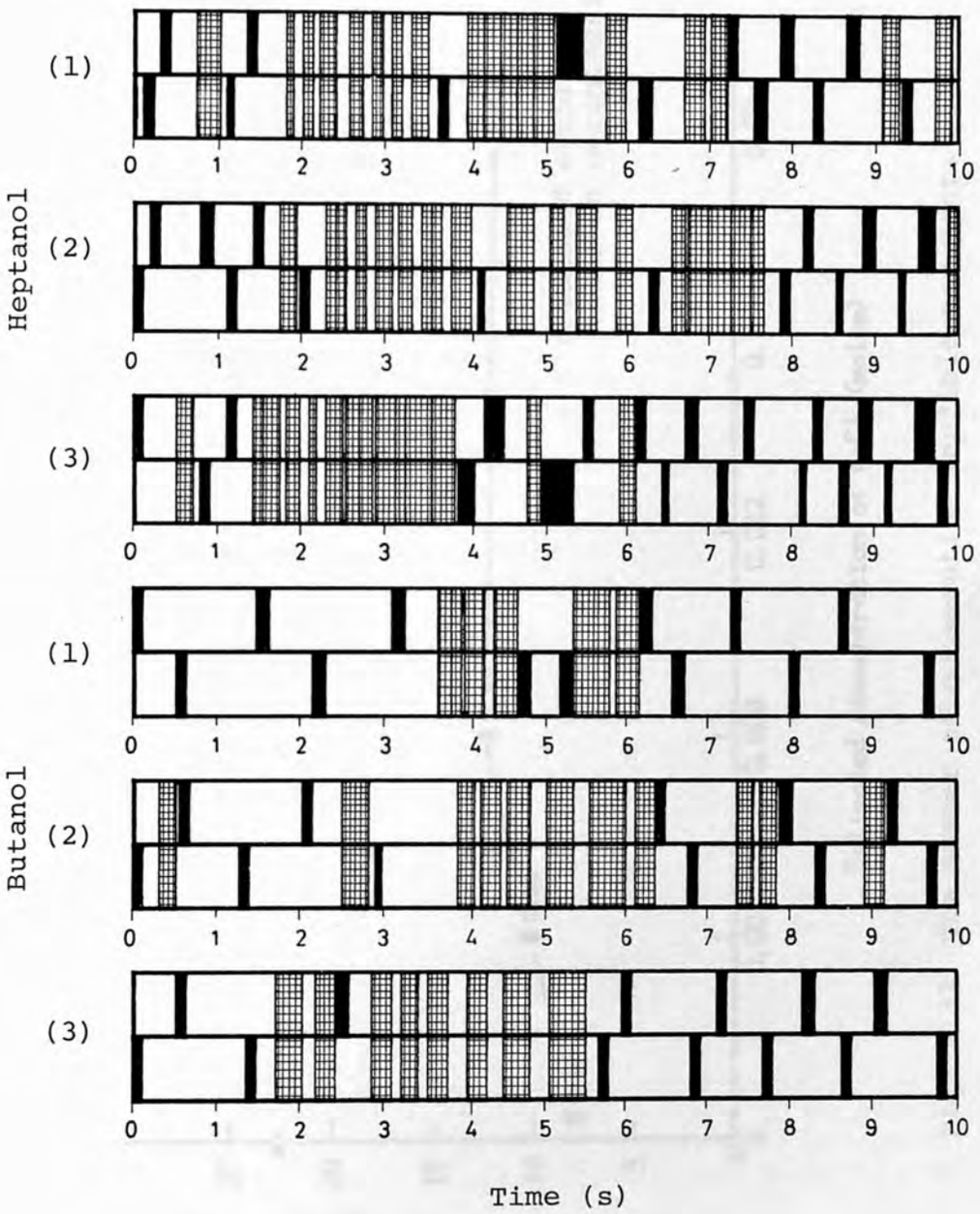


Figure 31 Examples of the responses of larvae to consecutive , one second, pulses of heptanol and butanol at an estimated concentration of 3×10^{-3}M. Water velocity 17cms^{-1}; water temperature $11.5^{\circ} \pm 1^{\circ}$C. See Table 4a for key to symbols.

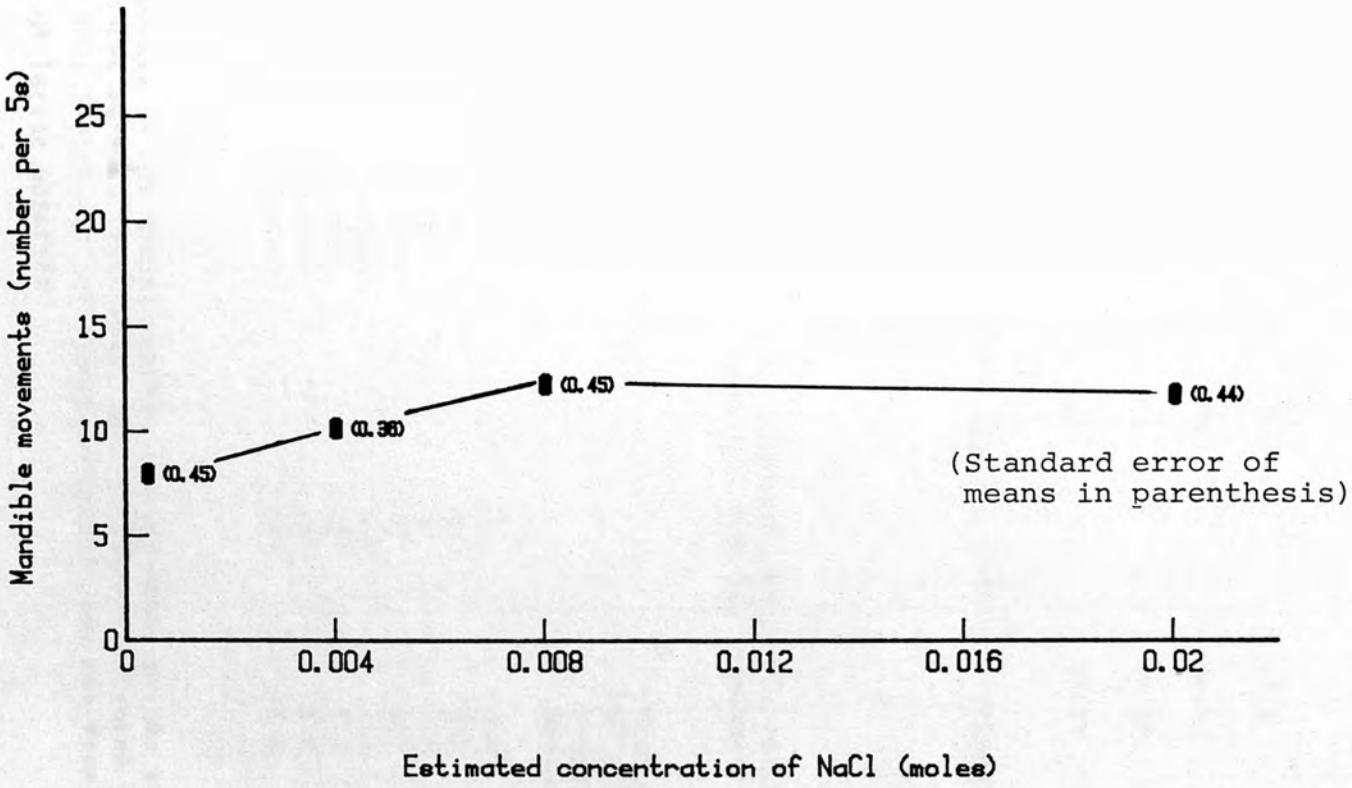


Figure 32 The number of consecutive mandible movements made in response to a one second pulse of NaCl. Values are means calculated from 5s extracts of behaviour (see text for further explanation). Water velocity 17cms^{-1}; water temperature 11.5^{o} $\pm$ 1^{o}C.

TABLE 21. The mean number of mandible movements in a burst made in response to pulses of NaCl at three concentrations.

Estimated concentration of NaCl (molar)	n	mandible movements (mean number in 5s)
0 (no NaCl)	29	7.9
0.004	34	10.1
0.008	38	12.2*
0.02	34	11.7*

Means marked by a * differ significantly from the remaining two (Student-Neuman-Keuls test).

n=no. of larvae observed

TABLE 22. The number of mandible movements made in a burst in response to pulses of a variety of chemical stimulants.

Compound	Concentration (molar)	n	Mean number of extra mandible movements	SE
NaCl	0.004	21	0.8	0.14
NaCl	0.008	34	3.4	0.52
NaCl	0.02	33	3.9	0.25
Aspartic acid	0.0075	16	3.7	0.48
HCl	0.00035	12	4.3	0.48
Butanol	0.035	17	5.3	0.79
Heptanol	0.0035	15	6.5	0.55
Amyl acetate	0.0035	12	7.6	0.49

See Appendix 11 for Kruskal-Wallis one-way ANOVA of the significance of differences between treatments. Most treatments differed significantly from 0.004M NaCl (which did not stimulate larvae) but not from each other.

n=no. of larvae observed

TABLE 23. The duration of each extra mandible movement made in a burst in response to pulses of a variety of chemical stimulants.
n=no. of larvae observed

Compound	Response type (see Table 17)	Concentration (molar)	n	Mean duration, s, of each extra mandible movement (± SE).	
Aspartic acid	1	0.0075	15	0.22*	(0.01)
HCl	-	0.00035	12	0.22*	(0.014)
Methylamine	2,4,5	0.0035	11	0.24*	(0.012)
Benzoic acid	1	0.0035	12	0.24*	(0.014)
NaCl	1	0.007	34	0.27	(0.018)
NaCl	1	0.02	33	0.27	(0.02)
NaOH	2,4	0.0075	11	0.26	(0.018)
Butanol	-	0.035	17	0.32	(0.024)
Amyl acetate	2	0.0035	15	0.32	(0.015)
Heptanol	3	0.0035	12	0.38	(0.025)

Means marked with a * were significantly different from all others (Student-Neuman-Keuls test).
n=no. of larvae observed

TABLE 24. The duration of bursts of mandible movements made in response to stimulation with one second pulses of various chemical stimulants.

Compound	Concentration (molar)	n	Mean duration of response, s (± SE)	
NaCl	0.007	34	0.96	(0.15)
NaCl	0.02	33	1.03	(0.075)
Butanol	0.035	17	1.88	(0.335)
Aspartic acid	0.0075	15	0.86	(0.125)
HCl	0.00035	12	1.00	(0.134)
Methylamine	0.0035	11	1.54	(0.154)
Benzoic acid	0.0035	12	0.88	(0.096)
Heptanol	0.0035	15	2.11	(0.23)
Amyl acetate	0.0035	12	2.96	(0.333)
NaOH	0.0075	11	2.33	(0.15)

Table 23 shows that the mean duration of each mandible movement (adduction and abduction) following stimulation varied from 0.22s to 0.38s. The first four means in Table 23 are significantly different from the rest (Student-Neuman-Keuls comparison of means test). With the exception of methylamine there was a general trend amongst the compounds listed in this table for Type 2 responses to be associated with longer individual mandible movements, perhaps confirming the qualitative definition of Type 2 movements in Table 16. The cause of this difference in the duration of mandible movements is unknown, although it may simply reflect the relative ability of diffeent compounds to stimulate the larvae. Although all stimulants listed here were delivered in 1s pulses bursts of mandible movements often lasted for more than one second, as is shown in Table 24. The mean length of bursts of movements varied from 0.96s to 2.33s.

The observations reported in this section show that there was a distinctive response to chemical stimulants. It is possible that similar responses could be initiated by contact with particles that had been removed from the cephalic fans and that they might normally be responible for the manipulation and ingestion of trapped particles. Although responses to particles and chemical compounds may serve the same function, comparison of Figures 30 and 31 with Figures 26 and 27 shows that there were differences between them. Diatomaceous earth stimulated longer periods of activity of the mandibles even though pulses were of the same duration as the pulses of pure chemical compounds. This was probably because larvae trapped particles of diatomaceous earth and therefore lengthened the period of stimulation.

Pulses of diatomaceous earth also caused a cumulative increase in the intensity of the response as the larvae were exposed to more pulses of particles (see Figures 26 and 27). There was no suggestion from observations of repeated stimulation with pure chemical compounds that larvae began to respond more intensely as more pulses of stimulant were delivered to them.

Apart from these two distinctions pure chemical compounds and inert particles appeared to have broadly similar effects on larval behaviour. Both stimulated mandible movements (and associated movements of the maxillae and labrum) and either caused no change in, or inhibited, the frequency with which the cephalic fans were cleaned. Clearly the presence of either inert particles or chemical compounds dissolved in the water does not immediately account for the considerable difference in fan cleaning frequencies observed in particle-free distilled water and unfiltered natural water.

Although pulses of diatomaceous earth and chemical compounds may initially cause the fans to be open for longer than before stimulation (by inhibiting the cleaning of the fans while extra mandible movements are made) this response is probably not a mechanism for gathering more food particles. In most cases the lengthening of the time for which the fans are open appears to be a prelude to the inhibition of feeding.

6.6 The effect of pulses of diatomaceous earth on larval feeding behaviour following the blocking of chemoreceptors with p-Chloromercuribenzoic acid (PCMB)

The observations reported in sections 6.3 and 6.5 suggested that physical and chemical stimulants initiated broadly similar behavioural responses in the larvae, stimulating mandible movements and inhibiting fan cleaning. However, it was necessary to obtain more evidence of the ways in which the effects of these two stimulant types were mediated by the sensory nervous system. It was not clear whether responses to diatomaceous earth were due to the stimulation of mechanoreceptors or chemoreceptors although the former was more likely. To investigate this larvae were exposed to pulses of diatomaceous earth following treatment with p-Chloromercuribenzoic acid (PCMB). A chemo-receptor blocker, PCMB is thought to bind to receptor proteins in sensilla, particularly at sulphur bridges (Shimada *et al*, 1974). Shimada *et al* found that, whilst experimenting on the sugar receptor in *P. regina*, PCMB also depressed the activity of the salt receptor. It seemed probable that PCMB could block most larval chemoreceptors.

A total of eight larvae were treated with PCMB in these experiments. The responses of these larvae to a series pulses of diatomaceous earth, following treatment with PCMB, are summarised in Figures 35a and b. The figures show the number of fan and mandible movements recorded in the ten seconds immediately following the injection of pulses of diatomaceous earth.

Larvae were exposed to a 10mM solution of PCMB in phosphate buffer for ten minutes, to block chemoreceptors. The flow of particle-free distilled water, in which the larvae had been acclimated inside the observation cell, was stopped and 5cm3 of PCMB solution injected into the cell around the larvae. After ten minutes the pumping of water through the cell was begun again to remove the PCMB from the cell.

Pulses of NaCl, at an estimated concentration of 0.0075M, were used to check that larvae had been treated successfully with PCMB, by confirming that they were no longer sensitive to this moderately strong stimulant concentration Figure 33 shows the effect of 0.0075M NaCl on untreated larvae. Examples of the responses of three larvae (two behavioural extracts for each larva, each showing the response to one pulse of NaCl) are shown in the figure. In most cases this concentration induced a burst of mandible movements. Figure 33 should be compared with Figures 34a to d which show the effect of the same concentration of NaCl on four larvae that were treated with PCMB in this experiment. In these examples each larva was exposed to three consecutive pulses of NaCl at an estimated concentration of 0.0075M.

Although some mandible movements were made there was no significant change in the number of mandible movements in the first 5s after the pulse when compared to unstimulated larvae. Larvae exposed to 1s pulses of NaCl at an estimated concentration of 0.0075M that had not been treated with PCMB showed a highly significant difference in the number of mandible movements in the 5s following stimulation (see Table 25). All eight larvae reported on here were unresponsive to NaCl by these criteria following treatment with PCMB.

It was assumed that the chemoreceptors of these larvae were blocked.

Kruskal-Wallis one-way analysis of variance was used to test the hypothesis that there was no difference in the numbers of fan and mandible movements in the ten second extracts of behaviour before and during the injection of pulses of diatomaceous earth. In this experiment the behaviour of the larvae during the post-PCMB test with NaCl (for sensitivity to a chemical stimulant) was chosen to represent 'pre-stimulation' behaviour. In the analysis of variance, behaviour during stimulation with NaCl (no diatomaceous earth) was compared with behaviour during stimulation (with pulses of diatomaceous earth). The intermediate steps in these tests are shown in Appendix 12.

Following treatment with PCMB there was still a significant increase in the number of mandible movements made in response to the injection of pulses of diatomaceous earth ($P<0.001$). This result is clearly seen in specimen 1, Figure 35a and specimens 5, 6 and 8, Figure 35b. In contrast to the results reported in section 6.3 however, there was no significant change in the number of fan beats following stimulation with diatomaceous earth. This shows that while the blocking of chemoreceptors altered some of the responses of the larvae to diatomaceous earth, mandible movements were initiated by mechanical stimulation alone.

The observations reported in Chapters 4, 5 and 6 show that larval behaviour is composed of two main groups of movements. The first of these, fan cleaning movements, proceed in conditions of moderate particle availability and low concentrations of chemical stimulants, conditions typified by unfiltered natural water and

particle-free distilled water. As food becomes more abundant (perhaps as refractory particles increase in concentration) this uninhibited pattern of behaviour is disrupted. Stimulation of mandible movements may occur, leading to temporary (such as is shown in Figures 30 and 31) or more prolonged inhibition (as is shown in Figure 26) of fan cleaning. These two groups of movements may be equated with 'food gathering' and 'food ingestion' respectively, the former being concerned with the filtering of particles from the water, the latter with their ingestion. This subdivision of the pattern of larval feeding behaviour is used in the discussion beginning in section 7.1.5 of the final chapter.

TABLE 25. The responses of larvae to 0.0075M NaCl before and after treatment with p-Chloromercuribenzoic acid (PCMB).

Treatment	n	Number of mandible moves in 5s		F	P
		Unstimulated	Stimulated		
0.0075M NaCl (no PCMB)	10	1.9	(n=20) 6.5	14.63	<0.001
0.0075M NaCl (with PCMB)	25	2.52	2.76	0.141	0.70

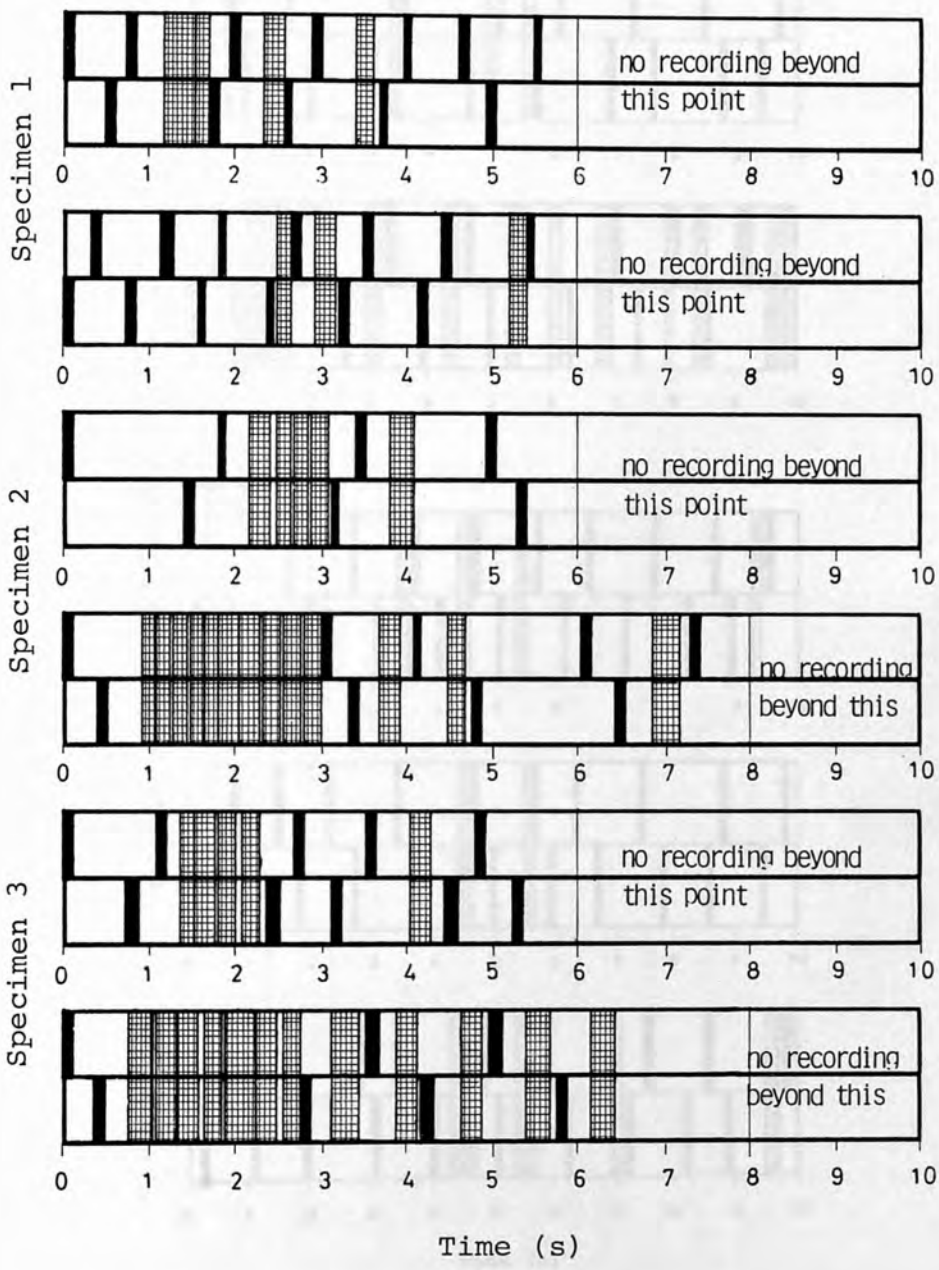


Figure 33 Examples of the responses of three larvae to one second pulses of NaCl at an estimated concentration of 7.5×10^{-3}M. Compare with PCMB treated larvae (Figure 34). Water velocity 17cms^{-1}; water temperature 11.5^{o} $\pm$ 1^{o}C. See Table 4a for key to symbols.

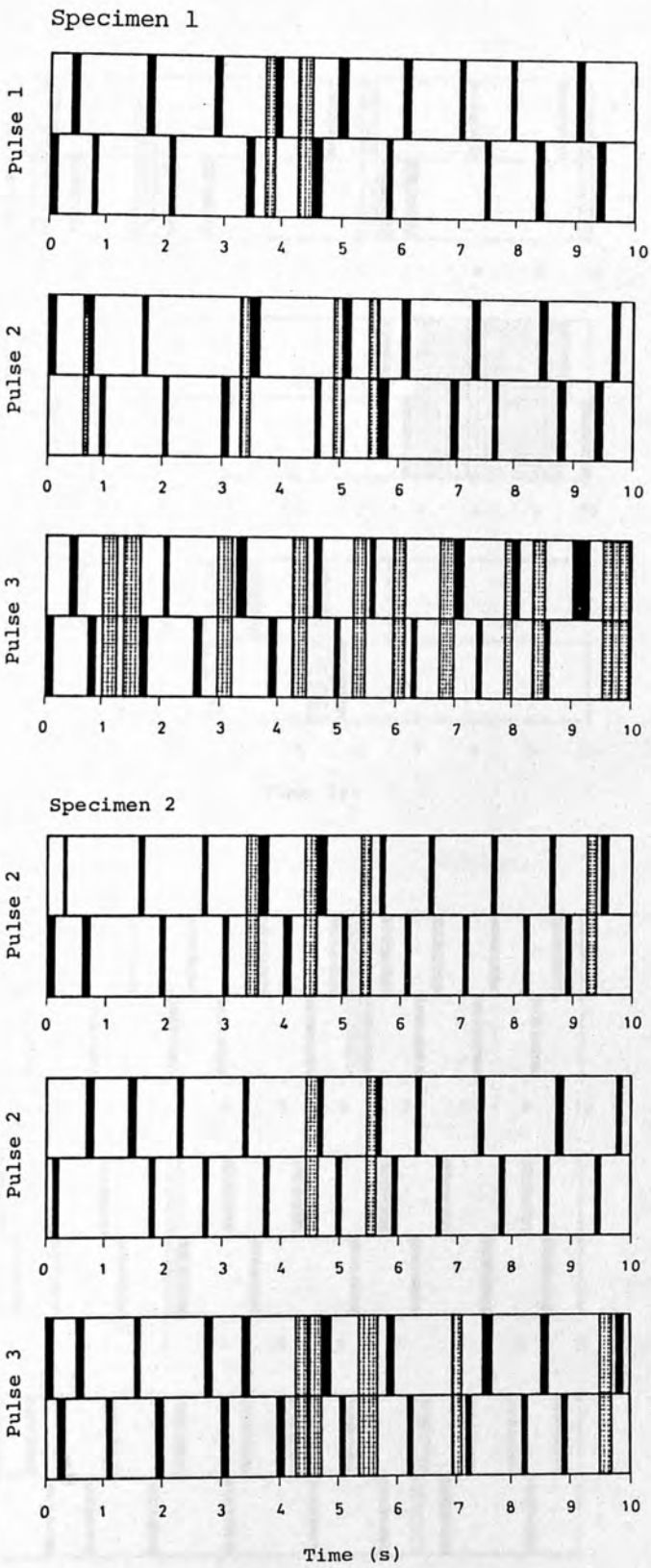
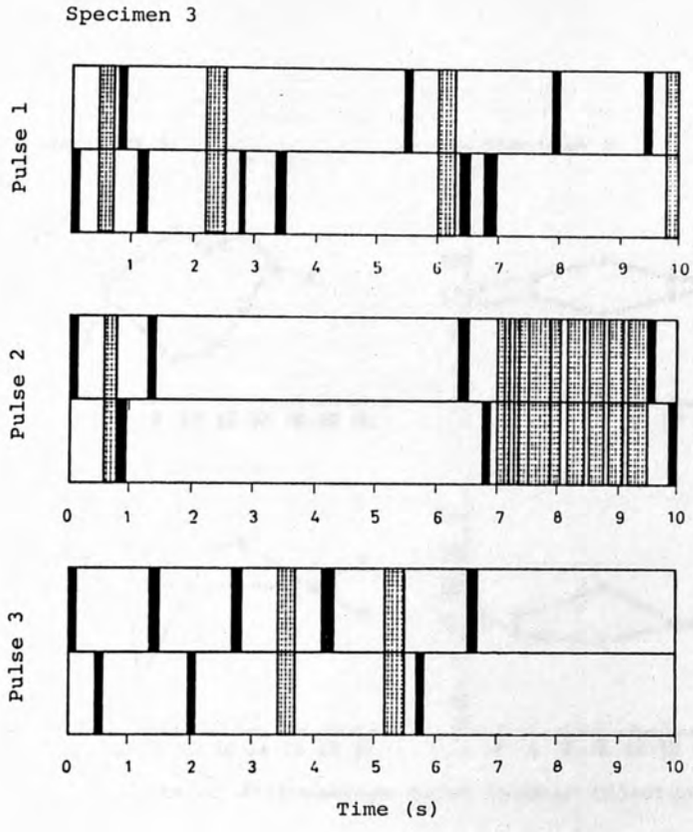


Figure 34 The response of an individual larva to three consecutive pulses of 7.5×10^{-3}M NaCl after treatment with PCMB. Compare with Figure 33. Water velocity 17cms^{-1}; water temperature $11^{\circ} \pm 1^{\circ}$C. See Table 4a for key to symbols.



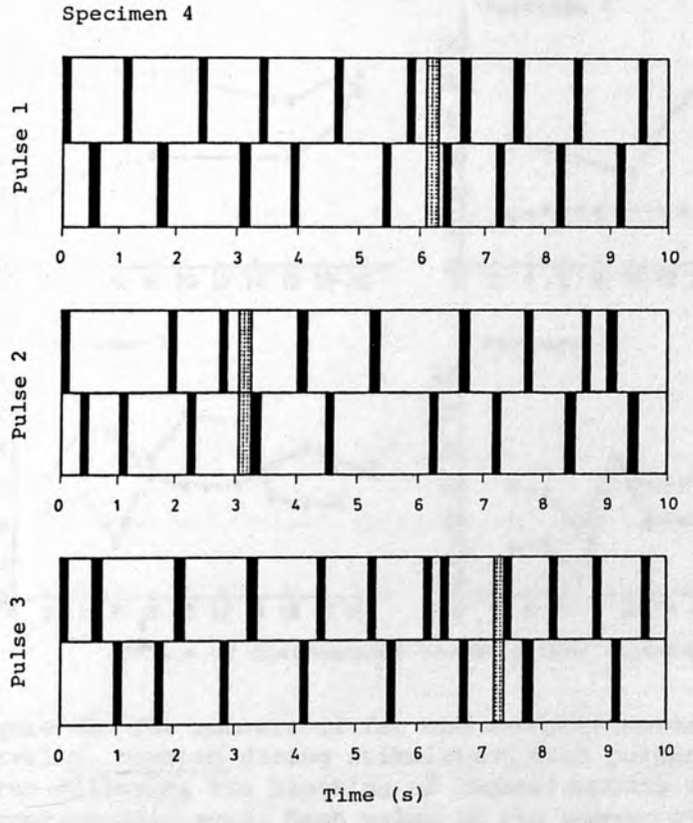


Figure 34 (continued) The response of an individual larva to three consecutive pulses of 7.5×10^{-3} M NaCl. after treatment with PCMB. Compare with Figure 33. Water velocity 17cms^{-1}; water temperature $11.5^{\circ} \pm 1^{\circ}$C. See Table 4a for key to symbols.

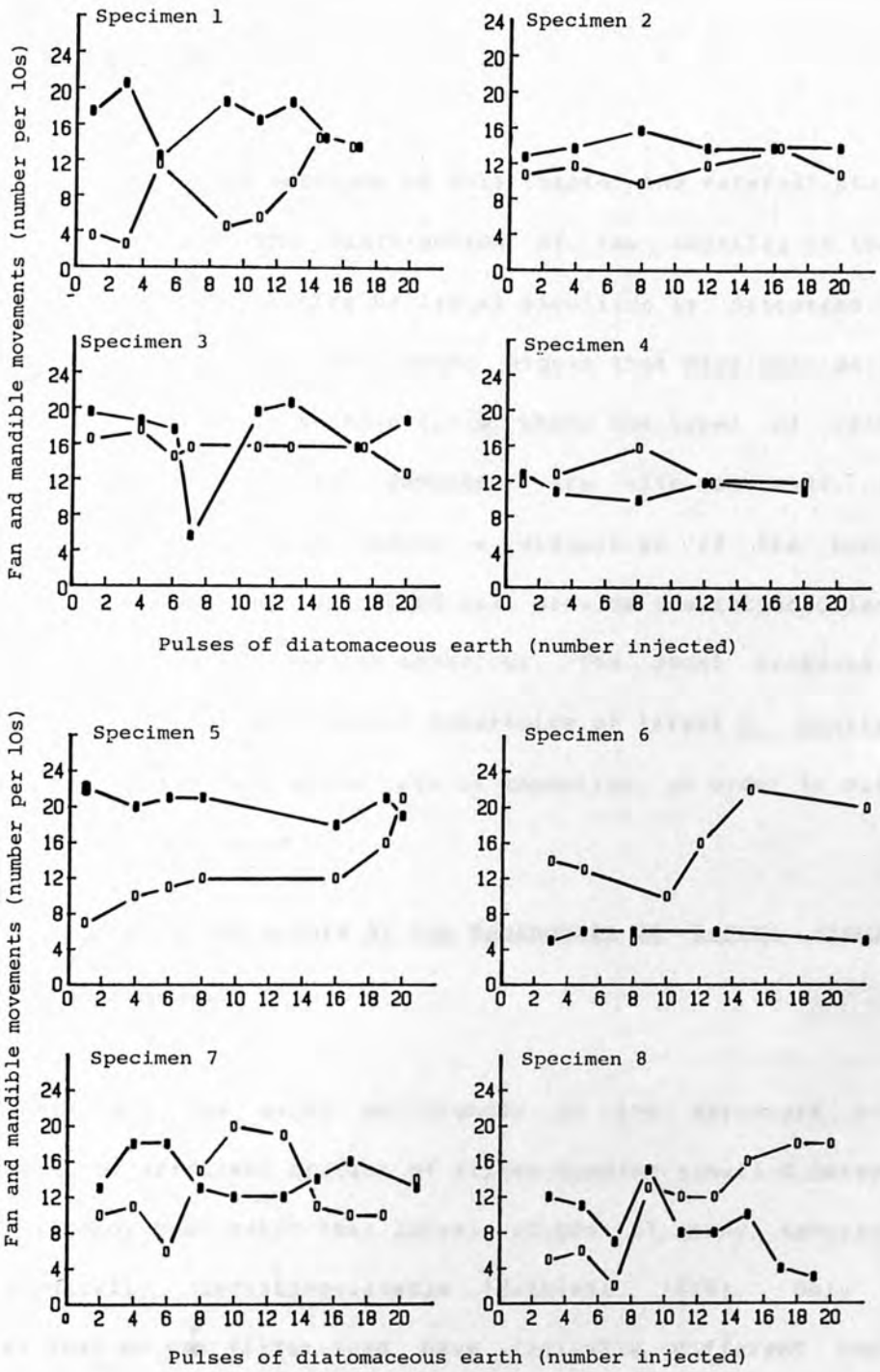


Figure 35. The numbers of fan and mandible movements made by larval S. ornatum during stimulation with pulses of diatomaceous earth following the blocking of chemoreceptors with p-Chloro-mercuribenzoic acid. Each value is the number of fan or mandible movements recorded during a ten second extract of behaviour; each ten second extract immediately followed the injection of a one second pulse of diatomaceous earth into the water. Graphs show selected responses of all larvae to a series of pulses of diatomaceous earth.

●=no. of fan movements
o=no. of mandible movements

CHAPTER 7 DISCUSSION

7.1.1 Introduction

In the first two sections of this Chapter the external structure of mouthparts and the distribution of the sensilla of the head capsule in different species of larval simuliids is discussed. The potential functions of the sense organs that have been described above are considered in section 7.1.5, where the types of stimulant that can influence larval behaviour are also discussed. In the remaining sections of this chapter a discussion of the behaviour patterns that have been described will provide the introduction to a simple model of larval feeding behaviour. The model proposes that many aspects of the behavioural repertoire of larval *S. ornatum* are concerned with regulating the rate of ingestion, in order to maintain it at some optimum value.

7.1.2 The external structure of the mouthparts of larval simuliids: introduction

There are few major differences in the structure of the mouthparts of different species of filter-feeding simuliid larvae and it has already been noted that larval stages of many species are morphologically indistinguishable (Rothfels, 1979). Only those species that do not filter-feed have radically different cephalic fans and mouthparts (Dumbleton, 1962). Craig (1977), Craig and Borkent (1980) and Chance (1970) all discussed the similarities in larval mouthpart structure. This similarity appears to extend to the distribution and types of external sense organs possessed by the larvae. Craig and Borkent (1980), describing the homologies of

larval maxillary palpal sensilla, incidentally demonstrated that there were relatively minor variations in the distribution and types of these sensilla within the tribe Simuliini.

Larvae of many species have characteristic habitats (Ladle, 1982) and it has been suggested that differences in distribution may be the result of larvae making use of different food sources (Carlsson et al, 1977, Ladle and Hansford, 1981). Therefore, despite the general similarities of external structure in the larvae it is worth examining the structure of their cephalic fans more closely as this may reveal ways in which the larvae of different species can specialise on different food sources.

7.1.3 Mouthpart structure: the cephalic fan as a filter

The cephalic fan of simuliids is a net with a great many, relatively large, holes in it. Despite this it is able to catch particles that are much smaller than the gaps between the fan filaments (Wotton, 1976) although it does so with a low efficiency (Kurtak, 1978). Most other aquatic insects that filter particles from flowing water, mostly chironomids and trichopterans, use a net, usually made of silk, with a mesh size close to the minimum particle-size that they capture (Wallace and Merrit, 1980).

Amongst 'structural' filter-feeder, the mayfly Isonychia sp, has filtering hairs on the legs, with fringing microtrichia, that are remarkably similar to the primary cephalic fan filaments of simuliids. However, unlike the simuliid fan filaments, these microtrichia overlap to form a distinct net (see Wallace and O'Hop, 1979), contrasting with those of simuliids which, except at the very bases of the fan filaments, are far too short to overlap.

The way in which the cephalic fan traps particles of any size from 0.1µm to 100µm with gaps between fan filaments up to 50µm across is still unclear. Although it has been known for several years that larvae secrete mucus onto the fan (Ross and Craig, 1980) the importance of mucus during filtering is assumed rather than known. Fine particles may simply stick to the mucous although it is possile that mucous is stretched across the fan filaments in some way, to form a net with a much smaller mesh size. Surprisingly the contents of the guts of larval simuliids have not been checked for the presence of mucous or silk that might be able to glue fine particles together (Ross and Craig, 1980, Kurtak, 1978, Schroeder, 1982).

Although particles may stick to silk on the fan it is possible that physical forces are also important in the operation of these filters, although there is as yet no evidence for this. It is known that the fan modifies the velocity and direction of water flow, considerably enhancing its ability to catch particles by reducing their velocity (Craig and Chance 1982). Although microtrichia on the filaments of the cephalic fan do not form a net their small size, and particularly the small gaps between them, suggests that they could be essential to the filtration process.

7.1.4 Mouthpart structure: the function of the microtrichia of the cephalic fans

Not only are microtrichia of the right size to remove very small particles from the water but their distribution and size differs between species, a fact that has been noted by most authors who have observed the structure of larval mouthparts (for example Grenier, 1949, Kurtak, 1978 and Chance, 1970,). Differences in the distribution of microtrichia on the fans of S. ornatum and S. noelleri have been noted here.

Kurtak (1978) suggested that these differences reflected the strength of the water current that larvae were normally exposed to. He suggested that larvae with stouter microtrichia were found in the streams with high water velocity, finer microtrichia being associated with lower water velocities. Although this might seem intuitively correct there have been no measurements of the forces to which the cephalic fans or microtrichia are exposed. The problem will not be resolved until more is known of the way in which the fans function, particularly as the role of the microtrichia in fine filtration is presently not understood.

Observations with the SEM suggested that there was little difference in the arrangement of microtrichia on the cephalic fans of S. ornatum and S. lineatum (personal observation). This may be related to the fact that both species are usually found in similar habitats. The main difference in the arrangement of the microtrichia of S. ornatum and S. noelleri is in the number of microtrichia in each subunit on the fan filaments. Each subunit is bounded by the shortest and the longest microtricha in a group, with microtrichia of

ascending size in between. Each subunit contains more microtrichia in S. noelleri than in S. ornatum and S. noelleri also has longer terminal hairs in each subunit.

The differences in microtrichia distribution and size between the two species are difficult to relate to differences in the habitats of the larvae. S. noelleri, a lake outlet species, was probably exposed to greater water velocities than S. ornatum but this does not appear to be manifested in a simple difference in the sturdiness of the microtrichia, the distinction suggested by Kurtak (1978) (see Plates 2.1 and 2.3).

The significance of differences in the size and distribution of the microtrichia could be examined experimentally; it may be possible to strip microtrichia from the fan filaments and observe the effect this has on the ability of the fan to trap particles. Comparative observations between species would confirm the significance, or otherwise, of differences in microtrichia distribution and size.

Unless such differences in fan structure are found to be highly significant it will be natural to look at behaviour yet more closely to see whether differences in the way in which larvae behave, in particular differences in feeding behaviour amongst the species, can help to explain some of the distributional differences amongst the species. To do this the main components of larval behaviour and the stimuli that influence those behaviour patterns must be known, allowing those parts of the environment of the greatest significance to the larvae to be recognised.

7.1.5 The external sensilla of the head capsule of larval simuliids

It was shown in Chapter 2 that larval S. ornatum have a relatively small population of external sensilla on the head capsule and its appendages. This confirms the observations of previous authors (Chance, 1970, Craig and Batz, 1980 and Craig and Borkent, 1982) who described parts of the sensory nervous system of larval simuliids. None of these authors reported that the larvae had any large concentrations of sensilla. As is typical of many larval endopterygotes their behavioural repertoire is mediated by a small number of sensilla (Chapman, 1982).

The external sensilla on the head capsule are nearly all either uniporous chemosensilla, in the sense of the classification proposed by Zacharuk (1980), or trichoid hairs that are probably mechanoreceptors. As Table 3 shows the only multiporous sensilla are those on the antennae and also possibly the ridged sensillum on the maxillary palp (Craig and Borkent, 1982). This suggests that the life of the larvae is dominated by contact chemoreception and mechanoreception. However, it should be noted that it may not be possible to predict the specific function of a sensillum from its external appearance (Lewis, 1970). This must apply particularly to larval simuliids as there have not been any physiological investigations of the function of the chemoreceptors of aquatic insects, while investigations of mechanoreceptor function in aquatic insects have been concerned with locomotion, orientation and pressure reception (see Dethier, 1976 and Chapman, 1982 for reviews). The role of sensilla in general in the regulation of feeding behaviour in aquatic insects is unknown.

The behaviour patterns in which sensilla, listed in Table 3, may be involved can be suggested by matching the environmental factors known to influence behaviour (see Figure 36, which is described in more detail in section 7.6) to the possible functions of the sensilla, deduced from their external form.

Three influences on behaviour appear to be important during 'food gathering'. Water temperature influences all aspects of behaviour by speeding physiological processes and so is unlikely to involve external sensilla. Water velocity and water quality, however, almost certainly influence behaviour through intermediary sense organs. Two external factors, the mechanical stimulation caused by particles and chemical stimulation caused by their surface chemistry, are probably the main influence on 'food ingestion' behaviour. The terms 'food gathering' and 'food ingestion', introduced at the end of Chapter 6, are discussed further in Section 7.4.

7.1.6 'Food gathering' behaviour: the influence of environmental factors and the role of external sensilla

'Food gathering', the movements associated with fan cleaning, was influenced by water velocity, the behavioural responses observed (see Chapter 5) probably being initiated by the stimulation of mechanoreceptors. The larger trichoid hairs on the frons, which are probably innervated by the cell bodies shown in Figures 7 and 8, may mediate this response. The relatively large group of sensory cell bodies immediately below the frons, could also be involved in the response to water velocity. Preliminary experiments, in which the

antennae were amputated, demonstrated that they did not mediate the response to water velocity. There is no evidence of a concentration of sensory cell bodies associated with the antennae, showing that larvae do not possess a Johnstons organ. Indeed the number of bipolar cell bodies associated with the antenna (see also Craig and Borkent, 1982) are enough only for the external sensilla that can be seen.

As specific sensilla sensitive to changing water velocity have not been identified the possibility remains that the stimulus for this response is a general increase in pressure on the body. Although external pressure receptors have been identified in other aquatic insects (eg Thorpe and Crisp, 1947) there is no evidence for their existence in larval simuliids, although internal stretch receptors could mediate this response.

That the response to natural water (which stimulated an increase in fan cleaning frequency in relation to particle-free distilled water) was due to the presence of natural food particles, was shown in Sections 4.5 and 6.3. The results suggest that the effect of water quality on larval behaviour (see Figure 36) was mediated by uniporous chemoreceptors, perhaps responding to the surface chemistry of natural food particles. The response to pure chemical compounds in solution was probably due to stimulation of the same sensilla. Although most larval chemoreceptors are uniporous they could still respond to stimulants in solution in the same way that some uniporous sensilla of P. regina respond to vapours (Dethier, 1972).

The response to pure chemical compounds differed from that to natural particles, initiating only extra mandible movements and not influencing fan cleaning frequency. Mechanical stimulation from particles also influenced 'food ingestion' behaviour (like pure chemical compounds, causing extra mandible movements, while inhibiting fan cleaning) but there was no evidence to suggest that inert particles could stimulate an increase in the frequency with which the fans were cleaned.

7.1.7 'Food ingestion' behaviour: the influence of environmental factors and the role of external sensilla

Both chemical and mechanical stimulation affected 'food ingestion' behaviour. 'Food ingestion', mandible movements without fan cleaning, was perhaps initiated by stimulation of contact chemoreceptors, such as those mentioned already, as well as stimulation of mechanoreceptors.

Extra mandible movements, with simultaneous inhibition of fan cleaning, were stimulated by a very wide range of chemical compounds (see Table 17). This range of sensitivity is not surprising considering the range of chemical compounds that larvae must be exposed to in their heterogeneous diet.

The way in which chemical stimulants might cause either an increase in the frequency of fan cleaning or initiate extra mandible movements is unclear. The intensity of stimulation may be responsible for this difference (pure chemical compounds perhaps causing more intense stimulation than natural food particles) with the more intense stimulation leading to extra mandible movements and

the inhibition of fan cleaning. Alternatively specific chemical compounds on the surface of algal cells, or other natural particles present in river water, could cause fan cleaning activity while non-specific stimulation caused mandible movements to be made. The mechanism of this dual response to chemical stimulation, possibly mediated by the same group of sensilla, requires further investigation.

As there appears to have been only one previous study of the sensitivity of an aquatic insect to stimulation with pure chemical compounds (Hodgson, 1951) it is not possible to tell whether the sensitivity of larval S. ornatum is typical of other aquatic insects. Comparisons have already been made in Chapter 6 (Tables 18 to 20) showing that S. ornatum larvae appear to be rather similar to P. regina and L. maculosus in their sensitivity to chemical compounds.

Of the sensilla listed in Table 3 the most likely to be involved with sensing the chemical qualities of food particles were those on the maxillae, the basal lobes of the labium and perhaps also the uniporous peg on the lobed area of the maxilla. It must be assumed that these and other sensilla, might also be sensitive to pure chemical compounds.

Like the stimulation caused by natural particles and pure chemical compounds in solution, mechanical stimulation of extra mandible movements is probably mediated by a relatively small number of mouthpart sensilla. These may include the apical teeth and perhaps also the apical hair pair of the mandible. The innervation of the apical teeth has not been reported previously in simuliids, although the innervation of mandibular teeth is known from studies of

other insect larvae (Ma, 1972). Apart from these two groups of sensilla, however, there are very few trichoid hairs that appear to be in a position on the mouthparts where they could be stimulated mechanically by captured particles.

The function of the sensilla on the tip of the maxillary palp and the marginal hairs of the hypostomium remains unclear, although both often come into contact with the substratum and are probably involved with sensing its qualities.

The possible functions of several remaining sensilla listed in Table 3 are difficult to speculate on. They are mostly in positions where they would appear to be unlikely to come into contact either with captured food particles or the substrate. They include the uniporous pegs on the aboral surface of the maxillary lobe and the trichoid hairs on the maxillary palp.

There are likely to be considerable technical problems in attempting to make electrophysiological measurements from the sensilla of small aquatic insects. This is particularly true of blackfly larvae because, once removed from water, they become dehydrated very rapidly unless kept in a moist environment. However, such difficulties could probably be overcome to make recordings from sensilla such as those on the tip of the maxillary palp which are clearly exposed.

7.2.1 The pattern of larval feeding behaviour: introduction

With the limited range of sense organs described in Chapter 5 it is not surprising to find that larval feeding behaviour is rather simple. Yet, despite this simplicity movements are made very rapidly and it is only with cine films or video tape recordings that behaviour can be observed closely. Consequently there have been few detailed observations made of the behaviour of blackfly larvae (Chance, 1970, 1977, Kurtak, 1978, Schroeder, 1980a and b, Craig and Chance, 1982 and this study) although many authors have commented, in passing, on larval behaviour.

The results reported here show that the feeding behaviour of larval S. ornatum is highly stereotyped, comprising a fan cleaning sequence repeated almost continuously except when disrupted by some form of disturbance, usually from another larva (personal observation) or from excess food (see Chapter 6 and below). In this work the significance of intra-specific interactions has not been considered. Close observations of behaviour have not been made in the field, but it seems unlikely that more elaborate behaviour patterns would be found.

There is no evidence from the the observations reported here to suggest that bouts of feeding, interspersed with bouts of inactivity, are a part of the endogenous pattern of larval behaviour. Observations of larvae feeding in unfiltered natural water have similarly failed to show any such regular bouts of feeding and resting, an observation supported by field observations where no regular changes in feeding rates have so far been recorded (Ladle et al, 1972, Mulla and Lacey, 1976). It will be argued below that the

most important interruptions to feeding are those caused by excessive peripheral stimulation from food particles.

The absence of regular meals during feeding distinguishes the feeding behaviour of simuliids from most other insects. Typically insects have bouts of feeding that appear to be tied to an endogenous rhythm, and that occur even when animals have constant access to food (Bernays and Simpson, 1982). This distinction probably reflects differences in the qualities of the foods of insects; simuliid larvae feed on a very heterogeneous mixture of food that can, perhaps, best be exploited by rapidly passing it through the gut, rather than attempting to digest it more fully. However, the nutritional requirements of simuliids remain unknown at the moment.

Whilst the observations reported here were being made there was little to suggest that larvae grazed food from the substrate. However, a number of authors have commented on the importance of grazing to blackfly larvae, including Dumbleton (1962) and Kurtak (1979). In a more natural environment larvae might reveal other aspects of their behaviour that were not seen in the laboratory. However, it is possible that the majority of reports of grazing are due to misinterpretation of the behaviour patterns that results from intense stimulation by particles. During the inhibition of feeding larvae often return from the characteristic feeding posture, when the head and thorax are rotated through up to 180°, so that the ventral surface of the head capsule is near to or in contact with the substrate. Movements of the head, often with repeated mandible movements while the fans are withdrawn, could be interpreted as grazing. These are in fact movements made in response to excessive stimulation and can be initiated by exposure to high concentrations

of particles. They are described in Table 16 as 'Type 4' movements.

In the next section the behaviour of larvae in particle-free distilled water, where feeding is uninhibited, is discussed. Uninhibited feeding is the basic, endogenous, behaviour pattern on which environmental factors act to produce the behaviour patterns observed in natural water. Following this the effect of stimulants, both physical and chemical, on feeding behaviour is discussed. These may promote or inhibit feeding behaviour, the observed effect probably depending on the depending on the quality of the stimulant.

7.2.2 The pattern of larval feeding behaviour: uninhibited feeding behaviour

In Section 4.3 the behaviour patterns described were those of uninhibited feeding. Typical examples of uninhibited feeding were shown in Figures 12 and 15. The former shows uninhibited feeding in several different individuals, while the latter shows it in four individuals over four hours. As can be seen from the figures, during uninhibited feeding larvae beat their cephalic fans repeatedly with few intervening mandible movements (the M-movements of Figure 11). The sequence of movements observed during fan cleaning in *S. ornatum* (and also in *S. equinum*) agrees almost exactly with the observations of Craig and Chance (1982) on *S. vittatum*. The range of movements that the larvae can make when cleaning the cephalic fans is inevitably very limited because of the relative simplicity of the mouthpart musculature and articulation (Puri, 1925).

As Section 4.3 showed, on each occasion that the fan was closed for cleaning there was only one associated set of mouthpart movements (mandible abduction and adduction, maxillary adduction and abduction and probably also movements of the labrum). During uninhibited feeding the cephalic fan was never raked more than once by the mandible when it was closed. Indeed, repeated raking of the closed fan in S. ornatum is indicative of advanced inhibition of feeding (see, for example, Figure 26), often immediately preceeding or accompanying extended closing of the fans.

As has been demonstrated in Section 4.5 uninhibited feeding behaviour may continue for several hours without significant changes in the frequency with which the fans are cleaned in a constant environment. The highly repetitive nature of the behaviour pattern suggests that there is a centrally generated rhythm, constantly repeated, that initiates the cleaning of the fans. This rhythm is generated whenever larvae are undisturbed (even in still water) and continues as long as they remain so. It is apparently without regular fluctuations that might cause periodicty in the activity of the larvae.

In particle free distilled water larvae had the minimum amount of stimulation possible in moving water. By using a low water velocity ($17 cms^{-1}$) it was also possible to minimise the stimulating effect of the water current. Since fan cleaning movements occurred with the lowest frequency in these conditions (that is, the greatest mean intervals between fan beats were recorded) it is reasonable to assume that the more stimulatory the environment of the larvae, up to certain limits to be discussed below, the greater the frequency of fan cleaning.

Larval behaviour during uninhibited feeding can be described in terms of two quantities: the interval between fan beats and the time taken to clean the cephalic fan. Extra mandible movements are rare enough to be ignored. In Chapter 4 the closing and cleaning of the cephalic fan was divided into three stages (Stages 2, 3 and 4), but they can be treated as a single quantity for more general descriptions of behaviour once their existence has been recognised. They would probably be of more importance in neurophysiological analyses of behaviour patterns.

7.2.3 Modifications of uninhibited feeding: the effect of temperature and water velocity

Uninhibited feeding behaviour may be modified in two general ways. In the first the interval between fan beats and duration of fan cleaning is modified but the basic pattern of the behaviour remains unchanged, with few extra mandible movements being made. This change results from changes in water temperature and velocity and water quality. In the second the basic pattern of behaviour is disrupted by the occurrence of extra mandible movements. The number of these that may be made is quite variable, depending om the stimulant, and the disruption may last for less than a second or may prevent normal fan cleaning for several minutes. Extra mandible movements can be initiated experimentally either by pure chemical stimulants or inert particles.

In Chapter 5 it was shown that temperature and water velocity modify uninhibited feeding behaviour. Increasing temperature and water velocity both led to reductions in the mean interval between

fan beats (and consequently an increase in the frequency of fan cleaning). The range of temperatures that larvae were exposed to experimentally was similar to that seen in a typical chalk stream, strongly suggesting that throughout the year there would be considerable variation in the frequency with which fans were cleaned in a natural environment. It is likely that temperature exerts a greater effect on the pattern of uninhibited behaviour than water velocity since at higher temperatures (both in particle-free distilled water and unfiltered natural water) there was no statistically significant difference in the response to water velocity (see Figure 24). This suggests that final instar larvae would be unlikely to select feeding sites on the basis of water velocity since this would have little effect on their feeding behaviour.

The time taken to clean the fans declined slightly in response to rising temperature but was not affected by water velocity. Both temperature and water velocity modified the frequency of fan cleaning by modifying the mean interval between fan beats suggesting that they may operate on a central generator that initiates the basic pattern of behaviour (see Figure 36).

7.2.4 Modifications of uninhibited feeding: the effect of particles and pure chemical stimulants

The second way in which the uninhibited pattern of behaviour may be modified has been demonstrated experimentally using pulsed stimulants in particle-free distilled water. Figures 26 and 27 and Appendix Figures 1a-d, for an inert stimulant particle, and Figures 30 and 31, for pure chemical compounds, show examples of stimulants that caused extra mandible movements to be made. At the same time these led to brief inhibition of normal fan cleaning whilst the mandible movements were being made.

Craig and Chance (1982) observed that larvae sometimes made extra mandible movements, without cleaning the fans, for 'no particular reason'. When seen in isolation these movements would probably appear to have no function. However, it will be argued in Section 7.3 that extra mandible movements may be fundamental to the ability of the larva to control the rate at which particles are ingested.

The responses to particles and chemical compounds described here are probably the behavioural basis of the feeding inhibition described by Gaugler and Molloy (1982). These responses are of considerable significance in the feeding behaviour of larval simuliids because they appear to be the only occasions when larvae stop gathering food from the fans. When feeding inhibition begins it probably passes through the same stages as the changes in behaviour shown in Figures 26 and 27 and Appendix Figures 1a to d, with increasing activity of the mandibles at the expense of fan cleaning. As more particles are delivered to the larvae the number of mandible

movements increases eventually leading to a reduction in the frequency of fan cleaning.

Feeding inhibition has not been investigated in the field so its significance, though potentially considerable, is still unknown. It is worth noting that blackfly larvae are characteristically associated with water low in suspended solids and it is possible that they may be excluded from rivers and streams carrying high concentrations of particles because of an inability to feed in these conditions. Although behavioural observations reported here, as well as those of Gaugler and Molloy (1982), suggest that feeding inhibition begins at relatively low particle concentrations, the work of Hart and Latta (1985) has shown that the ingestion rate continues to increase in particle concentrations up to $100mgl^{-1}$ in some circumstances.

This concentration is considerably greater than the $19mgl^{-1}$ that initiated feeding inhibition in S. ornatum in this study. It is also greater than the concentrations used by Schroeder (1980a) who observed behaviour very similar to feeding inhibition. Hart and Latta's result conflicts with the findings of Kurtak (1978) that the efficiency of ingestion declined with the increasing concentration of food in the water. It is possible that the food they used, the pollen of Corylus californica, was a feeding stimulant as there is evidence from the work reported here that natural food stimulates fan cleaning activity (see Table 8).

It has been suggested that feeding inhibition, and therefore the patterns of behaviour described here, are initiated by gut distension (Gaugler and Molloy, 1982). This seems unlikely since the gut is an apparently inflexible tube and particulates such as diatomaceous

earth appeared to influence behaviour only so long as they were fed to larvae; also there appears to be little change in the volume of the gut contents as they pass through the gut. In addition to this it is difficult to imagine how such a gut distension mechanism could work: once the gut is full the mechanism that allows it to empty (that is ingestion) is turned off. It seems more likely that inhibition is mediated by peripheral sense organs.

Both chemical compounds and inert particles had a similar effect on behaviour, although particles of diatomaceous earth had a longer lasting effect than stimulant chemicals. This is not surprising as particles, having been trapped by the fans, would be available to stimulate mouthpart sensilla for much longer than a transient chemical stimulant in the surrounding water. As the initiation of extra mandible movements was always accompanied by a brief cessation of fan cleaning it is suggested that 'mandible movements' are a dominant behaviour pattern to 'fan cleaning movements' (that is, 'food ingestion' dominates 'fan cleaning'). This suggests that there are at least two sensory pathways involved in controlling the feeding behaviour of larval blackflies. These pathways are considered further in Section 7.4.

7.3 Interspecific differences in the modification of the uninhibited pattern of behaviour

The results of studies that have considered larval behaviour in some detail (mainly those of Craig and Chance, 1982, Schroeder, 1980 and this study) suggest that there are few differences in the types of behaviour patterns observed during uninhibited feeding amongst different species. This reflects the morphological uniformity of simuliids which allows little variation in the way in which cephalic fans can be cleaned and food ingested. Yet differences in feeding behaviour may be expected because larvae are found in rivers with quite different particle regimes (Kurtak, 1979, Ladle and Hansford, 1981, Crosskey, 1982, 1985) and probably have some mechanism for coping with these differences. If differences exist in the feeding behaviour of different species, they will probably be found in differences in the frequencies or durations of various components of the behaviour pattern or in the ease with which uninhibited feeding is modified or disrupted.

A similar hypothesis has been put forward by Craig and Chance (1982), following their measurement of the time taken to clean the cephalic fan in S. vittatum. They suggested that variations in fan cleaning time amongst different species might explain the variations in the efficiency with which simuliid larvae gather food from the water, as had been observed by Kurtak (1978). As a result of the work described here the influence of variations in the mean interval between fan beats and in fan cleaning time on feeding efficiency may be considered.

The interval between fan beats varied considerably more than did the time taken to clean the fans. In S. ornatum there was, on average, an interval of about 0.5s to less than 0.2s between each fan beat, depending on the quality of the water and its temperature and velocity. This is roughly equivalent to a doubling of the frequency of fan cleaning; when the mean interval between fan beats is 0.5s there are about 100 fan beats min^{-1}, assuming that it takes 0.1s to clean the fan. With the same fan cleaning time, but an interval between fan beats averaging 0.2s there can be about 200 fan beats min^{-1}. The time taken to clean the fans, on the other hand, varies much less, from about 0.15s to 0.08s in all conditions. This suggests that changes in the interval between fan beats are more likely to be important in influencing the efficiency or rate, of ingestion than are changes in the time taken to clean the fans.

Although the duration of fan cleaning in S. ornatum appears to be quite similar to that of S. vittatum (Craig and Chance, 1982, found that S. vittatum cleaned its fans in 0.05s) such comparisons must be made with caution because sample sizes in other studies have been small and there have been no other accurate measurements of the mean interval between fan beats and the time taken to clean the fans in a variety of conditions.

However, there are clearly inter-specific differences in the frequency with which the fans are cleaned, and therefore probably in the mean intervals between fan beats as it is unlikely that variation in the time taken to clean the fan could alone account for the frequency variations observed. Observations reported by Schroeder (1980a) Hart and Latta (1985) and Lacey and Lacey (1983), as well as those reported here, all show this. Lacey and Lacey (1983) reported

field observations of an interval between fan beats of about 3 minutes in the Amazonian blackwater S. fulvinotum, the longest interval yet reported. The frequencies reported by the other authors suggested smaller differences although all await investigation in controlled laboratory conditions.

In this study observations of S. lineatum showed that there were no obvious differences in its behaviour when compared to S. ornatum, either in terms of the time taken to clean the fan or in the intervals between fan beats in different conditions. Since both species come from large, relatively warm, rivers, often occurring sympatrically, their behaviour patterns may be adapted to prevailing conditions and therefore quite similar.

In Hart and Lattas' (1985) study Prosimulium mixtum/fuscum showed fan cleaning frequencies in the range 25-100 beats min^{-1} at a temperature of 6.5°C and a water velocity of 41cms^{-1}. They did not present average values of fan cleaning frequencies but this was probably a significantly lower frequency than in S. ornatum, although direct comparison is difficult because S. ornatum larvae are only rarely exposed to such low temperatures. From the observations reported here it can be predicted from the regression equations in Figures 18 and 21 that S. ornatum larvae would, on average, beat their fans about 70 times min^{-1} at this temperature taking into account the effect on both fan cleaning time and mean interval between fan beats. This would be true of a velocity of 17cms^{-1} without natural food, so it would be expected that at 40cms^{-1}, with a natural food available the frequency would be greater.

Intra-specific variations in the frequency of fan cleaning, in response to variations in environmental quality, have been identified in several species (S. ornatum, S. lineatum and Prosimulium mixtum/fuscum) but their significance is not yet clear. However, results presented here, and also those of Gaugler and Molloy, (1982), Schroeder, (1980a) and Hart and Latta (1985) suggest that modifications to larval feeding behaviour mainly result from the need to regulate the rate of ingestion around some optimum.

7.4 The regulation of ingestion rates: a function for observed variations in feeding behaviour

It has been suggested that filter-feeders should optimise their rate of ingestion (Sibley, 1980). This presents larval blackflies with an unusual problem. Ingested food pushes existing gut contents through the gut, there being no peristalsis in the fore and mid-gut. It can be hypothesised that, as a consequence of this, as the amount of food gathered on the cephalic fans increases, the amount of time that food spends in the gut decreases. This could lead to less efficient use of collected food as a result of it spending a sub-optimal amount of time in the gut. Conversely, at low particle concentrations food could be in the gut for too long. This suggests that both high and low particle concentrations could reduce growth rates.

Field observations suggest that ingestion rates may indeed have important effects on larval growth rates. It has been observed that summer generations of S. ornatum pupate at a smaller size than winter generations (Ladle et al, 1977). This is likely to be disadvantageous since smaller adults probably produce fewer eggs, as

has been demonstrated in S. noelleri (Wotton, 1982). Since temperatures are generally higher in summer it is possible that increased ingestion rates, resulting from the effect of temperature on fan cleaning frequency, result in food generally spending too little time in the gut to be used most efficiently. In combination with increased respiration rates this could reduce the rate of growth.

If this hypothesis is correct the following behaviour patterns could be expected. At low food concentrations fan cleaning frequency will be low to allow the fans to be open and filtering for as long as possible. Ingestion rates in these conditions could be below the optimum. At high food concentrations, more frequent cleaning of the fans could prevent them from gathering too many particles. If greatly overloaded a temporary cessation of fan cleaning would regulate ingestion.

Gaugler and Molloy (1982) and Schroeder (1980a) showed that feeding inhibition could be initiated at relatively low particle concentrations (although Schroeder did not call the changes he observed feeding inhibition). In Schroeders experiments natural foods (algae) caused an increase in the amount of time for which the larvae stopped cleaning their fans as their concentration was increased (either by adding more particles to the water or by increasing the water velocity). Schroeder, it should be noted, dealt with realistically low concentrations of particles, something which not all authors have done.

Gaugler and Molloy (1982) using synthetic, but nutritious, particles found that particle concentrations of $10mgl^{-1}$ caused approximately 40% feeding inhibition (where inhibited feeding was partial or complete retraction of one or both fans) with higher concentrations causing correspondingly greater inhibition. The effects of diatomaceous earth described in this study suggest that it too could reduce the frequency of fan cleaning quite rapidly. As Figure 25 shows, any particle type or concentration that caused an increase in the the number of extra mandible movements would be likely to cause a reduction in the frequency of fan cleaning, perhaps with an associated reduction in the rate of ingestion.

If there was no regulation of the feeding, ingestion rates would be expected to rise as the concentration of particles in the water increased. At present there are few experimental data to test this hypothesis with. Hart and Latta (1985) found increasing fan cleaning rates as the concentration of food in the water increased, up to $200mgl^{-1}$ (a very high concentration to which larval blackflies would very rarely be exposed in the field). Following this they showed that in larvae starved for 120 minutes the volume of food ingested was positively related to the frequency of fan cleaning (in the range 25-125 beats min^{-1}), the two observations confirming that as food concentration rose the rate of ingestion also increased. This does not support the feeding regulation hypothesis. However, in larvae starved for only 10 minutes the increase in ingestion rate occurred only as fan beat frequency increased up to about 75 beats min^{-1}, with the same range of fan beat frequencies (25-125 beats min^{-1}).

Hart and Latta also found that the amount of food ingested per fan beat declined as the concentration of food increased in larvae starved for 10 minutes. The decline started at a higher food concentration in larvae that were starved for 120 minutes, but it still occurred, supporting a feeding regulation hypothesis.

The chemical quality of food particles may also influence the regulation of feeding. Exposing larvae to short pulses of a suspension of the alga Scenedesmus acutus, with a small quantity of diatomaceous earth added, (simulating natural food) caused an increase in fan cleaning frequency whilst the pulses were being injected (see Table 8). Of all experimental treatments used in the study reported here this was the only one which caused an increase in the rate of fan cleaning in particle-free distilled water. The result clearly mimics the difference in the fan cleaning frequency observed in unfiltered natural water when compared with particle-free distilled water. It also suggests that larvae respond differently to natural, probably nutritious, particles and inert particles. However, since the concentration of particles in the 'simulated food' mixture was less than that of the diatomaceous earth suspensions used in other experiments described here (about $10mgl^{-1}$ compared to about $19mgl^{-1}$) the result may be consistent with a feeding regulation hypothesis based on particle concentrations alone.

There may be two components to the behaviour pattern preventing the gathering of too much food. At low concentrations the increase in the amount of food gathered could be kept below the maximum possible by cleaning the fans more often, simply modifying uninhibited behaviour. At higher concentrations it might be necessary to reduce fan cleaning frequency or close the fans

altogether, the behaviour observed during feeding inhibition.

The ability of the larvae to compensate for high particle concentrations by beating the fans more frequently may be limited as the difference in the amount of time for which the fans are closed at different fan cleaning frequencies is relatively small. At a frequency of 100 beats min^{-1} (both fans), assuming that it takes 0.1s to clean the fan, each fan is open for 91% of the time (55 out of 60 seconds). As the frequency rises to 200 beats min^{-1}, with other conditions remaining the same, each fan is open for 83% of the time (50 out of 60 seconds). Larvae are unable to beat each fan more than about 120 times min^{-1} (240-250 beats min^{-1} combined) so even at the highest fan cleaning frequencies the fans are open for most of the time. If regulation of ingestion is to occur at the higher particle concentrations disruption of the uninhibited feeding pattern may be essential perhaps explaining the ease with which feeding inhibition may be initiated.

Further evidence supporting the feeding regulation hypothesis comes from field measurements of ingestion rates. These have shown a relatively small amount of variation in gut passage times amongst different species (Ladle *et al*, 1972, Mulla and Lacey, 1976, Elouard and Elsen, 1977, Lacey and Lacey, 1983), from about 20 minutes to 2hrs in all conditions. This suggests that there may be an optimum time for which food particles should be in the gut.

The two species in which the mechanics of feeding behaviour has been observed closely (*S. vittatum* and *S.ornatum*; *O. ornata* is a synonym for *S. ornatum*) come from moderately eutrophic waters. With abundant food available it is possible that they clean their fans relatively frequently to prevent the capture of too many particles.

In contrast to this Lacey and Lacey's (1983) Amazonian blackwater species was exposed to a very low concentration of particles. Despite (or perhaps because of) the very low frequency of cleaning of the fans that they observed, it was still found that larvae filled their guts in about thirty minutes, a quite typical value. Hart and Latta's (1985) results with P. mixtum/fuscum also showed relatively low fan cleaning frequencies; species of this genus are often found in cold, upland streams with low loads of suspended solids (Davies, 1968).

Variation in the frequency of fan cleaning may, therefore, reflect the need to pass food through the gut at a similar rate in all species. Such variations may be one way in which variations in the amount of food present in the water are counteracted. When food is scarce the fans must be kept open for as long as possible to prevent the ingestion rate falling below some optimum. If food is abundant, however, more frequent cleaning of the fans could prevent larvae from gathering too much food, to avoid exceeding the optimal ingestion rate. Because the mechanics of fan cleaning do not allow a very great reduction in the time for which the fans are open whilst feeding is uninhibited, feeding inhibition must also occur. This allows firstly a greater reduction in the frequency with which the fans are cleaned and if necessary temporary closing of the fans to prevent further gathering of food.

It has been demonstrated that there are two main components of larval feeding behaviour and it is suggested that they may be combined to regulate the rate of ingestion.

The first of these subdivisions has been called 'food gathering' and is concerned with the gathering of food particles on the fans, from the water, and all the processes that control the frequency of the cleaning of the fans, as long as feeding remains uninhibited. During feeding on low concentrations of particles each mandible movement associated with the cleaning of the fan is probably also enough to ingest all the particles that are captured.

The second subdivision of larval feeding behaviour nas been called 'food ingestion', and comprises extra mandible movements (and associated mouthpart movements). These movements occur when large quantities of food must be removed from the fans and pushed into the mouth. They are able to disrupt the normal pattern of uninhibited feeding behaviour. In section 7.5 a simple model of larval feeding behaviour is proposed that incorporates these two subdivisions of larval behaviour, indicates how they might combine to regulate larval feeding rates and suggests how some of the behavior patterns may be mediated by the sensory nervous system.

7.5 A model of the feeding behaviour of S. ornatum larvae

The model, shown in Figure 36, summarises the main behaviour patterns of S.ornatum larvae that have been described and the way in which these may regulate the rate of ingestion. When larvae are undisturbed they begin feeding, the frequency of fan cleaning reflecting the duration of the interval between fan beats, the time taken to clean the fans and the duration of any extra mandible movements, though the latter are small in number. In the uppermost box, 'Endogenous fan cleaning frequency', the mean interval between fan beats (IFB) and the mean time taken to clean the fan (FC) are shown for S. ornatum at a water velocity of 17cms^{-1} and a temperature of 10°C in particle-free distilled water; this may be regarded as the basic pattern of behaviour which is modified by outside influences.

Fan cleaning frequency is directly related to temperature, water velocity and water quality (shown at the top left) and negatively correlated with the number of extra mandible movements (shown at top right). The interaction of these four factors with the endogenously generated pattern of behaviour controls fan cleaning frequency. So far it has only been possible to measure the effect of the physical and chemical qualities of various particle types on behaviour very generally (using natural particles and diatomaceous earth). The combined influence of all these factors gives rise to the 'Observed fan cleaning frequency'. This is represented in the second box where the range of intervals between fan beats and times taken to clean the fan that have been observed (in all conditions, including particle-free distilled water and unfiltered natural water) are given.

The frequency of fan cleaning, the product of environmental influences on the endogenous behaviour pattern of the larvae, probably controls how much food is gathered by the larvae. This assertion is supported by the work of Hart and Latta (1985), described above. It has already been suggested that variations in the fan cleaning frequency may be one of two mechanisms to regulate ingestion rates. However, it is perhaps the less important of the two, and may only maintain a constant loading of the cephalic fans at relatively low ambient particle concentrations.

The amount of food handled by the mandibles during ingestion depends on the availability of particles and the behaviour of the larvae. Once particles have been gathered from the water it is possible for the second, and probably more important regulatory influence on the rate of ingestion to come into operation. This is 'food ingestion' when extra mandible movements may be made.

As the effect of particle concentrations on larval behaviour have not been quantified two hypothetical extreme cases are considered where "many particles" or "few particles" are captured to suggest a way in which this behaviour pattern could function to regulate ingestion rates. Central to this part of the model is the assumption that mouthpart sensilla are stimulated by captured particles in some way that is regularly related to particle concentration. It is suggested that the more stimulation the larvae receive the more additional mandible movements that are made and the more inhibited from cleaning the cephalic fans the larvae become.

To the left in Figure 36, where 'few particles' are trapped there will probably be relatively little stimulation of mechano- and chemoreceptors. This will lead to few extra mandible movements being stimulated. In the most extreme case no extra mandible movements will be needed and the fan raking movement made by the mandible associated with the fan beat will be enough to ingest those particles captured. In this case ingestion should proceed at a constant or greater rate and the behaviour pattern observed will be 'uninhibited'.

Alternatively, if a large number of particles are trapped on the fan there will be intense stimulation of mouthpart sensilla, leading to additional mandible movements (to the right in Figure 36). When this occurs the rate of ingestion may be reduced, though only briefly if the inhibition of fan cleaning is short-lived, as the fan cleaning frequency temporarily declines. However, should intense stimulation continue, ingestion could be prevented for some time. These two outcomes during feeding inhibition are indeeed observed when larvae are exposed to short pulses of particles (this study) or to water in which particles are permanently available (Gaugler and Molloy, 1982).

A simple feedback loop based on the stimulation of contact chemoreceptors or mechanoreceptors, such as those of the medial face of the maxillary lobe, the basal lobe of the labium or the apical teeth of the mandible could mediate this response. Once stimulation of these sense organs reaches a particular intensity, after mechanical or chemical stimulation, extra movements of the mandibles are initiated. Such movements continue until all the particles are ingested or lost to the current. Once stimulation falls below some threshold, uninhibited feeding resumes.

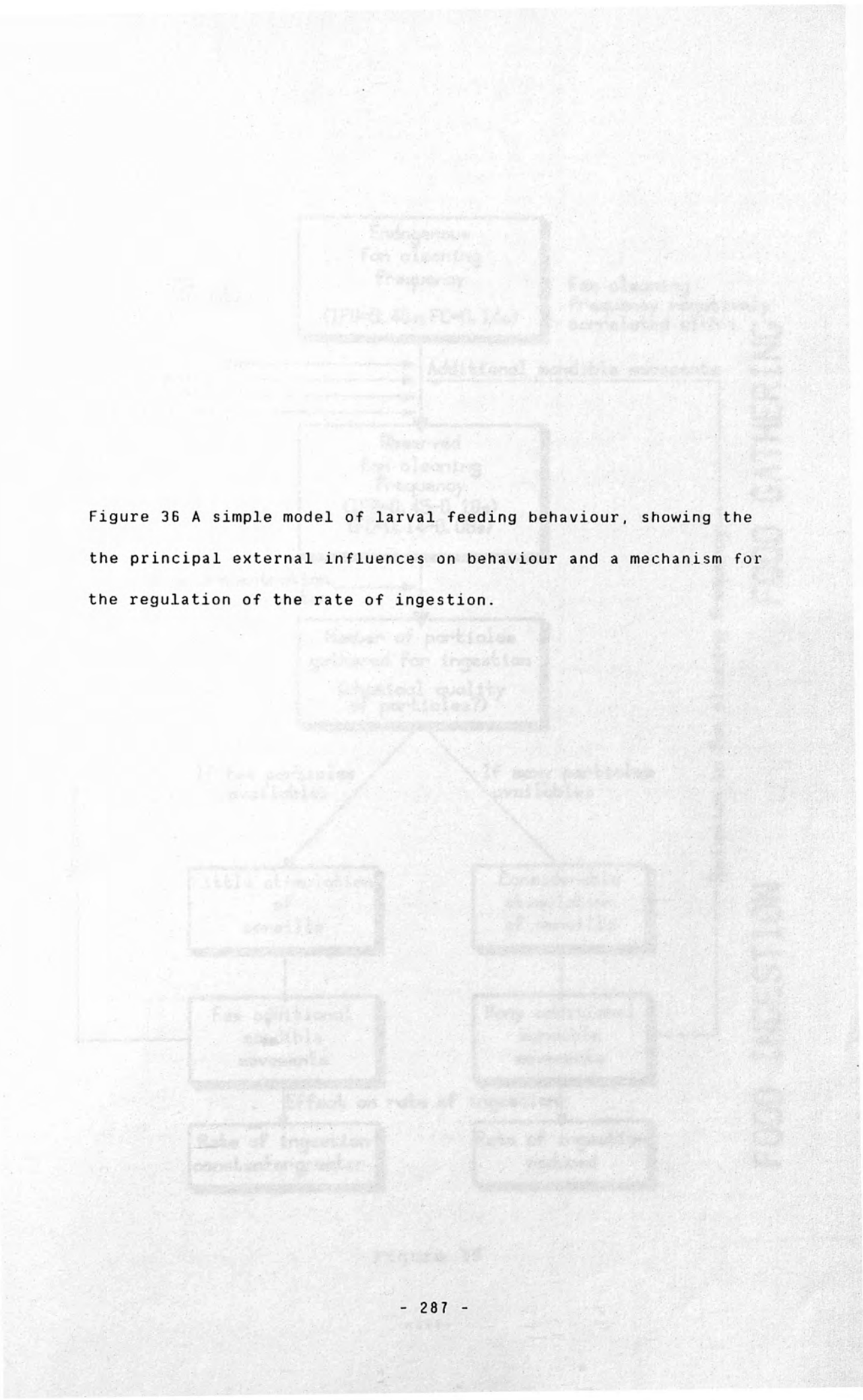


Figure 36 A simple model of larval feeding behaviour, showing the the principal external influences on behaviour and a mechanism for the regulation of the rate of ingestion.

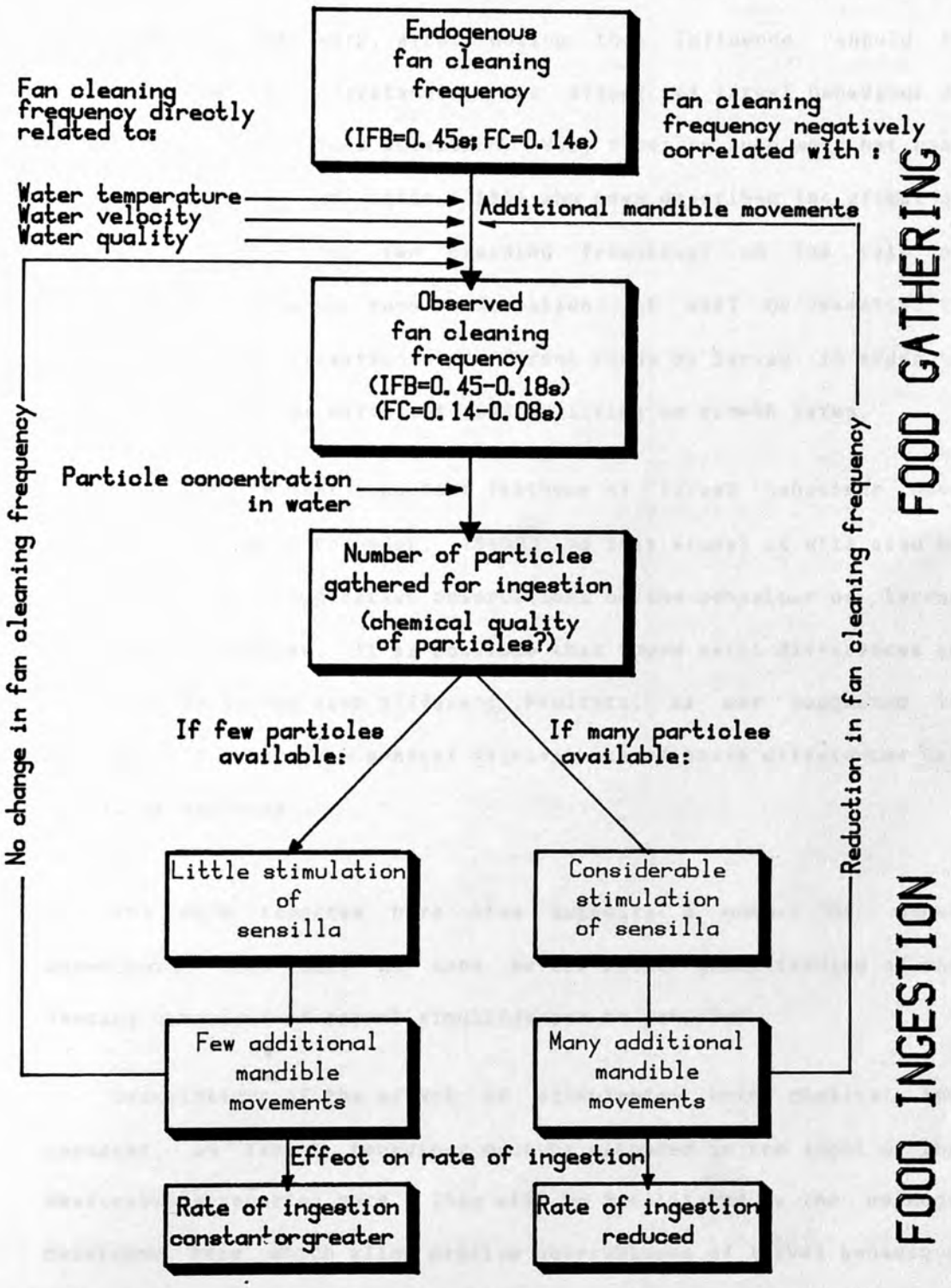

Endogenous fan cleaning frequency (IFB=0.45s; FC=0.14s)
Fan cleaning frequency directly related to:
Fan cleaning frequency negatively correlated with :
Water temperature
Water velocity
Water quality
Additional mandible movements
Observed fan cleaning frequency (IFB=0.45-0.18s) (FC=0.14-0.08s)
Particle concentration in water
Number of particles gathered for ingestion (chemical quality of particles?)
If few particles available:
If many particles available:
No change in fan cleaning frequency
Reduction in fan cleaning frequency
Little stimulation of sensilla
Considerable stimulation of sensilla
Few additional mandible movements
Many additional mandible movements
Effect on rate of ingestion
Rate of ingestion constant or greater
Rate of ingestion reduced
FOOD GATHERING
FOOD INGESTION

Figure 36

7.6 Conclusions

The work reported here contributes to our understanding of the the way in which the environment may modify the behaviour of larval simuliids. Further work, after noting this influence, should be directed towards understanding the effect of larval behaviour on ingestion rates and food selection. Very recently such work has been started by Hart and Latta (1985) who have described the effect of 'flick rate' (that is, fan cleaning frequency) on the rate of ingestion. Following such observations it will be essential to investigate the utilisation of different foods by larvae, in order to define the effect of different food qualities on growth rates.

Now that the most important features of larval behaviour have been identified (Schroeder, 1980a) and this study) it will also be possible to make comparative observations on the behaviour of larvae of different species. It is possible that there exist differences in behaviour of larvae from different habitats, as was suggested in section 7.4, but the general significance of these differences has yet to be explored.

The work reported here also suggests a number of other experiments that must be done before proper understanding of the feeding behaviour of larval simuliids can be achieved.

Descriptions of the effect of stimulants, both physical and chemical, on larval behaviour must be extended in the light of the observations reported here. This will be facilitated by the methods described here which allow precise observations of larval behaviour to be made. The effect of different particle concentrations on larval behaviour must be defined quantitatively as must their

influence on ingestion rates. The hypothesised significance of feeding inhibition as a behaviour pattern regulating ingestion rates must be investigated and the particle concentrations at which feeding inhibition begins, in a variety of environments, must be described. Finally the effect of the chemical qualities of particles in modifying the tendency for feeding to be inhibited must be explored more fully.

All these observations must be made in the laboratory, but it is equally important that the significance of feeding inhibition be confirmed in the field. Of considerable interest is the effect of seasonal variations in particle availability on larval behaviour. It is quite conceivable that, in the temperate zone at least, with generally high particle concentrations in flowing water in winter and associated low temperatures larvae may be inhibited from feeding for much of the time.

With good descriptions of ingestion rates in a variety of conditions, for the more abundant or important species, and with a sound understanding of larval feeding behaviour, the interaction between ingestion rates and growth rates may be investigated. This is perhaps the most important of the next group of experiments that must be done as there is presently only very general understanding of observed variations in growth rates, either within or between species.

All such advances in the understanding of larval biology may allow further improvements in the control of blackflies in those areas where they are disease vectors. It is possible that management techniques in which the value or availabilty of food to the larvae is reduced could be developed once there was a clearer understanding of

larval feeding biology.

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APPENDIX 1

The time taken to clean the fans in particle-free distilled water and unfiltered natural water; variation over 3.5 hours. All values are means derived from ten second extracts of behaviour.

The mean individual times taken to clean the cephalic fan after (a) 1 hour, (b) 1.5 hours, (c) 2.5 hours and (d) 3.5 hours in particle-free distilled water at a water velocity of 17cms^{-1} and a temperature of 12° ± 1°C. All values are derived from ten second extracts of the behaviour of each specimen.

(a) Specimen	n	Mean time taken to clean fan (±SE), s
1	6	0.140(±0.0085)
2	8	0.160(±0.0074)
3	13	0.270(±0.013)
4	9	0.152(±0.009)
5	7	0.122(±0.0021)
6	9	0.146(±0.011)
7	10	0.128(±0.005)
8	8	0.145(±0.007)
9	6	0.113(±0.0065)
10	7	0.171(±0.011)
11	16	0.115(±0.006)
12	10	0.128(±0.004)
Total	119	0.149(±0.012)

(b) Specimen	n	Mean time taken to clean fan (±SE), s
1	8	0.115(±0.0049)
2	8	0.140(±0.0074)
3	10	0.276(±0.017)
4	21	0.123(±0.0037)
5	10	0.132(±0.006)
6	8	0.165(±0.0088)
7	9	0.115(±0.0043)
8	10	0.128(±0.005)
9	5	0.128(±0.0076)
10	6	0.146(±0.0081)
11	13	0.116(±0.003)
12	9	0.133(±0.0066)
Totals	117	0.143(±0.012)

APPENDIX 1 continued

(c)

Specimen	n	Mean time taken to clean fan (±SE), s.
1	7	0.131(±0.0071)
2	8	0.145(±0.007)
4	9	0.200(±0.028)
5	9	0.137(±0.007)
6	7	0.125(±0.0056)
7	9	0.120(±0.0001)
8	9	0.137(±0.007)
9	8	0.130(±0.0063)
10	8	0.130(±0.0063)
11	11	0.116(±0.0063)
12	7	0.137(±0.0079)
Totals	92	0.137(±0.0066)

(d)

Specimen	n	Mean time taken to clean fan (±SE), s.
1	8	0.130(±0.0063)
2	8	0.170(±0.0063)
5	9	0.142(±0.007)
6	7	0.137(±0.0079)
7	10	0.132(±0.006)
8	9	0.142(±0.007)
9	8	0.135(±0.007)
10	7	0.125(±0.0056)
11	11	0.120(±0.0051)
12	7	0.142(±0.0079)
Totals	84	0.137(±0.0041)

APPENDIX 1 continued

The mean individual times taken to clean the cephalic fan in unfiltered natural water after (a) 0.75 hours, (b) 1.75 hours, (c) 2.5 hours and (d) 3.75 hours at a velocity of 17cms^{-1} and a temperature of 13° ± 1°C.

(a)

Specimen	n	Mean time taken to clean fan (±SE), s.
1	7	0.108(±0.0071)
2	15	0.104(±0.0051)
3	15	0.136(±0.0051)
4	15	0.144(±0.0051)
5	18	0.120(±0.003)
6	14	0.120(±0.0001)
8	14	0.111(±0.0045)
10	17	0.129(±0.0053)
Totals	115	0.121(±0.0049)

(b)

Specimen	n	Mean time taken to clean fan (±SE), s.
1	16	0.107(±0.0047)
2	13	0.123(±0.0052)
3	17	0.120(±0.0001)
4	19	0.120(±0.0052)
5	19	0.117(±0.002)
6	15	0.112(±0.0041)
8	13	0.113(±0.0041)
9	10	0.124(±0.0037)
10	17	0.115(±0.0031)
Totals	139	0.116(±0.0016)

APPENDIX 1 continued

(c)

Specimen	n	Mean time taken to clean fan (±SE), s.
1	15	0.106(±0.0049)
2	13	0.107(±0.0052)
3	17	0.120(±0.0001)
4	17	0.141(±0.0058)
5	17	0.115(±0.0046)
6	9	0.097(±0.007)
9	17	0.131(±0.0055)
10	14	0.140(±0.0069)
Totals	119	0.119(±0.0056)

(d)

Specimen	n	Mean time taken to clean fan (±SE), s.
1	15	0.112(±0.0041)
2	14	0.102(±0.0066)
3	17	0.129(±0.012)
4	17	0.115(±0.0031)
5	13	0.113(±0.0061)
6	13	0.110(±0.008)
7	14	0.114(±0.0056)
9	12	0.096(±0.0057)
Totals	115	0.111(±0.0031)

APPENDIX 2

The number and mean duration of extra mandible movements in (a) particle-free distilled water and (b) unfiltered natural water: variation over 3.5 hours. All values are derived from ten second extracts of the behaviour of each specimen.

(a) particle-free distilled water: water velocity 17 cms^{-1}; temperature 12° ± 1°C.

	Time (hours)			
	1	1.5	2.5	3.5
Specimen	Number of additional mandible movements in 10s			
1	2	2	1	7
2	3	2	0	2
3	2	10	-	6
4	5	4	9	-
5	1	3	2	3
6	0	1	2	0
7	1	10	8	4
8	0	0	3	1
Totals n	14	32	25	23
Mean duration of mandible movements (±SE), s.	0.254	0.236 (±0.0002)	0.262 (±0.0004)	0.250 (0.0004)

(b) unfiltered natural water: water velocity 17cms^{-1}; temperature 13° ± 1°C.

	Time (hours)			
	0.75	1.75	2.5	3.75
Specimen	Number of additional mandible movements in 10s			
1	15	1	6	4
2	5	12	4	4
3	9	3	9	3
4	8	3	6	3
5	3	1	1	11
6	11	2	14	8
7	7	-	-	7
8	5	2	-	-
Totals	63	24	40	40
Mean duration of mandible movements (±SE), s.	0.206 (±0.0003)	0.170 (±0.0001)	0.180 (±0.0002)	0.172 (±0.0002)

APPENDIX 3

The rate of feeding of *S. ornatum* on a mixture of diatomaceous earth and the alga *Scenedesmus acutus*. For each individual the upper row of values shows the time at which the observation of the position of the gut marker was made and the lower row the position of the gut marker. Each value describing the position of a gut marker refers to its position in relation to an abdominal segment. Thus "3.5" means "halfway through the third abdominal segment". At the boundary of the six and seventh segments the hind gut loops and progress of the gut marker beyond this point was not measured. The injection of food began at "0,0".

Specimen						
1	Time (min)	0	12	18	24	36
	Position	0	0.5	2	4	6.5
2	Time (min)	0	12	24	-	36
	Position	0	1	4	-	6
3	Time (min)	0	12	17	24	36
	Position	0	2.5	3.5	5.5	7
4	Time (min)	0	12	-	24	36
	Position	0	3	-	6.5	7
5	Time (min)	0	12	-	24	36
	Position	0	3	-	6.5	7
6	Time (min)	0	13	-	24	39
	Position	0	1	-	2	5

APPENDIX 3 continued

Specimen							
7	Time (min)	0	5	12	25	27	
	Position	0	1	3	5	6	
8	Time (min)	0	9	-	26	29	
	Position	0	1	-	4.5	6.5	
9	Time (min)	0	10	20	26	40	
	Position	0	2	5	5	6.5	
10	Time (min)	0	13	21	31	40	
	Position	0	1	3	3	6	
11	Time (min)	0	11	23	31	40	
	Position	0	1	3	4.5	6	
12	Time (min)	0	10	17	28	37	47	54
	Position	0	1	2.5	3	4.5	5.5	6

Note that only Specimens 1 to 9 were observed both before and during the provision of food.

APPENDIX 4.

The mean interval between fan beats at a water velocity of 17cms^{-1} before and after exposure to water velocities up to 68cms^{-1} (see Section 5.4). All observations in particle-free distilled water at a temperature of (a) 8°C, (b) 14°C and (c) 17°C.

(a) Mean interval between fan beats, s. (mean of intervals from 10s extract of behaviour).

Specimen	Before high velocities	After high velocities
2	0.61	0.65
3	0.76	0.88
4	0.94	-
6	1.04	0.53
7	0.42	-
8	0.49	0.47
9	0.90	0.55
x	0.74	0.61

df:1,10
F=0.977
n.s.

APPENDIX 4 continued

(b) Mean intervals between fan beats, s. (mean of intervals from 10s extract of behaviour).

Specimen	Before high velocities	After high velocities
1	0.36	0.39
2	1.00	0.33
3	0.39	-
4	0.29	0.62

df:1,5, F=0.081, n.s.

(c) Mean intervals between fan beats, s. (mean of intervals from 10s extract of behaviour)

Specimen	Before high velocities	After high velocities
1	0.48	0.62
4	0.54	0.66
5	0.88	-
6	0.38	0.37
7	0.22	0.31

df:1,7, F=0.007, n.s.

APPENDIX 5

(a) Covariance analysis of the regressions of the mean interval between fan beats against temperature in particle-free distilled water and unfiltered natural water in S. ornatum (see Figures 18 and 20).

Descriptive statistics

	Dependent variable		Covariate		
Water quality	Mean	Standard error	Mean	Standard error	n
PFDW	0.4764	0.03040	13.6692	0.50825	52
UFNW	0.3400	0.02992	12.2071	0.46170	28
Pooled	0.4287	0.02342	13.1575	0.37424	80

Water quality	Regression coefficient	Standard error	Intercept	t	df	2-tailed P
PFDW	-0.02605	0.0076	0.8325	-3.4204	50	0.01>P>0.001
UFNW	-0.03785	0.0103	0.8021	-3.6689	26	0.01>P>0.001
Pooled	-0.02295	0.0066	0.7307	-3.4811	78	P<0.001

The value of t comprises a test of HO (Slope = 0).

Water quality	r	r-squared
PFDW	-0.4355	0.1896
UFNW	-0.5841	0.3411
Pooled	-0.3667	0.1345

APPENDIX 5(a) continued

Analysis of variance in each of dependent variable and covariate

Source of variation	SS	df	MS	F	P
Dependent variable					
Treatments	0.34	1	0.34	8.448	0.01>P>0.001
Error	3.13	78	0.04		
Total	3.47	79			
Covariate					
Treatments	38.91	1	38.91	3.586	n.s.
Error	846.23	78	10.85		
Total	885.13	79			

Analysis of covariance

Source of variation	SS	df	MS	F	P
Adjusted means					
Treatments	0.55	1	0.55	17.510	P<0.001
Error	2.45	78	0.03		
Total	3.00	79			
Homogeneity of regression coefficients					
Treatments	0.02	1	0.02	0.568	n.s.
Error	2.43	76	0.03		
Total	2.45	77			

Adjusted treatment means

Water quality	Adjusted mean	Standard error
PFDW	0.4909	0.12537
UFNW	0.3131	0.12547

APPENDIX 5 continued

(b) Covariance analysis of the regressions of the time taken to clean the cephalic fan against temperature in particle-free distilled water and unfiltered natural water (see Figure 21a and b).

Descriptive statistics

	Dependent variable		Covariate		
Water quality	Mean	Standard error	Mean	Standard error	n
PFDW	0.1301	0.00518	13.7375	0.53422	48
UFNW	0.1217	0.00447	12.2071	0.46170	28
Pooled	0.1270	0.00368	13.1737	0.38535	76

Water quality	Regression Coefficient	Standard Error	Intercept	t	df	2-tailed P
PFDW	-0.00821	0.0008	0.2429	-10.7470	46	P<0.001
UFNW	-0.00840	0.0009	0.2242	-8.8790	26	P<0.001
Pooled	-0.00757	0.0007	0.2268	-11.2302	74	P<0.001

The value of t comprises a test of HO (Slope = 0).

Water qualtiy	r	r-squared
PFDW	-0.8457	0.7152
UFNW	-0.8672	0.7520
Pooled	-0.7939	0.6302

APPENDIX 5(b) continued

Analysis of variance in each of dependent variable and covariate

Source of variation	SS	df	MS	F	P
Dependent variable					
Treatments	0.00	1	0.00	1.228	n.s.
Error	0.08	74	0.00		
Total	0.08	75			
Covariate					
Treatments	41.42	1	41.42	3.807	n.s.
Error	804.99	74	10.88		
Total	846.41	75			

Analysis of covariance

Source of variation	SS	df	MS	F	P
Adjusted means					
Treatments	0.01	1	0.01	26.229	P<0.001
Error	0.02	74	0.00		
Total	0.03	75			
Homogeneity of regression coefficients					
Treatments	0.00	1	0.00	0.016	n.s.
Error	0.02	72	0.00		
Total	0.02	73			

Adjusted treatment means

Water quality	Adjusted Mean	Standard Error
PFDW	0.1348	0.01192
UFNW	0.1137	0.01193

APPENDIX 6

Covariance analysis of the regressions of mean interval between fan beats against water temperature in S. ornatum and S. lineatum

Descriptive statistics

Linear Regression

	Dependent varaible		Covariate		
Species	Mean	Standard error	Mean	Standard error	n
S. orn.	0.4764	0.03040	13.6692	0.50825	52
S. lin.	0.5143	0.03941	12.5071	0.41664	56
Pooled	0.4961	0.02509	13.0667	0.32970	108

Species	Regression coefficient	Standard error	Intercept	t	df	2-tailed P
S. orn.	-0.02605	0.0076	0.8325	-3.4204	50	0.01>P>0.001
S. lin.	-0.05110	0.0108	1.1535	-4.7185	54	P<0.001
Pooled	-0.03690	0.0065	0.9782	-5.7093	106	P<0.001

The value of t comprises a test of HO (Slope = 0).

Species	r	r-squared
S. orn.	-0.4355	0.1896
S. lin.	-0.5403	0.2919
Pooled	-0.4850	0.2352

Q_{10} =

SO 0.026 0.974 1.72

SL 0.0511 489 3.19

1.0060
0511
9489

APPENDIX 6 continued

Analysis of variance in each dependent variable and covariate

Source of variation	SS	df	MS	F	P
Dependent variable					
Treatments	0.04	1	0.04	0.566	n.s.
Error	7.23	106	0.07		
Total	7.27	107			
Covariate					
Treatments	36.41	1	36.41	3.165	n.s.
Error	1219.73	106	11.51		
Total	1256.14	107			

Analysis of covariance

Source of variation	SS	df	MS	F	P
Adjusted means					
Treatments	0.00	1	0.00	0.013	0.9082
Error	5.56	106	0.05		
Total	5.56	107			
Homogeneity of regression coefficients					
Treatments	0.19	1	0.19	3.649	0.0588
Error	5.37	104	0.05		
Total	5.56	105			

Adjusted treatment means

Species	Adjusted mean	Standard error
S. orn.	0.4988	0.16202
S. lin.	0.4936	0.16201

APPENDIX 7

Kruskal-Wallis analysis of variance of the effect of water velocity on the mean intervals between fan beats, at four temperatures in particle-free distilled water and three temperatures in unfiltered natural water, for *S. ornatum*.

(a) the effect of water velocity on the mean interval between fan beats at 8°C in particle-free distilled water.

Water velocity (cms^{-1})	Mean rank	Mean normal-score	n
17	17.21	0.720	7
34	12.80	0.105	5
51	8.67	-0.520	6
68	7.90	-0.488	5

Kruskal-Wallis H Statistic = 7.487 3 df P=0.0578
Correction term for ties = 0.9995
Normal-scores test W = 8.004 3 df P=0.04593

Test for monotonic trend over the four groups:
Kendall's Tau = -0.4195, 0.01>P>0.001

Pairwise contrasts	Based on ranks		Based on normal-scores	
Contrast	Lower confidence limit	Upper confidence limit	Lower confidence limit	Upper confidence limit
17 with 34cms^{-1}	-6.6847	15.5133	-0.8595	2.0894
17 with 51cms^{-1}	-1.9981	19.0933	-0.1609	2.6410
17 with 68cms^{-1}	-1.7847	20.4133	-0.2671	2.6818
34 with 51cms^{-1}	-7.3446	15.6113	-0.8997	2.1499
34 with 68cms^{-1}	-7.0883	16.8883	-1.0002	2.1850
51 with 68cms^{-1}	-10.7113	12.2446	-1.5575	1.4921

Any confidence interval that does not include zero
is significant at the 5% level (*).

APPENDIX 7 continued

(b) the effect of water velocity on the mean interval between fan beats at 10°C in particle-free distilled water.

Water velocity (cms^{-1})	Mean rank	Mean normal-score	n
17	23.54	0.603	12
34	18.00	0.096	10
51	11.08	-0.588	6
68	6.40	-0.933	5

Kruskal-Wallis H Statistic = 13.861 3 df 0.01>P>0.001
Correction term for ties = 0.9995
Normal-scores test W = 12.783 3df 0.01>P>0.001

Test for monotonic trend over the four groups:
Kendall's Tau = -0.4674, P<0.001

Pairwise contrasts	Based on ranks		Based on normal-scores	
Contrast	Lower confidence limit	Upper confidence limit	Lower confidence limit	Upper confidence limit
17 with 34cms^{-1}	-6.0294	17.1128	-0.5976	1.6105
17 with 51cms^{-1}	-1.0538	25.9705	-0.0987	2.4798
17 with 68cms^{-1}	2.7569	31.5264	0.1630	2.9080(*)
34 with 51cms^{-1}	-7.0386	20.8719	-0.6474	2.0157
34 with 68cms^{-1}	-3.2018	26.4018	-0.3832	2.4414
51 with 68cms^{-1}	-11.6807	21.0473	-1.2165	1.9063

Any confidence interval that does not include zero
is significant at the 5% level (*).

APPENDIX 7 continued

(c) the effect of water velocity on the mean intervals between fan beats at 14°C in particle-free distilled water.

Water velocity (cm^{-1})	Mean rank	Mean normal-score	n
17	12.00	0.662	4
34	9.38	0.151	4
51	7.25	-0.287	4
68	5.38	-0.522	4

Kruskal-Wallis H Statistic = 4.302 3 df n.s.
Correction term for ties = 0.9995
Normal-scores test W = 4.298 3 df n.s.

Test for monotonic trend over the four groups:
Kendall's Tau = -0.3785, 0.05>P>0.01

(d) the effect of water velocity on the mean interval between fan beats at 17°C in particle-free distilled water

Water velocity (cms^{-1})	Mean rank	Mean normal-score	n
17	13.40	0.688	5
34	9.67	0.026	6
51	6.50	-0.543	6
68	7.00	-0.336	1

Kruskal-Wallis H Statistic = 4.788 3 df n.s.
Correction term for ties = 1.00
Normal-scores test W = 5.451 3 df n.s.

Test for monotonic trend over the four groups:
Kendall's Tau = -0.3996, 0.05>P>0.01

(e) the effect of water velocity on the mean interval between fan beats at 9°C in unfiltered natural water

Water velocity (cms^{-1})	Mean rank	Mean normal-score	n
17	18.67	0.727	6
34	15.33	0.390	6
51	8.50	-0.492	6
68	7.50	-0.626	6

Kruskal-Wallis H Statistic = 10.447 3 df 0.05>P>0.01
Correction term for ties = 1.00
Normal-scores test W = 9.653 3 df 0.05>P>0.01

Test for monotonic trend over the four groups:
Kendall's Tau = -0.4912, 0.01>P>0.001

Pairwise contrasts	Based on ranks		Based on normal-scores	
Contrast	Lower confidence limit	Upper confidence limit	Lower confidence limit	Upper confidence limit
17 with $34cms^{-1}$	-8.0792	14.7458	-1.1207	1.7965
17 with $51cms^{-1}$	-1.2458	21.5792	-0.2395	2.6777
17 with $68cms^{-1}$	-0.2458	22.5792	-0.1056	2.8116
34 with $51cms^{-1}$	-4.5792	18.2458	-0.5773	2.3399
34 with $68cms^{-1}$	-3.5792	19.2458	-0.4434	2.4737
51 with $69cms^{-1}$	-10.4125	12.4125	-1.3247	1.5925

Any confidence interval that does not include zero
is significant at the 5% level (*).

(f) the effect of water velocity on the mean interval between fan beats at 12°C in unfiltered natural water.

Water velocity (cms^{-1})	Mean rank	Mean normal-score	n
17	20.00	0.945	7
34	14.20	0.199	5
51	8.57	-0.440	7
68	5.80	-0.905	5

Kruskal-Wallis H Statistic = 14.820 3 df 0.01>P>0.001
Correction term for ties = 0.9996
Normal-scores test W = 14.571 3 df 0.01>P>0.001

Test for monotonic trend over the four groups:
Kendall's Tau = -0.6312, P<0.001

Pairwise contrasts	Based on ranks		Based on normal-scores	
Contrast	Lower confidence limit	Upper confidence limit	Lower confidence limit	Upper confidence limit
17 with 34cms^{-1}	-5.7719	17.3719	-0.7331	2.22
17 with 51cms^{-1}	0.8649	21.9922	0.0347	2.7347(*)
17 with 68cms^{-1}	2.6281	25.7719	0.3706	3.3283(*)
34 with 51cms^{-1}	-5.9433	17.2005	-0.8399	2.1178
34 with 68cms^{-1}	-4.0991	20.8991	-0.4936	2.7011
51 eith 68cms^{-1}	-8.8005	14.3433	-1.0141	1.9436

Any confidence interval that does not include zero
is significan at the 5% level (*).

APPENDIX 7 continued

(g) the effect of water velocity on the mean interval between fan beats at 15°C in unfiltered natural water.

Water velocity (cms^{-1})	Mean rank	Mean normal-score	n
17	16.57	0.512	7
34	11.21	-0.149	7
51	9.83	-0.377	6
68	11.63	-0.070	4

Kruskal-Wallis H Statistic = 3.470 3 df n.s.
Correction term for ties = 0.9991
Normal-scores test W = 3.513 3 df n.s.

Test for monotonic trend over the four groups:
Kendall's Tau = -0.2314 n.s.

APPENDIX 8

The effect of water velocity on the time taken to clean the cephalic fan at three temperatures in particle-free distilled water and three temperatures in unfiltered natural water.

(a) particle-free distilled water, 8°C.

	Water velocity (cms^{-1})			
	17	34	51	68
Specimen	Time taken to clean the cephalic fan (s)			
2	0.182	0.153	0.153	0.145
3	0.160	0.2	0.12	0.151
5	-	0.16	0.18	0.193
6	0.25	0.172	0.176	0.173
8	0.195	0.24	0.149	0.164
9	0.19	-	0.235	-

(b) particle-free distilled water, 10°C

	Water velocity (cms^{-1})			
	17	34	51	68
Specimen	Time taken to clean the cephalic fan (s)			
1	0.215	0.177	0.104	0.126
2	0.153	0.16	0.144	0.168
3	0.175	0.148	-	-
4	0.186	0.154	0.111	0.095
5	0.14	0.16	0.154	0.151
6	0.16	0.154	0.133	0.152

APPENDIX 8 continued

(b) particle-free distilled water, 10°C

	Water velocity (cms^{-1})			
Specimen	17	34	51	68
7	0.17	0.133	-	-
8	0.148	0.16	0.17	-
9	0.194	0.173	-	-
10	0.166	0.192	-	-

APPENDIX 8 continued

(c) particle-free distilled water, 14°C.

	Water velocity (cms^{-1})			
	17	34	51	68
Specimen	Time taken to clean the cephalic fan (s)			
1	0.128	0.149	0.127	0.126
2	0.12	0.106	0.112	0.106
3	0.12	0.12	0.126	0.106
4	0.116	0.12	0.105	0.098

(d) unfiltered natural water, 9°C

	Water velocity (cms^{-1})			
	17	34	51	68
Specimen	Time taken to clean the cephalic fan (s)			
2	0.125	0.156	0.125	0.125
3	0.157	-	0.115	0.142
4	-	0.137	0.12	0.129
6	0.165	0.15	0.153	0.133
7	0.142	0.168	-	-
8	0.165	0.145	0.126	0.126
9	0.154	0.148	0.146	0.126

APPENDIX 8 continued

(e) unfiltered natural water, 12°C.

	Water velocity (cms^{-1})			
	17	34	51	68
Specimen	Time taken to clean the cephalic fans (s)			
1	0.14	0.127	0.127	0.124
2	0.126	-	0.164	0.168
3	0.13	0.128	0.116	0.105
4	0.12	0.123	0.114	-
5	0.128	0.135	0.127	0.115
6	0.125	0.113	0.098	-
7	0.12	-	0.146	0.097

(f) unfiltered natural water, 15°C

	Water velocity (cms^{-1})			
	17	34	51	68
Specimen	Time taken to clean the cepalic fan (s)			
1	0.104	0.102	0.116	0.1
3	0.089	0.082	0.089	-
4	0.091	0.084	-	-
5	0.087	0.081	0.082	0.09
6	0.087	0.077	0.077	0.07
7	0.1	0.094	0.074	-
8	-	0.101	0.1	0.098

APPENDIX 9

The responses of larval S. ornatum to a series of pulses of diatomaceous earth.

Each larva was exposed to up to twenty-five pulses of diatomaceous earth, with twenty seconds between pulses (see Section 6.3 and Figures 28a to d). The behaviour patterns of four selected larvae are shown, using block diagrams, in Appendix Figures 1a to d. A key to the symbols used is given in Table 4a. Specimen 1 in Appendix Figure 1a refers to Specimen 1 in Figure 28a, Specimen 3 in Appendix Figure 1a to Specimen 2 in Figre 28a, and so on.

Appendix Figure 1a. Specimen 1; responses to stimulation with pulses of diatomaceous earth.

Appendix Figure 1b. Specimen 3; responses to stimulation with pulses of diatomaceous earth.

Appendix Figure 1c. Specimen 5; responses to stimulation with pulses of diatomaceous earth.

Appendix Figure 1d. Specimen 12; responses to stimulation with pulses of diatomaceous earth.

Statistical analysis of results presented in Figures 28a to d.

(a) Kruskal-Wallis analysis of variance of the significance of differences in the number of fan cleaning movements made by larvae before and during their exposure to pulses of diatomaceous earth. Group 1 is the number of fan movements recorded during ten second extracts of behaviour before exposure to diatomaceous earth and group 2 the number of fan movements recorded in ten second extracts of behaviour during stimulation with diatomaceous earth. Each value of n refers to a ten second extract of behaviour.

Group	Mean rank	Mean normal-score	Sample size
1	51.60	0.493	15
2	37.94	-0.114	65

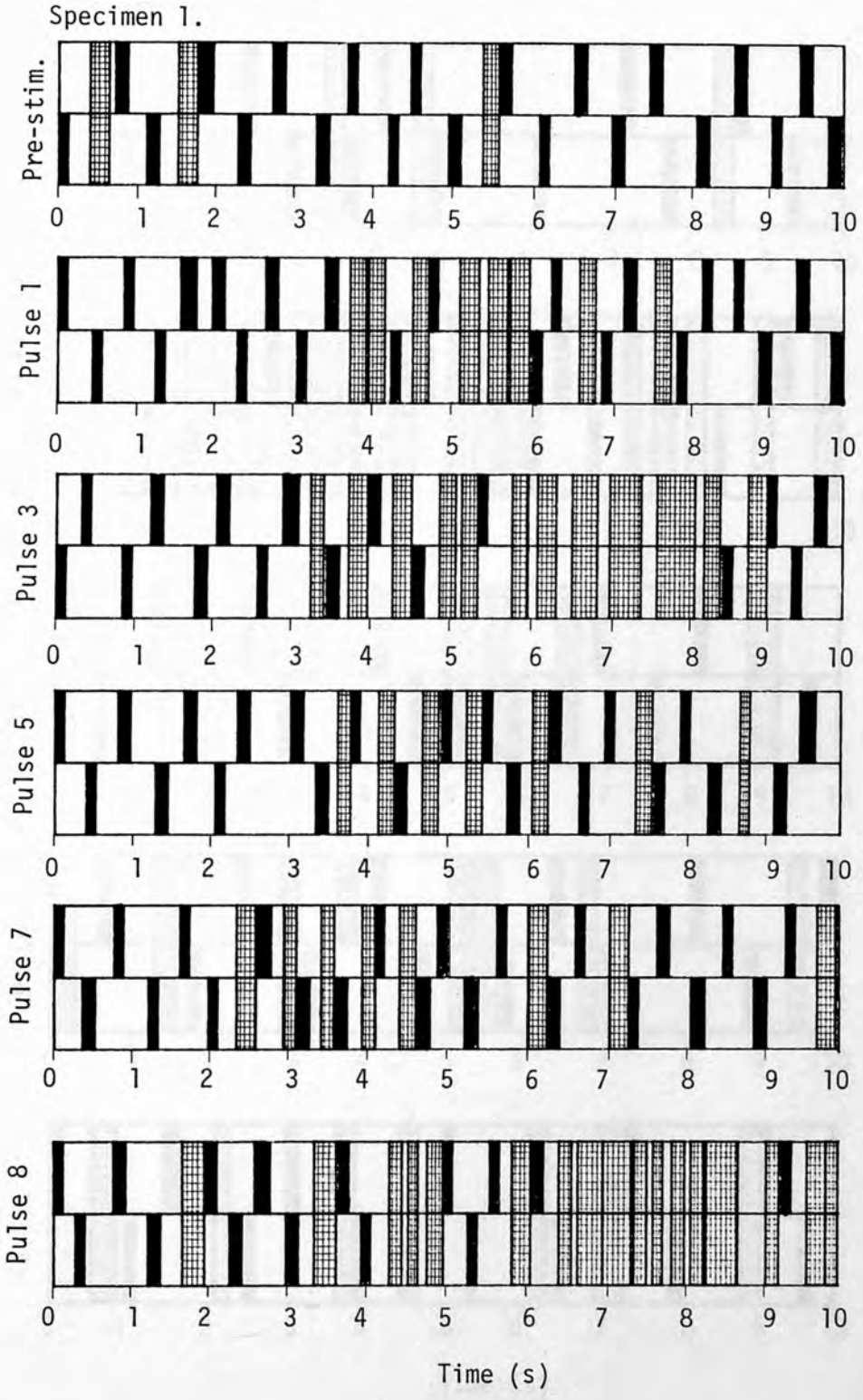
Kruskal-Wallis H Statistic = 4.243 1 df 0.05>P>0.01
Correction term for ties = 0.9927
Normal-scores test W = 4.951 1 df 0.05>P>0.01

(b) Kruskal-Wallis analysis of variance of the significance of differences in the number of mandible movements made by larvae before and during their exposure to pulses of diatomaceous earth. Group 1 is the number of mandible movements recorded during ten second extracts of behaviour before exposure to diatomaceous earth and Group 2 is the number of mandible movements recorded in ten second extracts of behaviour during exposure to diatomaceous earth. Each value of n refers to a ten second extract of behaviour.

Group	Mean rank	Mean normal-score	Sample size
1	12.23	-1.185	15
2	47.02	0.276	65

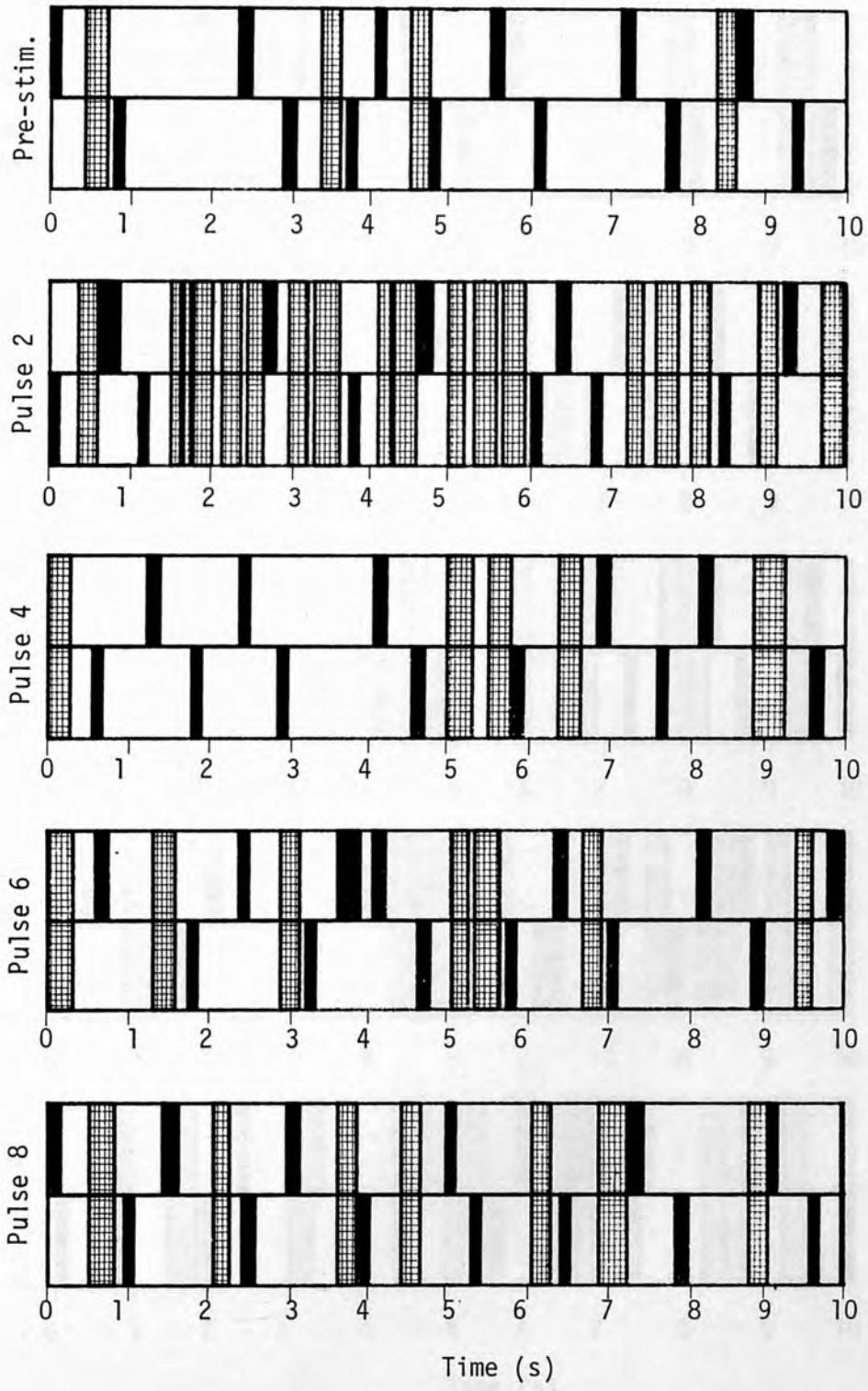
Kruskal-Wallis H Statistic = 27.407 1 df P<0.001
Correction term for ties = 0.9967
Normal-scores test W = 28.782 1 df P<0.001

Appendix Figure 1a (refer to Figure 28)



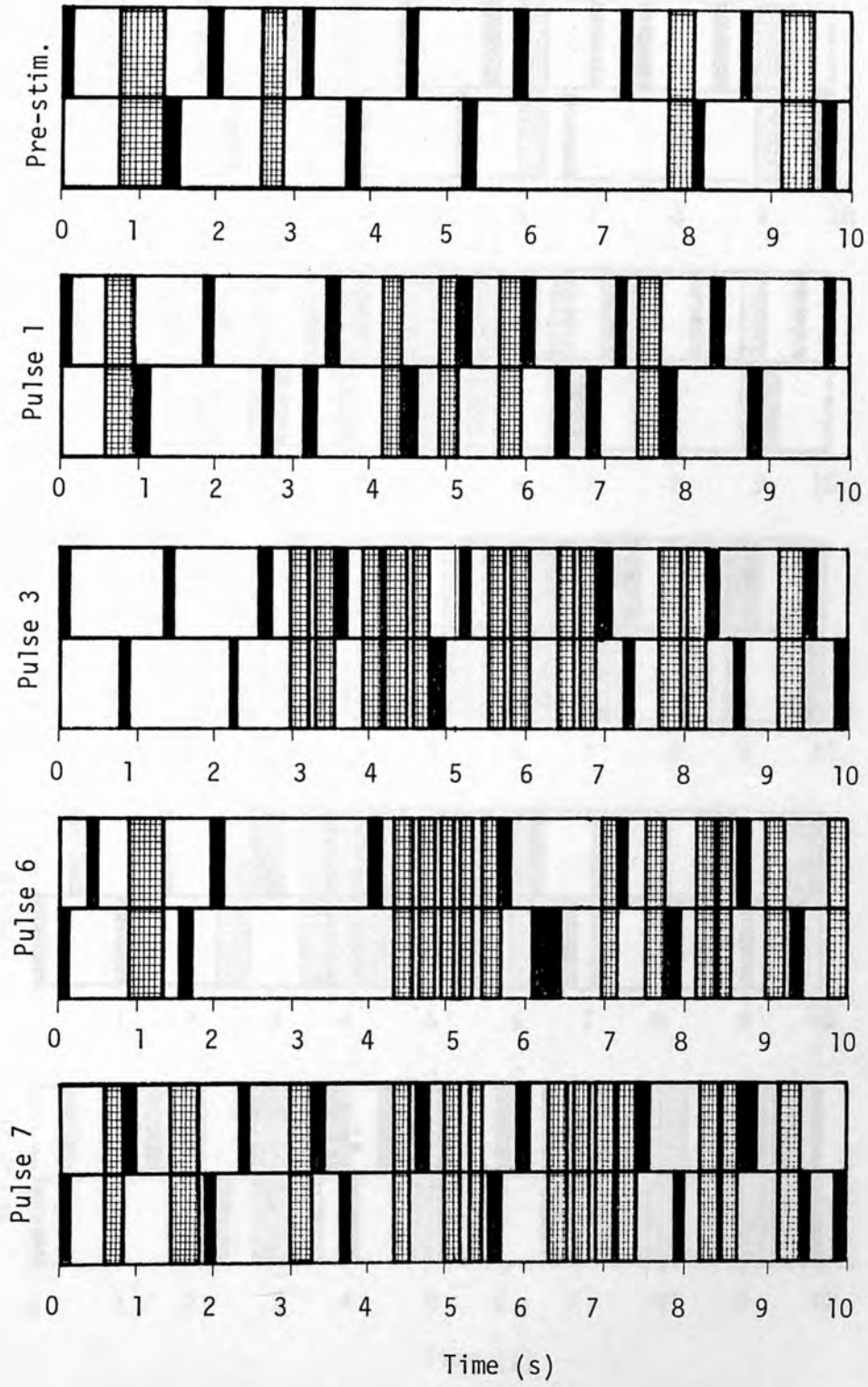
Appendix Figure 1b (refer to Figure 28)

Specimen 3



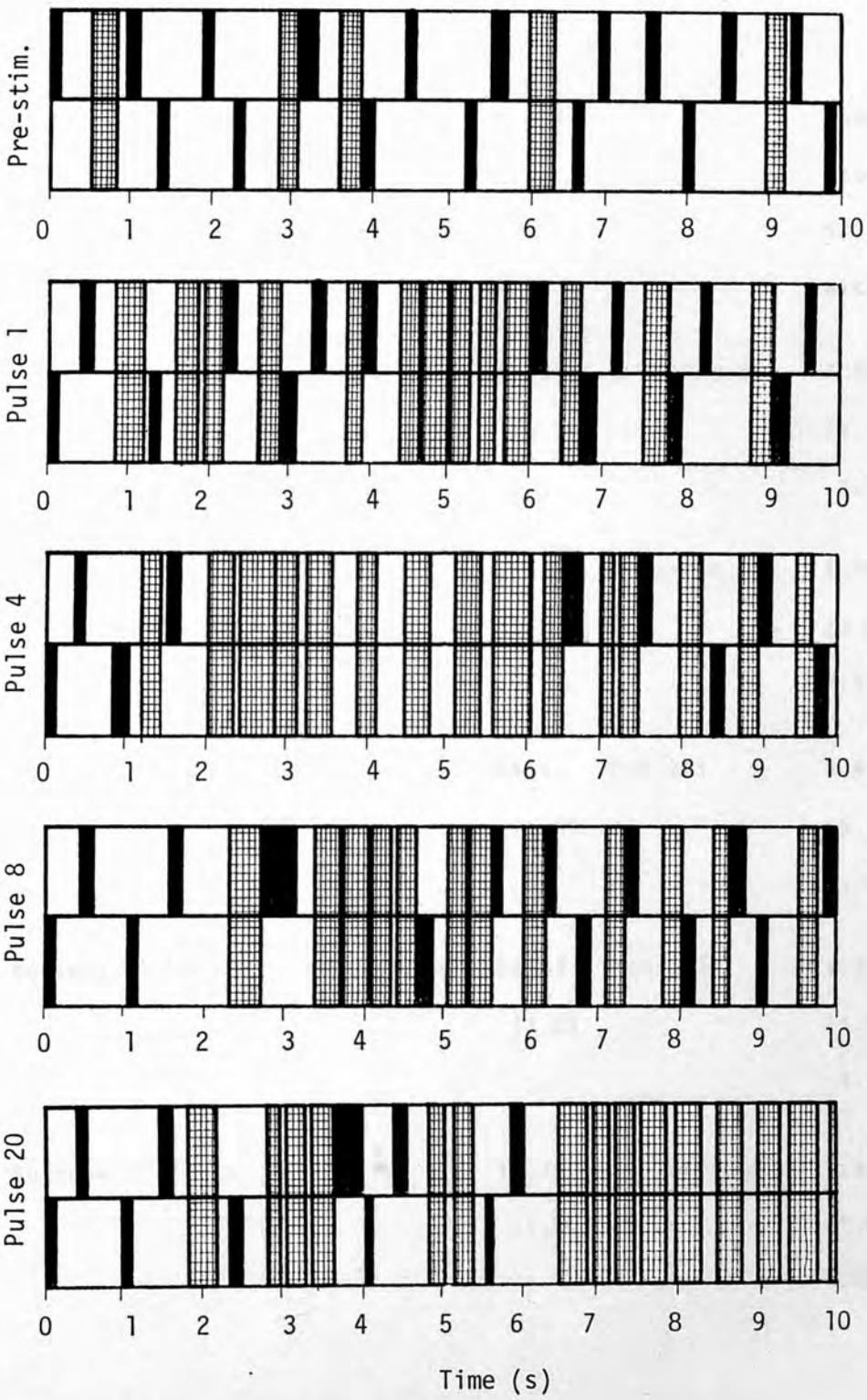
Appendix Figure 1c (refer to Figure 28).

Specimen 5



Appendix Figure 1d (refer to Figure 28)

Specimen 12



APPENDIX 10

Concentration response curves for S. ornatum larvae to HCl, NaCl, Butanol and sucrose. Statistics of regression lines presented in Figure 29.

Compound	Regression		F (df)	P	Concentration stimulating 50% (molar), with CL.
	Slope	Intercept			
HCl (1)	3.175	14.97	39.43 (1,3)	0.01>P>0.001	7.24x10-4 (1.38x10-4, 2.20x10-2)
HCl (2)	2.76	15.77	24.86 (1,6)	0.01>P>0.001	1.25x10-4 (3.38x10-4, 4.16x10-5)
NaCl	3.87	14.34	184.74 (1,3)	P<0.001	3.85x10-3 (5.42x10-3, 3.69x10-3)
Butanol	4.41	13.31	150.57 (1,3)	P<0.001	1.30x10-2 (1.02x10-2, 1.70x10-2)
Sucrose	4.44	7.12	13.72 (1,6)	0.01>P>0.001	$3.33x10^{-1}$ (1.67, 8.22x10-2)

Regression fitted to equation y = a + b (logx).

APPENDIX 11

Kruskal-Wallis one-way analysis of variance of the number of extra mandible movements in response bursts following stimulation with a variety of chemical compounds.

Group	Mean rank	Mean normal-score	n
1 (0.004 NaCl)	18.88	-1.307	21
2 (0.008 NaCl)	66.87	-0.202	34
3 (0.02 NaCl)	83.18	0.036	33
4 (0.0075 Aspartic acid)	90.04	0.155	12
5 (0.00035 HCl)	98.32	0.426	17
6 (0.035 Butanol)	122.77	0.830	15
7 (0.0035 Heptanol)	136.13	1.137	12
8 (0.0035 Amyl acetate)	77.38	-0.055	16

Kruskal-Wallis H Statistic = 74.241 7 df P<0.001

Correction term for ties = 0.9843

Normal-scores test W = 73.803 7 df P<0.001

APPENDIX 11 continued

Pairwise contrasts		Based on ranks		Based on normal-scores	
Contrast		Lower confidence limit	Upper confidence limit	Lower confidence limit	Upper confidence limit
Group 1 with	2	-95.8365	-0.1369	-2.0929	-0.1159(*)
Group 1 with	3	********	-16.1750	-2.3363	-0.3479(*)
Group 1 with	4	********	-8.7722	-2.7501	-0.1724(*)
Group 1 with	5	********	-23.1947	-2.8946	-0.5706(*)
Group 1 with	6	********	-45.6024	-3.3407	-0.9326(*)
Group 1 with	7	********	-54.8555	-3.7325	-1.1548(*)
Group 1 with	8	********	-1.2831	-2.4339	-0.0701(*)
Group 2 with	3	-58.4439	25.8156	-1.1080	0.6326
Group 2 with	4	-81.0631	34.7151	-1.5527	0.8391
Group 2 with	5	-82.6676	19.7558	-1.6861	0.4298
Group 2 with	6	********	-2.4597	-2.1362	0.0718(*)
Group 2 with	7	********	-11.3682	-2.5351	-0.1433(*)
Group 2 with	8	-62.7751	41.7604	-1.2274	0.9322
Group 3 with	4	-64.9773	51.2576	-1.3197	1.0815
Group 3 with	5	-66.6114	36.3280	-1.4538	0.6728
Group 3 with	6	-93.2715	14.1018	-1.9036	0.3146
Group 3 with	7	********	5.1743	-2.3022	0.0991
Group 3 with	8	-46.7137	58.3274	-0.9949	1.1751
Group 4 with	5	-73.2847	56.7209	-1.6142	1.0715
Group 4 with	6	-99.4969	34.0469	-2.0548	0.7040
Group 4 with	7	********	24.3004	-2.4364	0.4716
Group 4 with	8	-53.1713	78.5047	-1.1509	1.5693
Group 5 with	6	-85.5167	36.6304	-1.6657	0.8577
Group 5 with	7	********	27.2013	-2.0539	0.6318
Group 5 with	8	-39.1025	80.9996	-0.7600	1.7212
Group 6 with	7	-80.1302	53.4136	-1.6864	1.0724
Group 6 with	8	-16.5700	107.3534	-0.3954	2.1647
Group 7 with	8	-7.0880	124.5880	-0.1685	2.5518

Any confidence interval that does not include zero
is significant at the 5% level (*).

APPENDIX 12

The responses of larval *S. ornatum* to a series of pulses of diatomaceous earth following treatment with PCMB. The graphs in Figures 35a and b show the number of mandible movements and fan beats in the first ten seconds following each pulse of diatomaceous earth, in larvae with chemical sense organs blocked by PCMB. Each larva was exposed to series of up to twenty-five pulses of diatomaceous earth in a series, with twenty seconds between each pulse. The significance of differences in the numbers of fan and mandible movements before and during stimulation with diatomaceous earth were tested using Kruskal-Wallis one-way analysis of variance.

(a) Kruskal-Wallis one-way analysis of variance of the significance of differences in the number of fan movements before and during stimulation with diatomaceous earth, following PCMB treatment. Group 1, before stimulation with diatomaceous earth; Group 2, during with diatomaceous earth

Group	Mean rank	Mean normal-score	Sample size
1	55.10	0.299	25
2	44.75	-0.109	69

Kruskal-Wallis H statistic = 2.656 1 df n.s.
Correction term for ties = 0.9955
Normal-scores test W = 3.322 1 df n.s.

(b) Kruskal-Wallis one-wau analysis of variance of the significance of differences in the number of mandible movements before and during stimulation with diatomaceous earth, following treatment with PCMB. Group 1, before stimulation with diatomaceous earth; Group 2, during stimulation with diatomaceous earth.

Group	Mean rank	Mean normal-score	Sample size
1	22.50	-0.863	25
2	56.56	0.316	69

Kruskal-Wallis H statistic = 28.717 1 df P<0.001
Correction term for ties = 0.9961
Normal-scores test W = 27.935 1 df P<0.001